Crop competition as a strategy to control glyphosate-resistant *Chloris virgata* and application of biochar to control glyphosate mobility in Australian Soils

By

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Faculty of Science

The University of Sydney

January 2020

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Aman D. Sharma

ACKNOWLEDGEMENTS

I am very thankful to The University of Sydney for providing me with The University of Sydney International Scholarship (USydIS) for my PhD degree. I want to acknowledge radiation project support from Dr Rai Kookana, Principal Scientist, CSIRO, my advisors including Dr Balwant Singh, Dr Guy Roth, Dr Daniel Tan, Dr Peter Sharp, Dr Donna Lai and Dr Sheng Hua.

I appreciate guidance from Dr Helen Bramley and Dr Kedar Adhikari. I want to acknowledge my belated mother, who died of cancer. It was her wish that I should complete this journey. I appreciate my father and brothers, my family members including my wife Shaveta and children (Divya and Aryan).

No words can express my gratitude for laboratory and field staff from Australian Technology Park (ATP) and Plant Breeding Institute (PBI) Narrabri. I deeply acknowledge my friends to keep my spirit high during this journey.

BOOK CHAPTER / MANUSCRIPTS PUBLISHED

- SHARMA, AD (2019) 'Glyphosate resistance of *Chloris virgata* weed in Australia and glyphosate mobility are connected problems' in *Sorption* Intech Open, pp.1-17 **(Chapter 2).**

- SHARMA, AD (2019) Management of glyphosate-resistant *C. virgata* grass in mung bean using crop competition *Acta Scientific Microbiology* **2** (9) 108-113 **(Chapter 3).**
- SHARMA, AD and DANIEL K.Y. TAN (2019) Crop competition as a strategy to control *C.virgata* grass in sorghum *Acta Scientific Agriculture* **3** (9) 176-180 **(Chapter 4).**
- SHARMA, AD, LAI, D (2018) Sorption of radiolabelled glyphosate on biochar aged in contrasting soils. *Journal of Environmental Science and Health Part B* **53** (8) 1-5 **(Chapter 5)**.

CONFERENCES / MEETINGS

- Poster presentation- Crop competition as a strategy to control glyphosate-resistant *C. virgata*, Narrabri Campus, The University of Sydney, 26th September 2017.
- Oral presentation-Glyphosate-biochar-soil interactions on Australian soils of different composition for the New Zealand Society of Soil Science and Soil Science Australia Joint Conference, Queenstown, New Zealand, December 12-16th, 2016.
- Poster presentation-Glyphosate-biochar-soil interactions on Australian soils of different composition. The Veterinary Science Conference Centre, The University of Sydney, 10th October 2015.

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AUTHORSHIP ATTRIBUTION STATEMENT

Name - Aman D.Sharma

Publication details –1. Aman D.Sharma and Donna Lai (2018) Sorption of radiolabelled glyphosate on biochar aged in contrasting soils. *Journal of Environmental Science and Health Part-B* **53** (8) 1-5.

My contribution- Chapter 5 of this thesis (pages 64-69) is showing the abovementioned publication as a part of the thesis for a PhD degree. It was a radiation project in collaboration with the Commonwealth Scientific and Industrial Research Organisation (CSIRO). I designed the experiments following CSIRO protocol, conducted the batch sorption experiments at the Bosch Institute, School of Medical Sciences, The University of Sydney and wrote the draft of the manuscript. I was a chief investigator of this study, while Dr.Donna Lai was the lab in-charge.

2. Aman D.Sharma (2019) Management of glyphosate-resistant *Chloris virgata* grass in mung bean using crop competition *Acta Scientific Microbiology* **2** (9) 108-113.

My contribution- Chapter 3 of this thesis (pages 94-99, Appendix) is showing the abovementioned publication as a part of the thesis for a PhD degree. I designed the experiments, conducted the experiments at IA Watson Grains Research Centre, Narrabri, Australia and wrote the draft of the manuscript.

3. Aman D.Sharma and Daniel K Y Tan (2019) Crop competition as a strategy to control *C.virgata* grass in sorghum *Acta Scientific Agriculture* **3** (9) 176-180.

My contribution- Chapter 4 of this thesis (pages 100-104, Appendix) is showing the abovementioned publication as a part of the thesis for a PhD degree. I designed the experiments, conducted the experiments at IA Watson Grains Research Centre, Narrabri, Australia and wrote the draft of the manuscript, while Dr. Daniel Tan as an auxiliary supervisor reviewed the manuscript.

4. Aman D. Sharma (2019) 'Glyphosate resistance of *Chloris virgata* weed in Australia and glyphosate mobility are connected problems' in *Sorption* Intech Open, pp.1-17.

My contribution- Chapter 2 of this thesis (pages 105-121, Appendix) is showing the abovementioned publication as a part of the thesis for a PhD degree. I wrote the manuscript for this publication..

As a supervisor upon the candidature upon which this thesis is based, I can confirm the authorship attribution statements above are correct.

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Date 17/1/2020

ABSTRACT

The aim of the thesis is to provide solutions to the problems associated with the over-reliance of glyphosate herbicide application resulting in the emergence of resistant weeds like *Chloris virgata* grass and the consequent problem of glyphosate mobility in different soil environments. *Chloris.virgata* is an emerging problem in central Queensland and northern New South Wales. I hypothesised that mung bean and sorghum crops could suppress *C.virgata* at narrower row spacings and higher densities. Pot experiments were established for two seasons in a glasshouse. The data from experiments indicated maximum *C.virgata* biomass of 43-48g dry weight in both seasons, which was significantly reduced by 74 % when surrounded by 5-6 mung bean plants m^{-2}. Similarly, field experiments in two seasons indicated sorghum as a better competitor than *C.virgata*. Significant reductions in *C.virgata* biomass were observed when surrounded by 10 sorghum plants m^{-2} in rows spaced 50 cm apart in comparison to 75 and 100 cm spacings.

To study the problem of glyphosate mobility, the sorption behaviour of glyphosate in four contrasting soil types (Oxisol, Vertisol, Entisol, Inceptisol) amended with aged wood biochar was examined. Batch experiments were conducted using ^{14}C labelled glyphosate and liquid scintillation counting. Significant differences in biochar amended and unamended soils were only found in the Entisol soil system. The sorption behaviour of glyphosate in soil systems was the Oxisol>Vertisol>Entisol>Inceptisol. The Oxisol soil system was found to adsorb five times more glyphosate than the Inceptisol. Inceptisol soil systems showed the lowest amount of glyphosate sorption among all the soils. From the sorption studies, it was clear that the impact of biochar application was soil specific. Biochar can sorb glyphosate in Entisols and Inceptisols but application in Vertisols and Oxisols is ineffective. My thesis found that the strategies of biochar application and crop competition can contribute to overcoming some of the problems related to over application of glyphosate.

CHAPTER 1

INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine) is a non-selective post-emergent herbicide widely used in field crops, vegetable crops and in orchards, as it provides inexpensive and effective control of weeds (Baylis, 2000).It controls a broad spectrum of weeds in different agronomic situations by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is a key enzyme in the shikimate pathway in plants (Amrhein et al., 1980). EPSPS inhibition hinders the production of essential proteins and secondary metabolites controlling the energy pathways in plants (Bai and Ogbourne, 2016).

In Australia, Feathertop Rhodes Grass (FTR) (*Chloris virgata*) is a major glyphosate-resistant weed problem in cotton (*Gossypium hirsutum*) and grain crops of subtropical regions. Weeds like *C.virgata* can no longer be easily controlled with tillage due to regeneration and hence, the reliance on glyphosate has increased (Osten et al., 2007). Previous studies have reported glyphosate resistance in rigid ryegrass (*Lolium rigidum*) in Australia (Powles et al., 1998). *Chloris virgata* has been in the top 10 (national ranking) and top four (northern regional ranking) weed problems in Australia (Llewellyn et al., 2016). Major reasons suggested for *C.virgata* as a weed problem include the adoption of no till practices and the natural tolerance of *C.virgata* to glyphosate (Werth et al., 2013). *Chloris virgata* is responsible for 39,329 tonnes of production losses in all grain crops with a revenue loss of $7.7 million per year in Australia (Llewellyn et al., 2016).

Control of annual grasses with herbicides is difficult due to regeneration of annual grasses from the seed bank (Simmons, 2005). An increase in plant density of the threatened community can suppress the growth of an invasive plant (Simmons, 2005). The selective control of an exotic, invasive annual weed (*Rapistrumrugosum*) by oversowing with a competitive native species (*Gaillardia pulchella*) indicates that crop competition can be a better strategy than herbicide application to control invasive plants (Simmons, 2005).The use of crop row orientation to suppress weed growth has been reported in wheat and barley (Borger et al., 2010). Glyphosate use is an effective strategy for controlling weeds, however, over-reliance should be avoided and other weed management strategies like crop competition should be used in conjunction with glyphosate (Widderick et al., 2013). Effective strategies to avoid, delay or manage glyphosate resistance are of great value to growers for summer grasses (Thornby et al., 2013). Residual weeds have been observed at the end of the crop or fallow in the majority of the initially infested paddocks (Walker et al., 2005).Non-chemical options like crop competition should be incorporated into an integrated weed management program especially to control herbicide-resistant weeds (Wu et al., 2010). Crop competition can be used as a strategy to control glyphosate-resistant weeds like *C.virgata* (Sardana et al., 2017). It is hypothesised that two common grain crop competitors like sorghum (*Sorghum bicolor*) and mung bean (*Vigna radiata*) can control glyphosate resistant *C.virgata* at varying plant densities and row spacings.

In sorghum, *C.virgata* covers an area of 73,414 ha with a yield loss of 36,995 tonnes resulting in revenue loss of $7.0 million per year (Llewellyn et al., 2016), while for crops like canola and pulses, *C.virgata* covers an area of 19,590 ha with a yield loss of 503 tonnes and a revenue loss of $ 249,500 per year (Llewellyn et al., 2016). Sorghum farmers of the subtropical north-eastern region of Australia depend on key herbicides like atrazine, metolachlor, fluroxypyr, 2,4-D amine and glyphosate that are applied alone or mixed (Walker et al., 2005).Very few growers use tillage (0-17%) or crop competition (0-11%) to manage the weeds. The majority use pre-emergence (34-95%) and /or post-emergence (22-57%) herbicides without achieving effective weed control, particularly of grass weeds (Walker et al., 2005). Due to the problem of herbicide resistance, the cost of weed control has increased by \$ 55 ha^{-1}due to more herbicide applications (Bajwa et al., 2017). Thus, non-chemical management options like crop competition should be included to manage weeds for sustainable crop production in Australian conditions (Bajwa et al., 2017).

Mung beans another crop cultivated as a grain legume in central Queensland and northern New South Wales in Australia (Chauhan et al., 2017). It is also grown as a rotation crop due to its ability to fix nitrogen (Rachaputi et al., 2015). The lack of effective herbicides to control glyphosate resistant weeds, weed population, species shifts, increase in herbicide costs and concerns of environmental pollution has resulted in *C.virgata* becoming a major problem (Fernando et al., 2016, Ngo et al., 2017).

Over-reliance of glyphosate application by farmers in their fields to control weeds like *C.virgata* can pollute the aquatic environment through its movement from the soil systems. The widespread application of glyphosate has resulted in global issues of glyphosate transport and contamination risk of aquatic environments in Denmark (Kjaer et al., 2005), Hungary (Mortl et al., 2013), Brazil (Tzaskos et al., 2012) and U.S.A (Battaglin et al., 2014a). It is generally observed that glyphosate has limited risk of leaching due to its strong adsorption to soils and its subsequent rapid degradation by soil microbes (Busse et al., 2001, Borggaard and Gimsing, 2008). However, long-term use of glyphosate may result in pollution of surface , groundwater and of sediments (Kogan et al., 2003, Stewart et al., 2014). For the process of glyphosate adsorption, the type of clay is more important than its percent content (Glass, 1987). Hence, different behaviour patterns of glyphosate adsorption have been observed in different soils. Glyphosate can be extensively mobile in soil systems and a lack of retention of glyphosate for microbial degradation can result in glyphosate leaching into lower soil layers (Piccolo et al., 1994). Low sorption of glyphosate was shown to be the major reason for contamination of groundwater by herbicides in Western Australia (Gerritse et al., 1996).

However, few data have been published with respect to glyphosate behaviour in Australian soils.

Leaching of glyphosate is a global problem. In a study related to leaching of glyphosate and its degradation product AMPA (amino-methylphosphonic acid) in Danish agricultural field sites, strong soil sorption capacity and lack of macropores prevented the leaching of glyphosate and AMPA on sandy soil sites and hence less leaching of glyphosate and AMPA was observed (Kjaer et al., 2005). However, on loamy sites, glyphosate and AMPA were leached via macropores from the root zone to the aquatic environment in amounts exceeding the threshold values of drinking water suggesting a potential risk to the aquatic environment (Kjaer et al., 2005). Glyphosate residues have also been detected in surface waters from transmission from ground waters in catchment areas of Canada (Van Stempvoort et al., 2016) and in epilithic biofilms of southern Brazil (Fernandes et al., 2019). Very few studies related to glyphosate movement have been conducted on Australian soils (Gerritse et al., 1996). An effective strategy is needed to restrict the movement of glyphosate from soil systems to aquatic environments. Thus it is hypothesised that biochar incorporation into soils can be an effective strategy to adsorb organic compounds like glyphosate.

Biochar has large surface area per weight (Beesley et al., 2010) and is capable of improving the water holding capacity, nutrient storage capacities of soils, carbon content of soils and also helping stabilize organic chemicals such as pesticides in soil (Lou et al., 2011). However, these positive effects are dependent on the type of biochar as well as the crops, soil types and climatic conditions involved. There is a lack of knowledge concerning the behaviour of glyphosate in Australian soils in the presence of biochar.

1.1 CENTRAL RESEARCH QUESTION

How aged biochar can determine the fate of glyphosate in soils of different mineral composition and how crop competition can control glyphosate-resistant C.virgata?

Sub-questions

1. Can mung bean suppress *C.virgata* as a crop competitor? (Chapter 3)
2. Can sorghum suppress *C.virgata* as a crop competitor? (Chapter 4)
3. Does the sorption behaviour of glyphosate herbicide change in soils of different mineral composition and can the presence of biochar in soils of different mineral composition affect the sorption behaviour of glyphosate? (Chapter 5).

1.2 OBJECTIVES

The broad objective of the research for this thesis was to connect two major problems arising from the over-reliance of glyphosate application. First is the control of glyphosate-resistant *C.virgata* in two major grain crops and second is the sorption behaviour of glyphosate in different soils, as the two aspects are connected. To address this, the specific aims of the thesis include:

(i) to investigate the competition effect of varying row spacing and densities of mung bean on the growth of *C.virgata* (glasshouse studies for two seasons and field study for one season for competition and phenology aspects);

(ii) to identify the competition effect of varying row spacing and densities of sorghum on the growth of *C.virgata* (field studies for two seasons);

(iii) to quantify the extent of glyphosate sorption in soils of different composition in the presence and absence of biochar (batch experiments in the laboratory).

1.3 THESIS STRUCTURE

The thesis is presented in the format of Thesis Including Publication, which agrees with The University of Sydney's Thesis and Examination of Higher Degrees by Research Policy 2015. The body of the thesis consists of **Chapter 1** (Introduction), **Chapter 2** (Literature review), **and Chapter 6** (General discussion) are in manuscript format, while **Chapter 3** (Management of *C.virgata* in mung bean), **Chapter 4** (Crop competition as a strategy to control *C.virgata* in sorghum) and **Chapter 5** (Sorption of radiolabelled glyphosate) are in journal format.

Chapter 1 includes the introduction to the investigations. **Chapter 2** reviews the literature. Glasshouse studies related to the crop competition involving mung bean and *C.virgata*, including the weed phenology aspects are discussed in **Chapter 3. Chapter 4** investigates the competition between *C.virgata* and sorghum. The competition studies were completed at the Narrabri Campus, The University of Sydney. **Chapter 5** focuses on the adsorption behaviour of the glyphosate molecule in Australian soils of different composition. This study was completed in a series of batch experiments at the Bosch Institute, The University of Sydney. Finally, an overall discussion and conclusions based on the results are presented in **Chapter 6**.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The purpose of the literature review is to address two major aspects of glyphosate application on farmers' fields. The first aspect is glyphosate mobility, which is directly controlled by soil sorption processes and indirectly by molecule degradation processes. Glyphosate mobility is a global problem, as excessive glyphosate residues have been observed in groundwater, drinking water and urine of subsistence farmers from intensive agricultural localities (Osten and Dzul-Caamal, 2017).

In a study related to glyphosate sorption/desorption on biochars, biochar can limit glyphosate transport in soil and remediate contaminated water (Hall et al., 2018). In a similar study related to sorption and desorption of glyphosate in soils of Argentina, high glyphosate sorption and low desorption prevented groundwater contamination (Ortiz et al., 2017). Biochar can increase glyphosate sorption in the soil, although, the effect of biochar on glyphosate sorption depends on prevailing soil physicochemical properties (Kumari et al., 2016). Biochar application can be a potential strategy to control glyphosate leaching depending on sorption behaviour of glyphosate on Australian soils of different composition.

The second important aspect is the development of glyphosate resistance in weeds. Crop competition is a potential strategy to manage this problem. The literature review broadly focuses on the chemistry of the glyphosate molecule, its sorption behaviour in different soil systems, evolution of glyphosate resistance in weeds like *C.virgata* and approaches like biochar as a possible method to control glyphosate leaching and crop competition as a cultural method to control a glyphosate-resistant weed like *C.virgata*.

2.1.1 CHEMISTRYOF GLYPHOSATE

Glyphosate (N-(phosphonomethyl) glycine) is a non-selective post-emergence herbicide widely used in field crops, vegetable crops and in orchards. Glyphosate is absorbed by plants via leaves and shoots and is translocated throughout the plant. Its usual formulation is the salt of a deprotonated acid of glyphosate and a cation, e.g. isopropylamine or trimethylsulfonium. Its chemical structure has three groups (amine, carboxylate, and phosphonate) that form strong coordination bonds with metal ions to form bidentate and tridentate complexes (Fig.2.1). Hence it is a strong chelating herbicide (Subramaniam and Hoggard, 1988, McBride, 1991, Sundaram and Sundaram, 1997).

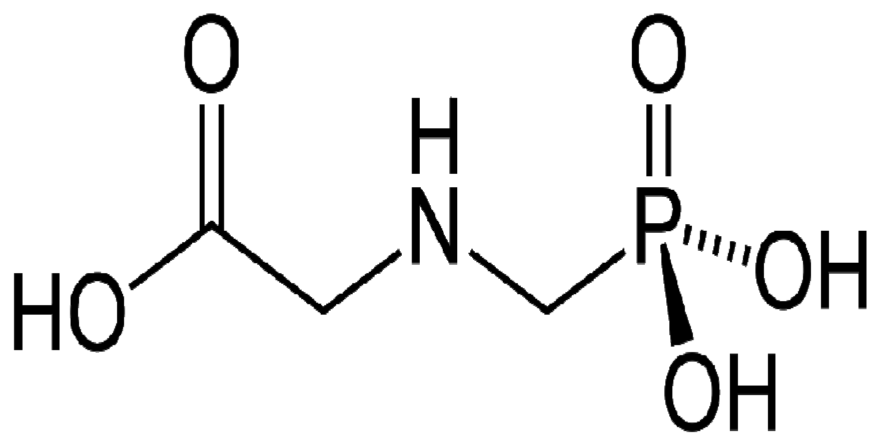

FIGURE 2.1 Chemical structure of glyphosate (Sundaram and Sundaram, 1997)

Chemically, glyphosate is a phosphonate (Borggaard and Gimsing, 2008). It is mainly the phosphonate group via which glyphosate is bonded to iron and aluminium oxides by ligand exchange with the formation of mononuclear, monodentate and/or binuclear, bidentate surface complexes (Gimsing and Borggaard, 2007). In a study related to the effect of pH on glyphosate adsorption, the pH of the solution influenced the electrical charge on glyphosate (Day et al., 1997).

2.1.2 GLYPHOSATE DEGRADATION

Among the micro-organisms, bacteria represent the majority of the glyphosate degrading organisms (Zhan et al., 2018). Bacteria degrade glyphosate by cleaving the C-N bond and convert glyphosate to AMPA which is further decomposed to methylamine. Glyphosate degradation can also occur via C-P lyase pathway to sarcosine, rather than AMPA (Sviridov et al., 2012). A bacterial strain *Bacillus subtilis* Bs-15 degraded 18% (12 h) to 67% (96 h) of glyphosate in sterile soil and 19% (12 h) to 72 % (96 h) in unsterilized soil. Glyphosate degradation was quantified using spectrophotometric method. It indicates that Bs-15 can significantly enhance glyphosate degradation and can be used for bioremediation of glyphosate contaminated soils (Yu et al., 2015).

2.1.3 MOBILITY OF GLYPHOSATE

The binding mechanisms of clay minerals and organic colloids results in non-occurrence of free glyphosate but leaching of glyphosate-soil organic matter complexes via preferential flow paths through the soil and transfer to waterways can occur, which could be a concern from the environmental pollution point of view. Quantum chemical bonding indicated that glyphosate interacted with soil organic matter via H-bond (Gros et al., 2017). In another study related to the desorption rate of glyphosate from goethite mineral surfaces, the rate of glyphosate desorption was mainly dependent on the breaking of the Fe-glyphosate bond via a dissociative or a dissociative interchange mechanism (Arroyave et al., 2017). Soil redox condition is also an important factor controlling the mobility of glyphosate. Microbial degradation and mineralization of glyphosate were slow in anoxic environments compared with oxic environments (Kanissery et al., 2015).

In US soils, glyphosate and AMPA have been detected together and found widely in the environment. The occurrence was more frequent in soils and sediments, ditches and drains, rivers and streams and less in lakes, ponds, wetlands, soil water and groundwater (Battaglin et al., 2014b). In western Switzerland, the surface runoff has been suggested as the major reason for the occurrence of glyphosate and AMPA in surface waters (Daouk et al., 2013). However, in a study related to Danish soils, limited leaching of glyphosate was reported in non-structured sandy soils, while sub-surface leaching to drainage systems was observed in a structured soil when high rainfall followed glyphosate application (Borggaard and Gimsing, 2008, Vereecken, 2005).

In a study related to ^{14}C glyphosate transport in undisturbed topsoil columns, due to large number of macropores, the amounts of glyphosate leached from the sandy loam were 50-150 times more than that from the sandy soil (de Jonge et al., 2000).

2.1.4 GLYPHOSATE RESIDUES

Glyphosate and its decomposition product AMPA have been reported in stream water samples in areas of Zurich, Switzerland with median concentrations of 0.11 and 0.20 μg/l. However, these compounds were not detected in groundwater (Poiger et al., 2017).

In a Canadian study, glyphosate residues were observed in both upland and wetland settings, although, the concentrations were well below the Canadian guidelines for drinking water quality (Van Stempvoort et al., 2016). Many other studies have reported glyphosate residues in streams and groundwater systems (Battaglin et al., 2014b, Daouk et al., 2013).

An enzyme-linked immunosorbent assay (ELISA) was used to determine glyphosate presence levels in Hungarian water samples. Few samples showed exceedingly high concentration (0.54-0.76 ng/ml) levels of glyphosate with this method (Mortl et al., 2013). The study showed that the glyphosate concentrations varied in samples due to different agricultural location. Liquid chromatography is another method that can be used for the detection of glyphosate residues in cereal, oilseed, and pulse crops (Wigfield and Lanouette, 1991).

2.1.5 SOIL PROPERTIES AND GLYPHOSATE MOBILITY

Data from sorption studies indicated that sorption coefficients are the most sensitive parameters for environmental risk assessment. Soil properties like pH and clay content govern the glyphosate adsorption in Argentinian soils (De Geronimo et al., 2018). In a related study in Argentina, high glyphosate sorption with low desorption in mollisols and ultisols indicated a low risk to groundwater contamination (Ortiz et al., 2017).

In another study on glyphosate mineralization in different agricultural soils, exchangeable acidity (H^+ and Al^{3+}), exchangeable Ca^{2+} ions and ammonium lactate extractable K were the key soil parameters governing mineralization (Nguyen et al., 2018). In a study related to glyphosate sorption with high soil phosphate levels, glyphosate sorption distribution constant K_d in soils ranged from 173-939 kg $^{-1}$ under very strong to strongly acidic conditions but the K_d was always < 100 kg $^{-1}$ under moderately acidic to slightly alkaline conditions suggesting that glyphosate may become mobile by water in soils with high phosphate levels (Munira et al., 2016). This is important with respect to the application of phosphatic fertilizers, as the phosphate ion would desorb glyphosate from adsorption sites resulting in mobility of glyphosate towards aquatic environments (de Jonge et al., 2001).

Generally, iron and aluminium oxides adsorb a greater amount of glyphosate and phosphates in comparison with layer silicates (Gimsing et al., 2004) indicating the role of soil mineralogy with respect to glyphosate sorption. As high phosphorus application can desorb glyphosate from sorption sites, application of char can be effective in these scenarios with respect to sorption of glyphosate. The rapid degradation of glyphosate in surface waters and its practically irreversible sorption indicated a low potential environmental risk (Maqueda et al., 2017).

An investigation on adsorption of the herbicide glyphosate and its main metabolite AMPA, found $pH_{(CaCl2)}$values, available phosphate and an amorphous iron and aluminium contents were the major parameters to predict the adsorption constants for these molecules (Sidoli et al., 2016). In a similar study, while examining the effect of humic acid (HA) on the adsorption/desorption behaviour of glyphosate on goethite minerals, glyphosate was desorbed by two parallel processes (i) a direct detachment from the surface, which is first order in adsorbed glyphosate (ii) a ligand exchange with HA molecules (Arroyave et al., 2016). Glyphosate adsorbs on humic acids via hydrogen bonding (Piccolo et al., 1995).

A laboratory study related to the fate of glyphosate and degradation in cover crop residues and underlying soil indicated that the differences in sorption and degradation levels were due to differences in the composition of the crop residues and availability to microorganisms (Cassigneul et al., 2016). In another study related to mobility of glyphosate in different soils under no-till and conventional tillage, adsorption of glyphosate was mainly controlled by the soil clay content and cation exchange capacity (CEC) and negatively correlated to pH and phosphorus. High Freundlich parameter (K_F) values obtained in isotherm studies were the dominant factor influencing glyphosate mobility. K_F values indicate the adsorption capacity of soil (Okada et al., 2016).

2.1.6 METHODS TO UNDERSTAND GLYPHOSATE MOBILITY

Sorption coefficients provide accurate information needed for reliable risk assessments of groundwater contaminants by pesticides (Paradelo et al., 2015). Empirical constants (K_F) of Freundlich sorption isotherm were 16.6 for the clay loam, 33.6 for the silty clay loam and 34.5 for the sandy clay loam indicating that it is the soil structure which dictates the glyphosate sorption behaviour (Al-Rajab et al., 2008). Leaching of glyphosate was dependent on hydrodynamic and biodegradation properties of soils (Al-Rajab et al., 2008). Application of char can be used as a strategy to increase the sorption of glyphosate (Hagner et al., 2015).

Movement of pesticides, their bioavailability and biotransformation are controlled by adsorption-desorption mechanisms operating at the interface between organic and inorganic soil colloids. High-resolution magic angle spinning, and nuclear magnetic resonance techniques can distinguish mobile and immobile phases of pesticides like glyphosate (Chamignon et al., 2008). Another study on glyphosate transport parameters suggested that glyphosate sorption is a kinetic process that depends on pore-water velocities and residence time of soil solution (Candela et al., 2007).

2.1.7 WHY GLYPHOSATE APPLICATION ON FIELD SITES IS A CONCERN

The International Agency for Research on Cancer (IARC) has reclassified that glyphosate is "probably carcinogenic to humans" (Myers et al., 2016), however, the United States Environment Protection Agency (US EPA) concluded that there is no convincing evidence that "glyphosate induces mutations" (Benbrook, 2019). The US EPA relied mostly on unpublished regulatory studies, 99% of which were negative, while IARC relied mostly on peer-reviewed studies,70% of which were positive (Benbrook, 2019). Glyphosate-based herbicides have been observed to pollute drinking water sources, air, and rainfall in agricultural regions (Myers et al., 2016). As the usage of glyphosate-based herbicides continues to increase, investment in epidemiological studies, biomonitoring, and toxicology studies based on the principles of endocrinology should be done (Myers et al., 2016). Apart from cancer, glyphosate has been found to be a potential factor causing chronic kidney disease due to drinking water faced by Sri Lankan farmers (Fig. 2.2) (Jayasumana et al., 2014). The role of drinking water has also been reported in another study which caused ill health in Indian farmers (Reddy and Gunasekar, 2013).

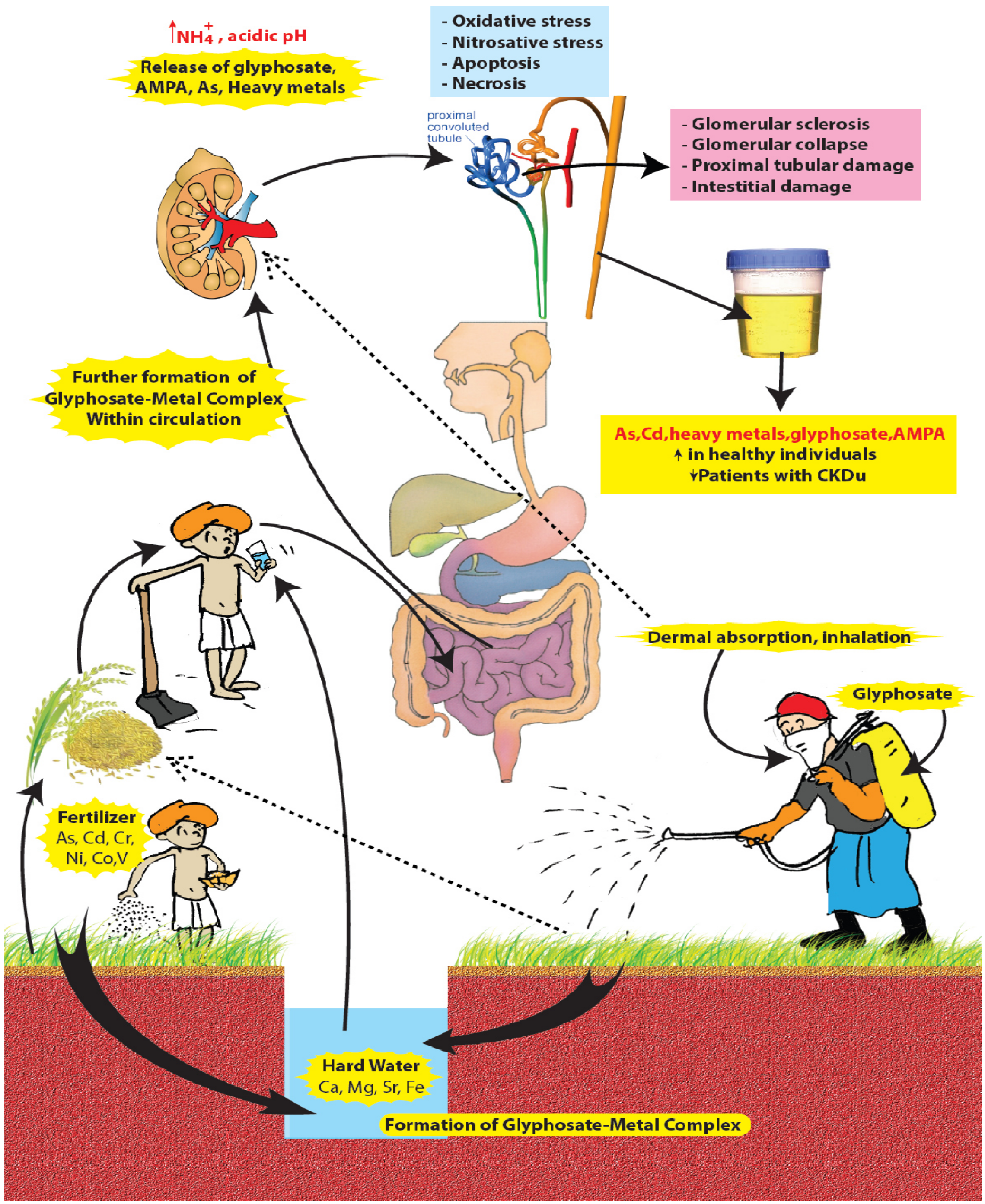

FIGURE 2.2 Glyphosate-metal-complexes, a potential reason for chronic kidney disease in Sri Lankan farmers (Jayasumana et al., 2014)

2.1.8 BIOCHAR’S POTENTIAL ROLE AS A SORBENT FOR ORGANIC POLLUTANTS LIKE GLYPHOSATE

Biochar can be defined as “the porous carbonaceous solid produced by the thermo chemical conversion of organic materials in an oxygen-depleted atmosphere that has physicochemical properties suitable for safe and long-term storage of carbon in the environment” (Shackley et al., 2014). Biochar and activated charcoal are similar with respect to production via pyrolysis, with medium to high surface areas (Cao and Harris, 2010). However, biochar is not activated or treated like activated charcoal (Ahmad et al., 2012, Cao and Harris, 2010). Crop residues are pyrolyzed at high temperature (> 500°C) in the absence of oxygen, followed by various activation processes to form activated charcoal (Cao and Harris, 2010). In comparison to activated charcoal, biochar has a non-carbonized fraction that interacts with soil contaminants like glyphosate. Soil minerals can increase the surface area and pore size of biochar, which in turn increase the adsorption capacity of biochars for organic pollutants like glyphosate (Zhao and Zhou, 2019). Biochar application can reduce the bioavailability and leachability of organic pollutants in soils through adsorption and other physicochemical reactions (Zhang et al., 2013). An increase in the surface area of biochars can increase the biochar’s ability to adsorb organic contaminants (Yu et al., 2006, Wang et al., 2010). The addition of phosphorus fertilizer to biochar amended soils can, however, remobilize glyphosate and damage non-target plants; therefore, improved understanding of this risk is important (Hall et al., 2018).

Factors affecting biochar’s performance for adsorption include pyrolysis temperature and surface area. Pyrolysis temperature is one of the factors directly affecting biochar’s performance. An increase in pyrolysis temperature of biochar generally increases the degree of carbonization and consequently surface area.

With an increase in surface area of biochars, sorption sites can be blocked by organic matter resulting in the diminished capability of aged biochars to adsorb organic contaminants (Yang and Sheng, 2003). The behaviour of biochar changes with time after its application to soil and this process is known as "ageing" (Martin et al., 2012). Ageing can alter the behaviour of biochar. To increase the remediation efficiency of biochar with respect to herbicides, more detailed research to explore the aspect of ageing is warranted.

2.1.9 BEHAVIOUR OF HERBICIDES IN A SOIL-BIOCHAR SYSTEM

In a study related to herbicide terbuthylazine-biochar-soil interaction, there was higher adsorption of herbicide in soil with low organic matter than in soil with the high organic matter. The reason for this result was attributed to a high concentration of organic molecules competing with herbicide for sorption sites in the soil having a high amount of organic matter (Wang et al., 2010). The sorption behaviour of glyphosate is similar to terbuthylazine in the presence of organic matter. Availability of herbicides can be greatly reduced with the application of biochar (Hildebrandt et al., 2009). Even a low application rate (0.1%) of biochar in the soil can appreciably reduce the availability of herbicides like diuron (Yang and Sheng, 2003).

In a comparative study (Song and Guo, 2012), 42 times higher hexachlorobenzene sorption by biochar than that by control soil was observed resulting in the reduction of volatilization and earthworm (*Eisenia foetida*) uptake of hexachlorobenzene from the soil. The extent of sorption of pesticides generally depends on the aromaticity of soil organic carbon. Properties that make biochars effective against herbicides are a high specific surface area, high microporosity, and high aromatic carbon.

2.1.10 THE BEHAVIOUR OF GLYPHOSATE IN A SOIL-BIOCHAR SYSTEM

Plant uptake of pesticides decreases markedly with increasing biochar content of the soil despite the greater persistence of the pesticide residues in biochar-amended soils (Yu et al., 2009). In a similar study related to the effects of biochar, wood vinegar and plants on glyphosate leaching and degradation, the addition of biochar to the soil decreased the leaching of glyphosate irrespective of plants. Hence, it was concluded that biochar can be used as an effective strategy to reduce the potential environmental risk to aquatic environments caused by glyphosate (Hagner et al., 2015).

In a study related to the effects of wood-based biochar on the leaching of pesticides chlorpyriphos, diuron, and glyphosate, it was concluded that biochar can be used as an adsorptive layer directly on or close to the soil surface to prevent losses of pesticides (Cederlund et al., 2017). In another study, biochar was found to limit glyphosate transport in soil systems. However, the addition of phosphatic fertilizer remobilized the glyphosate from biochar-amended soils. This phosphate- induced glyphosate desorption phenomenon is important to consider in soils having biochar amendment (Hall et al., 2018). Type of biochar also plays an important role, as hardwood biochars have been found to be ineffective sorbents of glyphosate in high-phosphate soils (Hall et al., 2018). Biochars produced at high temperature were found to be effective sorbents of glyphosate (Hall et al., 2018). Reduced glyphosate sorption on biochars was observed with the increase in pH from 6 to 9. Glyphosate adsorption was found to be maximum in the pH range of 5-6 (Herath et al., 2016, Mayakaduwa et al., 2016, Hall et al., 2018).

2.1.11 GLYPHOSATE-RESISTANT WEEDS

The second major aspect in this literature review is the evolution of glyphosate resistance in weeds due to heavy reliance on glyphosate. Glyphosate toxicity and glyphosate resistance are not different but connected problems, as glyphosate is applied to control weeds and its application results in movement of glyphosate to water bodies via soil systems affecting human health. When glyphosate contaminated drinking water is used for human consumption, it may potentially result in diseases like cancer or chronic kidney disease. However, frequent application of glyphosate not only results in its downward movement via soil systems but also results in the evolution of glyphosate resistance in weeds. Hence these problems are interconnected.

Thirty-eight weeds in total distributed over thirty-seven countries have shown resistance to glyphosate (Heap and Duke, 2018). These weeds represent the greatest threat to sustainable weed control practices (Heap and Duke, 2018).Weed surveys in the cotton-growing areas of New South Wales (NSW) and Queensland, Australia, showed the dominance of *Conyza bonariensis*, *Echinochloa colona* and *C.virgata* (Manalil et al., 2017).While assessing the weeds at risk of evolving glyphosate resistance in Australian sub-tropical glyphosate–resistant cotton systems, species with the highest risk to glyphosate resistance were *Brachiaria eruciformis*, *Conyza bonariensis*, *Urochloa panicoides*, *C.virgata*, *Sonchus oleraceus* and *Echinochloa colona* (Werth et al., 2011).

Chloris virgata is a high-risk species to glyphosate resistance in summer fallow (Werth et al., 2011). Glyphosate resistance in *C.virgata* populations in Australia has emerged due to transformation in Australian cropping systems from regular tillage and use of residual herbicides to minimum or no-tillage systems with a heavy reliance on glyphosate (Werth et al., 2013). This lack of tillage is the major reason for the emergence of weeds like *C.virgata* (Werth et al., 2013). A weed management system depending on only one tactic, for example, application of glyphosate, is the main driver for this species shift. With repeated use of glyphosate, *C.virgata* populations have become less susceptible to glyphosate formulations, especially after the early tillering stage (Werth et al., 2013).

Mechanisms involving resistance to glyphosate in weeds include (i) target-site alterations (target site mutation, target site gene amplification) (Baerson et al., 2002, Malone et al., 2016) (ii) Non-target site mechanisms involving reduced glyphosate uptake and/or reduced translocation of glyphosate (Lorraine-Colwill et al., 2002, Bostamam et al., 2012, Ghanizadeh et al., 2015). The alterations inhibit glyphosate binding or increase the effective dose needed for enzyme inhibition. Target site EPSPS- mutations have been found to be the primary mechanism conferring glyphosate resistance in populations of *C.virgata* (Baerson et al., 2002).

2.1.12 *Chloris virgata*

Chloris virgata as a glyphosate-resistant weed (Werth et al., 2011) has also been observed as a host for barley yellow dwarf and cereal yellow dwarf viruses (Hawkes and Jones, 2005). *Chloris virgata* can tolerate high salinity and alkalinity soil environments, *C.virgata* can form a dominant community in these environments (Li et al., 2009, Yang et al., 2009). *Chloris virgata* is tolerant to drought stress (Li et al., 2006). Many studies on *C.virgata* seed biology have been completed in China, India, Qatar, and Honduras (Li et al., 2006), while very few studies have been conducted in Australia (Fernando et al., 2016, Ngo et al., 2017).

Chloris virgata grass seed biology includes the study on dormancy, germination conditions, seed bank dynamics, growth and development (Mennan and Ngouajio, 2006). Dormancy mechanisms enable the seed to sense the optimum environmental conditions for the establishment of seedlings and hence play a pivotal role in control strategies for weedy grasses (Adkins et al., 2002). There are two types of seed dormancy mechanisms, those based in the tissues surrounding the embryo (seed coat based) or those found within the embryo (Adkins et al., 2002). The role of smoke in breaking the dormancy of plump windmill grass (C*hloris ventricosa.*), a related species to *C.virgata* grass (Read and Bellairs, 1999) has been reported; but no study related to dormancy break down of *C.virgata* grass by smoke has been reported. The seeds of *C.virgata* are triangular in shape, light in weight and hence shed easily from the heads making them good wind (anemochory) and water (hydrochory) dispersers (Fernando et al., 2016).

Seed germination is a major event in the growth of annual plants like *C.virgata* grass which is regulated by several environmental factors such as temperature and water potential

(Chachalis and Reddy, 2000, Alvarado and Bradford, 2002, Koger et al., 2004). High rainfall has been associated with *C.virgata* population outbreaks (Pezzani and Montana, 2006), suggesting that water plays an important role in the germination process. *Chloris virgata* grass possesses a C_4 photosynthesis mechanism and has better water use efficiency than grasses having the C_3 photosynthesis mechanism (Fernando et al., 2016). Among all the potential factors for *C.virgata* germination; light, salinity, and osmotic potential are the most critical factors (Fernando et al., 2016). A light requirement for germination has been observed among many small-seeded species and warm-season grasses (Grime et al., 1981, Adkins et al., 2002). In a study related to germination responses of *C.virgata* to temperature and reduced water potential, maximum germination percentages of *C.virgata* seeds were found at 15-25°C (Lin et al., 2016a). Germination of *C.virgata* seeds is affected by several factors, however, temperature and light play a significant role in the germination of *C.virgata* seeds (Lin et al., 2016b) . More studies on factors affecting *C.virgata* growth are needed due to the paucity of information.

In a study related to growth, development and seed biology of *C.virgata* in South Australia, *C.virgata* seedlings required 1,200 growing degree days from emergence to mature seed production (Ngo et al., 2017). Harvested seeds of *C.virgata* were dormant for a period of about 2 months and took 5 months of after-ripening to reach 50 % germination (Ngo et al., 2017). Seedling emergence of *C.virgata* was highest (76%) for seeds present on the soil surface and seedling emergence was significantly reduced by burial at 1 (57%), 2 (49%) and 5cm (9%) soil depth. Furthermore, *C.virgata* seeds buried in the soil persisted longer than those left on the soil surface (Ngo et al., 2017).

The thermal time to panicle emergence of *C.virgata* is similar to shattercane (*Sorghum bicolor*) (Donatelli et al., 1992). A related species of *C.virgata*, windmill grass (*Chloris truncata*) under irrigated field requires 21-23, 43-45 and 74-75 degree days from seedling emergence to reach tillering, panicle emergence and mature seed stage (Ngo et al., 2017). Maximum plant density and biomass in the case of windmill grass has been found to be 4.2-28.2 plants m^{-2} and 8.3-146.1g dry biomass m^{-2} depending on location (Borger et al., 2011).

Low rainfall over the summer months can delay the growth of *C.virgata* under rained conditions when compared with irrigated conditions (Ngo et al., 2017). Under irrigated conditions, 619 to 730 g of dry biomass m^{-2} of *C.virgata* (89 days after sowing) was observed, however, this value was much higher in comparison with one of its related species, windmill grass (*Chloris truncata*) (146 g m^{-2}) (Ngo et al., 2017).

Chloris virgata has several characteristics like rapid germination and low base temperature (2.1 to 3.0°C) for seed germination enabling it to survive rainfall events in spring, summer, and autumn in South Australia (Ngo et al., 2017). A base temperature is the temperature below which no development takes place.

2.1.13 EVOLUTION OF GLYPHOSATE RESISTANCE IN *Chloris virgata*

On a national ranking basis in Australia, *C.virgata*, as a herbicide-resistant weed ranks ninth, with a herbicide-resistant weed cost of $2.6 million (Llewellyn et al., 2016). In the northern region of Australia, it is the fourth top herbicide-resistant weed after ryegrass (*Lolium rigidum*), wild turnip (*Brassica rapa*), and barnyard grass (*Echinochloa crus-galli*) (Llewellyn et al., 2016).

The factors that aided the adoption of minimum tillage systems in Australian cropping systems include: machinery modifications, precision agriculture, controlled traffic farming, improved crop resistance, crop options and rotations, development of effective herbicides and the use of genetic modification technologies to breed herbicide-resistant crops (Thomas et al., 2007).

No tillage has increased the use of herbicides and consequently increased the rapid appearance of herbicide resistance in weeds (Ngo et al., 2017). Another reason for evolution is the introduction of glyphosate-tolerant crops since mid-1990s that has resulted in a sharp increase in the populations of *C.virgata* (Powles, 2008).

Glyphosate resistance was first reported in broadleaf *Conyza* (horseweed) species. The mechanism suggested for resistance was an altered sub cellular distribution resulting in sequestration of the glyphosate molecule away from the enzyme target site in the chloroplast (Kleinman and Rubin, 2017).Weeds receiving repeated exposure to a single mode of action of herbicide are the most likely candidates to develop resistance (Heap, 2014).

From the evolution point of view, no tillage along with reliance on glyphosate has contributed the most towards glyphosate resistance in *C.virgata*. The evolution of glyphosate resistance in *C.virgata* necessitates the need for diversity in weed management strategies for successful control of *C.virgata* and other *Chloris* species (Heap, 2014).

2.1.14 CROP COMPETITION AS A STRATEGY TO CONTROL *Chloris virgata*

Crop competition can be used as an effective strategy against *C.virgata*, especially when herbicides like glyphosate fail or underperform (Wu et al., 2010). Crop competition to control weeds has proven to be an effective cultural strategy in Australian cropping systems, aiming at suppression of weed biomass and fecundity resulting in crop yield gains (Lemerle et al., 2014). For crops having long critical periods, effective weed management at the early stages of approximately 6 to 10 weeks is required. Three major weed variables that affect crop-weed competition are:

(i) time of emergence of the weed relative to the crop; weeds that emerge later than the crop are much less competitive than the weeds that emerge before the crop;

(ii) weed seedling density is the second most important factor influencing weed-crop competition; and

(iii) differences in the competitive ability of weeds due to rapid leaf area development, high-density root systems and plant heights (Swanton et al., 2015).

Crop and weed plants compete for limited resources like water, nutrients, and light. Competition for nutrient uptake is dependent on intrinsic nutrient requirements and uptake efficiencies. Uptake efficiencies are further dependent on root length densities and nutrient membrane transporters. Species with a low nutrient requirement, extensive root systems, and effective membrane transporters will have a competitive advantage in a nutrient-limited system (Swanton et al., 2015).

Crop and weed plants compete for water, as water is required for plant growth. In the absence of water, a reduction in photosynthesis, wilting and nutrient deficiencies can occur. The length, magnitude and timing of the drought periods as well as soil attributes (water holding capacity, texture, structure and hydraulic conductivity), plant traits (root structure and density, drought tolerance, and water use efficiency) are the major factors that influence the competition for water availability between crop and weed plants (Swanton et al., 2015).

Light as a third major factor affects the growth of crop and weed plants (Odonovan et al., 1985). Different phenophases of both crop and weed plants are affected by light. Morphological changes in both crop and weed plants due to competition for light include an increase in stem elongation and reduction in stem diameter, the rate of leaf appearance and root and shoot biomass (Rajcan and Swanton, 2001, Page et al., 2010).

Crop competition studies under field conditions are mainly dependent on the environment, soil type, plant density, spatial arrangement, the proportion of each species and design of the experiment (Vila et al., 2004). The design of the crop competition experiment depends on the objective, as different objectives require different techniques (Cousens, 1991).

Crop species may out-compete weed species depending on factors such as crop density, crop planting pattern, crop vigour and weed vigour. Crop density or the number of plants per unit area is important for competition studies considering the relationship among plant yield, number of individuals and resources present in area (Radosevich, 1987). The competitiveness of a crop can be enhanced using competitive cultivars, higher plant densities, narrow row spacings and different row orientation (Borger et al., 2016).

Weed growth can be substantially reduced by shading weeds in the inter-row space by physical orientation of the crop rows (Borger et al., 2016). Competitive ability of the crops can also be increased by increasing plant density (Lemerle et al., 2014). The significant interaction between sorghum cultivars and planting densities in suppressing weed biomass has been observed (Al-Bedairy et al., 2013). A high- density crop can limit water and nutrients available to weeds more effectively than a low- density crop and high- density crops can result in the reduction of light available to weeds (Borger et al., 2016).

2.1.15 SUMMARY

In summary, the literature review covered two major problems associated with single reliance on glyphosate application for controlling weeds. The first one is glyphosate mobility via soil systems, a potential risk for aquatic environments, and there is no information on the fate of glyphosate on Australian soils from the last 22 years apart from a single study in Western Australia. This research gap prompted an investigation into glyphosate sorption behaviour in Australian soils of different mineral composition due to increased usage of glyphosate as a single strategy to control weeds. The second major problem is the evolution of glyphosate-resistant weeds like *C.virgata* in New South Wales and Queensland, Australia, a major threat to sustainable weed control strategies and due to paucity of information on the management of *C.virgata*, I hypothesised that cultural methods like crop competition can be used as a strategy to control glyphosate-resistant *C.virgata*.

CHAPTER 3

MANAGEMENT OF *Chloris virgata* IN MUNG BEAN USING CROP COMPETITION

3.1 SUMMARY

Chloris virgata, a glyphosate-resistant grass has been identified as a major weed problem in central Queensland and northern New South Wales. I hypothesised that mung bean as a crop competitor would suppress *C.virgata*. Pot experiments were established for two seasons in a glasshouse. Crop competition and phenology aspects of both mung bean and *C.virgata* were studied in the field. The data from the pot experiments indicated *C.virgata* biomass of 43-48g dry weight in both seasons was significantly reduced by 74 % when surrounded by 5-6 mung bean plants per pot. Phenology aspects indicated a different growth pattern of *C.virgata* when surrounded by six mung bean plants. Minimum *C.virgata* biomass was observed at 25 cm row spacing with a density of 35 crop plants m^{-2}. Our recommendation to farmers facing the problem of *C.virgata* is to sow mung bean plants 25 cm apart at a seeding rate of 35 crop plants m^{-2} to suppress *C.virgata* in Australian conditions.

Keywords: suppression, biomass, phenology, annual grass, density, row spacing, light

3.2 INTRODUCTION

Feathertop Rhodes grass (*Chloris virgata*) a native of North America, a major weed in cotton (*Gossypium hirsutum*) in the subtropical region of Australia has been found to be an emerging weed problem in central Queensland and northern New South Wales (NSW), Australia (Werth et al., 2013). The major reason for this problem has been suggested to be the adoption of zero tillage practices and tolerance of *C.virgata* to glyphosate, a widely used systemic herbicide (Werth et al., 2013). Across Australia, *C.virgata* causes yield losses in all crops of 39,329 tonnes with a revenue loss of $7.7 m (Llewellyn et al., 2016). *Chloris.virgata* is an in-crop weed of cotton (*Gossypium hirsutum*) and grain crops like wheat (*Triticum aestivum*), chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) (Ngo et al., 2017).

Crop competition involves the application of agronomic practices to suppress weed growth, including high crop density, weed competitive cultivars and high seeding rates (Chauhan et al., 2011). These practices are under-exploited, but if used properly, are environmentally benign weed management strategies (Sardana et al., 2017). Crop density or the number of plants per unit of area is an important decision for competition studies considering the relationship among plant yield and the number of individuals and resources present in an area (Radosevich, 1987, Borger et al., 2016). An increase in plant density can increase the competitive ability of the crop (Lemerle et al., 2014). Along with plant density, narrow row spacing can increase light interception by crops with a reduction in weed biomass and a consequent increase in crop yield (Sharma and Angiras, 1996).

Mung bean (*Vigna radiata*) is cultivated as a grain legume in central Queensland and northern New South Wales in Australia (Chauhan et al., 2017). Approximately 95% of the total mung bean production in Australia is exported to countries such as India, Vietnam, Philippines and China (Chauhan et al., 2017). Mung bean is generally grown with a wide row spacing of 1m in central and southern Queensland and northern New South Wales (Chauhan et al., 2017). In a crop like mung bean, narrow row spacings (25 and 50 cm) lowered weed biomass with a consequent increase in grain yield of mung bean (Chauhan et al., 2017). There is no information in the literature of using crop competition for *C.virgata* control. I hypothesised that mung bean would suppress *C.virgata* growth and my experiments had the following objectives (i) to study the crop competition between mung bean and *C.virgata* grass and (ii) to determine the effect of different densities of mung bean on *C.virgata* growth.

3.3 MATERIALS AND METHODS

3.3.1 GLASSHOUSE EXPERIMENTS

Glasshouse studies were established in two seasons at the GRDC glasshouse complex, International Grains Research Centre, Narrabri, Australia.

Experimental methodology

The pot experiment for *C.virgata* and mung bean was established on January 31, 2017, and repeated on November 27, 2017, with the same species and densities. The glyphosate resistant certified weed seeds of *C.virgata* were bought from Osten weeds consulting (Emerald, QLD, 4720). Seeds of both *C.virgata* and mung bean were sown in plastic trays filled with potting mix (Searles premium potting mix 65 L). The potting mix was composed of organic compost, peat, zeolite and trace elements. Minimum and maximum temperatures during the period of experiments were 20°C and 35°C, respectively. Ten days after sowing the two-leaf plants were transplanted to pots (26 cm length x 28 cm width x 26 cm height) filled with potting mix. Mung bean and *C.virgata* were co-established by using a seeding template in order to eliminate size biases (Hwang and Lauenroth, 2008, Barry and Dudash, 2015, Fernando et al., 2016, Walsh et al., 2009). The density treatments (mung bean plants-0-6 plants m^{-2}; *C.virgata* plants-0-1 plants m^{-2}) were arranged in a target neighbour design. The target neighbour design is an additive design in which one of the competing species is represented by a single individual (the target, *C.virgata*) and the density of surrounding individuals (the neighbours, mung bean) is manipulated (Gibson et al., 1999). The density of the target species was reduced to a single individual to preclude significant intraspecific interactions (Gibson et al., 1999).

Glasshouse study-measurements

For pot experiments, measurements included plant height (from the soil surface to the tip of the uppermost outstretched leaf) and the number of leaves per plant. Measurements were taken weekly for the entire season. Plants were watered as per moisture meter readings. The moisture meter/ hygrometer had a scale (1-10) with three different ranges (Dry, Moist, and Wet). The probe tip of the moisture meter was inserted into the root zone (10 mm) to measure soil moisture. If the probe indicated a moisture level of dry or moist, frequent waterings were done. Plants were watered up to the wet range. At the end of the experiment, aboveground biomass of all the plants was harvested and their dry weights were recorded. The plants were dried at 70°C for 48 hours in a dehydrator (Sterdium, Micro digital).

3.3.2 FIELD EXPERIMENT TO STUDY PHENOLOGY ASPECTS

To study phenological aspects, 6 mung bean plants (3 in each row with a plant to plant distance of 10 cm) and 1 *C.virgata* plant were co-established in the field with three replications by hand on medium clay soils at the International Grains Research Centre, The University of Sydney, Narrabri to study the interaction between mung bean and *C.virgata* following the same transplanting procedure outlined above for the glasshouse. Mung bean plants were transplanted by hand in rows 25 cm apart with a plant to plant spacing of 10 cm based on a previous study (Chauhan et al., 2017) with *C.virgata* plant at the centre between the rows on November 23, 2017, and harvested on January 26, 2018. For control treatments, *C.virgata* plants were not surrounded by mung bean plants. The root and shoot biomass of both mung bean and *C.virgata* plants were recorded by a scale and assessment of seed parameters (length of spike, seeds in each spike by manual counting, and the total weight of the spike for *C.virgata* plant were completed with a scale. The roots of both mung bean and *C.virgata* plants were excavated manually by spade to a depth of 50 cm. The roots after collection were washed and dried for 24 hours for the assessment of root weights separately for both mung bean and *C.virgata* plants.

3.3.3 FIELD EXPERIMENT TO STUDY MUNG BEAN-CHLORIS VIRGATA INTERACTION

Experimental Methodology

This competition study involved two different species: *C.virgata* and mung bean. The field experiment was initiated in the summer of 2017 to evaluate the effects of mung bean treatments on the growth of *C.virgata*. The field was prepared using recommended cultural practices of New South Wales, Department of Primary Industries for mung beans (Chauhan et al., 2017). The experiment was arranged as a randomised complete block with four replicates. Treatments consisted of three different row spacings (25, 50 and 75 cm) and plant densities (20, 28 and 35 mung bean plants m^{-2}). The mung bean was planted in 9 m x 2m experimental plots. *C.virgata* plants were transplanted on October 18th 2017 at the two leaf stage by hand in 1 m^2 (1 m x 1m) subplots at the centre portion of each experimental unit. Mung bean plant biomass was harvested at maturity on December 19th 2017.*Chloris virgata* and mung bean plants were dried at 70°C for 48 hours in a dehydrator (Sterdium, Micro digital).

Statistical analysis

A regression model using PROC GLM procedure (SAS 9.4) was used to test for treatment differences for the response variables of *C.virgata* and mungbean. *C.virgata* biomass was a dependent variable in the general linear model while row spacing and mung bean density were independent variables. Means at each harvest time were separated using Tukey's HSD at P = 0.05. The standard errors were calculated by the formula – Standard deviation / $\sqrt{n}$; where n represented number of replications.

3.4 RESULTS AND DISCUSSION

Biomass of C.virgata

Chloris virgata showed biomass of 43-48g dry weight in both seasons, which was reduced by 74 % when surrounded by 5-6 mung bean plants (Fig 3.1, Fig3.2). The findings demonstrated that *C.virgata* can be suppressed at higher densities of mung bean.

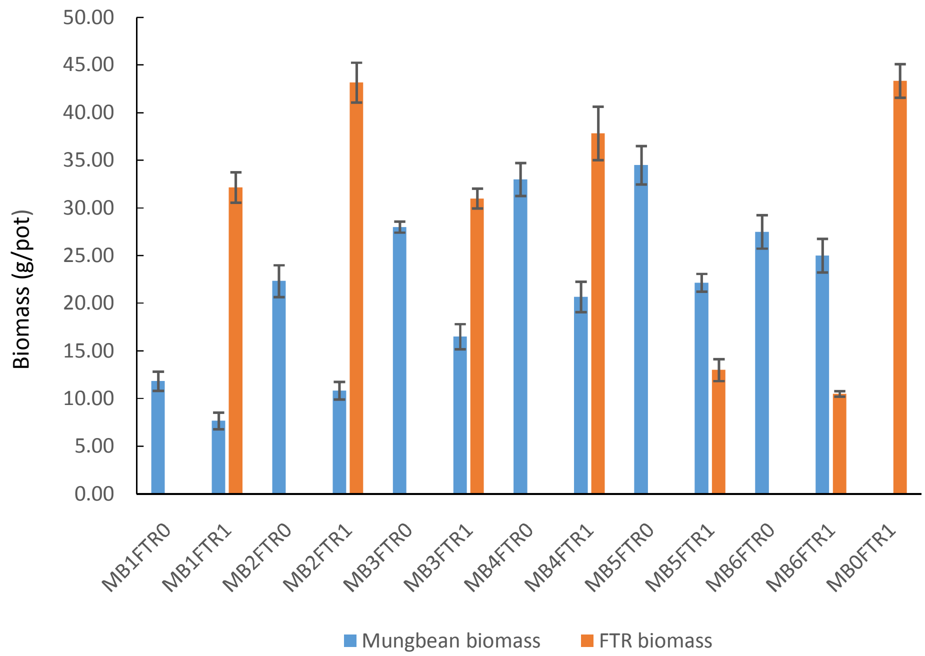

FIGURE 3.1 Mung bean (MB) and *C. virgata* (Feather Top Rhodes-FTR) biomass in January 2017. Error bars represent ± standard errors of the mean (n=3).The treatment abbreviations on the horizontal axis refer to MB (mung bean density; 0,1,2,3,4,5,6) and *C. virgata* (*C.virgata*; 0 and 1) in the glasshouse experiment.

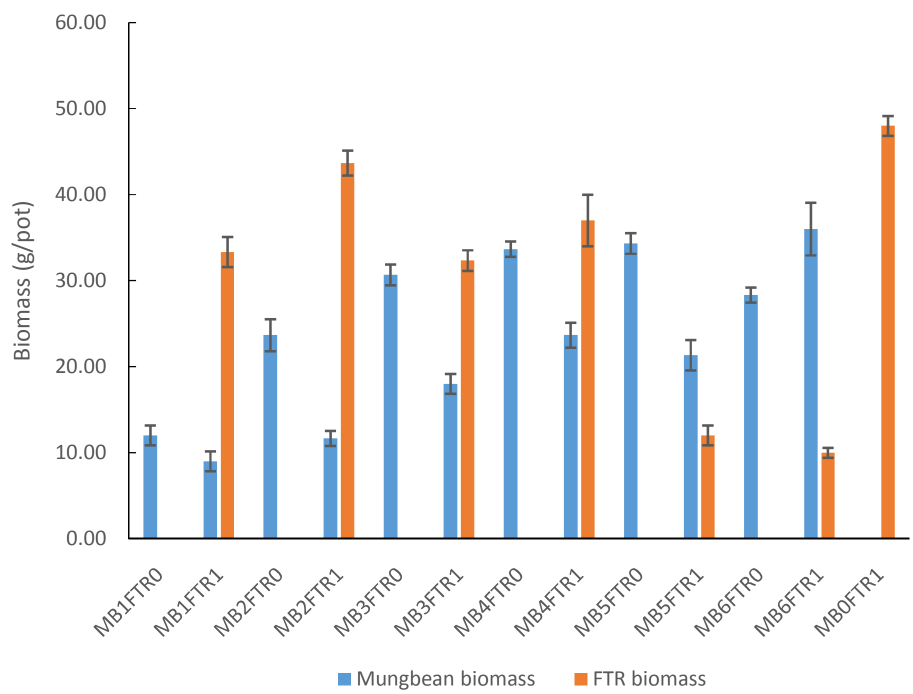

FIGURE 3.2 Mung bean (MB) and *C.virgata* (Feather Top Rhodes-FTR) biomass in January 2018. Error bars represent ± standard errors of the mean (n=3). The treatment abbreviations on the horizontal axis refer to MB (mung bean density; 0,1,2,3,4,5,6) and *C.virgata* (*C.virgata*; 0 and 1) in the glasshouse experiment.

The number of leaves per mung bean and C.virgata

The number of leaves of *C.virgata* per plant decreased 36-48% after 21 days but only in treatments with 5 and 6 mung bean plants (Table 3.1). There was no reduction in the number of leaves over time in other treatments. The maximum number of *C.virgata* leaves after 42 days was 13 when surrounded by 6 mung bean plants. The plausible reason is the greater competition from more mung bean plants that resulted in reduced resource acquisition and leaf production by *C.virgata.*

TABLE3.1 The number of leaves per plant for mung bean (MB) and *C.virgata* (Feather Top Rhodes-FTR) in two glasshouse experiments.SE(±) values represent standard errors with n=3.

Leaves (no.plant^{-1})										
Treatment	7 Days		14 Days		21 Days		28 Days		42 Days	
(No. of plants-0,1,2,3,4,5,6)	MB	FTR	MB	FTR	MB	FTR	MB	FTR	MB	FTR
MB1FTR0	2	0	5	0	11	0	17	0	17	0
MB1FTR1	2	2	5	11	10	26	15	59	15	59
MB2FTR0	2	0	5	0	11	0	16	0	16	0
MB2FTR1	2	2	5	10	10	26	15	51	15	51
MB3FTR0	2	0	5	0	11	0	14	0	14	0
MB3FTR1	2	2	5	9	9	24	11	58	11	58
MB4FTR0	2	0	5	0	9	0	13	0	13	0
MB4FTR1	2	2	5	9	10	23	12	37	12	37
MB5FTR0	2	0	5	0	9	0	12	0	12	0
MB5FTR1	2	2	5	8	8	16	12	16	12	16
MB6FTR0	2	0	5	0	8	0	12	0	12	0
MB6FTR1	2	2	5	7	7	13	12	13	12	13
MB0FTR1	0	2	0	17	0	25	0	60	0	60
SEs	(± 0.32)	(± 0.33)	(± 0.58)	(± 0.64)	(± 0.58)	(± 0.51)	(± 0.64)	(± 0.54)	(± 0.75)	(± 0.80)

The height of mung bean and C.virgata plants

In comparison with the control, a 32% reduction in height in *C.virgata* when surrounded by five or six mung bean plants at day 42 was observed (Table 3.2). This pattern of *C.virgata* height decrease with increasing density is similar to that of spiny amaranth weed, the height of which was suppressed due to a higher density of rice plants (Chauhan and Abugho, 2012).

TABLE 3.2 Mean plant height (cm) for mung bean (MB) and *C.virgata* averaged over two glasshouse experiments.SE (±) values represent standard errors with n=3.

Plant Height (cm)										
Treatment	7 Days		14 Days		21 Days		28 Days		42 Days	
(No. of plants-0,1,2,3,4,5,6)	MB	FTR	MB	FTR	MB	FTR	MB	FTR	MB	FTR
MB1FTR0	9.3	0.0	22.0	0.0	36.7	0.0	66.3	0.0	72.3	0.0
MB1FTR1	9.0	25.3	19.3	45.0	34.7	67.7	48.0	127.6	67.7	115.0
MB2FTR0	9.3	0.0	19.3	0.0	35.7	0.0	48.3	0.0	71.0	0.0
MB2FTR1	8.7	24.1	15.3	41.7	35.3	62.7	42.3	112.7	59.3	110.0
MB3FTR0	9.0	0.0	17.0	0.0	34.0	0.0	40.7	0.0	70.0	0.0
MB3FTR1	8.7	21.3	15.3	39.0	33.0	60.0	33.3	105.7	56.0	98.0
MB4FTR0	8.0	0.0	18.7	0.0	33.7	0.0	42.0	0.0	64.0	0.0
MB4FTR1	7.7	18.0	15.0	38.0	33.0	53.7	37.7	84.0	57.0	97.3
MB5FTR0	9.3	0.0	18.0	0.0	34.7	0.0	36.0	0.0	62.0	0.0
MB5FTR1	8.1	17.0	16.5	35.3	34.0	52.0	35.7	67.0	58.0	90.0
MB6FTR0	8.0	0.0	18.0	0.0	27.0	0.0	35.7	0.0	50.3	0.0
MB6FTR1	7.5	17.0	16.0	34.3	26.3	61.0	35.0	64.0	47.0	89.0
MB0FTR1	0.0	17.0	0.0	36.0	0.0	65.7	0.0	127.7	0.0	131.3
SEs	(± 0.33)	(± 0.29)	(± 0.58)	(± 0.80)	(± 0.58)	(± 0.58)	(± 0.33)	(± 0.80)	(± 0.33)	(± 0.36)

Seed production of C.virgata

Phenology aspects in the field indicated that on an average, the length of *C.virgata* spike was 7.9 cm (Table 3.3) when there was no mung bean competition.

TABLE 3.3 Shoot, root and seed parameters of *C.virgata* grass with and without competition with mung bean for the phenology aspects.The numbers in the parentheses are the standard error of the mean (n=3).

Treatment	Shoot weight (g/plot)	Root weight (g/plot)	Length of spike (cm)	Seeds in spike	Total weight of spike (g)
With competition	9.6 (±0.15)	0.6 (±0.01)	No spike	No spike	No spike
Without competition	68.0 (±0.15)	1.0 (±0.05)	7.9 (±0.05)	1806.0 (±2.85)	1.2 (±0.02)

Chloris virgata produced two spikes without competition, whereas, in competition with 6 plants, *C.virgata* had no spikes (Table 3.3). Weed seed production is an important aspect with respect to *C.virgata* from the sustainability point of view and any practice reducing the weed seed input to the soil seed bank can contribute substantially towards weed management approach related to the control of *C.virgata* (Gallandt, 2006).

The growth pattern of *C.virgata,* when grown alone in the phenology study was different from more vertical growth of *C.virgata* when surrounded by mung bean plants (Fig.3.3, Fig.3.4). The vertical growth of *C.virgata* in competition with mung bean plants represents an adaptation. Due to shading of mung bean plants, *C.virgata*, showed more upright growth to capture sunlight. When surrounded by 6 mung bean plants, the average *C.virgata* root biomass decreased by 40 percent and shoot biomass decreased by over 85 percent (Table 3.3, Fig.3.5).

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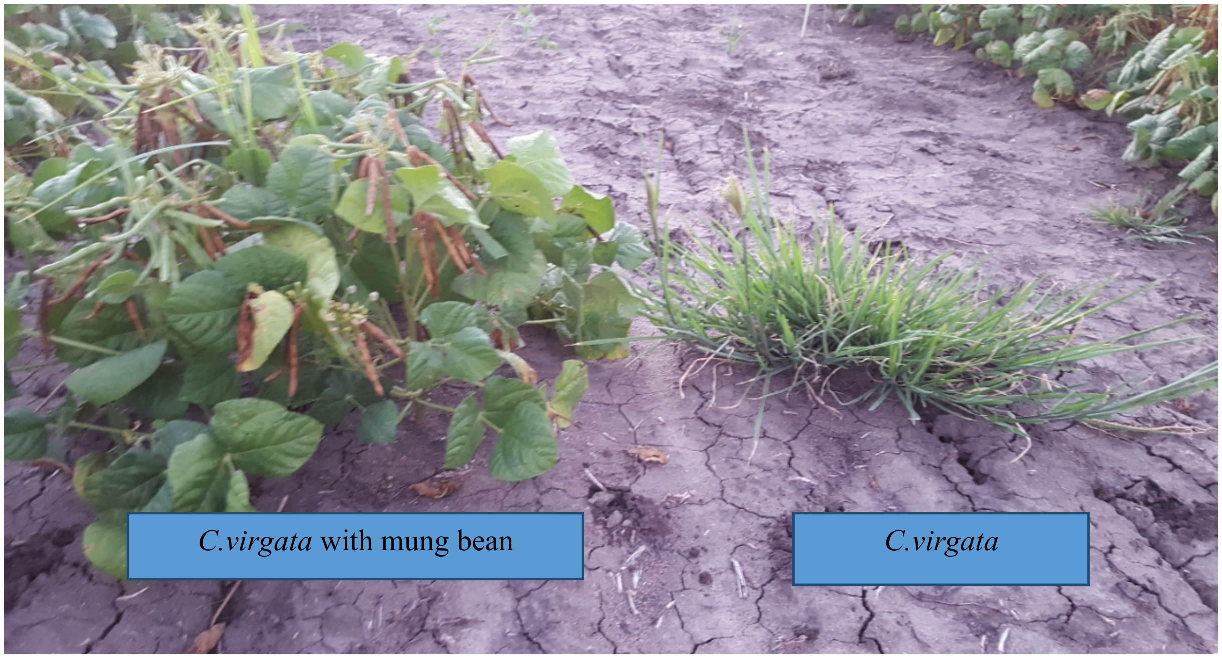


FIGURE 3.3 The reduction in biomass of *C.virgata* plant (left) after removal of six mung bean plants compared to *C.virgata* grown in the absence of mung bean.

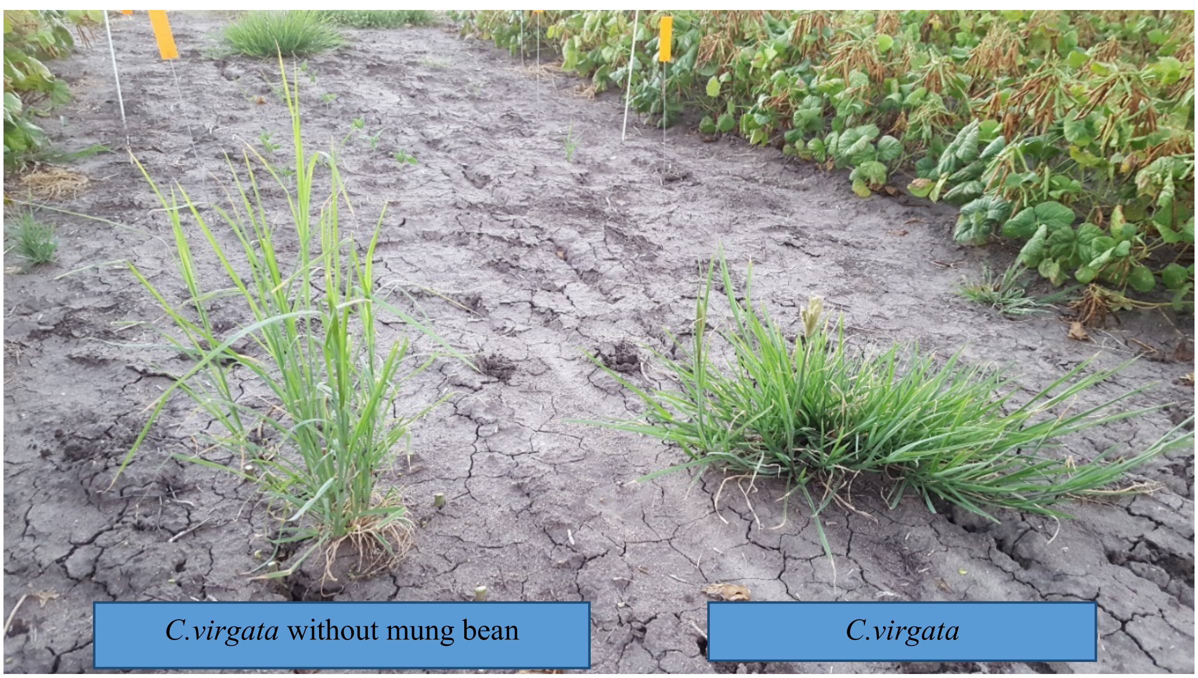


FIGURE 3.4 *C.virgata* plant with removal of mung bean (left) compared to *C.virgata* in the absence of mung bean (right)

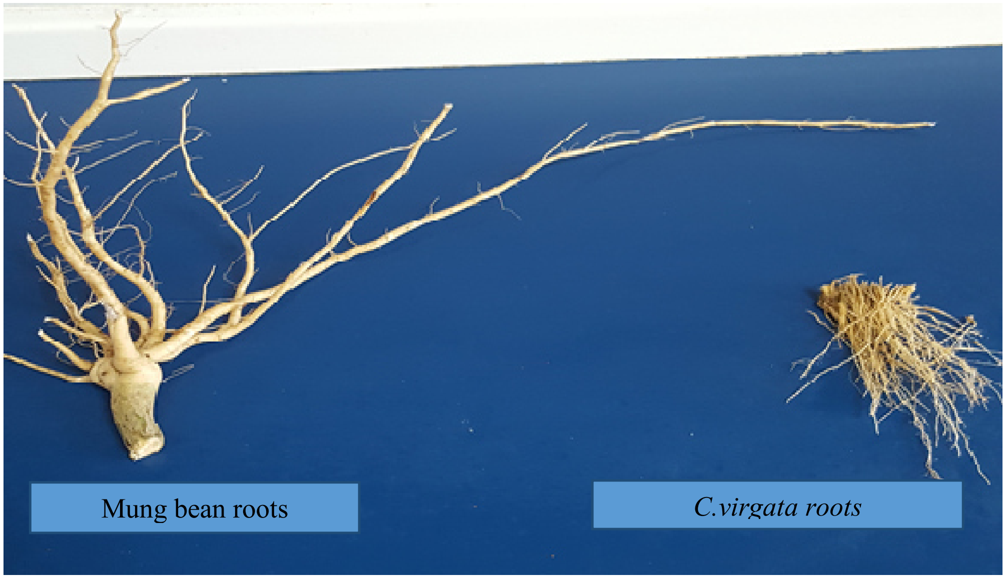


FIGURE 3.5 Roots of mung bean (left) and *C.virgata* plant (right) from the field excavation to study phenology aspects

Similarly, in competition with mung bean plants, the *C.virgata* average shoot weight decreased by seven times (Table3.3) due to competition effect.

It can be inferred from the phenology aspects that high root and shoot weights of mung bean resulted in the suppression of *C.virgata*. An increase in mung bean density suppressed *C.virgata*. The role of plant density of crop species has also been observed in previous studies to suppress weed species (Stapper and Fischer, 1990, Champion et al., 1998, Paynter and Hills, 2009). Increased beet density has been found to increase beet plant capacity to suppress weeds (Carvalho and Guzzo, 2008). Under drought stress, *Cyperus rotundus* was observed to be a superior competitor to mung bean, while *Eleusine indica* and *Synedrella nodiflora* were inferior (VanCaelenbergh et al., 1996), however, no information could be found related to *C.virgata*.

High-density crop plants can result in the reduction of water and nutrient availability to weeds more effectively than a low-density crop (Borger et al., 2016). It is likely that the major reason for the reduction in biomass of *C.virgata* was greater shoot and root biomass of mung bean planted at higher density. The finding is valuable in case of suppression of annual grass weeds like *C.virgata.* We advocate the option of crop competition to farmers, as it is environmentally friendly and does not require a significant increase in the cost of production (Sardana et al., 2017).

From the field experiment, it was observed that with the increase in mung bean densities, *C.virgata* biomass was decreased (Fig.3.6).

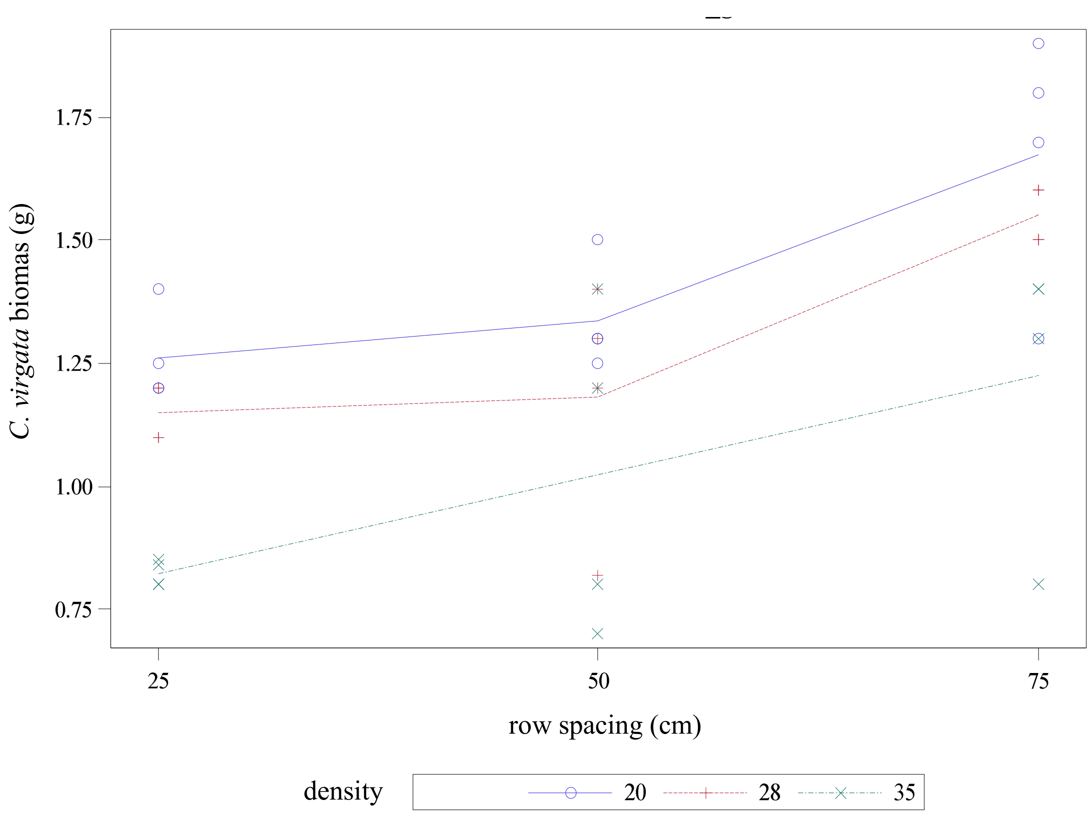


FIGURE 3.6 Interaction plot of *C.virgata* biomass (g) at varying plant densities (20, 28 and 35 plants / m^2) and row spacings (25, 50 and 75 cm) of mung bean plants.

Fig.3.6 shows that the minimum *C.virgata* biomass was observed at 25 cm row spacing with a density of 35 crop plants per meter square. High-density crop plants result in the reduction of water and nutrient availability to weeds more effectively than a low-density crop, which was the major reason for suppression of *C.virgata* by mung bean plants. *Chloris.virgata* was outcompeted when surrounded by mung bean plants in the field demonstration. The option of crop competition to farmers is recommended, as it is environmentally friendly and does not require a significant increase in the cost of production. The implication of this study would be a recommendation to farmers facing the problem of *C.virgata* in mung bean crops is to sow mung bean in rows 25 cm apart with a crop plant density of 35 plants m^{-2} to suppress the growth of *C.virgata*. This agronomic practice will not affect the time of sowing for mung bean plants.

CHAPTER 4

CROP COMPETITION AS A STRATEGY TO CONTROL *Chloris virgata* GRASS IN SORGHUM BICOLOR

4.1 SUMMARY

Chloris virgata grass covers an area of 73,414 ha in sorghum crops with a yield loss of 36,995 tonnes, resulting in revenue loss of \$7.0 million year^{-1} in Australia. We hypothesised that sorghum at high densities, when grown at narrow row spacings can suppress *C.virgata*. Field experiments for two successive years were conducted to study the effect of varying row spacings (50, 75, and 100 cm) and sorghum densities (5, 7.5, and 10 plants m^{-2}) on the growth of *C.virgata*. A significant reduction in *C.virgata* biomass was observed when surrounded by 10 sorghum plantsm^{-2} spaced 50 cm apart. It is recommended that farmers growing sorghum facing problems with *C.virgata* should increase sorghum plant densities to 10 sorghum plants m^{-2} and rows be planted 50 cm apart.

Keywords: suppression, light, crop canopy, plant height, phenology, biomass, weed

4.2 INTRODUCTION

Sorghum (*Sorghum bicolor*)is an important rotational crop in subtropical north-east Australia with a cropping area of approximately 0.82 Mha (Wu et al., 2010). Early weed interference in sorghum can result in large yield losses (Werle et al., 2016). *Chloris virgata* has been identified as one of the top weeds of sorghum along with awnless barnyard grass (*Echinochloa colona* L.) and sweet summer grass (*Brachiaria eruciformis* Sm.). In sorghum, *C.virgata* covers an estimated area of 73,414 ha with a total yield loss of 36,995 tonnes resulting in a revenue loss of \$7.0 millionyear^{-1} (Llewellyn et al., 2016, Werth et al., 2013). The presence of herbicide-resistant weeds like *C.virgata* resistant to glyphosate, the limited number of registered herbicides in a crop, and crop rotational restrictions when using some of these herbicides have created a challenging environment for weed control in sorghum crops (Grichar et al., 2004).

Sorghum growers of the subtropical north-eastern region of Australia depend on key herbicides like atrazine, metolachlor, fluroxypyr, 2,4-D, and glyphosate that are applied alone or in tank mixture (Walker et al., 2005). Few growers use tillage (0-17%) or crop competition (0-11%) to manage the weeds. Utilization of crop competition has been observed only in wheat crops, where 17-20 % of growers use high seeding rates (Walker et al., 2002). This has prompted an urgent investigation into non-chemical control options like crop competition as a better option for sorghum weed control in comparison to chemical methods.

Correct prediction of weed phenological development, especially in the case of *C.virgata* is essential for weed-crop competition studies (Deen et al., 1998). Studies related to phenology provide important information on functional rhythms of plants and plant communities (Ralhan et al., 1985). Suppressive activity of grain sorghum against weeds increased with sorghum plant density (Al-Bedairy et al., 2013). Considering sorghum as a crop competitor for *C.virgata,* I aimed to determine the: (i) effect of density of sorghum and row spacing on the biomass of *C.virgata* and (ii) competition effect of sorghum plants on the phenology of *C.virgata*.

4.3 MATERIALS AND METHODS

Field studies were established on medium clay Vertosol soil in 2016 (pH-8.8; EC-0.13 dSm^{-1}; OC- 0.61%) and in 2017 (pH-7.2; EC-0.09 dSm^{-1}; OC- 0.45%) at the International Grains Research Centre, Narrabri. The certified weed seeds of glyphosate resistant *C.virgata* were purchased from Osten Weeds Consulting Pty Ltd (Emerald, QLD, 4720).

Experimental methodology

The competition study involved two different species: *C.virgata* and sorghum (Variety MR-43). Fields were prepared with a field cultivator and recommended cultural practices for sorghum production were used (Chauhan et al., 2017). The experiment was arranged as a randomized complete block with four replicates. Treatments consisted of three different row spacings (50, 75, and 100 cm) and plant densities (5, 7.5, and 10 sorghum plants m^{-2}) based on the recommended package of practices by the New South Wales State Government (Chauhan et al., 2017). In 2016, *C.virgata* was broadcasted in a subplot of 2 m^2 (2 m length x 1 m width) on 14th October while in 2017, *C.virgata* was transplanted in a subplot of 1 m^2 (1 m length x 1 m width) on 18thOctober with the objective of reducing the soil weed seed bank at the same density as of 2016. Competition between sorghum and *C.virgata* plants was established by transplanting *C.virgata* seedlings at the two-leaf stage in the subplots of the experimental units. Time of competition was the same in both years and at similar growth stages.

Sorghum plants were harvested at maturity. *C.virgata* and sorghum samples were dried at 70°C for 48 hours in a dehydrator (Sterdium, Micro digital). The dry weight biomass of both sorghum and *C.virgata* were recorded. The phenology of *C.virgata* was studied separately in the presence and absence of sorghum. Sorghum and *C.virgata* plants were transplanted at the two-leaf stage and co-established in the field. Six sorghum plants in three replicates were spaced 50 cm apart based on the results of the previous year with one *C.virgata* plant at the centre between the rows. The field experiment for phenology of two species was initiated along with the crop competition study to evaluate the phenology of *C.virgata* in the presence or absence of sorghum. At bi-weekly intervals, plant height (height from the ground to the flag leaf) of *C.virgata* was recorded using a metre ruler and the number of leaves of *C.virgata* were recorded by visual counting in the presence and absence of sorghum. Roots of both sorghum and *C.virgata* were excavated with spade, washed and dried for 24 hours. Root and shoot weights of both species were measured on scales (PS 3500 R2 RADWAG®).

Statistical analysis

Base R statistical package indicated no significant interactions between treatments and experimental runs. Therefore, the data were pooled for each field experiment for further statistical analysis. A regression model using PROC GLM procedure (SAS 9.4) was used to test for treatment differences for the response variables of *C.virgata* and sorghum. Means at each harvest time were separated using Tukey's HSD at $P = 0.05$.

4.4 RESULTS AND DISCUSSION

Significant differences ($P < 0.005$) among the treatments indicated that 10 sorghum plants m^{-2} spaced 50 cm apart can reduce *C.virgata* biomass (Fig.4.1) in comparison with other combinations of plant densities and row spacings. This finding is important for the sorghum farmers facing the problem of *C.virgata*. It clearly implies that narrow row spacing with high sorghum plant densities can reduce *C.virgata* biomass. Sorghum plants were able to suppress *C.virgata* due to their height and a higher rate of growth (Fig.4.2, 4.3).

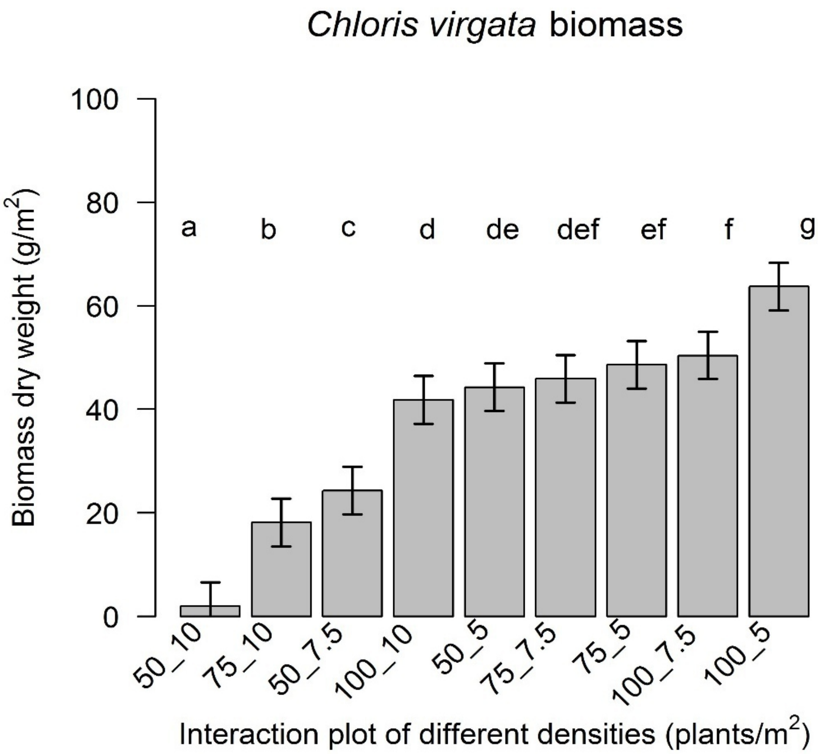


FIGURE 4.1 Interaction plot for *C. virgata* biomass at different densities and row spacings of sorghum for the seasons 2016-2017 and 2017-2018.[In figure 4.1, 50 cm, 75 cm, and 100 cm represent different row spacings, while 5, 7.5, and 10 represent the number of sorghum plants per metre square. The vertical bars are the 95% confidence intervals and the letters are a compact letter display of Tukey's HSD].

FIGURE 4.2 *C.virgata* was broadcasted into sorghum crop in 2016-2017 season

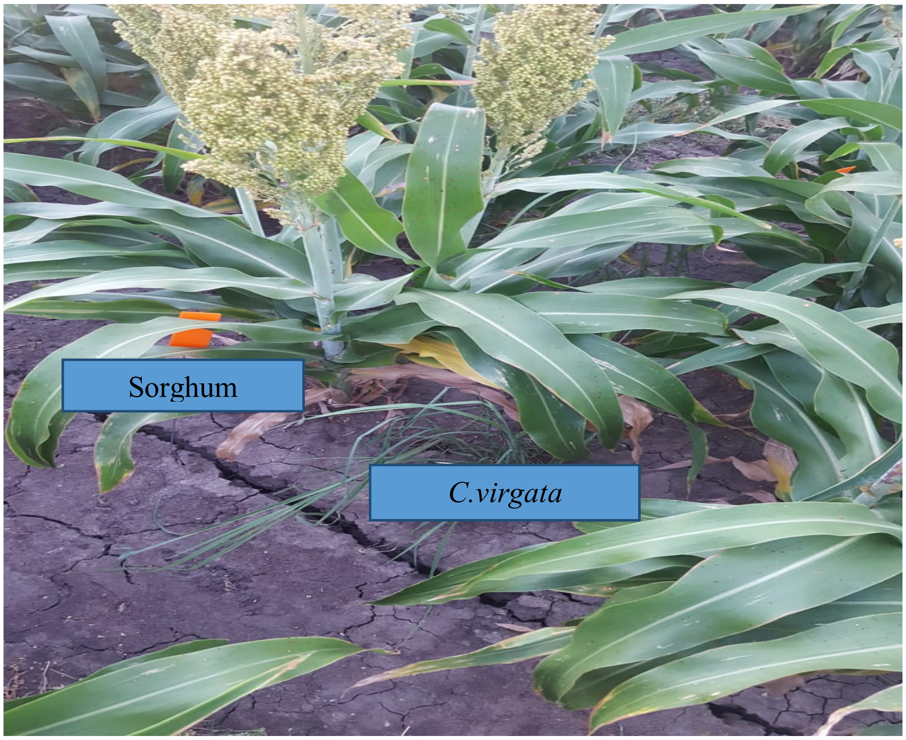


FIGURE 4.3 *C.virgata* was transplanted into sorghum crop in 2017-2018 season

The phenology study indicated that *C.virgata* plants without competition from sorghum showed more lateral growth (Fig.4.4), while the *C.virgata* in competition with sorghum plants showed more vertical growth (Fig.4.7). This is an adaptation of *C.virgata* plant due to competition by sorghum plants. Sorghum plants suppressed the growth of *C.virgata* by shading and this decreased the growth of *C.virgata*, while the possibility of competition for water/nutrients or even the release of allelochemicals cannot be negated to affect the growth of *C.virgata as* reported in other studies, where brassica species has been found to be phytotoxic by releasing chemicals like glucosinolates (Rehman et al., 2019*)*.

There was no change in total leaf production in *C.virgata* plants facing competition from sorghum after 28 days (Table 4.1) while leaf production continued to increase in *C.virgata* plants in the absence of sorghum plants.

A substantial increase in the height of *C.virgata* plants in competition with sorghum was observed (Table 4.2) in comparison with control plants without sorghum. This appears to be an adaptation by the *C.virgata* plant for the competition for light with sorghum plants. There was approximately a seven-fold decrease in shoot weight of *C.virgata* with sorghum compared without sorghum and 14 times decrease in the root weight of *C.virgata* with sorghum compared without sorghum (Table 4.3).

FIGURE 4.4 Phenology experiment to study sorghum-*C.virgata* interaction

Grain yield of sorghum was increased with the decrease in *C.virgata* (Feathertop Rhodes, FTR) biomass (Fig.4.5). Highest biomass and grain yield of sorghum was observed at 75 cm spacing when surrounded by 10 plants / m^2 (Fig.4.6, Fig.4.7). Interaction plots for sorghum biomass (Fig.4.6) indicated 28% increase in sorghum biomass in the absence of *C.virgata.* Significant interactions ($p < 0.001$) between row spacing and densities of sorghum plants with respect to biomass and grain production of sorghum were observed. Differences in harvest index (0.12 with *C.virgata* and 0.10 without *C.virgata*) (Fig.4.8) demonstrated the negative effect of *C.virgata* on the biomass and grain yield of sorghum.

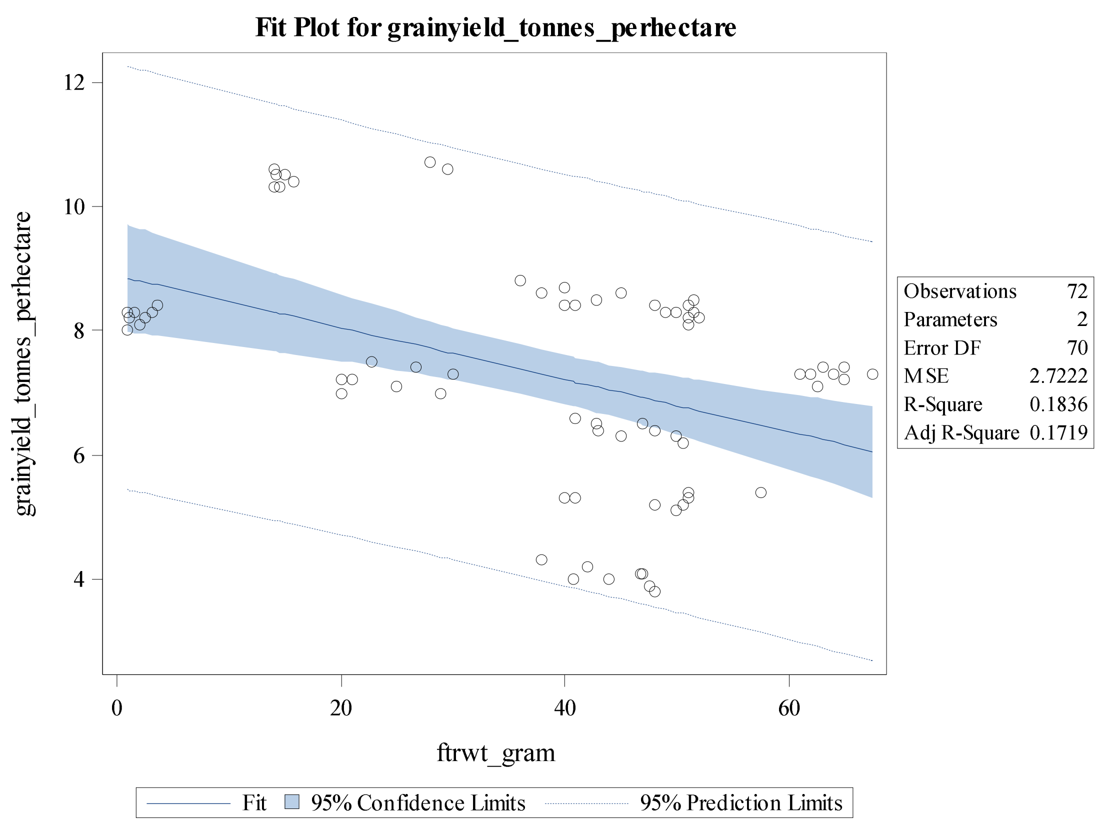


FIGURE 4.5. Interaction plot shows a decrease in *C.virgata* biomass (g) with the increase in grain yield (tonnes/ha) of sorghum

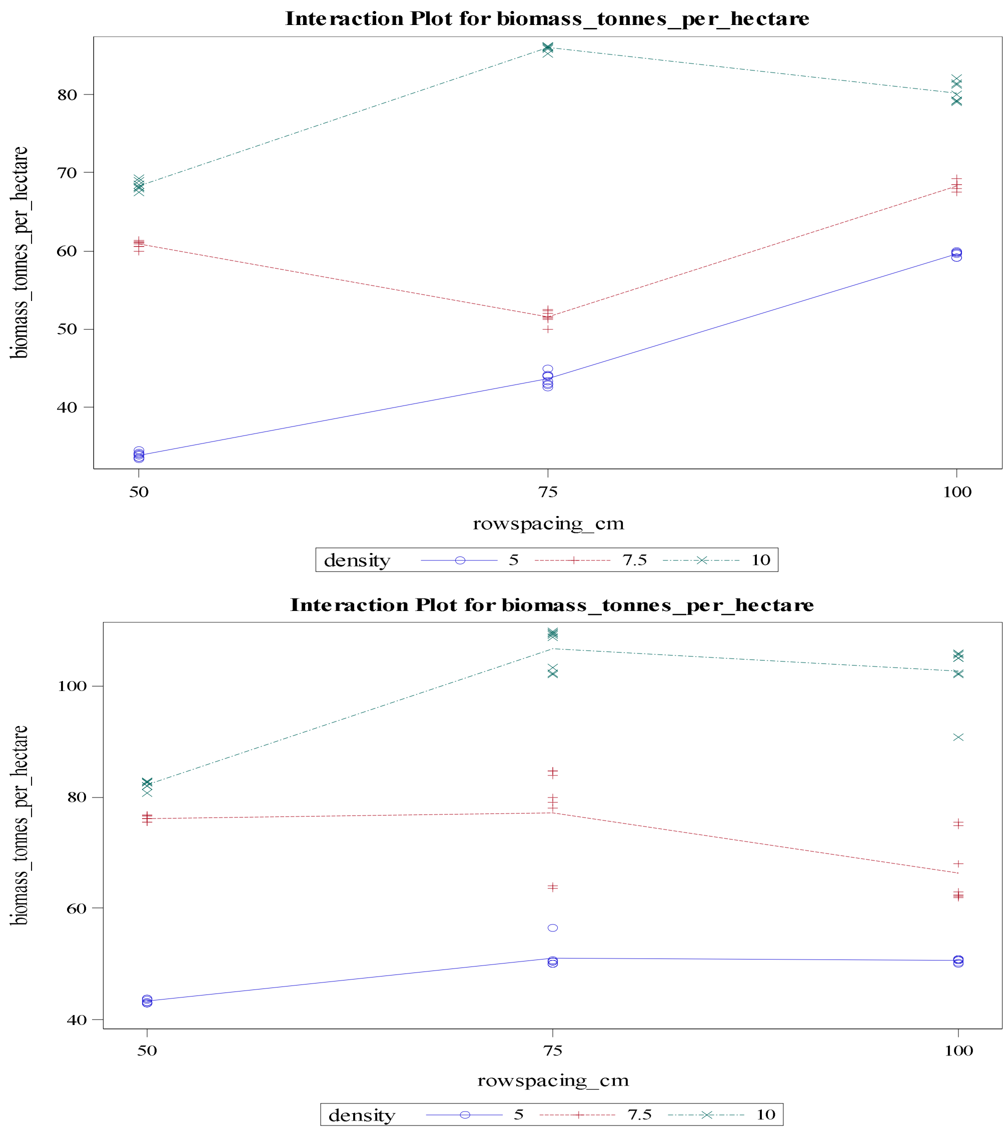


FIGURE 4.6. Interaction plot for biomass (tonnes/ha) of sorghum at varying row spacings (50 cm, 75 cm, and 100 cm) and densities of sorghum plants (5, 7.5, and 10 plants per metre square) in the presence (above) and absence (below) of *C.virgata*.

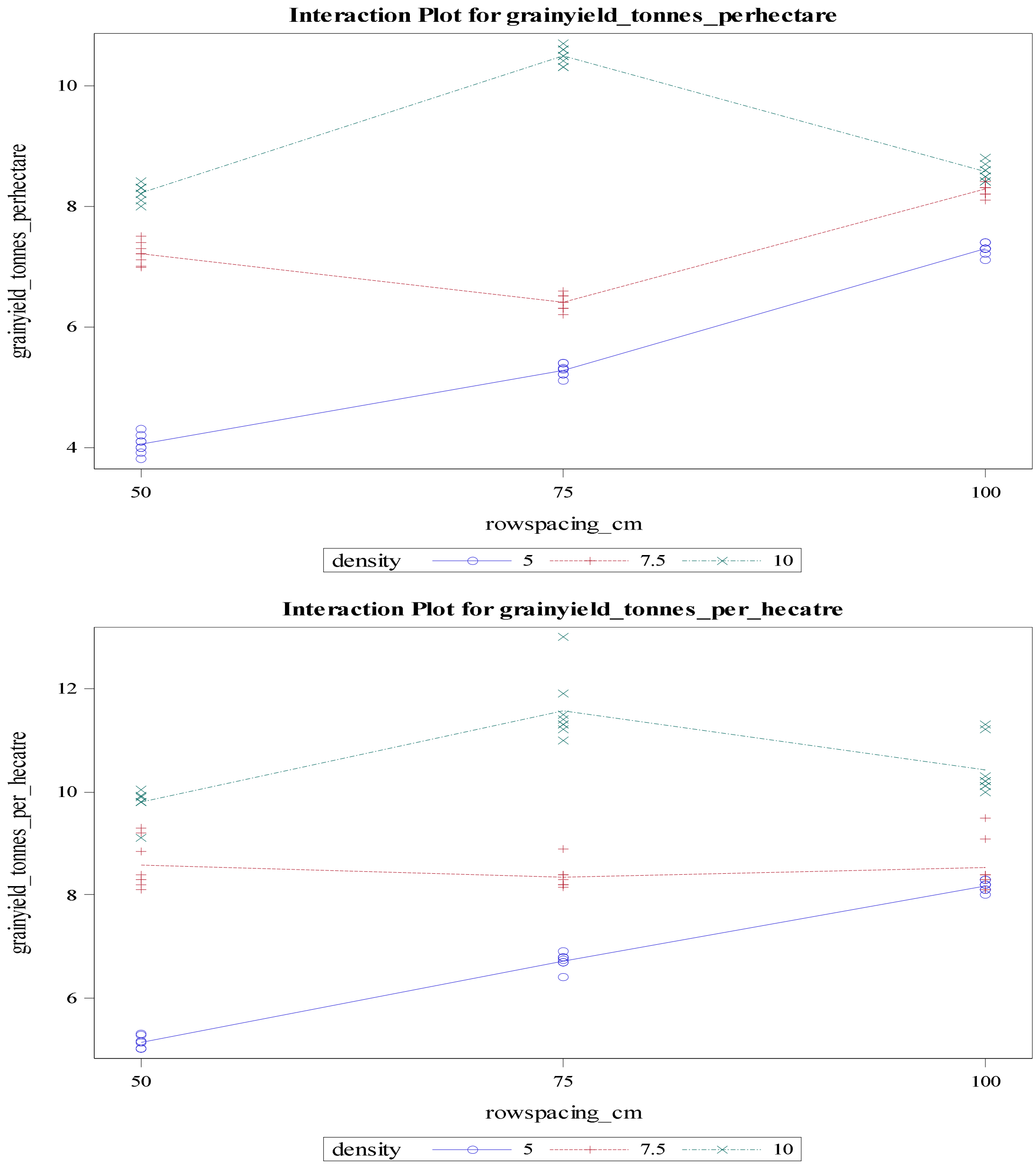
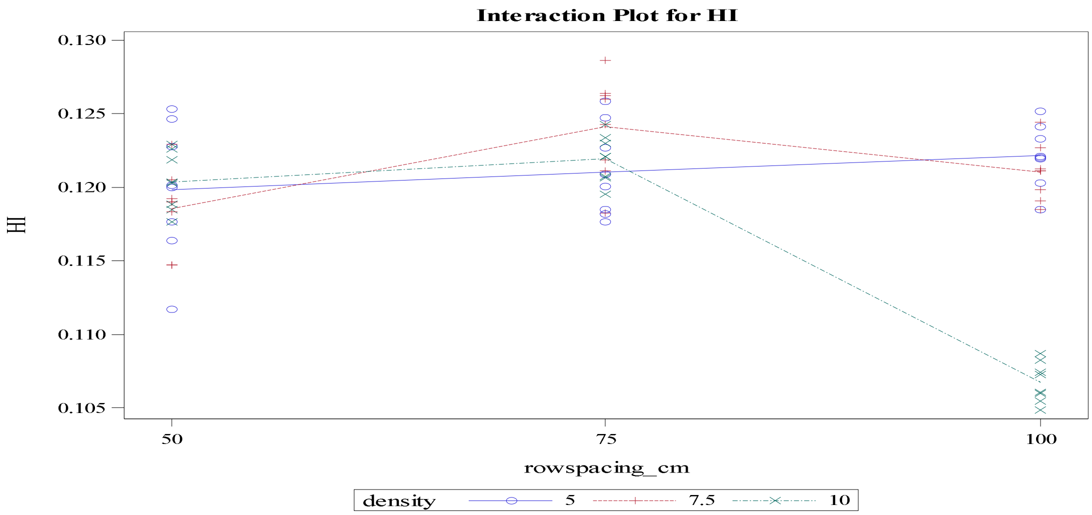


FIGURE 4.7. Interaction plot for grain yield (tonnes/ha) of sorghum at varying row spacings (50 cm, 75 cm, and 100 cm) and densities of sorghum plants (5, 7.5, and 10 plants per metre square) in the presence (above) and absence (below) of *C.virgata*.



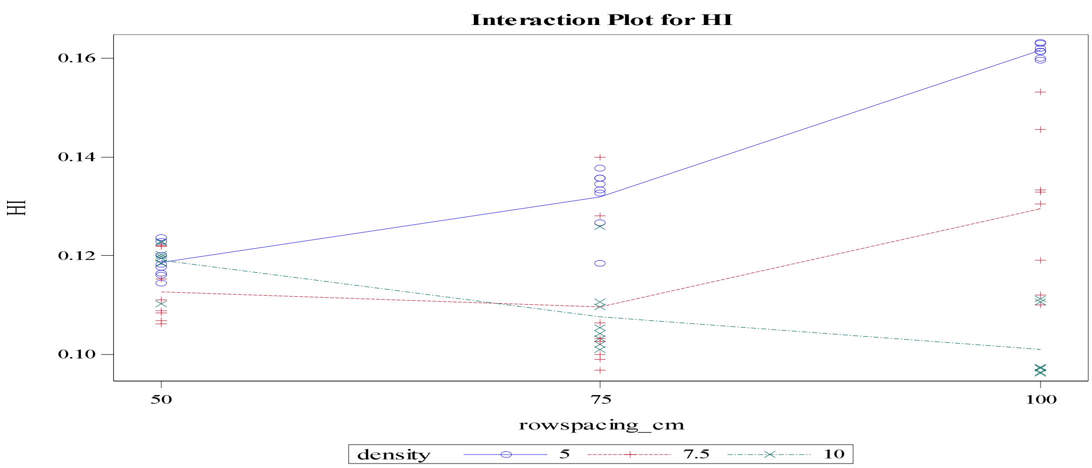


FIGURE 4.8. Interaction plot for Harvest index (HI) of sorghum at varying row spacings (50 cm, 75 cm, and 100 cm) and densities of sorghum plants (5, 7.5, and 10 plants per metre square) in the presence (above) and absence (below) of *C.virgata*.

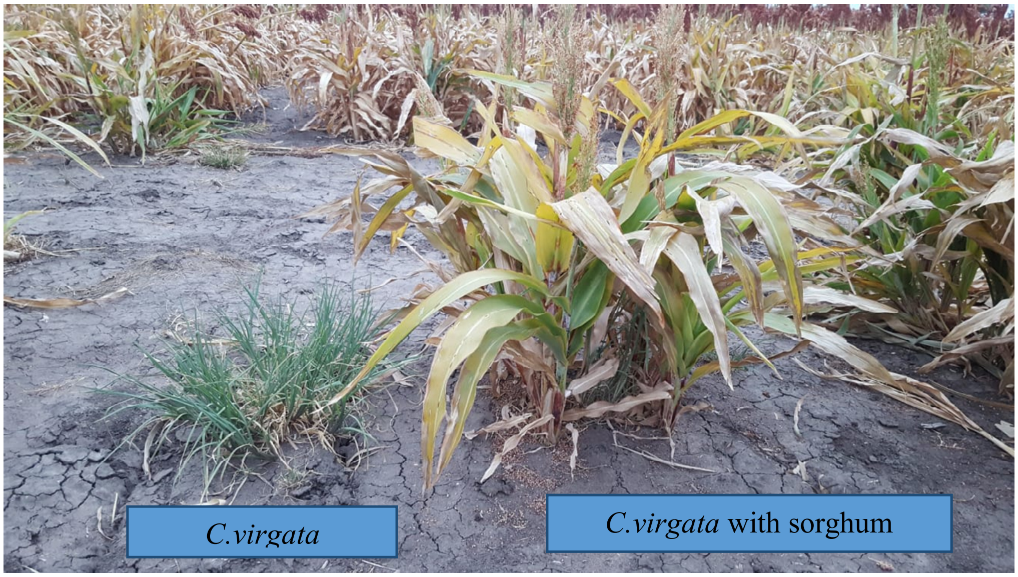


FIGURE 4.9 *C.virgata* plant without the removal of sorghum plants (right) showed vertical competitive growth, however, the *C.virgata* (left) plant showed more lateral growth when grown individually.

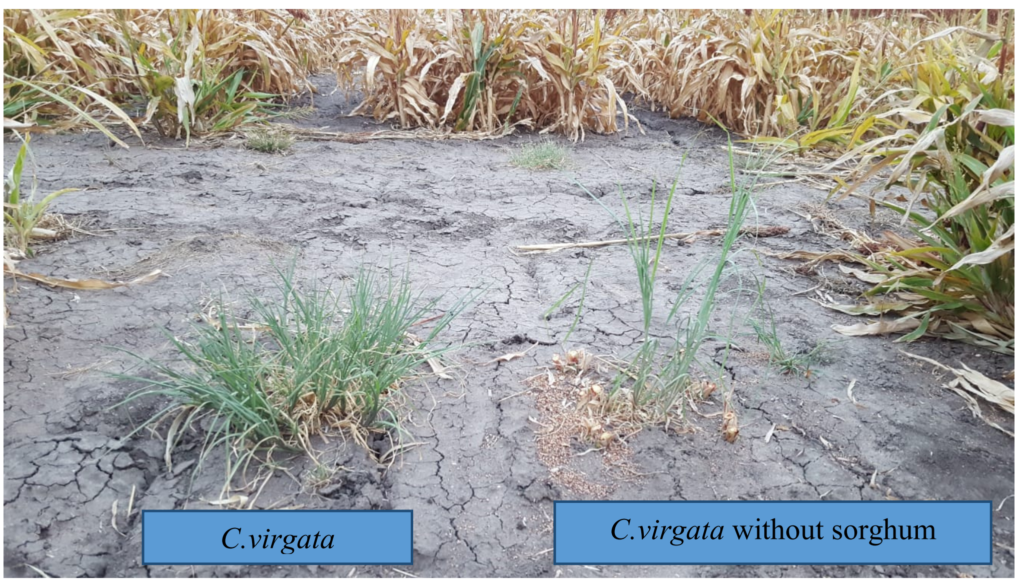


FIGURE 4.10 A *C.virgata* plant (right) after removal of sorghum plants showed a decrease in growth due to competition

TABLE 4.1 The number of leaves of *C.virgata* in competition with sorghum

Number of Leaves of *Chloris virgata*	14 days	28 days	42 days	56 days
Control	17 (±0.33)	55 (±0.57)	242 (±0.57)	281 (±0.57)
With sorghum	11(±0.33)	36 (±0.33)	36 (±0.33)	36 (±0.33)

The numbers in the parentheses are the standard error of the mean (n=3)

TABLE 4.2 The height of *C.virgata* in competition with sorghum

Height of *Chloris virgata* (cm)	14 days	28 days	42 days	56 days
Control	15 (±0.33)	32 (±0.33)	35 (±0.33)	42 (±0.33)
With sorghum	10 (±0.57)	47 (±0.57)	77 (±0.57)	78 (±0.33)

The numbers in the parentheses are the standard error of the mean (n=3)

TABLE 4.3 Shoot and root weight of *C.virgata* with and without competition with sorghum

Parameter	Shoot weight (g/plot)	Root weight (g/plot)
Control	160.0 (±2.52)	4.1 (±0.05)
With sorghum	23.5 (±0.17)	0.3 (±0.005)

The numbers in the parentheses are the standard error of the mean (n=3)

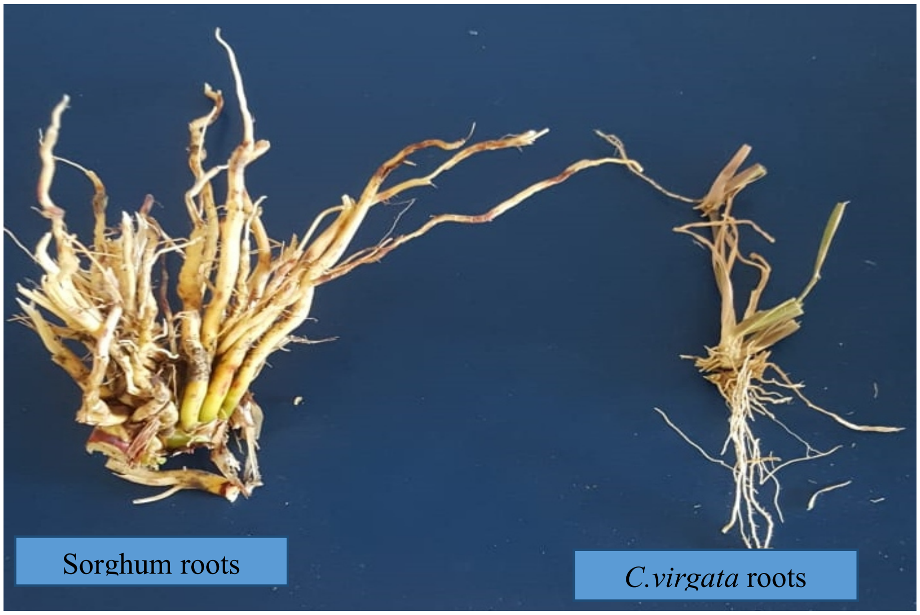


FIGURE 4.11 Roots of sorghum and *C.virgata* plant from the field excavation

Higher sorghum densities (10 plants m^{-2}) at narrow row spacing (50 cm) resulted in the suppression of *C.virgata*, which is in agreement with previous studies (Andrade et al., 2002, Mashingaidze et al., 2009). It clearly implies that narrow row spacing with high sorghum plant densities can reduce *C.virgata* biomass. These findings are in agreement with a previous study in which sorghum yield from a 45-cm row spacing was considerably higher compared with 60 and 90 cm row spacings due to a reduction in weed biomass (Bishnoi et al., 1990). Narrow rows (45 cm) yielded 7262, 7135 and 3395 kg ha^{-1} in no-till after clover, no-till after wheat and conventional tillage respectively, while 90 cm rows yielded 4,832, 4,589 and 2,197 kg ha^{-1} from no-till after clover, no-till after wheat and conventional tillage respectively (Bishnoi et al., 1990). The reduction in weed biomass of *C.virgata* observed here was due to narrowly spaced sorghum plants which resulted in narrow leaves and long stems that increased sorghum plant biomass (Bullock et al., 1988, Kasperbauer and Karlen, 1994). It implies that sorghum can be sown at narrow row spacings with higher densities to suppress the growth of *C.virgata*.

Higher densities of sorghum in field trials resulted in the reduction of *C.virgata* biomass due to non-penetration of light (Ramesh et al., 2017, Grichar et al., 2004). As plants mature and the canopy closes, the competition for photosynthetically active radiation (PAR) becomes intense (Holt, 1995) for crop and weed plants. Sorghum can absorb incoming light more efficiently than weeds (Grichar et al., 2004) and absence of light due to canopy closure of sorghum plants resulted in the reduction of *C.virgata* biomass.

The phenological study of *C.virgata* indicated no further production of leaves in *C.virgata* plants after four weeks (Table 4.1). This indicates strong competition for resources (light, water, and nutrients) between sorghum and *C.virgata* plants. An increase in the height of *C.virgata* in the presence of sorghum plants (Table 4.2) to study phenology aspects was an adaptation to capture light. About seven-fold decrease in shoot weight was observed for *C.virgata* in the presence of sorghum (Table 4.3). Competition for light was the major reason for the reduction in weed biomass in previous studies (Ballare and Casal, 2000), which implies that maximising light interception in sorghum crop canopies with high densities at narrow spacings can suppress *C.virgata*.

CHAPTER 5

SORPTION OF RADIOLABELLED GLYPHOSATE ON BIOCHAR AGED IN CONTRASTING SOILS

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Published: 30 October 2018

In **Journal of Environmental Science and Health, Part B**

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
https://doi.org/10.1080/03601234.2018.1531658

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Sorption of radiolabelled glyphosate on biochar aged in contrasting soils

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ABSTRACT

Glyphosate mobility from terrestrial to aquatic environments has raised concerns about it. Utilizing soil's inherent properties along with sorption properties of aged biochar, we hypothesized that selective application of biochar would be more effective in economic terms for glyphosate sorption on contrasting soils. To test this hypothesis, batch experiments and liquid scintillation counting for ^{14}C labeled glyphosate were used. The sorption behavior of glyphosate was examined in four contrasting Australian soil types (Oxisol, Vertisol, Entisol, and Inceptisol) amended with aged biochar to determine glyphosate concentrations by measuring ^{14}C activity using liquid scintillation counting. Freundlich parameters were calculated for soil-soil/biochar combinations. The pattern of glyphosate sorption was Oxisol > Vertisol > Entisol > Inceptisol. Oxisol adsorbed approximately five times more glyphosate compared with Inceptisol. Oxisol soil system adsorbed maximum amount of glyphosate principally due to the presence of iron-aluminum oxides exhibiting variable charges which got increased due to the presence of aged biochar. Considering all the soil/soil-biochar systems, Inceptisol soil system showed the least adsorption of glyphosate. A significant contribution of char was observed only in the Entisol soil system and the finding is valuable as char can be applied in Entisol soil systems to control glyphosate mobility.

KEYWORDS

Glyphosate; adsorption; isotherms; biochar; liquid scintillation counting

Introduction

Glyphosate (*N*-phosphonomethylglycine, $C_3PNO_5H_8$) is an active ingredient in broad- spectrum herbicides used for controlling weeds.[1] Glyphosate is very soluble in water (12 g L^{-1}).[2] The World Health Organization's International Agency for Research on Cancer has confirmed that glyphosate is "probably carcinogenic to humans."[3] Glyphosate-based herbicides often contaminate drinking water sources, air, and precipitation in agricultural regions.[3] Apart from cancer, the role of glyphosate has been confirmed in chronic kidney disease faced by Sri Lankan farmers.[4] This is a global problem, as excessive glyphosate residues in groundwater, drinking water and the urine of subsistence farmers from intensive agricultural localities have been reported, which can pose a risk to human health.[5]

Glyphosate has three functional groups: amine, carboxylate and phosphonate and these are responsible for strong coordination with metal ions.[6] Glyphosate is bonded to iron and aluminum oxides,[6] mainly via the phosphonate group in the adsorption process. In general, iron and aluminum oxides adsorb a greater amount of glyphosate and phosphates compared to layer silicates,[7] supporting the role of soil mineralogy in glyphosate sorption.

Previous studies have shown the efficiency of biochar to sorb herbicides. In an interesting comparison, wheat char sorbed ~400 to 2500 times more diuron than a silty loam soil within a concentration range of 0–6 mg L^{-1} clearly indicating higher sorption of herbicide by char compared to the soil.[8] Biochar, undergoes different biogeochemical reactions when applied to the soil during a process known as "ageing."[9]

An increase in the surface area of biochar has been observed to increase the ability of biochar to adsorb organic contaminants such as glyphosate.[10,11] The high specific surface area of biochar dictates most soil–biochar interactions. Even with an increase in the surface area of biochar, sorption sites can be blocked by organic matter, which is the likely cause for the diminished capability of aged biochar to adsorb organic contaminants.[8]

There are contrasting reports about the use of biochar to sorb glyphosate in different soil systems. Enhanced sorption of glyphosate was observed in soils amended with birch wood biochar but the sorption was dependent on physical and chemical properties of soils.[12] During ^{14}C glyphosate transport in undisturbed topsoil columns, the amount of glyphosate leached from the macroporous sandy loam was 50–150 times greater than that from the sandy soil, which suggests the different behavior of soil with respect to glyphosate.[13] In a related study on the adsorption and mobility of glyphosate in different soils under no-till and conventional tillage, the adsorption of glyphosate was influenced by the soil clay content and cation exchange capacity (CEC) and negatively related to pH and phosphorus. High

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Table 1. Physico-chemical properties of different soil systems.[15]

Property Location WRB order (FAO, 2006)	Inceptisol Western Australia (WA) (35°89S, 116°38E) Arenosols	Entisol South Australia (SA) (33°14S, 134°72E) Calcisol	Oxisol New SouthWales (NSW) (28°50S, 153°25E) Ferralsol	Vertisol Queensland (28°13S, 152°6E) Vertisol
pH (1:5 H_2O)	5.70 (±0.01)	8.77 (±0.01)	5.65 (±0.01)	7.89 (±0.01)
Organic carbon (%)	0.95 (±0.02)	2.53 (±0.09)	4.39 (±0.02)	2.25 (±0.04)
Sand / (%)	97.5 (±0.1)	66.3 (±0.1)	24.3 (±2.0)	27.3 (±0.7)
Silt / (%)	1.2 (±0.1)	12.2 (±0.2)	31.6 (±1.4)	28.5 (±0.8)
Clay / (%)	1.3 (±0.1)	21.5 (±0.4)	44.1 (±0.6)	44.2 (±0.1)
CEC (mmolc kg^{-1})	24.4 (±2.5)	99.1 (±3.3)	119.9 (±2.9)	265.4 (±3.1)
Minerals in the clay fraction	kaolinite	Illite	goethite, gibbsite, kaolinite and hematite	smectite, kaolinite

The numbers in parentheses are the standard error of the mean ($n = 4$).

K_F values obtained in isotherm studies were the major indicator of glyphosate mobility.[14]

There are very few studies in which the sorption of glyphosate to aged biochar in different soil types has been studied. Using the inherent properties of soil along with the sorption properties of aged biochar, we hypothesized that (i) the sorption behavior of glyphosate will vary in Australian soils of different composition; and (ii) aged biochar will affect the sorption behavior of glyphosate.

Materials and methods

Soil and soil–biochar samples were obtained from a previous incubation experiment conducted at the University of Sydney.[15] Soil samples (without biochar) and soil-biochar mixtures were incubated for 12 months at 20 °C. The four soils, that is, Inceptisol, Entisol, Oxisol, and Vertisol, were surface samples (0–15 cm) that were collected from different field locations in Australia (Table 1). The wood biochar was produced at 550 °C using slow pyrolysis (5–10 °C min^{-1} heating rate, 40 min residence time) from ^{13}C-depleted woody biomass of *Eucalyptus saligna*.[15] Biochar was sieved through a 2 mm sieve. The CEC (mmolc) and specific surface area (m^2 g^{-1}) for 550 °C biochar was 54 (mmolc) and 228.3 (m^2 g^{-1}), respectively.[15]

Radiotracer adsorption study

Batch equilibration sorption experiments using the radiotracer technique were conducted. For this analysis, 0.5 g of soil with and without char was weighed (in triplicate) in 2 mL Eppendorf tubes, except for the New South Wales soils (0.25 g of soil with and without char). To check for any sorption on the Eppendorf tubes, two sets of controls were prepared. From one set, 0.5 mL of supernatant was taken and analyzed for the initial count of disintegration per minute (dpm) and the second set was shaken with other samples to obtain the final count. A measure of 0.75 mL of 0.01 M mercuric chloride was added to each tube including the control tubes to avoid microbial degradation. Suspensions were shaken for 24 h at 20 ± 1 °C and samples were then equilibrated with 0.25 mL of spiking solution of cold concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 mg L^{-1}) and a constant hot concentration of 0.67 kBq mL^{-1} for 24 h. Samples were subsequently centrifuged (Eppendorf centrifuge 5415 D) for 10 min at 13,000 rpm. The supernatant solution (0.5 mL) was combined with 4 mL of scintillation cocktail (Optiphase high safe 3 / Perkin Elmer) in super polyethylene vials. The glyphosate concentrations were determined by measuring the ^{14}C activity,[16] in a liquid scintillation counter (Tricarb-4810 / Perkin Elmer). The effect of quenching was automatically adjusted by liquid scintillation counting (LSC).[16] LSC involves the conversion of energy from radioactive decay into photons of light and the radioactive carbon was monitored using LSC.[17] The amount of glyphosate adsorbed (mg kg^{-1}) was calculated from the loss of radioactivity that is, from the differences between the solution concentrations before and after equilibrium as disintegration per minute (dpm). We calculated sorption by comparing the initial concentration (initial dpm count) against the final concentration in solution after the sorption equilibration. The glyphosate sorption data were fitted to the nonlinear form of the Freundlich equation. The Eq. (1). can be expressed as-

$$x = k_F C^n \quad (1)$$

where x is the amount adsorbed (mg kg^{-1}), C is the equilibrium concentration (mg L^{-1}), k_F is the Freundlich sorption coefficient reflecting the number of adsorption sites and n is a constant. Adsorption coefficients from batch experiments are an important tool for understanding contaminant behavior.[18]

Statistical analysis

The batch experiments were repeated, data were pooled as no significant interactions between treatments and experiments were observed. The data were analyzed using Analysis of Variance (GenStat version 18) and the means were separated by Tukey's 5% confidence intervals.

Results and discussion

Oxisol soil system showed the highest sorption of glyphosate among all the soil systems (Fig. 1). Significant differences ($P < 0.001$) were observed with respect to glyphosate sorption in all the soil systems. The pattern of glyphosate sorption was as follows: Oxisol (NSW) > Vertisol (QLD) > Entisol (SA) > Inceptisol (WA) (Table 2). Oxisol (NSW) adsorbed approximately five times more glyphosate compared with Inceptisol (WA).

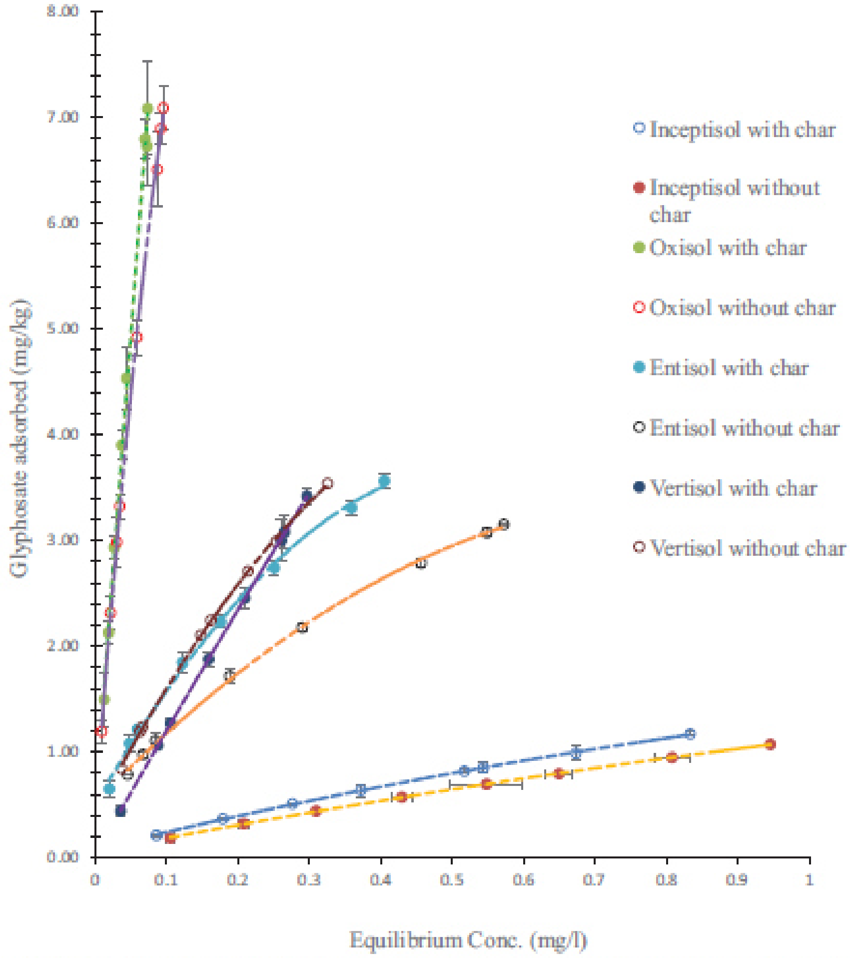


Figure 1. Glyphosate adsorption isotherms for the soil / soil-biochar systems. **(a)** Glyphosate adsorption isotherms for the Oxisol soil / soil-biochar system. **(b)** Glyphosate adsorption isotherms for the Vertisol soil / soil-biochar system. **(c)** Glyphosate adsorption isotherms for the Entisol soil / soil-biochar system. **(d)** Glyphosate adsorption isotherms for the Inceptisol soil / soil-biochar system.

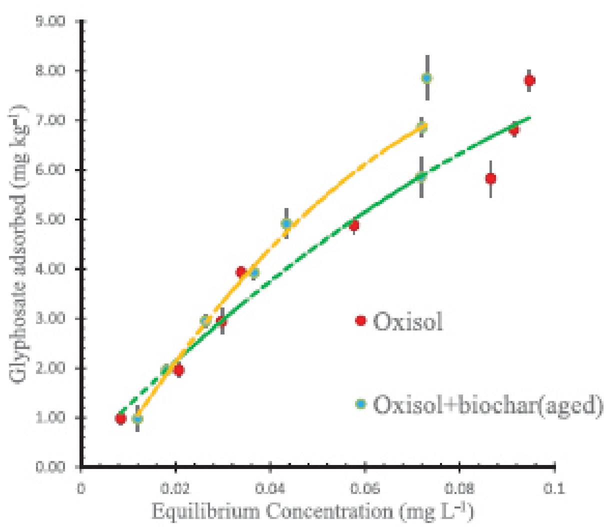
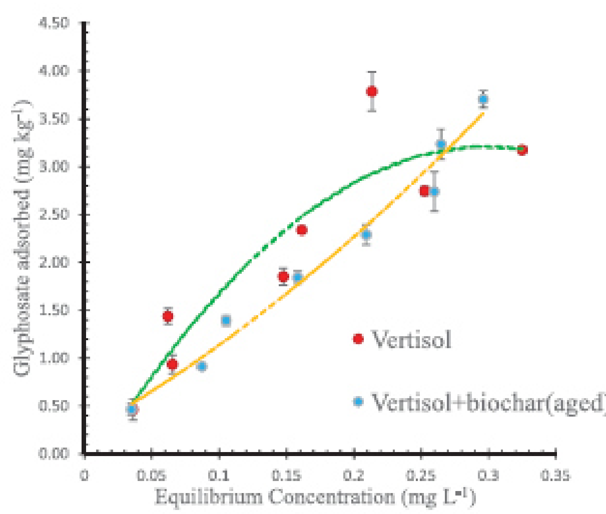


Figure 1. (continued)



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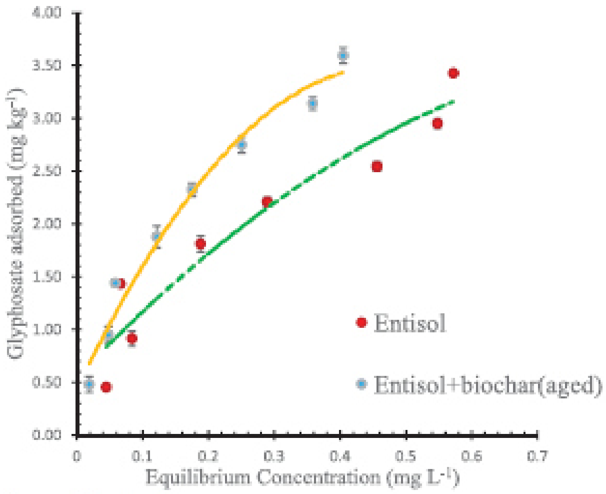

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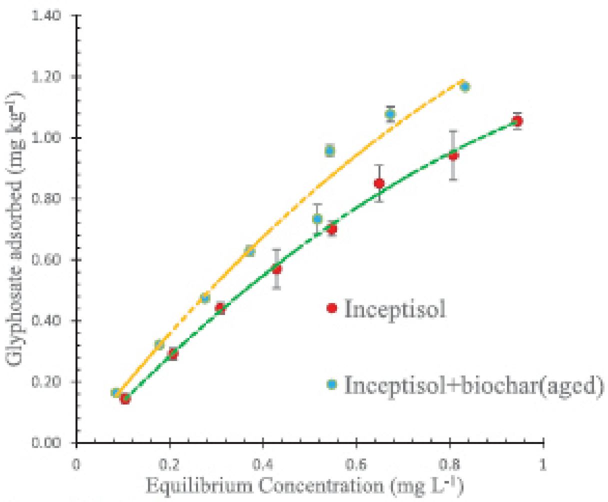

Figure 1. (continued)

An examination of the Freundlich K_F values (Table 2) clearly shows that the Oxisol (NSW) adsorbed the maximum amount of glyphosate; followed by Vertisol (QLD). The least adsorption of glyphosate was observed in Inceptisol (WA). Maximum sorption of glyphosate was observed in the Oxisol soil system (Fig. 1a) compared to other soil systems mainly due to the presence of clay, especially iron oxides (goethite, gibbsite, kaolinite, and hematite) and aged biochar.

Iron oxides exhibit a variable charge compared to clay minerals such as smectite which exhibit a fixed charge. The binding mechanism of glyphosate molecules involves an exchange of the hydroxyl of the iron hydration sphere by the P–OH group of the phosphoric moiety of the glyphosate.[19] In a similar study related to the behavior of glyphosate in a reservoir and the surrounding agricultural soils, the highest glyphosate adsorption was observed on amorphous oxides.[6] Our results also clearly show that the variable charge minerals such as iron oxides adsorb more glyphosate compared to clay minerals with a fixed charge. This finding is in agreement with other studies,[6,20] in which variable charge minerals were found to be effective glyphosate sorbents, whereas soils dominated by permanent charge minerals such as smectite were found to adsorb less glyphosate. Compared to the Oxisol soil system, the Vertisol soil system (Fig. 1b) showed less sorption of glyphosate which demonstrates that the mineral composition of the clay fraction affects glyphosate adsorption. The main reason for this behavior was the suppression of the char effect due to the presence of smectite minerals. This implies that the application of char will have a lesser effect in the Vertisol soil systems due to the presence of smectite-type clay. The significant contribution of char towards glyphosate adsorption was observed in the Entisol soil system with illite as the major clay mineral (Table 1, Fig. 1c).

Table 2. Freundlich parameters for glyphosate adsorption data for the soil and soil-biochar systems

Soil system	k_F (±s.e.)	n (±s.e.)	R^2
Oxisol with char (NSW)	5.3445 ± 0.27^c	1.0512 ± 0.08^c	0.99
Oxisol without char (NSW)	5.3135 ± 0.17^c	0.9611 ± 0.05^c	0.99
Vertisol with char (QLD)	2.1225 ± 0.05^b	0.9430 ± 0.10^b	0.99
Vertisol without char (QLD)	2.1344 ± 0.28^b	0.9411 ± 0.12^b	0.99
Entisol with char (SA)	1.7909 ± 0.10^a	0.8521 ± 0.04^a	0.99
Entisol without char (SA)	1.0844 ± 0.10^b	0.8734 ± 0.19^b	0.98
Inceptisol with char (WA)	1.1296 ± 0.04^d	0.9435 ± 0.03^d	0.98
Inceptisol without char (WA)	1.0641 ± 0.03^d	0.9730 ± 0.02^d	0.99

The numbers in parentheses are the standard error of the mean ($n = 4$). Here a, b, c, and d represent different pairs of treatment means at the 0.05% significance level.

The higher values of K_F for Entisol amended with char compared to systems without char amendment suggests that biochar creates more adsorption sites with a consequent increase in glyphosate adsorption. Isotherms were curvilinear in the Entisol soil system with illite; whereas they were linear for Inceptisol with kaolinite, which is in agreement with previous findings.[21] The Inceptisol (WA) soil system (Fig. 1d) showed the lowest adsorption of glyphosate among all the soil/soil-biochar systems.

The possible explanation for this behavior is a low clay content (Table 1) and the presence of Kaolinite as a major mineral in the clay fraction. This finding is important, as low sorption of glyphosate has been found to be the major reason for the contamination of groundwater by herbicides in Australian soils.[22] It has been generally observed that glyphosate has a limited risk of mobility due to its strong adsorption and rapid degradation,[1,23], however, long-term use of glyphosate may result in the surface, groundwater and sediment pollution.[24,25] Glyphosate can be very mobile in soil systems and the lack of retention of the molecule for microbial degradation can result in the movement of glyphosate to lower soil layers.[2,19]. Our study indicates that char can sorb glyphosate at higher concentration levels in the Entisol soil systems.

Conclusions

Significant differences were observed among the different soil systems with respect to glyphosate adsorption. The effect of aged biochar was found to be the highest in Entisol and the lowest in the Vertisol soil systems. Inceptisols demonstrated the lowest amount of glyphosate sorption among all the soils and are therefore the most vulnerable soil systems with respect to glyphosate mobility.

Disclosure statement

There is no potential conflict of interest declared by the authors.

Funding

The authors deeply appreciate funding in the form of the University of Sydney International Scholarship (USydIS, student ID- 440059389) from the University of Sydney and project support from the CSIRO.

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CHAPTER 6

GENERAL DISCUSSION

The aim of this thesis was to investigate the problems associated with the control of glyphosate-resistant weeds like *C.virgata* and the consequent problems of glyphosate mobility in different soil environments. The findings from this research enhanced the understanding of these problems and solutions in the form of strategies related to biochar application and crop competition.

The central research question was:

How aged biochar can determine the fate of glyphosate in soils of different mineral composition and how crop competition can control glyphosate-resistant C.virgata?

The general introduction in **Chapter 1** and the literature review in **Chapter 2** introduced the problems of control of glyphosate-resistant *C.virgata* and global problem of mobility of glyphosate in different soil systems. The following includes a discussion of the main findings of the research chapters and their practical implications.

Chapter 3 was related to the **'Management of *C.virgata* in mung bean using crop competition'.** The **key findings** were1.*The data from the pot experiments indicated C.virgata biomass of 43-48 g dry weight in both seasons was significantly reduced by 74 % when surrounded by 5-6 mung bean plantsm*$^{-2}$.

2. *Phenology aspects indicated a different growth pattern of C.virgata in the field when surrounded by six mung bean plants spaced 25 cm apart.*

Chapter 3 addressed the emerging problem of *C.virgata* grass in mung bean crops which are cultivated as grain legume in central Queensland and northern New South Wales in Australia (Chauhan et al., 2017). No studies have been reported the impact of mung bean on *C.virgata.*

The objective of the study was to examine the suppressive ability of mung bean on *C.virgata*. This study is valuable to farmers facing problems with *C.virgata*. Mung bean was a better competitor than *C.virgata* in my study, however, *Eleusine indica* and *Synedrella nodiflora* were better competitors than mung bean in previous studies (VanCaelenbergh et al., 1996). Our recommendation to farmers facing the problem of *C.virgata* is to sow mung bean plants 25 cm apartwith a density of 35 plants $m^{-2}$to suppress *C.virgata* in northern New South Wales.

Chapter 4: Crop competition as a strategy to control *C.virgata* grass in *Sorghum bicolor*

Key Findings: 1**.** *A significant reduction in C.virgata biomass was observed when surrounded by 10 sorghum plants* m^{-2} *spaced 50 cm apart.*

Chapter 4 discussed the impact of sorghum on *C.virgata* in sorghum. The results indicated that *C.virgata*, when surrounded by 10 sorghum plantsm^{-2} in row spacings of 50 cm can suppress *C.virgata*. It is in agreement with a previous study in which sorghum yield from 45 cm row spacing was higher compared with 60 and 90 cm row spacings due to the reduction in weed biomass (Bishnoi et al., 1990). Narrowly spaced sorghum plants sown in proximity resulted in narrower leaves and longer stems increasing sorghum plant biomass (Bullock et al., 1988, Kasperbauer and Karlen, 1994). Yield responses to narrow crops depends on increased radiation interception (Andrade et al., 2002). Maize crop planted in narrow rows (60 cm) showed less yield reduction from hoe weeding than those planted in wider rows (75 cm or 95 cm) in previous studies (Mashingaidze et al., 2009). Apart from aspects of plant density and crop spacing, crop row orientation as a part of crop competition has been a useful weed control strategy in wheat and barley (Borger et al., 2016). The reduction in weed biomass in wheat and barley crops was due to increased light interception by crops oriented east-west compared with north-south crops (Borger et al., 2016). My studies on sorghum and mungbean showed that crop competition can be a useful strategy for farmers to control summer grasses like *C.virgata* considering the economic and environmental impacts. Narrow row spacing in mung bean can provide more ground cover, high light interception resulting in more leaf area index (LAI) (Drews et al., 2009), major factors involved in suppression of weeds like *C.virgata*. The results could be extrapolated to similar *Chloris* species like windmill grass (*Chloris truncata*).

Chapter 5: Sorption of radiolabelled glyphosate on biochar aged in contrasting soils

Key Findings: 1**.** *The pattern of glyphosate sorption in soil systems was the Oxisol>Vertisol>Entisol>Inceptisol.*

2. *Significant contribution of biochar was observed only in the Entisol soil system.*

Chapter 5 addressed the phenomenon of glyphosate adsorption. The objectives of the study were to study the sorption behaviour of glyphosate in soils of different composition in the presence and absence of biochar. The present study is valuable considering the information gap for herbicide mobility on Australian soils. The finding is important considering the vulnerability of Inceptisol soil systems of Western Australia (Gerritse et al., 1996). The implication of sorption study could be useful for soils of Western Australia having high organic matter. Soils having less organic matter reported more glyphosate adsorption and hence less mobility towards water bodies in previous studies (Gerritse et al., 1996). Organic matter was found to be a major competitor for glyphosate on sorption sites in previous studies (Gerritse et al., 1996).

Freundlich (K_F) values of sorption experiments indicated that Inceptisols would adsorb the lowest amount of glyphosate, which suggested that the glyphosate mobility will be higher in these systems and consequently a higher risk to aquatic systems, as observed by other researchers for soils having less clay content (de Jonge et al., 2001, Gimsing and Borggaard, 2007).

My experiment revealed that biochar application and incorporation would be effective in these systems. The maximum contribution of char was observed in Entisol soil systems. This finding is consistent with those of other researchers (Kumari et al., 2016) about the role of biochar. Hence biochar based mitigation strategies can be used for glyphosate adsorption (Hall et al., 2018). Strong sorption of glyphosate on biochar, however, weak desorption by dilute $CaCl_2$ indicated that hardwood biochars are ineffective sorbents for glyphosate in high phosphate soils (Hall et al., 2018). Glyphosate sorption and pH are generally negatively correlated (McConnell and Hossner, 1985, Gimsing et al., 2004) in soils and a reduction in glyphosate sorption to biochar has been observed, when pH was increased from 6 to 9 (Herath et al., 2016, Mayakaduwa et al., 2016). Biochar application as per the findings, should be soil specific. From practical implications point of view, it is of no use in Oxisol and Vertisol soil systems but can be applied in Inceptisol and Entisol soil systems. Batch experiments following CSIRO protocol clearly showed that Entisol soil systems are the most responsive with respect to biochar application to reduce the mobility of glyphosate.

The sorption behaviour of glyphosate indicated maximum sorption in Oxisol and Vertisol soil systems due to high clay content. It implies that type of clay plays a major role than the amount of clay with respect to glyphosate sorption. Char application would be ineffective in these systems due to high clay content. The previous studies related to the study of sorption mechanisms of glyphosate indicated $\pi - \pi$ electron donor-acceptor interactions, H-bonding and heterogeneous chemisorption through biochar surface functional groups(Herath et al., 2016, Mayakaduwa et al., 2016, Cederlund et al., 2017). The practical implications are soil specific. Biochar properties vary according to the type of biochar. Biochar used in my study was produced from slow pyrolysis at 550°C from *Eucalyptus salinga*, however, biochar produced from another source can show different behaviour, an aspect that need to be explored. Mathematical models like pesticide root zone model (PRZM) can be used to measure the dissipation rate of glyphosate. In the field study, glyphosate was found to be more persistent than metazachlor but less persistent than trifluralin. AMPA was found to be more persistent than glyphosate (Mamy et al., 2008). Further investigation is needed on this aspect in case of Australian soils.

The detailed investigations answered the central research question that mung bean and sorghum crops can suppress the growth of *C.virgata* under field conditions and sorption behaviour determined the fate of glyphosate in different soil systems. Over application of glyphosate has not only resulted in the evolution of glyphosate-resistant weeds like *C.virgata* in Australian environments but also its mobility depending on physio-chemical conditions of soils which could be a risk to aquatic environments. Hence studies related to glyphosate mobility and crop competition complement each other.

6.1 FUTURE DIRECTIONS OF RESEARCH

This thesis has generated knowledge for the research communities, farmers, growers, and students interested in the behaviour of glyphosate under field conditions and the consequences of the over-application of glyphosate resulting in the evolution of glyphosate resistance in grass weeds like *C.virgata* and the consequent problem of glyphosate mobility. Future research priorities should include:

- ❖ extensive field trials and economics based on the recommendations in this thesis to control *C.virgata* in mung bean and sorghum;
- ❖ use of Models like Hydrus 1D, LeachP and PRZM can be the future research priorities to study glyphosate mobility; and
- ❖ glyphosate residue studies from soil and water samples from different ecosystems in Australia. Any biochar application should be soil specific with respect to glyphosate as per the findings of this thesis.

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Appendix

ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 9 September 2019

Research Article

Management of Glyphosate-Resistant *Chloris Virgata* Grass in Mung Bean Using Crop Competition

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Received: August 06, 2019; **Published:** August 19, 2019

Abstract

Chloris virgata, a glyphosate-resistant grass has been identified as a major weed problem in central Queensland and northern New South Wales. It was hypothesised that mung bean as a crop competitor would suppress *Chloris virgata*. For this purpose, pot experiments were established for two seasons in a glasshouse. Phenology aspects of both mung bean and *Chloris virgata* were studied in the field. The current study results indicated *Chloris virgata* biomass of 43-48 g dry weight in both seasons was significantly reduced by 74% when surrounded by 5-6 mung bean plants. Phenology aspects showed a different growth pattern of *Chloris virgata* when surrounded by six mung bean plants spaced 25 cm apart. Our recommendation to farmers facing the problem of *Chloris virgata* is to sow mung bean plants 25 cm apart to suppress *Chloris virgata* in Australian conditions.

Keywords: Suppression; Biomass; Phenology; Annual Grass; Density; Row Spacing; Light

Introduction

Feathertop Rhodes grass (*Chloris virgata*) a native of North America, a major weed in cotton (*Gossypium hirsutum*) in the subtropical region of Australia has been found to be an emerging weed problem in central Queensland and northern New South Wales (NSW), Australia [1]. The major reason for this problem has been suggested to be the adoption of zero tillage practices and tolerance of *Chloris virgata* to glyphosate, a widely used systemic herbicide [1]. Across Australia, *Chloris virgata* cause yield losses in all crops of about 39,329 tonnes with a revenue loss of $7.7 m [2].

Crop competition involves the application of agronomic practices to suppress weed growth, including high crop density, weed competitive cultivars and high seeding rates [3]. These practices are under-exploited, but if used properly, are environmentally benign weed management strategies [4]. Crop density or the number of plants per unit of area is an important decision for competition studies considering the relationship among plant yield and the number of individuals and resources present in an area [5,6]. An increase in plant density can increase the competitive ability of the crop [7]. Along with plant density, narrow row spacing can increase light interception by crops with a reduction in weed biomass and a consequent increase in crop yield [8].

Mung bean (*Vigna radiata*) is cultivated as a grain legume in central Queensland and northern New South Wales in Australia [9]. Approximately 95% of the total mung bean production in Australia is exported to various countries like India, Vietnam, Philippines and China [9]. Mung bean is generally grown with a wide row spacing of 1per meter in central and southern Queensland and Northern New South Wales [9]. In a crop like mung bean, narrow row spacings (25 and 50 cm) lowered, weed biomass with a consequent increase in grain yield of mung bean [9]. There is no information in the literature of using crop competition for *Chloris virgata* control. I hypothesised that mung bean would suppress *Chloris virgata* growth and my experiments had following objectives (i) to study the crop competition between mung bean and *Chloris virgata* grass and (ii) the effect of different densities of mung bean on *Chloris virgata* growth.

Materials and Methods

Glasshouse studies were established in two seasons at the GRDC glasshouse complex, International Grains Research Centre, Narrabri, Australia.

Experimental methodology

The pot experiment for *Chloris virgata* and mung bean was established on January 31, 2017, and repeated on November 27, 2017, with the same species and densities. The seeds of *Chloris virgata* were brought from Osten weeds consulting (Emerald, QLD, 4720). Seeds of both *Chloris virgata* and mung bean were sown in plastic trays filled with potting mix (Searles premium potting mix

65 L). The potting mix was composed of organic compost, peat, zeolite and trace elements. Minimum and maximum temperatures during the period of experiments were 20^0C and 35^0C, respectively. Ten days after sowing the two-leaf plants were transplanted to pots (26 cm length x 28 cm width x 26 cm height) filled with potting mix. Mung bean and *Chloris virgata* were co-established by using a seeding template in order to eliminate size biases [10-13]. The density treatments [mung bean plants- 0-6 plants m-2; *Chloris virgata* plants- 0-1 plants m-2) were arranged in a target neighbour design. The target neighbour design is an additive design in which one of the competing species is represented by a single individual (the target, *Chloris virgata*) and the density of surrounding individuals (the neighbours, mung bean) is manipulated [14]. The density of the target species was reduced to a single individual to preclude significant intraspecific interactions [14].

Glasshouse study-measurements

For pot experiments, measurements included plant height (from the soil surface to the tip of the uppermost outstretched leaf) and the number of leaves per plant. Measurements were taken weekly for the entire season. Waterings were done as per the moisture meter. The moisture meter/ hygrometer had a scale (1-10 cm) with three different ranges (Dry, Moist, and Wet). The probe tip of the moisture meter was inserted into the root zone (10 mm) to measure soil moisture. If the probe indicated a moisture level of dry or moist, lots of frequent watering were done to reduce run-off. Plants were watered up to the wet range. At the end of the experiment, aboveground biomass of all the plants was harvested and their dry weights were recorded. The plants were dried at 70°C for 48 hours in a dehydrator (Sterdium, Micro digital).

Phenology aspects

To study phenology aspects 6 mung bean plants (3 in each row with a plant to plant distance of 10 cm) and 1 *Chloris virgata* plant were co-established in the field with three replications by hand following the same transplanting procedure outlined above for the glasshouse. Mung bean plants were transplanted by hand in rows 25 cm apart with a plant to plant spacing of 10 cm based on a previous study [9] with *Chloris virgata* plant at the centre between the rows on November 23, 2017, and harvested on January 26, 2018. For control treatments, *Chloris virgata* plants were not surrounded by mung bean plants. The root and shoot biomass of both mung bean and *Chloris virgata* plants were recorded by a scale and assessment of seed parameters (length of spikelet, seeds in each spikelet by manual counting, and the total weight of spikelet for *Chloris virgata* plant were completed with a scale. The roots of both mung bean and *Chloris virgata* plants were excavated manually by spade. The roots after collection were washed and dried for the assessment of root weights separately for both mungbean and *Chloris virgata* plants.

Results

Biomass of Chloris virgata, Feathertop Rhodes (FTR)

There was a significant reduction in *Chloris virgata* biomass in the glasshouse when surrounded by 5 or 6 mung bean plants (Figure 1 and Figure 2).

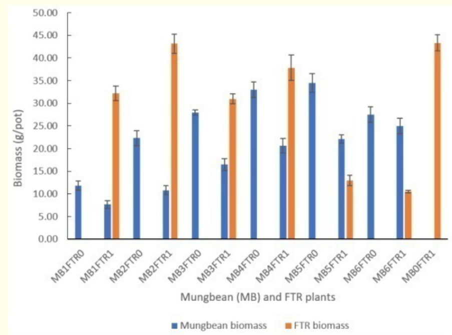

Figure 1: Mung bean (MB) and FTR biomass in the January of the 2016-2017 season.

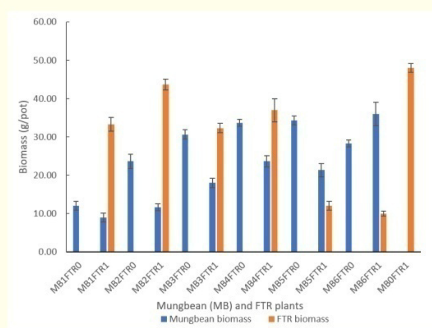

Figure 2: Mung bean (MB) and FTR biomass in the January of the 2017-2018 season.

Citation: Aman D Sharma., *et al.* "Management of Glyphosate-Resistant *Chloris Virgata* Grass in Mung Bean Using Crop Competition". *Acta Scientific Microbiology* 2.9 (2019): 108-113.

Error bars represent ± standard errors of the mean (n=3). The treatment abbreviations on the horizontal axis refer to MB (mung bean density; 0,1,2,3,4,5,6) and FTR (Feathertop Rhodes density; 0 and 1) in the glasshouse experiment

Error bars represent ± standard errors of the mean (n = 3). The treatment abbreviations on the horizontal axis refer to MB (mung bean density; 0,1,2,3,4,5,6) and FTR (Feathertop Rhodes density; 0 and 1) in the glasshouse experiment *Chloris virgata* was of 43-48g dry weight in both seasons, which was significantly reduced by 74% when surrounded by 5-6 mung bean plants. (Figure 1, Figure 2). Our findings demonstrated that *Chloris virgata* can be suppressed at higher densities of mung bean.

The number of leaves per mung bean and FTR plants

The number of leaves of *Chloris virgata* per plant were decreased 36-48% after 21 days but only in treatments with 5 and 6 mung bean plants (Table 1). There was no reduction in the number of leaves over time in other treatments. The maximum number of *Chloris virgata* leaves after 42 days were 13 when surrounded by 6 mung bean plants. The plausible reason was the greater competition from more mung bean plants that resulted in reduced resource acquisition and leaf production by *Chloris virgata*.

Leaves (no. plant^{-1})										
Treatment (No. of plants - 0,1,2,3,4,5,6)	7 Days		14 Days		21 Days		28 Days		42 Days	
	MB	FTR	MB	FTR	MB	FTR	MB	FTR	MB	FTR
MB1FTR0	2	0	5	0	11	0	17	0	17	0
MB1FTR1	2	2	5	11	10	26	15	59	15	59
MB2FTR0	2	0	5	0	11	0	16	0	16	0
MB2FTR1	2	2	5	10	10	26	15	51	15	51
MB3FTR0	2	0	5	0	11	0	14	0	14	0
MB3FTR1	2	2	5	9	9	24	11	58	11	58
MB4FTR0	2	0	5	0	9	0	13	0	13	0
MB4FTR1	2	2	5	9	10	23	12	37	12	37
MB5FTR0	2	0	5	0	9	0	12	0	12	0
MB5FTR1	2	2	5	8	8	16	12	16	12	16
MB6FTR0	2	0	5	0	8	0	12	0	12	0
MB6FTR1	2	2	5	7	7	13	12	13	12	13
MB0FTR1	0	2	0	17	0	25	0	60	0	60
SEs	(± 0.32)	(± 0.33)	(± 0.58)	(± 0.64)	(± 0.58)	(± 0.51)	(± 0.64)	(± 0.54)	(± 0.75)	(± 0.80)

Table 1: The number of leaves per plant for mung bean (MB) and Feathertop Rhodes (FTR) in two glasshouse experiments. SE (±) values represent standard errors with n=3.

The height of mung bean and FTR plants

In comparison to the control, a 32% reduction in height in *Chloris virgata* when surrounded by five or six mung bean plants at day 42 was observed (Table 2). This pattern of *Chloris virgata* was similar to that of spiny amaranth weed, the height of which was suppressed due to a higher density of rice plants [15].

Seed production of Chloris virgata (FTR)

Phenology aspects in the field indicated that on an average, the length of Chloris virgata spikelet was 7.9 cm (Table 3) when there was no mung bean competition.

Chloris virgata produced two spikelets without competition, whereas, in competition with 6 plants, Chloris virgata produced no spikelets (Table 3). Weed seed production is an important aspect with respect to Chloris virgata from the sustainability point of view and any practice reducing the weed seed input to the soil seed bank can contribute substantially towards weed management approach related to the control of Chloris virgate [16].

The growth pattern of Chloris virgata, when grown alone in the phenology study was different from more vertical growth of Chloris virgata when surrounded by mung bean plants (Figure 3, Figure 4).

Treatment (No. of plants -0,1,2,3,4,5,6)	Plant Height (cm)									
	7 Days		14 Days		21 Days		28 Days		42 Days	
	MB	FTR	MB	FTR	MB	FTR	MB	FTR	MB	FTR
MB1FTR0	9.3	0.0	22.0	0.0	36.7	0.0	66.3	0.0	72.3	0.0
MB1FTR1	9.0	25.3	19.3	45.0	34.7	67.7	48.0	127.6	67.7	115.0
MB2FTR0	9.3	0.0	19.3	0.0	35.7	0.0	48.3	0.0	71.0	0.0
MB2FTR1	8.7	24.1	15.3	41.7	35.3	62.7	42.3	112.7	59.3	110.0
MB3FTR0	9.0	0.0	17.0	0.0	34.0	0.0	40.7	0.0	70.0	0.0
MB3FTR1	8.7	21.3	15.3	39.0	33.0	60.0	33.3	105.7	56.0	98.0
MB4FTR0	8.0	0.0	18.7	0.0	33.7	0.0	42.0	0.0	64.0	0.0
MB4FTR1	7.7	18.0	15.0	38.0	33.0	53.7	37.7	84.0	57.0	97.3
MB5FTR0	9.3	0.0	18.0	0.0	34.7	0.0	36.0	0.0	62.0	0.0
MB5FTR1	8.1	17.0	16.5	35.3	34.0	52.0	35.7	67.0	58.0	90.0
MB6FTR0	8.0	0.0	18.0	0.0	27.0	0.0	35.7	0.0	50.3	0.0
MB6FTR1	7.5	17.0	16.0	34.3	26.3	61.0	35.0	64.0	47.0	89.0
MB0FTR1	0.0	17.0	0.0	36.0	0.0	65.7	0.0	127.7	0.0	131.3
SEs	(± 0.33)	(± 0.29)	(± 0.58)	(± 0.80)	(± 0.58)	(± 0.58)	(± 0.33)	(± 0.80)	(± 0.33)	(± 0.36)

Table 2: Mean plant height (cm) for mung bean (MB) and Feathertop Rhodes (FTR) averaged over two glasshouse experiments. SE (±) values represent standard errors with n=3.

Treatment	Shoot weight (g)	Root weight (g)	Length of spikelet (cm)	Seeds in spikelet	Total weight of spikelet (g)
With competition	9.6 (±0.15)	0.6 (±0.01)	No spikelet	No spikelet	No spikelet
Without competition	68.0 (±0.15)	1 (±0.05)	7.9 (±0.05)	1806 (±0.85)	1.2 (±0.02)

Table 3: Shoot, root and seed parameters of *Chloris virgata* (FTR) grass with and without competition with mung bean for the phenology aspects. The numbers in the parentheses are the standard error of the mean (n=3).

The vertical growth of *Chloris virgata* in competition with mung bean plants represents an adaptation. Due to shading of mung bean plants, *Chloris virgata*, showed more upright growth to capture sunlight. When surrounded by 6 mung bean plants, the average *Chloris virgata* root biomass decreased by 40 percent and shoot biomass decreased by over 85 percent (Table 3).

Figure 3: *Chloris virgata* plant (Left) without removal of mung bean

Figure 4: The reduction in biomass of *Chloris virgata* plant (Left) after removal of mung bean plants. The *Chloris virgata* plant on the left is now exposed after the six mung bean plants have been removed.

Citation: Aman D Sharma., *et al.* "Management of Glyphosate-Resistant *Chloris Virgata* Grass in Mung Bean Using Crop Competition". *Acta Scientific Microbiology* 2.9 (2019): 108-113.

Figure 5: Roots of mung bean (Left) and *Chloris virgata* plant (Right) from the field excavation to study phenology aspects..

Similarly, in competition with mung bean plants, the *Chloris virgata* average shoot weight reduced about seven times (Table 3) due to competition effect.

It can be inferred from the phenology aspects that high root and shoot weights of mung bean resulted in the suppression of *Chloris virgata*.

Discussion

An increase in mung bean density was found to be the reason of suppression of *Chloris virgata* population. The role of plant density of crop species has also been observed in previous studies to suppress weed species [17-19]. Increased beet density has been found to increase beet plant capacity to suppress weeds [20]. Under drought stress, *Cyperus rotundus* was observed to be a superior competitor to mung bean, while Eleusine indica and Synedrella nodiflora were inferior [21], however, no information related to *Chloris virgata* has been observed [22].

High- density crop plants can result in the reduction of water and nutrient availability to weeds more effectively than a low- density crop [6]. It is likely that the major reason for the reduction in biomass of *Chloris virgata* was greater shoot and root biomass of mung bean planted at higher density. The finding is valuable in case of suppression of annual grass weeds like *Chloris virgata*. We advocate the option of crop competition to farmers, as it is environmentally friendly and does not require a significant increase in the cost of production [4].

Acknowledgements

Authors deeply appreciate funding in the form of USydIS (University of Sydney International Scholarship) (Student Id- 440059389) from the University of Sydney and project support from IA Watson Grains Research Centre, Narrabri.

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Volume 2 Issue 9 September 2019

ACTA SCIENTIFIC AGRICULTURE (ISSN: 2581-365X)

Volume 3 Issue 9 September 2019

Research Article

Crop Competition as a Strategy to Control Feathertop Rhodes Grass in Sorghum

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Received: July 24, 2019; **Published:** August 26, 2019

Abstract

In sorghum, Feathertop Rhodes (FTR) (*Chloris virgata*) covers an area of 73,414 ha with a yield loss of 36,995 tonnes resulting in revenue loss of $7.0 million/year. Crop competition has been suggested as a benign strategy due to weed population shifts, increase in herbicide costs and concerns of environmental pollution. We hypothesised that sorghum at high densities, when grown at narrow row spacings can suppress FTR. We aim to investigate the influence of sorghum row spacing and plant density on Feathertop Rhodes (FTR) grass growth and development. Field experiments for two successive years were conducted to study the effect of varying row spacings (50, 75 and 100 cm) and sorghum densities (5, 7.5 and 10 plants/m^2) on the growth of FTR. Significant reduction in FTR weight was observed when surrounded by 10 sorghum plants/m^2 spaced 50 cm apart. The study is valuable for sorghum farmers facing the problem of FTR.

Keywords: Feathertop Rhodes Grass; Sorghum; Crop Competition; Biomass

Introduction

Sorghum is an important rotational crop in subtropical north-east Australia with a cropping area of approximately 0.82 Mha [1]. Early weed interference in sorghum can result in large yield losses [2]. Feathertop Rhodes (FTR) grass (*Chloris virgata*) has been identified as one of the top weeds of sorghum (*Sorghum bicolor L.*) along with awnless barnyard grass (*Echinochloa colona*) and sweet summer grass (*Brachiaria eruciformis*). In sorghum, FTR covers an area of 73,414 ha with a yield loss of 36,995 tonnes resulting in revenue loss of $7.0 million/year [3,4]. Presence of herbicide-resistant weeds like FTR, limited registered herbicides, and crop rotational restrictions have created a challenging environment for weed control in sorghum [5].

Sorghum growers of the subtropical north-eastern region of Australia depend on key herbicides like atrazine, metolachlor, fluroxypyr, 2,4-D amine and glyphosate that are applied alone or mixed [6]. Very few growers use tillage (0-17%) or crop competition (0-11%) to manage the weeds. Although the majority of growers use pre-emergence (34-95%) and/or post-emergence (22-57%) herbicides without achieving effective weed control, commonly used wide-row and skip- row planting configurations have escalated the problem [6]. This has prompted the urgent need for non-chemical control options like crop competition to control weeds in sorghum.

Studies related to phenology provide important information on functional rhythms of plants and plant communities [7]. Correct prediction of weed phenological development, especially in the case of FTR is a prerequisite of weed- crop competition studies [8]. Considering sorghum as a crop competitor for FTR, we aimed to determine the: (i) effect of density of sorghum and row spacing on the biomass of FTR and (ii) the influence of sorghum plants on the phenology of FTR.

Materials and Methods

Field studies were established in 2016 on medium clay Vertosols (pH-8.8; EC-0.13 dS/m; OC- 0.61%) and in 2017 on medium clay Vertosols (pH-7.2; EC-0.09 dS/m; OC- 0.45%) at the International Grains Research Centre, Narrabri.

Experimental methodology

The competition study involved two different species: Feathertop Rhodes (FTR) (*Chloris virgata* Sw.) and Sorghum (*Sorghum bicolor*; Var MR-43). In the year 2016, sorghum was sown in a 24 m^2 plot, while FTR was broadcasted in a subplot of 2 m^2. In the year 2017, sorghum was sown in 18 m^2 plot, while FTR was transplanted in a subplot of 1 m^2.

Fields were prepared with a field cultivator and recommended cultural practices for sorghum were used similarly to previous studies [9,10]. The experiment was arranged as a randomised complete block with four replicates. Treatments consisted of three different row spacings (50, 75 and 100 cm) and plant densities (5, 7.5 and 10 sorghum plants/m^2). In the year 2016, Feathertop seeds were broadcast by hand in 2 m^2 subplots at the centre portion of each experimental unit similar to a previous study [11] and thinned by hand to achieve a target plant density of 5 plants/m^2, however, in the year 2017, Feathertop Rhodes seedlings were transplanted by hand in 1 m^2 subplots at the centre portion of each experimental unit with the objective of reduction in soil weed seed bank [11]. Competition between sorghum and FTR plants was established by transplanting FTR seedlings at two- leaf stage in the subplots of the experimental units.

Measurements

Sorghum plants were harvested at maturity. FTR and sorghum samples were dried at 70°C for 48 hours in a dehydrator (Sterdium, Micro digital). Seed heads were weighed and threshed, dry matter and grain yield were recorded as per the previous studies [9].

The phenology of FTR was studied in the presence and absence of sorghum. Sorghum and FTR plants were transplanted at the two- leaf stage and co-established in the field. Six sorghum plants in three replicates were spaced 50 cm apart with the FTR plant at the centre. The field experiment for phenology of two species was initiated along with the crop competition study to evaluate the phenology of FTR in the presence or absence of sorghum [10]. At a bi-weekly interval, plant height (height from the ground to the flag leaf) of FTR was recorded using a meter ruler and the number of leaves of FTR were recorded by visual counting in the presence and absence of sorghum.

Stat analysis

No significant interactions between treatments and experimental trials were observed. Therefore, the data were pooled for each field trial for further statistical analysis. A 3 factor-factorial regression model using PROC GLM procedure (SAS 9.4) was used to test for treatment differences for the response variables of FTR and sorghum.

Results

Significant reduction in FTR weight was observed when surrounded by 10 sorghum plants/m2 spaced 50 cm apart. Significant differences ($p < 0.005$) among the treatments indicated that 10 sorghum plants per square metre, if spaced 50 cm apart can decrease FTR biomass (Figure 1). The finding is important for the sorghum farmers facing the problem of FTR. It clearly implies that narrow row spacing with high sorghum plant densities can reduce FTR biomass. Sorghum plants were able to suppress FTR due to their height and increased rate of growth.

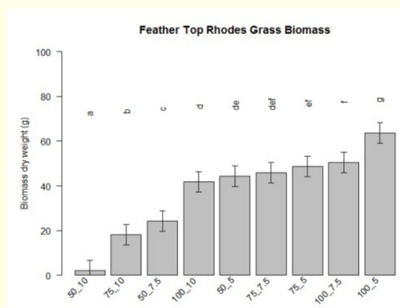

Figure 1: Interaction plot for FTR biomass at different densities and row spacings of sorghum for the years 2016 and 2017. In this figure (50 cm, 75 cm and 100 cm) represent different row spacings, while (5, 7.5 and 10) represent number of sorghum plants per metre square.

Grain yield of sorghum was increased with the decrease in FTR biomass (Figure 2).

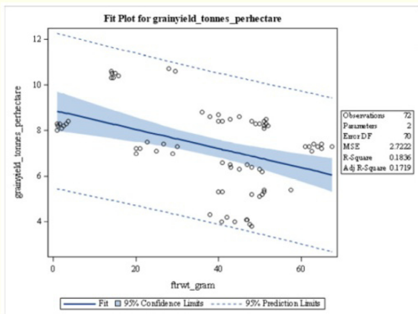

Figure 2: Interaction plot shows a decrease in FTR biomass (g) with the increase in grain yield (tonnes/ha) of sorghum.

Highest biomass and grain yield of sorghum was observed at 75 cm spacing when surrounded by 10 plants/m^2 (Figure 3, Figure 4). Interaction plots for sorghum biomass (Figure 3) indicated 28% increase in sorghum biomass in the absence of FTR, while there was 80% increase in grain yield (Figure 4) of sorghum in the absence of FTR. Significant interactions ($p < 0.001$) between row spacing and densities of sorghum plants with respect to biomass and grain production of sorghum were observed. Differences in harvest index (0.12 with FTR and 0.10 without FTR) (Figure 5) demonstrated the negative effect of FTR on the biomass and grain yield of sorghum.

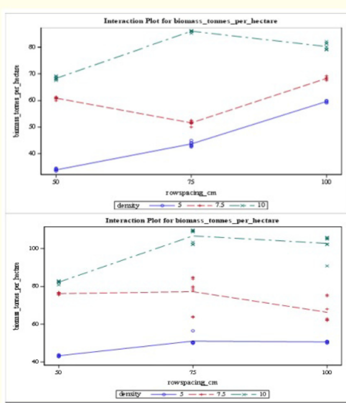

Figure 3: Interaction plot for biomass (tonnes/ha) of sorghum at varying row spacings (50 cm, 75 cm and 100 cm) and densities of sorghum plants (5, 7.5 and 10 plants per metre square) in the presence (above) and absence (below) of FTR.

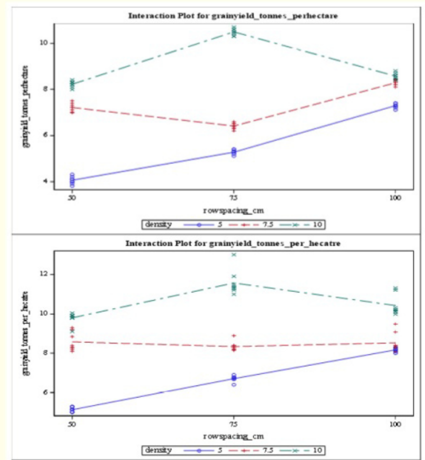

Figure 4: Interaction plot for grain yield (tonnes/ha) of sorghum at varying row spacings (50 cm, 75 cm and 100 cm) and densities of sorghum plants (5, 7.5 and 10 plants per metre square) in the presence (above) and absence (below) of FTR.

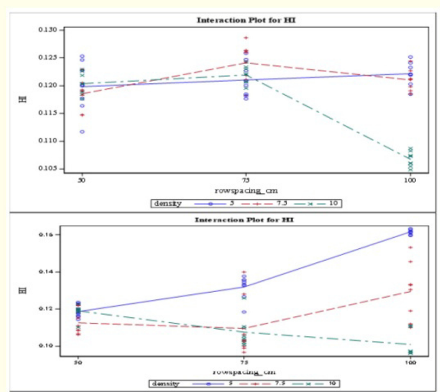

Figure 5: Interaction plot for Harvest index (HI) of sorghum at varying row spacings (50 cm, 75 cm and 100 cm) and densities of sorghum plants (5, 7.5 and 10 plants per metre square) in the presence (above) and absence (below) of FTR.

Phenology of FTR with and without competition with sorghum

FTR plant without competition with sorghum showed more lateral growth, while the FTR in competition with sorghum plants showed more vertical growth. This is an adaptation of FTR plant due to competition by sorghum plants. Sorghum plants suppressed the growth of FTR by shading effect. The absence of light due to shading effect of sorghum resulted in decreased growth of FTR. After 28 days, there was no leaf production in FTR plants facing competition from sorghum (Table 1) while leaf production continued in FTR plants in the absence of sorghum plants. An increase in height of FTR plants in competition with sorghum was observed (Table 2) in comparison to control plots. This is an adaptation by the FTR plant for the competition for light with sorghum plant. There was an eight-fold decrease in shoot weight of FTR compared with shoot weight of sorghum and four times decrease in the root weight of FTR compared with root weight of sorghum in the phenology study. This finding clearly indicates that sorghum is a better competitor than FTR.

Number of Leaves of FTR	14 days	28 days	42 days	56 days
Control	17 (±0.33)	55 (±0.57)	242 (±0.57)	281 (±0.57)
With sorghum	11(±0.33)	36 (±0.33)	36 (±0.33)	36 (±0.33)

Table 1: Phenological aspects of FTR in competition with sorghum.

The numbers in the parentheses are the standard error of the mean (n=3); Here FTR = Feather top Rhodes

Height of FTR (cm)	14 days	28 days	42 days	56 days
Control	15 (±0.33)	32 (±0.33)	35 (±0.33)	42 (±0.33)
With sorghum	10 (±0.57)	47 (±0.57)	77 (±0.57)	78 (±0.33)

Table 2: Phenological aspects of FTR in competition with sorghum.

The numbers in the parentheses are the standard error of the mean (n=3); Here FTR = Feather top Rhodes

Discussion

From our experiments, it was observed that higher sorghum densities (10 plants/m^2) at narrow row spacing (50 cm) resulted in the suppression of FTR, which is in agreement with previous studies [12,13]. The decrease in grain yield of sorghum was observed with the increase in FTR biomass, which is in agreement with another study in which Japanese millet (*Echinochloa esculenta*) adversely reduced the seed production of sorghum [1].

The findings are in agreement with a previous study in which sorghum yield from 45-cm row spacing was considerably higher compared with 60 and 90 cm row spacings due to the reduction in weed biomass [14]. The reason for the reduction in weed biomass of FTR was narrowly spaced sorghum plants sown in proximity which resulted in narrow leaves, long stems and root biomass that increased the sorghum plant biomass [15,16]. It implies that sorghum can be sown at narrow row spacings with higher densities to suppress the growth of FTR. Higher sorghum densities mean a higher seeding rate while the costs of seed would be compensated with a reduction in weed control costs (i.e., chemicals, fuel, machinery, and labour) [17].

Higher densities of sorghum in field trials resulted in the reduction of FTR biomass due to non-penetration of light [5,18]. It has been observed that as plants mature and the canopy closes, the competition for photosynthetically active radiation (PAR) becomes intense [19] for crop and weed plants. Sorghum can absorb incoming light more efficiently than weeds [5] and absence of light due to canopy closure of sorghum plants resulted in the reduction of FTR biomass.

Conclusion

Phenological aspects of FTR indicated non-production of leaves in FTR plants after four weeks (Table 1), while facing competition from sorghum. It clearly indicates the competition for resources (light, water, and nutrients) between sorghum and FTR plants in which sorghum was found to be a strong competitor. An increase in height of FTR in the presence of sorghum plants (Table 2) was an adaptation to capture light. Competition for light was the major reason for the reduction in weed biomass in previous studies [20], which implies that by maximising light interception in sorghum crop canopies with high densities at narrow spacings can suppress FTR.

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Chapter

Glyphosate Resistance of *Chloris virgata* Weed in Australia and Glyphosate Mobility Are Connected Problems

Aman D. Sharma

Abstract

The purpose of this review paper is to address two major aspects of glyphosate application on farmers' fields. The first aspect is the development of glyphosate resistance in weeds like *Chloris virgata*, and the second aspect is glyphosate mobility, which is directly controlled by soil sorption processes and indirectly by molecule degradation processes. This is a global problem, as excessive glyphosate residues in groundwater, drinking water, and urine of subsistence farmers from intensive agricultural localities have been reported, which can pose a risk to human health. Approaches like biochar as a possible strategy to control glyphosate leaching and crop competition as a cultural method to control glyphosate-resistant weed like *Chloris virgata* can be the potential solutions of the glyphosate resistance and glyphosate mobility.

Keywords: resistance, glyphosate, mobility, biochar, crop competition

1. Introduction

1.1 Chemistry of glyphosate

Glyphosate (N-(phosphonomethyl) glycine) is a non-selective post-emergence herbicide widely used in field crops, vegetable crops, and orchards. Glyphosate is absorbed by plants via leaves and shoots and is transported throughout the whole plant. Its usual formulation is salt of a deprotonated acid of glyphosate and a cation, e.g. isopropylamine or trimethylsulfonium. Its chemical structure has three groups (amine, carboxylate, and phosphonate) that form strong coordination bonds with metal ions to form bidentate and tridentate complexes (**Figure 1**). Hence it is a strong chelating herbicide [1].

Chemically, glyphosate is a phosphonate. It is mainly the phosphonate group via which glyphosate is bonded to iron and aluminum oxides by ligand exchange with the formation of mononuclear, monodentate, and/or binuclear, bidentate surface complexes [2].

1.2 Glyphosate degradation

Among the microorganisms, bacteria represent the majority of the glyphosate-degrading organisms [3]. Bacteria degrade glyphosate by cleaving the C-N bond and

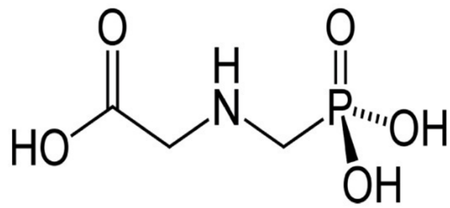

Figure 1.
Chemical structure of glyphosate [1].

converting glyphosate to AMPA (amino-methyl phosphonic acid) which is further decomposed and finally excreted to the environment. Glyphosate degradation can also occur via C-P lyase pathway to sarcosine, rather than AMPA. A bacterial strain *Bacillus subtilis* Bs-15 degraded 18% (12 h) to 67% (96 h) of glyphosate in sterile soil and 19% (12 h) to 72% (96 h) in unsterilized soil. It indicates that Bs-15 can significantly enhance glyphosate degradation.

1.3 Mobility of glyphosate

The binding mechanisms of clay minerals and organic colloids result in non-occurrence of free glyphosate, but leaching of glyphosate complexes via preferential flow paths through the soil and transfer to waterways can occur, which could be a concern from the environmental pollution point of view [4]. In another study related to the desorption rate of glyphosate from goethite mineral surfaces, the rate of glyphosate desorption is mainly controlled by the breaking of the Fe-glyphosate bond through a dissociative or a dissociative interchange mechanism [5]. Soil redox condition is also an important factor controlling the mobility of glyphosate. Microbial degradation and mineralization of glyphosate were slow in anoxic environments compared with oxic environments [6].

In US soils, glyphosate and AMPA have been detected together and found widely in the environment. The occurrence was more frequent in soils and sediments, ditches and drains, and rivers and streams and less in lakes, ponds, wetlands, soil water, and groundwater [7]. In western Switzerland, the surface runoff has been suggested as the major reason for the occurrence of glyphosate and AMPA in surface waters [8]; however, in a study related to Danish soils, limited leaching of glyphosate was reported in non-structured sandy soils, while subsurface leaching to drainage systems was observed in a structured soil when high rainfall followed glyphosate application [9].

In a study related to ^{14}C glyphosate transport in undisturbed topsoil columns, the amounts of glyphosate leached from the macroporous sandy loam were 50–150 times larger than that from the sandy soil [10].

1.4 Glyphosate residues

Glyphosate and its decomposition product AMPA have been reported in stream water samples in areas of Zurich, Switzerland, with median concentrations of 0.11 and 0.20 μg/l; however, these compounds were not detected in groundwater [11].

In a Canadian study, glyphosate residues were observed in both upland and wetland settings; however, the concentrations were well below the Canadian guidelines for drinking water quality. Many other studies have reported glyphosate residues in streams and groundwater systems [8].

An enzyme-linked immunosorbent assay (ELISA) was used to determine glyphosate presence levels in Hungarian water samples. Few samples showed exceedingly high concentration levels of glyphosate with this method [12]. Liquid chromatography is another method that can be used for the detection of glyphosate residues in cereal, oilseed, and pulse crops [13].

1.5 Soil properties and glyphosate mobility

Data from sorption studies indicated that sorption coefficients are the most sensitive parameters for environmental risk assessment and soil properties like pH and clay content govern the glyphosate adsorption in Argentinian soils. In a related study in Argentina, high glyphosate sorption with low desorption in mollisols and ultisols indicated a low risk of groundwater contamination [14].

In another study on glyphosate mineralization in different agricultural soils, exchangeable acidity (H^+ and Al^{3+}), exchangeable Ca^{2+} ions, and ammonium lactate extractable K were the key soil parameters governing mineralization [15]. In a study related to glyphosate sorption with high soil phosphate levels, glyphosate sorption distribution constant K_d in soils ranged from 173 to 939 l Kg $^{-1}$ under very strong to strongly acidic conditions, but the K_d was always <100 l Kg $^{-1}$ under moderately acidic to slightly alkaline conditions suggesting that glyphosate may become mobile by water in soils with high phosphate levels [16]. This is important concerning the application of phosphatic fertilizers, as the phosphate ion would desorb glyphosate from adsorption sites resulting in the mobility of glyphosate towards aquatic environments [17].

Generally, iron and aluminum oxides adsorb a greater amount of glyphosate and phosphates in comparison to layer silicates [18] supporting the role of soil mineralogy concerning glyphosate sorption. As high phosphorus application can desorb glyphosate from sorption sites, application of char can be effective in these scenarios concerning sorption of glyphosate. The rapid degradation of glyphosate in surface waters and its practically irreversible sorption indicated a low potential environmental risk [19].

An investigation on adsorption of the herbicide glyphosate and its main metabolite AMPA found that $pH_{(CaCl2)}$ values, available phosphate, and amorphous iron and aluminum contents were the major parameters to predict the adsorption constants for these molecules [20]. In a similar study, while examining the effect of humic acid (HA) on the adsorption/desorption behaviour of glyphosate on goethite minerals, the herbicide was desorbed by two parallel processes: (i) a direct detachment from the surface, which is first order in adsorbed glyphosate, and (ii) a ligand exchange with HA molecules, which is first order in adsorbed glyphosate and first order in dissolved humic acid [21]. Glyphosate is adsorbed by humic acids via hydrogen bonding [22].

A laboratory study related to the fate of glyphosate and degradation in cover crop residues and underlying soil indicated that the differences in sorption and degradation levels were due to differences in the composition of the crop residues and availability to microorganisms [23]. In a related study of adsorption and mobility of glyphosate in different soils under no-till and conventional tillage, adsorption of glyphosate was influenced by the soil clay content and cation exchange capacity (CEC) and negatively related to pH and phosphorus. High Freundlich parameter (K_F) values obtained in isotherm studies were the dominant factor influencing glyphosate mobility. K_F values indicate the adsorption capacity of the soil [24].

1.6 Methods to understand glyphosate mobility

Sorption coefficients provide accurate information needed for reliable risk assessments of groundwater contaminants by pesticides [25]. In a study related to sorption and leaching of ^{14}C-glyphosate in agricultural soils, non-extractable glyphosate residues become available eventually and take part in biodegradation and leaching. Empirical constants (K_F) of Freundlich sorption isotherm were 16.6 for the clay loam, 33.6 for the silty clay loam, and 34.5 for the sandy clay loam indicating that it is the soil structure which dictates the glyphosate sorption behaviour [26]. Leaching of glyphosate was dependent on hydrodynamic and biodegradation properties of soils [26]. Application of char can be used as a strategy to increase the sorption of glyphosate [27].

Movement of pesticides and their bioavailability and biotransformation are controlled by adsorption/desorption mechanisms operating at the interface between organic and inorganic soil colloids. High-resolution magic angle spinning and nuclear magnetic resonance techniques can distinguish mobile and immobile phases of pesticides like glyphosate [28]. Another study on glyphosate transport parameters suggested that glyphosate sorption is a kinetic process that depends on pore-water velocities and residence time of soil solution [29].

1.7 Why is glyphosate application on field sites a concern?

The International Agency for Research on Cancer (IARC) has reclassified that glyphosate is "probably carcinogenic to humans" [30]; however, the United States Environment Protection Agency (US EPA) concluded that there is no convincing evidence that "glyphosate induces mutations" [31]. The US EPA relied mostly on unpublished regulatory studies, 99% of which were negative, while IARC relied mostly on peer-reviewed studies, 70% of which were positive [31]. Glyphosate-based herbicides often contaminate drinking water sources, air, and precipitation in agricultural regions [30]. As the usage of glyphosate-based herbicides continues to increase, investment in epidemiological studies, biomonitoring, and toxicology studies based on the principles of endocrinology should be done [30]. Apart from cancer, glyphosate has been found to be a potential factor causing chronic kidney disease due to drinking water faced by Sri Lankan farmers [32]. The role of drinking water has also been reported in another study which caused ill health in Indian farmers [33].

1.8 Biochar's potential role as a sorbent for organic pollutants like glyphosate

Biochar can be defined as "the porous carbonaceous solid produced by the thermochemical conversion of organic materials in an oxygen-depleted atmosphere that has physicochemical properties suitable for safe and long-term storage of carbon in the environment" [34]. Biochar and activated charcoal are similar concerning production via pyrolysis, with medium to high surface areas [35]; however, biochar is not activated or treated like activated charcoal [35, 36]. Crop residues are pyrolyzed at high temperature (>500°C) in the absence of oxygen, followed by various activation processes to form activated charcoal [35]. In comparison to activated charcoal, biochar has a non-carbonized fraction that interacts with soil contaminants like glyphosate. Soil minerals can increase the surface area and pore size of biochar, which in turn increase the adsorption capacity of biochars for organic pollutants like glyphosate [37]. Biochar application can reduce the bioavailability and leachability of organic pollutants in soils through adsorption and other physicochemical reactions [38]. An increase in the surface area of biochars has been observed to increase the biochar's ability to adsorb organic contaminants [39, 40]. The addition of phosphorus fertilizer to biochar-amended soils can, however, remobilize glyphosate and damage non-target plants; therefore, improved understanding of this risk is important (**Figure 2**) [41].

The soil environment is a three-dimensional structure of water-filled pores, gas-filled pores, and soil particulates (organic matter, sand, silt, and clay) [42]. Biochar can be used as a sorbent for organic pollutants due to its highly aromatic

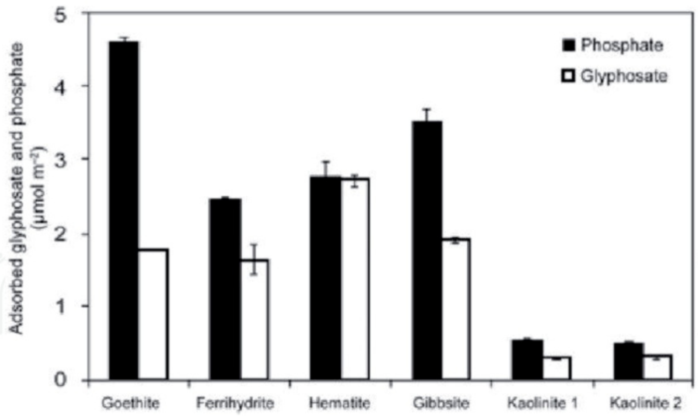

Figure 2.
Phosphate and glyphosate adsorption by minerals [5].

nature, high surface area, micropore volume, and abundance of polar functional groups [43]. Factors affecting biochar's performance for adsorption include pyrolysis temperature and surface area. Pyrolysis temperature is one of the factors directly affecting biochar's performance. An increase in pyrolysis temperature of biochar generally increases the degree of carbonization and consequently surface area.

Even with the increase in surface area of biochars, sorption sites can be blocked by organic matter, and this is the likely cause for the diminished capability of aged biochars to adsorb organic contaminants [44]. The behaviour of biochar changes with time after its application to soil, and this process is known as "aging". Aging can alter the behaviour of biochar. To increase the remediation efficiency of biochar concerning herbicides, more detailed research to explore the aspect of aging is warranted.

1.9 Behaviour of herbicides in a soil-biochar system

In a study related to herbicide terbuthylazine-biochar-soil interaction, there was higher adsorption of herbicide in soil with low organic matter than in soil with the high organic matter. The reason for this result was attributed to a high concentration of organic molecules competing with herbicide for sorption sites in the soil having a high amount of organic matter [40]. Availability of herbicides can be greatly reduced with the application of biochar [45]. Even a low application rate (0.1%) of biochar in the soil can appreciably reduce the availability of herbicides like diuron [44].

In a comparative study [46], 42 times higher hexachlorobenzene sorption by biochar than that by control soil was observed, resulting in the reduction of volatilization and earthworm (*Eisenia foetida*) uptake of hexachlorobenzene from the soil. The extent of sorption of pesticides generally depends on the aromaticity of soil organic carbon. Properties that make biochars effective against herbicides are a high specific surface area, high microporosity, and high aromatic carbon.

1.10 The behaviour of glyphosate in a soil-biochar system

Plant uptake of pesticides decreases markedly with increasing biochar content of the soil despite the greater persistence of the pesticide residues in biochar-amended soils [47]. In a similar study related to the effects of biochar, wood vinegar, and plants on glyphosate leaching and degradation, the addition of biochar to the soil decreased the leaching of glyphosate irrespective of plants. Hence, it was concluded that biochar can be used as an effective strategy to reduce the potential environmental risk to aquatic environments caused by glyphosate [27].

In a study related to the effects of wood-based biochar on the leaching of pesticides chlorpyrifos, diuron, and glyphosate, it was concluded that biochar can be used as an adsorptive layer directly on or close to the soil surface to prevent losses of pesticides [48]. In another study, biochar was found to limit glyphosate transport in soil systems; however, the addition of phosphatic fertilizer remobilized the glyphosate from biochar-amended soils. This phosphate-induced glyphosate desorption phenomenon is important to consider in soils having biochar amendment [41]. The type of biochar also plays an important role, as hardwood biochars were ineffective sorbents of glyphosate in high-phosphate soils [41]. Biochars produced at high temperature were effective sorbents of glyphosate [41]. Reduced glyphosate sorption on biochars was observed with the increase in pH from 6 to 9 [41, 49, 50].

2. Glyphosate-resistant weeds

The second major aspect in this review paper is the evolution of glyphosate resistance in weeds due to heavy reliance on glyphosate. Glyphosate toxicity and glyphosate resistance are not different but connected problems, as glyphosate is applied to control weeds and its application results in movement of glyphosate to water bodies via soil systems affecting human health. When glyphosate-contaminated drinking water is used for human consumption, it may potentially result in diseases like cancer or chronic kidney disease; however, frequent application of glyphosate not only results in its downward movement via soil systems but also results in the development of glyphosate resistance in weeds. Hence these problems are interconnected.

While assessing the weeds at risk of evolving glyphosate resistance in Australian subtropical glyphosate-resistant cotton systems, species with the highest risk to glyphosate resistance were *Brachiaria eruciformis*, *Conyza bonariensis*, *Urochloa panicoides*, *Chloris virgata*, *Sonchus oleraceus*, and *Echinochloa colona* [51]. Thirty-eight weeds in total distributed over 37 countries have shown resistance to glyphosate [52]. These weeds represent the greatest threat to sustainable weed control practices [52]. Weed surveys in the cotton-growing areas of New South Wales (NSW) and Queensland, Australia, indicated the dominance of *Conyza bonariensis*, *Echinochloa colona*, and *Chloris virgata* [53].

Chloris virgata is a high-risk species to glyphosate resistance in summer fallow [51]. Glyphosate resistance in *Chloris virgata* populations in Australia has emerged due to transformation in Australian cropping systems, particularly unirrigated cotton systems, from regular tillage and use of residual herbicides to minimum or no-tillage systems with a heavy reliance on glyphosate [54]. This lack of tillage is the major reason for the emergence of weeds like *Chloris virgata* that are small-seeded and emerge at or close to the surface [54]. A weed management system depending on only one tactic, for example, application of glyphosate, is the main driver for this species shift. With repeated use of glyphosate, *Chloris virgata* populations have become less susceptible to glyphosate formulations, especially after the early tillering stage [54].

Mechanisms involved in providing resistance to glyphosate in weeds include (i) target-site alterations (target site mutation, target site gene amplification) [55, 56] and (ii) non-target site mechanisms involving reduced glyphosate uptake and/or reduced translocation of glyphosate [57–59]. The alterations inhibit glyphosate binding or increase the effective dose needed for enzyme inhibition. Target site EPSPS mutations are the primary mechanism conferring glyphosate resistance in populations of *Chloris virgata* [55].

2.1 *Chloris virgata* (feathertop Rhodes grass)

Chloris virgata as a glyphosate-resistant weed [51] has also been identified as a host for barley yellow dwarf and cereal yellow dwarf viruses [60]. As *Chloris virgata* can tolerate high-salinity and high-alkalinity soil environments, *Chloris virgata* can form a dominant community in these environments [61, 62]. *Chloris virgata* is tolerant to drought stress [63]. Many studies on *Chloris virgata* seed biology have been completed in China, India, Qatar, and Honduras [63], while very few studies have been conducted in Australia [64, 65].

Chloris virgata grass seed biology includes the study on dormancy, germination conditions, seed bank dynamics, growth, and development [66]. Dormancy mechanisms enable the seed to sense the optimum environmental conditions for the establishment of seedlings and hence play a pivotal role in control strategies for weedy grasses [67]. There are two types of seed dormancy mechanisms, those based in the tissues surrounding the embryo (seed coat based) or those found within the embryo [67]. The role of smoke in breaking the dormancy of plump windmill grass (*Chloris ventricosa*), a related species to *Chloris virgata* grass [68], has been reported; but no study related to dormancy breakdown of *Chloris virgata* grass by smoke has been reported. The seeds of *Chloris virgata* are triangular in shape and light in weight and hence shed easily from the heads making them good wind (anemochory) and water (hydrochory) dispersers [64].

Seed germination is a key event in the growth of annual plants like *Chloris virgata* grass which is regulated by several environmental factors such as temperature and water potential [69–71]. High rainfall has been associated with *Chloris virgata* population outbreaks [72], suggesting that water plays an important role in the germination process. *Chloris virgata* grass possesses the C_4 photosynthesis mechanism and has better water use efficiency than grasses having the C_3 photosynthesis mechanism. Among all the potential factors for *Chloris virgata* germination; light, salinity, and osmotic potential are the most critical factors [64]. A light requirement for germination has been observed among many small-seeded species and warm-season grasses [67, 73]. In a study related to germination responses of *Chloris virgata* to temperature and reduced water potential, maximum germination percentages of *Chloris virgata* seeds were found at 15–25°C [74]. Germination of *Chloris virgata* seeds is affected by several factors; however, temperature and light play a significant role in the germination of *Chloris virgata* seeds. More studies on factors affecting *Chloris virgata* growth are needed due to the paucity of information.

In a study related to growth, development, and seed biology of *Chloris virgata* in South Australia, *Chloris virgata* seedlings emerging after summer rainfall events under field conditions needed 1200 growing degree days from emergence to mature seed production [65]. Harvested seeds of *Chloris virgata* were dormant for a period of about 2 months and took 5 months of after-ripening to reach 50% germination [75]. Seedling emergence of *Chloris virgata* was highest (76%) for seeds present on the soil surface and seedling emergence was significantly reduced by burial at 1 (57%), 2 (49%), and 5 cm (9%) soil depth. Furthermore, *Chloris virgata* seeds buried in the soil persisted longer than those left on the soil surface [75].

The thermal time to panicle emergence of *Chloris virgata* is similar to shattercane (*Sorghum bicolor*) [76]. A related species of *Chloris virgata*, windmill grass (*Chloris truncata*) under irrigated field requires 21–23, 43–45, and 74–75 days from seedling emergence to reach tillering, panicle emergence, and mature seed stage [75]. Maximum plant density and biomass in case of windmill grass have been found to be 4.2–28.2 plants m^{-2} and 8.3–146.1 g dry biomass m^{-2} depending on location [77].

Water stress due to extremely low rainfall over the summer months was the reason for the delayed growth of *Chloris virgata* under rained conditions when compared to irrigated conditions [75]. Under irrigated conditions, 619 to 730 g of dry biomass m^{-2} of *Chloris virgata* (89 days after sowing) was observed; however, this value was much higher than one of its related species, windmill grass (*Chloris truncata*) (146 g m^{-2}) [75].

Chloris virgata has several characteristics like rapid germination and low base temperature (2.1 to 3.0°C) for seed germination enabling it to survive rainfall events in spring, summer, and autumn in South Australia [75].

2.2 Evolution of glyphosate resistance in *Chloris virgata*

On national ranking basis in Australia, *Chloris virgata*, as an herbicide-resistant weed, ranks ninth, resulting in herbicide-resistant weed cost of $2.6 million [78]. In the northern region of Australia, it is the top fourth herbicide-resistant weed after ryegrass (*Lolium rigidum*), wild turnip (*Brassica rapa*), and barnyard grass (*Echinochloa crus-galli*) [78].

Minimum tillage due to its benefits like reduced soil erosion and improvement in moisture conservation has resulted in the reduction of soil disturbance in grain cropping fields. The factors that aided the adoption of minimum tillage systems in Australian cropping systems include machinery modifications that allow greater flexibility in the cropping systems, precision agriculture and refinement of controlled traffic farming, improved crop resistance or tolerance to plant diseases associated with stubble retention, availability of more crop options and rotations, development of a broader spectrum of effective herbicides, and the use of genetic modification technologies to breed herbicide-resistant crops [79].

Minimum tillage has increased the use of herbicides and consequently increased the rapid appearance of herbicide resistance in weeds [75]. Another reason for evolution is the introduction of glyphosate-resistant crops in the mid-1990s that has resulted in a sharp increase in the populations of *Chloris virgata* [80].

Glyphosate resistance was first reported in broadleaf *Conyza* (horseweed) species. The mechanism suggested for resistance was an altered subcellular distribution resulting in sequestration of the glyphosate molecule away from the enzyme target site in the chloroplast [81]. Weeds receiving repeated exposure to a single mode of action of herbicide are the most likely candidates to develop resistance [82].

From the evolution point of view, minimum tillage along with reliance on glyphosate has contributed the most towards glyphosate resistance in *Chloris virgata*. The evolution of the glyphosate resistance in *Chloris virgata* highlights the need for diversity in weed management strategies for successful control of *Chloris virgata* and other *Chloris* species [82].

2.3 Crop competition as a strategy to control *Chloris virgata*

Crop competition can be used as an effective strategy against *Chloris virgata*, especially when herbicides like glyphosate fail or underperform [83]. Crop competition to control weeds has proven to be one of the most effective cultural strategies in Australian cropping systems, aiming at suppression of weed biomass and fecundity resulting in crop yield gains [84]. Three major weed variables that affect crop-weed competition are:

- Time of emergence of the weed relative to the crop and weeds that emerge later than the crop are much less competitive than the weeds that emerge before the crop.

- Weed seedling density is the second most important factor influencing weed-crop competition.
- Differences in the competitive ability of weeds due to rapid leaf area development, high-density root systems, and plant heights [85].

Crop and weed plants compete for limited resources like water, nutrients, and light. Competition for nutrient uptake is dependent on intrinsic nutrient requirements and uptake efficiencies. Uptake efficiencies are further dependent on root length densities and nutrient membrane transporters. Species with a low nutrient requirement, extensive root systems, and effective membrane transporters will have a competitive advantage in a nutrient-limited system [85].

Crop and weed plants compete for water, as water is required for plant growth. In the absence of water, a reduction in photosynthesis, wilting, and nutrient deficiencies can occur. The length, magnitude, and timing of the drought periods as well as soil attributes (water holding capacity, texture, structure, and hydraulic conductivity), plant traits (root structure and density, drought tolerance, and water use efficiency) are the major factors that influence the competition for water availability between crop and weed plants [85].

Light as a third major factor affects the growth of crop and weed plants [86]. Different phenophases of both crop and weed plants are affected by light. Morphological changes in both crop and weed plants due to competition for light include an increase in stem elongation and reduction in stem diameter, the rate of leaf appearance, and root and shoot biomass [87, 88].

Crop competition studies under field conditions are mainly influenced by the environment, soil type, plant density, spatial arrangement, the proportion of each species, and design of experiment [89]. The design of the crop competition experiment depends on the objective, as different objectives require different techniques [90].

Crop species may outcompete weed species depending on factors such as crop density, crop planting pattern, crop vigor, and weed vigor. Crop density or the number of plants per unit of area is important for competition studies considering the relationship among plant yield and the number of individuals and resources present in the area [91]. The competitiveness of a crop can be enhanced using competitive cultivars, higher plant densities, narrow row spacings, and different row orientation [92].

Weed growth can be substantially reduced by shading weeds in the inter-row space by physical orientation of the crop rows [92]. Competitive ability of the crops can also be increased by increasing plant density [84]. The significant interaction between sorghum cultivars and planting densities in suppressing weed biomass has been observed [93]. A high-density crop can limit water and nutrients available to weeds more effectively than a low-density crop, and high-density crops can result in the reduction of light available to weeds [92].

3. Summary

In summary, the review paper covered two major problems associated with single reliance on glyphosate application for controlling weeds. The first one is glyphosate mobility via soil systems, a potential risk for aquatic environments, and there is no information on the fate of glyphosate on Australian soils from the last 22 years apart from a single study in Western Australia. This research gap prompted an investigation into glyphosate sorption behaviour in Australian soils of the different mineral composition due to increased usage of glyphosate as a single strategy to control weeds. The second major problem is the evolution of glyphosate-resistant weeds like *Chloris virgata* in New South Wales and Queensland, Australia, a major threat to sustainable weed control strategies, and due to paucity of information on the management of *Chloris virgata*, we hypothesized that cultural methods like crop competition can be used as a strategy to control glyphosate-resistant *Chloris virgata*.

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