Weed profiling fields of herbicide tolerant maize in the Mthatha region, Eastern Cape Province

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I can do all things through Christ who strengthens me. Philippians 4:13

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Appendix A: List of abbreviations

Table A: List of abbreviations relevant to this dissertation

Acronym	Description
Bt	*Bacillus thuringiensis*
CBD	Convention of Biological Diversity
CT	Centre Transect
EC	Eastern Cape
ET	Edge Transect
EU	European Union
FTs	Functional Types
GM crops	Genetically Modified crops
GM	Genetic Modification
GMOs	Genetically Modified Organisms
HT	Herbicide Tolerant
LMOs	Living Modified Organisms
M	Field Margin
MF	Maize Fields
NEMBA	National Environmental Management Biodiversity Act
NMDS	Non-metric multidimensional scaling
PAST	Paleontological Statistics software
PERMANOVA	Permutational Multivariate Analysis of Variance
PFTs	Plant Functional Types
RDA	Redundancy Analysis
RT	Reference Transect
SA	South Africa
SANBI	South African National Biodiversity Institute
SIMPER	Similarity Percentage Analysis
SIMPROF	Similarity Profile
UPGMA	Unweighted Pair Group Method with Arithmetic Mean clustering

Abstract

Maize cultivation has been a historical agricultural practise of most indigenous people in southern Africa for centuries. Although the origins of maize are rooted to south-central Mexico in North-America, it has for centuries been an important subsistence and economic crop for southern Africa. In South Africa, maize provides a source of income for thousands of commercial and smallholder farmers countrywide. The continued increased demand for maize production has led to the development of different cultivars to assist farmers to achieve better crop management towards good yields, and subsequent, profitable margins. At the same time, weed management and control, among others, remain a big challenge for maize farmers. Although weeds associated with herbicide tolerant (HT) maize have been studied to a greater degree globally, South Africa still lacks fundamental research to better understand their existence and prevalence in HT maize agroecosystems. This calls for the development of knowledge to determine what weeds interfere with HT maize cropping systems. This will enable farmers to choose best practice and methods for weed control that would minimize or even prevent negative impacts on the environment, while maintaining their fields for sustainable maize production. The study aimed to profile HT maize weed populations in rural farming communities of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province, South Africa. This was done by documenting and comparing their composition (abundance, diversity, and richness) and functional traits between maize fields and their margins. In addition, the different herbicide protocols administered to control weeds in the HT maize fields were also profiled. Field surveys were conducted during the 2017/18, 2018/19 and 2019/20 planting seasons. The HT maize varieties planted were NK603xMON810 (2017/18-season one) and MON89034 (2018/19; 2019/20-seasons two and three, respectively). Weed surveys were conducted at four sites, namely Baziya Makaula, Baziya Mission, Baziya Jojweni and Tsolo. The farming method or practice was the same in all four sites in terms of HT maize varieties used and herbicide protocols followed across the three seasons. The sampling in each field and the field margin (where possible) was carried out in 3x3 m quadrats at intervals of 10 m along a transect line. A total of 88 weed species, representing 31 families, were recorded from 244 plots for both maize fields (MF) and field margins (M) combined. The three families that contributed the most weed species were Asteraceae, Fabaceae and Poaceae. There were three weed species which contributed individual dissimilarity of >5% between maize fields and field margins, and all being dominant in the latter (*Cynodon dactylon* (L.) Pers, *Richardia brasiliensis* Gomes and *Sonchus nanus* O.Hoffm). Weed species trait composition differed significantly ($p=0.031$) between most sites - and pre-ploughing, post-ploughing and seed sowing surveys ($p<0.001$) - indicating that local rather than regional factors affect trait composition. However, trait richness for pre-ploughing, post-ploughing and seed sowing surveys did not differ significantly ($p>0.05$). Weed species richness differed significantly over the three seasons ($p<0.05$). The findings confirm that maize fields and field margins are characterised by different weed assemblages and diversity. No similarity in species composition was confirmed ($p<0.05$). Similarity in trait richness was confirmed as it did not differ significantly ($p>0.05$). Weed species and functional type richness differed significantly with lower richness in post-ploughing than pre-ploughing surveys. Therefore, the herbicide application effectiveness was supported. No clear evidence was gathered to suggest that herbicide application contributes to weed shift, as weed species and functional types did not differ significantly in composition ($p>0.05$) for pre-ploughing, post-ploughing and seed sowing surveys. By studying the weed composition in HT maize, the study has successfully provided preliminary evidence of the traits that could be characteristic of HT maize weeds with the ability or predisposition to become herbicide tolerant. The findings suggest that effective weed control using herbicides in HT maize fields is possible, although this still needs further testing across numerous seasons. Furthermore, additional investigation and monitoring of various environmental parameters likely to be responsible for some of the unexplained observations is necessary to fully understand weed mechanisms, both spatially and temporally.

Keywords: Agroecosystem, Diversity, Functional types, Maize, Baziya, Tsolo, Pre- and Post-ploughing surveys, Seed sowing survey, Weeds, Weed traits

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CHAPTER 1: Introduction

1.1 Background and rationale

Maize is important for the South African economy because it is a source of income for thousands of commercial and smallholder farmers. The South African maize industry was deregulated in 1997 (Greyling and Pardey, 2018) and has since been operating in a free market environment, meaning that producers may sell to whomever they wish. The South African maize industry is important for foreign exchange through exports, mainly to China, Iran, Japan, Kenya and Venezuela (FAO, 2019).

Maize production varies widely across the globe depending on factors such as climate (Msowoya *et al*., 2016), crop management practices (Mutuku *et al*., 2020) and market demands (DAFF, 2018). Maize yield is high in intensively cultivated temperate regions such as the United State (US) and western Europe, followed by China, Argentina, and SA (Tigchelaar *et al*., 2018). Maize is a global staple food for millions of people and feed for animals (Romney *et al*., 2003). Furthermore, maize is also used to produce fuel and other materials such as enzymatic products (Green *et al*., 2015). Total grain production worldwide amounted to more than 1.11 billion metric tons in 2018/19 (Statista, 2021). It was estimated that the global annual maize harvest for 2019/20 would show a decrease by a rate of 3.5% due to factors such as climate change and weed infestation (McCormick, 2020).

1.1.1 Maize production in South Africa

South Africa produces approximately 8 million tonnes of maize annually on 3.1 million hectares (DAFF, 2018). In SA, maize is the most important grain crop grown in all nine provinces (Ray *et al*., 2013). South Africa produced approximately 1.1 million hectares of yellow maize and 1.3 million hectares of white maize for commercial purposes in 2019, and a further 0.7 million hectares of non-commercial yellow and white maize (BFAP, 2019). The major maize producing provinces are Free State, North West, Mpumalanga, Gauteng for production in order of contribution (Statista, 2020; DAFF, 2017). Maize farming in the EC is essential for food production and income generation for rural farmers. Yellow and white genetically modified (GM) and conventional (non-GM) maize cultivars are grown in the province.

Successful maize production for most commercial farmers and a few smallholder farmers is dependent on technological advancement (Rapsomanikis, 2015). It is also dependent on sustainable agricultural inputs such as use of chemicals, well-structured soil, balanced essential plant nutrients, sufficient and regular rain, and mean temperatures of approximately 20ºC (Tuğrul, 2019). The world population is predicted to exceed 9 billion people in the near future, which means an increase in demand for available food and arable land. Food safety issues, postharvest losses, regulation inconsistencies and consumer attitude are the challenges that need to be met in order to maintain food security and sustainability. Therefore, possible solutions to feed the world in the near future include advancement in food processing technologies such as genetic modification technology with the use of genomic approaches (Jenny *et al*., 2016).

1.1.2 Use of genetic modification technology for maize production

Genetic modification technology has brought about an added advantage of traits that are acquired from the bacterium, *Bacillus thuringiensis* (Bt), for insect resistance (Gewin, 2003), and *Agrobacterium* sp. strain CP4, for herbicide tolerance (HT) (Funke *et al*., 2006). *Bacillus thuringiensis* maize, as it is commonly known, produces insecticidal proteins that resist maize stem borer, whereas herbicide tolerant crops have traits to tolerate herbicides that kill weeds. Glyphosate resistant plants carry the gene coding for the resistance of glyphosate. Glyphosate herbicides target an enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase required for the production of amino acids (Holländer-Czytko and Amrhein, 1987). Once the gene coding for a glyphosate-insensitiveness is incorporated into the genome of a plant, the CP4 EPSP synthase confers crop resistance to glyphosate (Funke *et al*., 2006).

The introduction of a herbicide-resistant gene into maize in 1998 has led to the so called "Roundup Ready" crops (Perry *et al*., 2016). These herbicide tolerant crops are designed to tolerate a specific broad-spectrum herbicide, which kills target weeds, but leaves the cultivated crop intact. The utilisation of these crops has led to a disparity of articulated benefits and risks. In particular, the increase in herbicide use, the rise in herbicide-resistant weeds, weed shift and numerous environmental, social and human health impacts (Gage *et al*., 2019; Schütte *et al*., 2017). At the same time, herbicide tolerant crops have helped to enable weed management practices that have improved yield and profitability, while better protecting the environment in terms of soil preservation (Green, 2012). For example, selective herbicides that target only certain weed species are widely favoured, because only a few weeds in agricultural fields interfere with the productivity of crop plants through competition (Pieterse, 2010).

The adoption of the genetic modification technology has enhanced farming practices and management, even for smallholder farming (Raman, 2017). The widespread adoption of genetically modified crops has changed agricultural practices, resulting in considerable changes in how weed communities are managed (Raman, 2017). Good practices have allowed for effective weed control and enhanced crop performance/yield (Kairovin *et al*., 2019). However, this technology may also lead to potential adverse effects on the environment. One such potential problem is related to herbicide applications to weeds, as overuse or incorrect application of herbicides can affect soil fertility, microorganisms and chemical leach into water-bodies (Kalia and Gosal, 2011). It is therefore important to target weed species, in terms of diversity and composition, to know precisely which herbicide should be applied and at what concentrations in herbicide tolerant maize fields. Poor practices have led to herbicide tolerance developing in weeds, such as *Conyza canadensis* (L.) Cronquist in soybean and cotton systems, *Amaranthus palmeri* S. Watson in cotton systems and *Ambrosia trifida* L. in soybean systems (Gage *et al*., 2019).

1.1.3 Weeds and their management in agriculture

Weeds are undesirable plant species that grow where they are not wanted, especially in agricultural fields (Hewlett, 1948). They constitute one of the major economic important problems for maize growers, because they can reduce yield up to 86% (Kannan *et al*., 2013). However, it is worth noting that the value of any plant is determined by the perceptions of its viewers (Potgieter *et al*., 2019). Certain plants in cultivated crop fields may be unwanted by farmers as they compete with crops for resources, but they may provide other valuable ecosystem services (Smith *et al*., 2020). Even though they are considered as main constraints to crop production, they can also be important in supporting biodiversity within the agroecosystem. For example, reduction in abundance of host plants (weeds) may affect other taxa and insects as they depend on them for nesting and food sources (Marshall *et al*., 2003). Many field weeds are beneficial as indigenous food plants (Williams and Murthy, 2011). They are also beneficial to human and animals for medicinal purposes (DEA, 2011), and provide habitat for various animals for pollination services and pest control (Smith *et al*., 2020). At the same time, weeds need to be managed so as to protect crops from being outcompeted for nutrients, food, water and sunlight that support their growth for better yield.

There are various weed management methods applied worldwide, including chemical and mechanical management (Beckie, 2006). Weed control in agriculture dates back centuries before advanced genetic modification technology was in place. At the time, the common techniques included manual weeding. Mechanical weed management involves manual removal of weeds and it is very laborious and inefficient, hence weeding is often not done on time. Manual techniques such as hand hoeing, pulling and cutting are the preferred alternatives mostly by those who cannot afford to buy herbicides (Jabran *et al*., 2015; Pieterse, 2010). In commercial farming, herbicide use has become popular for the majority of weed management problems (Tesfay *et al*., 2014) and proper selection of herbicides is essential for successful weed control (Kannan *et al*., 2013). Herbicides are preferred and have been shown to be one of the most effective means to control weeds that infest crops, killing approximately 90% of the targeted weed species (Kraehmer *et al*., 2014). Consequently, the range of targeted weed species by herbicide application has increased rapidly (Gaines *et al*., 2020). Erroneous applications have made some weed species to evolve and become resistant over time in response to herbicide use (Pacanoski, 2017), as well as a decline in efficacy of herbicides (Palou *et al*., 2008). The herbicides mostly used in herbicide tolerant crops are roundup, trifluralin, acetochlor, s-metolachlor, fomesafen, pendimethalin, 2,4-Dichlorophenoxyacetic acid, diuron and pyrithiobac (Brookes and Barfoot, 2014).

One of the most effective weed management approaches is now based on the introduction of new herbicides with new modes of action (Busi *et al*., 2019). It is also based on applying the strategy of rotating herbicides to reduce the chance of weed resistance development (Baghestani *et al*., 2007). Most herbicides have become reliable due to the advanced development of herbicide tolerant genetically modified crops. When herbicides are applied to herbicide tolerant crops, they remain unaffected while weeds are killed. This approach is much more flexible in controlling weeds and has the potential to replace tillage techniques, which often lead to soil erosion and degradation (Green, 2015). Machine vision technology such as robotics, which is an automated machine to recognise crop row patterns to remove intra-row weeds is also considered as an effective weed management technique (Fennimore *et al*., 2016; Wu *et al*., 2020). However, where there are large and dense weed populations, automated machines cannot effectively differentiate weeds from crops (Wu *et al*., 2020). Chemical companies continue with the search for new herbicides while automated weed removal devices continue to become more effective (Fennimore *et al*., 2016; Wu *et al*., 2020).

Due to the adoption of herbicide tolerant crops, chemical weed management has become a common practice (Pieterse, 2010). Different herbicides are used at different cultivation stages, either before planting, after planting, before the crops have emerged (pre-emergence) and after emergence (post-emergence). This is done to control weeds during the initial stages of crop growth effectively. Hence, it enables highly productive and profitable farming if administered properly. However, herbicides are toxic to the environment if used inappropriately (Ferreira *et al*., 2020) and, therefore, education and training is crucial in guiding the proper use of herbicides for effective results. At the same time, the use of herbicides may facilitate weed shifts (Owen, 2008), and this necessitates the combination of different herbicides in different years.

1.1.4 Knowledge gaps in herbicide tolerant maize weeds and their control in South Africa

Although weeds associated with HT maize have been studied globally, South Africa still lacks actual research to better understand their existence and prevalence in HT maize agroecosystems. There is lack of a basic checklist of weed species in HT maize cropping systems. From the literature, it is evident that we need to learn more about weed composition and diversity of HT maize fields and margins. This will enable the identification of weed species that are/could become tolerant to herbicides in the Oliver Tambo District Municipality of the Eastern Cape Province. Studying these species will provide evidence of the traits that could be characteristics of weeds with the ability or predisposition to become herbicide tolerant. Therefore, to ensure that possible effects of HT maize are monitored, more information on weed species diversity patterns, spatially and temporally, is needed. In addition, herbicides used to control/suppress the various weed species in HT maize need to be evaluated in relation to weed diversity and documented according to their effectiveness.

In line with various legislation, frameworks and protocols in South Africa that seek to promote environmental and health safety, it is important to conduct research on genetically modified crops and their interactions within the agroecosystem (Raman, 2017; Peterson *et al*., 2000). Such legislations that are directly related to the issue of monitoring Genetically Modified Organisms (GMOs) are: GMO Act No. 15, 1997 and the National Environmental Management Biodiversity Act No. 10 of 2004. In response to these acts, and to assist poor farmers in rural areas and commercial farmers, it is imperative to develop a better understanding of weed diversity patterns and herbicide use in HT maize to fill the knowledge gap and establish scientifically credible baseline information for future comparisons, and to propose science-based management strategies to reduce weed impact.

1.2 Aim and objectives of the study

1.2.1 Aim

The main aim of the study is to provide a profile of weed populations in HT maize fields of rural farming communities in the selected areas of Oliver Tambo District Municipality, Eastern Cape Province, by comparing the composition and diversity of weed species and traits between HT maize fields and margins, and over time.

1.2.2 Objectives

The main objectives of the study were to:

- Establish a reference collection for weeds in the HT maize cropping systems of the selected areas of Oliver Tambo District Municipality of the Eastern Cape;

- Determine the weed species composition and diversity of fields and associated reference sites (field margins);
- Determine the weed species composition and diversity of fields for pre-ploughing, post-ploughing and seed sowing surveys; and
- Determine the traits and functional groups of weeds for pre-ploughing, post-ploughing and seed sowing surveys;
- Establish a herbicide use profile for effective weed control.

1.3 Hypotheses

The main research questions are whether weeds in HT maize fields share similar traits and whether the use of herbicides does control these species, based on their richness and composition. To answer these questions, the following hypothesis were tested:

H1) Herbicide tolerant maize fields have the same weed species composition and diversity across fields. This hypothesis tests whether site specific effects occur under similar herbicide applications.

H2) Weeds in HT maize fields share similar traits. The hypothesis tests whether there are specific weed traits and functional types that are prone to the development of HT.

H3) Herbicide application in HT maize fields is effective in controlling/suppressing weeds. This hypothesis tests whether herbicide application rotations are limiting the chance of weeds to develop resistance to certain herbicides.

H4) Herbicide use leads to a weed shift over time as some weed species become dominant under herbicide rotation in the fields. This hypothesis tests whether any weed species are becoming tolerant to combinations of applied herbicides.

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CHAPTER 2: Literature review

2.1 Genetically modified crops in agriculture

Agriculture is the most extensive land use practice, but, loss of arable land due to climate change, urbanisation and soil degradation has led to the adoption of technologically advanced approaches for the production of high yield crops with the use of less land (Govers *et al*., 2017). This involved the development of genetically modified crops. In 2019, 191.7 million hectares of genetically modified crops were cultivated in 26 countries by up to 17 million farmers (ISAAA, 2019). Top five countries producing genetically modified crops at an adoption rate of above 94% are shown in Table 2.1.

Table 2.1 Top five countries producing genetically modified crops in 2019.

Countries	Adoption rate	Area (Million Hectares)
United States of America (USA)	95%	71.50
Brazil	94%	52.80
Argentina	100%	24.00
Canada	90%	12.50
India	94%	11.90

Source: ISAAA, 2019.

Population growth has led to high food demand which threatens food security (WWF, 2019). Food security exists when people have access to sufficient, safe, and nutritious food. However around 900 million people are undernourished, meaning that they are undersupplied with nutrients. It is estimated that for South Africa to feed the predicted population of 73 million people in 2050, it will have to produce 50% more food on top of what it is already producing (WWF, 2019). Genetically modified crops can be an important component in addressing food security, and growing GM crops may influence farmers income including their economic access to food. Most people suffer from nutritional deficiencies due to insufficient intake of micronutrients. To eradicate hunger, GM crops are mentioned as the solution, while others consider this as a further risk to food security (WWF, 2019).

South Africa introduced its first commercial GM maize in 1997 (Table 2.2). This was Monsanto's MON810 Yield Guard insect resistant maize. Since then, there has been cotton and soybean approvals in the years 1997 and 2001, respectively (ISAAA, 2018). Those approvals are based on various traits for food, feed and cultivation. The dominant traits are herbicide tolerance, including glyphosate herbicide tolerant (HT), glufosinate HT, 2,4-D HT and insect resistance. Wide adoption of maize cultivars with these genetic traits (Table 2.3), such as herbicide resistance, has allowed the increased use of herbicides, whereas insect resistance has reduced the use of insecticides (Rother *et al*., 2008). Known adoption rates by farmers for the respective years are also shown in Table 2.2, with those years according to the available records.

Table 2.2 First authorisation of genetically modified crops by the South African government and adoption rates by farmers.

Crop	First authorisations		Known adoption rate by farmers for the respective latest years	
	Cultivation	Food and Feed	Year	Rate
Maize - *Zea mays* L.	1997	2001	22016/17	92%
			2017/18	93%
			2018/19	87%
Cotton - *Gossypium hirsutum* L.	1997	1997	2016/17	100%
			2017/18	100%
			2018/19	100%
Soybean - *Glycine max* L.	2001	2001	2016/17	95%
			2017/18	95%
			2018/19	95%

Source: ISAAA, 2019; Africabio, 2021

Table 2.3 General release of maize commercial traits and years of approval in South Africa.

Event name	Commercial trait	Company	Year approved
MON810 / Yieldgard	Insect resistance	Monsanto	1997
NK603	Herbicide tolerance	Monsanto	2002
Bt11	Insect resistance	Monsanto	2007
MON810 x NK603	Insect resistance Herbicide tolerance	Monsanto	2007
MON89034	Insect resistance	Monsanto	2010
MON89034 x NK603	Insect resistance Herbicide tolerance	Monsanto	2010
GA21	Herbicide tolerance	Syngenta	2010
BT11xGA21	Insect resistance Herbicide tolerance	Syngenta	2010
TC1597	Insect resistance Herbicide tolerance	Du Pont Pioneer	2012
TC1507 x MON810	Insect resistance Herbicide tolerance	Du Pont Pioneer	2014
TC1507 x MON810 x NK603	Insect resistance Herbicide tolerance	Du Pont Pioneer	2014
MON87460	Drought tolerance Antibiotic resistance	Monsanto	2015
MON89034 x TC1507 x NK603	Insect resistance Herbicide tolerance	Dow AgroSciences SA	2018

Source: ISAAA, 2018

2.1.1 Maize production in South Africa

Zea mays L. is a cereal grain in the Poaceae (grass) family and originates from south-central Mexico in central America where it was domesticated 1500 BC (Mangelsdorf, 1954). It is globally known as maize (from Spanish: *maíz* after Taino: *mahiz*). In some parts of the world it is referred to as 'corn'. Maize cultivation has been a historical agricultural practise of most indigenous people in southern Africa for centuries (van Zyl, 1989). In South Africa (SA), it is referred to as 'mealie' (English) and *mielie* (Afrikaans) from Portuguese: *milho*. In the local languages, maize goes by many names. For instance, in the Eastern Cape (EC) it is called *umbona* (isiXhosa), and in adjacent KwaZulu-Natal it is *umbila* (isiZulu). In Limpopo Province, it is called *mavhele* (Tshivenda), *lefela* (Sepedi) and *xifaki* (Xistsonga). For provinces such as Gauteng, North West and Free State, where Setswana/Southern Sotho are the common languages, maize is referred to as either *mmidi* and/or *poni*. For the Northern Cape Province, were Setswana is also spoken, maize is known as *leheya*. In Mpumalanga Province, where Sepedi, Xitsonga and siSwati are the dominant languages, maize is referred to as *lefela* (Sepedi), *xifaki* (Xistsonga) and *mbila/mbhila* (siSwati).

In SA, maize is the main grain crop produced (Statista, 2020). More than 9,000 farmers produce maize and that also includes smallholder farmers (GrainSA, 2015). Total maize production was 32,261,000 tonnes in 2016/17. The highest maize producing provinces are Free State, North West, Mpumalanga and Gauteng in order of contribution (Statista, 2020; DAFF, 2017). Among the nine provinces, Western Cape and Eastern Cape are the least producers (Table 2.4) and the production was measured in hectares (Statista, 2020).

Table 2.4 Maize production in South Africa by province in 2021 arranged in ascending order.

Province	**Hectares**
Western Cape	4,000
Eastern Cape	24,000
Limpopo	37,500
Northern Cape	44,400
KwaZulu-Natal	105,000
Gauteng	113,000
Mpumalanga	525,000
North West	573,000
Free State	1,325,000

Source: Statista, 2021

The Eastern Cape Province maize farming is categorised in such a way that all maize produced in the province is by the commercial and non-commercial sector (DAFF, 2013). Maize produced in the Eastern Cape constitutes approximately 55% of the total maize produced by the non-commercial sector in South Africa (DAFF, 2013). Yield in the province for both white and yellow maize has an average production of 7.03 t/ha (Statista, 2021) due to factors such as weed infestation, infertile soil, insect pests and poor cultivars (Fanadzo, 2012; Nel and Davies, 1999). Therefore, the Eastern Cape government has come up with an approach to support smallholder farmers through a cropping programme support scheme that is implemented under the Eastern Cape Provincial Growth and Development Plan (Eastern Cape Rural Development Agency, 2013). The farmers' participation on the programme is on condition that they should pay 18% of the cost of the production per hectare, with the government covering 82% (Kadango *et al*., 2020). Government appoints private service providers to supply equipment for planting, fertilisers, herbicides and insecticides. Genetically modified maize was first introduced to small scale farmers in the Eastern Cape during the 2001/02 cropping season (Gouse, 2012).

2.1.2 Concerns on the adoption of genetically modified crop

Genetically modified crops have generated a great deal of controversy and their adoption has had substantial socio-economic impacts (Garcia-Yi *et al*., 2014; Nurliza, 2014). Farmers have different socio-economic motivations for adopting GM crops with regards to expected benefits and uncertainties. This suggests that the adoption of GM crops could have different impacts on poor versus wealthy farmers (Azadi *et al*., 2015). For example, the impacts associated with social and ethical aspects, both in short and long term (Azadi *et al*., 2015). A more practical example would be the changing views on irreversible effects such as interbreeding with wild types, competition with natural species and increased human health diseases (Prakash *et al*., 2011; Davis and Mitoh, 2003). A change in acceptability of GM crops may imply a change in values. At the same time, a direct impact on cultural aspects. For example, GM seeds need to be purchased, having the potential to cause a disturbance in the traditional exchange of seeds among indigenous farmers (Garcia-Yi *et al*., 2014). Genetically modified crop adoption is also expected to influence farmers' income as technology improves as they must buy seeds and pesticides. Other aspects that may be affected include health issues associated with change in pesticide use and nutritional status if bio-fortified crops are cultivated and consumed (Garcia-Yi *et al*., 2014). While GM crops might have great impact on ensuring future food security, concerns about their negative impacts are increasing. The critics on GM crops have concerns regarding safety, toxicity and the environment (Bawa and Anilakumar, 2012).

Across the globe, there are questions as to what role, if any, GMOs play in helping to address issues of agriculture, nutrition, and climate challenges. Concerns have also been raised over environmental and health impacts of GMOs (EFSA, 2009). In addition, laws around seed patents are considered a hinderance to farmer's seed access in a widely competitive market (EFSA, 2009). Governments are trying to respond to those aspects in different ways. For example, by banning GMOs completely or embracing them, while others are continuously attempting to balance the concerns and the needs of all sides (González-Vaqué *et al*., 2017). Currently, most governments are trying to finalise biosafety regulations and policies that will facilitate for the advanced research and development of GMOs including the adoptions and commercialisation for various uses (Hartung and Schaub, 2018). Some countries are realising the potential of GM crops as they seek to expand their export markets, address food insecurity and climate change. This expands the debate on genetic engineering as GMOs have come to the fore in food and agricultural policy worldwide (Mathur *et al*., 2017). Food security, climate change and local economies play a role in governments' favourable consideration for GMOs (Lusk and McCluskey, 2018). The general argument is that it provides improved yield and reduced production costs to help both smallholder farmers and commercial export markets.

2.1.3 Benefits associated with genetically modified crops

Farmers have adopted GM crops for better weed management, cost savings, and ease of use of chemicals to control weeds (Raman, 2017). This has made the adoption of GM crops attractive to farmers because when first introduced, managing GM crop fields resulted in fewer weeds in HT crops compared to conventional crops (Duke and Powles, 2009). One of the advantages of GM crops is their potential promise for food security in developing countries with small scale agriculture. Although the latter is in contrast to arguments in several countries, citing patent hinderences and financial burdens for small scale farmers (Azadi *et al*., 2015).

Genetically modified crops are considered safe, while contributing towards the improvement of food quality with extended shelf life benefiting farmers, consumers and the environment (Wisniewski *et al*., 2002). Genetically modified foods are cheaper and more stable with more quality and better taste (Azadi and Ho, 2010). Examples include sweet corn and redder tomatoes, and tender-stemmed broccoli or seedless watermelons (Wesseler, 2002). Another example would be that of Mexican farmers. Cotton farmers in Mexico have saved 30% on production costs due to reduced pesticide application (ISAAA, 2016). Honduras has enhanced farming income from GM maize by 3 million US dollars in the year 2015 (Brookes and Barfoot, 2017).

2.2 Brief overview of genetically modified crops regulations

The first 21 years of GM crop cultivation has delivered substantial agronomic, environmental, health and social benefits to consumers (ISAAA, 2019). Europe grows less than 0.5% of the world's GM crops due to European Union (EU) regulations, as well as the refusal of European consumers to buy GM crops (Akumo *et al*., 2012). More than 18 million farmers who have planted GM crops have derived benefits from the higher productivity and this has contributed to global food and feed security (ISAAA, 2019). With the rise of GM crop varieties, there has been political drivers and pressures involving economic, ethical, environmental, health safety and other legal questions in agriculture (Cohen and Paarlberg, 2004).

The potential impact of GMOs, also referred to as living modified organisms (LMOs), for economic growth is an important consideration for most countries (Shukla *et al*., 2018). For African nations, the EU was the number one trade destination in 2016, accounting for more than a quarter of all exports (Shukla *et al*., 2018). The imports of GMOs to the EU, including labelling standards and commercialisation, proved costly since the EU has strict regulations for approvals and imports (Tao and Shudong, 2003). However, in countries such as China and USA, which have fewer regulatory restrictions on the import of GMOs, the trade of GM crops provides increasing economic opportunities (Tao and Shudong, 2003).

African countries remain obliged to adhering to the international declarations they have signed onto (Leonelli, 2019). Since 2003, 121 nations have signed the Cartagena Protocol on Biosafety, requiring countries to protect biological diversity from the potential risks posed by LMOs (Leonelli, 2019). The Biosafety Protocol is rooted in the Convention of Biological Diversity (CBD), which sets up procedures in the safe transfer, handling and use of LMOs that may have adverse effects on biodiversity and takes account of human health risks (Leonelli, 2019). Each party, signatory to the protocol, facilitates and promotes public awareness and education in support of the Protocol. Signatory governments, including Kenya, Nigeria, and South Africa, have had to implement policies and legislation creating a safe framework for the possible research for GMO introduction(s) in their country (Morris, 2010; Mtui, 2012). Forty African countries signed the Maputo Declaration which committed them to investing 10% of their national budgets into agricultural development that is guided by both legislative and research protocols (Mtui, 2012; Snyman *et al*., 2018).

There are key legislations that are directly related to the issue of compliance of GMOs in terms of environmental impact and those are: GMO Act No. 15, 1997 and the National Environmental Management Biodiversity Act (NEMBA) No. 10 of 2004 (Hartung and Schaub, 2018). The GMO, Act No. 15 of 1997, aims in provision for the measures that promote the development, production, use and application of GMOs or to regulate the responsible use of GMOs in SA (Leonelli, 2019). This is to ensure that all the activities involving the GMO such as importation, production, release, and distribution are carried in such a way that minimise possible harmful environmental impacts. It also establishes common measures for the evaluation of potential risks that arises out of GMOs by laying down the requirements and criteria for risk assessment (Leonelli, 2019). It is also aimed to establish appropriate procedures that notify specific activities involving the use of GMO with the provision for matters connected to it (SAFLII, 1997).

The GMO Act is implemented by Directorate Biosafety and administered by the GMO Registrar. Various legislations related to GMOs in South Africa are shared by various government departments that are guided by the Cartagena Protocol on Biosafety (Figure 2.1). It also establishes common measures for the evaluation of potential risks that arises out of GMOs by laying down the requirements and criteria for risk assessment. It is also aimed to establish appropriate procedures that notify specific activities involving the use of GMOs with the provision for matters connected to it (SAFLII, 1997).

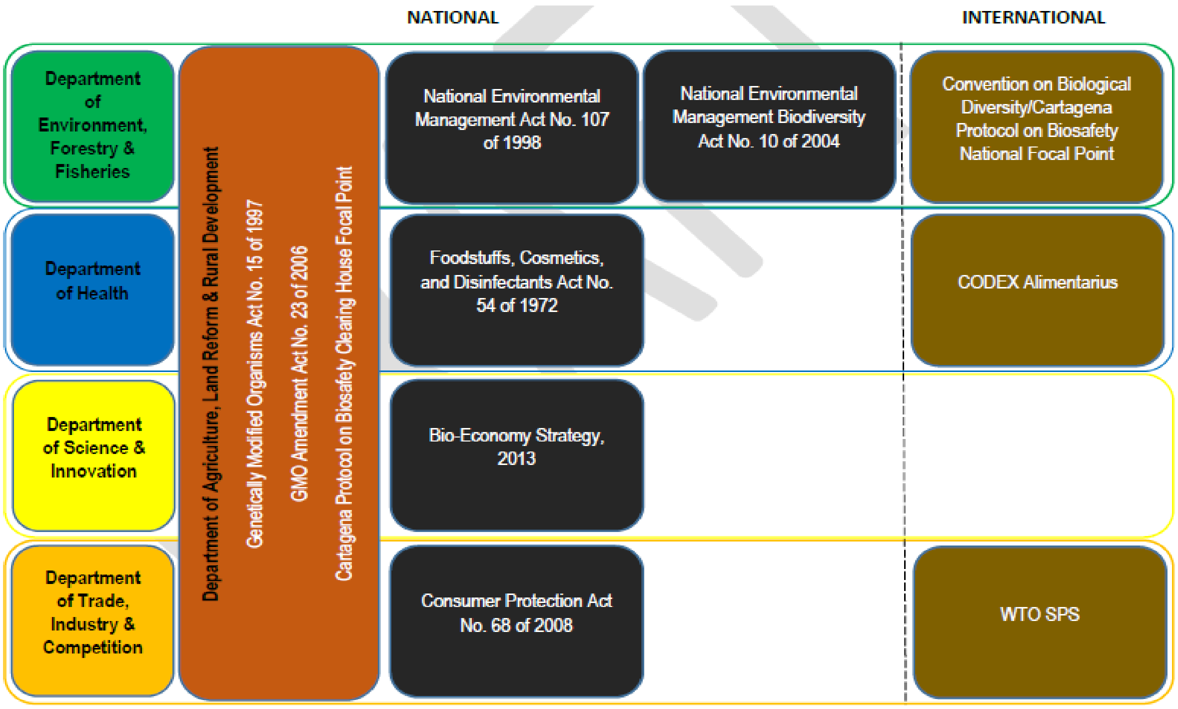

Figure 2.1 South African's National Biosafety Framework illustrating the Acts involved in Genetically Modified Organisms. Source: ERA Framework (unpublished).

The NEMBA provides for the management of SA's biodiversity within the framework of the National Environmental Management Act (NEMA), 1998. It promotes the protection of species and ecosystems, fair and equitable sharing of benefits arising from bioprospecting involving indigenous biological resources for the South African National Biodiversity Institute (SANBI). The NEMBA with regards to GMOs, confers the SANBI the responsibility to monitor and report on the environmental impacts of GMOs released into the environment in South Africa. NEMBA relates to GMOs by regulating products, activities, and developments of GMOs to ensure that they are economically, socially, and environmentally sustainable. Under the NEMBA act, the environmental release of GMOs is one of the listed activities.

2.2.1 Aspects of the Genetically Modified Oganism Act relating to non-target organisms

South Africa has recognised the potential benefits of using biotechnology and it has adopted a National Biotechnology Strategy in 2001 for sustainable development (Motari *et al*., 2004). South Africa has developed a biosafety regulatory system to ensure the utilisation of biotechnology in a way that minimises disruption to the environment whilst focusing on sustainable development goals and laws of the country (Eckerstorfer *et al*., 2019). The SA government believes that the various applications of biotechnology, including GMOs, may contribute to mitigating the environmental impacts of agriculture (Brookes and Barfoot, 2017). Genetically modified crop breeding and commercialisation in South Africa relies on a few, yet complementary GMO legislations (Bothma *et al*., 2010). Even with these legislative instruments in place, the different approaches applied towards the potential risks of GMOs (i.e. crops) to human health, socio economic, biodiversity and non-target organisms remain debatable, globally (Prakash *et al*., 2011).

The potential effects of GM crops on non-target organisms and biodiversity has been a concern since their adoption and use (Poppy, 2000). However, the potential adverse effect that may rise from GM crops is hindered by the lack of basic checklists of species in the cropping ecosystems to fully assess the possible impacts on non-target species (Dutton, *et al*., 2002; Naranjo, 2009). Non-target organisms are those that are not intentionally targeted by a certain management strategy. An example that describes non-target effects on the above ground organisms inside a cropping system include the impact of GM crops on other plants and insects (Lang *et al*., 2019). To ensure that all the possible effects of GM crops are monitored, more information on the diversity of other species such as arthropods and weeds is needed (Russell, 2007). For example, arthropod diversity patterns of richness and diversity as non-target organism provide information to evaluate possible impacts of GM crops at different levels (Botha *et al*., 2015). The criteria for ranking non-target species in each functional type to facilitate selection of species to evaluate in non-target tests is consistent with Annex 3 of the Cartagena Protocol. This includes maximum possible exposure, potential adverse effects, and potential exposure (Andow and Hilbeck, 2004). Ecologically speaking, species have significant functions such as biological control, pollination, and decomposition. Hence, species losses are very likely to have an economic impact if the abundance of species changes (Andow and Hilbeck, 2004).

2.3 Development and use of herbicide tolerant crops

Advances in biotechnology have enabled the development of crops which resist selective and non-selective herbicides. The incorporation of genes that resist herbicides has made weed management easy in agricultural systems (Green, 2012). However, herbicide reliance in cropping systems for weed control has also contributed to the lack of variation in weed control practices (Johnson *et al*., 2009). This limitation in weed management has been shown to favour the evolution and domination of herbicide resistant weed populations (Beckie, 2006). This happens because the benefits of HT crops enable farmers to choose not to diversify in weed management (Duke and Powles, 2008). In 2009, five different genetically modified HT crops were grown in the USA (i.e. canola, sugar beet, cotton, soybean and maize) and some of these were also grown in other countries such as

Argentina, India and SA (Duke and Powles, 2008). Refer to Table 2.5 a full list and respective years of approval in each country.

Table 2.5 Herbicide tolerant genetically modified crops and their first years' of approval (ISAAA, 2017).

Crop	Countries approved	First year approved
Alfalfa	USA	2005
Canola	Australia	2003
	Canada	1995
	USA	1995
Cotton	Argentina	2001
	Australia	2002
	Brazil	2008
	Colombia	2004
	Costa Rica	2008
	Mexico	2000
	Paraguay	2013
	SA	2000
	USA	1994
Maize	Argentina	1998
	Brazil	2007
	Canada	1996
	Colombia	2007
	Cuba	2011
	EU	1998
	Honduras	2001
	Paraguay	2012
	Philippines	2002
	SA	2002
	USA	1995
	Uruguay	2003
Soybean	Argentina	1996
	Bolivia	2005
	Brazil	1998
	Canada	1995
	Chile	2007
	Costa Rica	2001
	Mexico	1996
	Paraguay	2004
	SA	2001
	USA	1993
	Uruguay	1996
Sugar Beet	Canada	2001
	USA	1998

2.4 Common herbicides used in genetically modified maize crops

Herbicidal weed control is regarded an ideal way to control weeds, but proper selection of herbicides is essential for successful weed control (Kannan *et al*., 2013). Consequently, the range of targeted weed species by herbicide has increased rapidly and this has made the weed communities to rapidly change over time and space in response to herbicide use. There are various herbicides used to control weeds in GM maize (Table 2.6). The most preferred herbicides used in genetically modified maize are acetochlor, diuron, fomesafen, glyphosate, halosulfuron methly, mesotrione, nicosulfuron, pendimenthalin, pyrithiobic, s-metolachlor, topramezone, trifluralin and 2,4-D (Brookes and Barfoot, 2015).

Table 2.6 Herbicides used in genetically modified maize fields with time of application studied in different regions of the world.

Active Ingredients	Time of application	Selection	Weed category	Source
2,4-Dichlorophenoxyacetic	Pre- and post-emergence	Selective	Broadleaf and Grass	Brookes and Barfoot, 2017
Acetochlor	Pre-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Atrazine	Pre- and post-emergence	Selective	Broadleaf and Grass	Chikoye *et al*., 2005
Bromoxynil	Post-emergence	Selective	Broadleaf	GrainSA, 2015
Cynazine	Pre- and post-emergence	Selective	Broadleaf and Grass	Moyer and Blackshaw, 1993
Dicamba	Pre- and post-emergence	Selective	Broadleaf	Ganie and Jhala, 2017
Glyphosate	Post-emergence	Non-selective	Broadleaf and Grass	Dill, 2005
Halosulfuron	Post-emergence	Selective	Broadleaf	Nandula *et al*., 2007
Mesotrione	Pre- and post-emergence	Selective	Broadleaf and Grass	Maseldzija and Dudic, 2018
Metolachlor	Pre- and post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Nicosulfuron	Post-emergence	Selective	Broadleaf and Grass	Maseldzija and Dudic, 2018
Primagram gold	Post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
S-metolachlor	Pre- and post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Sorgomil	Pre- and post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Tembotrione	Post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Terbuthylazine	Pre-emergence	Selective	Broadleaf and Grass	Maseldzija and Dudic, 2018
Terbuthylazine	Post-emergence	Selective	Broadleaf and Grass	GrainSA, 2015
Topramezone	Post-emergence	Selective	Broadleaf and Grass	Zhang *et al*., 2007
Trifluralin	Post-emergence	Selective	Broadleaf and Grass	Brookes and Barfoot, 2015

2.4.1 Weeds associated with maize crop fields: not specific to herbicide tolerant crops

Maize weed species differ from region to region because weeds have their own adaptability to the prevailing environmental factors in an area. Some regions have weeds that become introduced to the environment after natural disasters or agricultural disturbance (Bagavathiannan and Davis, 2018). Weed ecology has been studied in developed countries better than the rest of the world. For example, USA and Serbia are some of the largest maize producing countries that have conducted extensive studies on weeds in maize and soybean fields (Lee and Thierfelder, 2017). Table 2.7 lists weeds associated with maize crop fields.

Table 2.7 Weed species studied in maize crop fields.

Family	Weed species	Source
Malvaceae	*Abutilon theophrasti* Medik.	Vyn *et al*., 2006
Asteraceae	*Ageratum conyzoides* (L.) L.	Masum *et al*., 2013
Amaranthaceae	*Amaranthus* L.	GrainSA, 2015
Amaranthaceae	*Amaranthus palmeri* S.Watson	Brookes and Barfoot, 2015; Janak and Grichar, 2016; Vyn *et al*., 2006, GrainSA, 2015
Amaranthaceae	*Amaranthus retroflexus* L.	Maseldzija and Dudic, 2018
Amaranthaceae	*Amaranthus tuberculatus* (Moq.) Sauer.	Bernards *et al*., 2012; Heap, 2015; Peterson *et al*., 2016; Brookes and Barfoot, 2015
Asteraceae	*Ambrosia artemisiifolia* L.	Maseldzija and Dudic, 2018
Asteraceae	*Ambrosia trifida* L.	GrainSA, 2015
Gramineae	*Avena fatua* L.	Maseldzija and Dudic, 2018
Asteraceae	*Bidens pilosa* L.	Rashid *et al*., 2020
Poaceae	*Brachiaria fasciculata* (Sw.) Parodi	Janak and Grichar, 2016
Poaceae	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb.	Janak and Grichar, 2016
Cannabaceae	*Cannabis sativa* L.	Maseldzija and Dudic, 2018
Poaceae	*Cenchrus tribuloides* L.	Yaduraju *et al*.,2015
Chenopodiaceae	*Chenopodium album* L.	Bernards *et al*., 2012; Heap, 2015; Peterson *et al*., 2016; Glowacka, 2011; Maseldzija and Dudic, 2018
Chenopodiaceae	*Chenopodium hybridum* L.	GrainSA, 2015; Maseldzija and Dudic, 2018
Poaceae	*Chloris virgata* Sw.	Rashid *et al*., 2020
Asteraceae	*Cirsium arvense* (L.) Scop.	Maseldzija and Dudic, 2018; Glowacka, 2011
Commelinaceae	*Commelina benghalensis* L.	GrainSA, 2015; Osipe *et al*., 2017; Chikoye *et al*., 2005
Asteraceae	*Conyza canadensis* (L.) Cronquist	Brookes and Barfoot, 2015
Asteraceae	*Conyza abbreviata* Wall.	Osipe *et al*., 2017
Cornaceae	*Cornus canadensis* L.	Yaduraju *et al*.,2015
Fabaceae	*Crotalaria sphaerocarpa* DC.	Rashid *et al*., 2020
Poaceae	*Cynodon dactylon* (L.) Pers.	Maseldzija and Dudic, 2018; Masum *et al*., 2013
Boraginaceae	*Cynoglossum officinale* L.	Yaduraju *et al*.,2015
Cypearceae	*Cyperus cyperoides* (L.) Kuntze	Masum *et al*., 2013
Cyperaceae	*Cyperus esculentus* L.	GrainSA, 2015; Rashid *et al*., 2020
Cyperaceae	*Cyperus rotundus* L.	Masum *et al*., 2013
Cyperaceae	*Cyperus* L.	Masum *et al*., 2013
Solanaceae	*Datura ferox* L.	GrainSA, 2015
Solanaceae	*Datura stramonium* L.	Maseldzija and Dudic, 2018
Apiaceae	*Daucus carota* L.	Bernards *et al*., 2012; Heap, 2015; Peterson *et al*., 2016
Amaranthaceae	*Digera muricata* (L.) Mart.	Masum *et al*., 2013
Poaceae	*Digitaria* L.	Vyn *et al*., 2006
Poaceae	*Digitaria sanguinalis* (L.) Scop.	Masum *et al*., 2013
Poaceae	*Echinochloa* L.	Vyn *et al*., 2006
Poaceae	*Echinochloa colona* (L.) Link	Masum *et al*., 2013
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Janak and Grichar, 2016; Glowacka, 2011; Masum *et al*., 2013
Asteraceae	*Galinsoga parviflora* Cav.	Maseldzija and Dudic, 2018; Glowacka, 2011
Asteraceae	*Helianthus annuus* L.	Maseldzija and Dudic, 2018
Malvaceae	*Hibiscus trionum* L.	Maseldzija and Dudic, 2018
Poaceae	*Imperata cylindrica* (L.) Raeusch.	Rashid *et al*., 2020

Table 2.7 Weed species studied in maize crop fields continued.

Family	Weed species	Source
Convolvulaceae	*Ipomoea nil* (L.) Roth	Osipe *et al*., 2017
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	Rashid *et al*., 2020
Cyperaceae	*Kyllinga squamulata* Vahl	Chikoye *et al.*, 2005
Poaceae	*Lolium rigidum* Gaudin	Masum *et al*., 2013
Lamiaceae	*Mentha arvensis* L.	Maseldzija and Dudic, 2018
Poaceae	*Panicum maximum* Jacq.	Masum *et al*., 2013
Asteraceae	*Parthenium hysterophorus* L.	Masum *et al*., 2013
Plantaginaceae.	*Plantago minor* Garsault	Maseldzija and Dudic, 2018; Masum *et al*., 2013
Polygonaceae	*Polygonum aviculare* L.	Maseldzija and Dudic, 2018
Portulacaceae	*Portulaca oleracea* L.	Yaduraju *et al*.,2015
Brassicaceae	*Raphanus raphanistrum* L.	Bernards *et al*., 2012; Heap, 2015; Peterson *et al*., 2016
Rubiaceae	*Richardia brasiliensis* Gomes	Osipe *et al*., 2017
Poaceae	*Rottboellia cochinchinensis* (Lour.) Clayton	Rashid *et al*., 2020
Polygonaceae	*Rumex maritimus* L.	Yaduraju *et al*.,2015
Polygonaceae	*Rumex patientia* L.	Maseldzija and Dudic, 2018
Poaceae	*Setaria viridis* (L.) P.Beauv.	Yaduraju *et al*.,2015
Solanaceae	*Solanum carolinense* L.	Yaduraju *et al*.,2015
Solanaceae	*Solanum nigrum* L.	Maseldzija and Dudic, 2018
Asteraceae	*Sonchus arvensis* L.	Maseldzija and Dudic, 2018
Poaceae	*Sorghum halepense* (L.) Pers.	GrainSA, 2015
Asteraceae	*Tagetes minuta* L.	Rashid *et al*., 2020
Compositae	*Taraxacum campylodes* G.E. Haglund	Maseldzija and Dudic, 2018
Asteraceae	*Tridax procumbens* (L.) L.	Yaduraju *et al*.,2015
Poaceae	*Brachiaria texana* (Buckley) S.T. Blake	Janak and Grichar, 2016
Plantaginaceae	*Veronica persica* Poir.	Maseldzija and Dudic, 2018
Violaceae	*Viola arvensis* Murray	Yaduraju *et al*.,2015
Asteraceae	*Xanthium strumarium* L.	Maseldzija and Dudic, 2018

A South African study by Botha *et al*. (2015) showed that plant species richness in maize fields was lower compared to rangeland. However, weeds continued to be present and abundant in cropped fields due to their pre-adaptive traits to intensive cropping systems (Botha *et al*., 2017), dynamic nature of weeds (Batlla *et al*., 2020), management practices including herbicide application (Pieterse, 2010), and shifts in weed species (Owen, 2008). Therefore, the dynamic nature of weeds necessitates the continuous monitoring thereof on agricultural fields to maintain agricultural productivity and environmental health. It is more likely for weeds to emerge continuously and become resistant due to their dynamic nature (Chauhan *et al*., 2017) and hence, management plays a role to combat the development of weeds that are resistance towards herbicides (Pieterse, 2010).

2.4.2 Potential for weed facilitation in herbicide tolerant crops through gene flow

Gene flow is the transfer of genetic variation from one population to another where, if the rate of gene flow is high, the two populations become an effective single population (Pesole, 2008). It can happen through vertical gene transfer or horizontal gene transfer (Choudhuri and Kotewicz, 2014). Vertical gene flow is the transfer of genetic material from parents to offspring whereas horizontal gene flow involves movement of genetic material from a donor to a recipient of another offspring (Choudhuri and Kotewicz, 2014). Gene transfer also depends on various factors such as type of vector, coexistence of the crop and its close relatives, their biology and physiology (Mallory-Smith and Zapiola, 2008). This is irrespective of whether the plant species is genetically modified or non-genetically modified, as gene flow is common naturally. For the context of GM crops, an example would be gene flow from herbicide tolerant sunflower crops to weedy sunflower *Helianthus annuus* L. (Bozic *et al*., 2015). *H. annuus* is an invasive species that is widely distributed in several regions and occurs in many different forms (varieties) in agricultural systems, including cultivated sunflower fields. It is commonly controlled by chemicals such as imidazolinone or sulfonylurea (Bozic *et al*., 2015).

Gene flow is often promoted by loss of crop seed during harvest time and dispersal into margins where it crosses with the weedy taxon (He *et al*., 2014). This results in crop to weed gene flow, and herbicide tolerant hybrids to weedy relatives. This type of natural crossing is a normal process which has been occurring between crops and wild relatives for centuries, as well as between relative populations, from before the invent of GM crops (He *et al*., 2014). Gene flow has played an important role in the evolution of weed species (Campbell *et al*., 2006). On the other hand, it can lead to an extinction or dominance of certain weed species related to the crops, or even an increase to new weed forms which are aggressive (Garcı´a-Ruiz *et al*., 2018). An example of the influence of morphological factors is illustrated by a study done in China by Lu *et al*. (2014) about gene flow from glufosinate-resistant rice to weedy rice of which the flow of genes was found less in taller rice recipients than in shorter ones.

2.4.3 Possibility of weed shift in herbicide tolerant cropping systems

Agricultural practices lead to selection pressure on weed communities which result in weed population shifts (Owen, 2001). Some species are killed by weed management practices, but others are not affected or remain dormant during application of management practices (Shahzad *et al*., 2021). Weed shift is the change in species composition or relative frequencies of weeds in a weed community in response to natural or human-made environmental changes in an agricultural system (Shahzad *et al*., 2021). One of the main contributors to weed shift is disturbance through tillage and use of herbicides. It has been shown that changes in weed communities owing to species that do not respond to glyphosate-based management are rapidly increasing (Owen, 2008). Examples of weed population shifts in glyphosate-resistant crops include common water hemp (*Amaranthus tuberculatus* (Moq.) Sauer.), horseweed (*C. canadensis*) and giant rag weed (*A. trifida*) (Owen, 2008). Weed shifts occur when weed management practices do not control an entire weed community. Weed shifts also occur with agronomic practices such as crop rotation, herbicide rotation and use of mixed active herbicides (Owen, 2001).

The number of herbicide-active ingredients commercially available has decreased in terms of the effectiveness. Therefore, this has increased the ecological implications on the agroecosystem, such as reducing the biodiversity of arable land, and facilitating weed population shifts in weed communities (Firbank, 2000; Watkinson *et al*., 2000; Powles, 2010). With the decline of herbicides effectiveness, it has led to the use of combined herbicides and crop rotation. Crop rotation allows for the use of alternative weed management strategies including herbicide rotation. Crop rotation has the potential to reduce weed population densities and maintain lower weed species diversity, which can then have an impact on weed population shift (Liebman and Dyck, 1993). The increased diversification of weeds in crop rotation dilutes the selection pressure that favours specific weed populations and subsequently reduces the potential for weed population shifts (Weisberger *et al*., 2019).

2.5 Weeds in agriculture and their characteristics in general

Weeds are undesirable plant species recognised worldwide as growing where they are not wanted. They constitute one of the major economically important challenges for maize growers and other crops because they can reduce yield by up to 86% (Kannan *et al*., 2013).

Weeds are characterised in many ways. They are very competitive, persistent, and mostly resilient to certain weed control methods (Beckie, 2006). Weeds have germination requirements that are very adaptive to most environments with continuous seed production (Batlla and Benech-Arnold, 2014). The continuous seed production makes them disperse easily over short and long distances. When evaluating success of species over time, it is important to consider number of individuals in certain habitats because species could be wiped out by a single event (Travlos *et al.*, 2018). The abundance of weeds is more of a concern than their presence. For example, farmers are less concerned by a few occurring individuals which can be selectively managed, than a vast occupation on fields (Potgieter *et al*., 2019).

2.5.1 Weed establishment, traits and persistence

Plant functional traits are any morphological and physiological feature that is measurable on an individual level and is used in ecosystem ecology to address the responses of species to environmental changes (Martin and Isaac, 2015). It provides a basis for understanding how environmental changes affect plant species filtering and composition (Lavorel and Garnier, 2002). Plant traits are therefore widely used to address questions in natural ecosystems at various scales. Functional plant trait research has led to better understanding of the impacts of biodiversity in the ecosystem, but it has not been widely applied to agroecosystems (Wood *et al*., 2015). Hence, plant functional traits should be measured across agricultural ecosystem gradients to predict how ecosystems vary with farm practices such as weed management (Botha *et al*., 2017).

Plant traits can be measured quantitatively and qualitatively (Martin and Isaac, 2015). The main reason for investigating traits is to predict weed response to biotic and abiotic disturbances such as crop variety effect, herbicide application and grazing. Plants disperse to new sites and become established, and thereafter, they become persistent. Both establishment and persistence are determined by the way that plants tolerate a wide range of biotic and abiotic conditions of a site (Lavorel and Garnier, 2002). In terms of persistence due to herbicide use, it involves traits that resist specific levels and spectrums of herbicide application; hence, this involves traits enabling the herbicide-weed interaction. Two sets of traits have been identified and this includes the “hard” and “soft” traits (Belluau and Shipley, 2018). Then there are those traits measured for dispersal purposes such as seed mass and shape, and those for establishment, such as leaf area and plant height, flowering and resprouting ability (Belluau and Shipley, 2018).

2.5.2 Weed dispersal and infestation

Seed dispersal is divided into two components, namely dispersal in space and time. Small seeds are associated with large seed production that enhances dispersal, while seed mass and shape are indicators of longevity (Giles, 1990). When seeds deposit at a new site, it must develop into a new seedling, therefore seedling size is critical for successful establishment of weeds (Cernac *et al*., 2006). Species with heavy seeds tend to have better establishment due to higher seedling fitness when competing with neighbours that have low seed mass (Duncan *et al*., 2019). After establishment, a plant must persist, and the life span of a plant enhances population persistence. Life span can be measured as annual or perennial (Bender *et al*., 2000). It is therefore important to also consider the method for measurement to give a better accuracy of traits. It is critical to know how to choose and identify a plant, and which plant to choose for measurements. Important aspects to consider is the life stage, phenology, and habitat (Iversen *et al*., 2014). Sample size is also an important factor to consider in providing accurate assessments of dispersal, establishment, and persistence (Eriksson, 2000) These types of studies are conducted at the species level and not community level because species populations depend on ecological mechanisms and respond strongly to disturbance; whereas community level properties emerging from the species populations are less sensitive to disturbance as they depend on regional mechanisms (Supp and Ernest, 2014).

A study by Fried *et al*. (2015) reported that weed dispersal is driven by agricultural management at multiple scales. Distribution of weeds in the crop fields and margins is affected by both field and boundary management (Petit *et al*., 2012). Field margins accommodate higher diversity and abundance of weed species than the core of cultivated fields (Romero *et al*., 2008; Kovacs-Hostyanszki *et al*., 2011). This is because disturbance imposed by farming practices are harsh in the core of the crop fields whereas the constraints are often relaxed at the margin and the management becomes less efficient (Kleijn and van der Voort, 1997). Weed dispersal between the crop fields and margins occur in both directions (Marshall, 2002) and short distance weed dispersal from margins to crop fields are perceived by farmers as a source for crop infestation (Cordeau *et al*., 2011).

2.5.3 Trait based approach towards understanding crop-weed interactions

The mechanism of plant community assembly may provide evidence to improve crop management and offer better understanding as to how weeds impact on crop yields. Plant functional trait diversity can be used to analyse a crop-weed interaction with different levels of weed species and how they respond to resource competition (Pakeman *et al*., 2015). Functional traits underpin species responses to environmental conditions, and they can be used to understand the response of weeds to crop management (Cernac *et al*., 2006, Botha *et al*., 2017). Plant functional traits can provide insight on the process of weed species community assembly to understand the role played by both biotic and abiotic factors (Shipley *et al*., 2006; Schob *et al*., 2012). Functional traits can also be used to understand plant to plant interaction in cropping systems to protect biodiversity by reducing the use of pesticides (Schob *et al*., 2012). Attempts need to be made to apply a functional trait-based approach to explore the crop-weed dynamics of arable plant communities (Navas, 2012), by investigating the different trait responses to shifts in competitiveness as indicators of crop-weed interactions (Pakeman *et al*., 2015).

2.6 Weed management in agriculture

Weed scientists have come up with a list of recommendations for various herbicide resistant weed management practices involving several techniques including chemical and mechanical use for effective and safe weed control (Norsworthy *et al*., 2012). Recommendations are based on a thorough understanding of several concepts to survey and identify resistant weed populations. Data is obtained through field monitoring to prevent weed seed production and dispersal from field to field or field to margins. Recommendations also involve growing competitive crops that can suppress weed species (Hmielowski, 2019), and application of herbicide rotations with herbicide compounds with different modes of action to control similar weeds (Busi *et al*., 2019). A further recommendation is to apply the correct dosage and application times for certain types of weed species. It is also recommended that new herbicides should replace herbicides to which resistance has developed (Norsworthy *et al*., 2012). To further develop recommendations, it is important to promote research and publish guidelines for better access and management strategies worldwide.

Mechanical strategy includes the prevention of physical movement of weed seeds to uninfested areas. This could be achieved by using non-contaminated seeds while preventing the movement of plant reproductive structures carrying seeds (Melander *et al*., 2005). Other methods include cultural control such as crop rotation and crop cover with plants that can suppress weeds (Liebman and Dyck, 1993). It also involves other plants in general that supress weed species by chemicals that they release as well as manipulation of crop planting dates (Nichols *et al*., 2015). For example, maize (*Zea mays*) and rice (*Oryza sativa*) have been identified to produce a chemical such as benzoxazinoid hydroxamic acid which can kill some of the weeds (Kato-Noguchi and Ino, 2003; Niemeyer, 2009). In general, crop rotation is one of the methods that improve weed control by periodically changing the weed community (Nichols *et al*., 2015), because different crops differ in planting and harvest date, competitive ability, fertility requirements, and therefore, favouring different weed communities which allows the use of herbicides of different modes of action (Garcı´a-Ruiz *et al*., 2018). Planting seasons and tillage can further disrupt the growth and interactions of weeds (Nichols *et al*., 2015).

2.6.1 Chemical weed management classification

Herbicides are chemicals that inhibit the growth of plants and kill them (Belz and Duke, 2014). They have several classifications and can be classified by crop type, for example maize herbicides, and by their application timing, such as pre- and post- to crop or weed emergence (Hakoomat *et al*., 2017). They can also be classified by their chemical family, for example sulfonylureas or dinitroanilines, and by their mode of action, for example photosystem II inhibitors or acetolactate synthase (ALS) inhibitors (Hakoomat *et al*., 2017). Mode of action is the most relevant classifier, but less practical, as it describes how the herbicide imposes selection pressure on weeds (Vencill *et al*., 2012). They can also be classified according to the application method, such as foliar or soil applied herbicides (Vats, 2015).

2.6.1.1 Soil applied herbicide classification

Soil applied herbicides must persist in the soil for effectiveness to kill the weeds while allowing the emergence of desired crop. When the herbicide is applied before the crop is planted, it is referred to as pre-plant herbicide (Vats, 2015). Pre-plant herbicides are applied a few days before crop sowing depending on the type of crop to be planted as well as the soil type. Pre-plant and pre-emergence herbicides can be applied in the crop row to control weeds between the rows (Hakoomat *et al*., 2017). A soil herbicide can also be applied after the crop has established to lengthen the weed control in the crop. For example, in glyphosate resistant crops, metachlor can be sprayed post-emergence in a tank mixture with glyphosate. Metachlor is a herbicidal chemical that has no activity on emerged plants (Zemolin *et al*., 2014), but provides residual control between applications of broad-spectrum herbicides like glyphosate or glufosinate. Soil herbicides are important because they prevent weeds from emerging with the crop. Due to resistance in weeds to foliar herbicides, the use of soil applied herbicides has gradually increased (Hakoomat *et al*., 2017). For example, a test of resistance in *Amaranthus palmeri* indicated that resistance could be delayed with the use of soil applied herbicides (Ward *et al*., 2020).

2.6.1.2 Foliar applied herbicide classification

Foliar herbicides are applied to weed foliage with or without contact of the spraying machine with the crop (Vats, 2015). They are effective against weed seedlings. These are post-emergence herbicides and can be applied after crop emergence in HT crops. Post-emergence herbicides have sufficient weed control due to their broad spectrum of activity, convenience, and flexibility (Gower *et al*., 2003). Hence, the above-mentioned necessitates the production and testing of selective post-emergence herbicides in maize crops. Glyphosate is one of the broad-spectrum post-emergence herbicides that can control dicotyledonous weeds, annual and perennial grasses (Franz *et al*.,1997). Post-emergence herbicides can be applied over the HT crop and weeds. Foliar herbicides are used for controlling emerged weeds present at planting; and they are usually called burndown herbicides (Doublet *et al*., 2009). They are also called contact herbicides when only the treated part of the plant is affected. They are called translocated when the herbicide enters the plant and moves within it to the site of herbicide action through phloem or xylem (Vats, 2015).

2.7 Multiple versus single herbicide use in genetically modified crops

The use of multiple modes of herbicides in weed management has increased due to the increasing number of weeds developing resistance towards the use of single mode of herbicides (Green *et al*., 2007). Many biotic and abiotic factors determine the effectiveness of each tank mix of multiple mode herbicides. However, each component of a herbicide tank mix is not necessarily effective under all applications for every weed species (Green *et al*., 2007). For example, broadleaf species, giant ragweed, summer annual weeds and large seeded weeds can be difficult to control with a single herbicide because of their extended germination period and emergence characteristics. Therefore, farmers tend to use multiple herbicides to control such weeds. Herbicides containing multiple active ingredients are available for farmers who want to reduce the selection for herbicide resistance in weeds (Bagavathiannan and Norsworthy, 2016). Morevover, each active ingredient should have similar efficacy against the target weed species in order to be effective (Kraehmer *et al*., 2014). Multiple herbicides such as the use of chloroacetamide and atrazine can be used to successfully control weeds and this has been demonstrated for waterhemp that has developed resistance in some regions in the USA (Gaines *et al*., 2020).

A study done in central and south-central Texas by Janak and Grichar (2016) showed that maize yield was high when herbicide treatments used contained more than one active ingredient, which means more weeds were suppressed. Acetochlor and pendimethalin used alone was reported as the most consistent control of annual grass including *Brachiaria reptans* (L.) C.A. Gardner and C.E. Hubb (Sprawling signal grass), *Echinochloa crus-galli* (Barnyard grass), *Panicum fasciculatum* Sw. (Brown top panicum), *Urochloa texana* (Buckley) R.D.Webster (Texas millet).

Control of *Amaranthus palmeri* was reported to be 90% when the herbicides acetochlor, metolachlor, and combinations of atrazine plus acetochlor or fluthiacet-methyl plus pyroxasulfone was used (Janak and Grichar, 2016). Of all the weeds encountered in the corn field, Hophornbeam copperleaf (*Acalypha ostryifolia* Riddel ex J.M. Coult.) was found difficult to control, but with a mixture of acetochlor, saflufenacil or pyroxasulfone it was possible to control 90% of it. Common sunflower (*H. annuus*) was also 90% controlled by acetochlor or sulflufenacil mix (Janak and Grichard, 2016). A study on the effect of different pre- and post-emergence herbicides application at Ambo and Guder sites in Ethiopia by Janak and Grichard (2016) showed that nicosulfuron herbicide with silwet gold organosilicon adjuvant was effective in controlling weeds.

2.8 Development of weed resistance to herbicides

Herbicide resistance is generally defined as the decreased response of a plant species towards herbicides (Sanbagavalli *et al*., 2000), or survival of weed species following a herbicide dose lethal to that species. In other words, it is the ability of a weed species to survive herbicide treatment. It is also described as the inherited ability of weeds to survive and reproduce following the exposure to a dose of herbicide that is normally lethal to the other weeds (Vencill *et al*., 2012). Resistance development may be natural or induced by techniques such as genetic engineering (Romeis *et al*., 2019). For most weed species, resistance to herbicides could be a normal outcome of natural selection, and hence, any mutation that confers herbicide resistance could already exist in weed species before any exposure to herbicides (Gaines *et al*., 2020). Therefore, mutations may increase over time after herbicide applications and weed species may become dominant carriers of resistant genes (Heap, 2014).

Weed resistance to herbicides has over the years become a major concern affecting farmers using herbicide tolerant crops. Currently, herbicides resistant weeds have been reported in 93 crops across 70 countries (Powles and Yu, 2010). There are more than 30 herbicide resistant weed species confirmed in North America and Western Europe combined (Heap, 2014). Countries to have confirmed high numbers of herbicide resistant weeds include Germany, Israel, Japan, Spain and USA (Heap, 2014). De Egea *et al*. (2016) reported nine of the most herbicide resistant weeds globally found in high abundance in crop fields of different ecoregions of Paraguay: *Amaranthus hybridus* L., *B pilosa*, *Commelina erecta* L., *Conyza bonariensis* (L.) Cronquist, *Digitaria insularis* (L.) Mez ex Ekman, *Eleusine indica* (L.) Gaertn., *Euphorbia heterophylla* L., *Richardia brasiliensis* (Gomes) Klotzsch and *Solanum americanum* Mill. In 2013, 14 weed species resistant to glyphosate were reported in the USA and ten were found to exhibit similar modes of resistance in Australia (Brookes and Barfoot, 2015).

2.8.1 Weed resistance to glyphosate

Glyphosate is a non-selective herbicide that can control all weed species in GM crop ecosystems through post-emergent application. Glyphosate herbicide is highly synthetic and translocated to both below and above ground meristems to minimise the re-growth of weed species (Galon *et al*., 2013). Glyphosate was developed by Monsanto in 1974, as a non-selective herbicide that contains the active ingredient N-(phosphometil) glycine (Peillex and Pelletier, 2020). Glyphosate is the molecule that inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (Schonbrunn *et al*., 2001). It is an effective broad-spectrum herbicide and declared environmentally friendly post-emergence with low soil toxicity (Baylis, 2000; Duke and Powles; 2008). Glyphosate is effective in killing a wide variety of broadleaf herbs, grasses and woody plants. It is soluble and absorbed into the soil as its residues are poorly mobile and it is readily degraded by microbes. Glyphosate was the most used herbicide in the agricultural sector and second most used in garden, home, and commercial application in 2007 (Pieterse, 2010), hence it remains one of the most preferred chemical for weed control by farmers (Brookes and Barfoot, 2017).

The main characteristics of glyphosate, such as its broad weed spectrum in terms of annual and perennial application, and in addition to its ability to control both monocot and dicot weeds, has made it the most widely used herbicide (Baylis, 2000). Due to it being non-selective, selectivity has been achieved by timing the application thereof, such as pre-plant or pre-emergence control of weed species. Glyphosate has been declared to have a low risk for the evolution of weed resistance, but this has been disputed (Beckie, 2006). Rotating herbicide applications with other weed control methods has been proposed to reduce and/or delay the rate of weed resistance development to glyphosate (Pieterse, 2010). Some glyphosate resistant weed species related to maize cropping systems in the USA are *A. palmeri* (Palmer Pigweed), *A. tuberculatus* (Waterhemp) and *C. canadensis* (Marestail). A study conducted in India by Yaduraju *et al*. (2015) revealed weeds resistant to herbicides, such as *Cenchrus tribuloides* L., *Cynoglossum officinale*, *Solanum carolinense* L. and *Viola arvensis* Murray. As farmers are faced with the challenge of weed resistance to glyphosate in HT crops, they are being advised to combine other herbicides with glyphosate to minimise the potential for weeds to develop resistance (Beckie, 2006).

2.8.2 Weed resistance to 2,4-Dichlorophenoxyacetic acid herbicide

Herbicide 2, 4-D is an organic compound which selectively kills broadleaf weeds (Gervais *et al*., 2008). Herbicide 2, 4-D has been widely used to control weeds in a variety of crops and has been applied as a foliar herbicide (Gervais *et al*., 2008). Hence, it is important to understand its activity when applied on crop fields, as well as its associated adverse effects - if any. Since 1991, aerial application has been banned in KwaZulu-Natal and totally prohibited in the magisterial districts of Camperdown, Pietermaritzburg, and Richmond (DAFF, 2017). It was withdrawn from all agricultural use in the Western Cape in 1980 (Islam *et al*., 2018). It is a non-volatile dry crystalline solid and slightly soluble in water, therefore, it must be changed into preferable ratio that disperse easily (Gervais *et al*., 2008). There are factors that affect the translocation of 2, 4-D uptake by plants such as growth stage of the plant. Plant age affects herbicide uptake, for example, translocation from young leaves may differ from mature leaves. Uptake can also be affected by the type of species. Depending on the formulation of 2, 4-D herbicide, an adjuvant chemical can either increase or decrease uptake (Varshney and Singh, 1990).

Herbicide 2, 4-D is effective in controlling broadleaf weeds and can have limited soil residual activity. There were 32 weed species reported to be resistant to 2, 4-D (Heap, 2015). Those weed species include: *A. tuberculatus* (Waterhemp), *C. album* (Lambs quarters), *Daucus carota* L. (Wild carrot), *Raphanus raphanistrum* L. (Wild radish) and *Sonchus oleraceus* (Sowthistle) (Bernards *et al*., 2012; Heap, 2015; Peterson *et al*., 2016). Post application of 2, 4-D can control annual broadleaf weeds including, *A. tuberculatus* (Water hemp), Ipomoea species (Morning glory), *Ambrosia* species (Ragweed), *C. album* (Lambs quarters) and *A. palmeri* (Pigweed) that are common in maize fields. The application rate of 2, 4-D is 280 to 560 g/ha, with the lower rate being the most used to avoid crop injury (Cernac *et al*., 2006). A study by Osipe *et al*., (2017) evaluated the effectiveness of 2, 4-D in controlling weeds and found that *C. benghalensis*, *Conyza* species, *Ipomoea nil* (L.) Roth and *R. brasiliensis* showed tolerance to the herbicide. And a mixture of more than one herbicide inclduing 2, 4-D and glyphosate showed better weed control than when only 2, 4-D was used (Osipe *et al*., 2017).

2.8.3 Weed resistance to Triazine herbicides

The herbicide triazine was discovered in 1952 in Basel, Switzerland (Muller, 2008) and it has played a positive role in the promotion of crop production (Chomas and Kells, 2004). Atrazine is a member of triazine, which is a pre- and post-emergence herbicide that is applied on more than 60% of USA maize (Chomas and Kells, 2004). It is commonly used for *Amaranthus palmeri* weed control in maize crops (Vyn *et al*., 2006), but its use has decreased over time due to weed resistance development. Of all the triazines, atrazine is one of the most used herbicides in agriculture for the control of monocot and dicot weeds such as *C. arvense*, *D. stramonium*, *D. sanguinalis*, *Setaria* species, *Sonchus* species and *X. strumarium* (Chomas and Kells, 2004). It is widely used in maize and sorghum crops. The intensive use of Triazines was reported to have resulted in two cases of resistance to Atrazine by *Amaranthus* species (Chomas and Kells, 2004), due to high use of herbicide mixtures (Brulinski, 2019). Another study of weed resistance to atrazine conducted in Serbia showed resistance of *A. retroflexus* and *C. hybridum*, and other resistant weed species confirmed were *Abutilon theophrasti* Medik., *C. album, D. stramonium, E. crus-galli, Setaria viridis* (L.) P. Beauv., and *Sorghum halepense* L. (Vrbničanin *et al*., 2020).

2.8.4 Weed resistance to Chlorsulfuron herbicide

The first commercial herbicide of chlorsulfuron was introduced in 1982 by DuPont in small grain crops (Ray, 1984). Chlorsulfuron is an effective herbicide at low use rate (O'Sullivan and Kirkland, 1984). It is applied as pre- and post-emergent herbicide and controls both monocot and dicot weed species in crops. The first resistance of weeds to chlorsulfuron was reported in the USA in 1987 by Shaner (1992) on *A. retroflexus*, *Cornus canadensis* L. and *Lolium rigidum* Gaudin. This herbicide is used as foliar herbicide and it controls monocot weed species in rice and cereals. The frequent use of these herbicides has led to the confirmation of further resistant weed species such as *E. crus-galli* and *Lolium* species (Heap, 2015).

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CHAPTER 3: Study area and methodology

3.1 Study area

The study was conducted at Baziya and Tsolo, which fall under the Oliver Tambo District Municipality, Eastern Cape Province. Baziya is part of the King Sabata Dalindyebo Local Municipality, whereas Tsolo is under the Mhlontlo Local Municipality. Tsolo can be described as a small town about 41km from Mthatha and Baziya is a village situated about 40km from Mthatha. Although both areas are referred to locals as falling under the town of Mthatha, they are not specifically a greater part of Mthatha. For the purpose of this study, both these areas are considered to be independent of the Mthatha but fall within the same District Municipality. Baziya lacks climatic details in terms of research and for the purpose of this study, climatic details given is that of Mthatha and Tsolo. The study area is about 54.97 km² in size, with an estimated population of 96,114 (1,748.34 per km²) (Census, 2011). The Oliver Tambo District is rated among the most financially challenged districts in terms of all living measures in the Eastern Cape Province (Meth, 2020). The number of people living in most financially challenged state is 64.6%, with an unemployment rate of 65.5% and literacy rate of 42.2% (Stats SA, 2019). Majority of the EC's population are black African, and they are predominantly speakers of Xhosa, a Nguni language (KSD, 2018/19).

The district is predominantly rural, hence smallholder agriculture and livestock raising are the primary economic activities in the area (Kibirige *et al*., 2019). Oliver Tambo District Municipality has some secondary industries that produce textiles, wood products, foodstuff, and processed tobacco (Meth, 2020). Wheat, maize, sorghum, and vegetables are grown in the area (Kibirige *et al*., 2019).

The climate in the region is mild, generally warm and temperate (Mucina and Rutherford, 2006; sawx.co.za). The province gets progressively wetter from west to east, where Mthatha (744 mm) and Tsolo (801 mm) receive a substantial amount of rainfall during the year, even for the driest months (Table 3.1). The average annual temperature in Mthatha is 17ºC and for Tsolo it is 16.2ºC (Table 3.1). Winter frost is more common for Tsolo (approximately 11 days per annum) than Mthatha (4 days per annum).

Baziya and Tsolo regions have fertile soils, with a clay fraction of organic matter and minerals. This resembles a more of tropical and subtropical soils (Mandiringana *et al*., 2007). In terms of vegetation, the Mthatha and Tsolo regions are largely thornveld, subtropical evergreen forest and grassland (Table 3.1), with some forest on the ravines forest in Pondoland (Mucina and Rutherford, 2006). The grassland and savanna are dominated by a large variety of grasses and forbs, with few shrubs and trees (Bredenkamp *et al*., 1998).

Table 3.1 Climate details for the biomes, vegetation units and the regions of Mthatha: (Mucina and Rutherford, 2006).

Biome	Vegetation unit	Bioregion	Subregion	MAP	MAT	MFD
Grassland	Dry Coast Hinterland Grassland	Sub-Escarpment Grassland Bioregion	Tsolo	887	14.6	26
Grassland	Mthatha Moist Grassland	Lowveld Bioregion	Tsolo, Mthatha	743	16.2	5
Grassland	Eastern Valley Bushveld	Lowveld Bioregion	Tsolo, Mthatha	773	17.8	3
Savanna	Zululand Lowveld	Lowveld Bioregion	Mthatha	717	17.0	3

MAP= Mean annual precipitation (mm); MAT= Mean annual temperature (ºC); MFD= Mean frost days per annum)

3.2 Site selection

Study sites were selected with the assistance of GrainSA's Mthatha office. Areas with little to no knowledge in weed species records for HT maize fields in the region were prioritised. Smallholder farmers chosen for the study fall within the GrainSA development programme whereby they receive various levels of training, resources, and mentorship for maize farming. In addition, GrainSA is responsible and facilitates the spraying protocols (for different herbicides) used by farmers in the region to manage/control weeds in HT maize fields.

The following sites were selected for sampling in Baziya, namely Makaula, Jojweni and Baziya Mission (Figure 3.1). The sampling sites in Tsolo are presented in Figure 3.2. In Makaula, ten maize fields were selected for surveys, in Jojweni five and six in Mission. In total, Baziya had 21 maize fields selected for surveys which constituted 28 ha. In Tsolo, seven maize fields were selected for surveys with a total area of 7 ha. Maize field size, in hectares, was measured using a Global Positioning System (GPS) by moving from a starting point around the field back to the starting point.

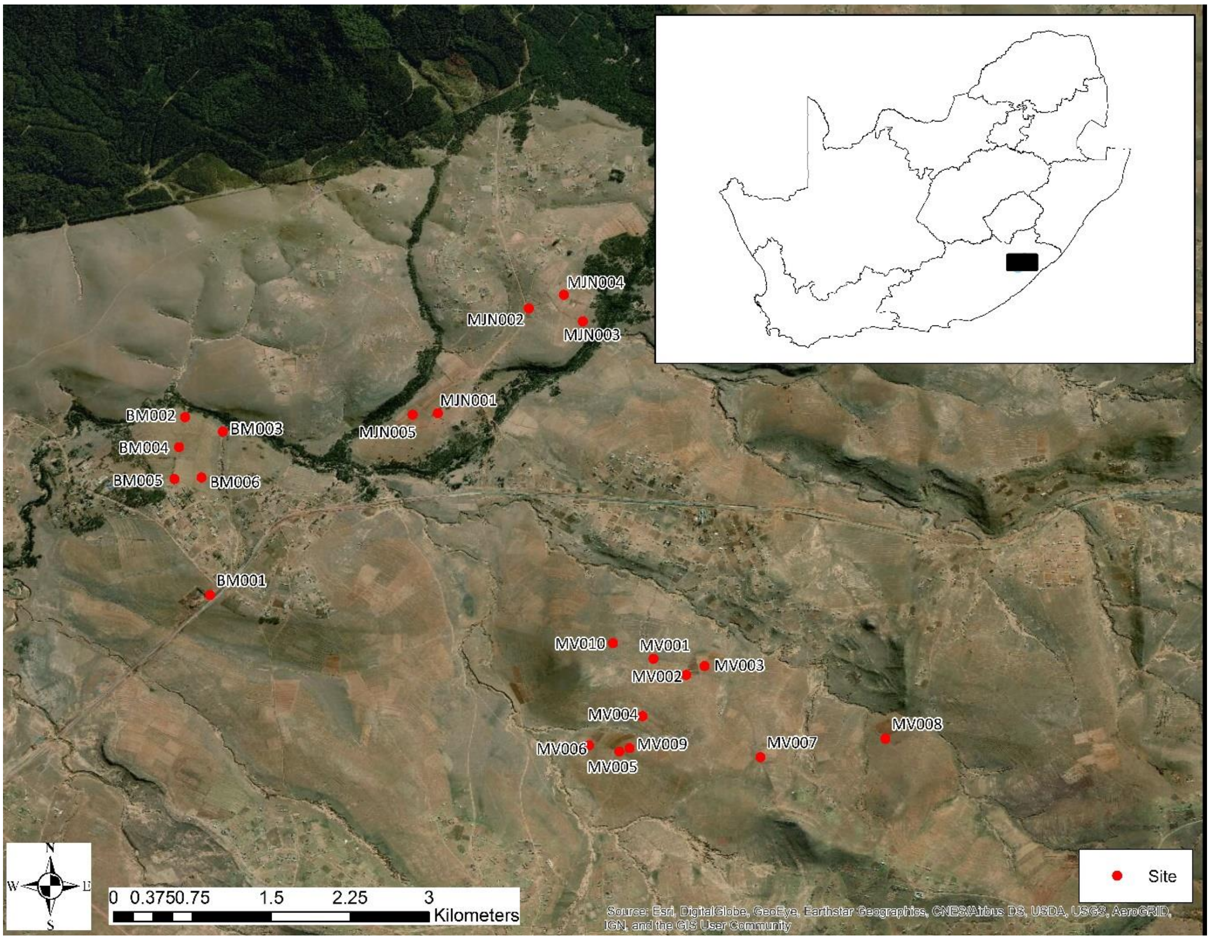

Figure 3.1 Localities of Baziya study sites (red dots) in the Oliver Tambo District of the Eastern Cape (is indicated by a black rectangle on the insert map of South Africa). Maize fields of different sites are: MJN= Jojweni; MV= Makaula Village; and BM= Mission.

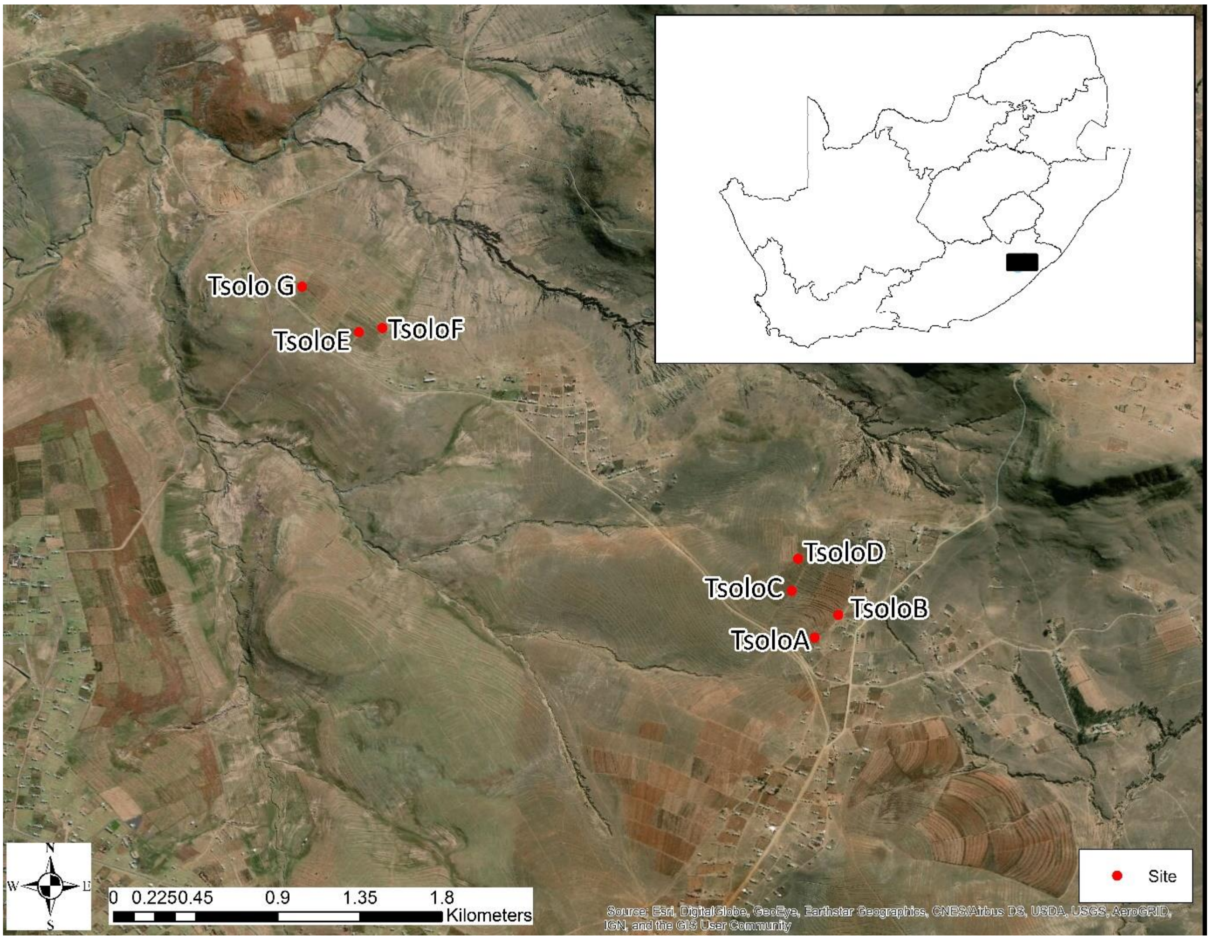

Figure 3.2 Locality of Tsolo study site (red dots) in the Oliver Tambo District of the Eastern Cape (is indicated by a black rectangle on the insert map of South Africa). Maize fields of different sites are presented.

3.3 Sampling design

The sampling criteria was adopted from a protocol established during a 2017/18 pilot study (from here on referred to as season one). The sampling design was adopted, with modifications, from Botha *et al*. (2015). The lack of uniformity in maize fields (i.e. smaller size; length and width) informed modifications, leading to three zones for sampling (Reference Transect=RT, Edge Transect=ET and Centre Transect=CT). Criteria were developed to determine where the different transects would be sampled (Figures 3.3, 3.4 and 3.5). In the sampling criteria, a control transect to sample conventional or non-HT maize was not accounted for during the study. This is because no comparable sites with non-HT maize or of similar field sizes were present in the surrounding area. Although it would have been an option to have a control in a different region within the same province, this was not considered as the geology, soil and microclimate in the respective regions were likely to be highly variable. This would have possibly been an inconclusive deciding factor for the study outcomes.

The number of times (repeats) that each maize field could be surveyed was heavily dependent on each farmer's preference in terms of farming inputs for possible ploughing/planting and environmental factors. In addition, the famer's willingness to be guided/mentored on what time is best for the ploughing and herbicide application timeframes and protocols. October to November were targeted for pre-ploughing surveys before ploughing, or disking of fields took place. Also, no herbicides were applied at this stage.

Table 3.2 Overview of the terms used throughout the thesis in terms of pant surveys and herbicide application.

	Pre-ploughing survey	Post-ploughing and seed sowing survey
Ploughing	No	Yes
Planting	No	Yes
Pre-emergence herbicide	No	Yes
Seedling emergence	No	Yes
Post-emergence herbicide	No	Yes/No

After ploughing, a pre-emergence herbicide was sprayed between three to seven days after planting, followed by post-emergence spraying five to six weeks after germination of the maize crop. The pre-ploughing surveys were conducted before ploughing, seed sowing and herbicide application. The post-ploughing and seed sowing surveys were conducted after ploughing, pre-emergence herbicide application and maize crop emergence. Post-emergence herbicide was either not yet applied or in the process of being applied. In some instances, the post-emergence herbicide was never applied.

Post-ploughing and seed sowing surveys were conducted on or before the period of post-emergence herbicide application (5^{th}-6^{th} week), in late January to early February depending on when the planting season started. Field transects were surveyed based on the three criteria and included combinations of the CT in the middle of the fields, ET at 50 m from the field margin and the RT outside the fields in the field margin. The different transects referred to here, are defined and illustrated in Figure 3.4.

A typical herbicide tolerant maize agroecosystem comprises of two main areas, namely the maize field and the maize field margin. The zone inside the maize fields, bordering the field margins is called the field edge. The zone of the field margins bordering the maize fields is called the field boundary (Figure 3.3).

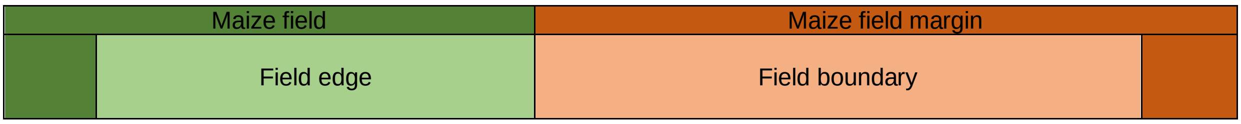

Figure 3.3 The structure of the field zones in the maize agroecosystem regarding the terms: edge and boundary.

3.3.1 Centre Transect (CT)

These transects were sampled in all the maize fields surveyed (minimum requirement). The positioning was determined by the longest side of the maize fields, with the CT parallel to the longest side (Figure 3.3). CTs were surveyed in the middle of the maize crop and twice in a planting season, for pre-ploughing, post-ploughing and seed sowing surveys, and over three seasons. From here onwards, the mention of three seasons refer to 2017/18, 2018/19 and 2019/20.

3.3.2 Edge Transect (ET)

If maize fields had sides >100m long, then they were large enough to incorporate ETs. Two ETs were placed in such fields to supplement the CT survey and to be more representative of the field (Figure 3.4). However, these transects were not a minimum requirement. ETs were located on each end of the longest side of the maize fields (inward) at 50 m from the field edge. Edge Transects were surveyed twice in a planting season, for pre-ploughing, post- ploughing and seed sowing surveys, and over three seasons.

3.3.3 Reference Transect (RT)

RTs were sampled depending on the positioning of the maize fields within the landscape. The RTs were placed in the field boundary of the M, directly adjacent to the field. Not all maize fields had large enough field boundaries to be sampled, but where possible, two transects at opposite ends of the field were surveyed (Figure 3.5). RTs were only surveyed once, pre-ploughing survey in the first season, as they were not ploughed or planted and were considered a reference base of what the field would ideally look like in terms of weed species composition in the absence of exogenous disturbance. It should be noted that in some maize fields, herbicide drift and/or grazing was observed taking place in the field boundary, suggesting that the reference transects were affected by endogenous disturbances. The survey in the RTs was only conducted during one season no follow up surveys.

3.3.4 Placement of transects

Transects were placed per field according to three criteria which considered: i) field length and width, ii) field margin vegetation to accommodate RTs, and iii) space to construct three transects where possible. Compass direction did not dictate the placing of transects, as field length was most important. Transect placement was handled as follows:

- RTs were placed in the field M at each end of a field, inside the field boundary. The sampling in each transect was carried out in 3x3 m quadrats, at an interval of 10 m along the transect line parallel to the field ranging between 80 and 220 m. Not all fields had a suitable boundary to act as reference sites.
- Up to three transects (one CT and two ETs) were placed inside the field parallel to one another and the longer side of the field. The reason for three transects was to capture enough variability in plant species composition. Quadrats were sampled every 10 m along the transect lines.
- The length of the CTs differed between fields as it was dependent on the length of the field. For a field of 100 to 120 m in length, the CT would typically be 50 or 60 m long to allow for the 50 m spacing from the field edge.
- The ETs were positioned 50 m into the field from each edge, also comprising 3x3 m quadrats at an interval of 10 m along the transect line of the field ranging between 80 and 220 m in length.

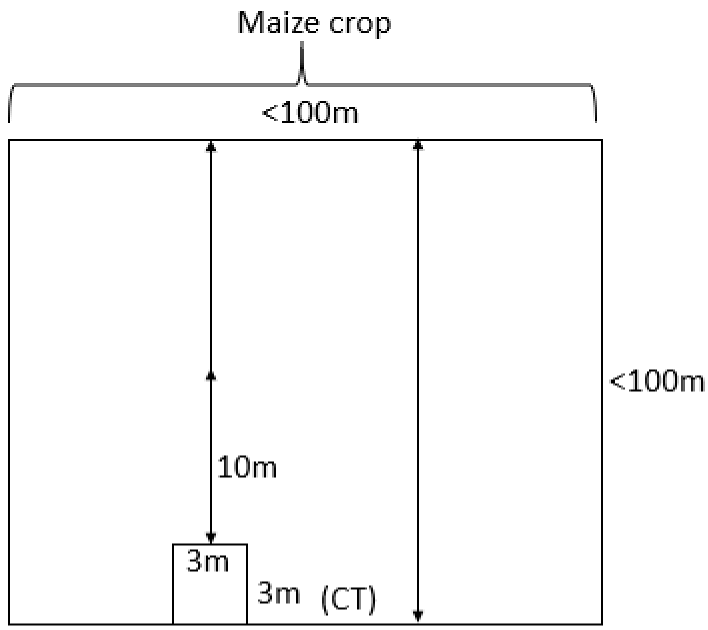

Figure 3.4 Criterion one for weeds sampling. When both sides (length and width) of a maize field were less than 100 m long, then only a Centre Transect could be sampled. Edge Transect and Reference Transect are absent. At 10 m intervals, 3x3 m quadrats/plots were sampled.

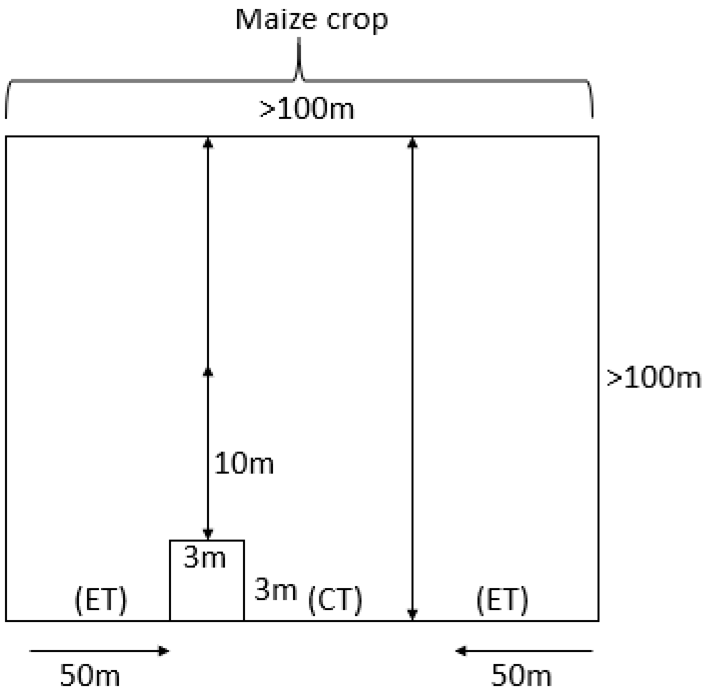

Figure 3.5 Criterion two for weeds sampling. When at least one side (length and width) of a maize field was more than 100 m long, then a Centre Transect and two Edge Transects were sampled. Reference Transects are absent. At 10 m intervals, 3x3 m plots were sampled.

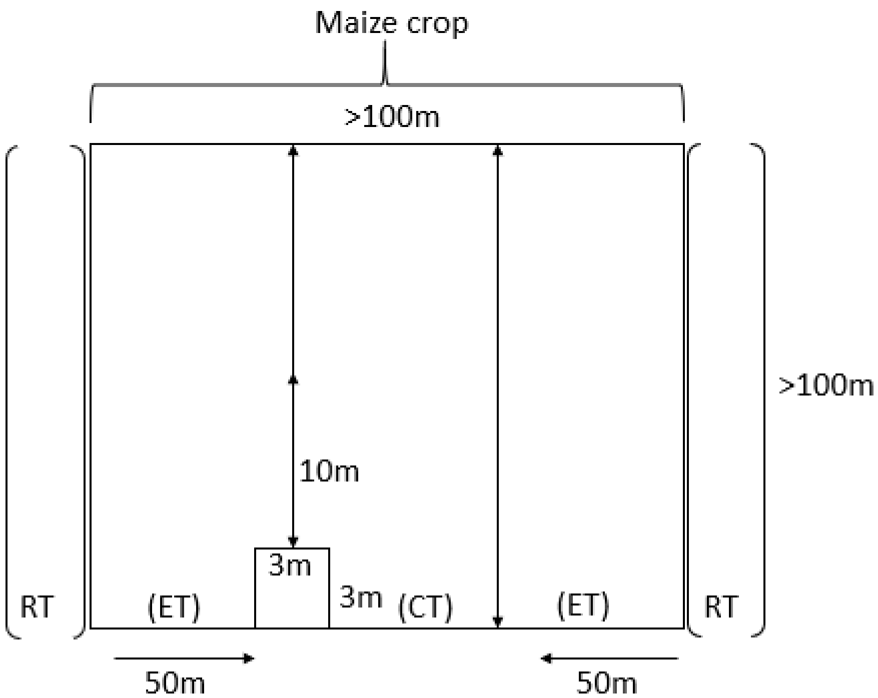

Figure 3.6 Criterion three for weeds sampling. When at least one side (length and width) of a maize field was more than 100 m long, and a field margin was present, then a Centre Transect, two Edge Transect and two Reference Transect were sampled. At 10 m intervals, 3x3 m plots were sampled.

3.3.5 Sampling of transects

Maize fields planted with HT maize were sampled. The maize cultivar used in season one was different to that of seasons two and three. Season one (2017/18) used NK603XMON810 maize, but seasons two and three (2018/19 and 2019/20) used MON89034. The change in maize cultivars had no effect on this study because both maize traits (NK603XMON810 and MON89034) confer insect resistance and herbicide tolerance. This was important as it allowed the study to still focus on the main objectives regarding herbicide use for weed control in HT maize fields.

The target weed species were those found inside the maize fields and associated uncultivated field boundary (reference transects). The RT represent endogenous disturbance only related to agricultural practices such as herbicide drift and grazing.

Weed species presence and abundance per quadrat, per transect, per field were recorded to species level (Germishuizen *et al.,* 2006; Fish *et al*., 2015). In each field, every 3x3 m quadrant was measured out with a measuring tape, and the four corners were marked with metal droppers (1.5 m in height). The weed species and total number of individuals were recorded using a pre-designed data sheet. When species could not be identified, they were collected, pressed, and taken to either Compton Herbarium, Kirstenbosch Research Centre or the AP Goossens Herbarium, North-West University for identification.

3.3.6 Selection of the weed traits

Weed traits with a known response to factors associated with agricultural disturbance were selected, Table 3.3 (Cornelissen *et al*., 2003; Aronson *et al*., 2007). Functional traits information that demonstrate functional response to the agroecosystem management were gathered from a variety of sources in the literature including Brooks *et al*. (2012); Fitter and Peat, (1994); Germishuizen *et al*. (2006); Hawes *et al*. (2009); Pakeman (2011); South African National Biodiversity Institute ("PlantZAfrica", 2020).

Table 3.3 Selected weed traits in herbicides tolerant maize fields for Baziya and Tsolo.

Weed traits	**Description of weed trait categories and code score**	**Weed trait units**	**Literature references**
Photosynthetic pathway	1=Photosynthetic pathway CAM 2=Photosynthetic pathway C4 3=Photosynthetic pathway C3	Categorical	Crayne *et al*. (1999); Sage, (2001)
Carbon storage in reserve organ	0=No specialised carbon storage organs 1=Specialised carbon storage organs	Binary	Janeček *et al*. (2011); Poorter & Bergkotte, (1992)
Life form	1= Chamaephyte life form 2= Therophyte life form 3= Hemicryptophyte life form 4= Phanerophyte life form 5=Geophyte life form	Categorical	Germishuizen & Meyer (2003); Cornelissen *et al*. (2003)
Life span	1=Annual life span 2=Biennial life span 3=Perennial life span	Categorical	Cornelissen *et al*. (2003); Lavorel *et al*. (1997)
Flowering season	0=Flowering season (Unspecified) 1= Flowering season (All year) 2= Flowering season (Spring) 3= Flowering season (Summer) 4= Flowering season (Autumn) 5= Flowering season (Winter)	Categorical	Liu *et al*. (2014)
Seed size	1=Seed size length <2mm 2=Seed size length 2-<4mm 3=Seed size length 4-10mm 4=Seed size length >10mm	Categorical	Latzel *et al*. (2010)
Seed dispersal mode	0=Seed dispersal mode (No obvious agent) 1=Seed dispersal mode (Wind) 2=Seed dispersal mode (Animals with low mobility) 3=Seed dispersal mode (Animals with high mobility)	Categorical	Liu *et al*. (2014); Latzel *et al*. (2010)
Number of cotyledons	1= Monocotyledonous 2= Dicotyledonous	Binary	Chandler, (2008); Conner and Agrawal, (2005)
Nitrogen fixing ability	0=Absent nitrogen fixing ability 1=Present nitrogen fixing ability	Binary	Botha *et al*., (2017); Naisbitt *et al*. (1992); Cornelissen *et al*. (2003); Yelenik *et al*. (2006)
Spinescence	0= Absent spinescence 1= Present spinescence	Binary	Botha *et al*. (2017)
Growth form	1=Rosette growth form 2=Prostrate growth form 3=Erect growth form 4=Tussock growth form 5=Creeper growth form 6=Tufted growth form	Categorical	Cornelissen *et al*. (2003); Martínková *et al*. (2020)

Table 3.4 Details of the sampling frequencies across Baziya Makaula, Baziya Mission, Baziya Jojweni and Tsolo for three seasons.

Site Name	Maize Field name	Total number of transects	Complete data for season one (2017/18)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Complete data for season two (2018/19)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Complete data for season three (2019/20)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Reference transects
Baziya Makaula	MV001	3	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
	MV002	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	MV003	3	Yes	Yes	Yes	No	Yes	No	No	No	No	Yes
	MV004	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	MV005	3	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
	MV006	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	MV007	3	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
	MV008	3	Yes	Yes	Yes	No	Yes	No	No	No	No	Yes
	MV009	3	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	MV010	3	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Baziya Mission	BM001	3	No	Yes	No	No	No	No	No	No	No	No
	BM002	3	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes
	BM003	3	Yes	Yes	Yes	No	Yes	No	No	No	No	Yes
	BM004	3	Yes	Yes	Yes	No	No	No	No	No	No	Yes
	BM005	3	Yes	Yes	Yes	No	No	No	No	No	No	Yes
	BM006	3	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Site Name	Maize Field name	Total number of transects	Complete data for season one (2017/18)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Complete data for season two (2018/19)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Complete data for season three (2019/20)	Pre-ploughing survey	Post-ploughing and seed sowing survey	Reference transects
Baziya Jojweni	MJN001	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	MJN002	3	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes
	MJN003	1	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No
	MJN004	1	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No
	MJN005	1	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Tsolo	TA	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	TB	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	TC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	TD	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	TE	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	TF	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	TG	1	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Yes= represent that sampling was conducted for that season; No= represent the opposite; Pre= represents pre-ploughing survey and Post= represents post-ploughing and seed sowing survey

3.4 Herbicide classification and effectiveness

Herbicide identification across the three seasons, for both pre- and post-emergence herbicide application (protocols) was done with the assistance of designated GrainSA extension officers on site. This was done to establish a reference for all herbicides used for weed control in the Baziya and Tsolo HT maize cropping systems. The product labels were also used for cross referencing and verification. Application protocols for each maize field were requested for filing and seasonal protocols were documented across all the fields across the three seasons. Herbicide water mixing ratio per protocol was 200 litres for both pre- and post-emergence herbicide spraying. The mixed herbicides were applied per one hectare of the maize field. Where the protocol changed in terms of herbicide application, changes had to be applied to all the fields and the entire sampling site, otherwise the different fields on the same site where not going to be comparable over time. The respective herbicide labels were also used to determine what weeds are targeted by each of the herbicide(s) or for control (e.g. selective versus non-selective herbicdes) according to manufacture's recommendation. Documented weeds were cross referenced with globally available list for invasiveness and potential for resistance development (Health, 2020; Heap, 2014).

3.5 Data analysis

A single data matrix was compiled in Excel (Windows 2013) and updated seasonally for all fields. The data matrix consisted of weed species and individual counts for each transect, for pre-ploughing, post-ploughing and seed sowing surveys, and over three seasons. Data was formatted to be imported into PRIMER 6 (Clarke and Gorley, 2006) to check whether enough weed species data had been sampled for analysis. Sample based rarefaction curves showing the observed number of weed species using PRIMER 6 were constructed as estimates of total species richness, based on all recorded weed species (Sobs), Chao's estimator based on number of rare species (Chao1), Chao's estimator using presence-absence data (Chao2) and a Bootstrap estimator based on proportion of quadrats containing each species. Occurrence data was imported into PRIMER 6 to analyse species richness, abundance, Shannon diversity and Pielou's Evenness. To test for differences across maize fields, One-way analysis of variance (ANOVA) was applied in Statistica version 13.3 packages. Indicator species analysis (IndVal) was done in RStudio to determine indicator species for each field (Dufrêne and Legendre, 1997).

3.5.1 Weed species composition

The data matrix consisting of weed species and individual counts for each site was used to explore the composition. To determine the compositional similarity of weed species between HT maize fields and field margins, Non-metric multidimensional scaling (NMDS) analysis was applied in PRIMER 6. NMDS is an unrestricted ordination technique for the evaluation of the relationships among sampling sites (Bliss *et al*., 2017). This analysis was performed using Bray-Curtis similarity and square root transformation to balance the contribution of highly abundant and rare species (Kent, 2012). Bray-Curtis similarity distance measures are recommended for ordinations. Its ordinations are based on the species space measures and plots similar sites closer together and dissimilar sites further apart (Gaines and Gratton, 2010) with the results presented as two-dimensional plots. Stress values can be interpreted as follows: i) a stress value of <0.05 gives an excellent representation with no misinterpretation; ii) stress value of <0.1 corresponds to a good ordination; iii) stress value of

<0.2 gives a potentially good and useful two-dimensional picture; iv) >0.3 can be considered poor and difficult to interpret (Clarke & Warwick, 2001).

PERMANOVA (permutations = 999; type III sums of squares) was selected in PRIMER 6 to test for significant difference in weed species composition between the two treatments (pre-ploughing, post-ploughing and seed sowing survey) and individual sites (MV, MJN, BM and T) over the three seasons. PERMANOVA is suitable for abundance data, since response data within complex experimental designs are analysed based on permutations of dissimilarity matrices to make analysis independent of distribution (Hunter, 2017). Furthermore, PERMANOVA does not require specific assumptions for normality (Linstadter *et al*., 2016). To distinguish between significant different treatments, pairwise comparison of PERMANOVA analysis was performed.

To determine as to which weed species were responsible for the changes in composition and to calculate the contribution of each species percentage to the similarity between sites, a Similarity Percentage Analysis (SIMPER) was carried out in PRIMER 6. SIMPER determines and compares the contribution of each weed species to site (s) similarity (Hunter, 2017). The same data matrix used for NMDS was used for SIMPER to determine the species percentage contribution. SIMPER was applied to the data in Paleontological Statistics software (PAST) (Hammer *et al*., 2001).

3.5.2 Quantifying weed species diversity

The data set containing abundance of weeds with corresponding HT maize fields were used to calculate diversity indices per site over three seasons in PRIMER 6. This was done to determine weed species diversity of the HT maize fields and associated field margins. Univariate diversity indices were calculated for Total species (S), Total individuals (N), Margalef's species richness (d), Pielou's evenness (J'), Shannon diversity (H') and Simpson's diversity (1-Lambda) (Clarke & Gorley, 2006). Simpson's index of diversity is considered a robust and meaningful diversity index. It is also known as dominance or evenness measure, since it is weighted towards the most abundant species in the sample and less sensitive to species richness (Magurran, 2004; Pielou, 1975). Equations are indicated in Table 3.5. To provide a full overview of weed species richness and diversity patterns (Magurran, 2004), these matrices were used in combination as they display different aspects of diversity. Species richness is the number of individual species present in a defined area (Begon *et al*., 2005).

Table 3.5 Equations used to calculate indices of weed species diversity.

Indices	Equation	Description
Species richness (S)	_	Number of species per field.
Abundance (N)	_	The number of individuals per field.
Margalef's species richness (d)	$(d)=\frac{(s-1)}{\ln N}$	S is the number of species and N is the number of individuals in the sample.

Indices	Equation	Description
Simpson's diversity index (Ď)	$(\check{D}) = \frac{\Sigma ni(ni-1)}{N(N-1)} - 1$	N is the total number of individuals for the species, ni is the number of individuals for the i'th species.
Shannon-Wiener diversity index (H')	(H') = −Σpi.lnpi	pi is the relative abundance of the i'th species.
Pielou's evenness index (J')	(J')= $H'/H'max = \frac{H'}{\ln S}$	H' is the Shannon-Wiener diversity index. S is the species richness.

3.5.3 Quantifying weed functional traits

The indicator value index (IndVal) proposed by Dufrene and Legendre (1997) was calculated in RStudio using the IndVal function under the labdsv package to identify the existence of the indicator weed species able to persist under agricultural effects of herbicide application in HT maize fields. The significant levels were set at $p<0.05$. NMDS of functional traits was done in PRIMER 6 to determine traits composition using weed species traits. The trait-species matrix of forty-two weed species and eleven traits were multiplied with the species-abundance matrix using the MMult function in Microsoft Excel (2016) to produce a matrix from which trait diversity index calculation (richness) was performed in PRIMER 6 (Hanke *et al*, 2014). The data for trait richness was transformed and tested for normality in Statistica version 13.3, and the untransformed data was used to draw graphs for representation of normal distribution.

3.5.3.1 Hierarchical clustering of weed functional traits

Hierarchical clustering analysis with an Unweighted Pair Group Method with Arithmetic Mean (UPGMA)-clustering algorithm and Gower distance measure appropriate for mixed (categorical and binary) data types was created in PRIMER 6. This was done to detect groupings of weed species based on their trait scores (Botha *et al*., 2017). The Gower distance measure was used because it is appropriate for mixed data types (Gower, 1971; Botha *et al*., 2017). Similarity Profile (SIMPROF) test was also applied as an objective method for the identification of significant groupings compared to subjective cut-off levels. Each life form (annual versus perennial) was analysed separately to identify, describe, and discuss the plant functional types (PFTs) across HT maize fields. PFT clusters were identified according to the grouping of weed species based on their trait scores, which revealed PFTs at different hierarchical levels, following the approach of Linstädter *et al*. (2016). Since life form remains the strongest indicator of agricultural disturbance (Pérez-Harguindeguy *et al*, 2013), effects of underlying important disturbance traits may be weakened when all PFTs are analysed collectively (Lavorel *et al,* 1997).

Using the MMult function in Microsoft Excel, a functional type-plot matrix was compiled by multiplying a functional type-species matrix (with presence and absence data) with the species-abundance matrix. Furthermore, a treatment-plot matrix was compiled in Microsoft Excel, in which data for pre-ploughing, post-ploughing and seed sowing survey were given binary scores which were then used as environmental data (Botha *et al*., 2017). Patterns in functional assemblages of pre-ploughing, post-ploughing and seed sowing survey were analysed with Canoco for Windows version 5 for Redundancy Analysis (RDA). RDA was done for the multivariate analysis of a set of response variables (PFTs) to the complete species-trait matrix.

RDA was also done to test for clustering based on specific traits in ordinal space to quantify the effect of pre-ploughing, post-ploughing and seed sowing survey on functional type assemblage. This was done to evaluate patterns in variation of PFTs assemblage and demonstrate redundancy relationships between PFT and pre-ploughing, post-ploughing and seed sowing survey (Kent & Coker, 1994).

3.5.4 Weed species and functional traits analysis of variance

Data for weed species and functional types (for traits analysis) was imported into PRIMER 6 for NMDS analysis to check the composition to determine if the use of different herbicide protocols over three seasons leads to weed shift. Data for weed species and functional types diversity indices (abundance and richness) from PRIMER 6 were imported as excel sheet into Statistica and tested for normality. Where the data was not normally distributed, it was log transformed. The normality was represented in a form of Histogram and described as p values to show the significant difference between the seasons of the HT maize fields pre-ploughing, post-ploughing and seed sowing survey. Kolmogorov-Smirnov test was done to quantify a distance and report the maximum differences between the cumulative distribution function of the sample sizes. The Shapiro-Wilk test was also selected because it is considered a highly effective test, for all sample sizes and distribution types to test for normality in the sample size (Razali and Wah, 2011).

One-way ANOVA was performed in Statistica, using weed species and functional types abundance and richness diversity index data to determine the effect of the use of different herbicide protocol application for weed control in HT maize fields for pre-ploughing, post-ploughing and seed sowing survey over three seasons. Where significant differences were obtained, Post-hoc tests were done with Tukey honest significant difference (HSD) to determine as to what season(s) the difference(s) had occurred. And to test the difference(s) of the effect of herbicide specifically between pre-ploughing, post-ploughing and seed sowing survey, T-test was done in Statistica using abundance and richness diversity of weed species and functional types dataset.

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CHAPTER 4: Weed species composition and diversity of maize fields and margins

Abstract

Weeds are undesirable plant species that grow where they are not wanted as they often outcompete crops and displace natural biodiversity. Over the years, several methods (chemical and mechanical) have been developed to either control, suppress or eliminate weeds completely. The adoption and applicability of these methods depend on farmers' preferences and resource availability for weed control. This study presents a comparative survey of species composition and diversity of weed communities in fields planted with herbicide tolerant (HT) maize, and associated field margins, in the Oliver Tambo District Municipality of the Eastern Cape. It is important to profile weed communities (problematic or otherwise) of maize as they pose a threat to the production of the most important staple grain of South Africa. Weed surveys were conducted at four sites, namely Baziya Makaula, Baziya Mission, Baziya Jojweni and Tsolo. Fields at these sites received the same herbicide protocol per season. The sampling in each field was carried out in 3x3m quadrants at intervals of 10 m along a transect line. A total of 88 weed species, representing 31 families, were recorded from 244 plots for both maize fields (MF) and field margins (M) combined. Maize fields and margins, together, were comprised of 48 indigenous and 40 alien species. Higher numbers of indigenous (35) and alien (39) weed species were found in maize fields than in field margins. However, an equal number of families (25) were represented in both. The three families that contributed the most weed species were Asteraceae, Fabaceae and Poaceae. There were three weed species which contributed individual dissimilarity of >5% between maize fields and field margins, and all being dominant in the latter. The fields also shared 53% of the weed species confirming the hypothesis that maize fields share similar weed species. Non-metric Multidimensional Scaling grouped maize fields and field margins separately based on weed species composition ($p<0.001$), but with a high stress value of 0.28. Maize fields and margins differed significantly in terms of Simpson ($p=0.044$) and Shannon diversity ($p=0.003$), being higher in the fields. This was also confirmed in terms of composition and diversity where some fields did not generally differ significantly. However, when maize fields and field margins were both specifically compared, difference was expected because maize fields are exposed to annual exogenous agricultural disturbance that supports high weed diversity, whereas field margins had less severe endogenous disturbance and was dominated by fewer, but persistent species of high abundance. This study confirms that maize fields do share similar composition and diversity of weed assemblages, but different from field margins.

Keywords: Agroecosystem, Biodiversity, *Cynodon dactylon*, Disturbance, Herbicide, *Richardia brasiliensis*

4.1 Introduction

Weeds are plant species that grow in areas where they are not wanted. Such areas may include crop fields and roadsides. Weeds compete with most crops for resources and reduce harvest yields (Marwat and Hashim, 2002). Weeds compete for water, sunlight and nutrition needed for crop growth and productivity. Consequently, a need for weed control and eradication has existed from before, and persist even after, technological advancements (Marwat and Hashim, 2002). Weed invasions within landscapes have received considerable attention globally (Vilà and Ibáñez, 2011) and brought about different views on their potential impacts on various productive systems (Hadi *et al.*, 2014). However, not all weeds are detrimental to the environment where they occur (Ramesh *et al*., 2017). For example, some weeds can be important for various ecosystem services in agricultural lands, such as providing habitat and supporting prey species for higher trophic levels (Zhang *et al*., 2007; Marshall, 2001). They are primary producers and therefore important to the food provision of most animal species (Marshall *et al*., 2003).

Different crop fields vary in the diversity of weed species composition, which is dependent on local historical agronomic practices like soil tillage, agro-chemical use, and the prevailing climate, soil type and topography (Marshall *et al*., 2003; Sims *et al*., 2018). Persistent seedbanks facilitate weed diversity and reflects historical management practices of a particular field (Hadi *et al.*, 2014). Studies by Hadi *et al*. (2014) and Khan *et al*. (2017), showed that weed species commonly found inside maize crop fields, in general, belong to plant families such as Apiaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Oxalidaceae, Plantaginaceae, Poaceae, Polygonaceae and Solanaceae.

Unlike crop fields themselves, there are other factors that influence weed species composition and diversity in the maize fields adjacent to the margins. Agricultural dynamics and changes in the structure of habitat patches are known to affect species composition and distribution in the landscape (Baessler and Klotz, 2006). In addition, field margins are also likely to be dominated by alien species due to prevailing disturbance, such as grazing, with animals able to transfer seeds of weedy plants from one area to another (Khan *et al*., 2017). Subsequently, maize field margins are considered more diverse in weed species than maize fields (Botha *et al*., 2017).

Given that the study aim is to profile weeds associated with HT maize in a single region, the hypothesis proposes that maize systems will have similar weed composition and diversity across fields but will differ between maize fields and field margins. This assumption is based on existing literature, which indicates that weed assemblages in the maize fields will be those assemblages of species pre-adapted to tolerate field-specific herbicide use (Boutin and Jobin, 1998). In addition, the difference between maize fields and margins would be ascribed to differences in management practices, as the field margins are less disturbed compared to fields that experience exogenous disturbance such as soil tillage (Tokarska-Guzik *et al*., 2014).

The assessment and profiling of weeds has always been an important task to guide management of agricultural activities to protect crop productivity and prevent invasion of the surrounding environment (MacLaren *et al*., 2020). This study will present the first record of weeds associated with HT maize fields and their margins in the selected areas of Oliver

Tambo District Municipality. This is a significant milestone, given that very little knowledge and baseline data exist for the region. Furthermore, this information will benefit maize farmers and future researchers working on weeds in Sub-Saharan Africa, when it comes to the development, implementation and prioritisation of various herbicide-based weed management protocols and measures towards ensuring maximum crop productivity.

4.2 Materials and methods

Four sites in the selected areas of Oliver Tambo District Municipality of the Eastern Cape Province were surveyed for this study. Sample plots constituted 208 for maize fields and 36 for field margins. Detailed information and description of the study area and site selection is provided in Chapter 3, sections 3.1 and 3.2. The sampling design is also described in Chapter 3, section 3.3. Weed species diversity and abundance per quadrat, per transect, per maize fields and field margins (different quadrats for respective transects) were recorded for both (i) pre-ploughing and (ii) post-ploughing and seed sowing surveys (Raza *et al*., 2019). Sample based rarefaction curves showing the observed number of weed species using PRIMER 6 (Clarke and Gorley, 2006) were constructed as estimates of total species richness, based on all weed species discovered (Sobs), Chao's estimator based on number of rare species (chao 1), Chao's estimator using presence-absence data (chao2) and a Bootstrap estimator based on proportion of transects containing each species (refer to Chapter 3, section 3.6). Non-metric multidimensional scaling (NMDS) for weed species composition was performed in PRIMER 6 to determine the compositional similarity (refer to Chapter 3, section 3.6 for a detailed description). Significant difference in weed species diversity among the sites was tested in Statistica software version 13.3. To determine the species percentage contribution for average dissimilarity and mean abundance, SIMPER was performed in PAST (Hammer *et al*., 2001). Categorized box and whiskers graphs were drawn in Statistica.

4.3 Results

4.3.1 Rarefaction and richness estimator for the herbicide tolerant maize fields and margins

Sampling based rarefaction curve estimates of maize fields and field margins indicated sufficient sampling effort for maize fields sites and insufficient sampling for field margins (Figure 4.1 (a) and (b)). Less sufficient sampling for field margins occurred because some fields were fenced and very close to one another in a way that it was impossible to sample margins in these areas. Field margins surveys were defined as reference transects in the methods chapter (see Chapter 3, section 3.3).

Out of 208 maize field plots sampled, 74 weed species were recorded (Table 4.1) and the richness estimators suggested richness in the community to be 80 for all four estimators (Sobs, Chao1, Chao2 and Bootstrap) (Figure 4.1 (a)). However, in the 36 sampled plots for M, 61 weed species were recorded (Table 4.2) and the richness estimator suggested the richness in the community to be 60+ and increasing for the three estimators (Sobs, Chao1, and Bootstrap), except for Chao2 which was 80+ (Figure 4.1 (b)).

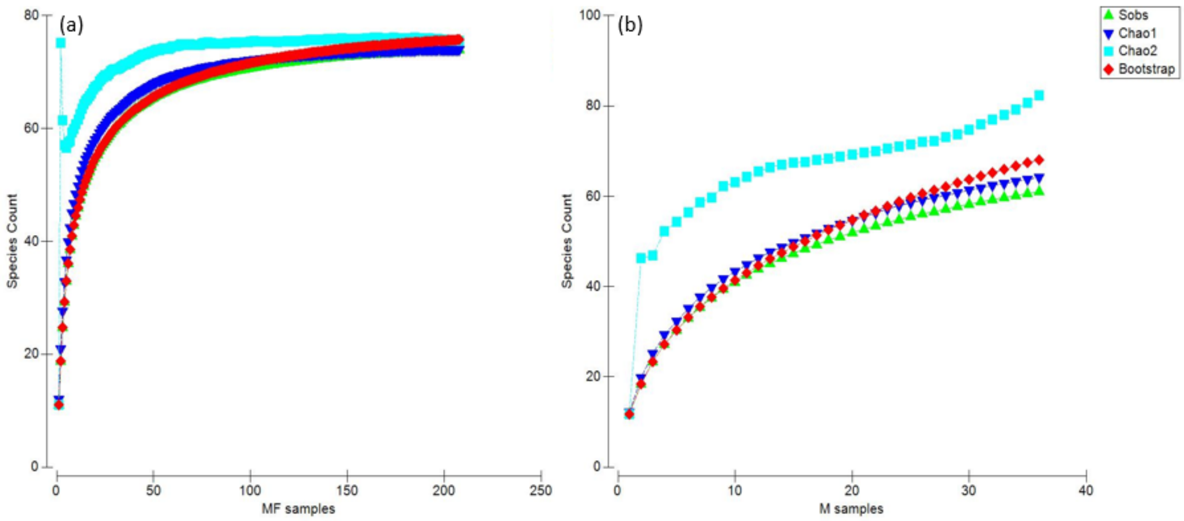

Figure 4.1 Sample based rarefaction curves for HT maize fields (MF) surveys (a) and margins (M) (b), with species richness estimates based on all weed species discovered (Sobs), Chao's estimator based on number of rare species (Chao1), Chao's estimator using just presence-absence data (chao2) and a Bootstrap estimator based on proportion of transects containing each species.

4.3.2 Taxa in maize fields and field margins

More forbs than grasses were observed in the agroecosystem, and a higher number of indigenous species than alien species (Table 4.1). The dominant families in both maize fields and field margins were Poaceae (30), followed by Asteraceae (28), Fabaceae (14) and then Cyperaceae (9) (Table 4.2).

Table 4.1 List of species recorded for maize fields and margins in Baziya and Tsolo.

Family	Scientific Name	Common Name	Growth form	Indigenous/Alien	Maize fields (MF)	Field margins (M)
Amaranthaceae	*Amaranthus viridis* L.	Spiny pigweed	Forb	Alien	Present	Present
Apiaceae	*Daucus carota* L.	Wild carrot	Forb	Alien	Present	Present
Asparagceae	*Asparagus species* L.	Asparagus	Forb	Indigenous	Absent	Present
Asteraceae	*Arctotheca calendula* K. Lewin	Capeweed	Forb	Indigenous	Present	Present
Asteraceae	*Berkheya onopordifolia* (DC.) O.Hoffm. Ex	Purple berkheya	Forb	Indigenous	Present	Absent
Asteraceae	*Bidens bipinnata* L.	Spanish needles	Forb	Alien	Present	Present
Asteraceae	*Bidens pilosa* L.	Jersey cudweed	Forb	Alien	Present	Present
Asteraceae	*Cirsium vulgare* (Savi) Ten.	Spear thistle	Forb	Alien	Present	Absent
Asteraceae	*Conyza bonariensis* (L.) Cronquist	Argentine fleabane	Forb	Alien	Present	Present
Asteraceae	*Cotula heterocarpa* DC.	Water buttons	Forb	Indigenous	Present	Present
Asteraceae	*Galinsoga parviflora* Cav.	Galant soldier	Forb	Alien	Present	Absent
Asteraceae	*Helichrysum asperum* (Thunb.) Hilliard & B.L.Burtt	Hairy everlasting	Forb	Indigenous	Present	Present
Asteraceae	*Helichrysum griseolanatum* Hilliard	Fluffy white	Forb	Indigenous	Present	Present
Asteraceae	*Oncosiphon piluliferum* (L.f.) Källersjö	Pineapple weed	Forb	Indigenous	Present	Absent
Asteraceae	*Pseudognaphalium luteoalbum* (L.) Hilliard & B.L.Burtt	Jersey cudweed	Forb	Indigenous	Present	Present
Asteraceae	*Sonchus asper* (L.) Hill	Prickly sow-thistle	Forb	Alien	Present	Present
Asteraceae	*Sonchus nanus* Sond. ex Harv.	Sowthistle	Forb	Alien	Present	Present
Asteraceae	*Tagetes minuta* L.	Khaki weed	Forb	Alien	Present	Present
Asteraceae	*Xanthium spinosum* L.	Spiny cocklebur	Forb	Alien	Present	Present
Brassicaceae	*Rorippa nudiuscula* Thell.	Yellowcresses	Forb	Alien	Present	Present
Campanulaceae	*Lobelia erinus* L.	Garden lobelia	Forb	Indigenous	Present	Present
Campanulaceae	*Wahlenbergia stellarioides* Cham.	Tufted bluebell	Forb	Indigenous	Present	Present
Chenopodiaceae	*Chenopodium carinatum* R.Br.	Keeled goosefoot	Forb	Alien	Present	Present
Cleomaceae	*Cleome monophylla* L.	Rocky mountain beeplant	Forb	Indigenous	Present	Absent
Commelinaceae	Commelina benghalensis	Benghal dayflower	Forb	Alien	Present	Present
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	Common morning glory	Forb	Alien	Present	Present
Convolvulaceae	*Ipomoea spp.* L.	Bindweed	Forb	Alien	Present	Absent
Cucurbitaceae	*Cucumis myriocarpus* Naudin	Gooseberry cucumber	Forb	Indigenous	Present	Present
Cunoniaceae	*Callicoma serratifolia* Andrews	Black wattle	Forb	Alien	Present	Present
Cyperaceae	*Bulbostylis humilis* (Kunth) C.B.Clarke	Bushy heads grass	Sedge	Indigenous	Present	Absent
Cyperaceae	*Cyperus congestus* Vahl	Nut grass	Sedge	Indigenous	Present	Absent

Cyperaceae	*Cyperus esculentus* L.	Tigernut	Sedge	Indigenous	Present	Absent
Cyperaceae	*Cyperus* spp. L.	Sedges	Sedge	Indigenous	Present	Present
Euphorbiaceae	*Chamaesyce prostrata* (Aiton) Small	Green creeping spurge	Forb	Alien	Present	Present
Euphorbiaceae	*Ricinus communis* L.	Castor bean	Forb	Alien	Present	Absent
Fabaceae	*Alysicarpus rugosus* (Willd.) DC.	Red moneywort	Forb	Indigenous	Present	Present
Fabaceae	*Chamaecrista biensis* (Steyaert) Mendonca & Torre	Locust weed	Forb	Indigenous	Present	Present
Fabaceae	*Indigofera spicata* Forsk.	True indigo	Forb	Indigenous	Present	Absent
Fabaceae	*Lespedeza pilosa* (Thunb.) Siebold & Zucc.	Carlifonia bush clover	Forb	Alien	Present	Absent
Fabaceae	*Medicago polymorpha* L.	California burclover	Forb	Indigenous	Present	Present
Fabaceae	*Tephrosia capensis* (Jacq.) Pers.	Tephrosia fern	Forb	Alien	Present	Present
Fabaceae	*Trifolium repens* L.	White clover	Forb	Alien	Present	Present
Fabaceae	*Zornia capensis* Pers.	Caterpillar bean	Forb	Indigenous	Present	Present
Gisekiaceae	*Gisekia pharnacioides* L.	Sand herbage	Forb	Alien	Present	Present
Hypoxidaceae	*Hypoxis villosa* L.f.	Golden winter star	Forb	Indigenous	Present	Present
Iridaceae	*Aristea abyssinica* Pax	Blue-eyed grass	Forb	Indigenous	Present	Absent
Malvaceae	*Hibiscus nigricaulis* Baker f.	Wild stockrose	Forb	Indigenous	Present	Absent
Malvaceae	*Hibiscus trionum* L.	Flower-of-an-hour	Forb	Indigenous	Present	Present
Onagraceae	*Oenothera parodiana* Munz	Evening primrose	Forb	Alien	Present	Present
Papaveraceae	*Argemone mexicana* L.	Mexican prinkly poppy	Forb	Alien	Present	Absent
Papaveraceae	*Papaver aculeatum* Thunb.	Orange poppy	Forb	Indigenous	Present	Absent
Plantaginaceae	*Diclis reptans* Benth.	Toadflax	Forb	Indigenous	Present	Present
Plantaginaceae	*Plantago virginica* L.	Dwarf plantain	Forb	Alien	Present	Present
Poaceae	*Andropogon eucomus* Nees	Snowflake grass	Grass	Indigenous	Absent	Present
Poaceae	*Arundinella nepalensis* var. contracta Ohwi	Tambuki grass	Grass	Indigenous	Absent	Present
Poaceae	*Brachiaria deflexa* (Schumach.) Robyns	Congo signal grass	Grass	Indigenous	Absent	Present
Poaceae	*Brachiaria eruciformis* var. divaricata Basappa & Muniy.	Velvet bushwillow	Grass	Indigenous	Absent	Present
Poaceae	*Bromus diandrus* Roth	Great brome	Grass	Alien	Present	Absent
Poaceae	*Cynodon dactylon* (L.) Pers.	Couch grass	Grass	Alien	Present	Present
Poaceae	*Digitaria argyrograpta* (Nees) Stapf	Silver finger grass	Grass	Indigenous	Absent	Present
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Cockspur grass	Grass	Alien	Present	Absent
Poaceae	*Eleusine coracana* (L.) Gaertn.	Goose grass	Grass	Indigenous	Present	Present
Poaceae	*Elionurus muticus* (Spreng.) Kuntze	Wire lemon grass	Grass	Indigenous	Absent	Present
Poaceae	*Enneapogon scaber* Lehm.	Enneapogon	Grass	Alien	Present	Present
Poaceae	*Eragrostis curvula* (Schrad.) Nees	Love grass	Grass	Indigenous	Present	Absent
Poaceae	*Eragrostis plana* Nees	Eragrostis	Grass	Indigenous	Present	Present
Poaceae	*Lolium perenne* L.	Perennial ryegrass	Grass	Alien	Absent	Present
Poaceae	*Melinis repens* (Willd.) Zizka	Rose Natal grass	Grass	Indigenous	Absent	Present
Poaceae	Panicum ecklonii Nees	Bajiho	Grass	Indigenous	Absent	Present
Poaceae	*Panicum natalense* Hochst.	Natal buffalo grass	Grass	Indigenous	Absent	Present
Poaceae	*Panicum schinzii* Hack.	Land grass	Grass	Indigenous	Present	Absent
Poaceae	*Paspalum dilatatum* Poir.	Paspalum spp	Grass	Alien	Present	Absent
Poaceae	*Pennisetum thunbergii* Kunth	Fountain grass	Grass	Indigenous	Absent	Present
Poaceae	*Sporobolus africanus* (Poir.) Robyns & Tournay	Rush grass	Grass	Indigenous	Absent	Present
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth	Stipagrostis spp	Grass	Alien	Present	Absent
Poaceae	*Themeda triandra* Forssk.	Red oat grass	Grass	Indigenous	Present	Present
Poaceae	*Urochloa panicoides* P.Beauv.	Panic liverseed grass	Grass	Indigenous	Present	Present
Polygalaceae	*Polygala amatymbica* Eckl. & Zeyh.	Clump forming plant	Forb	Indigenous	Present	Absent
Portulacaceae	*Portulaca oleracea* L.	Pigweed	Forb	Alien	Present	Present
Rubiaceae	*Richardia brasiliensis* Gomes	Tropical Mexican clover	Forb	Alien	Present	Present
Scrophulariaceae	*Jamesbrittenia aurantiaca* (Burch.) Hilliard	Terracotta gazania	Forb	Indigenous	Absent	Absent
Solanaceae	*Datura stramonium* L.	Jimson weed	Forb	Alien	Present	Absent
Solanaceae	*Solanum retroflexum* Dunal	Earleaf nightshade	Forb	Alien	Present	Present
Solanaceae	*Solanum rigescens* Jacq.	Flannel weed	Forb	Alien	Present	Present
Urticaceae	*Urtica urens* L.	Dwarf nettle	Forb	Alien	Present	Absent
Verbenaceae	*Chascanum hederaceum* (Sond.) Moldenke	White trumpets	Forb	Indigenous	Present	Present
Verbenaceae	*Verbena bonariensis* L.	Tall verbena	Forb	Alien	Present	Present
Zygophyllaceae	*Tribulus terrestris* L.	Tack weed	Forb	Indigenous	Present	Absent

Maize fields had a higher number of total weed species and alien species than field margins (Table 4.2), with the caution that field margins were undersampled. Both maize fields and field margins had the same number of families presented, which was 25 each (Table 4.2). A Venn diagram (Figure 4.2) indicated that maize fields comprised 31% unique species compared to field margins (16%). Shared species between maize fields and field margins was 53% (Figure 4.2). Maize fields had a higher number of alien (39) than indigenous species (35) (Table 4.2).

Table 4.2 Total number of indigenous and alien species found per each family for maize fields and margins.

Family	Indigenous		Alien		Total	
	MF	M	MF	M	MF	M
Amaranthaceae	0	0	1	1	1	1
Aspiaceae	0	0	1	1	1	1
Asparagaceae	0	1	0	0	0	1
Asteraceae	7	5	9	7	16	12
Brassicaceae	0	0	1	1	1	1
Campanulaceae	2	2	0	0	2	2
Chenopodiaceae	0	0	1	1	1	1
Cleomaceae	1	0	0	0	1	0
Commelinaceae	0	0	1	1	1	1
Convolvulaceae	0	0	2	1	2	1

Table 4.2 Total number of indigenous and alien species found per each family for maize fields and margins continued.

Family	Indigenous		Alien		Total	
	MF	M	MF	M	MF	M
Cucurbitaceae	1	1	0	0	1	1
Cunoniaceae	0	0	1	1	1	1
Cyperaceae	4	1	4	0	8	1
Fabaceae	5	4	3	2	8	6
Euphorbiaceae	0	0	0	1	0	1
Gisekiaceae	0	0	1	1	1	1
Hypoxidaceae	1	1	0	0	1	1
Iridaceae	1	0	0	0	1	0
Malvaceae	2	1	0	0	2	1
Onagraceae	0	0	1	1	1	1
Papaveraceae	1	0	1	0	2	0
Plantaginaceae	1	1	1	1	2	2
Poaceae	6	15	6	3	12	18
Polygalaceae	1	0	0	0	1	0
Portulacaceae	0	0	1	1	1	1
Rubiaceae	0	0	1	1	1	1
Scrophulariaceae	0	1	0	0	0	1
Solanaceae	0	0	3	1	3	1
Urticaceae	0	0	1	0	1	0
Verbenaceae	1	1	1	0	2	1
Zygophyllaceae	1	0	0	0	1	0

MF= Maize fields; M= Field margins

Table 4.3 Total number of all weed families and species (indigenous and alien) in maize fields and field margins.

Totals	MF & M	MF	M
Families	31	25	25
All species	88	74	61
Indigenous species	48	35	34
Alien species	40	39	27

MF= Maize fields; M= Field margins

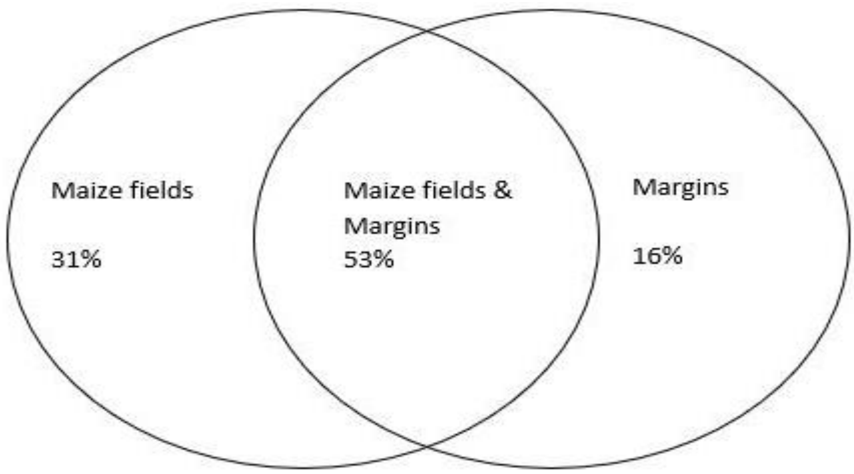

Figure 4.2 Venn diagram indicating the percentage of unique and shared species between the maize fields and field margins.

4.3.3 Weed species composition of maize fields and margins

Non-metric multidimensional scaling analysis showed separate groupings for maize fields and field margins (Figure 4.3). The grouping indicated that the field margins had (mostly) a different species composition than maize fields. There was certainly a medium to low level of similarity with a few field margin plots overlapping with maize fields. Stress value of ≤ 0.28 for the NMDS analysis was high, which indicated that the ordination was arbitrary (Clarke and Gorley, 2006). However, Permutational Multivariate Analysis of Variance (PERMANOVA) confirmed that the species composition differed significantly between maize fields and field margins (p<0.001).

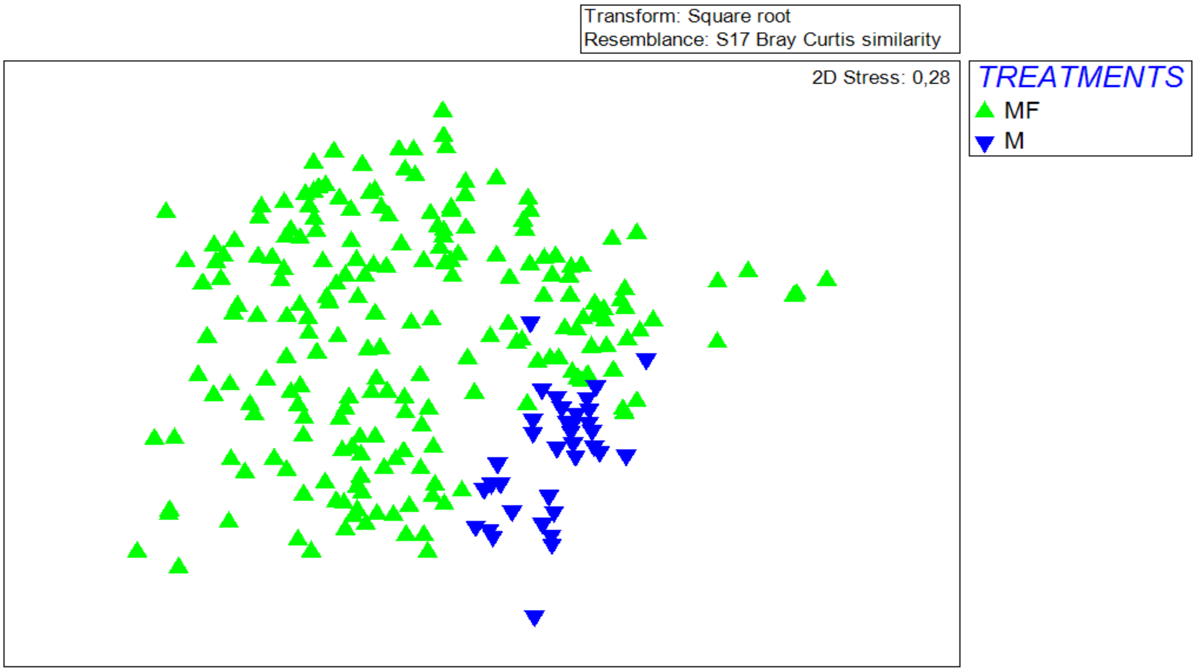

Figure 4.3 NMDS scaling analysis of weed species at all the sampled sites for the maize fields (MF) and margins (M).

Non-metric Multidimensional Scaling results comparing maize fields of the four sites are presented in Figure 4.4. Results showed that some of the more distant Tsolo (T) maize fields grouped further away from the other field sites, which means that they differed slightly in weed species composition. In contrast, the other three sites clustered, showing broad similarity in composition. These three sites occur within the same locality (Baziya Makaula to Baziya Mission was 5.6 km, Baziya Makaula to Baziya Jojweni was 3.8 km and Baziya Mission to Baziya Jojweni was 3 km). However, Baziya and Tsolo are 60 km apart. The weed species of each site were further divided into distant clusters (Figure 4.4) and this separation is best explained by pre-ploughing, post-ploughing and seed sowing surveys. For example, BM plots (blue triangles) grouped more readily with other sites, according to pre-ploughing, post-ploughing and seed sowing surveys, rather than grouping all its field surveys together. The pre-ploughing, post-ploughing and seed sowing survey split was confirmed by T-test in Statistica (refer to chapter 5, section 5.3.4, Table 5.6).

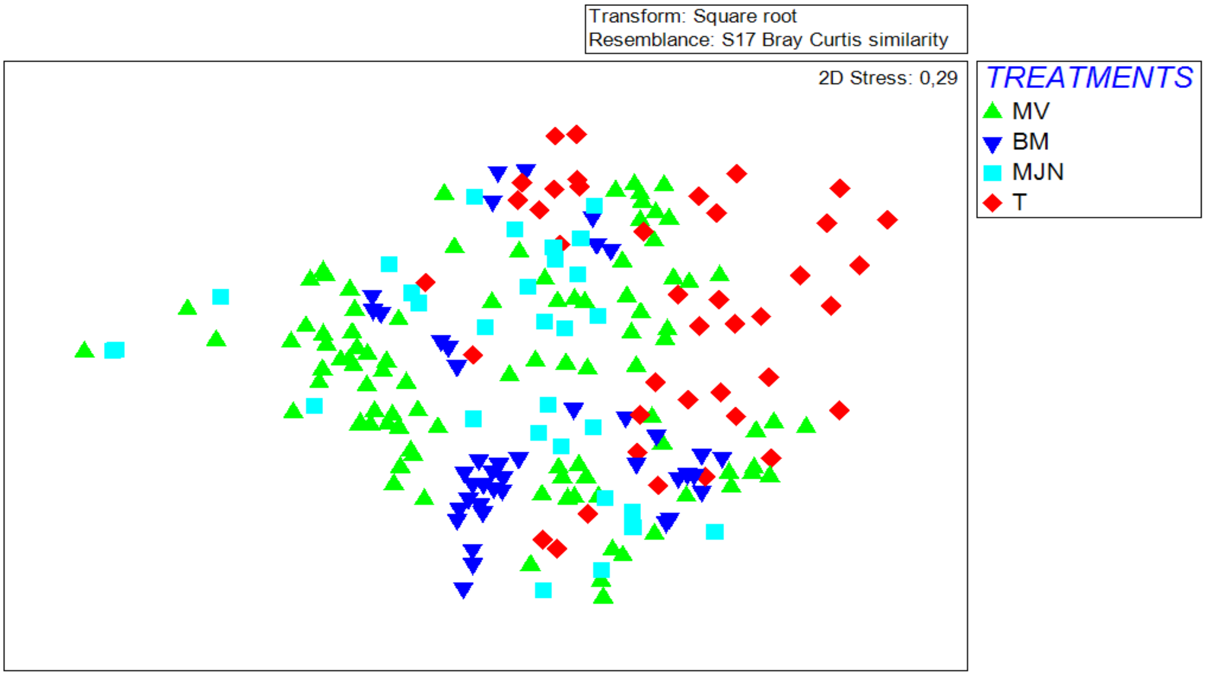

Figure 4.4 NMDS analysis of all weed species for the sampled maize fields. Baziya Mission (MV), Baziya Makaula (BM), Baziya Jojweni (MJN) and Tsolo (T).

In M, there was a division of plots per site based on weed species composition for BM and MV (Figure 4.5). Field margins of T were grouped on the right side and MJN was only found on the left side. The split observed for the weed assemblages of T and MJN could be due to the distance between the sites, or a pre-ploughing, post-ploughing and seed sowing survey (herbicide drift) effect for BM and MV. The pre-post herbicide application split was confirmed by T-tests in Statistica (refer to chapter 5, section 5.3.4, Table 5.6).

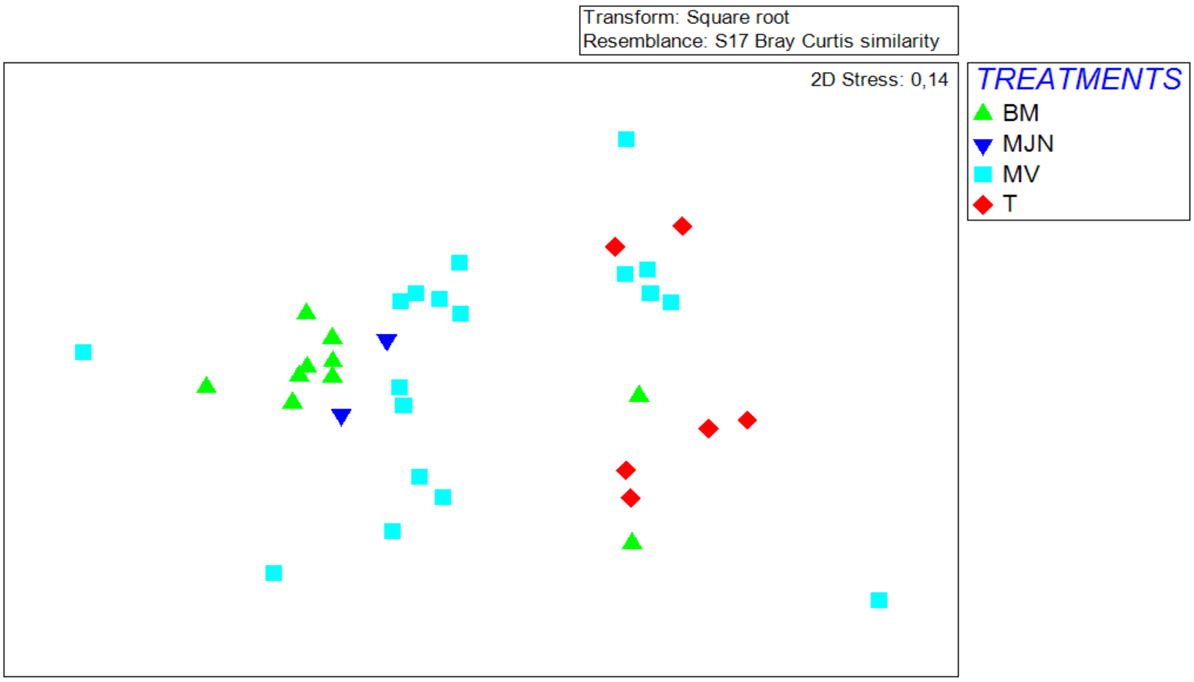

Figure 4.5 NMDS analysis for all weed species of the sampled field margins (M). Baziya Mission (MV), Baziya Makaula (BM), Baziya Jojweni (MJN) and Tsolo (T).

4.3.4 Weed species responsible for community dissimilarities

Weed species responsible for differentiation between communities were those that made a percentage contribution of more than 5% to community dissimilarity. High abundance was observed for *C. dactylon*, *Richardia brasiliensis* Gomes and *S. nanus* in M and contributed the most to dissimilarity between maize fields and field margins (Table 4.4a). Weed species with high mean abundance in the maize fields compared across sites were *C. carinatum*, *C. dactylon* and *H. griseolanatum* in the MV site (Table 4.4b-d), *Amaranthus viridis*, *Cyperus* spp. and *C. dactylon* in the BM site (Table 4.4b, e, f), *Cyperus* spp. for MJN (Table 4.4c, e, g) and *H. asperum* for T (Table 4.4d, f, g). Weed species with high mean abundance in M compared across sites were *Arctotheca calendula* (L.) Levyns, *H. griseolanatum* and *C. dactylon* in MJN (Table 4.4h, k, l), *C. dactylon* and *S. nanus* in BM (Table 4.4h-j), *R. brasiliensis* for MV (Table 4.4i, k, m) and *R. brasiliensis* and *S. nanus* for T (Table 4.4j, l, m). *Cynodon dactylon* is the only species that was found in all the sites as part of the weed species that contributed more than 5% of the average dissimilarity across the fields. Only 11 weed species for the maize fields combined with field margins contributed >5% to dissimilarity between sites.

Table 4.4 Similarity Percentage Analysis (SIMPER) of the average percentage dissimilarity of weed species making more than 5% contribution between a) fields and margins, and among b-g) maize fields and h-m) margins of the four sites.

Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean abundance (1st)	Mean abundance (2nd)
a) Maize fields (MF) vs Margin (M)					
R. brasiliensis	32.08	34.17	34.17	18.5	**1,410**
***Cynodon dactylon* (L.) Pers.**	25.65	27.33	61.49	74.9	**942**
***Sonchus nanus* O.Hoffm.**	5.17	5.5	67	13.6	**143**
b) MF: MV vs BM					
***Amaranthus viridis* L.**	20.19	21.8	21.8	7.28	**309**
C. dactylon	11.36	12.27	34.08	99.1	99.6
***Cyperus* spp.**	7.41	8.01	42.09	26.7	**84.3**
***Helichrysum griseolanatum* Hilliard**	6.58	7.11	49.2	**191**	6.43
***Chenopodium carinatum* R.Br.**	5.58	6.03	55.23	**67.6**	35.3
c) MF: MV vs MJN					
C. dactylon	13.21	14.57	14.57	**99.1**	50.6
H. griseolanatum	9.64	10.64	25.21	**191**	45.3
***Cyperus* spp.**	7.58	8.36	33.57	26.7	**59.5**
***Ipomoea purpurea* (L.) Roth**	7.41	8.16	41.74	49.3	**74.3**
C. carinatum	5.74	6.33	48.07	**67.6**	3.27
d) MF: MV vs T					
H. griseolanatum	10.13	10.64	10.64	**191**	71
C. dactylon	9.63	10.11	20.76	**99.1**	9.07
***Helichrysum asperum* (Thunb.) Hilliard & B.L.Burtt**	6.37	6.69	27.45	1.71	**103**
C. carinatum	5.47	5.74	33.2	**67.6**	9.53

Taxon	**Average dissimilarity**	**Contribution %**	**Cumulative %**	**Mean abundance (1st)**	**Mean abundance (2nd)**
e) MF: BM vs MJN					
A. viridis	20.44	22.38	22.38	**309**	25.4
C. dactylon	8.68	9.51	31.89	**99.6**	50.6
***Cyperus* spp.**	8.63	9.45	41.35	**84.3**	59.5
f) MF: BM vs T					
A. viridis	18.6	19.82	19.82	**309**	27.7
***Cyperus* spp.**	6.22	6.62	26.45	**84.3**	11.6
H. asperum	6.17	6.57	33.03	20.4	**103**
C. dactylon	5.33	5.68	38.71	**99.6**	9.07
g) MF: MJN vs T					
H. asperum	7.26	7.82	7.82	18	**103**
H. griseolanatum	6.24	6.72	14.54	45.3	**71**
***Cotula heterocarpa* DC.**	6.19	6.66	21.21	**66.7**	54
***Cyperus* spp.**	6.05	6.51	27.73	**59.5**	11.6
C. dactylon	5.71	6.15	33.88	**50.6**	9.07
h) M: BM vs MJN					
R. brasiliensis	9.07	18.79	72.22	531	**728**
H. griseolanatum	3.14	6.51	78.73	1.6	**181**
S. nanus	2.99	6.21	84.94	**212**	100
i) M: BM vs MV					
C. dactylon	33.19	48.08	48.08	**2,213**	520
R. brasiliensis	19.9	28.82	76.9	531	**1,883**
S. nanus	3.96	5.74	82.64	**212**	88.1
j) M: BM vs T					
C. dactylon	40.8	52.16	52.16	**2,213**	**0**
R. brasiliensis	19.85	25.38	77.54	531	**1,713**
S. nanus	6.23	7.97	85.51	**212**	207
k) M: MJN vs MV					
R. brasiliensis	23.12	39.94	39.94	728	**1,883**
C. dactylon	18.04	31.17	71.11	**1,223**	520
H. griseolanatum	3.79	6.55	77.67	**181**	5.33
A. calendula	3.44	5.95	83.62	**151**	17.4
l) M: MJN vs T					
C. dactylon	31.21	42.65	42.65	**1,223**	**0**
R. brasiliensis	24.98	34.14	76.79	728	**1,713**
S. nanus	5.30	7.24	84.03	100	**207**
A. calendula	4.06	5.54	89.58	**151**	**0**
m) M: MV vs T					
R. brasiliensis	36.36	52.24	52.24	1,880	1,710
C. dactylon	14.17	20.36	72.6	520	0
S. nanus	6.44	9,26	81.86	88.1	207

MV= Baziya Makaula; MJN= Baziya Jojweni; BM= Baziya Mission; T= Tsolo; Red= Indicates high mean abundance.

4.3.5 Weed species diversity across maize fields and margins

Total weed individuals were significantly more ($p<0.001$) in field margins than maize fields (Figure 4.6a). Pielou's evenness showed a significantly higher evenness ($p=0.001$) for maize fields (Figure 4.6b), meaning there are less weeds dominating in maize fields compared to field margins. Overall, a low evenness value of less than 0.6 was recorded for both maize fields and field margins, suggesting that weed species did not occur at equal proportions in either maize fields or field margins, which is indicative of disturbance. Simpson and Shannon diversity (Figure 4.6c, d) both showed a significantly higher value ($p=0.044$; $p=0.003$) for maize fields compared to field margins, suggesting that MF had higher diversity of weed species present. Overall, the diversity index numbers were very low at less than 1.5 for both maize fields and field margins, which suggested that the fields and margins were poorly colonised probably due to annual disturbance. Total species (S) and Margalef's species richness (d) are not presented here as they showed no significant difference ($p>0.05$) between maize fields and field margins.

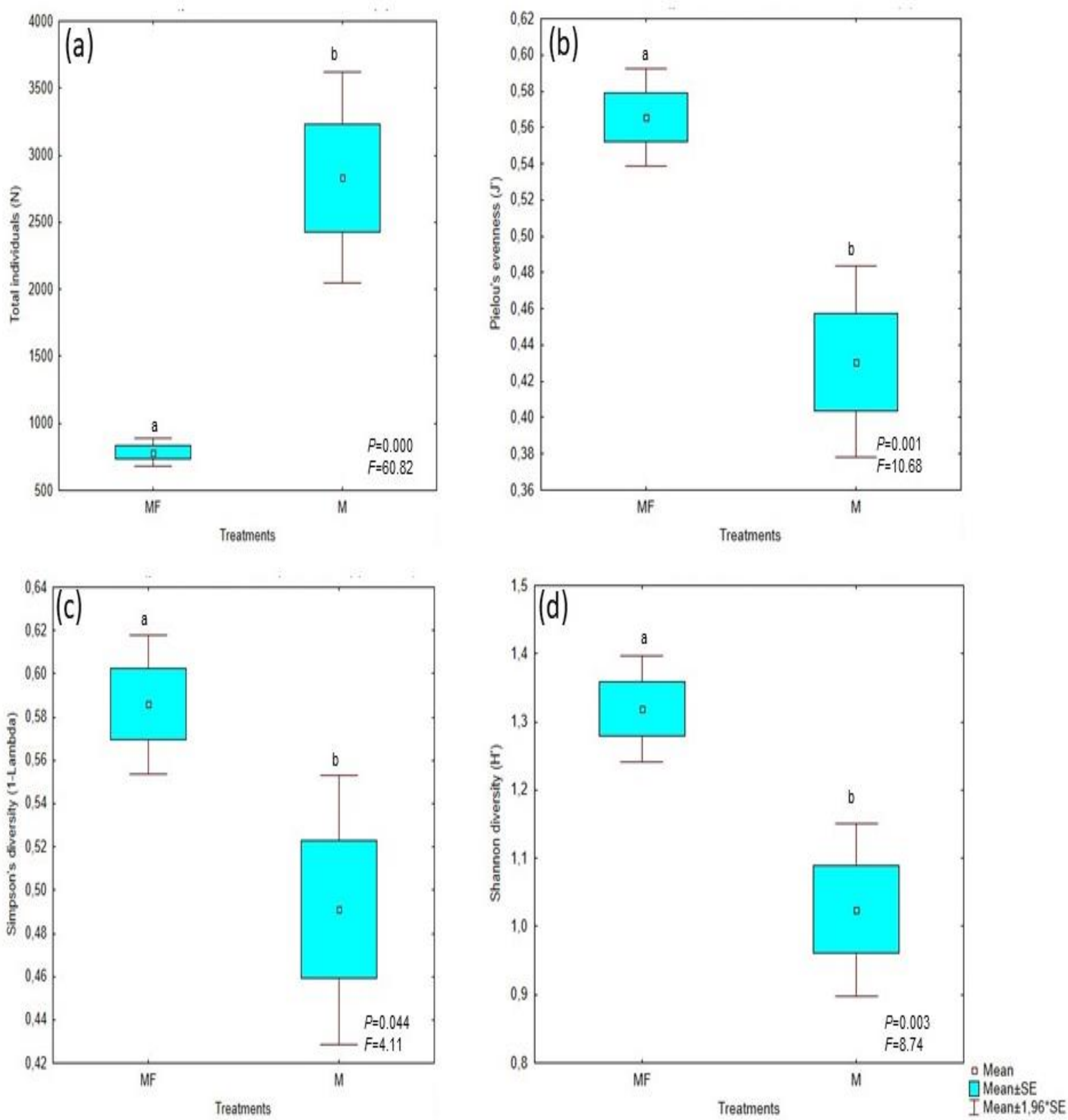

Figure 4.6 Mean weed individuals (a), Pielou's evenness (b), Simpson's index of diversity (c) and Shannon-Wiener diversity (d) index of maize fields versus field margins. Different letters on top of the boxes represent significant differences (p<0.05).

Total weed individuals in maize fields differed significantly (p=0.007) between two sites (MV vs BM) (Figure 4.7a). For the remaining measures (Figure 4.7 b-d), significant differences were recorded only between MV and T, for Simpson index of diversity (p=0.01), Shannon-Wiener diversity index (p=0.01). And for Margalef species richness index it did not differ (p=0.08) statistically, although visually it looks as if there was a difference. Therefore, the maize fields sites were considered varying in diversity. As T did not differ significantly from BM and MJN, the difference observed is probably a result of MV-specific management practices and unknown abiotic factors. Total species (S) and Pielou's evenness (J') are not presented because they showed no significant difference (p>0.05) between sites.

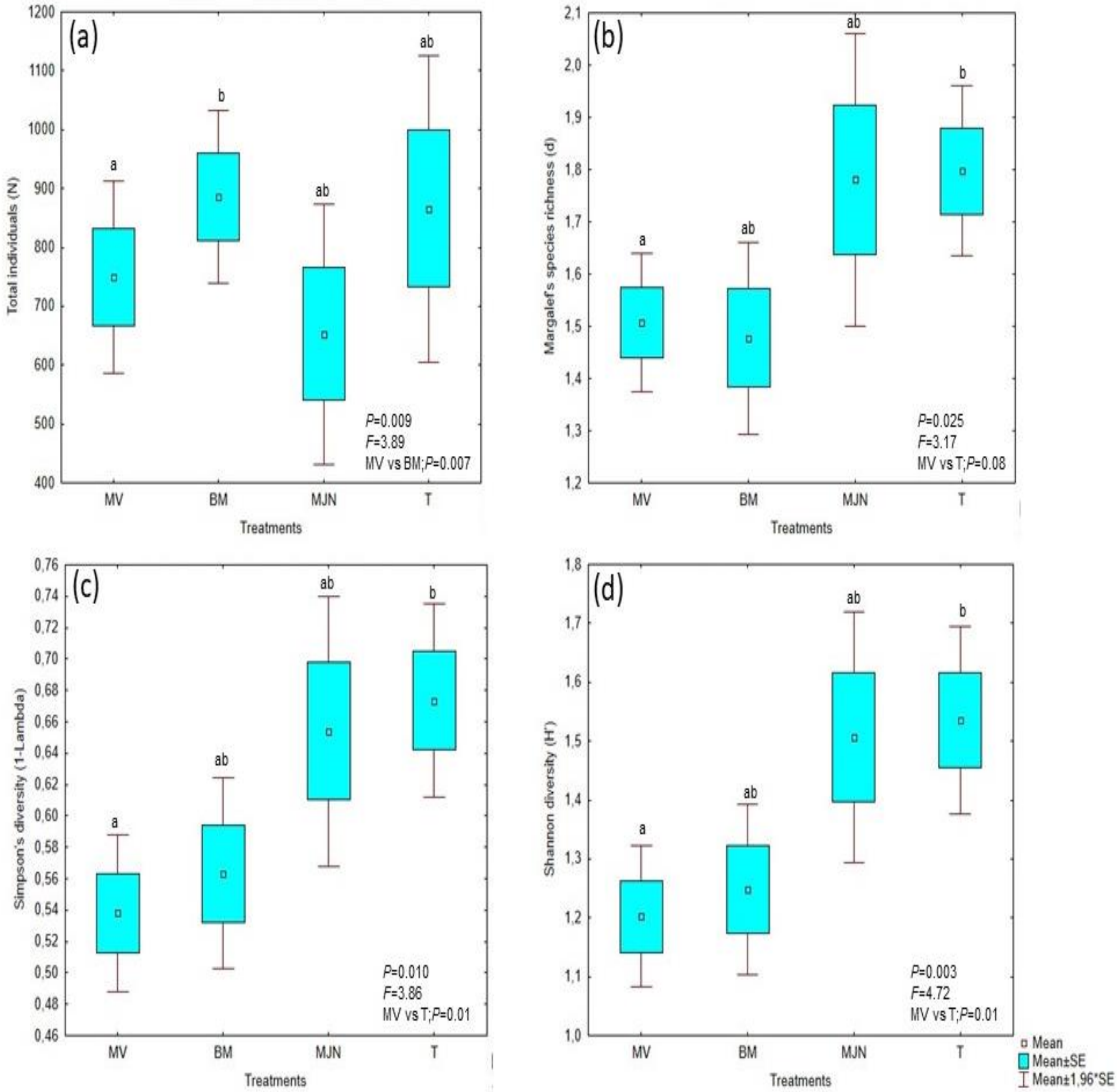

Figure 4.7 Mean weed individuals (a), Margalef's species richness (b), Simpson's index of diversity (c) and Shannon-Wiener diversity (d) indices of maize fields for the four sites Baziya Makaula (MV), Baziya Mission (BM), Baziya Jojweni (MJN) and Tsolo (T). Different letters represent a significant difference.

The difference of the richness and abundance for field margins were not presented here because they showed no significant difference ($p>0.05$) as tested for the six indices (Total species (S), Total individuals (N), Pielou's evenness (J'), Margalef's species richness (d), Simpson's index of diversity and Shannon-Wiener diversity index). Therefore, the field margin sites were considered equal in diversity independent of site, but it must be noted that reference sites were undersampled (Figure 4.1b).

4.4 Discussion

4.4.1 Weed species of maize fields and margins

Weeds are adapted to habitats which are disturbed by crop cultivation (Erskine, 1984). Different weed species are usually associated with a certain crop and always inhabit that cropland depending on the local climate and management conditions (Gage *et al*., 2019;

MacLaren *et al*., 2020). A major concern is that the disturbance associated with crop conversion may have effects on diversity patterns inside fields. Therefore, maize field plant diversity differs from margins because of differences in disturbance intensities (Botha *et al*., 2015). According to literature, diversity of plants is higher inside maize fields than field margins (Botha *et al*., 2017; Janse van Rensburg *et al*., 2020). And the species assemblage patterns for plants has distinct communities in maize fields and field margins. Hence, field margins are rich in plant diversity because they are less transformed compared to MF.

Weed species found in maize fields and field margins differ because of the disturbance intensities, including ploughing and chemical applications in the former. Hence, species that survive in the maize fields are those that have a rapid colonisation mechanism (Fried *et al*., 2019). In the margins of this study, *Cynodon dactylon, R. brasiliensis* and *S. nanus* had a higher mean abundance than in maize fields. This suggests that weed species can respond well towards agricultural disturbance of margins in the absence of regular ploughing or herbicide applications which regularly suppresses weed establishment within maize fields. Additional weed species that had higher mean abundance in margins included *A. calendula*, *Tagetes minuta* L. and *Themeda triandra* Forssk. Those species with higher mean abundance in the maize fields included *A. viridis, Chamaesyce prostrata* Aiton., *Chenopodium carinatum* R.Br., *Cyperus* species, *H. griseolanatum* and *Ipomoea purpurea* (L.) Roth. This means that they have a rapid colonisation trait, which allows them to rapidly invade or survive a ploughed or sprayed area. Therefore, different species react differently to the environment due to disturbance. Weed species of field margins, such as *T. triandra*, were more dominant in the field margins and near absent from the maize fields. A study by Snyman *et al.* (2013) supports the finding of this study as they reported that *T. triandra* is a native species that underperforms in disturbed areas.

For the BM and T sites that had *Cyperus* species as a dominant weed, despite herbicide application, suggests that it was able to adapt very well and resist herbicides used in MF (Naczi & Ford, 2008; Peerzada, 2017). Weed species that were dominant in M across sites were *A. calendula* in the MJN, *Cynodon dactylon* and *S. nanus* in BM and, *R. brasiliensis and S. nanus* in T. Only *C. dactylon* was found dominant in the maize fields and field margins of BM, suggesting that it adapted better to both disturbed systems. This random effect of adaptation of weeds to different disturbance (Erskine, J. 1984; Norris, 1997) is well known, for instance Głowacka (2012) reported *Echinochloa crus-galli* (L.) P. Beauv., *Chenopodium album* L., *Galinsoga parviflora* Cav., and *Cirsium arvense* L. as dominant weeds in maize fields of Lublin, Poland. These species are also part of the weed flora of the Eastern Cape, but were not locally dominant.

Examples of maize fields associated weeds in this study were *A. viridis*, *C. prostrata*, *C. carinatum*, *C. dactylon*, *Cyperus* species, *Helichrysum asperum*, *Helichrysum griseolanatum*, *Ipomoea purpurea*, *R. brasiliensis* and *Sonchus nanus* (refer to Table 4.4). This is in line with the report of weed species associated with maize crops by Botha *et al*. (2007) and Maseldzija and Dudic (2018). GrainSA (2015) has also reported weed species in the genera found in this study, namely species of *Amaranthus*, *Conyza*, *Cynodon, Cyperus* and *Ipomoea*.

4.4.2 Weed species composition across maize fields and margins

Maize fields and field margins were both characterised by an abundance of weed species. This study indicated a visible difference of weed species assemblages between maize fields and field margins. However, more than half of weed species in maize fields were shared with those of field margins, although there was only a slight nesting of weed species assemblages between the maize fields and field margins. Marshal (2003), reported that in wheat and barley agroecosystems of Europe, 30% of the weed species found in field margins were also found in maize fields. However, in this study, the shared weed species between maize fields and field margins was 53%, suggesting that in the presence and absence of exogenous disturbance, maize fields and field margins still harboured similar weed species composition. This supported the hypothesis that HT maize fields have the same weed species composition and diversity across fields. It also indicates that there was a shift of weed species between maize fields and field margins (Botha *et al*., 2015). This would be expected considering exogenous and endogenous disturbance in maize fields, versus endogenous only in field margins (Ferrero *et al*., 2017).

When sites were compared for maize fields only, some of T fields were compositionally different from the other sites. This slightly different weed species composition, compared to other sites, was due to different local soil and climatic conditions (Mandiringana *et al*., 2005). In contrast, the other three sites (MV, BM and MJN) showed broad similarity in composition among themselves and were also in closer proximity suggesting similar environmental parameters (Głowacka, 2012). In field margins, there was a division of plots per site based on weed species composition for BM and MV. Margins of T were substantially separated from MJN in terms of species composition suggesting the influence of abiotic disturbance such as climatic factors (Raza *et al*., 2019) in the fields as there was no herbicide application in M.

4.4.3 Weed species diversity across maize fields and margins

The main aim of most agricultural systems is to achieve better yield production by reducing weed species (Shafi, 2018). Findings in this study showed that maize fields had significantly lower weed individuals, but higher evenness and diversity than the field margins. These effects are ascribed to agricultural practice that target and subdue weed species accumulation within the maize fields. Hence, crop fields are unfavourable environments for most weed species because of high levels of agricultural disturbance involving weed management, such as ploughing, herbicide applications, use of fertilisers and crop rotation (Hatfield and Prueger, 2015; Reynolds *et al*., 2015). Chemical control of weed species has been shown to successfully reduce the abundance and diversity of weeds (Puricelli and Tuesca, 2005; Santin-Montanyá *et al.,* 2013). It is also important to note that weeds often continue to be present and abundant in crop fields due to their pre-adaptive traits to intensive cropping systems (see Chapter 5 of this thesis; Botha *et al*., 2017), dynamic nature of weeds (Batlla *et al*., 2020), management practices, including herbicide application (Pieterse, 2010), and shifts in weed species (Botha *et al*., 2017).

The maize fields had significantly higher evenness than field margins, despite being more heavily disturbed. This could be attributed to herbicide application in the fields which suppress any weeds becoming dominant and due to selective grazing in field margins which favours palatable species which in turn enhance the numbers of unpalatable species. This contrasted with the study by Janse van Rensburg (2020), finding soybean crop fields to have significantly lower evenness than the boundary, and Izquierdo, *et al*. (2009) who reported a decrease in diversity and evenness of crop fields. The factors that influenced their findings was also chemical application and grazing disturbance. Similarly, in vineyards, lower evenness of weed species was observed under herbicide treatment (Sanguankeo and León, 2011). This contradicts the findings of this study. Hence, it is important to consider the contrasting finding regarding evenness in this study, since the stability and function of the ecosystem increases with increasing diversity and evenness (Frank and McNaughton, 1991; Tilman, 1996; Maestre *et al.,* 2012).

Agricultural disturbance may also affect plant diversity in the field margins due to grazing and herbicide drift (Felsot *et al*., 2010). In this study, that was the case because diversity was lower in field margins, meaning that grazing and probable herbicide drift contributed towards the decrease in diversity. This is common in other studies, for example, a study by Jobin *et al*. (1997) observed lower plant diversity in the field margins adjacent to crop fields that were treated with herbicides. Schmitz *et al*. (2014) observed the same due to fertiliser drift from the fields into the margin. Therefore, higher plant diversity in the maize fields might be ascribed by its support of species that perform an important ecosystem service that is beneficial to the ecosystem.

Conclusion

In this study the aim was to profile weeds associated with HT maize in the selected areas of Oliver Tambo District Municipality, Eastern Cape, to establish a reference collection for weeds in the HT maize cropping systems and to determine the weed species composition and diversity of maize fields and associated field margins. Relating to the hypothesis of this study, HT maize systems have similar weed composition and diversity across fields but differ between maize fields and field margins. In response to agricultural disturbance, maize fields had lower abundance of weeds, but higher diversity than field margins. Weed species assemblage composition differed between maize fields and field margins. Therefore, the hypothesis was supported. The difference between maize fields and field margins would be ascribed to differences in management practices, as the field margins are less disturbed compared to maize fields that experience exogenous disturbance in various ways such as herbicide application and grazing. There is a large overlap in weed species of maize fields and field margins. Maize fields have more total number of weeds and much more alien weed species than field margins, meaning that weed species are able to colonise and persist in the maize fields after disturbance. Three families that contributed most weed species were Asteraceae, Fabaceae and Poaceae. Weed species most responsible for dissimilarity of >5% between maize fields and field margins are: *Amaranthus viridis, Chenopodium carinatum, Cotula heterocarpa, Cynadon dactylon, Cyperus* species*, Helichrysum asperum, Helichrysum griseolanatum, Ipomea purpurea, Richardia brasiliensis* and *Sonchus nanus*. Therefore, this information will benefit maize farmers and future researchers working on weeds in HT maize crops of the Oliver Tambo District Municipality, and in Sub-Saharan Africa, when it comes to the development, implementation and prioritising of various herbicide-based weed management protocols and measures towards ensuring maximum crop productivity. It is important to remember that poor management practices can lead to herbicide resistance developing in weeds.

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CHAPTER 5: Plant functional types composition and richness of indicator weeds in herbicide tolerant maize fields

Abstract

The reaction of weeds to agricultural disturbance may not always be expressed by the species composition and diversity of the entire weed community. This is becuase individual weed responses are more often linked to traits that enable or hinder their persistence in disturbance prone agroecosystems. In this study, specific functional traits of indicator weed species associated with herbicide tolerant maize in the Oliver Tambo District of the Eastern Cape were described. The aim of this study was to investigate the traits and functional types of weed species able to persist in herbicide tolerant maize fields under the influence of herbicide applications. This was done by exploring the abundance patterns, composition and richness of specific weed traits and functional types. Plant functional traits include the morphological, anatomical, biochemical, physiological or phenological features measurable at the individual level. Plant functional types are the sets of species sharing traits in response to the environment factors. Frequency measures (%) were used for indicator species analysis and significance levels were set at $p<0.05$ for selection purposes. Plant functional traits information were gathered from a variety of sources in the literature that demonstrate functional response to the agroecosystem management. A data set comprising of 42 indicator weed species and 11 predefined disturbance traits from 28 herbicide tolerant fields were considered for functional analysis. Indicator weed species were divided into annual, with 24 species, and perennial/biennial, with 18 species. Clusters were identified according to the grouping of weed species based on their trait scores which revealed ten plant functional types (PFTs) at different cut-off level distance of up to 0.35. After clustering of the traits, PFTs were compared between the sites pre-ploughing, post-ploughing and seed sowing surveys to see if they share similar traits as they are under similar disturbance of herbicide use. Weed species trait composition differed significantly ($p=0.031$) between most sites, but the more distant site (Tsolo) showed much similarity with the Bayiza sites indicating that local, rather than regional factors, affect trait composition. Weed traits composition for pre-ploughing, post-ploughing and seed sowing surveys differed significantly ($p=0.003$), indicating that disturbances associated with post-emergence (after ploughing, sowing and herbicide application) act as filters selecting for weed species with specific adaptations to colonise fields after such disturbances. Trait richness across maize fields generally did not differ significantly. Although trait composition differed at post-ploughing and seed sowing survey, trait richness for pre-ploughing, post-ploughing and seed sowing survey did not differ significantly ($p>0.05$) because filtering allowed for the dominance of a new set of traits which allow weeds to survive under these types of disturbance. T-tests results showed a significant difference for four PFTs (1, 2, 3 and 5) pre-ploughing, post-ploughing and seed sowing surveys. Only PFTs 1, 3 and 5 that differed significantly, increased for post-ploughing and seed sowing surveys, whereas PFTs 2 decreased. Increases of a PFT is indicative of species that have traits which make them tolerant to a combination of ploughing and herbicides. This study therefore highlights the species types and traits that need to be targeted to enhance the weed control protocol of the Oliver Tambo District Municipality in the Eastern Cape.

Keywords: Agricultural disturbance, Diversity, Frequency, Indicator weeds, Plant functional types, Ploughing, Weed traits

5.1 Introduction

The transformation of natural vegetation in cultivated agroecosystems negatively affects plant diversity and composition (Muller *et al*., 2021). This is because a larger proportion of diversity is found in farmlands, and due to rapid expansion of croplands, diversity is lost (Marshall and Moonen, 2002; Wessels *et al*., 2003). For the management of diverse and functional ecosystems within farmlands, it has become important to understand the potential effects that respective farming activities might have on plant communities since agricultural practices and activities are known to cause disturbance to plant communities (Dale and Polasky, 2007). In disturbed areas, plant species can adapt differently, and the response may be directly linked to functional traits that enable or hinder their persistence (Kraft *et al*., 2008; Poorter *et al*., 2009). Plants colonising disturbed areas may be defined in terms of their functional types, based on similar traits and similar responses to disturbance (Lavorel *et al*., 1997).

Plant functional traits are studied to better understand plant species based on ecosystem dynamics (Lavorel *et al*., 1997; Lavorel and Garnier, 2002; Díaz *et al.*, 2007; Linstädter *et al.*, 2014; Kimball *et al.*, 2016). Traits are morphological, phenological or physiological features that impact the fitness of individual species. However, to date, principles of functional trait ecology have not been widely applied to agricultural research and management (Martin and Isaac, 2015). Therefore, making it important to identify and better understand plant functional traits in agroecosystems for the advancement of agricultural and environmental management research (Martin and Isaac, 2015). Functional traits are affected by agricultural activities, which can be considered as limiting filters, for which individuals bearing specific traits of response persist in a community (Keddy, 1992). Plant functional traits may therefore be used to define species as functional types with similar features, as they are likely to respond similarly to climatic conditions and/or disturbances (Grime, 1979; Lavorel *et al.*, 1997; Díaz *et al.*, 2001; Linstädter *et al.*, 2014).

Agricultural activities range from rangelands for grazing to intensive crop production and maize is one of the most widely grown crops worldwide. Genetically Modified maize development and cultivation, particularly for herbicide tolerant cultivars, has led to more frequent use (often dependent) of herbicides for weed control. A study by Fried *et al*. (2019), showed that most abundant weeds in maize crops are species that germinate in spring-summer, flower late, and are likely to have the same life cycle as maize. Botha *et al*. (2017) also reported that weed functional traits differ between grassy biomes in disturbed agroecosystems of South Africa, suggesting that climatic conditions, in addition to site-specific herbicide application and grazing, all have an influence. Hence, traits that are related to high colonisation capacity, involving wind dispersal and seed longevity, are beneficial to species exposed to exogenous disturbances such as ploughing. Traits related to specific seasonal adaptation (such as late flowering) and the C4 photosynthetic pathway are better adapted to endogenous disturbances such as herbicide use because some weeds respond by exhibiting similar traits as crop plants to avoid hazards. Traits which predict the ability of weeds to persist after agricultural disturbance include growth form (Cornelissen *et al*., 2003), life span (Germishuizen and Meyer 2003; Martínková *et al*., 2020), flowering phenology, pollination mode, flowering season (Crayne *et al*., 1999), N-fixating ability (Botha *et al*., 2017) and photosynthetic pathway (Fried *et al*., 2018) are considered.

Plant communities with high functional trait diversity are more stable and resilient after a disturbance. This is because there is a large pool of traits that allow plant species response across a wider range of disturbance (Díaz and Cabido, 2001). To understand weed dynamics, it is important to know which weed traits play an important role in species' ability to cope with disturbance associated with agricultural activities. Disturbances impact some functional traits more than others (Liira *et al*., 2008). For example, functional traits that are more resilient to agricultural activities may serve as indicators in land use monitoring studies to assess the transformation of natural landscape into agricultural systems (Liira *et al*., 2008). Therefore, it is important to understand weed traits in agroecosystem to inform better planning and execution of agricultural practices. Protection of field margins enhances trait diversity, especially within the natural habitat, towards the enhancement of functional diversity that provide a wide array of ecosystem services (Wratten *et al*., 2012). Although high trait diversity is good for agriculture, caution is needed as some traits associated with species in these marginal areas can contribute to their weediness. This makes it important to assess whether there are specific functional traits that make weed species to persist in HT maize, and establish which species are likely to develop tolerance to herbicides.

This study aimed to determine weed traits that enable species to persist in herbicide tolerant maize fields after herbicide application (pre and post-emergence herbicides). The objectives of this study were to: 1) identify indicator weed species able to persist after herbicide application; 2) classify weed species into functional types using disturbance traits; 3) compare the composition of weed traits pre-ploughing, post-ploughing and seed sowing surveys as to provide the traits that allow persistence in spite of herbicide use; 4) compare the richness of weed traits pre-ploughing, post-ploughing and seed sowing surveys; and 5) compare PFTs increase from before to after herbicide application as it shows what combination of shared traits allow for survival after herbicide application and which species are favoured by these traits.

To address the knowledge gap in the South African HT maize agroecosystems in terms of functional traits, this study considers changes/shift in weed functionality in a transformed habitat exposed to herbicide application disturbance. The hypothesis states that weeds in HT maize fields share similar traits. Considering the contribution of functional traits to weed species persistence, the expectation is to find similar weed species traits across HT maize fields because the weed management system of herbicide application was identical per season. The second hypothesis states that weeds in HT maize fields pre-ploughing, post-ploughing and seed sowing surveys will not share similar traits. Different weed species traits are expected, because pre-ploughing surveys were conducted before ploughing and herbicide application, whereas post-ploughing and seed sowing refers to when germination and herbicide application has already occurred. Hence, the filtering effect will be visible in the selection of traits that allow persistence after herbicide application.

5.2 Material and methods

5.2.1 Functional traits identification and adaptation to herbicides disturbance analysis

Functional traits that have known responses to factors associated with agricultural disturbance (Cornelissen *et al*., 2003; Aronson *et al*., 2007) were selected based upon reported and published data from different databases (refer to Chapter 3, section 3.3.6,

Table 3.2). The indicator value index (IndVal) proposed by Dufrene and Legendre (1997) was calculated to determine the indicator weed species in HT maize fields. Overall abundance of all weed species recorded from the HT maize fields during pre-ploughing were combined. Frequency measures (%) were used for indicator species analysis performed in RStudio using the IndVal function under the labdsv package and significance levels were considered at $p<0.05$. A hierarchical cluster analysis with an Unweighted Pair Group Method with Arithmetic Mean (UPGMA)-clustering algorithm and Gower distance measure appropriate for mixed (categorical and binary) data types was used in PRIMER 6 to detect groupings of weed species. Similarity Profile (SIMPROF) test was done as an objective method for the identification of significant groupings compared to subjective cut-off levels (Clarke *et al*., 2006), refer to Chapter 3, section 3.6.5 for more details. Redundancy Analysis (RDA) was done to extract and summarise the variation in a set of response variables in Canoco5. It was applied to the complete species-trait matrix to test for clustering based on specific traits in ordinal space. Non-metric Multidimensional Scaling (NMDS) of weed functional traits were done in PRIMER 6 to determine functional traits composition using weed indicator species traits for all plots per site across maize field data. The trait-species matrix of 42 weed (indicator) species and 11 traits were multiplied with the species-abundance matrix using the MMult function in Microsoft Excel to produce a matrix from which trait diversity index calculations (richness) was performed in PRIMER 6. The data for trait richness was transformed and tested for normality in Statistica, and the untransformed data was used to draw graphs for representation of normal distribution. Significant difference of weed species diversity among the sites and for pre-ploughing was tested in Statistica version 64. T-test was done in Statistica using PFTs pre-ploughing survey and post-ploughing and seed sowing survey data to test for differences between the means of the two data groups collected at different times in the same maize fields. Top ten most abundant weed indicator species were selected manually from the IndVal results and their average sum for pre-ploughing survey was also calculated in Excel. These weed indicator species where selected according to the highest total individuals to see if their individual numbers increased or decreased when comparing pre-ploughing, post-ploughing and seed sowing surveys with the selected traits per PFT.

5.3 Results

The results presented in this chapter depict the response effects of weed species due to herbicide application by assessing shifts of traits composition, richness, and plant functional types (PFTs) across herbicide tolerant maize fields for pre-ploughing, post-ploughing and seed sowing survey.

5.3.1 Indicator weed species of herbicide tolerant maize

Indicator weed species analysis identified 42 weed species and the dominant families were Asteraceae (most dominant), Cyperaceae and Fabaceae (Table 5.1). Indicator weed species with the highest indicator value (IndVal), greater or equals to 0.3, were nine in total (Table B2). The species with the highest IndVal value of 0.558 and highest frequency of occurrence (recorded in 114 plots) was *Amaranthus viridis* L. (Table 5.2).

Table 5.1 Family names ranked according to number of weed indicator species (≥2 to qualify for inclusion here).

Family	Indicator weed species
Asteraceae	11
Fabaceae	5
Cyperaceae	4
Malvaceae	2
Poaceae	2
Solanaceae	2

Table 5.2 Indicator weed species with value (≥0.3).

Family	Indicator species	Indicator Value	*P-value	^Frequency
Amaranthaceae	*Amaranthus viridis* L.	0.558	0.001	114
Apiaceae	*Daucus carota* L.	0.323	0.003	49
Asteraceae	*Bidens bipinnata* L.	0.433	0.001	13
Asteraceae	*Cirsium vulgare* (Savi) Ten.	0.339	0.001	44
Asteraceae	*Conyza bonariensis* (L.) Cronquist	0.321	0.001	23
Asteraceae	*Sonchus nanus* Sond. ex Harv.	0.348	0.003	103
Chenopodiaceae	*Chenopodium carinatum* R.Br.	0.397	0.002	112
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	0.342	0.001	54
Portulacaceae	*Portulaca oleracea* L.	0.329	0.001	30

* P-values indicate significant indicator species at P<0.05
^Frequency refers to the number of surveyed plots the species were recorded from

5.3.2 Description of plant functional types

Annual weed species were 24 in total (Figure 5.1), compared to 18 perennials (Figure 5.2) and providing a 4:3 split. Abbreviated weed names are shown as full species names in Appendix B; Table B3. A total of ten PFTs were recognized for the 42 weed indicator species based on 11 disturbance tolerance traits (Table 5.3).

Unweighted Pair Group Method with Arithmetic Means (UPGMA) analysis identified six PFTs consisting of annual species (Figure 5.1). In annual species, the first level was initiated by two splits: 1) specialized, and 2) no specialized carbon storage organ, which was an important variable for the separation. PFT 1 comprised of prostrate and erect dicotyledon Non-N-Fixers with specialized carbon storage. PFT 2 consisted of Non-N-Fixers with unassisted seed dispersal and specialized carbon storage organ. PFT 3 had similar traits as PFT 2, except that it had N-Fixer trait for those species. PFT 4 included Non-Spinescent N-Fixers with specialized storage carbon. PFT 5 comprised of C3 and C4 Non-Spinescent Non-N-Fixer species with specialized carbon storage organ. PFT 6 had species with spinescent, assisted and non-assisted form of dispersal with specialized carbon storage organ. An outlier species HIBNIG (*Hibiscus nigricaulis*) was grouped with PFT 6 because they shared some of the traits such as spinescent, N-Fixer and assisted seed dispersal (Figure 5.1; Appendix B, Table B3).

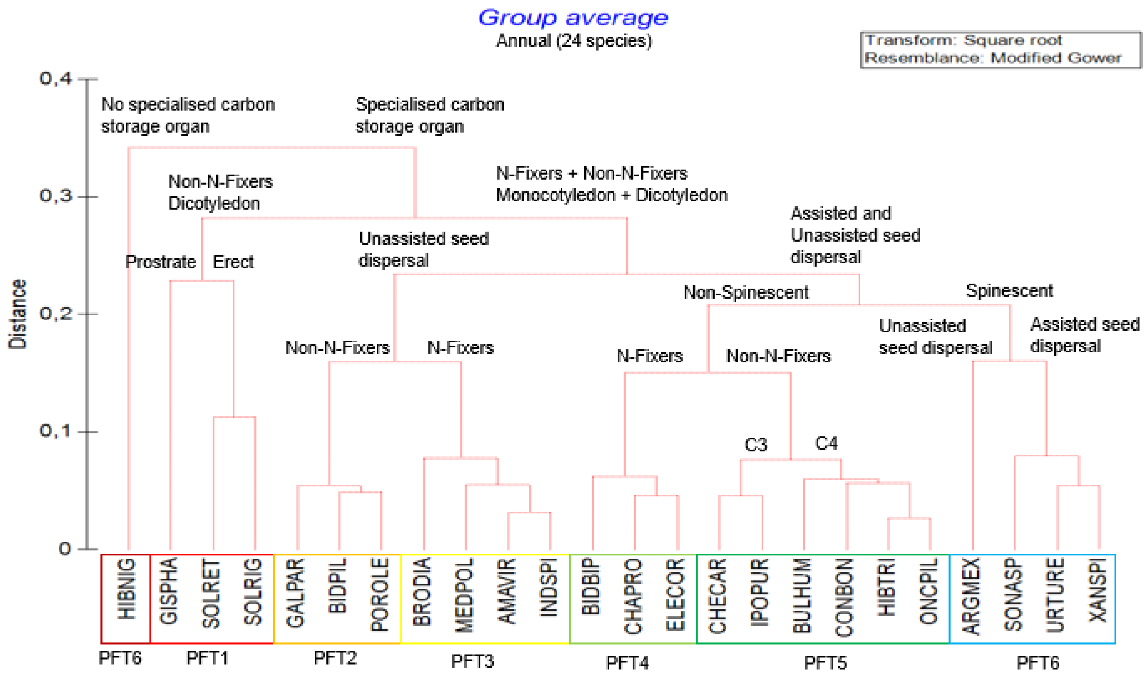

Figure 5.1 Cluster analysis (UPGMA) based on Gower distance measure proposing six major plant functional types (PFTs) from the trait composition for annual species recorded from herbicide tolerant maize fields. Red lines in the dendrogram indicate branching with significant structure, as determined by SIMPROF analysis.

Unweighted Pair Group Method with Arithmetic Means (UPGMA) analysis identified four PFTs consisting of perennial species (Figure 5.2). In perennial species, the first level was initiated by flowering seasons and split into unspecified flowering season and specified flowering season. PFT 7 represented species with assisted seed dispersal, no specialized carbon storage organ with specified flowering season. PFT 8 comprised of N-Fixers and Non-N-Fixers, Non-Spinescent with specialized carbon storage organ and specific flowering season. PFT 9 comprised of species that are spinescent nitrogen fixers and Non-N-Fixers and have assisted seed dispersal mode with specialized carbon storage and specified flowering season. PFT 10 had many shared traits with species that comprised of Non-Spinescent N-Fixers and Non-N-Fixers with assisted seed dispersal and specialized carbon storage and specified flowering season. An outlier species RORNUD (*Rorippa nudiuscula*), was grouped with PFT 10 species because they shared some of the traits such as specialized carbon storage organ, assisted seed dispersal without spinescent and nitrogen fixing ability (Figure 5.2).

All PFT's, except PFT 7, had specialized carbon storage organs as the main trait. PFT 7 with no specialized carbon storage organs included *Callicoma serratifolia* Andrews, *Chascanum hederaceum* (Sond.) Moldenke and *Cotula heterocarpa* DC.

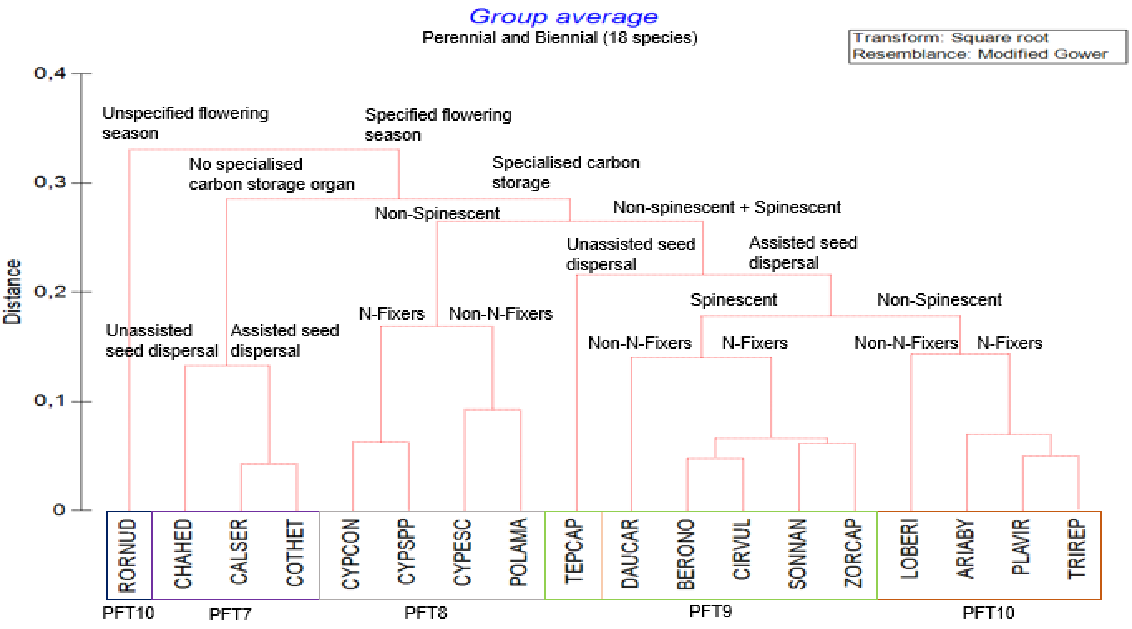

Figure 5.2 Cluster analysis (UPGMA) based on Gower distance measure proposing four major plant functional types (PFTs) from the trait composition for perennial species recorded from HT maize fields. Red lines in the dendrogram indicate branching with significant structure, as determined by SIMPROF analysis.

Table 5.3 Description of the ten plant functional types (PFTs) identified by means of cluster analysis.

Plant Functional Types	Life form	Species names	Functional Traits description
PFT1	Annual	• *Gisekia pharnacioides* L. • *Solanum retroflexum* Dunal • *Solanum rigescens* Jacq.	Annual, dicotyledon, prostrate and erect, Non-nitrogen-fixing weed species with specialised carbon storage organs.
PFT2	Annual	• *Bidens pilosa* L. • *Galinsoga parviflora* Cav. • *Portulaca oleracea* L.	Annual, monocotyledon and dicotyledon, Non-Nitrogen fixing weed species with unassisted seed dispersal and specialised carbon storage organs.
PFT3	Annual	• *Amaranthus viridis* L. • *Bromus diandrus* Roth • *Indigofera spicata* Forsk. • *Medicago polymorpha* L.	Annual, monocotyledon and dicotyledon, Nitrogen fixing weed species with unassisted seed dispersal and specialised carbon storage organs.
PFT4	Annual	• *Bidens bipinnata* L. • *Chamaesyce prostrata* (Aiton) Small • *Eleusine coracana* (L.) Gaertn.	Annual, monocotyledon and dicotyledon, Nitrogen-fixing and non-spinescent weed species following an assisted seed dispersal and specialised carbon storage organs.

Table 5.3 Description of the ten plant functional types (PFTs) identified by means of cluster analysis continued.

Plant Functional Types	Life form	Species names	Functional Traits description
PFT5	Annual	• *Bulbostylis humilis* (Kunth) C.B.Clarke • *Chenopodium carinatum* R.Br. • *Conyza bonariensis* (L.) Cronquist • *Hibiscus trionum* L. • *Ipomoea purpurea* (L.) Roth • *Oncosiphon piluliferum* (L.f.) Källersjö	Annual, monocotyledon and dicotyledon, Non-Nitrogen-fixing and non-spinescent weed species following an assisted seed dispersal and specialised carbon storage organs with a C3 and C4 photosynthetic pathway.
PFT6	Annual	• *Argemone mexicana* L. • *Hibiscus nigricaulis* Baker f. • *Sonchus asper* (L.) Hill • *Urtica urens* L. • *Xanthium spinosum* L.	Annual, monocotyledon and dicotyledon, Nitrogen and Non-Nitrogen-fixing spinescent weed species with assisted and unassisted seed dispersal following a specialised carbon storage organ.
PFT7	Perennial/Biennial	• *Callicoma serratifolia* Andrews • *Chascanum hederaceum* (Sond.) Moldenke • *Cotula heterocarpa* DC.	Perennial and biennial, assisted and unassisted seed dispersal weed species with specified flowering season and no specialised carbon storage organs.
PFT8	Perennial/Biennial	• *Cyperus congestus* Vahl • *Cyperus esculentus* L. • *Cyperus* spp. L. • *Polygala amatymbica* Eckl. & Zeyh.	Perennial and biennial, Nitrogen and Non-Nitrogen fixing weed species with Non-Spinescent, specified flowering season and specialised carbon storage organs.
PFT9	Perennial/Biennial	• *Berkheya onopordifolia* (DC.) O.Hoffm. Ex • *Cirsium vulgare* (Savi) Ten. • *Daucus carota* L. • *Sonchus nanus* Sond. ex Harv. • *Tephrosia capensis* (Jacq.) Pers. • *Zornia capensis* Pers.	Perennial and biennial, Nitrogen and Non-Nitrogen fixing weed species following Non-Spinescent and spinescent with assisted seed dispersal, specified flowering season and specialised carbon storage organs.
PFT10	Perennial/Biennial	• *Aristea abyssinica* Pax • *Lobelia erinus* L. • *Plantago virginica* L. • *Rorippa nudiuscula* Thell. • *Trifolium repens* L.	Perennial and biennial, Nitrogen and Non-Nitrogen fixing non-spinescent with assisted seed dispersal following specified flowering season with specialised carbon storage organs.

5.3.3 Weed trait assemblages across maize field sites in pre-ploughing, post-ploughing and seed sowing survey

Weed assemblages differed significantly between most sites ($p<0.005$), but Tsolo showed similarity to some of the Bayiza sites (Table 5.4). The difference was supported by the NMDS which indicated strong clustering of most fields, but the variation in species composition per field at MV (elongated clustering - substantial species turnover) made it different from the other sites (Figure 5.3). With reference to chapter 4, section 4.3.2 (Figure 4.6), where site T showed significant differences in weed species composition because it is located furthest away from the other sites and was expected to have different soil and climate conditions. However, in terms of functional traits, site T showed similarity to the other sites including BM and MJN specifically (Figure 5.5). This means that there are indeed traits assemblages that are shared between sites, even those that differ the most in terms of environmental conditions, possibly suggesting a management effect. NMDS of pre-ploughing, post-ploughing and seed sowing surveys trait composition did not separate conspicuously into clear groups (Figure 5.4), but had significant difference in composition ($p=0.003$; Table 5.4).This means that there was a clear weed species trait shift between the fields for pre-ploughing survey versus the post-ploughing and seed sowing survey as would be expected considering the ploughing and herbicide application taken place before the post-emergence surveys. A fair stress value of 0.1 was recorded for the maize field sites pre-ploughing, post-ploughing and seed sowing surveys, giving an acceptable representation of weed traits in the ordination space.

Table 5.4 PERMANOVA results of dissimilarity in weed trait assemblages across maize field sites at P<0.05.

MF sites	T	P (perm)
MV vs BM	3.417	0.001
MV vs MJN	2.135	0.002
MV vs T	3.025	0.001
BM vs MJN	2.001	0.004
BM vs T	1.541	0.057
MJN vs T	1.317	0.115
Pre vs Post emergence	-	0.003

MF= Maize fields; MV= Baziya Makaula; MJN=Baziya Jojweni; BM= Baziya Mission; P-values in red indicate significant difference at P<0.05.

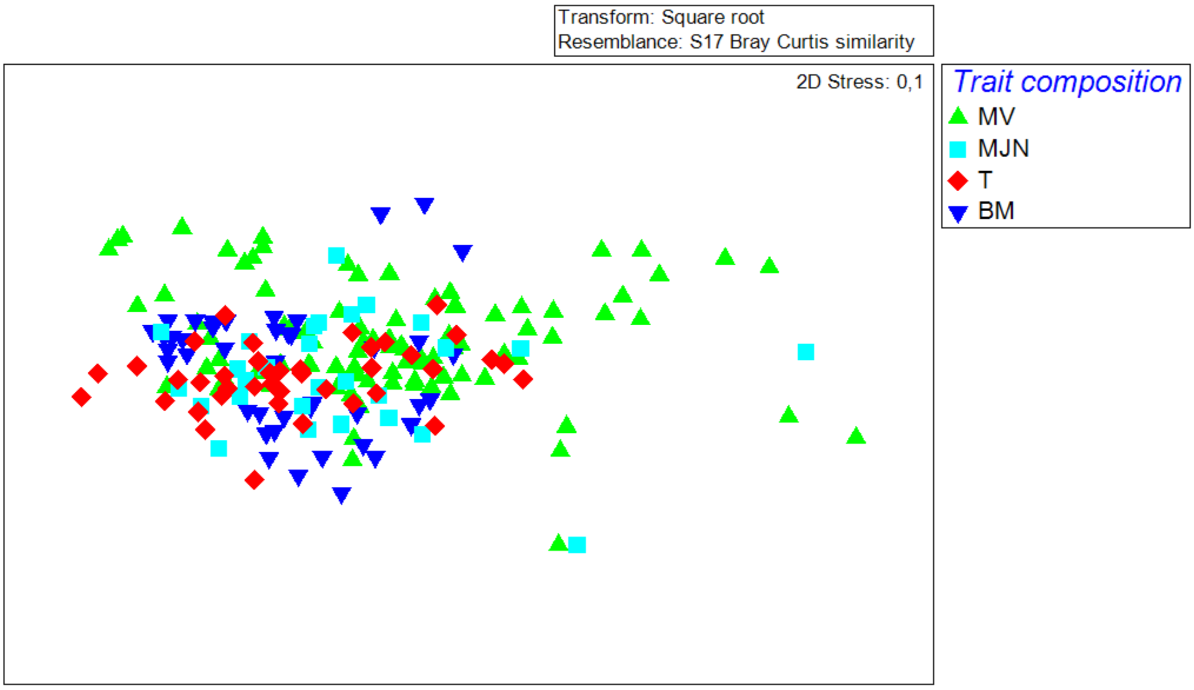

Figure 5.3 Non-metric Multidimensional scaling (NMDS) ordination of the weed functional traits composition illustrating variation between the maize field sites. The significant difference was p=0.001.

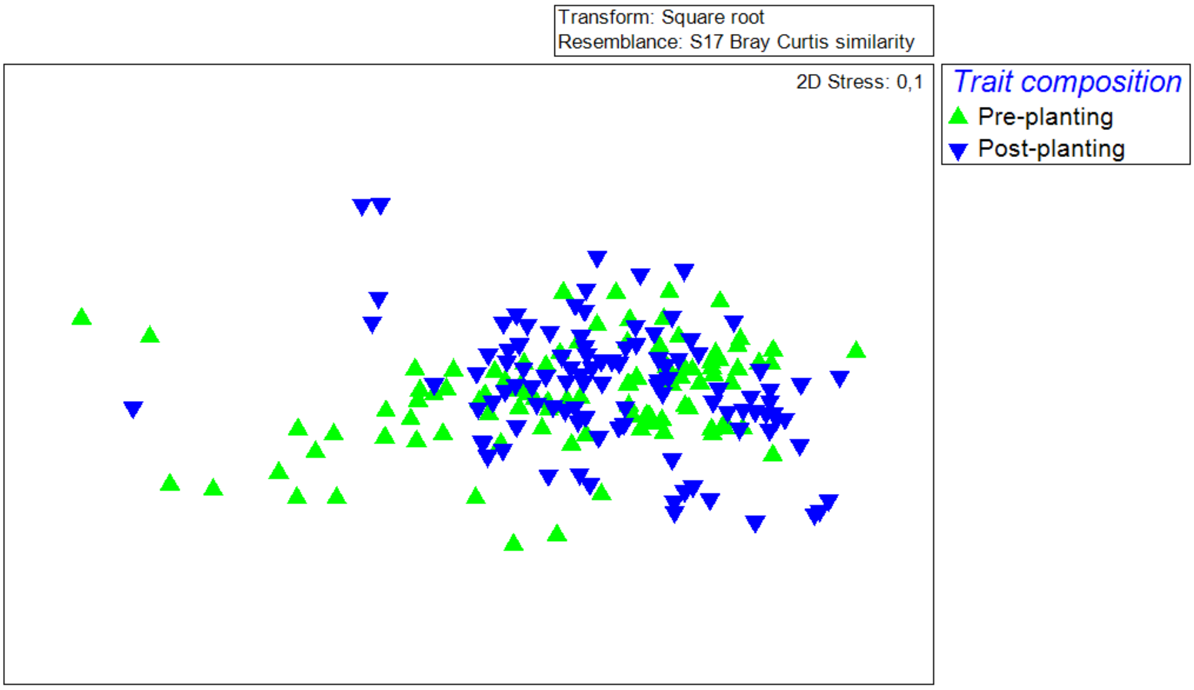

Figure 5.4 Non-metric Multidimensional scaling (NMDS) ordination of the weed functional trait composition illustrating grouping of the maize fields for pre-ploughing survey (pre-planting) and post-ploughing and seed sowing survey (post-planting).

5.3.4 Weed traits richness across maize field sites for pre-ploughing, post-ploughing and seed sowing survey

Weed traits richness between maize field sites showed a significant difference ($p<0.05$) between MV and T sites only (Figure 5.5). T site had the highest species trait richness compared to MV with the lowest. However, the other sites did not differ significantly (Figure 5.5). No significant difference ($p>0.05$) in trait richness was observed for pre-ploughing, post-ploughing and seed sowing survey (Figure 5.6). This was predicted as lower trait richness was expected for pre-ploughing surveys because the fields had not been ploughed and no herbicides had been applied which dilutes the species pool and associated traits.

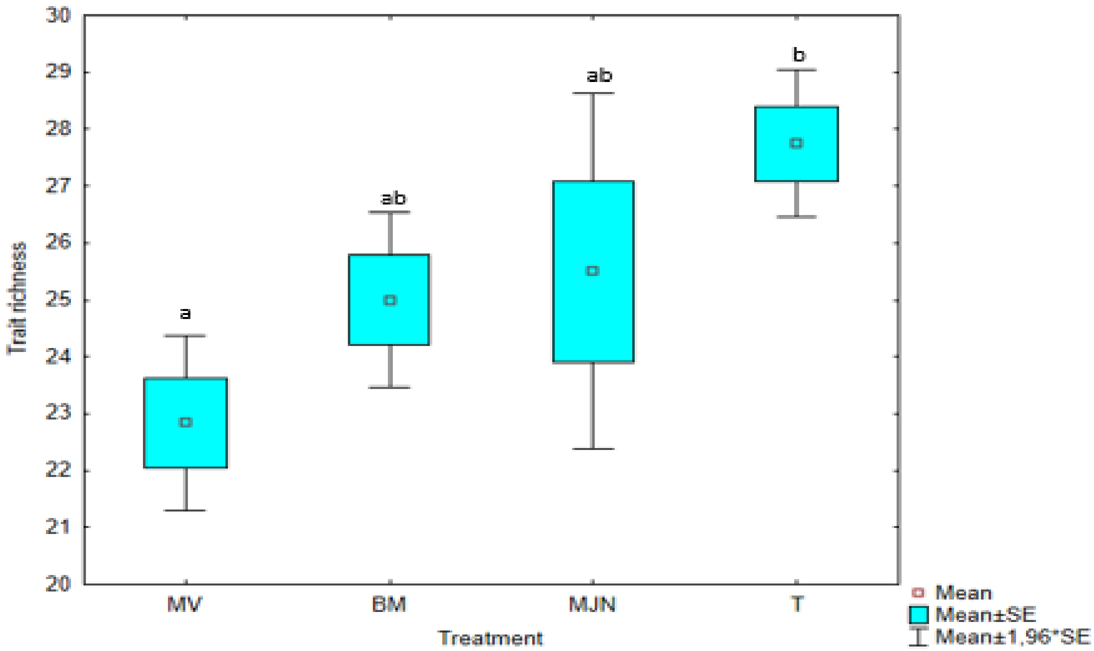

Figure 5.5 Weed trait richness of maize field sites. Different letters represent significant difference at p<0.05.

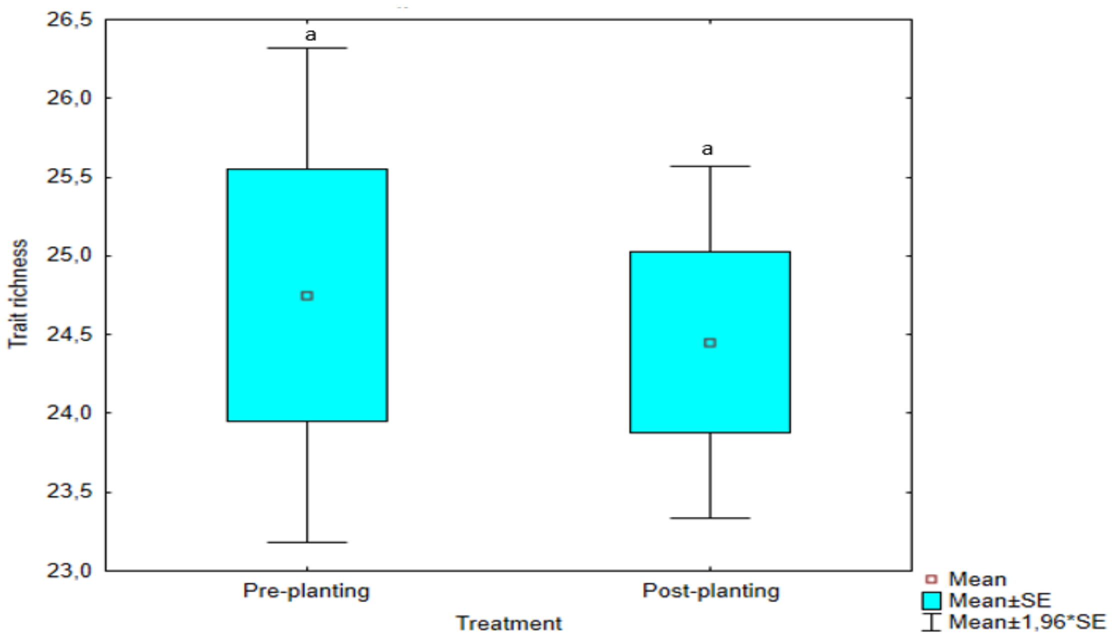

Figure 5.6 Weed traits richness of pre-ploughing survey (pre-planting) and post-ploughing and seed sowing survey (post-planting) in maize fields. Different letters represent significant difference at p<0.05.

5.3.5 Response of the weed species traits towards disturbance in terms of persistence

Redundancy Analysis (RDA) of the ten PFTs in response to herbicide use of pre-ploughing, post-ploughing and seed sowing surveys revealed a strong correlation of PFTs 9 and 10 towards pre-planting (Figure 5.7). PFTs 3 and 5 showed a strong correlation to post-planting (Figure 5.7). PFTs 3 and 5 had the longest vector towards post-ploughing and seed sowing survey (post-planting) and they consisted of annual species. They both have specialised carbon storage organs with unassisted seed dispersal traits. PFTs 9 and 10 were closely related and linked towards pre-ploughing survey (pre-planting) and they consisted of perennial species. They both have specialised carbon storage organs with assisted seed dispersal as the main shared traits.

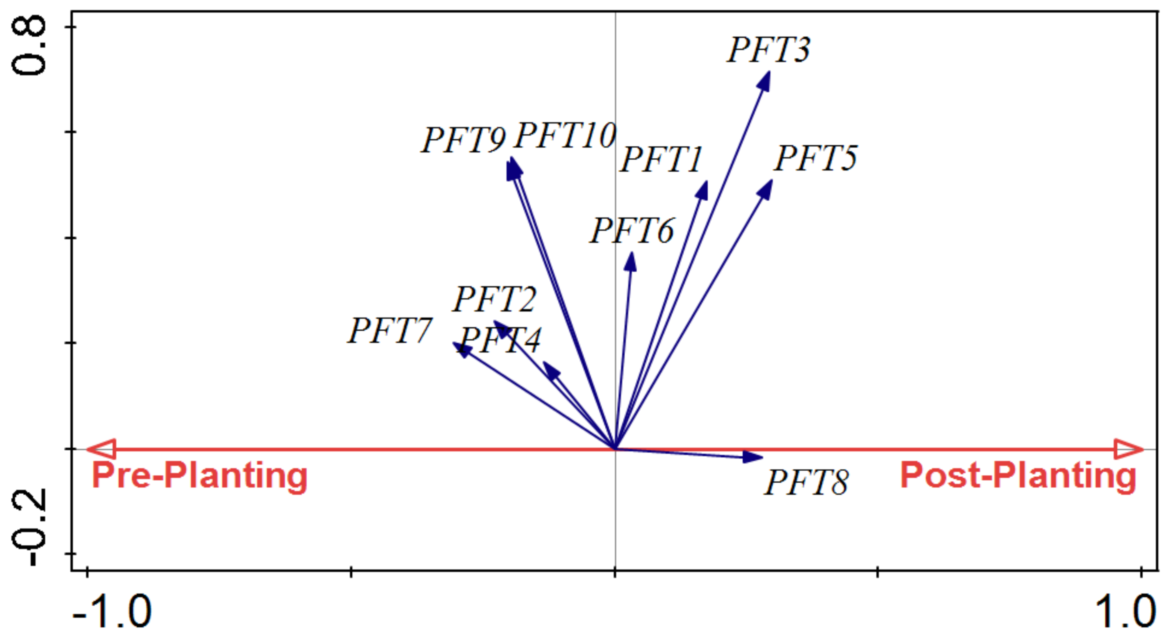

Figure 5.7 Redundancy Analysis (RDA) of plant functional types data in response to pre-planting and post-planting which refers to pre-ploughing, post-ploughing and seed sowing survey.

Trait and environmental data used for the RDA (Figure 5.7) had response and explanatory variables of 10 and 2 respectively, with types composition and general analysis. The results showed a total variation of 5827.91 and explanatory variables accounted for 5.8% (Table 5.5), however, adjusted explained variation was 5.3% with a constrained analysis. The test on all axis had pseudo-F=12.7, P=0.002.

Table 5.5 Eigenvalues for axis 1 and axis 2 with explained variations for both axis respectively.

Statistic	Axis 1	Axis 2
Eigenvalues	0.06	0.21
Explained variation (% cumulative)	5.8	27.19
Pseudo-canonical correlation	0.61	<0.005

To better understand the effect of herbicide application in weeds, the abundance of species in each PFT were compared between pre-ploughing, post-ploughing and seed sowing surveys. Annual plant species count was substantially higher for PFTs 1 (P=0.009), 3 (P=0.017) and 5 (P=0.003), and lower for PFT 2 (P=0.008) during the post-ploughing and seed sowing survey (Table 5.6; Figure 5.8). However, perennial weed species counts showed that PFT 8 had greater number of individuals in post-ploughing and seed sowing survey, although non-significant (Table 5.7; Figure 5.9). All the other perennial PFTs showed a non-significant decrease in weed abundance. The species of PFTs 1, 3, 5 and 8 can therefore be considered to hold traits that enable them to overcome both ploughing and herbicide application post-emergence (Table 5.7). Considering that significant increases were only reported for annual weeds, the following species are considered as tolerant, or best adapted, to ploughing and herbicide application post-emergence: *Amaranthus viridis* L., *Bromus diandrus* Roth, *Bulbostylis humilis* (Kunth) C.B.Clarke, *Chenopodium carinatum* R.Br., *Conyza bonariensis* (L.) Cronquist, *Gisekia pharnacioides* L., *Hibiscus trionum* L., *Indigofera spicata* Forsk., *Ipomoea purpurea* (L.) Roth, *Medicago polymorpha* L., *Oncosiphon piluliferum* (L.f.) Källersjö, *Solanum retroflexum* Dunal and *Solanum rigescens* Jacq.

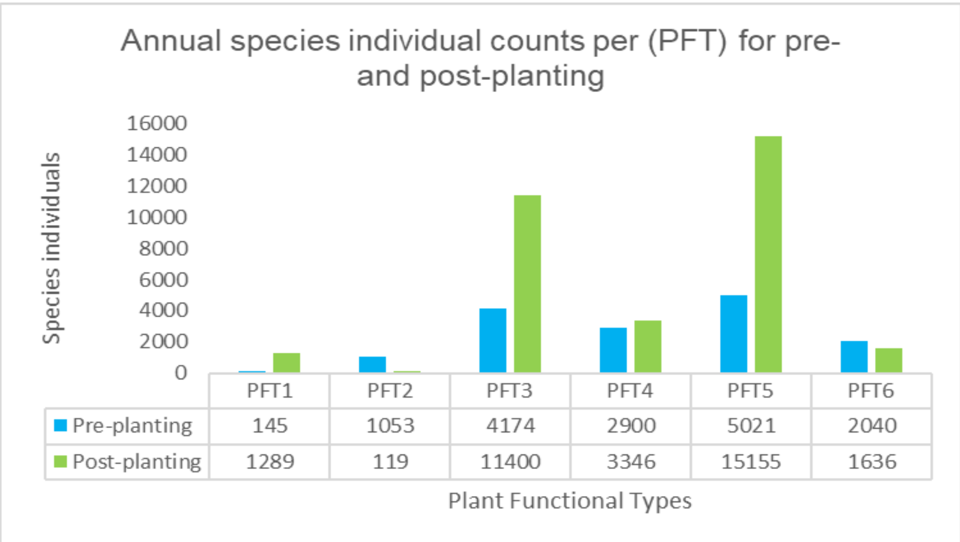

Figure 5.8 Annual species individual counts per plant functional type for pre-planting (pre-ploughing survey) and post-planting (post-ploughing and seed sowing survey).

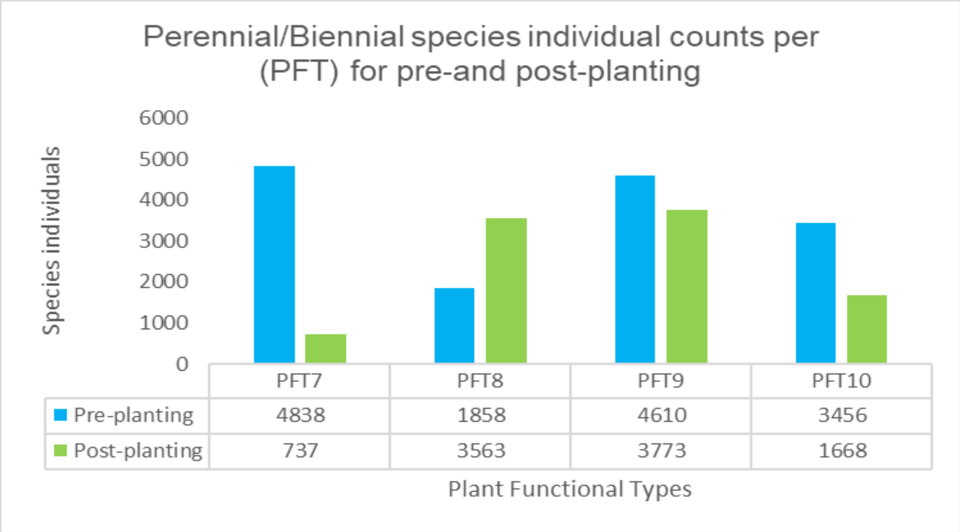

Figure 5.9 Perennial weed species individual counts per plant functional type for pre-planting (pre-ploughing survey) and post-planting survey (post-ploughing and seed sowing survey).

Table 5.6 Plant functional types of T-test results for pre-ploughing, post-ploughing and seed sowing survey in Statistica.

Variable	Mean	t-value	df	P	Valid N	Std.Dev.	F-ratio Variance	P Variance
PFT1-Pre	1.394	-2.601	206	0.009	104	7.683	30.517	<0.01
PFT1-Post	12.394				104	42.446		
PFT2-Pre	10.125	2.663	206	0.008	104	34.077	54.299	<0.01
PFT2-Post	1.144				104	4.625		
PFT3-Pre	40.134	-2.385	206	0.017	104	108.438	6.503	<0.01
PFT3-Post	109.615				104	276.539		
PFT4-Pre	11.471	1.567	206	0.118	104	42.59	2.806	<0.01
PFT4-Post	3.846					25.42		
PFT5-Pre	48.278	-2.922	206	0.003	104	100.933	10.346	<0.01
PFT5-Post	145.721					324.660		
PFT6-Pre	19.615	0.421	206	0.674	104	74.676	1.699	<0.01
PFT6-Post	15.730					57.281		
PFT7-Pre	62.932	1.155	206	0.249	104	169.276	1.058	0.773
PFT7-Post	35.413					174.168		

Table 5.6 Plant functional types of T-test results for pre-ploughing, post-ploughing and seed sowing survey in Statistica continued.

Variable	Mean	t-value	df	p	Valid N	Std.Dev.	F-ratio Variance	P Variance
PFT8-Pre	17.865	-1.819	206	0.070	104	63.501	1.093	0.650
PFT8-Post	34.259					66.409		
PFT9-Pre	44.326	0.673	206	0.501	104	74.706	1.659	<0.01
PFT9-Post	36.278					96.237		
PFT10-Pre	33.231	1.507	206	0.133	104	108.620	6.831	<0.01
PFT10-Post	16.038					41.557		

PFT= Plant functional types; Std.Dev= Standard deviation; A negative t-value means a reversal in the directionality of the effect, which has no bearing on the significance of the difference between types.

Table 5.7 T-test outcomes of the increase or decrease of species abundances of Plant Functional Type from pre-ploughing, post-ploughing and seed sowing surveys and as supported by significance.

Plant Functional Type (PFT)	Increase/Decrease	Significant difference P<0.05
PFT1	Increase	√
PFT2	Decrease	√
PFT3	Increase	√
PFT4	Increase	X
PFT5	Increase	√
PFT6	Decrease	X
PFT7	Decrease	X
PFT8	Increase	X
PFT9	Decrease	X
PFT10	Decrease	X

√ = represent a significant difference; X= represent a non-significant difference

Ten weed indicator species having high abundance are considered in terms of the three important traits known to be problematic in crop fields (Table 5.8). *Amaranthus viridis* had the highest average sum of 62.77, followed by *Chenopodium carinatum* with an average sum of 36.83 (post-ploughing and seed sowing survey). When average sum of pre-ploughing, post-ploughing and seed sowing survey were compared, eight out of ten weed species showed an increase for post-ploughing and seed sowing survey and flagged as herbicide resistant (Table 5.8).

Table 5.8 Top ten most abundant weed species in herbicide tolerant maize fields and the traits associated with pre-ploughing, post-ploughing and seed sowing surveys average sum.

Scientific name	Average sum	Ave Sum Pre	Ave Sum Post	Herbicide resistant	PFT	Traits		
						Carbon storage	Nitrogen fixing ability	Life span
***Amaranthus viridis* L.**	62.77	33.61	91.93	√	3	Specialised storage organ	N-Fixer	Annual
***Bromus diandrus* Roth**	10.70	6.31	15.08	√	3	Specialised storage organ	N-Fixer	Annual
***Chamaesyce prostrata* (Aiton) Small**	22.38	16.44	28.32	√	4	Specialised storage organ	N-Fixer	Annual
***Bulbostylis humilis* (Kunth) C.B.Clarke**	16.69	9.95	23.44	√	5	Specialised storage organ	Non-N-Fixer	Annual
***Chenopodium carinatum* R.Br.**	36.83	10.94	62.73	√	5	Specialised storage organ	Non-N-Fixer	Annual
***Ipomoea purpurea* (L.) Roth**	32.59	12.14	53.04	√	5	Specialised storage organ	Non-N-Fixer	Annual
***Sonchus asper* (L.) Hill**	13.67	16.86	10.48	×	6	Specialised storage organ	Non-N-Fixer	Annual
***Cotula heterocarpa* DC.**	20.14	40.20	0.09	×	7	Specialised storage organ	N-Fixer	Perennial
***Cyperus* spp. L.**	25.36	17.33	33.39	√	8	Specialised storage organ	N-Fixer	Perennial
***Tephrosia capensis* (Jacq.) Pers.**	16.60	7.75	24.45	√	9	Specialised storage organ	Non-N-Fixer	Perennial

√= represents the ability to resist herbicides; ×= represents the inability to resist herbicides

5.4 Discussion

5.4.1 Weed traits composition in herbicide tolerant maize fields

Ecosystem functions are known to be affected by species composition (Liu *et al*., 2018; Carrick and Forsythe, 2020), functional trait composition of the community and trait diversity (Liu *et al*., 2016; Botha *et al*., 2017). This study found that weed species trait composition differed significantly between majority of the maize field sites for the pre-ploughing, post-ploughing and seed sowing surveys. This trend is consistent with Hejda *et al*. (2019), as their study revealed disturbance to be one of the main influences on species traits composition. Differences in traits can maintain diversity in community by reducing competition (Kraft *et al.,* 2015). However, where the similarities and dissimilarities were detected, these can be viewed to be unexpected. Firstly, some sites were quite similar, in this case, sites that were far apart and probably expected to differ considering the different soil and climate conditions at these sites (Hejda *et al*., 2019). This could be because the correct herbicide protocol was followed, and worked, therefore overriding the environmental parameters. Although soil and climate were different, these sites harboured plant traits that were similar compositionally. This means that plants in these fields had specific, shared traits that enabled persistence in fields despite the herbicide disturbance effect.

Secondly, assemblages that differed between sites in close proximity were also unexpected, as these maize field sites used identical herbicides. They should have similar traits, hence, their traits to persist in the face of the disturbance of herbicide application should have been similar but were not. A possible reason to explain this difference could be the incorrect and overuse of herbicides applied in the fields in terms of litres per hectare (Gage *et al*., 2019). Also, the mixing ratio, time and weather do matter and influence the response of plant species towards herbicide application (Gage *et al*., 2019). Therefore, a more tangible reason for differences could be that the herbicides were applied in different days or weeks apart per site, with some erratic weather in between. Therefore, the tracking of exact times and weather conditions during herbicide applications is recommended in future surveys. This could assist in understanding the skewed, yet interesting patterns shown by the current findings.

The NMDS analysis for the maize fields pre-ploughing, post-ploughing and seed sowing surveys showed a significant difference of weed trait composition. This was expected because pre-ploughing surveys sampled when fields were less disturbed as it happened before ploughing, and no herbicides applied, and therefore weeds with traits associated with a more stable system would be present (Vencill *et al*., 2012). Post-ploughing and seed sowing surveys sampled when germination and herbicide application have been applied, selecting for species with either tolerance (Vencill *et al*., 2012) of rapid colonisation traits (Franzén *et al*., 2019). Therefore, weed species traits enabling weeds to persist after herbicide application would be beneficial and lead to a shift in trait composition in the fields post-ploughing and seed sowing.

5.4.2 Weed traits richness in herbicide tolerant maize fields

Traits richness between pre-ploughing, post-ploughing and seed sowing surveys did not differ significantly. Meaning that the richness of plant traits does not depend on whether herbicide use disturbance has occurred or not. Species in the disturbed environment are related to the impact on trait richness (Travlos *et al*., 2018) as there was a difference between disturbed and undisturbed environment in the study by Hejda *et al*. (2019). Findings of this study contradicts a study by Botha *et al*. (2017) who reported higher traits richness inside the maize fields. Therefore, an increase in trait richness in maize fields can be ascribed to the number of traits measured or is due to an increase in abundance of species supported by specific traits such as carbon storage organs.

5.4.3 Plant functional types in herbicide tolerant maize fields

The findings of this study showed a higher number of annual weed functional types compared to perennial weeds. This is because when perennials are periodically disturbed by herbicides and suppressed, they are replaced by fast-reproducing annual species that colonize in abundance (Franzén *et al*., 2019). In contrast, annual weeds bloom continuously throughout the season, but only live (survive) when conditions are favourable (Nichols *et al*., 2015). Hence, they are dominant in herbicide disturbed fields because they use resources efficiently, flower quickly, set seed and disperse between ploughing and herbicide application events (Petit *et al*., 2012). Botha *et al*. (2017) also found that maize fields favoured annual species with long range dispersal trait. These findings are supported by the results from Siebert *et al*. (2021), that reported an annual, nitrogen fixing ability of forbs as favourable after abiotic disturbance such as fire, herbivory, and drought stress. Botha *et al*. (2017) found that maize fields were characterised by a greater relative abundance of herbaceous growth forms without nitrogen-fixing ability compared to the higher numbers of nitrogen-fixers in marginal and rangeland vegetation. The differentiation in relative abundance of nitrogen-fixing taxa may be the result of fertilizer application in maize fields which overrides the competitive advantage that would favour nitrogen-fixers. Nitrogen fixing is usually associated with species colonising nutrient-poor systems. This is because it is a strategy that plants use for nutrient uptake via symbiosis with nitrogen fixing bacteria present in the soil (Cornelissen *et al.,* 2003; Mus *et al*., 2016; Goyal *et al*., 2021). But plant species that have traits that are herbicide tolerant are filtered into fertilised fields, which means that the nitrogen-fixing trait does not provide a competitive advantage. Most herbicides anyways suppress nitrogen-fixing bacteria (Tian *et al*., 2010; Potera, 2007), which further removes the value of the trait but still it must be considered.

Most weed species that persisted in HT maize fields in this study had carbon storage organs. Carbon storage organs are important in the adaptability of the plant species in response to disturbances on fields. A study by Shen *et al*. (2016) showed that disturbance contributed to regulate carbon storage in plants which contributed to a diversity of traits associated with carbon storage. Plants take up carbon dioxide and turn it into sugar that becomes leaves and roots, and after plants die, they decompose. This allows them to resprout after the foliage dies out. However, this could also depend on the life cycle of the plant.

Assisted seed dispersal is one of the traits that was found to be common in this study and possibly is the reason why in the post-ploughing and seed sowing survey some PFTs still had higher abundance. Maize crop fields are dominated by weeds that spread their seeds through wind dispersal (Fried *et al*., 2019). Mechanism for wind dispersal was found in association with high disturbance levels. A study by Tainton (1999) showed that adaptation of the dispersal ability is a known characteristic of pioneer species that often occupy disturbed sites. This is one of the traits that represent an adaptive strategy evolved under disturbance events of intensive management associated with maize agriculture including agro-chemical application (Liira *et al*., 2008). Petit *et al*. (2012) also concluded that weed dispersal is driven by agricultural management at multiple scales.

Predominant weed species traits in maize revealed a positive relationship between regional frequency and local abundance (Siebert *et al*., 2020). Maize crop fields were dominated with weeds that have the C4 photosynthetic pathway (Fried *et al*., 2019). This agrees with the findings of this study where some of the weed indicator species followed C4 photosynthesis, such as *B. humilis*, *C. bonariensis*, *H. trionum* and *O. piluliferum*. Therefore, they can use the gathered compounds to extract more carbon dioxide from the air, helping them to prevent water loss. C4 pathway trait is common for the mentioned weeds and maize (Fried *et al*., 2019), suggesting that C4 weed species should be able to adapt to the disturbance in a similar way to the dominant crop (Fried *et al*., 2019).

A study by Fried *et al*. (2019) showed that species of the Poaceae, and those with triazine-resistant populations, were more abundant in maize crop fields. This is because most grasses can disperse seeds for germination at a high rate. In this study, only two indicator weeds were from the Poaceae, namely *B. diandrus* and *E. coracana.* The two species also had specialised carbon storage organs as the other main trait. Lososová *et al*. (2008) reported specified flowering season as one of the main advantageous weed traits in cultivated fields of Czech Republic. This is in line with this study were perennial PFT's have specified flowering seasons (summer, autumn, winter, or spring). Similarly, to a study by Fried *et al*. (2019) for perennials.

5.4.4 Herbicide tolerant weeds in maize fields

Fried *et al*. (2019) reported the top five species, *C. album, E. crus-galli* (L.) Beauv, *Persicaria maculosa* Gray, *Persicaria lapathifolia* (L.) Delarbre and *A. retroflexus* to have traits adaptive to herbicides. Although these weed species were not found in this study, they showed similarity in plant families, namely Amaranthaceae, Poaceae and Polygonaceae, and traits reported in the maize fields of this study. These species showed common traits like those of this study, and they included dicotyledon, annual life form, C4 photosynthetic pathway and specified flowering season. Findings of this study showed an increase in number of weed species traits from pre-ploughing, post-ploughing and seed sowing surveys. This confirms the filtering of species with a wider range of traits for persistence under agricultural disturbance, in this case which is herbicide application (Kang *et al*., 2016). Traits responsible for the strong correlation towards post-ploughing and seed sowing in this study for perennial species included the nitrogen fixing trait, specialised carbon storage and assisted seed dispersal. Some of the documented weeds species were correlated to post-ploughing and seed sowing in this study were: *A. viridis, B. diandrus, B. humilis, C. bonariensis, C. carinatum, C. congestus*. *C. esculentus C. species, P. amatymbica, H. trionum, I. purpurea, I. spicata, M. polymorpha, O. piluliferum*. *S. retroflexum*, *S. rigescens*. This suggests that some weed species had traits that are adapted to the disturbance and can persist after herbicide application and they have not yet been confirmed to be resistant to certain herbicides by other studies. A list of species with similar genus and family to the species found in this study is given in Chapter 2, section 2.4.1 (Table 2.7). The section details species studied in maize fields and some were found to be resistant to herbicides.

Conclusion

Maize fields are agroecosystems that involve diverse disturbances through ploughing and herbicide applications. This study described the specific patterns of plant functional types and specific weed functional traits associated with maize fields pre-ploughing compared to post-ploughing and seed sowing. Relating to the hypothesis of this study, weed species in HT maize fields share similar traits and there are no observations of specific functional types that are prone towards the development of herbicide tolerance, because similar herbicide protocol for weed control per season was used. In response to agricultural disturbance, HT maize fields did not share similar functional traits throughout, but fields generally shared some similarity in traits. However, where these similarities and dissimilarities were detected, respectively, it contradicted the initial expectations for sites that were located far from each other. Ideally, these should have differed considering the different soil and climate conditions at the respective sites. Although the mechanisms responsible for this observation are not fully understood, it is likely that the use of the correct herbicide protocol was followed; and this contributed to the suppression of environmental parameters that would have dictated otherwise. Weed traits pre-ploughing differed compositionally from post-ploughing and seed sowing. This was expected because pre-ploughing surveys were less disturbed as it is when the fields are not ploughed, and no herbicides applied. Therefore, weeds with traits associated with a less disturbed system would be present. Hence, the hypothesis of this study was supported. Future studies could focus more on a robust seasonal approach, supplemented by constant monitoring of various environmental variables to allow for a thorough understanding of trait composition and richness patterns of weeds in HT maize fields.

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CHAPTER 6: The effect of herbicide application for weed control in fields planted with herbicide tolerant maize

Abstract

Weeds are one of the major constraints in agroecosystems, and they need to be controlled for sustainable and profitable crop production. This study was conducted in selected herbicide tolerant (HT) maize fields in the Oliver Tambo District, Eastern Cape Province. The study covers three HT maize planting seasons to evaluate herbicide application for weed control. Field surveys were conducted during 2017/18, 2018/19 and 2019/20 to study the effect of herbicide use on weed dynamics and functionality in terms of traits in HT maize varieties NK603xMON810 (season one) and MON89034 (seasons two and three). These are stacked varieties and confer insect resistance and herbicide tolerance. Per season, more than four types of herbicides were used in a 200L mixing ration for application on the fields. 200L was used per 1ha of HT maize planted. Glyphosate was used as a pre- and post-emergence herbicide on growing weed species because it is a non-selective herbicide. Maize varieties were not the same across the seasons and the herbicide protocol was also not consistent across the three seasons. Season one used different combinations compared to the other two seasons, for both pre- and post-emergence herbicide applications. Seasons two and three differed in that season three did not apply post-emergence herbicides. The study findings are therefore not based on a controlled field experiment, but merely assesses weed control over three seasons according to the protocols applied. The three seasons differed significantly ($p<0.05$) overall in species richness when tested with One-way ANOVA analysis. Season one differed significantly from season two ($p<0.01$), and it also differed significantly from season three ($p<0.01$). However, when we consider pre-ploughing surveys separately, then weed species richness and functional types (FTs) differed significantly ($p<0.01$) for season one compared to seasons two and three (these seasons showed no significant differences). This can be ascribed to the latter seasons having a similar herbicide protocol. Post-ploughing and seed sowing for seasons one and three differed significantly ($p<0.01$) in terms of weed species richness and FTs. This is best explained by season three not having a post-emergence herbicide application, leading to possible increase in weed species abundance. T-test results showed a significant difference ($p<0.01$) between season two and three with decreasing mean species richness in post-ploughing and seed sowing surveys, but the abundance was still high. Results showed that the herbicide mixture and rotation influenced the total species richness and abundance. As this study sought to test two hypotheses: 1) herbicide application rotation control weeds effectively; and 2) herbicide application leads to a weed shift over time. Given the findings, the first hypothesis was rejected as herbicide application in HT maize fields were found not to be controlling/suppressing weeds. NMDS analysis did not show significant differences for weed species or FT composition after either pre-ploughing, post-ploughing and seed sowing surveys for all three seasons. Hence, the second hypothesis was also rejected. Therefore, changing herbicide protocol every season does not assist in controlling weed species.

Keywords: Agroecosystem, Herbicide, HT maize, Pre- and post-ploughing, Seed sowing surveys, Weed shift

6.1 Introduction

Increased human population growth globally has led to significant agricultural land use expansion as more land for cultivation is needed (Koerner *et al.*, 2014; Veblen *et al.*, 2016; Zerbo *et al.*, 2016). Abiotic factors, such as drought, fire, overgrazing and herbicide use, to the above-mentioned expansion are predicted to act as disturbances in agricultural lands. Understanding the effects of these disturbances on agricultural lands and weed species that compete for resources with agricultural crop production over seasons is essential for the management of agroecosystem functionality (Trichard *et al*., 2013). Agroecosystem functionality can only be achieved if farmers have access and are able to make use of the mechanical and chemical methods to control and suppress weeds (Beckie, 2006) and the tested protocols to overcome herbicide resistance in weeds (Schütte *et al*., 2017).

Mechanical methods, such as hand weeding and hoeing, are the simplest forms of weed control (Brand *et al*., 2007). Hand weeding is only effective in small fields, but it is not practical on large areas because it is laborious and time consuming (Gollojeh *et al*., 2013; Jabran *et al*., 2015). However, chemical control such as herbicide application is a better alternative to manual weeding of large fields as it is affordable and gives better control (Chikoye *et al*., 2002, 2004). Herbicide use has therefore become popular for the majority of weed management problems (Tesfay *et al*., 2014) and proper selection of herbicides is essential for successful weed control (Kannan *et al*., 2013). However, the strong reliance on herbicide use for weed control has led to an increase in herbicide application (Benbrook, 2012). In some instances, this has resulted in a rise in herbicide resistant weeds, weed shift and numerous negative environmental impacts (Schütte *et al*., 2017; Gage *et al*., 2019). The use of herbicides can eliminate weeds at an early stage because different herbicides are used at different cultivation stages. For example, before planting, after planting, before the crops have emerged and after emergence (Reddy and Jha, 2016). For this study, the focus is only on pre-ploughing, post-ploughing and seed sowing survey.

Herbicide use provides an important weed control option to crop fields by suppressing weed species development that negatively affect crop yield. Therefore, with the use of herbicides, weed species composition, functionality and diversity is managed (Odadi *et al.*, 2011; Veblen *et al.*, 2016). Weeds are also known to develop resistance to herbicides through adaptive traits which allow them to persist (Baucom, 2019). One of the common practices to delay and combat weed resistance has been to rotate different herbicides after every season (Baghestani *et al*., 2007). However, limited research has been done to study how herbicide application practices affect herbicide resistance in weeds. Despite rotating herbicides every season, herbicide resistance has increased through the years with some weeds developing resistance to more than one herbicide (Vrbničanin, 2020). Some studies have found herbicide rotation effective, but other studies have found them ineffective against resistance, such as the case of waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer) (Beckie and Reboud, 2009; Norsworthy *et al*., 2012). The effectiveness of herbicide use in weed control remains less studied in SA. At the same time, this remains an important topic as research on the effectiveness of herbicides and rotation for weed control will allow for better prediction of weed response to herbicide change under different seasons. This could potentially allow for better management of weeds in agricultural fields for maximum crop production and biodiversity protection (Gao *et al.*, 2009; Porensky *et al.*, 2013; Treydte *et al.*, 2013; Swemmer *et al.*, 2018).

Another practice, independent of rotation, is the mixing of multiple herbicides (mix use). Mixed use of herbicides such as chloroacetamide and atrazine was reported to successfully control weeds, such as waterhemp, that has developed resistance in some regions of the US (Gaines *et al*., 2020). Some farmers believe that the mixed use of herbicides is more effective than rotations, in suppressing resistance evolution through herbicide selection. Most effective weed management is now based on the introduction of combinations of new herbicides with new modes of action (Busi *et al*., 2019).

The aim of this study was to quantify weed control and shifts due to herbicide application on HT maize fields in the selected areas of the Oliver Tambo District Municipality of the Eastern Cape Province. This was not an experimentally controlled study but based on quantitative observations made over three growing seasons. The specific objectives were to detect changes in weed species and functional type (FT) diversity (weed control) and composition (weed shifts) due to the existing pre- and post-emergence herbicide application protocols (not fixed seasonally). The hypothesis of this study was that herbicide application rotation control weeds effectively (Anderson, 2007). Another hypothesis states that herbicide application leads to a weed shift over time (Qasem, 2013).

This study compares weed species and FTs over seasons in response to a three-season herbicide application protocols. Therefore, it is predicted that diversity and composition of weeds and FTs will differ across seasons due to annual changes affected by the variable herbicide application protocols. Season one is expected to differ from seasons two and three because the herbicide application protocol was changed, but season two and season three will also differ from one another because the post-emergence herbicide application was not implemented in season three.

6.2 Materials and methods

6.2.1 Inventory of herbicides applied over three seasons

An inventory of herbicides (spraying protocol) was obtained from designated extension officers from the study area's Mthatha GrainSA office. The same herbicide application protocol and spraying schedule from GrainSA was followed across all sites (maize fields). Identification of herbicides was also carried out on site using the product labels, with assistance provided by the GrainSA personnel. Table 6.1 outlines the herbicides applied at Baziya and Tsolo in HT maize fields for the three seasons (2017/18; 2018/19; 2019/20). It is important to note that in some instances, insecticides and wetting agents were used in the protocols. Each herbicide spraying protocol was administered with a mixing ratio of 200L of water, for each hectare sprayed - using a tractor lodged boom spray. Table 6.2 gives an overview of the total hectares of HT maize fields sprayed at post-ploughing and seed sowing survey (herbicide protocols across the three seasons).

Table 6.1 Detailed listing of pre- and post-emergence herbicides applied in herbicide tolerant maize fields over three seasons (2017/18; 2018/19; 2019/20). Insecticides in italics.

Seasons	**Pre-emergence**		**Post-emergence**		**Selective (S) / Non-selective (NS)**
	Active ingredient	**Trade name**	**Active ingredient**	**Trade name**	
2017/18	Primagram gold	Primagram gold	Primagram gold	Primagram gold	S
	2,4-D Amine	2-4-D Amine 480 SL	2,4-D Amine	2-4-D Amine 480 SL	S
	Halosulfuron	Halo 750 WDG	Halosulfuron	Halo 750 WDG	S
	Glyphosate	PowerMax	Glyphosate	PowerMax	NS
	Sorgomil	Sorgomil	Sorgomil	Sorgomil	S
2018/19	*Alpha-cypermethrin*	*Alpha-thrin 100 SC*	*Alpha-cypermethrin*	*Alpha-thrin 100 SC*	Insecticide
	Metolachlor	Metolachlor 915 EC	Metolachlor	Metalochlor (^) EC	S
	Glyphosate	PowerMax	Glyphosate	PowerMax	NS
	Mesotrione	Cantron 480 SC	Halosulfuron	Halo 750 WDG (50g water soluble bag)	S
			Terbuthylazine	Terbuweed 600 WDG	S
			2,4-D Amine	2,4-D Amine 480 SL	S
			Adjuvant	Villa 51	Wetting agent
2019/20	*Alpha-cypermethrin*	*Alpha-thrin 100 SC*	Post-emergence herbicides were not applied		Insecticide
	Metolachlor	Metolachlor 915 EC			S
	Mesotrione	Cantron 480 SC			S
	Glyphosate	PowerMax			NS

Table 6.2 Total herbicide ratio applied into the herbicide tolerant maize fields per hectare over the three seasons.

Field names	Total number of maize fields	Total hectares sprayed	Mixing water ratio 200 L/Hectare
Baziya Makaula (MV)	10	17	3400
Baziya Mission (BM)	6	6	1200
Makaula Jojweni (MJN)	5	7	1400
Tsolo (T)	7	7	1400
Total	**28**	**37**	**7400**

6.2.2 Weed control analysis

The data set containing abundance of weed species with corresponding fields were used to calculate diversity indices per site over three seasons in PRIMER 6. This was done to determine diversity of the weed species and FT in the HT maize fields. The diversity indices were calculated for Total individuals (N) and Margalef's species traits richness (d), T-test was done in Statistica using diversity of weed species and FTs dataset for pre-ploughing, post-ploughing and seed sowing surveys to test for differences between the means of the two data groups collected at different seasons. This was done to evaluate how weeds and FT respond to different herbicide applications over the seasons in pre-ploughing, post-ploughing and seed sowing surveys. The aim was not to compare the seasons, but the different weed responses for pre-ploughing and post-ploughing in terms of abundance and richness. One-way ANOVA in Statistica with a Tukey honest significant difference (HSD) post hoc tests established the difference in weed abundance and richness diversity index values across seasons for pre- and post-emergence surveys.

6.2.3 Weed shift analysis

To illustrate how different seasons compared in terms of weed species and FT composition, NMDS, based on the Euclidean distance matrix, was performed in PRIMER 6, followed by PERMANOVA using abundance data of the 42 indicator weed species (see Chapter 5). The PERMANOVA analysis was performed with 999 permutations and type III sums of square. This was done to assess the significant differences in weed species and FTs composition between different seasons for pre- and post-emergence surveys.

6.3 Results

6.3.1 Weed and functional type diversity response to pre- and post-emergence herbicide application over seasons

Weed species richness (Figure 6.1) and FTs richness (Figure 6.2) for pre-ploughing surveys differed significantly ($p<0.05$) between some seasons (Table 6.3). Species richness for season one differed significantly from seasons two and three where a different combination of herbicides was applied ($p<0.05$), but with no difference between seasons two and three (Figure 6.1a). Species richness for post-ploughing and seed sowing survey differed significantly ($p<0.05$) between seasons one and three where no (post-emergence, Table 6.1) herbicide was applied (Figure 6.1b). And the richness for FTs of seasons one and two were significantly less than season three (Figure 6.2). In these comparisons, herbicide application was considered across seasons for post-ploughing and seed sowing surveys. However, it is also important to understand how abundance and richness of weed species and FTs change within a season during pre-ploughing, post-ploughing and seed sowing surveys.

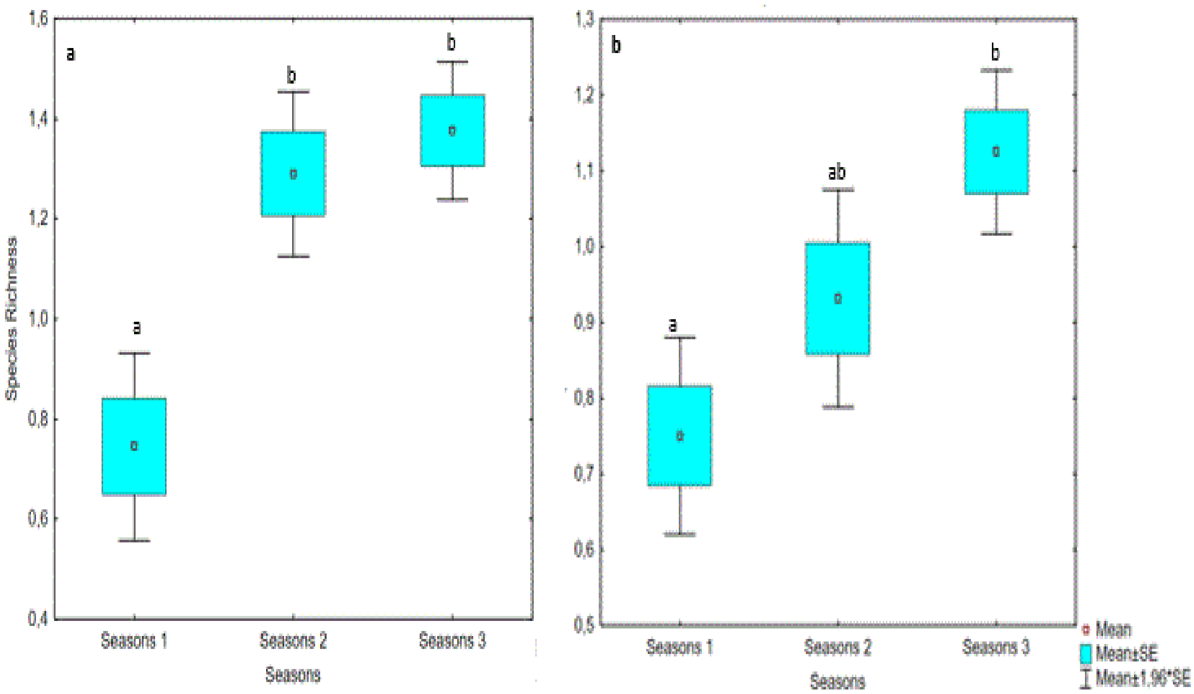

Figure 6.1 One-way ANOVA test for weed species richness pre-ploughing survey (a) and post-ploughing and seed sowing survey (b) over three seasons in response to herbicide use.

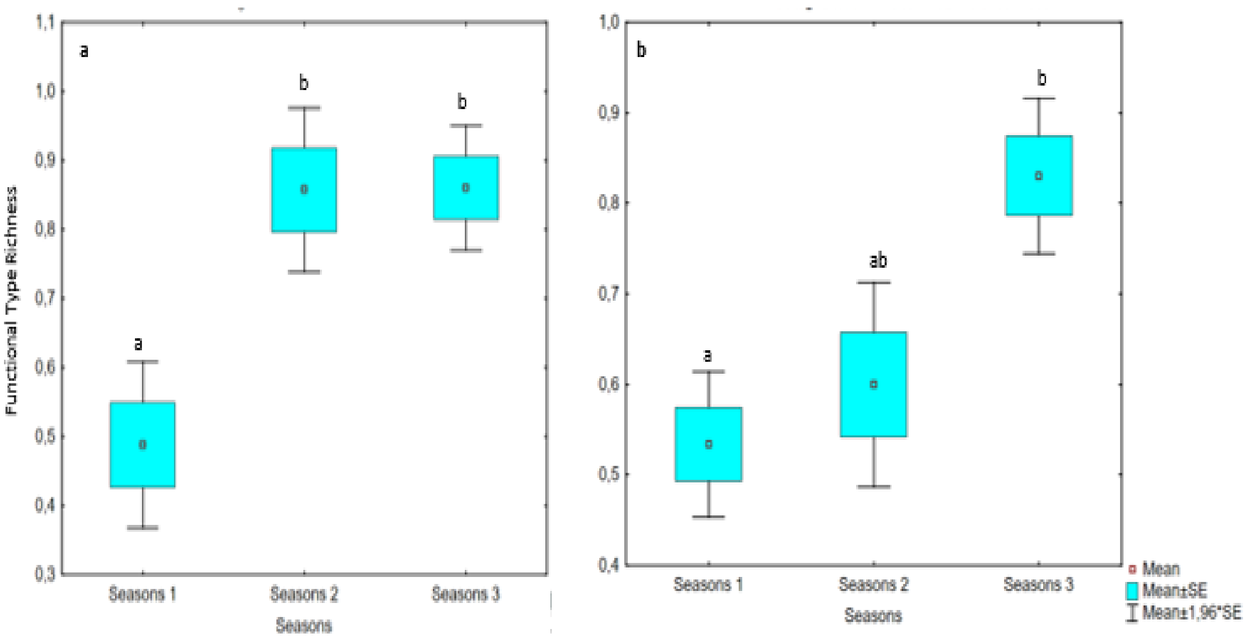

Figure 6.2 One-way ANOVA test for functional type richness pre-ploughing survey (a) and post-ploughing and seed sowing survey (b) over three seasons in response to herbicide use.

Weed species richness of seasons two and three differed significantly ($p<0.01$) for both surveys, namely pre-and post-ploughing surveys, with higher richness in pre than in post (Figure 6.3) and indicating that herbicide application was able to suppress weed species richness in the fields.

Functional types of pre- and post-ploughing differed significantly for season two, with higher trait richness in pre- than post- surveys (Figure 6.4), indicating that herbicide application had reduced FT richness, and therefore functionality, of the fields.

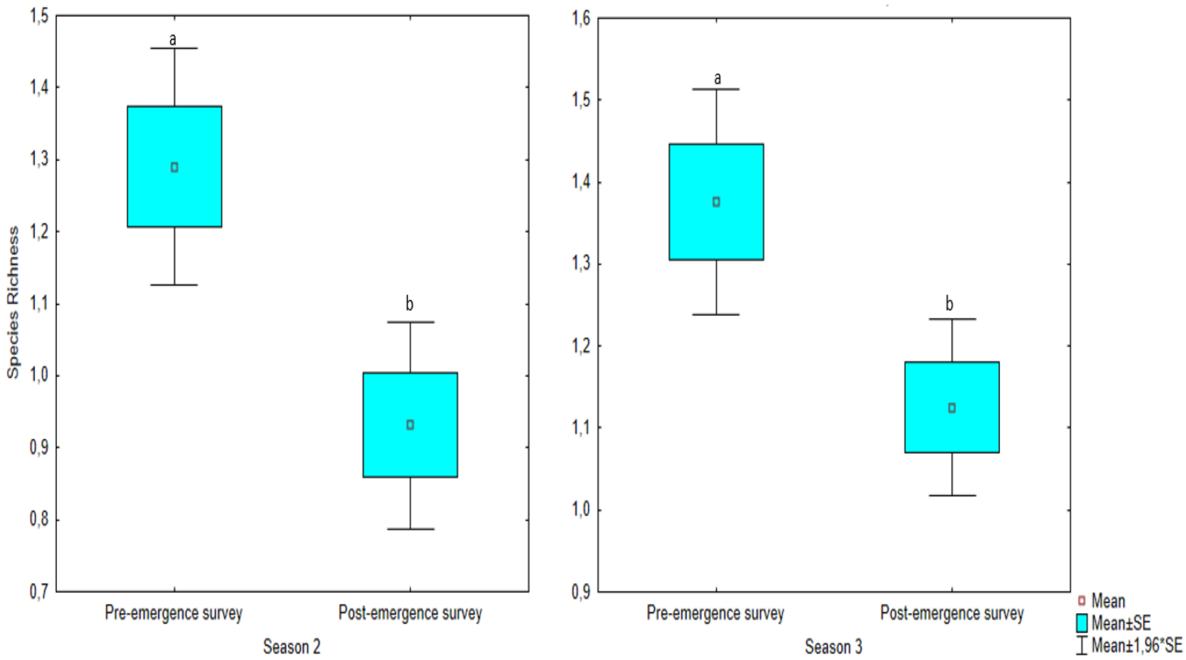

Figure 6.3 One-way ANOVA analysis of species richness for pre- and post-emergence in seasons two and three in response to herbicide application. Pre-emergence surveys represent pre-ploughing surveys, whereas post-emergence surveys represents post-ploughing and seed sowing surveys. Different letters indicate significant differences (p<0.05).

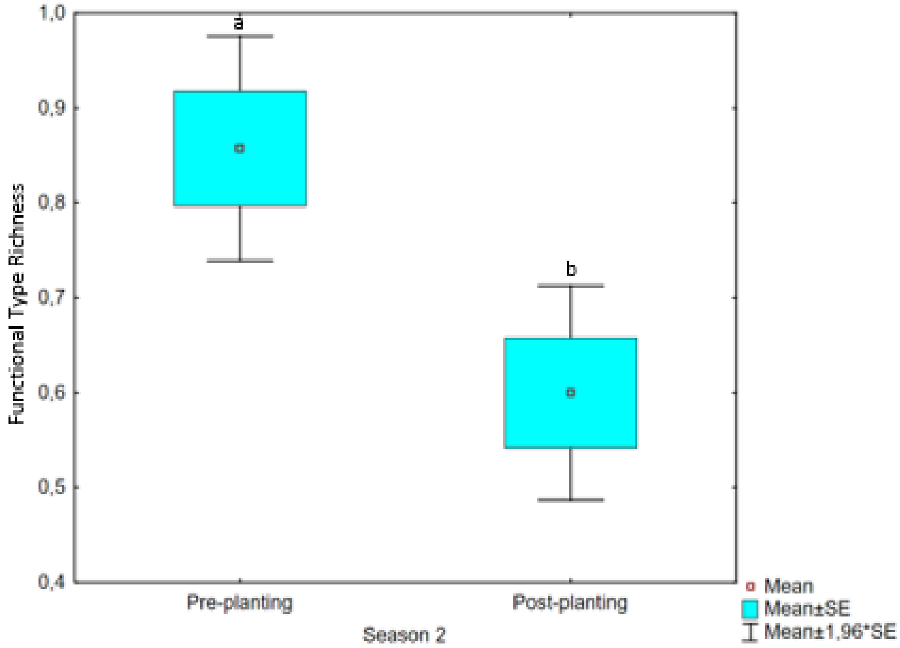

Figure 6.4 One-way ANOVA analysis of FT richness for pre- and post-emergence for season two in response to herbicide application. Pre-emergence survey represents pre-ploughing survey whereas post-emergence survey represents post-ploughing and seed sowing surveys. Different letters indicate significant differences (p<0.05).

Functional types abundance (which is the number of individuals of weed species and functional types) were similar and did not differ significantly between pre-ploughing, post-ploughing and seed sowing surveys (p>0.05; Table 6.3). However, weed abundance for the pre-ploughing survey was lower than that of post-ploughing and seed sowing survey. The negative t value for the seasons in Table 6.3 below showed an effect of herbicide use (weed abundance increased), even though the p-values did not show any significant difference. The results also suggested that herbicide use has limited effectiveness, because there was an increase in the mean values of weed abundance during post-emergence surveyed compared to pre-emergence.

Table 6.3 T-test of abundance and richness of the weed species and functional type pre-ploughing, post-ploughing and seed sowing survey over the three seasons.

Weed species richness	**Mean**	**t-value**	**Df**	**P**	**Std.Dev**	**F-ratio Variances**	**P Variances**
S1 Pre-ploughing survey	0.74	-0.04	84	0.96	0.62	2.08	0.01
S1 Post-ploughing and seed sowing survey	0.75				0.43		
S2 Pre-ploughing survey	1.29	3.21	66	0.001	0.48	1.30	0.44
S2 Post-ploughing and seed sowing survey	0.93				0.42		
S3 Pre-ploughing survey	1.37	2.81	52	0.01	0.36	1.63	0.21
S3 Post-ploughing and seed sowing survey	1.12				0.28		
Functional type richness							
S1 Pre-ploughing survey	0.48	-0.61	84	0.53	0.40	2.26	0.01
S1 Post-ploughing and seed sowing survey	0.53				0.26		
S2 Pre-ploughing survey	0.85	3.08	66	0.01	0.35	1.11	0.76
S2 Post-ploughing and seed sowing survey	0.59				0.33		
S3 Pre-ploughing survey	0.86	0.47	52	0.63	0.23	1.10	0.79
S3 Post-ploughing and seed sowing survey	0.83				0.22		
Weed abundance							
S1 Pre-ploughing survey	237.51	-1.45	84	0.15	301.48	2.28	0.01
S1 Post-ploughing and seed sowing survey	358.46				455.47		
S2 Pre-ploughing survey	359.64	-1.32	66	0.19	422.27	2.15	0.03
S2 Post-ploughing and seed sowing survey	529.61				620.23		
S3 Pre-ploughing survey	283.48	-0.62	52	0.53	324.25	1.33	0.46
S3 Post-ploughing and seed sowing survey	343.14				375.12		

Red font represents seasons with significant differences

6.3.2 Weed and functional type composition for pre-ploughing, post-ploughing and seed sowing survey over three seasons

NMDS analysis of weed and FT composition (Figures 6.5 and 6.6) had a relatively fair stress value of less than 0.2. NMDS analysis of weed composition for pre-ploughing (Figure 6.5a) and post-ploughing and seed sowing surveys (Figure 6.5b) over three seasons had similar groupings. Similarly, NMDS analysis of FT composition for pre-ploughing (Figure 6.6a) and post-ploughing and seed sowing surveys (Figure 6.6b) over three seasons also had similar groupings. PERMANOVA confirmed that the three seasons did not differ significantly in terms of weed species and FT composition (Table 6.4).

Table 6.4 Summary of the p values of the weed and functional type composition of pre-ploughing, post-ploughing and seed sowing survey.

Seasons	**Pre-ploughing survey p values**	**Post-ploughing and seed sowing survey p values**
Species composition		
Season one, Season two	0.662	0.830
Season one, Season three	0.654	0.941
Season two, Season three	0.574	0.997
Trait composition		
Season one, Season two	0.612	0.624
Season one, Season three	0.376	0.624
Season two, Season three	0.126	0.405

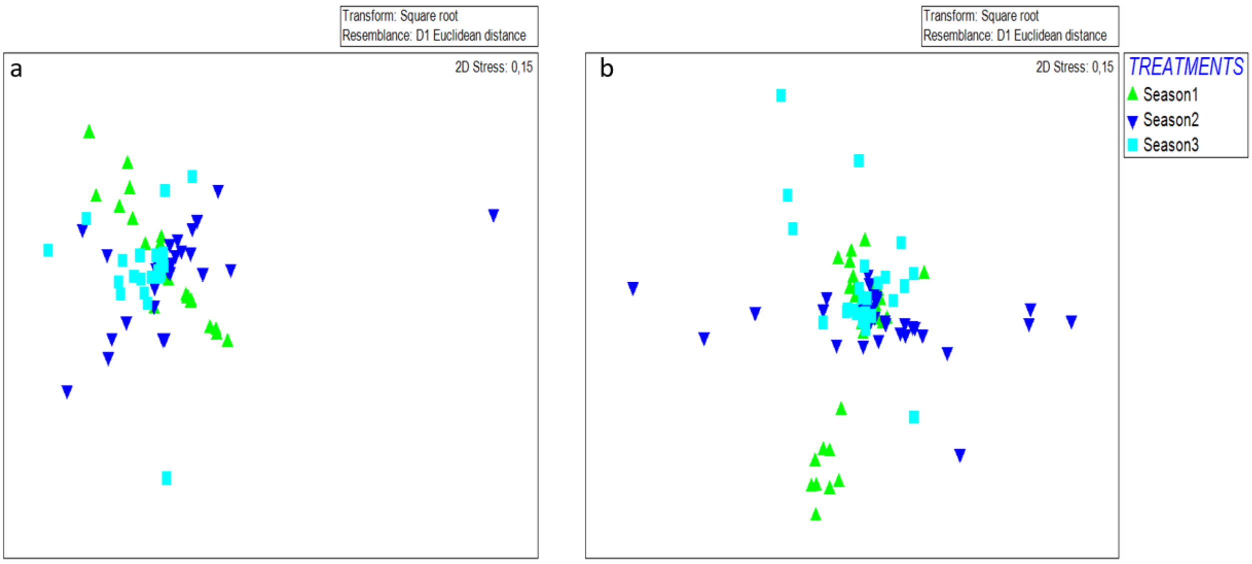

Figure 6.5 NMDS analysis of weed species composition of (a) pre-ploughing survey and (b) post-ploughing and seed sowing survey over three seasons.

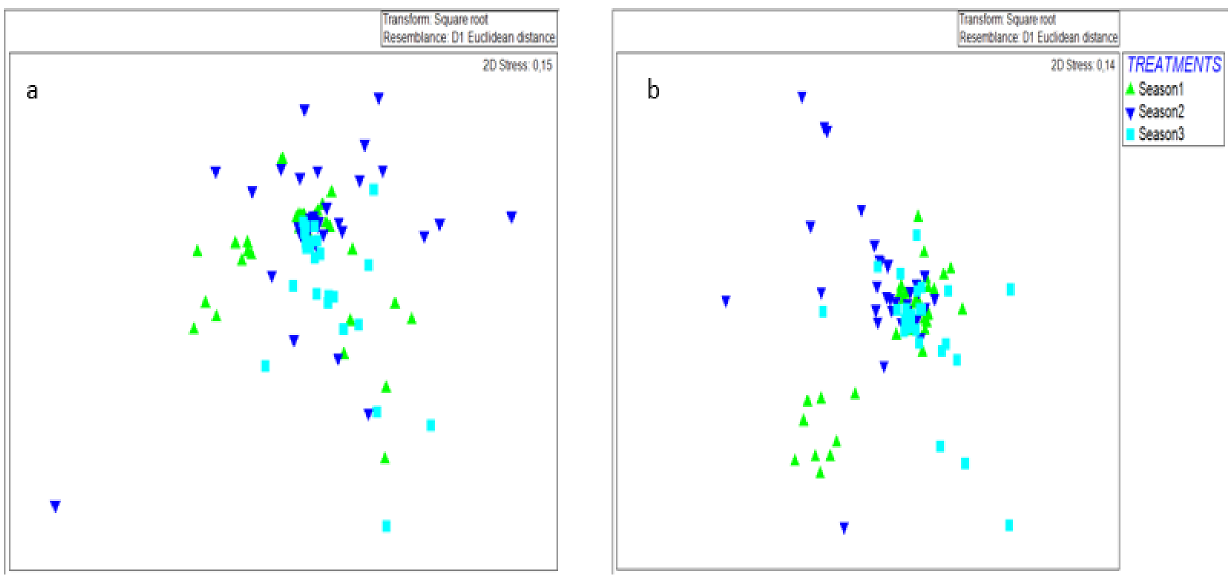

Figure 6.6 NMDS analysis of functional types composition of (a) pre-ploughing survey and (b) post-ploughing and seed sowing survey over three seasons.

6.4 Discussion

Studies of HT maize with intensive herbicide application suggest that systems with a similar herbicide application should have similar weeds in terms of composition (Norsworthy *et al*., 2012). In this study, the use of a variable herbicide application protocol over three seasons has resulted in different responses of weeds and FTs towards herbicides. Observation over three seasons showed that differing applications of herbicides was not able to control weeds effectively. Here, the expectation was that change of herbicide application protocol over seasons would result in selection against certain species that might become resistant. In contrast, it appears that some weed species might be better adapted or even showing some level of resilience towards the current variable herbicide protocol. Thus, contributing to higher abundance of certain weeds in some seasons, although this trend was not consistent for species across all three seasons.

However, no weed shifts across the maize fields in the three seasons was clear based on species composition. Hence, this farming system, which is governed by a variable three season herbicide protocol, showed a negative feedback as it did not result in consistent weed decreases over three seasons. This is because when herbicides were applied in first season, initial weed abundances decreased, but when herbicide rotation was implemented during the second season, the abundance of certain weed species increased. Therefore, suggesting that the herbicide protocol used is more geared for the controlled selection of certain weeds, and select for traits that are most advantageous for weed establishment such as specialised carbon organs, life form (annual) and specialised seed dispersal (Chapter 5). Which means that other selective herbicides are selecting for specific weed traits in the fields.

It was expected that herbicide use would significantly affect weed species composition in HT maize fields. However, results showed no significant difference over the three seasons with the use of a variable herbicide protocol. The nesting of weed species assemblages meant that the fields harboured similar weed species composition. Therefore, there was no weed shift over the three seasons due to the application of a different herbicide protocol per season. Rotation of herbicide might have prevented the occurrence of a weed shift, which means that the protocol was able to maintain a broad, inclusive weed community for the study period. This agrees with a study by Marochi *et al*. (2018), who found that rotating herbicides with different modes of action was effective in controlling weed communities. However, continuous application of the same herbicides over seasons may facilitate weed shifts (Owen, 2008), and necessitates the combination of different herbicides in different seasons (Beckie and Reboud, 2009) to prevent this. Various studies have also shown that similar herbicides become less effective when used repeatedly (Beckie and Reboud, 2009) and they could be more effective when they are rotated (use of different herbicides across seasons over time) (Norsworthy *et al*., 2012). However, this study couldn't account for this as the study period was not long enough to make a meaningful assessment of species and FTs composition.

This study showed high mean abundance of weeds in post-ploughing surveys, meaning that weed species were able to re-colonise maize fields efficiently after herbicide application. This is in contrast with a study on the effect of different pre- and post-emergence herbicides application protocols in maize in Ethiopia (Tesfay *et al*., 2014), which showed that nicosulfuron herbicide with silwet gold organosilicon adjuvant was effective in controlling weeds post-emergence. This suggests that weed species of the Oliver Tambo District Municipality had the required traits to overcome herbicide effects. Although abundances increased over time, species richness for post-ploughing and seed sowing surveys was the least in season three. This means that although the species became less, the persistent species had the required traits to overcome herbicide effects and become even more abundant. When a different herbicide protocol was used in the second season, weed species abundance decreased. However, when the same herbicides were not applied in season three, the number of weed species increased. This is an indication of weed recovery and trait response to the herbicide application protocol. Although it is not known, it is highly likely that if the same protocol continues to be used without alternating with a new protocol, weeds will become more prevalent and recover better (Schütte *et al*., 2017; Gage *et al*., 2019). However, this would require further investigation (Gressel and Segel, 1990).

Although weed species and FTs richness decreased during a single season, resulting in lower functionality, the FTs richness increased consistently over the seasons, suggesting that the weed community became more functional over time under the current herbicide use protocol. However, weed species and functional types richness for pre-ploughing, post-ploughing and seed sowing survey decreased from pre-ploughing to post-ploughing for all three seasons. Suggesting that a few weeds were able resprout and persist under herbicide application, which is advantageous towards crops in the fields as the competition could be less.

The study findings also corroborate other findings (Lagator *et al*., 2013) in terms of the effectiveness of the herbicides when it relies on more than one herbicide per season. For example, Janak and Grichar (2016) found that maize yield was higher when herbicide treatments contained more than one active ingredient. This means that more weeds were suppressed by herbicides and this resulted in less competition with crops. The finding by Janak and Grichar (2016) to control *Amaranthus palmeri* was reported to be 90% effective when the herbicides acetochlor, metolachlor, and combinations of atrazine plus acetochlor or fluthiacet-methyl plus pyroxasulfone were used. Therefore, using different herbicides with different modes of action are recommended to suppress resistant weed species. For this study, this was somewhat true for season one, going into season two, but not the case for season three as herbicides were not applied post-emergence.

Overall, important observations were made in this study in terms of the various herbicide applications/protocols. Most importantly, was the findings after each season, which suggest a different, yet unpredictable, response in both weed species and community recovery. One factor that was beyond the control of this study, was the way the herbicide mixing ration is carried out, and how the boom spray was calibrated and administered. In addition, the weather conditions could have varied considerably when the spraying was carried out. All these factors could have also played a part in the study findings.

Conclusion

The aim of this study was to quantify weed control and shifts due to herbicide application on HT maize fields. It must be noted that this was not an experimentally controlled study but based only on the observations made over three growing seasons. Weed species and functional type richness differ significantly in pre-ploughing, post-ploughing and seed sowing surveys, and across the three seasons. Pre-ploughing surveys were expected to differ from post-ploughing and seed sowing surveys, and that post-ploughing will be occupied with less weed species richness because herbicide is applied to control weeds. The richness of weeds and FTs decreased from pre- to post-ploughing suggesting a greater selection of herbicides applied into the fields were effective. This finding was expected as all the herbicides used were selective except for glyphosate. It is evident that the richness was lower in the first season and increased consequently from season one to season three as the herbicide protocol continued to be adjusted. Therefore, weed species and functional types became adapted and resilient to the herbicide protocol over seasons two and three. Relating to the first hypothesis that herbicide rotation is effective in controlling weeds

(Anderson, 2007), it can be concluded that this protocol of rotating herbicides is effective within a single season, but not across seasons. Therefore, the hypothesis is partially rejected.

Relating to the second hypothesis, it was predicted that composition of weeds and FTs will differ across seasons due to annual changes affected by the variable herbicide application protocol. Season one was expected to differ from seasons two and three because the herbicide application protocol was changed, but season two and season three were also expected to differ from one another because the post-emergence herbicide application was not implemented in season three. There was no weed shift detected, because weeds and FT composition did not differ across the three seasons. It also should be noted that the study was of too short duration, even though it covered three seasons, to make a meaningful assessment of weed species and FT composition and richness. Hence, more surveys with greater monitoring of herbicide application protocol are needed in the future for better assessment towards the trend of weed species and FTs response in herbicide protocol rotation across seasons. As it stands the second hypothesis was rejected

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CHAPTER 7: Study synthesis and limitations

7.1 Synthesis of the study

In the introduction (Chapter 1), the rationale for carrying out this study was outlined – citing the critical knowledge gap for the selected study areas when it comes to weed profiling in HT maize fields. To assist farmers towards feasible weed control, it is important to develop a better understanding of weed species diversity patterns and herbicide use in HT maize to fill the knowledge gap and establish scientifically credible baseline information for future comparisons, and to propose science-based management strategies to reduce weed impact. The main aim of the study was to provide a profile of weed populations in HT maize fields of rural farming communities in the selected areas of Oliver Tambo District Municipality, Eastern Cape Province, by comparing the composition and diversity of weed species and traits between fields and margins, and over time. This study provided evidence of the traits that could be characteristic of weeds with the ability or predisposition to become herbicide tolerant.

Maize fields are highly disturbed by ploughing and herbicides applied to control weed species, whereas M are less disturbed because no ploughing or herbicide application takes place. The first hypothesis stated that HT maize systems have the same weed species composition and diversity across fields. The results showed compositional similarity in some of the sites and the hypothesis was supported when maize fields sites were compared on their own and field margin sites on their own. This was expected because the sites were applied with similar herbicide protocol across the fields. When maize fields and field margins were compared, differences between the fields were expected because maize fields are exposed to annual exogenous agricultural disturbance that supports high weed diversity, whereas field margins had less severe endogenous disturbance and was dominated by fewer, but persistent species of high abundance. This finding is supported in a way that weeds compete with most crops for resources, hence in maize fields there is a competition between weeds and crops. Therefore, weeds of these different zones must differ in adaptation and response to disturbance. The findings of this study confirm that maize fields and field margins are characterised by different weed assemblages and diversity supporting the first hypothesis that maize fields and field margins have different weed assemblages and diversity.

The second hypothesis stated that weeds in HT maize fields share similar traits. The hypothesis that maize fields share similar traits was rejected in terms of similarity in composition. This was not expected as the fields used similar herbicide protocols and those fields were in proximity. However, the hypothesis was supported for the similarity in trait richness. Herbicides are meant to control weed species where they are applied, meaning that diversity (at times abundance) must be higher where herbicides were not applied and lower where herbicides were applied to show that indeed they are effective. This observation is further explained by the testing of hypothesis three, as it provides better context to the possible reason for the rejection of hypothesis two based on composition similarity and the support of richness similarity.

The third hypothesis stating that herbicide application is effective in weed control was partially supported. Weed species richness for pre-ploughing survey was higher than the richness of the post-ploughing and seed sowing survey. The results suggested that herbicides applied in the maize fields were able to suppress specific weed species. However, when the effectiveness of herbicide rotation over the three seasons was assessed, the outcome was different because the abundance varied across the three seasons. The abundance decreased in season one, increased in season two and then decreased in season three. Therefore, the effectiveness of the current herbicide rotation protocol, from season one, two, with no change in season three, cannot be confirmed. A more robust approach for data collection with extended seasonal observations is needed to further account for any response(s) of weed species towards herbicide rotation protocols.

Lastly, the fourth hypothesis that herbicide application leads to weed shift was not supported. There was no observed weed shift as weeds and functional types composition did not differ across the three seasons. It should also be noted that the study was limited to only three seasons, therefore restricted in terms of deriving the much-needed assessment for weed species functional type composition and richness. Hence, more surveys with greater monitoring of herbicide application protocols are needed in the future for better assessment towards the trend of weed species and functional types response in herbicide protocol rotation across seasons. Ultimately, the data presented here sets a good baseline that may be widely applicable towards the management of weed species in HT maize fields. This knowledge contributes to the current existing HT maize cultivation management strategies when it comes to the understanding and practical approach towards proactive action when dealing with disturbances and their subsequent impacts on HT maize agroecosystems.

7.2 Limitations encountered during the study

Although the limitations outlined below were encountered during the three seasons of the study, the results presented across the various chapters were achieved confidently. In addressing these limitations, all data per season was sampled to represent a great degree of variation and accommodated both nested and unequal sampling approaches. The limitations encountered during the study are as follows:

- The plant (weed) surveys were not consistent for all sites across the seasons as explained in Chapter 3. This affected some maize fields and field margins – season dependent.
- Regions were unequally sampled as this was dependent on the availability of fields and farmers' willingness to participate in the GrainSA programme – subsequent, this study.
- Fields were not equal in size – hectares, length and width. However, the survey protocol used accommodated this variation for a standardized approach across the different fields and sites.
- Both maize fields and margin transects were different in lengths. Therefore, the number of individuals and number of species per plot were calculated as relative abundance and relative richness.
- The use of different herbicides, at different application times, in different seasons.
- The herbicide mixing ration, boom spray admiration and calibration was solely the responsibility of the farmers and contractors involved in the respective farming projects.

Appendix B: Supplementary weed species data supporting chapter 5

Table B1: Weed species and their growth form as well as category for the indigenous and alien sampled in the herbicide tolerant maize fields and field margins of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province.

Family	Scientific Name	Common Name	Growth form	Indigenous/Alien	Maize fields (MF)	Field margins (M)	Both (MF & M)
Amaranthaceae	*Amaranthus viridis* L.	Spiny pigweed	Forb	Alien	Present	Present	Present
Apiaceae	*Daucus carota* L.	Wild carrot	Forb	Alien	Present	Present	Present
Asparagceae	*Asparagus species* L.	Asparagus	Forb	Indigenous	Absent	Present	Absent
Asteraceae	*Arctotheca calendula* K. Lewin	Capeweed	Forb	Indigenous	Present	Present	Present
Asteraceae	*Berkheya onopordifolia* (DC.) O.Hoffm. ex Burtt Davy var. *onopordifolia*	Purple berkheya	Forb	Indigenous	Present	Absent	Absent
Asteraceae	*Bidens bipinnata* L.	Spanish needles	Forb	Alien	Present	Present	Present
Asteraceae	*Bidens pilosa* L.	Jersey cudweed	Forb	Alien	Present	Present	Present
Asteraceae	*Cirsium vulgare* (Savi) Ten.	Spear thistle	Forb	Alien	Present	Absent	Absent
Asteraceae	*Conyza bonariensis* (L.) Cronquist	Argentine fleabane	Forb	Alien	Present	Present	Present
Asteraceae	*Cotula heterocarpa* DC.	Water buttons	Forb	Indigenous	Present	Present	Present
Asteraceae	*Galinsoga parviflora* Cav.	Galant soldier	Forb	Alien	Present	Absent	Absent
Asteraceae	*Helichrysum asperum* (Thunb.) Hilliard & B.L.Burtt	Hairy everlasting	Forb	Indigenous	Present	Present	Present
Asteraceae	*Helichrysum griseolanatum* Hilliard	Fluffy white	Forb	Indigenous	Present	Present	Present
Asteraceae	*Oncosiphon piluliferum* (L.f.) Källersjö	Pineapple weed	Forb	Indigenous	Present	Absent	Absent
Asteraceae	*Pseudognaphalium luteoalbum* (L.) Hilliard & B.L.Burtt	Jersey cudweed	Forb	Indigenous	Present	Present	Present
Asteraceae	*Sonchus asper* (L.) Hill	Prickly sow-thistle	Forb	Alien	Present	Present	Present
Asteraceae	*Sonchus nanus* Sond. ex Harv.	Sowthistle	Forb	Alien	Present	Present	Present
Asteraceae	*Tagetes minuta* L.	Khaki weed	Forb	Alien	Present	Present	Present
Asteraceae	*Xanthium spinosum* L.	Spiny cocklebur	Forb	Alien	Present	Present	Present

Table B1: Weed species and their growth form as well as category for the indigenous and alien sampled in the herbicide tolerant maize fields and field margins of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province continued.

Family	**Scientific Name**	**Common Name**	**Growth form**	**Indigenous/Alien**	**Maize fields (MF)**	**Field margins (M)**	**Both (MF & M)**
Brassicaceae	*Rorippa nudiuscula* Thell.	Yellowcresses	Forb	Alien	Present	Present	Present
Campanulaceae	*Lobelia erinus* L.	Garden lobelia	Forb	Indigenous	Present	Present	Present
Campanulaceae	*Wahlenbergia stellarioides* Cham.	Tufted bluebell	Forb	Indigenous	Present	Present	Present
Chenopodiaceae	*Chenopodium carinatum* R.Br.	Keeled goosefoot	Forb	Alien	Present	Present	Present
Cleomaceae	*Cleome monophylla* L.	Rocky mountain beeplant	Forb	Indigenous	Present	Absent	Absent
Commelinaceae	Commelina benghalensis	Benghal dayflower	Forb	Alien	Present	Present	Present
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	Common morning glory	Forb	Alien	Present	Present	Present
Convolvulaceae	*Ipomoea spp.* L.	Bindweed	Forb	Alien	Present	Absent	Absent
Cucurbitaceae	*Cucumis myriocarpus* Naudin	Gooseberry cucumber	Forb	Indigenous	Present	Present	Present
Cunoniaceae	*Callicoma serratifolia* Andrews	Black wattle	Forb	Alien	Present	Present	Present
Cyperaceae	*Bulbostylis humilis* (Kunth) C.B.Clarke	Bushy heads grass	Sedge	Indigenous	Present	Absent	Absent
Cyperaceae	*Cyperus congestus* Vahl	Nut grass	Sedge	Indigenous	Present	Absent	Absent
Cyperaceae	*Cyperus esculentus* L.	Tigernut	Sedge	Indigenous	Present	Absent	Absent
Cyperaceae	*Cyperus* spp. L.	Sedges	Sedge	Indigenous	Present	Present	Present
Euphorbiaceae	*Chamaesyce prostrata* (Aiton) Small	Green creeping spurge	Forb	Alien	Present	Present	Present
Euphorbiaceae	*Ricinus communis* L.	Castor bean	Forb	Alien	Present	Absent	Absent
Fabaceae	*Alysicarpus rugosus* (Willd.) DC.	Red moneywort	Forb	Indigenous	Present	Present	Present
Fabaceae	*Chamaecrista biensis* (Steyaert) Mendonca & Torre	Locust weed	Forb	Indigenous	Present	Present	Present
Fabaceae	*Indigofera spicata* Forsk.	True indigo	Forb	Indigenous	Present	Absent	Absent
Fabaceae	*Lespedeza pilosa* (Thunb.) Siebold & Zucc.	Carlifonia bush clover	Forb	Alien	Present	Absent	Absent

Table B1: Weed species and their growth form as well as category for the indigenous and alien sampled in the herbicide tolerant maize fields and field margins of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province continued.

Family	**Scientific Name**	**Common Name**	**Growth form**	**Indigenous/Alien**	**Maize fields (MF)**	**Field margins (M)**	**Both (MF & M)**
Fabaceae	*Medicago polymorpha* L.	California burclover	Forb	Indigenous	Present	Present	Present
Fabaceae	*Tephrosia capensis* (Jacq.) Pers.	Tephrosia fern	Forb	Alien	Present	Present	Present
Fabaceae	*Trifolium repens* L.	White clover	Forb	Alien	Present	Present	Present
Fabaceae	*Zornia capensis* Pers.	Caterpillar bean	Forb	Indigenous	Present	Present	Present
Gisekiaceae	*Gisekia pharnacioides* L.	Sand herbage	Forb	Alien	Present	Present	Present
Hypoxidaceae	*Hypoxis villosa* L.f.	Golden winter star	Forb	Indigenous	Present	Present	Present
Iridaceae	*Aristea abyssinica* Pax	Blue-eyed grass	Forb	Indigenous	Present	Absent	Absent
Malvaceae	*Hibiscus nigricaulis* Baker f.	Wild stockrose	Forb	Indigenous	Present	Absent	Absent
Malvaceae	*Hibiscus trionum* L.	Flower-of-an-hour	Forb	Indigenous	Present	Present	Present
Onagraceae	*Oenothera parodiana* Munz	Evening primrose	Forb	Alien	Present	Present	Present
Papaveraceae	*Argemone mexicana* L.	Mexican prinkly poppy	Forb	Alien	Present	Absent	Absent
Papaveraceae	*Papaver aculeatum* Thunb.	Orange poppy	Forb	Indigenous	Present	Absent	Absent
Plantaginaceae	*Diclis reptans* Benth.	Toadflax	Forb	Indigenous	Present	Present	Present
Plantaginaceae	*Plantago virginica* L.	Dwarf plantain	Forb	Alien	Present	Present	Present
Poaceae	*Andropogon eucomus* Nees	Snowflake grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Arundinella nepalensis* var. contracta Ohwi	Tambuki grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Brachiaria deflexa* (Schumach.) Robyns	Congo signal grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Brachiaria eruciformis* var. divaricata Basappa & Muniy.	Velvet bushwillow	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Bromus diandrus* Roth	Great brome	Grass	Alien	Present	Absent	Absent

Table B1: Weed species and their growth form as well as category for the indigenous and alien sampled in the herbicide tolerant maize fields and field margins of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province continued.

Family	**Scientific Name**	**Common Name**	**Growth form**	**Indigenous/Alien**	**Maize fields (MF)**	**Field margins (M)**	**Both (MF & M)**
Poaceae	*Cynodon dactylon* (L.) Pers.	Couch grass	Grass	Alien	Present	Present	Present
Poaceae	*Digitaria argyrograpta* (Nees) Stapf	Silver finger grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Cockspur grass	Grass	Alien	Present	Absent	Absent
Poaceae	*Eleusine coracana* (L.) Gaertn.	Goose grass	Grass	Indigenous	Present	Present	Present
Poaceae	*Elionurus muticus* (Spreng.) Kuntze	Wire lemon grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Enneapogon scaber* Lehm.	Enneapogon	Grass	Alien	Present	Present	Present
Poaceae	*Eragrostis curvula* (Schrad.) Nees	Love grass	Grass	Indigenous	Present	Absent	Absent
Poaceae	*Eragrostis plana* Nees	Eragrostis	Grass	Indigenous	Present	Present	Present
Poaceae	*Lolium perenne* L.	Perennial ryegrass	Grass	Alien	Absent	Present	Absent
Poaceae	*Melinis repens* (Willd.) Zizka	Rose Natal grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	Panicum ecklonii Nees	Bajiho	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Panicum natalense* Hochst.	Natal buffalo grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Panicum schinzii* Hack.	Land grass	Grass	Indigenous	Present	Absent	Absent
Poaceae	*Paspalum dilatatum* Poir.	Paspalum spp	Grass	Alien	Present	Absent	Absent
Poaceae	*Pennisetum thunbergii* Kunth	Fountain grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Sporobolus africanus* (Poir.) Robyns & Tournay	Rush grass	Grass	Indigenous	Absent	Present	Absent
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth	Stipagrostis spp	Grass	Alien	Present	Absent	Absent
Poaceae	*Themeda triandra* Forssk.	Red oat grass	Grass	Indigenous	Present	Present	Present
Poaceae	*Urochloa panicoides* P.Beauv.	Panic liverseed grass	Grass	Indigenous	Present	Present	Present
Polygalaceae	*Polygala amatymbica* Eckl. & Zeyh.	Clump forming plant	Forb	Indigenous	Present	Absent	Absent

Table B1: Weed species and their growth form as well as category for the indigenous and alien sampled in the herbicide tolerant maize fields and field margins of the selected areas of Oliver Tambo District Municipality, Eastern Cape Province continued.

Family	Scientific Name	Common Name	Growth form	Indigenous/Alien	Maize fields (MF)	Field margins (M)	Both (MF & M)
Portulacaceae	*Portulaca oleracea* L.	Pigweed	Forb	Alien	Present	Present	Present
Rubiaceae	*Richardia brasiliensis* Gomes	Tropical Mexican clover	Forb	Alien	Present	Present	Present
Scrophulariaceae	*Jamesbrittenia aurantiaca* (Burch.) Hilliard	Terracotta gazania	Forb	Indigenous	Absent	Absent	Absent
Solanaceae	*Datura stramonium* L.	Jimson weed	Forb	Alien	Present	Absent	Absent
Solanaceae	*Solanum retroflexum* Dunal	Earleaf nightshade	Forb	Alien	Present	Present	Absent
Solanaceae	*Solanum rigescens* Jacq.	Flannel weed	Forb	Alien	Present	Present	Present
Urticaceae	*Urtica urens* L.	Dwarf nettle	Forb	Alien	Present	Absent	Absent
Verbenaceae	*Chascanum hederaceum* (Sond.) Moldenke	White trumpets	Forb	Indigenous	Present	Present	Present
Verbenaceae	*Verbena bonariensis* L.	Tall verbena	Forb	Alien	Present	Present	Present
Zygophyllaceae	*Tribulus terrestris* L.	Tack weed	Forb	Indigenous	Present	Absent	Absent

Table B2: Indicator weed species with the p value and frequency.

Family	Indicator Weed species	Indicator Value	P-value	Frequency
Amaranthaceae	*Amaranthus viridis* L.	0.558	0.001	114
Apiaceae	*Daucus carota* L.	0.323	0.003	49
Asteraceae	*Berkheya onopordifolia* (DC.) O.Hoffm. Ex	0.133	0.001	4
Asteraceae	*Bidens bipinnata* L.	0.433	0.001	13
Asteraceae	*Bidens pilosa* L.	0.264	0.002	29
Asteraceae	*Cirsium vulgare* (Savi) Ten.	0.339	0.001	44
Asteraceae	*Conyza bonariensis* (L.) Cronquist	0.321	0.001	23
Asteraceae	*Cotula heterocarpa* DC.	0.205	0.011	37
Asteraceae	*Galinsoga parviflora* Cav.	0.103	0.042	15
Asteraceae	*Oncosiphon piluliferum* (L.f.) Källersjö	0.140	0.012	17
Asteraceae	*Sonchus asper* (L.) Hill	0.212	0.034	50
Asteraceae	*Sonchus nanus* Sond. ex Harv.	0.348	0.003	103
Asteraceae	*Xanthium spinosum* L.	0.081	0.019	6
Brassicaceae	*Rorippa nudiuscula* Thell.	0.299	0.001	16
Campanulaceae	*Lobelia erinus* L.	0.230	0.002	35
Chenopodiaceae	*Chenopodium carinatum* R.Br.	0.397	0.002	112
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	0.342	0.001	54
Cunoniaceae	*Callicoma serratifolia* Andrews	0.166	0.002	9
Cyperaceae	*Bulbostylis humilis* (Kunth) C.B.Clarke	0.273	0.019	60
Cyperaceae	*Cyperus congestus* Vahl	0.071	0.019	3
Cyperaceae	*Cyperus esculentus* L.	0.067	0.03	2
Cyperaceae	*Cyperus* spp. L.	0.255	0.008	80
Euphorbiaceae	*Chamaesyce prostrata* (Aiton) Small	0.239	0.001	25
Fabaceae	*Indigofera spicata* Forsk.	0.160	0.003	20
Fabaceae	*Medicago polymorpha* L.	0.190	0.001	10
Fabaceae	*Tephrosia capensis* (Jacq.) Pers.	0.244	0.021	62
Fabaceae	*Trifolium repens* L.	0.204	0.047	61
Fabaceae	*Zornia capensis* Pers.	0.205	0.002	23

Table B2: Indicator weed species with the p value and frequency continued.

Family	Indicator Weed species	Indicator Value	P-value	Frequency
G isekiaceae	*Gisekia pharnacioides* L.	0.175	0.004	17
Iridaceae	*Aristea abyssinica* Pax	0.059	0.049	5
Malvaceae	*Hibiscus nigricaulis* Baker f.	0.116	0.043	20
Malvaceae	*Hibiscus trionum* L.	0.196	0.008	29
Papaveraceae	*Argemone mexicana* L.	0.197	0.002	11
Plantaginaceae	*Plantago virginica* L.	0.092	0.034	10
Poaceae	*Bromus diandrus* Roth	0.275	0.001	11
Poaceae	*Eleusine coracana* (L.) Gaertn.	0.136	0.01	17
Polygalaceae	*Polygala amatymbica* Eckl. & Zeyh.	0.075	0.011	3
Portulacaceae	*Portulaca oleracea* L.	0.329	0.001	30
Solanaceae	*Solanum retroflexum* Dunal	0.143	0.001	6
Solanaceae	*Solanum rigescens* Jacq.	0.186	0.003	25
Urticaceae	*Urtica urens* L.	0.1	0.003	4
Verbenacea	*Chascanum hederaceum* (Sond.) Moldenke	0.067	0.028	2

Table B3: Indicator weed species with their functional traits in the herbicide tolerant maize fields.

Acronym	Scientific name	Family	Photosynthetic pathway	Carbon storage in reserve organ	Life form	Growth form	Life span	Seed size
AMAVIR	Amaranthus viridis	Amaranthaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
ARGMEX	*Argemone mexicana* L.	Papaveraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Rosette growth form	Annual life span	Seed size length <2mm
ARIABY	*Aristea abyssinica* Pax	Iridaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Geophyte life form	Tufted growth form	Perennial life span	Seed size length 2-<4mm
BERONO	*Berkheya onopordifolia* (DC.) O.Hoffm. Ex	Asteraceae	Photosynthetic pathway C4	Specialised carbon storage organs	Geophyte life form	Erect growth form	Perennial life span	Seed size length <2mm
BIDBIP	*Bidens bipinnata* L.	Asteraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length >10mm
BIDPIL	*Bidens pilosa* L.	Asteraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length >10mm
BRODIA	*Bromus diandrus* Roth	Poaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Tufted growth form	Annual life span	Seed size length >10mm
BULHUM	*Bulbostylis humilis* (Kunth) C.B.Clarke	Cyperaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Tussock growth form	Annual life span	Seed size length <2mm
CALSER	*Callicoma serratifolia* Andrews	Cunoniaceae	Photosynthetic pathway CAM	No specialised carbon storage organs	Phanerophyte life form	Erect growth form	Perennial life span	Seed size length <2mm
CHAPRO	*Chamaesyce prostrata* (Aiton) Small	Euphorbiaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Creeper growth form	Annual life span	Seed size length <2mm
CHAHED	*Chascanum hederaceum* (Sond.) Moldenke	Verbenaceae	Photosynthetic pathway CAM	No specialised carbon storage organs	Chamaephyte life form	Erect growth form	Perennial life span	Seed size length <2mm
CHECAR	*Chenopodium carinatum* R.Br.	Chenopodiaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length 4-10mm
CIRVUL	*Cirsium vulgare* (Savi) Ten.	Asteraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Hemicryptophyte life form	Erect growth form	Biennial life span	Seed size length 4-10mm
CONBON	*Conyza bonariensis* (L.) Cronquist	Asteraceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Rosette growth form	Annual life span	Seed size length <2mm
COTHET	*Cotula heterocarpa* DC.	Asteraceae	Photosynthetic pathway CAM	No specialised carbon storage organs	Chamaephyte life form	Erect growth form	Perennial life span	Seed size length <2mm
CYPCON	*Cyperus congestus* Vahl	Cyperaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Hemicryptophyte life form	Tussock growth form	Perennial life span	Seed size length <2mm
CYPESC	*Cyperus esculentus* L.	Cyperaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Hemicryptophyte life form	Erect growth form	Perennial life span	Seed size length >10mm
CYPSPP	*Cyperus* spp. L.	Cyperaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Hemicryptophyte life form	Erect growth form	Perennial life span	Seed size length >10mm
DAUCAR	*Daucus carota* L.	Apiaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Erect growth form	Biennial life span	Seed size length 2-<4mm
ELECOR	*Eleusine coracana* (L.) Gaertn.	Poaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
GALPAR	*Galinsoga parviflora* Cav.	Asteraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
GISPHA	*Gisekia pharnacioides* L.	Gisekiaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Prostrate growth form	Annual life span	Seed size length <2mm

Acronym	Scientific name	Family	Photosynthetic pathway	Carbon storage in reserve organ	Life form	Growth form	Life span	Seed size
HIBNIG	*Hibiscus nigricaulis* Baker f.	Malvaceae	Photosynthetic pathway CAM	No specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
HIBTRI	*Hibiscus trionum* L.	Malvaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
INDSPI	*Indigofera spicata* Forsk.	Fabaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Creeper growth form	Annual life span	Seed size length <2mm
IPOPUR	*Ipomoea purpurea* (L.) Roth	Convolvulaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Creeper growth form	Annual life span	Seed size length 4-10mm
LOBERI	*Lobelia erinus* L.	Campanulaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Perennial life span	Seed size length <2mm
MEDPOL	*Medicago polymorpha* L.	Fabaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Prostrate growth form	Annual life span	Seed size length 2-<4mm
ONCPIL	*Oncosiphon piluliferum* (L.f.) Källersjö	Asteraceae	Photosynthetic pathway C4	Specialised carbon storage organs	Hemicryptophyte life form	Erect growth form	Annual life span	Seed size length <2mm
PLAVIR	*Plantago virginica* L.	Plantaginaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Perennial life span	Seed size length 2-<4mm
POLAMA	*Polygala amatymbica* Eckl. & Zeyh.	Polygalaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Chamaephyte life form	Erect growth form	Perennial life span	Seed size length 4-10mm
POROLE	*Portulaca oleracea* L.	Portulacaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Prostrate growth form	Annual life span	Seed size length 4-10mm
RORNUD	*Rorippa nudiuscula* Thell.	Brassicaceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Erect growth form	Perennial life span	Seed size length <2mm
SOLRET	*Solanum retroflexum* Dunal	Solanaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Phanerophyte life form	Erect growth form	Annual life span	Seed size length 4-10mm
SOLRIG	*Solanum rigescens* Jacq.	Solanaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Phanerophyte life form	Erect growth form	Annual life span	Seed size length 4-10mm
SONASP	*Sonchus asper* (L.) Hill	Asteraceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Rosette growth form	Annual life span	Seed size length 2-<4mm
SONNAN	*Sonchus nanus* Sond. ex Harv.	Asteraceae	Photosynthetic pathway C4	Specialised carbon storage organs	Therophyte life form	Rosette growth form	Perennial life span	Seed size length 2-<4mm
TEPCAP	*Tephrosia capensis* (Jacq.) Pers.	Fabaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Chamaephyte life form	Erect growth form	Perennial life span	Seed size length <2mm
TRIREP	*Trifolium repens* L.	Fabaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Hemicryptophyte life form	Creeper growth form	Perennial life span	Seed size length <2mm
URTURE	*Urtica urens* L.	Urticaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length <2mm
XANSPI	*Xanthium spinosum* L.	Asteraceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Erect growth form	Annual life span	Seed size length >10mm
ZORCAP	*Zornia capensis* Pers.	Fabaceae	Photosynthetic pathway C3	Specialised carbon storage organs	Therophyte life form	Creeper growth form	Perennial life span	Seed size length 2-<4mm