EFFECT OF ROUNDUP® (*Glyphosate*) ON DIVERSITY AND ABUNDANCE OF WEEDS IN COTTON FIELDS IN MWEA, KENYA

BY

JANET NJERI KIMUNYE (B.Sc. Nairobi)

A THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI, SCHOOL OF BIOLOGICAL SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT ECOLOGY

JULY, 2011

DECLARATION

I, JANET NJERI KIMUNYE, declare that this thesis is my original work and has not been presented for a degree in any other university to the best of my knowledge.

Signed.....

Date: 2/8/2011

This thesis has been submitted for examination with our approval as university supervisors

Professor Jenesio I. Kinyamario

Signed.....

Date.....2/8/2011.....

Dr. Nelson O. Amugune

Signed.....

Date.....2/8/2011.....

DEDICATION

To my loving husband Martin Thiong'oh and daughters Melannie and Mikalynn Thiong'oh, my dear parents Mr. and Mrs. Ephantus Kimunye and Mrs. Pauline Thiong'oh and all those who believe in and work towards sustainable agricultural development.

ACKNOWLEDGEMENTS

I extend my gratitude to my supervisors Prof. Jenesio I. Kinyamario and Dr. Nelson O. Amugune for their support, advice and encouragement. This study would not have been possible without their tireless effort.

To Martin, Melannie, Mikalynn and my entire family thank you for your moral support and for being there for me, my friends and all who contributed in every way to the success of this study, I thank you from the bottom of my heart.

I thank the director and staff KARI Mwea station for the permission to carry out the research in their farm and providing the seeds, Mutiso (University of Nairobi Herbarium) for weed species identification, Dr. Niels for assistance on the use of R-Program and my field assistant for their assistance during my field work.

I finally thank the BiosafeTrain Project for funding my studies and the research work and Prof. Kinyamario for coordinating the program without whom this work would not have been possible.

ABSTRACT

Weeds interfere with cotton production by competing for important resources including light and nutrients. Several approaches are taken to control weeds in order to achieve maximum yields. Where genetically modified cotton exhibiting resistance to Roundup® have been commercialised, Roundup® has been extensively used in controlling weeds. There, are however, reports of weeds developing resistance to Roundup® and interference with weed diversity where Roundup® has been used for long periods. In this study we tried to mimic a glyphosate tolerant field by covering convectional cotton variety Hart 89M with polythene papers to protect cotton plants from herbicide effect during spraying. The objective of this study was to investigate the effect of glyphosate on weed diversity and abundance, the presence of glyphosate tolerant species and effect of weeds on growth of cotton. This study was important because the country is embarking on reviving the cotton industry by use of modern technology like Bt (*Bacillus thuringiensis*) cotton and Herbicide resistant cultivars that would reduce the amount of labour for weed control. Information on effect of Roundup® on weed diversity is not available in Kenya so this study will help in determining if it is feasible to popularise the use of herbicides particularly Roundup® for weed control. Weed diversity was sampled using three 0.5 x 0.5m quadrats before and after treatments in each subplot. The treatments included in this study were Roundup® spray (2l/ha), hand weeding untreated check and natural vegetation. The experimental design was a split plot design with the treatments as the main plots and the timing of treatment as the subplots.

The R-program was used for data analysis and diversity was analysed using the Renyi diversity index. A total of 43 weed species was recorded before spraying but this reduced to 30 species after spraying. The most abundant species were *Euphorbia geniculata, Spermacoce laevis, Digitaria velutina and Bidens pilosa* while the others were trace. The diversity and abundance of weeds decreased significantly after spraying with Roundup® 9 weeks after germination. The results obtained show that Roundup® is effective in controlling most of the weeds in Mwea where only *Commelina benghalensis* exhibited tolerance 21days after spraying. Early hand weeding and spraying with Roundup® reduced weed density which in turn resulted in

taller cotton plants (108.14±0.687 and 104.39±0.950cm), more squares (8.19 and 6.43 per plant) and higher productive bolls at (8.26 and 6.13 per plant).

From this study it is evident that early weed removal is necessary if a farmer is to realize maximum yields. Spraying with Roundup® reduced the diversity and abundance of most weeds but the time of herbicide application is also important.

Key words: Roundup®, *Weeds, Tolerance, bolls*

TABLE OF CONTENTS

LIST OF FIGURES

Pages

LIST OF TABLES

Pages

APPENDICES

Pages

LIST OF ACRONYMS

GRCs-Glyphosate Resistant Crops

EPA-Environmental Protection Agency

KARI- Kenya Agricultural Research Institute

CBD-Convention on Biological Diversity

Bt- *Bacillus thuringiensis*

2,4-D- 2,4-dichlorophenoxy acetic acid

MCPA- 4-chloro-2-Methylphenoxy acetic acid

PP- Pre-plant

PPI- Pre-plant incorporated

PRE- Pre-emergence

POST- Post emergence topical

PDIR- Post emergence directed herbicides

CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

In Kenya, cotton is mainly grown in the semi-arid regions of Eastern, Central, Nyanza, Coast, Western and Rift Valley provinces. Its production has however been characterized by low yields for decades. This has been attributed to high costs of production occasioned by weeds, pests and diseases. In Kenya, *Bacillus thuringiensis* (*Bt*) and Herbicide tolerant (*Ht*) cotton are being considered for introduction to farmers as part of Government strategy for the revival of the collapsed cotton industry.

Weeds significantly affect cotton production as they compete for moisture, nutrients and sunlight thus reducing crop yield. Effect of weed on yield reduction depends on weed species, weed density, distribution and duration of competition (Papamilchail *et al.,* 2002; Kohel and Lewis, 1984; Thornton *et al.,* 1990; Wiles *et al.*, 1993). According to Hillocks (1998), weed control is an important crop protection practice since uncontrolled weed growth especially during the early stages of establishment can greatly decrease the final yield.

Glyphosate is a non selective post emergence herbicide. Genetically modified crops have been modified for resistance against this herbicide including cotton, corn, soybean and canola. Following repeated use of a single herbicide regime, some weeds are reported to have become resistant to glyphosate due to the intense selection pressure.

Glyphosate was used as a means of achieving these objectives since cotton has already been modified to exhibit tolerance to it and the trials on this technology are set to start in the country. Hence information on its effects on weed diversity and a list of

species tolerant to it is necessary but currently lacking in Kenya.

1.2 LITERATURE REVIEW

1.2.1 Origin of cultivated cotton

The word 'cotton' refers to four species in the genus *Gossypium* (Malvaceae) —*G. Hirsutum* L., *G. Barbadense* L., *G. Arboretum* L. and *G. Herbaceum* L. — that were domesticated independently as source of textile fibre (Brubaker *et al.*, 1999). Globally, the *Gossypium* genus comprises about 50 species (Brubaker *et al.*, 1999). The place of origin of the genus is not known, however the primary centres of diversity for the genus are west-central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). DNA sequence data from the existing *Gossypium* species suggests that the genus arose about 10 – 20 million years ago (Wendel and Albert, 1992). The cultivated cotton in Kenya is mostly *G. hirsutum* L., variety Hart 89M which was developed through multiline crossing of local varieties. It is a non-Bt cotton variety developed for the environments South of Rift valley in Kenya (Waturu *et al.*, 2007).

1.2.2 Biology of cotton

Cotton is a perennial plant with an indeterminate growth habit, so that vegetative and reproductive growth occurs at the same time; but four main growth stages can be distinguished, namely (i) germination and seedling establishment; (ii) leaf area and canopy development; (iii) flowering and boll development; and (iv) maturation. The cotton-growing season varies from 100 to over 190 days according to climatic conditions and plant variety (Beltrao, 2002). Under favourable conditions, the cotton radicle emerges within 2-3 days. While a substantial root system develops in the first month, the growth of the stem and leaves

above ground is relatively slow. During germination and seedling establishment root growth dominates the growth of the cotton plant. The taproot may be as deep as 25.4cm by the time the cotyledons emerge. Cotton emerges quickest from warm, moist soil. As the cotton plant grows, the radicle that originally emerged from the seed becomes a taproot, from which lateral roots begin to form and grow. Lateral roots and the taproot make up the basal root system. Other roots then develop from this basal root system and they have a functional life of about 3 weeks. The main stem leaves are the first vegetative structures that appear on the main stem. Main stem leaves and branches form at the nodes. A fruiting bud, called a square, begins to form at the initiation of the fruiting branch. The first square produced on a fruiting branch is known as a first position square. As a cotton plant develops, new leaves appear and expand, increasing sunlight interception. Flowering begins around 50 days after seedling emergence and continues until 120 days or longer (Fuzzato, 1999). Cotton flowers open at or near dawn and remain open for only a single day, closing near sunset. Cotton plants have indeterminate flowers and continue producing flowers until changes in the weather cause mature leaves to shed. Chemical defoliants are applied when about 50-60% of the balls open to avoid green stains on the lint during harvesting and also hasten maturity.

1.2.3 Weediness of cotton

Cotton has been grown for centuries throughout the world without any reports that it is a serious weed pest. No *Gossypium* species are recognised as problematic weeds agriculturally or environmentally (Tothill *et al.*, 1982) and neither does cotton have relatives that are problematic weeds (Keeler *et al.*, 1996). Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds, such as seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance

dispersal of seeds (Keeler, 1989). *G. Hirsutum* and *G. Barbadense* may occur as escapes from agriculture and/or as small populations of naturalised exotic species (Lazarides *et al.*, 1997). Where such populations have established, however, they are not considered to threaten agricultural productivity or native biodiversity.

1.2.4 Constraints to cotton production

Cotton is grown by small scale farmers in various agro-ecological zones in Kenya. These include Rift Valley, Central, Coast, Eastern, Nyanza and Western provinces. There has been a decrease in cotton production (Ikiara and Ndirangu, 2002) due to various constraints, including a high incidence of pests and diseases, lack of certified seeds, collapse of extension services and high costs of weed management. In Africa, yield losses due to weeds range from 25% to total (100%) crop failure. In Kenya, average yield losses due to uncontrolled weed growth are around 50–60% (Mwanda, 2000).The majority of small holder farmers identify weeding as the major constraint in their farming systems (Vissoh *et al.*, 2004). Of all the labour in crop production 50-70% of it is spent weeding (Chikoye *et al.*, 2007).

1.2.5 Effect of weeds on cotton production

According to Anderson (1996), a weed is any plant growing where it is not wanted, and in general, adversely affects the use, economic value, and aesthetic aspect of the land and waters it infests.

Weeds are humans' worst pest organisms, interfering with food production everywhere and reducing production, economic growth, and food security (Milberg and Hallgren, 2004; Jones *et al.*, 2005).Weeds significantly affect cotton production as they compete, for moisture, nutrients and sunlight thus reducing the yield. Effect of weed on yield

reduction depends on weed species, weed density and distribution (Papamilchail *et al.*, 2002; Kohel and Lewis, 1984; Thornton *et al.*, 1990; Wiles *et al.*, 1993). Lint quality is also lowered by thrash and lint staining from live weeds at harvest from crushed leaves, stem and fruits (Garner and Bowen, 1961). Weeds have also been found to harbour cotton insect pests and diseases organisms thus complicating their management (Kohel and Lewis, 1984, Anderson, 1983). In addition, weeds exert stress to the cultivated crops through their all allopathic and parasitism effect and the crop need to be kept free of weeds during the critical stages of growth to prevent crop yield loss (Knezevic *et al.*, 2002).

Weeds can be detrimental to crop production due to competition for water, nutrients, and sunlight. Weeds consume 5 to 6 times Nitrogen, 5 to 12 times Phosphorous and 2 to 5 times Potassium more than cotton crop at the early growth stages and thus reduces cotton yield by 54-85% (Jain *et al.*, 1981). Cotton weed not only reduces the number of mature bolls per plant but also lowers lint quality (Abernathy and McWhorter, 1992). Weed and crop competition can be defined as the situation where two or more plants grow in close proximity to each other and draw on the same limited-supply resource pool (Coble and Byrd, 1992). The weed species, density, and duration of the population determine the competitive damage to crop. The more competitive species with the greatest density and longest duration will cause the most significant reduction in crop production. Cotton must be kept weed-free for a period of two months after emergence in order to avoid crop loss (Gale and Earl, 1970). This critical period occurs when both the crop plants and weeds are in their active vegetative stage of growth. The more competitive the weed species, the longer the weed-free period must be (Coble and Byrd, 1992).

1.2.6 Weed control in cotton

According to Hillocks (1998), weed control is an important crop protection practice since uncontrolled weed growth especially during the early stages of establishment can greatly decrease the final yield. Good weed control should be economical and sustainable, be able to prevent weed interference to the crop, reduce weed seed bank and prevent weed resistance.

Changes in cotton farming systems over the last decade have led to a change in weed spectrum. Weed control has moved from relying fully on tillage that is more expensive and more labour intensive to systems that rely on the use of herbicides with minimum tillage (Young *et al.*, 1994). Herbicides have proved to be effective in cotton weed management than in any other major crop especially in United States of America (Mcwhorter and Bryson, 1992) where different herbicides have been developed for weed management for pre- and post- emergence of either the weed or the crop. With the advent of herbicides, changes in the dominant weed species in agricultural land has been rapid and dramatic (Anderson, 1996).

Cultural weed control utilizes practices that are less favourable for weeds, yet more advantageous for crops. This includes narrow row spacing and crop selection favourable for a critical weed-free period, as well as crop rotations and use of smother crops. Narrow-row spacing creates canopy closure early in the season causing low-light conditions that prevent newly emerging weed seedlings from developing (Gunsolus, 1990). According to Bussan *et al.* (1993), selection of a crop variety that emerges and quickly produces leaf canopy increases its ability to compete with and suppress weeds.

Biological weed control uses natural enemies, or biotic agents, such as herbivorous animals, insects, nematodes, and pathogens to help reduce weed populations. Mechanical weed control is the physical removal or prevention of weeds by hand-pulling, hoeing, mowing, flooding, smothering, burning, and machine tilling.

Chemical weed control utilizes phytotoxic chemicals, referred to as herbicides, to kill or suppress weeds. An increased use in herbicides began in 1944 with the discovery of 2,4-dichlorophenoxy) acetic acid (2,4-D) and 4-chloro-2-methylphenoxy) acetic acid (MCPA). Use of herbicides has dramatically increased in time with new herbicide developments (Anderson, 1996). Herbicides can be applied in cotton as preplant (PP), preplant incorporated (PPI), preemergence (PRE), post emergence-topical (POST), and post emergence-directed (PDIR) herbicides. Herbicides that are applied PPI are sprayed prior to planting and incorporated into the soil in order to control germinating weeds and in some cases reduce herbicide degradation and volatility.

Roundup®, the trade name for Glyphosate has isopropyl amine salt of glyphosate as the main active ingredient. It is a very effective non-selective herbicide. Before introduction of Glyphosate resistant crops (GRCs), glyphosate was used in non-crop situations, (Anderson, 1996) before planting the crop, or with specialized application equipment to avoid contact with the crop (Duke, 1998; Franz *et al.*, 1997). The herbicide inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved in the synthesis of the amino acids tyrosine, tryptophane and phenylalanine (Duke *et al.*, 2003). Glyphosate is absorbed through the foliage and translocated to the growing points. It has therefore been the herbicide of choice in controlling weeds in cotton fields.

1.2.7 Glyphosate resistant cotton

Glyphosate inhibits aromatic amino acid biosynthesis at the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway; however, cotton tolerance was developed by inserting a gene that encodes for a glyphosate-resistant EPSPS enzyme (Ganesh *et al.*, 1992; Suh *et al.*, 1993). Glyphosate-tolerant cultivars of cotton were introduced in 1997, allowing glyphosate to be applied POST until the four-leaf cotton growth stage. After the four leaf stage, glyphosate must be applied PDIR to prevent crop injury (Jones and Snipes, 1999; Kalaher *et al.*, 1997; Light *et al.*, 2003; Pline *et al.*, 2002).The adoption of GRCs has changed agronomic practices like the increased use of glyphosate at the expense of other herbicides, manner and frequency that glyphosate is used and the amount of tillage conducted (Young, 2006).

One fear with the development of herbicide resistant plants is that these plants might out-cross with their wild relatives, for example the case of *G. barbadense* and *G. mustelinum* Brubaker *et al.*, (1993), resulting in superweeds that are more competitive and difficult to control than the common weeds. This would not only complicate weed management but make it more chemical intensive. If the superweeds develop, they are likely to crowd out the indigenous plants thus interfering with local biodiversity. According to Freckleton *et al.*, (2004), glyphosate resistant crops would lead to increased weediness on agricultural land and invasiveness of unmanaged areas. However this idea has been opposed by Watkinson *et al.*, (2000) who suggested that weed control in herbicide tolerant crops would clean up previously weedy fields leading to a decline in weeds and wildlife depending on them. Other risks attributable

to GRCs include weed population shifts, introgression of the trait to volunteer crops and weeds and evolution of weeds resistant to glyphosate (Zelaya *et al.*, 2007; Owen, 2005; Gealy *et al.*, 2007). Sanyal *et al.*, (2008) also points that farmers who adopt GRCs may ignore the principles of integrated weed management which has implications on agricultural profitability and sustainability.

1.2.8 Herbicide resistance

Herbicide resistance refers to the ability of a plant biotype to survive and reproduce under a normally lethal dose of herbicide. There has been an increase in the number of weeds showing herbicide resistance during the past two decades. More than 300 biotypes of weeds have evolved resistance to one or more of all the major groups of herbicides among which, resistance to glyphosate is currently of greatest concern. The widespread adoption of herbicide resistant crops, such as Roundup®-Ready™ soybean, corn, cotton and oilseed rape has greatly improved the effectiveness of weed management. However, greater glyphosate usage has played a role in the evolution of glyphosate resistance in weedy species (Dill, 2005). It was originally expected that resistance to glyphosate would evolve slowly or not at all Watkinson *et al.*, (2000).However this belief has been dispelled in recent years (Dill, 2005). Once resistance is significantly frequent within a population, it might spread rapidly to other populations by pollen or seed, and potentially can be transmitted to other species via hybridization (Rieger *et al.*, 2002). An increase in the application frequency of a particular herbicide will probably be accompanied by commensurate resistance to that herbicide (Owen and Zelaya, 2005).

Due to repeated use of a single herbicide regime, some weeds are reported to have become resistant to glyphosate due to the intense selection pressure. Some of the

weeds reported to show resistance to Roundup® in the USA include, horseweed (*Conyza Canadensis* L), Pigweed, common water hemp (*Amaranthus rudis*), Johnsons grass (*Sorghum halepense*) Palmer amaranth (*Amaranthus palmeri*) among others (Bridges, 1992). Other glyphosate resistant weeds that have been reported include, annual ryegrass (*Lolium rigidum*) in Australia (Powles *et al.*, 1998), goose grass (*Eleusine indica*) in Malaysia (Baerson *et al.*, 2002) Italian ryegrass (*Lolium multiflorum*) in Chile (Perez and Kogan, 2003), and hairy fleabane (*Conyza bonariensis*) in South Africa. In Kenya, no weeds have been reported to show resistance to glyphosate but *Bidens pilosa* was reported to be resistant to D/22 herbicides (Njoroge, 1991).

1.2.9 Justification

In the developed world, weeds are a major cost to crop production and, even so, still significantly reduce yield, while in developing world, weeds cause starvation, poverty and loss of human potential (Gianessi, 2009). In Africa, yield losses due to weeds range from 25% to total crop failure. In Kenya, average yield losses due to uncontrolled weed growth are around 50–60% (Mwanda, 2000).The majority of small holder farmers identify weeding as the major constraint in their farming systems (Vissoh *et al.*, 2004) since50-70% of all the labour in crop production is spent on weeding (Chikoye *et al.*, 2007). Herbicides greatly reduce the needed labour but unfortunately only 3-5% African small holder farmers are using herbicides in their fields (Lagoke *et al.*, 1992). Hand weeding in cotton fields takes up to 254 h/ha, while spraying with a back pack sprayer would take 8 h/ha (Lagoke *et al.*, 1992) while chemical weeding cost a third of the two hand weedings in maize study in Kenya (Maina *et al.*, 2003).

In Kenya, *Bt* and *Ht* cotton are being considered for introduction to farmers as part of

Government strategy for the revival of the collapsed cotton industry. Lack of knowledge is the most limiting factor in the adoption of herbicide technology. If the smallholder farmers are given technical support, they would take advantage of herbicide technology and improve crop production (Makanganise *et al.*, 1999). However before popularization of the herbicide technology, studies relating to the long term effect of the herbicides on the environment and particularly the biodiversity are crucial.

In other parts of the world, where glyphosate has been extensively used, studies have reported glyphosate to have adverse effect on weed diversity and at the same time common weeds are known to develop resistance towards it. In this regard and with plans of reviving the Kenyan cotton industry using modern biotechnology, it is of great importance that a study should address weed diversity and effect of glyphosate on weed composition dynamics and biological diversity. Biological diversity supports and comprises ecological functions that are vital for natural ecosystems and crop production in sustainable agricultural systems (Convention on Biological Diversity (CBD), 1992). Changes in biological diversity can have adverse effect on natural and agricultural ecosystems (US EPA, 1998).

To avoid the risk of introducing super weeds that would in turn have negative effects on biodiversity, a study is essential to identify weed species that are tolerant to glyphosate with potential of becoming resistant with subsequent use over the years thereby causing biodiversity population shifts. These studies are lacking in Kenya. This study seeks to investigate, 1) the effects of glyphosate on weed diversity and abundance given that an acceptable level of weed diversity in and around crop fields has been documented by Altieri, (1994) to play important ecological roles such as enhancement of biological insect pest control, better soil cover reducing erosion and 2) the extent of weeds showing tolerance

to glyphosate which potentially may become resistant to the substance if it is used repeatedly. This will help the decision makers in determining the feasibility and sustainability of herbicide resistant crops if introduced in the country.

1.3 Objectives of the study

The main objective of this study was to determine the effect of Roundup® (Glyphosate) on weed diversity and abundance in cotton fields and monitor the presence of weeds that are tolerant to glyphosate.

1.3.1 Specific objectives

The specific objectives were:

1. Investigate the diversity and abundance of weeds in cotton fields,
2. Determine the effect of weeds on the growth of cotton, and
3. Determine the presence of weeds tolerant to Roundup®.

1.3.2 Hypotheses

Roundup® has no effects on the diversity and abundance of weeds in cotton fields and may have no influence on cotton growth.

CHAPTER TWO

2. STUDY AREA AND METHODS

2.0 Study area

The study was conducted at Kenya Agricultural Research Institute (KARI) station at Mwea, Kirinyaga District in Central Province of Kenya. The research station is located about 100 km northeast of Nairobi, Kenya. Mwea Division lies at the base of Mt Kenya at an altitude of approximately 1,200 m above sea level. Several perennial rivers flow through the flat terrain of the poorly drained Mwea division. These conditions have formed swamps and wetlands that have led to the development of the largest rice irrigation scheme in Kenya, known as Mwea Tebere Rice Irrigation Scheme.

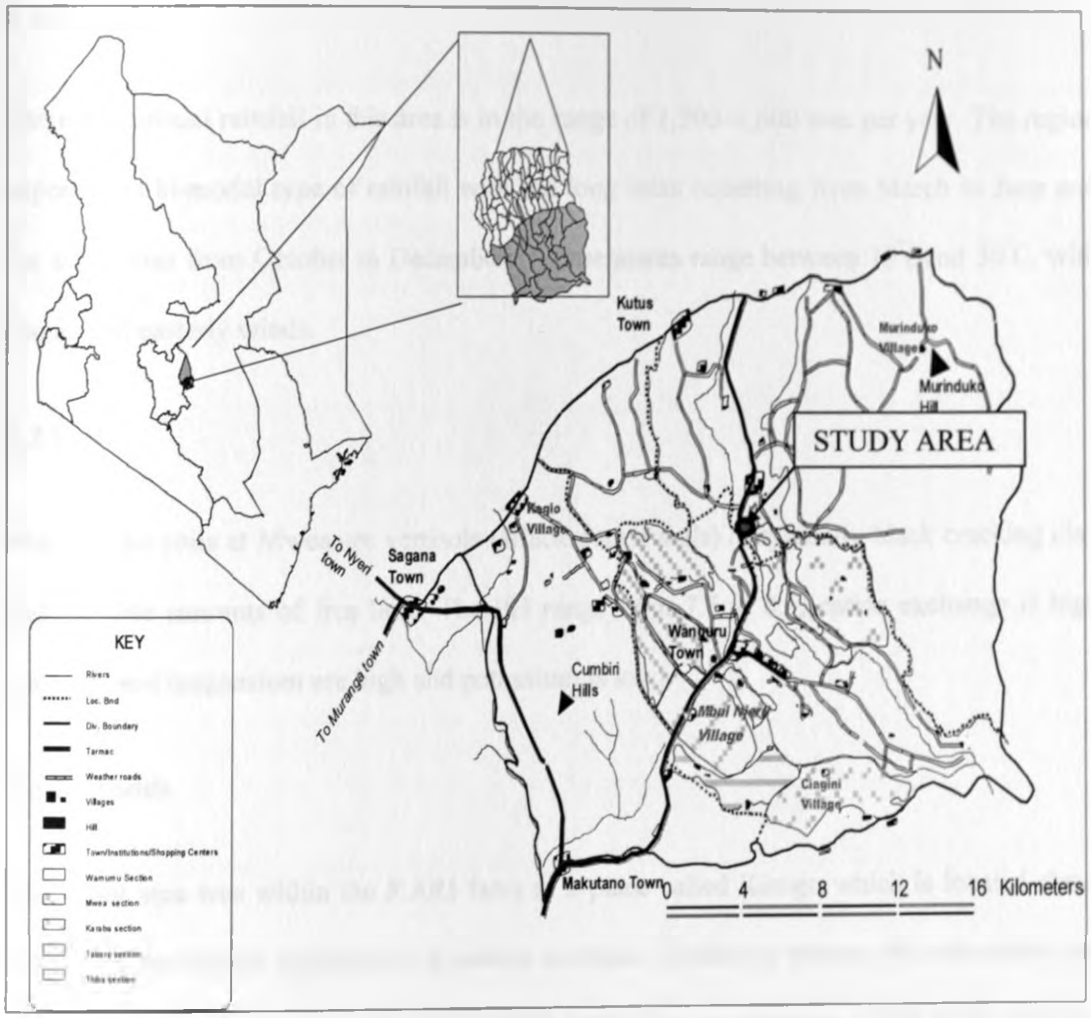


Figure 2.1: Map of the study area, Mwea Kenya

2.1Climate

The mean annual rainfall in this area is in the range of 1,200–1,600 mm per year. The region experiences bi-modal type of rainfall with the long rains occurring from March to June and the short rains from October to December. Temperatures range between 10°Cand 30°C, with occasional easterly winds.

2.2 Soils

Much of the soils at Mwea are vertisols (Black cotton soils) of typically black cracking clay and variable amounts of free lime. The PH range from 7.5 to 8.5; cation exchange is high (calcium and magnesium are high and potassium is low).

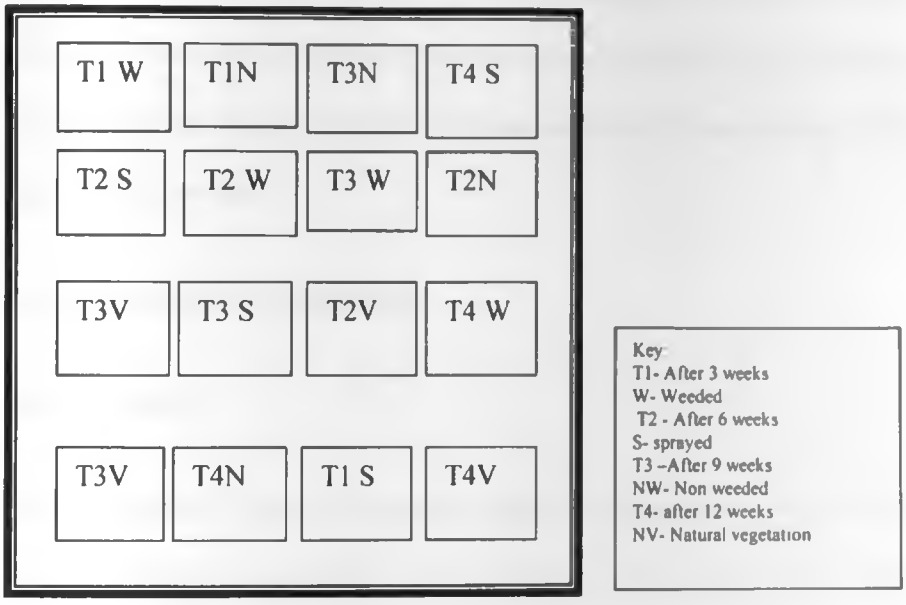
2.3 Methods

The study area was within the KARI farm at a place called Kirogo which is located about 5km from the Kenya Agricultural Research Institute (KARI) in Mwea. Site allocation and initial weed survey was carried out in October 2009. The other aspects of this study (sowing, weed control and data collection) ran from November 2009 and ended on March 2010.

2.3.1 Field layout

The design used was a split plot with the treatments as the main plots and time of application as the sub-plots. The treatments used in this study included four herbicide (Roundup®) and weeding sessions done at three weeks intervals. The study also included two controls, a non-weeded control and natural vegetation. Each of the treatment plots was divided into four subplots measuring 5mx5m representing the four treatment intervals i.e. three, six, nine and twelve weeks after germination respectively. Each of the subplots was separated from the

next with a 1m wide pathway while replicates were separated from each other by a 2m pathway. Each replicate had sixteen subplots measuring 5mx5m and three replicates were used for the study and they all were in the same topographical region. The subplots were randomly allocated to each replicate.



NB: Diagram not drawn to scale

Figure 2.2: Field layout of the experimental plots

2.3.2 Preliminary Study

An initial survey was done before the land was prepared for sowing so as to identify the entire initial flora, representing the baseline data as described by Lep and Milauer, (2003). This was done during the dry season just before the seasonal rains. Observations on weed density were recorded with the help of the quadrate method in the subplots. Specifically three quadrats measuring 0.5mx 0.5m was used in the study. Weed species occurring within the plots were identified and counted to establish their abundance and species diversity.

2.3.3 Land preparation

Land preparation was done before the seasonal rains. The subplots assigned to the treatments namely weeding, spraying and non-weeding were first slashed to the ground to clear all the vegetation. They were later prepared by hand digging in readiness for sowing. Cotton variety Hart 89 M was sown in all the subplots that were prepared as above using the spacing of 1mx 0.3m while the natural vegetation remained untouched. Since cotton in Mwea is rainfed, this study relied on rains.

2.3.4 Counts of individual weeds

Sprayed plots

Since Roundup® is non-selective, cotton plants were covered to protect them from the effect of the herbicide using polythene bags. The plots to be sprayed were sheltered using a large polythene paper to prevent spray drift into neighbouring plot, spraying was done early in the morning when the wind speed was slow, and a special nozzle was fitted to the sprayer that minimized drift. Roundup® was used at the rate of 2l/ha (the recommended concentration for spot application by the manufacturer).

Counts of individual plants were done using three quadrats measuring 0.5m x 0.5m that were randomly placed within each subplot. All the weeds rooted within each quadrat were identified into species and counted for use in plotting of the diversity indices and rank abundance curves. Weed species that were not identified in the field were pressed and brought to the University of Nairobi herbarium for identification. The first count was made before herbicide (Roundup®) was applied in the subplots marked as time one (T1) sprayed. This procedure was repeated in all the subsequent spraying times in the respective plots. A

post herbicide count was made in all plots after the last application of herbicide (after a delay of 21 days to allow for mortality in the plots sprayed lastly).

Weeded plots

Weed counts in these plots was done as in the sprayed plots above (Section 2.3.4) before weeding at the assigned time and the plots maintained weed free there after by manually removing the weeds as they germinate.

Non –weeded plots

In these plots there was no weed control and cotton was allowed to compete with weeds throughout the season. Data on weeds were collected as in the other plots using quadrats (see Section 2.3.4)

Natural vegetation plots

In these control plots, land was not tilled and cotton was not sowed i.e. the plots remained untouched. Weed counts were done at each treatment session as in (Section 2.3.4).

2.3.5 Effect of weeds on growth of cotton

Ten cotton plants were randomly selected from each subplot and marked. These plants were used for all the subsequent measurements on cotton growth including; plant height, number of squares (the first fruiting part) and number of mature bolls. The length from the soil surface to the tip was taken as the height of cotton while all the open bolls were counted.

2.3.6 Effect of glyphosate on weeds

Cotton plants were covered using polythene bags to guard against glyphosate

phytotoxicity. All the weed species present in the plots were noted and their response to glyphosate application recorded. Glyphosate at the rate of (2l/ha) was carefully evenly sprayed covering all the weed plants. Effect of the herbicide on the weeds was observed after 7 days, 14 days and 21 days and the weed species remaining at each observation recorded. The response was characterized by wilting, yellowing and eventual death of susceptible species.

2.3.7 Data analysis

All data were analysed using R program version 2.10.1. (R Development Core Team, 2009).Anova was carried out to test for any significant differences between and within treatments and treatment times. When F test was significant ($p<0.05$) means were separated using Tukeys test. Diversity was analysed using Renyi entropy as suggested by (Renyi, 1961) while weed abundance and rank frequency were determined using PC-ORD version 5 (McCune and Mefford, 1997). Evenness was determined from the slope of the curve while abundance was obtained from the rank abundance of each species.

$$H_\alpha = \frac{1}{1-\alpha}\log\sum p_i^\alpha, \quad \alpha \geq 0, \alpha \neq 1$$

H= Index of species diversity

α= Scale parameter , $\alpha = \infty$

Pi= Proportion of total sample belonging to the i^{th} species

Renyi diversity index belongs to the one parametric index families that allows the diversity of a community to be characterized by a scale dependent profile instead of a numerical value

(Patil and Taillie, 1982). This method takes into account the rare and the abundant species thus when the scale parameter is 0, the method is extremely sensitive to the rare species, at 1 the Renyi diversity index is similar to the Shannon diversity and less sensitive to the rare species and when the scale parameter changes to 2, Renyi diversity is equivalent to Simpson diversity and hence sensitive to the frequent than rare species.

CHAPTER THREE

3. RESULTS

3.0 Preliminary survey study

During the initial survey, a total of 18 weed species was recorded representing 8 families. Among them, *Indigofera colutea* and *Digitaria velutina* were the most abundant species while the least abundant species were *Lantana camara* and *Panicum maximum*. The fields were however characterised by high evenness as shown in Table 3.1below.

Table 3.1: Family, Abundance and Rank abundance of species recorded during the preliminary survey

Family	Species	RankAbundance	Log(SumAbundance)
Fabaceae	*Crotalaria polysperma*	8	2.05
	Indigofera colutea	1	2.41
	Desmodium ramosissimum	3	2.32
	Indigofera ambelacensis	4	2.28
Poaceae	*Sorghum verticilliflorum*	12	1.95
	Setaria verticillata	5	2.25
	Panicum maximum	17	1.43
	Digitaria velutina	2	2.36
	Eragrostis suber	14	1.82
	Cyperus spp	15	1.68
	Digitaria abyssynica	13	1.91
Capparaceae	*Cleome monophylla*	10	1.98
	Gynandra gynandropsis	16	1.65
Verbenaceae	*Lantana camara*	18	1.40
Rubiaceae	*Spermacoce laevis*	18	1.40
Euphorbiaceae	*Euphorbia geniculata*	9	2.02
Compositae	*Bidens pilosa*	7	2.09
Boraginaceae	*Trichodesma zeylanicum*	11	1.96

3.1 Weed diversity before treatments

The number of weed species recorded before treatment was 43 representing 17 families. Family Poaceae had the highest number of representatives followed by Fabaceae and

composite respectively. The other families were represented by few species Table 3.2. At three weeks after germination non-weeded plots had the highest species richness while the natural vegetation had the lowest species richness (Figure 3.1 below). The plots were however similar in terms of the more common species. After six weeks weeded plots were slightly higher in species richness while the sprayed plots had the lowest species diversity. However the natural vegetation plots were more diverse with respect to the more common species Figure 3.2. In the plots that were treated after nine weeks, weeded plots were the most diverse while the non-weeded plots were the least diverse Figure 3.3 but at sampling time 4, i.e. 12 weeks after germination the reverse was observed where the non-weeded plots were the most diverse as compared to the rest as in Figure 3.4.

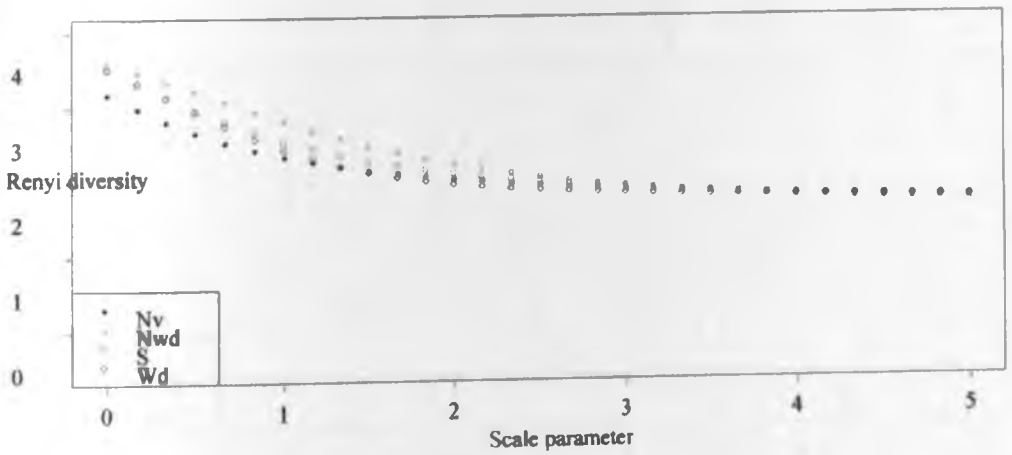


Figure 3.1: Diversity profiles of the Renyi diversity index for all the treatments (3 weeks after germination) NV; Renyi profile in natural vegetation, NW; Non-weeded plots; Sprayed plots and Wd; Weeded plots

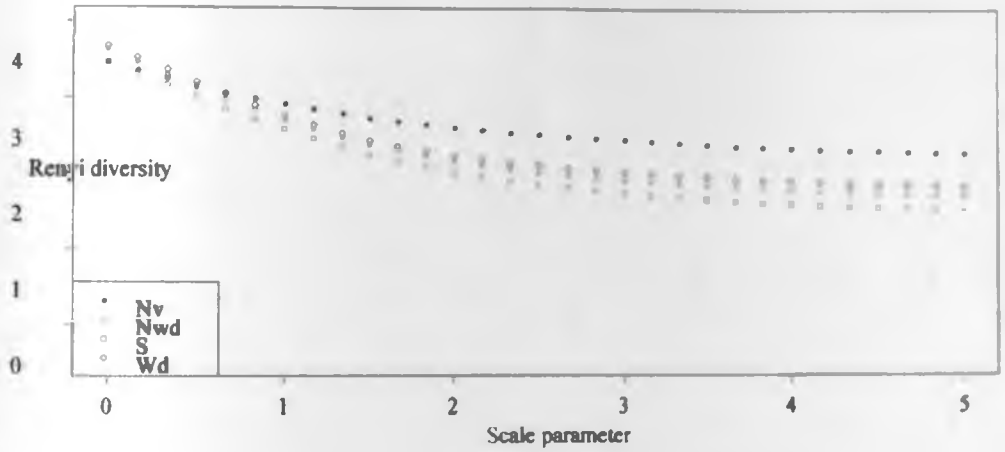


Figure 3.2: Diversity profiles of the Renyi diversity index for all the treatments (6weeks after germination) NV; Renyi profile in natural vegetation, NW; Non-weeded plots; Sprayed plots and Wd; Weeded plots.

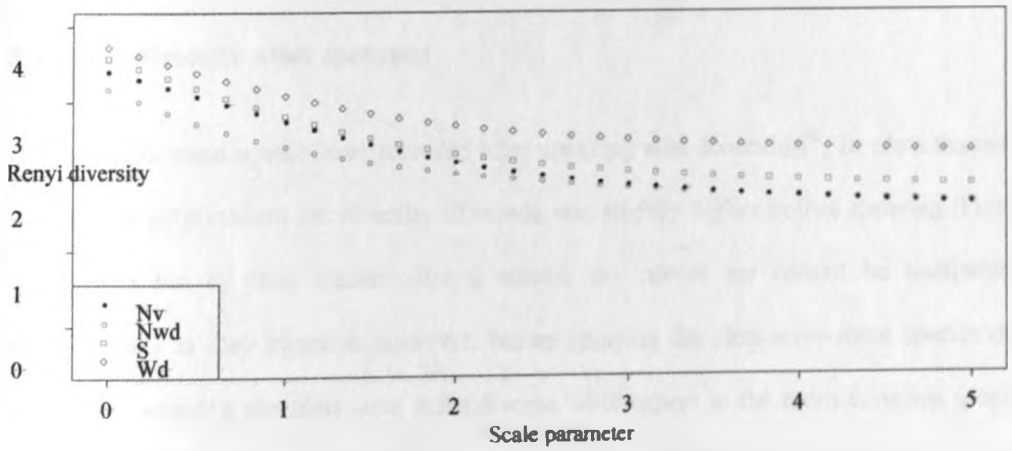


Figure 3.3: Diversity profiles of the Renyi diversity index for all the treatments (9weeks after germination) NV; Renyi profile in natural vegetation, NW; Non-weeded plots; Sprayed plots and Wd; Weeded plots.

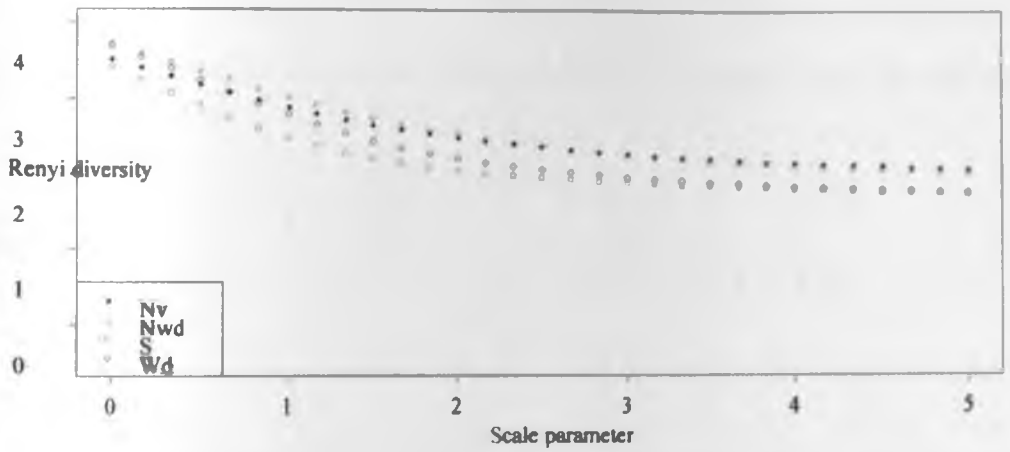


Figure 3.4: Diversity profiles of the Renyi diversity index for all the treatments (12weeks after germination) NV; Renyi profile in natural vegetation, NW; Non-weeded plots; Sprayed plots and Wd; Weeded plots.

3.2 Weed diversity after spraying

A total of 30 weed species was recorded after spraying with Roundup®. In plots treated 3 weeks after germination, the diversity of weeds was slightly higher before spraying (Figure 3.5 below) but in plots treated after 6 weeks, the curves are cannot be comparable unequivocally as they intersect. However, before spraying the plots were more species rich while after spraying the plots were more diverse with respect to the more common species (Figure 3.6). The change in Renyi diversity was more drastic in plots treated after nine weeks as in (Figure 3.7) the diversity is much higher before spraying.

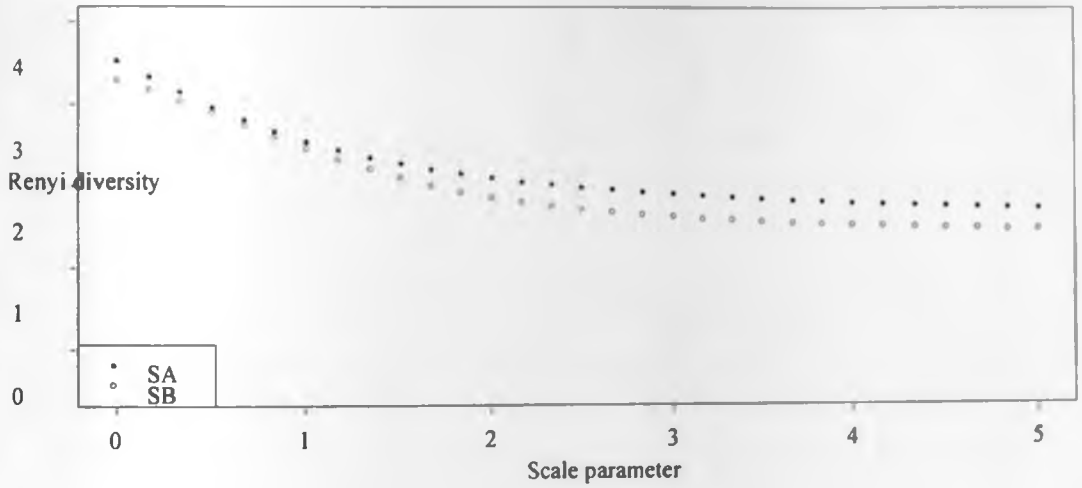


Figure 3.5: Diversity profiles of the Renyi diversity index for the pre and post spraying (3weeks after germination) SA; Renyi profile before spraying, SB; Renyi profile after spraying

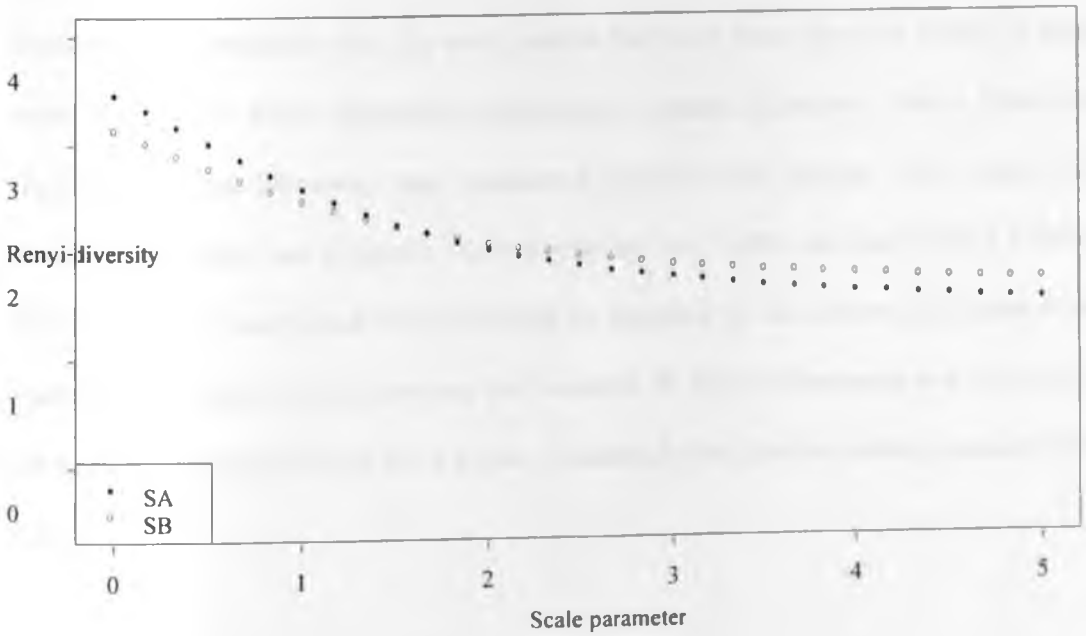


Figure 3.6: Diversity profiles of the Renyi diversity index for the pre and post spraying (6 weeks after germination) SA; Renyi profile before spraying, SB; Renyi profile after spraying

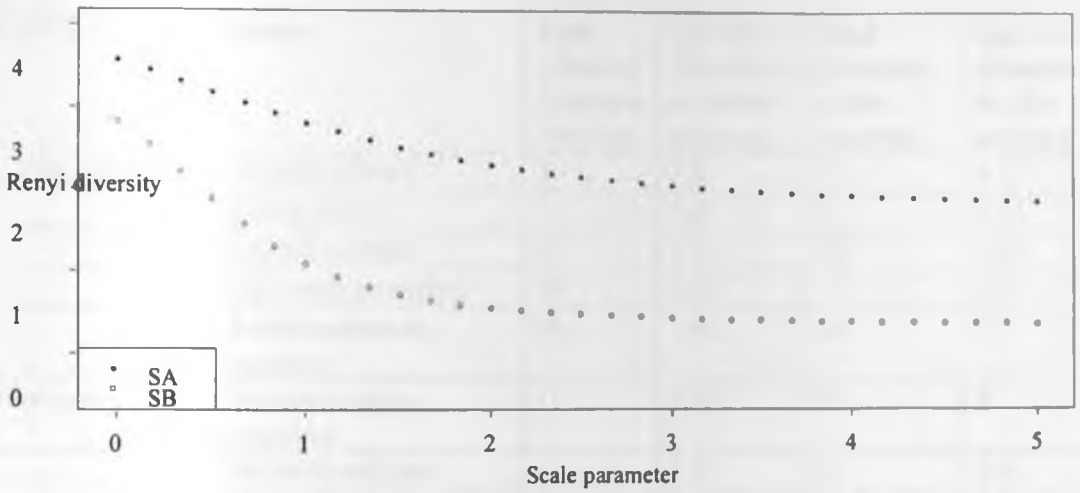


Figure 3.7: Diversity profiles of the Renyi diversity index for the pre and post spraying (9 weeks after germination) SA; Renyi profile before spraying, SB; Renyi profile after spraying

3.3 Weed abundance

From the rank abundance list, the weed species that were most abundant before spraying were; *Spermacoce laevis, Euphorbia geniculata, Portulaca oleraceae, Bidens pilosa and Digitaria velutina.* However, their abundance reduced after spraying with species like *Portulaca oleraceae* and *Digitaria velutina* reducing to <1 after spraying Table 3.2 below. Species evenness was higher before spraying as indicated by the species abundance in the Table below. However, after spraying low evenness in species abundance was observed as the high ranking species have much higher abundances than the low ranking species (Table 3.2)

Table 3.2: Families, Abundance and Rank Abundance of species recorded before and after spraying

Family	Species	Rank Abundance before spraying	Log (Sum Abundance) before spraying	Rank Abundance after spraying	Log (Sum Abundance after spraying
Compositae	*Targetes minuta*	21	1.81	33	0.7
	Bidens pilosa	3	3.03	3	1.95
	Launea cornuta	17	1.96	10	1.56
	Galinsoga parviflora	10	2.40	14	1.3
	Acanthospermum hispidum	18	1.95	26	0.9
Poaceae	*Dacteloctenium aegyptica*	11	2.39	41	0
	Digitaria velutina	6	2.55	9	1.61
	Digitaria abyssynica	9	2.46	23	1.04
	Setaria verticillata	4	3.02	21	1.04
	Eragorastis suber	38	1.50	43	0
	Sorghum verticilliflorum	15	2.01	30	0.7
	Cynodondactylon	13	2.33	17	1.23
	Eleusineindica	38	1.50	19	1.08
Euphorbiaceae	*Eurphorbia geniculata*	2	3.11	2	2.78
	Euphorbia hirta	22	1.81	25	0.9
Portulacaceae	*Portulaca oleraceae*	5	2.74	32	0.7
Rubiaceae	*Spermacoce laevis*	1	3.18	1	2.84
Fabaceae	*Indigofera ambelacensis*	16	1.99	24	1
	Indigofera colutea	7	2.49	7	1.65
	Desmodium ramosissimum	6	2.55	8	1.61
	Crotoraria brevidens	31	1.67	31	0.7
	Cassia mimmosoides	36	1.58	42	0
	Crotoraria spinosa	33	1.62	28	0.85
	Glycine wightii	34	1.61	40	0
Commelinaceae	*Commelina benghalensis*	12	2.38	4	1.9
Amaranthaceae	*Amaranthus graecizans*	28	1.71	15	1.3
	Amaranthus hybridicus	24	1.77	12	1.41
Cyperaceae	*Cyperus spp*	14	2.24	39	0
Boraginaceae	*Trichodesma zeylanicum*	32	1.63	13	1.38
Nyctaginaceae	*Boerhavia erecta*	29	1.69	6	1.73
Malvaceae	*Cocculus hirsutus*	43	1.14	11	1.48
	Corcochurus tridens	31	1.67	36	0
	Sida acuta	33	1.62	16	0

	Waltheria indica	13	2.33	38	1.28
Asteraceae	*Xanthium pungens*	41	1.23	37	0
Capparaceae	*Cleome monophylla*	30	1.69	27	0
Zygophyllaceae	*Tribulus terrestis*	32	1.63	29	0.9
Polygonaceae	*Oxygonum sinuatum*	35	1.58	22	0.78
	Fallopia convulvus	20	1.90	5	1.04
Verbenaceae	*Lantana camara*	39	1.34	35	1.82
Solanaceae	*Nicandra physalodes*	25	1.77	20	0.48
	Hypharia	42	1.18	18	1.04

3.4 Round up tolerant species

During the first treatment (3 weeks after germination) Roundup® controlled the entire weed species present within 7 days but during the subsequent treatments more time was required before death of weed species. During the third treatment (after 9 weeks), only 76.52% of weeds were controlled 7 days after treatment. *Commelina benghalensis* is the only species that showed tolerance to Roundup® at the rate used. Growth of *Commelina* was only suppressed and regenerated after sometime (See Table 3.3 below).

Table 3.3: Percentage of weed species controlled by Roundup® during each herbicide application interval and number of days after application

Number of Week	DAY7	DAY 14	DAY 21
	Percentage Controlled		
3	79.07	100	
6	88.37	95.34	97.67
9	90.7	93.02	97.67
12	74.44	86.04	97.67

3.5 Cotton growth characteristics

3.5.1 Cotton height

There was significant (F=28.96, d.f=2, p<0.001) difference in the mean cotton height among the three treatments and among the different treatment times (F=17.88, d.f=3, p<0.001) Appendix 1. There was however no significant (p>0.05) difference in cotton height between the weeded and the sprayed plots. Similarly, plots treated 3weeks after sowing showed no significant (p> 0.05) difference in height with plots treated after 6 weeks while those treated after 9 weeks had no significant(p> 0.05) difference with those treated after 12 weeks. The tallest plants were found in plots treated in time I while the shortest were in non-weeded plots and plots treated at time 4 in the plots weeded at time I had the tallest plants while (Table 3.4).

Table 3.4: Mean height ± se (cm) of cotton in the different treatments and treatment times; Time1; plots treated 3weeks after germination, Time2; treated after 6 weeks; Time3; treated after 9 weeks and Time4 treated after 12 weeks

Treatment	Time1	Time2	Time3	Time4
Weeded	108.14±0.687	99.09±4.129	56.91±10.377	32.49±2.469
Sprayed	104.39±0.950	89.08±1.538	36.55±2.190	29.1±0.721
Non-weeded	27.93±2.736	26.810±0.26	28.23±1.355	26.93±2.171

There was change in height of cotton after the treatment (weeding and spraying) at the different treatment times. This increase was however observed until six weeks after which the effect of weed removal on cotton height was not significant (Figure. 3.8 and 3.9). In the non-weeded plots there was an increase in height of cotton up to six weeks after which no

significant increase was observed (Figure 3.10)

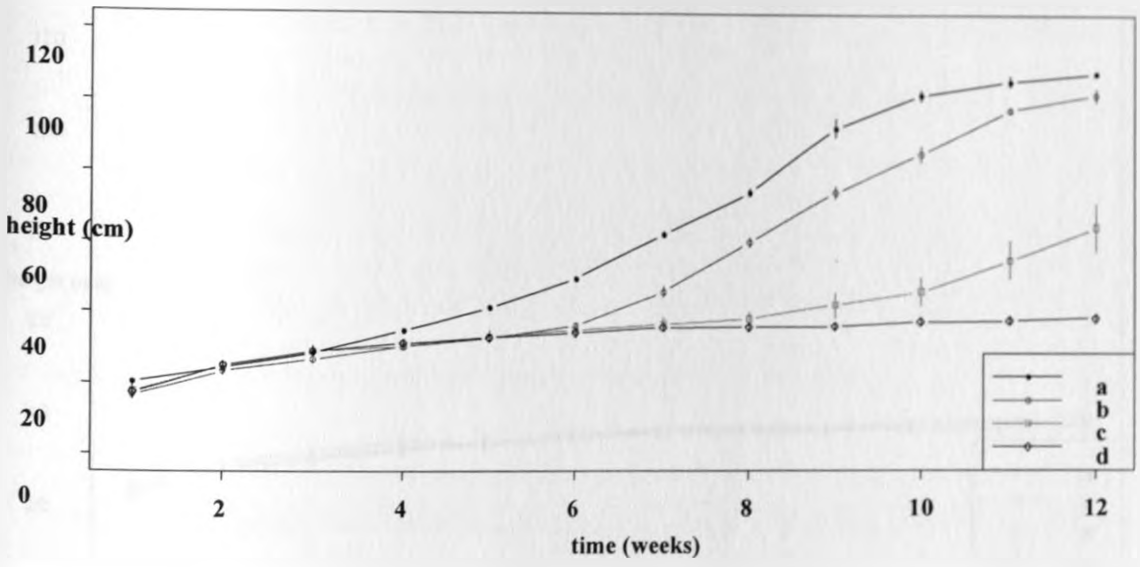


Figure 3.8: Time trends in the height of cotton showing an increase in height of cotton after weeding a) weeded three weeks after germination, b) weeded after six weeks, c) weeded after nine weeks, d) weeded after 12weeks

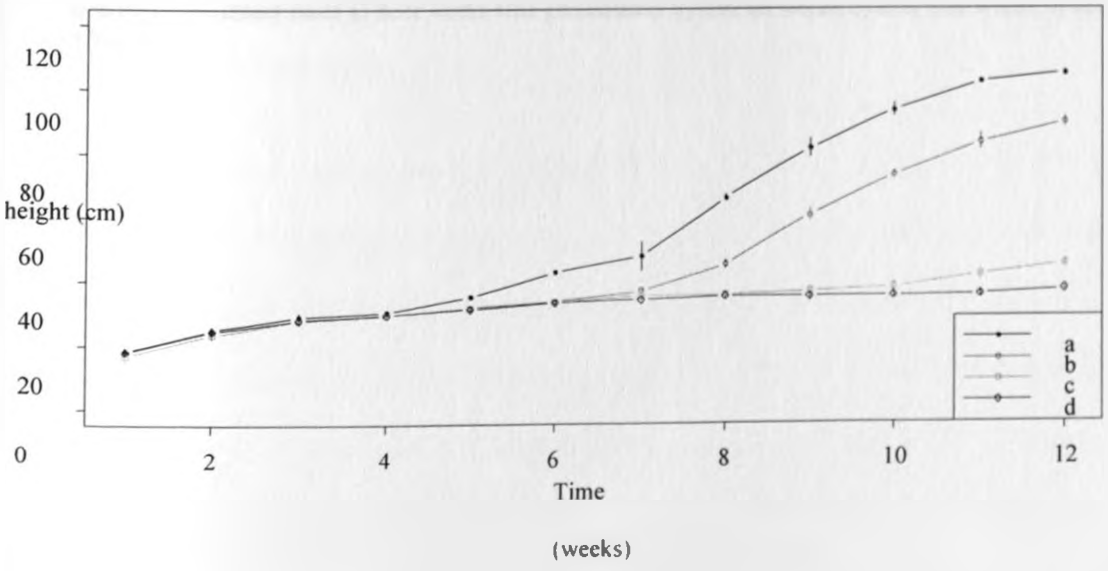


Figure 3.9: Time trends in the height of cotton showing an increase in height of cotton after Spraying a)Sprayed three weeks after germination, b) Sprayed after six weeks, c) Sprayed after nine weeks. d) Sprayed after 12weeks

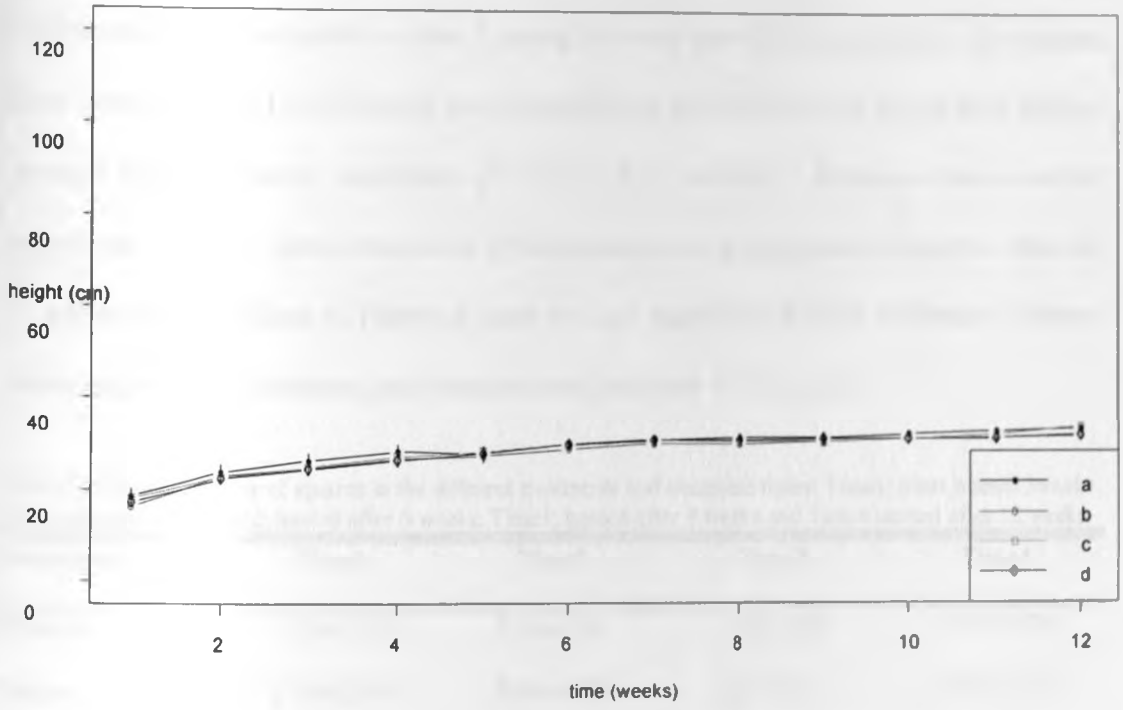


Figure 3.10: Time trends in the height of cotton showing an increase in height of cotton in non-weeded plots at different treatment times a) three weeks after germination, b) after six weeks, c) after nine weeks, d) after 12weeks

3.5.2 Number of squares

The number of recorded squares differed significantly ($F=17.03$, $d.f=2$, $p<0.001$) among the treatments with plots weeded at time 1 having the most number of squares and non-weeded plots with the least. The difference was not significant ($p>0.05$) between the sprayed and the weeded plots. Similarly, significant ($F=12.47$, $d.f=3$, $p<0.001$) difference was observed among the treatment times where time 1 had the most and least number of squares observed in plots treated at time 4. However there was no significant $p>0.05$ difference between treatment time1 and time two, and between time3 and time 4 (Table. 3.5).

Table 3.5: Mean number of squares in the different treatments and treatment times; Time1; plots treated 3weeks after germination, Time2; treated after 6 weeks; Time3; treated after 9 weeks and Time4 treated after 12 weeks

Treatment	Time1	Time2	Time3	Time4
Weeded	12.27±2.212	9.53±0.321	4.90±1.08	1.33±0.126
Sprayed	8.70±0.736	8.90±0.200	2.83±0.583	0.80±0.173
Non-weeded	1.00±0.132	1.10±0.132	1.37±0.189	1.03±0.028

3.5.3Number of mature bolls

The number of mature bolls counted differed significantly ($F=11.51$, $d.f=2$, $p<0.001$) among the different treatments but the difference was not significant between the sprayed and the weeded plots $p>0.05$. Weeded plots had the highest number of mature bolls while non-weeded plots had the lowest< 1. Similarly a significant ($F=10.62$, $d.f=3$, $p<0.001$) difference was observed among the treatment times however there was no significant $p>0.05$ difference between treatment time1 and time two, time 3 and time2 and between time3 and time 4 (Table 3.6).

Table 3.6: Mean number of mature bolls in the different treatments and treatment times; Time1; plots treated 3weeks after germination, Time2; treated after 6 weeks; Time3; treated after 9 weeks and Time4 treated after 12 weeks

Treatment	Time1	Time2	Time3	Time4
Weeded	21.30±3.619	18.27±1.308	4.47±1.183	0.40±0.500
Sprayed	18.17±4.136	8.03±2.968	1.83±1.171	0.20±0.087
Non-weeded	0.50±0.218	0.33±0.126	0.37±0.058	0.27±0.029

CHAPTER FOUR

4. DISCUSSION AND CONCLUSION

4.0 Discussion

This study has shown that cotton growth characteristics (plant height) and yield (from number of mature bolls recorded) varied by treatment and with treatment time and indicated that weeds affected cotton growth. Plots that remained weed free from week three had the tallest plants and produced the most bolls. There was marked increase in height of cotton after weed removal indicating that weeds do compete with cotton. Comparable results were obtained in previous studies by Robinson (1976), Nobrega *et al.*, (1998), and Mahar *et al.*, (2007) where non-weeded plots produced both shortest plants and the lowest number of mature bolls. The duration of weed competition also determines the competitive damage to crop. This is illustrated in this study where plots that were treated 9 weeks after germination produced no yields since weeds overshadowed the cotton plants (Robinson, 1976). As observed in this study, and by others Gale and Earl (1970), weed competition was more critical during the first two months as plots treated 3weeks and 6weeksafter germination recorded higher yields than the rest.

The land used in this study has been tilled continuously and this could explain the presence of perennial dicots and grasses during the initial survey. The number of weed species recorded before tillage was much lower as compared to weeds recorded after tillage. This is can be explained by the fact this survey was conducted after the previous harvest and during the dry season. In such a case, most of the annuals had already matured and dried up. Some of these species however did not appear after tillage for example *Panicum maxima*. There was no significant difference in diversity of weeds among the plots indicating that the plots were

homogeneous in terms of species composition and management practices applied. This is in agreement with Zelaya (1998) who showed that continued tillage reduces weed diversity and promotes reproduction of perennial plants.

This study has demonstrated a lower diversity in the natural vegetation and surprisingly in sprayed plots before treatments. Soil disturbance is an important vegetation selection factor according to Froud (1987), and any form of cultivation practises usually has consequences on the composition and density of weed floras. The lower diversity in natural vegetation could be as an effect or lack of disturbance. However the lower diversity in sprayed could not be clearly established and may have been due to chance.

A lower diversity of weeds was observed in this study after spraying. The decline was more pronounced when spraying was done 9 weeks after germination but when done 6 weeks after germination diversity of the rare species was low but that of more common species was higher than before spraying. In a previous study by Watkinson *et al.*, (2000) their results suggested that weed control in herbicide tolerant crops would clean up weedy fields leading to a decline in weeds. This is in agreement with findings of the current study that recorded a marked decline in diversity and abundance of weeds after spraying with Roundup®. The decline in abundance of some weed species like *Digitaria velutina* and *Portulaca oleraceae* could be a reflection of the effectiveness of round up in controlling weeds thus with continued use some of these weeds may eventually disappear from the agricultural lands. However some species seem to be favoured by glyphosate use like *Euphorbia geniculata* and this could explain the slightly lower diversity observed after spraying since the fields were dominated by *Euphorbia geniculata*. Thus continued use of Roundup® could probably lead to weediness of fields by such species as Freckleton *et al.*, (2004) had indicated.

In this study we tried to mimic a Glyphosate resistant (GR) field by covering cotton plants with polythene bags to protect the crop. From the results of this study, Roundup® has proved effective in controlling 97.67% of all the weeds present within 21 days with the exception of *Commelina benghalensis* (Tropical spiderwort) that did not die. However its growth was suppressed by Roundup® application and these plants took a long period to resume growth and even then their growth was much slower as compared to other *Commelina benghalensis* plants that were not treated with Roundup®. Similar observations have been made elsewhere where Roundup® only controlled 53% of tropical spiderwort 21 days after treatment with Roundup® (Culpepper *et al.*, 2004). In this study glyphosate was only 100% effective (see Table 3.1) when applied 3 weeks after germination (when weeds are young). This is in agreement with Culpepper *et al.* (2004) and Prostko *et al.*, (2005) who found that glyphosate alone was not effective in managing spiderwort because of herbicide tolerance and continuous germination throughout the growing season. Continuous germination was however, not observed during this study probably because the season was characterized by dry spells and spiderwort grows well when the moisture content is plentiful (Kaul *et al.*, 2002). Thus, to effectively manage older tropical spiderwort additional management practises are required as Webster *et al.*, (2006) had suggested.

4.1Conclusions and recommendations

From this study it is evident that weeds interfere with the growth of cotton and thus weed control is necessary especially during the early stages of growth. Chemical weed control like (Roundup®) is effective in controlling weeds and is less labour intensive as compared to manual weeding. However spraying with Roundup® reduced the diversity and abundance of weeds in Mwea thus interfering with the weed species composition. The timing of Roundup® application is of importance since application nine weeks after germination had the most

drastic effect on diversity. Thus if Roundup® is to be adopted as a means of weed control, farmers need to be sensitized on the best time to apply it. Continued use of glyphosate in cotton fields is likely to have a negative effect on the diversity and abundance of weeds in cotton fields. However, glyphosate has proved to be effective in controlling most weeds in cotton fields with the exception of *Commelina benghalensis* that has exhibited tolerance to the recommended rates for spot application. This weed has a potential of becoming a problematic (resistant) weed with continued use of glyphosate. Before the introduction of GR cotton, additional management practises towards older *Commelina benghalensis* need to be considered to avoid the risk of it becoming a superweed.

Since this was a one off treatment that used only one rate of Roundup®, the results obtained is a reflection of the short term dynamics in weed species composition thus, more research is required to examine the extent of *Commelina's* tolerance to different rates of Roundup®, establish the presence of resistance gene in the species and effects of Roundup® application below ground biodiversity micro organisms and nematodes. This is because recent studies have shown that Roundup® has residual effects in the soil contrary to what its manufacturers had indicated. Thus, the effect on below ground biodiversity would be of great concern.

REFERENCES

Abernathy JR and McWhorter CG. 1992. Evolution of weed control in cotton. *In* C.G. McWhorter and J.R. Abernathy (eds.) Weeds of Cotton: Characterization and Control. The Cotton Foundation. Memphis, TN, 1-8.

Anderson WP. 1983. Weed Crop competition. Weed science principles. 2nd edition, West Pub. Co. MN, 15.

Anderson WP. 1996. Weed sciences. Principles and application 3rd edition, West Pub. Co., MN, 39-64.

Baerson SR, Rodriguez DJ, Tran M, Feng YM, Biest NA, Dill GM. 2002. Glyphosate-resistant goosegrass: Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Plant physiology*, **129**: 1265-1275.

Beltrao NE. 2002. Que planta e esta? Cultivar (Agosto de 2002), 17-18.

Bridges DC. 1992. Crop losses due to weeds in the United States. Champaign, Ell.: Weed Science Society of America.

Brubaker CL, Bourland FM, Wendel JE. 1999. The origin and domestication of cotton. In C.Smith, J.T Cothren, eds Cotton: Origin, History, Technology, and Production. John Wiley and Sons, Inc., New York, pp 3-31.

Brubaker CL, Koontz JA and Wendel JF. 1993. Bidirectional cytoplasmic and nuclear introgression in the New World cottons, *G. barbadense* and *G. hirsutum*. *American Journal of Botany*, **80:** 1203-1208.

Bussan AJ, Burnside OC and Orf JH. 1993. Selection for weed competitiveness in soybean cultivars. *Proc. Weed Sci. Soc.*, 48:76.

Chikoye D, Ellis-Jones J, Riches C and Kanyomeka L. 2007. Weed management in Africa: experiences, challenges and opportunities. *XVI International Plant Protection Congress*, 652-653.

Coble HD and Byrd JD. 1992. Interference of weeds with cotton. In C.G. McWhorter and J.R. Abernathy (eds.) Weeds of Cotton: Characterization and Control. The Cotton Foundation. Memphis, TN. pp 73-84.

Culpepper AS, Flanders JT, York AC, and Webster TM. 2004. Tropical spiderwort (Commelina benghalensis) control in glyphosate-resistant cotton. *Weed Technol.*, **18**: 432-436.

Dill GM. 2005. Glyphosate-resistant crops: History, status and future. *Pest Manag Sci.*, **61(3)**: 219-224.

Duke SO. 1998. Herbicide-resistant crops—their influence on weed science. *J Weed Sci Technol.*, 43: 94- 100.

Franz JE, Mao MK, and Sikorski JA. 1997. Toxicology and environmental properties of glyphosate. In Glyphosate: A Unique Global Herbicide. American Chemical Society Monograph 189. Washington, D.C. *American Chemical Society*, Pp. 103–141.

Freckleton RP, Stephens PA, Sutherland WJ and Watkinson AR. 2004. Amelioration of biodiversity impacts of genetically modified crops: predicting transient versus long-term effects. *Proceedings of the Royal Society of London*, **271**: 325- 331.

roud-Williams RJ, Chancellor RJ., Drennan, DSH. 1987. Potential changes in weed floras associated with reduced-cultivation systems for cereal production in temperate regions. *Weed Res.*, **21**: 99–109.

Fuzzato M. 1999. Melhoramento genetico do algodoeiro. In: Cia, E., Freire, E.C. and Santos, W.J. dos (eds) Cultural do Algodeiro. Piracicaba, potafos, Brazil, pp. 15-34.

Gale AB and Earl RB. 1970. Influence of weed competition on cotton. *Weed Sci.*, **18**: 149-154.

Ganesh MK, Padgette SR and Fraley RT. 1992. History of herbicide tolerant crops, methods of development and current state of the art - emphasis on glyphosate tolerance. *Weed Technol.*, **6**: 626-634.

Garner TH and Bowen HD. 1961. Influence of crab grass on the quality of mechanically harvested cotton. Trans. ASAE, **4**: 210-214.

Gealy DR, Bradford KJ, Hall L, Hellmich R, Raybould A, Wolt J. 2007. Implications of gene flow in the scale-up and commercial use of biotechnology-derived crops: Economic and policy consideration (CAST Issue Paper 37). Ames, IA: Center for Agriculture Science and Technology (CAST).

Gianessi LP. 2005. Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Manage Sci.*, **61**: 241–245.

Gunsolus JL. 1990. Mechanical and cultural weed control in corn and soybeans. *J. Altern. Agric.*, **5**: 114-119.

Hillocks RJ. 1998. Potential benefits of weeds with reference to small holder

Agriculture in Africa. *J. Biomed & Life Sci.*, **3**: 155-167.

kiara MM and Ndirangu, LK. 2003. Developing a revival strategy for Kenya's cotton - textile industry. A value chain approach. KIPPRA Working paper Number 8. Kenya Institute for Public Policy Research and Analysis (KIPPRA), Nairobi, Kenya, 104.

Jain SC, Iyer BG, Jain HC. and Jain NK. 1981. Weed management and nutrient losses in upland cotton under different ecosystems of Madhya Pradesh. Proceeding of the 8th Asian-Pacific *Weed Sci. Soc.*, Bangalore, India, pages 103-107.

Jones RE, Vere DT, Medd RW and Alemseged Y. 2005. Estimating the economic costs of weeds in Australian annual winter crops. *Agric. Econ.*, **32**: 253–265.

Jones MA and Snipes CE. 1999. Tolerance of transgenic cotton to topical application of glyphosate. *J. Cotton Sci.*, **3**: 19-26.

Kalaher CJ, Coble HD and York AC. 1997. Morphological effects of Roundup® application timings on Roundup®-Ready cotton. Proc. Belt. Cotton Conf, pages 204.

Kaul V, Sharma N, and Koul AK. 2002. Reproductive effort and sex allocation strategy in *Commelina benghalensis* L., a common monsoon *weed. Bot. J. Linnean Soc.*, **140:** 403-413.

Keeler KH, Turner CE and Bolick MR. 1996 .Movement of crop transgenes into wild from glyphosate-resistant crops. *Weed Technol.*, **20**: 301-307.

Keeler KH. 1989. Can genetically engineered crops become weeds? *Biotech.*, 7: 1134-1139.

Keeley PE and Thullen RJ. 1989. Growth and competition of black nightshade (*Solanum*

nigrum) and Palmer amaranth (*Amaranthus palmeri*) with cotton (*Gossypium hirsutum*). *Weed Sci.*, **27**: 326-34.

Klee HJ, Muskopf YM and Gasser CS. 1987. Cloning of an Arabidopsis thaliana gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. *Mol. Gen.*, **210:** 437-442.

Knezevic SZ, Evans SP, Blankenship EE, Van Acker RC and Lindquis JL. 2002. Critical period for weed control. The concept and data analysis. *Weed Sci.*, **50**: 773-786.

Kohel RJ and Lewis CF. eds. 1984. Cotton Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WI. 605

Lagoke STO, Adejonwo KO, Choudhary AH and Ogungbile AO. 1992. Herbicide evaluation in the hoe-farming system cotton production in Northern Nigeria. *Tropical Pest Management*, **38***(3)*: 286-294.

Lep J and Milauer P. 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge.

Light GG, Baughman TA, Dotray PA, Keeling JW and Wester DB. 2003. Yield of glyphosate-tolerant cotton as affected by topical applications on the Texas high plains and rolling plains. *J. Cotton Sci.*, **7**: 231-235.

Mahar GM, Ouad FC, Buriro UA, and Solangi GS. 2007. Effect of post emergence herbicides on the growth and yield of up-land cotton. *Asian J. of Plant Sci.*, **6(8)**: 1282-1286.

Maina JM, Kivuva BM, Mburu MWK, Murdoch AJ, Njuguna JM, and Mwangi DM. 2003. Weed management options for resource poor maize-dairy farmers in Central Kenya. *The BCPC International Congress, Crop Sci. and Tech.*, 993-998.

Makanganise A, Mabasa S, Jasi L, and Mangosho E. 1999. Farmer participation and evaluation of their perceptions on integrated weed control methods with specific reference to herbicide technology in communal areas of Zimbabwe. *17th East African Biennial Weed Science Conference Proceedings*, 97-107.

McCune B and Mefford MJ. 1997. PC-ORD. Multivariate analysis of ecological data, Version 2.0. MJM Software Design, Gleneden Beach.

McWhorter CG and Bryson CT. 1992. Herbicide use trends in cotton. Weeds of Cotton: Characterization and Control Memphis, TN 233–294.

Milberg P and Hallgren E. 2004. Yield loss due to weeds in cereals and its large-scale variability in Sweden. *Field Crops Res.*, **86**: 199–209.

Mwanda CO. 2000. A note on weed control in Machakos District,Kenya. Published in: Starkey P and Simalenga T (eds), *Animal Power for Weed Control*. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, Holland.

Njoroge JM. 1991. Tolerance of *Bidens pilosa* and *Parthenium hysterophorus* L to paraquat (Gramoxone) in Kenya coffee. Kenya Coffee, **56**: 999-1001.

Nobrega LB, Vieira DJ, Beltrao NE, Azevedo DM and Araujo JD. 1998. Chemical weed

control in up-land cotton. Revista de oleaginosas-e-fibrosas, Brazil, **2**: 61-69.

Owen MDK. 2000. Current use of transgenic herbicide-resistant soybean and corn in the USA. *Crop Prot.*, **19**: 765-771.

Papamichail D, Eleftherohorinos I, Froud-Williams R, and Gravanis F. 2002. Critical period of weed competition in cotton in Greece. *Phytoparasitica*, **30**: 105–111.

Patil G and Taillie C. 1982. Diversity as a concept and its measurement. Journal of the American Statistical Association, **77**: 548–561.

Perez A and Kogan M. 2003 Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. plants. *In* Herbicide Resistant Plants. CRC Press Inc.

Pline WA, Edmisten KL Wilcut JW, Wells R and Thomas J. 2003. Glyphosate- induced reductions in pollen viability and seed set in glyphosate-resistant cotton and attempted remediation by gibberellic acid (GA3). *Weed Sci.*, **51**: 19-27.

Powles SB, Lorraine-Colwill DF, Dellow JF and Preston C. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci.*, **46**: 604 –607.

Prostko EP, Culpepper AS, Webster TM, and Flanders JT. 2005. Tropical spiderwort identification and control. *Weed Res.*, **43**: 12-19.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Renyi A. 1961. On measure of entropy and information. in Neyman, J. (ed.). Proceedings of the 4th Berkeley Symposium on Mathematical Statistics and Probability, Vol. I. Univ.

California Press, Berkeley. Pages 547-561

Rieger MA, Lamond M, Preston C, Powles SB and Roush RT. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, **296**: 2386–2388

Robinson EL. 1976. Effect of weed species and placement on seed cotton yields. *J. Weed Sci.*, **24**: 353-355.

Sanyal D, Bhwomiik PC, Anderson RL and Shrestha A. 2008. Revisiting the perspective and progress of integrated weed management. *Weed Sci.*, **56**: 161-167.

Secretariat of the Convention on Biological Diversity. 2000. Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes. Montreal: Secretariat of the Convention on Biological Diversity.

Suh H, Hepburn AG, Kriz AL, and Widholm JM. 1993. Structure of the amplified 5-enolpyruvylshikimate-3-phosphate synthase gene in glyphosate-resistant carrot cells. *Plant Mol. Biol.*, **22**: 195-205.

Thornton PK, Fawcett RH, Dent JB, and Perkins TJ. 1990. Spatial weed distribution and economic thresholds for weed control. *Crop Prot.*, **9**:337-342.

Tothill JC, Mott JJ and Gillard P. 1982. Pasture weeds of the tropics and subtropics with special reference to Australia. In Biology and Ecology of Weeds. Dr W. Junk Publishers, The Hague, 403-427.

US-EPA. 1998. Principles of Environmental Impact Assessment Review: Appendix D: Contents of specific Environmental Impact Assessment Tools.

Vissoh PV, Gbehounou G, Ahanchede A, Kuyper TW and Roling NG. 2004. Weeds as Agricultural Constraint to Farmers in Benin: Results of a Diagnostic Study. *NJAS.*, **52**: 305-329.

Watkinson AR, Freckleton RP, Robinson RA and Sutherland WJ. 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science*, **289**: 1554-1556

Waturu CN, Kambo CM, Ngigi RG, and Muthoka G. 2007. Field evaluation of transgenic *Bt* cotton varieties DP4488 and DP 404BG for efficacy on target and non-target pests and environment. KARI publication, 57.

Webster TM, Burton MG, Culpepper AS, Flanders JT, Grey LT, and York AC. 2006. Tropical spiderwort (Commelina benghalensis L.) Control and Emergence patterns in preemergence Herbicide System. *J. of Cotton Sci.*, **10**: 68-75.

Wendel JF and Albert VA. 1992. Phylogenetics of the Cotton genus (*Gossypium)*: Character-state weighted parsimony analysis of chloroplast-DNA restriction site data and its systematic and biogeographic implications. *Syst. Bot.*, **17**: 115-143.

Wiles LJ, Gold HJ, and Wilkerson GG. 1993. Modeling the uncertainty of weed density estimates to improve post-emergence herbicide control decisions. *Weed Res.*, **33**: 241–252.

Young BG. 2006. Changes in herbicide use patterns and production practices resulting data and its systematic and biogeographic implications. *Syst. Bot.*, **17**: 115-143.

Young FL, Ogg Jr AG, Papendick RI, Thill DC, Alldredge JR. 1994. Tillage and weed management affects winter wheat yield in an integrated pest management system. *Agron. J.*, **86**: 147-154.

Zelaya I.A, Owen MDK, and VanGessel MJ. 2007. Transfer of glyphosate Resistance: Evidence of hybridization in Conyza (Asteraceae). *Am. J. of Bot.*, **94**: 660-673

APPENDICES

Appendix1: Anova summary table of heights in both the treatments and timings and Tukeys test to separate the mean

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Timing	3	12888.8	4296.3	17.884	7.62E-07
Control	2	13915.1	6957.5	28.962	9.89E-08
Residuals	30	7206.9	240.2		
Timing	diff	lwr	upr	p adj	
b-a	-7.673	-27.540	12.194	0.722	
c-a	-34.479	-54.346	-14.612	0.000	
d-a	-46.231	-66.098	-26.364	0.000	
c-b	-26.806	-46.673	-6.939	0.005	
d-b	-38.558	-58.425	-18.691	0.000	
d-c	-11.752	-31.619	8.115	0.389	
Treatment	diff	lwr	upr	p adj	
S-Nwd	36.1875	20.58831	51.78669	0.000009	
Wd-Nwd	45.61167	30.01247	61.21086	1E-07	
Wd-S	9.424167	-6.17503	25.02336	0.310179	

Appendix2: Summary Anova table for the number of squares in both the treatments and timings and Tukeys test separating the means

	df	Sum Sq	Mean Sq	F value	Pr(>F)
Timing	3	238.35	79.45	12.472	1.82E-05
Control	2	217	108.5	17.032	1.14E-05
Residuals	30	191.11	6.37		
Timing	diff	lwr	upr	p adj	
b-a	-0.81111	-4.04632	2.424101	0.90323	
c-a	-4.28889	-7.5241	-1.05368	0.005829	
d-a	-6.33333	-9.56855	-3.09812	5.32E-05	
c-b	-3.47778	-6.71299	-0.24257	0.031411	
d-b	-5.52222	-8.75743	-2.28701	0.000356	
d-c	-2.04444	-5.27966	1.190768	0.332237	
Treatment	diff	lwr	upr	p adj	
S-Nwd	4.183333	1.643113	6.723554	0.000919	
Wd-Nwd	5.833333	3.293113	8.373554	1.06E-05	
Wd-S	1.65	-0.89022	4.190221	0.260692	

Appendix3 : Summary Anova table for the number of mature bolls in both the treatments and timings and Tukeys test separating the means

	df	Sum sq	Mean sq	F Value	Pr(>F)
Timing	3	977.93	325.98	10.622	6.38E-05
Control	2	706.26	353.13	11.506	0.000196
Residuals	30	920.7	30.69		
Timing	diff	lwr	upr	p adj	
b-a	-4.44444	-11.5454	2.656539	0.340453	
c-a	-11.1	-18.201	-3.99902	0.001043	
d-a	-13.0333	-20.1343	-5.93235	0.000135	
c-b	-6.65556	-13.7565	0.445427	0.072501	
d-b	-8.58889	-15.6899	-1.48791	0.012998	
d-c	-1.93333	-9.03432	5.16765	0.880018	
Treatment					
S-Nwd	6.691667	1.116124	12.26721	0.016003	
Wd-Nwd	10.74167	5.166124	16.31721	0.000137	
Wd-S	4.05	-1.52554	9.625543	0.189856	