Associated impacts of common weeds on *Bacillus thuringiensis* (*Bt*) cotton: a case study of Mwea, Kenya

By

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DECLARATION

This thesis is my original work and has not been submitted to any other university for award of a degree.

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This thesis has been submitted for examination with our approval as University of Nairobi supervisors

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DEDICATION

This thesis is dedicated to my wife Mrs. Dorcas Jepleting Ngetich, my daughter Daisy Chepkosgei Ngetich and my parents the late Mr. Benjamin Mibey and Mrs. Priscilla Mibey. Their love, belief and wishes have helped me realise my potential.

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TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

Bt - Bacillus thuringiensis

MOA - Ministry of Agriculture

CaMv - Cauliflower Mosaic virus

KARI - Kenya Agricultural Research Institute

NBC - National Biosafety Committee

At - Agrobacterium tumefaciens

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

KEPHIS - Kenya Plant Health Inspectorate

ABSTRACT

Cotton (*Gossypium hirstum* L.) is an important cash crop in Kenya but its yields are low due to various constraints including weeds, pests and diseases. Cotton is especially sensitive to weed competition because it grows relatively slowly in the early stages, and does not reach full ground cover until eight or more weeks after germination. The effects of weeds on the cotton crop can be caused by competition for light, water and nutrients, and will depend on the type and density of weed growth. Weeds also act as alternative host for insect pests that attact the cotton plants. The African bollworm, if not properly controlled, is an important pest responsible for close to 100% yield loss. Although *Bt* cotton is known to affect insect pests, not much is known about its competition with weeds. It is however postulated that unlike conventional cotton, *Bt* cotton may outcompete weeds as it is believed that it has a competitive advantage than conventional cotton over weeds because of the fact that it would not be attacked by pests and would have a head start in the early growth stages.

The main objective of this study was to establish the effect of weeds on cotton growth and to evaluate the competitive ability of *Bt* cotton against common weeds in Kenya. This study therefore sought to find whether and when this competitive advantage was achieved under field conditions, and the role of weeds in harbouring pests of cotton. The experiment used a completely randomized block sampling regime using weeded and non-weeded plots. Growth parameters (namely plant heights, number of bolls, number of leaves and number of attacked leaves) of *Bt* and conventional cotton plants were assessed under both weedy and weed free conditions during the growing season.

There was no significant ($p>0.05$) difference in number of leaves, bolls and pest damage between *Bt* and non-*Bt* cotton (HART89M) varieties. HART89M had higher ($P<0.05$) final height compared to *Bt* cotton variety. The difference in plant growth parameters between weeded and non-weeded plots was found to be significant ($P<0.05$). The common weeds found were *Commelina benghalensis* (mean density = 50.041±1.79), *Waltheria indica* (mean density = 49.416±1.34) and *Cleome monophylla* (mean density = 49.25±0.85). Generally it was concluded that *Bt* cotton had no competitive advantage over weeds compared to conventional cotton. This conclusion has implications in cotton stewardship programmes in that farmers need to understand that *Bt* cotton, thuogh is not attacked by bollworms, requires the necessary number of weedings in order to avoid losses caused by weeds.

Keywords: *Bacillus thuringiensis, Bt* cotton, weeds.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The word "cotton" refers to four species in the genus *Gossypium* (Malvaceae) - *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L.- that were domesticated independently as sources of textile fibre (Brubaker *et al.,* 1999a). Globally, the *Gossypium* genus comprises about 50 species (Brubaker *et al.,* 1999a). The place of origin of the genus is not known, however, the primary centers 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; Seelanan *et al.,* 1997).

Cotton lint was spun and woven into cloth even before 3000 B. C. (Gulati and Turner, 1928 cited in McGregor, 1976). Most commercially cultivated cotton is derived from two species, *G. hirsutum* (Upland cotton, 90% of world plantings) and *G. barbadense* (Pima, or Long-staple cotton). The species *G. hirsutum* is the most widely planted in Kenya.

The crop was introduced in Kenya in the early 18th century by the British colonialists (Lutrell *et al.*, 1994). Cotton is an important fiber crop that provides a source of income to farmers and fiber to the textile industries. The seeds provide an important source of oil and feed cake for humans and livestock (Lutrell *et al.*, 1994). In Kenya, cotton is mainly grown in the semi-arid regions of Eastern, Central, Nyanza, Coast, Western and Rift Valley provinces.

For decades, cotton production in Kenya has been characterized by low yields. In 2007, the country produced 45,000 bales of lint (Ministry of Agriculture, 2007) falling short of

the annual potential of 300,000 bales. Pests, diseases and weeds are the major constraints to cotton production. According to Fontes *et al.* (2006) the group of pests attacking cotton is known to comprise at least 30 species of insects and three species of mites. In Kenya, the major pests causing low yield and poor quality cotton include the African bollworm (*Helicoverpa armigera*), Cotton stainer (*Dysdercus* spp.), Cotton aphid (*Aphis gossypii*) and Cotton red spider mite (*Tetranychus telarius*) (Waturu, 2001). The African bollworm is the most important pest in cotton fields especially during the reproductive phase, appearing at the squaring stage and may cause up to 100% yield loss if unchecked.

Cotton is sensitive to weed competition because it grows relatively slowly in the early stages, and does not reach full ground cover until eight or more weeks after germination; and often under poor conditions, full ground cover is never achieved. To keep the crop free of weeds until the cotton plants meet across the rows is expensive, and it is important to know whether and when weed control can be relaxed without seriously affecting yields. Many experiments have been carried out to determine the period in which weed control is most critical, but the results of these experiments have often been contradictory (Hawtree, 1980).

Crother (1943) in Sudan found that the seventh and eighth week after sowing were the most critical, but Thomas (1969) considered that the first six weeks were the most important for weed control. Arnold *et al.* (1976) compared two successive seasons in Uganda, and found that the results were markedly affected by seasonal conditions. The effects of delayed weeding were much greater in the drier season, when the young cotton was adversely affected by water stress. Thomas and Schwerzel (1968) found in Zimbabwe that the critical period for weed competition was between two and four weeks after crop emergence, but they postulated that the critical

period would be longer in wetter seasons. On the Kafue flats in Zambia, Kerkhoven (1964) found that the competition between weeds and cotton was mostly for nitrogen; and that while weeding up to six weeks was important to reduced competition, subsequent weeding had an even greater effect on yield until the cotton plants met across the rows. In Nigeria, Lawes (1964) pointed out that the process of weed eradication may be confounded by the effect of cultivation on soil surface, and hence by infiltration of water into the soil.

The effect of weeds on the cotton crop can be caused by competition for light, water and nutrients, and will depend on the type and density of weed growth as well as on the type of soil, rainfall and the level of fertility (Papamichail *et al.,* 2002). Hawtree (1980), reviewing numerous studies on the effects of weed competition on the growth and yield of cotton in various parts of the world, concluded that the weight of evidence indicate that weed competition during the first two months of the crop's life was more injurious than during the second two months.

Once full ground cover has been achieved, cotton can compete satisfactorily with most common species of weeds, but some tall and climbing weeds can still present a problem, not only because of their effect on yield of cotton, but because they interfere with picking and other field operations. Climbing weeds hinder access to the crop for spraying and picking; weed foliage can impede the full impact of a spray intended to give full coverage to the leaves of the cotton plants. Late weed growth can interfere with mechanical harvesting and cause staining of the lint, while grasses and other species of weeds which shed their seeds on the open bolls add more labour of clearing trash from the seed cotton (Hawtree,1980). Weed seeds produced at this time can add to the weed problems in the subsequent crops.

Genetic modification of organisms to incorporate useful genes, such as in the case of *Bt* cotton, has been advocated as a promising technology for the future development of sustainable agricultural systems (Vadakattu and Watson, 2004). Transgenic *Bt* cotton varieties are grown in United States of America (USA), Australia, China (Shelton *et al.,* 2002) India, Pakistan and South Africa (Bennett *et al.,* 2006), greatly reducing insecticide dependence in these countries. *Bt* cotton are plants engineered to express insecticidal proteins (*cry1Ac* and *cry2Ab2*) produced by the bacterium *Bacillus thuringiensis* that are toxic to major lepidopteran pests (Derek, 2007). The plants are transformed by *Agrobacterium*-mediated transformation. The plasmid vector contains gene expression cassette(s) that codes for the *Bt* insecticidal protein under the control of the cauliflower mosaic virus (CaMV)35S promoter (Derek, 2007). When incorporated into plants, *Bt* proteins are much more persistent and effective therefore greatly reducing the need for application of broad-spectrum insecticides.

In Kenya, *Bt* cotton is being considered for introduction to farmers as part of the Government's strategy for the revival of the collapsed cotton industry. The introduction of *Bt* cotton in Kenya was initiated through an application by Kenya Agricultural Research Institute (KARI) to import Bollgard *Bt* cotton in 2001. In 2002, the National Biosafety Committee (NBC) now the National Biosafety Authority (NBA) allowed requests for the importation and testing of *Bt* cotton by KARI in contained facilities and this was followed by approval by the National Council of Science and Technology (NCST) in 2003.

Bt cotton is expected to be released for use by farmers once approved by National Biosafety Authority (Waturu, 2007). While numerous studies conducted on *Bt* cotton in Kenya have focused on non-targets such as arthropods (Waturu *et al.*, 2007), literature on weeds biodiversity

is scanty. For any transgenic crop to be introduced into the country, it is a requirement that the National Biosafety Authority (NBA) be provided with relevant biosafety data. Pre-release evaluation of most genetically engineered plants focuses on genetic stability of the inserted gene and agronomic aspects of the plants. This is done in order to demonstrate the safety of genetically engineered crops. Presently, there are limited quantitative data on weed competition with *Bt* cotton in Kenya.

1.2 Literature review

1.2.1 Biology of cotton

Cotton is a perennial plant with an indeterminate growth habit; vegetative and reproductive growth occurs at the same time; but four main growth stages can be distinguished: (i) germination and seedling establishment; (ii) leaf area and canopy development; (iii) flowering and boll development and (iv) maturation. The length of cotton-growing season varies from 100 to over 190 days according to climatic conditions and plant variety (Beltrao, 2002). Germination begins with the entry of moisture into the seed and embryo via the chalazal aperture, at the seeds' apex (Christiansen and Moore, 1959). The seed/embryo then begins to swell as it absorbs moisture. Under favourable conditions, the radicle (root tip) emerges within 2 - 3 days from the seed and newly germinated seedlings emerge above the soil 5 - 10 days after emergence of the radicle (Oosterhuis and Jernstedt, 1999). However, growth of 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 faster in warm and moist soils. As the cotton plant grows, the radicle that originally emerged from the seed becomes a taproot, from which lateral roots begin to grow.

Lateral roots and the taproot make up the basal root system. Other roots which develop from this basal root system have a functional life of about 3 weeks (Oosterhuis and Jernstedt, 1999).

1.2.2 Leaf and canopy development

Following germination, cotton plant growth continues with the development of a central, main stem that bears the first true leaves spirally, along its axis. Leaves are typically 10 -15 cm wide, palmately-lobed, with 3 - 5 lobes on each leaf. Branching of the main stem occurs initially from axillary buds of the main stem leaves. Either vegetative (monopodial) or fruiting (sympodial) branches are produced. Both branch types bear true leaves, but about 5 - 6 weeks after planting the total area of leaves born on fruiting branches exceeds that of the main stem and vegetative branches, constituting about 60% of the total leaf area at maturity (Oosterhuis and Jernstedt, 1999).

1.2.3 Reproduction and dispersal

In cotton plants, reproductive maturity is reached about 4 - 5 weeks after planting, with the formation of floral buds ("squares"). It takes about 25 days between the initial appearance of a square and anthesis (flower opening) (Oosterhuis and Jernstedt, 1999). Under normal crop conditions, about 60% of squares and immature fruit are abscised prematurely. Mature flowers are not usually shed before pollination (Oosterhuis and Jernstedt, 1999). Cotton flowers anthese at or near dawn and remain open for only one day. At anthesis, the petals of *G. hirsutum* are creamy white. They turn pink-red within about one day of pollination, after which they abscise.

The first square produced on a fruiting branch is known as a first position square. As the 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 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 (Oosterhuis and Jernstedt, 1999).

1.2.4 Pollen and Pollination

Soon after anthesis, the anthers of cotton flowers dehisce, discharging their pollen. Cotton pollen is relatively large and heavy, and not easily dispersed by wind (Jenkins, 1992). Cotton is a facultative self-pollinator, and an opportunistic out-crosser when insect pollinators are present (Oosterhuis and Jernstedt, 1999). Cotton pollen remains viable for about 12 hours (Govila and Rao, 1969). Fertilisation of ovules occurs about 12 - 30 hours after pollination.

1.2.5 Fruit development

The growth and development of cotton fruit, known as "bolls", begins immediately following fertilisation although the most rapid period of growth occurs after about 7 - 18 days (Oosterhuis and Jernstedt, 1999). During development, the bolls are spherical to ovoid and pale green. Maximal boll size is achieved about 25 days after fertilisation, with full maturity achieved approximately 20 days later. Mature bolls are thick and leathery, and dry rapidly to become brittle and brown. Such fruit often split open, revealing the seeds and associated fibres.

1.2.6 Uses of cotton and its by-products

Cotton is currently the leading plant fibre crop worldwide and is grown commercially in the temperate and tropical regions in more than 50 countries (Smith, 1999). Specific areas of production include countries such as USA, India, China, America, the Middle East and Australia, where climatic conditions suit the natural growth requirements of cotton, including periods of hot and dry weather and where adequate moisture is available, often obtained through irrigation. Cotton is primarily grown as fibre crop. It is harvested as "seed cotton" which is then "ginned" to separate the seed and lint. The long "lint" fibres are further processed by spinning to produce yarn that is knitted or woven into fabrics.

The ginned seed is covered in short, fuzzy fibres, known as "linters". These must be removed before the seed can be used for planting or crushed for oil, and are used in a variety of products including foods. The linters are produced as first-cut or second-cut linters. The first-cut linters have a longer fibre length and are used in the production of mattresses, furniture upholstery and mops. The second-cut linters have a much shorter fibre length and are a major source of cellulose for both chemical and food uses. They are used as a cellulose base in products such as high fibre dietary products as well as a viscosity enhancer (thickener) in ice cream, salad dressings and toothpaste. In the chemical industry the second-cut linters are used with other compounds to produce cellulose derivatives such as cellulose acetate, nitrocellulose and a wide range of other compounds (Gregory *et al.*, 1999).

The delinted cotton seed can be processed to produce oil, meal and hulls. Cotton seed oil has been in common use since the middle of the nineteenth century and achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act

because of its common use prior to 1958 (Anzfa, 2002). It is used in a variety of products including edible vegetable oils and margarine, soap, and plastics. Cotton seed, or meal, flour or hulls derived from it, are also used in food products and for animal feed, but this is limited by the presence of natural toxicants in the seeds (gossypol and cyclopropenoid fatty acids). Human consumption of cotton seed meal is reported mainly in central American countries and India where it is used as a low cost, high quality protein ingredient (Franck 1989; Ensminger *et al.*, 1990).

1.2.7 Constraints of cotton production in Kenya

Cotton is grown by small scale farmers in various agro-ecological zones in Kenya. These zones include areas 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, weeds, lack of certified seeds, collapse of extension services, lack of credit, low producer prices and marketing uncertainties. Lepidopteran pests such as the African bollworm also limit cotton production.

1.2.8 Pests and diseases of cotton in Kenya

More than 1,326 species of insects have been reported in commercial cotton fields worldwide but only a small proportion are pests (Matthews andTunstall, 1994). Of the 30 pests of cultivated *G. hirsutum*, the most important are the caterpillars of *Helicoverpa armigera* and *H. punctigera,* and the spider mite *Tetranychus urticae* (Shaw 2000; Pyke and Brown, 2000).

Important cotton pests in Kenya include the African, pink and spiny bollworms; cotton leaf worm; red spider mite; jasid; aphids; whiteflies and cotton stainers (Waturu, 2001). These pests

cause huge yield losses and cotton farmers in Kenya have to spend fifty seven percent of production costs on pest control (Wakhungu and Wafula, 2004).

The main viral disease experienced is "blue disease" which is transmitted by *Aphis gossypii* (Bell, 1999). It causes stunted growth due to shortening of the internodes, leaf rolling, an intensive green colour of the foliage and vein yellowing. Three important fungal diseases attack cotton roots. Fusarium wilt is a soil and seed-borne fungal disease caused by *Fusarium oxysporum* f. sp. *vasinfectum* that can enter the root via damage caused by root knot nematode (*Meloidogyne incognita*) infestation.

1.2.9 Transgenic (*Bt*) cotton

Transgenic *Bt* cotton has been genetically engineered by inserting *cry1Ac* gene either singly or in combination with *cry2Ab2* gene (Perlak *et al.*, 1990). The two genes are from a soil dwelling bacterium known as *Bacillus thuringiensis* subsp *Kurstaki*. The protein toxins *cry1A*c and *cry2Ab2* expressed in the engineered plants are toxic to key lepidopteran pests including the African bollworm, the most important pest of cotton in Kenya. Other genes that have been used in genetic transformation of cotton include the *vip3A* and the *cry1Fa* genes. *Bt* cotton plants engineered with the *vip3A* gene have been shown to be effective against *H. armigera* (Cloud *et al.*, 2004) while those containing the *cry1Fa* genes are effective against *Heliothis virescens* and *Pectinophora gossypiella*. The transgenic cotton varieties Bollgard I (DP 404BG and DP 448B varieties) and Bollgard II (06Z604D) are currently grown in confined field trials in Kenya. Bollgard I carries the *cry1Ac* gene while Bollgard II carries *cry1Ac* and *cry2Ab2* genes for the control of the African bollworm.

1.2.10 Locus structure of the *Bt* gene in Bollgard I and II

Each transgenic cotton variety has a specific plasmid vector, transgenic locus structure, transgene expression and transmission (Perlak *et al.*, 1990). Bollgard I cotton was produced through *Agrobacterium*-mediated transformation using a plasmid vector containing two plant gene expression cassettes. One cassette codes for the *Bt* insecticidal protein *cry1Ac,* and the second cassette codes for the selectable marker gene *nptII* under the control of the cauliflower mosaic virus *(CaMV)35S* promoter and the non-translated region of the 3' region of the nopaline synthase gene (Perlak *et al.*, 1990) The vector also contains a fragment with the *aad* gene that confers resistance to the antibiotics spectinomycin and streptomycin. The transgenic locus contains two copies of the T-DNA insert with one insert containing a full length *cry1Ac* gene and the *nptII* gene, and the second insert contains an inactive 3' portion of the *cry1Ac* gene. The two inserts are linked and seem to segregate as a single locus (Agbios, 2002).

Bollgard II transgenic locus contains a single DNA insert into the Bollgard I genome. The plasmid vector used contains two plant gene expression cassettes and a bacterial kanamycin resistance gene and the origin of replication. The first cassette contains the *cry2Ab2* gene encoding the insecticidal protein and the second cassette contains the *uidA* gene encoding the beta-glucurodinase reporter protein (Jefferson *et al.*, 1986). The *cry2Ab2* and *uidA* genes are under the control of the enhanced *CaMV 35S* promoter and the 3' region of the nopaline synthase gene from *Agrobacterium tumefaciens.*

1.2.11 Effect of weeds on cotton production

Weeds can be detrimental to crop production due to competition for water, nutrients, and sunlight. 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 that it infests. In cotton, competition with weeds results in fewer and less mature bolls per plant and lower lint quality (Abernathy and McWhorter, 1992). Weed-crop competition is one of the major causes of crop yield loss (Cao *et al.*, 2007). Competition can be defined as two or more plants growing in close proximity to each other and drawing on the same limited-supply resource pool (Coble and Byrd, 1992). According to Coble and Byrd1 (992), the weed species, density, and duration of the population determine the competitive damage to cotton. The more competitive species with the greatest density and longest duration will cause the most significant reduction in cotton production. The extent of yield losses depend on weed density (Fischer and Ramirez, 1993), type of weedy plants (Diarra *et al.*, 1985). Cotton must be kept weed-free for a period after emergence in order to avoid crop loss. The more competitive the weed species is, the longer the weed-free period must be (Coble and Byrd, 1992). In 1991, cotton worth about $4.1 billion was lost due to weeds in the United States (Bridges, 1992). In Kenya the cost of weeding accounts for 12% of the cost of cotton production (Ikiara and Ndirangu, 2002).

According to Oerke and Dehne (2004), loss potentials caused by weeds worldwide average at 34%. As many as 30 genera of annual and perennial grasses, sedge, and broadleaf weed species have been identified in cotton fields across the United States. Over 100 of these species are considered troublesome weeds in cotton (Holm *et al.*, 1977). Among these species, five genera;

Ipomoea, *Amaranthus*, *Cyperus*, *Xanthium*, and *Senna* were reported to have caused the greatest percentage cotton crop loss in the United States in 2002 (Byrd, 2003).

1.2.12 Weed-Crop Interactions

Weeds are the most universal of all crop pests, proliferating each year on every farm in Africa (Obuo *et al.*, 1997). African soils contain 100 to 300 million buried weed seeds per hectare of which only a fraction germinate and emerge each year (Chikoye *et al.*, 1997). The soil seed population in a Nigerian experiment was estimated at 20,130 seeds per square meter (200 million per hectare) (Chikoye *et al.*, 1997). A review of crop pests in sub-Saharan Africa indicated that weeds are the most important pest to control in all zones studied (Sibuga, 1997). Over 286 species of common weeds have been identified in crop fields in some West African countries (Njoku, 1996). A total of 263 weed species belonging to 38 families were found in crop fields in West Africa (Chikoye and Ekeleme, 2001). Broad-leaved weeds (72%) and grasses (24%) dominated the total weed spectrum, whereas sedges (4%) were minor. Mean weed species richness per field was similar across all agroecological zones and averaged about 16 per field (Chikoye and Ekeleme, 2001). A survey of smallholder wheat fields in Ethiopia found that the weed population reached 743 weeds per square meter in contrast to a crop stand of only 149 wheat plants per square meter (Tanner and Sahile, 1991). Unweeded fields in Nigeria produced between 17 and 30 tons per hectare of fresh weed weight (Adigun *et al.*, 1991). Weed problems are more severe in African tropical regions than in Europe and North America because weeds grow more vigorously and regenerate more quickly because of the heat and higher light intensity. High humidity and high temperature, conditions characteristic of sub-Saharan Africa, favour rapid and excessive weed growth (Akobundu, 1980b). Weeds compete with crops for nutrients,

space, light and water thus reducing crop yields. Numerous studies have documented the negative effects on yield of season-long weed competition in Africa. Under unweeded conditions, crop losses have been measured; for maize (55 - 90%), common bean (50%), sorghum (40 - 80%), cowpea (40-60%), rice (50-100%), cotton (80%), wheat (50 - 80%), groundnut (80%), and cassava (90%) repectively (Ambe *et al.*, 1992; Akobundu, 1987; Olowe *et al.*, 1987; Ishaya *et al.*, 2007; Ngouajio *et al.*, 1997; Chikoye *et al.*, 2004; Dadari and Mani, 2005). Weeds need to be cleared from a field prior to planting a crop and weeds need to be removed from the field during the growing season for optimal yields to be achieved. Weed competition is most serious when the crop is young. The critical period of crop-weed competition is approximately equal to the first one-third to one-half of the life cycle of the crop. In weed-crop competition studies, the "critical period" is the stage after which weed growth does not affect crop yields. Keeping the crop free of weeds for the first third of its life cycle usually assures near maximum productivity (Doll, 2003). African crops that have been studied at experimental farms in order to define the weed-free period required to prevent yield reduction and the weed-free period in days required after planting for various crops have shown the following as critical weed free days; maize, 56; rice, 42; sorghum, 35; cassava, 84; cowpea, 40 (Obuo *et al.*, 1999; Akobundu, 1987; Ambe *et al.*, 1992).

1.2.13 Weed control in cotton

Weed management systems must be developed to reduce the economic losses caused by weed competition in cotton. There are many methods of weed control including cultural, biological, mechanical, and chemical (Anderson, 1996). Cultural weed control utilizes practices that are less favourable for weeds, yet more advantageous for crops. These include row spacing and crop

selection favorable for a critical weed-free period, as well as crop rotations and planting of smothers 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 increases its leaf area might increase 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. However, mechanical weed control is the physical removal or prevention of weeds by hand-pulling, hoeing, mowing, flooding, smothering, burning, and machine tilling. One form of machine tilling used in cotton production is cultivation.

Chemical weed control utilizes phytotoxic chemicals, referred to as herbicides, to kill or suppress weeds. An increased use of herbicides began in 1944 with the discovery of 2,4-D [(2,4-dichlorophenoxy) acetic acid] and 4-chloro-2-methylphenoxy acetic acid (MCPA), and has dramatically increased in time with new herbicide developments (Anderson, 1996). Herbicides can be applied in cotton as pre-plant (PP), pre-plant incorporated (PPI), pre-emergence (PRE), post-emergence-topical (POST) or as 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.

Before the introduction of herbicide-tolerant cotton, herbicides were applied either as selective POST herbicides, or as non-selective PDIR herbicides. Selective herbicides kill weeds with little to no effect on the crop, allowing them to be applied topically. Non-selective herbicides injure or kill both the weed and crop; therefore, they must be directed under the crop canopy rather than

topically applied. Although POST herbicides applications tend to provide better weed coverage and require less equipment than PDIR herbicides applications, a limited number of broad-spectrum, selective herbicides available for POST applications make PDIR herbicides a viable option for weed control in non-transgenic cotton.

Glyphosate [*N*-(phosphonomethyl) glycine] is a non-selective, systemic herbicide that controls a variety of annual and perennial broadleaf, grass, and sedge weeds. The development of transgenic crops that are resistant to glyphosate allows glyphosate to be applied as a POST herbicide. 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; Klee *et al.,* 1987; Suh *et al.,* 1993; Thompson *et al.,* 1987).

1.2.14 Hand weeding

Hand weeding is the predominant weed control practice on smallholder farms in many developing countries (Vissoh *et al.*, 2004). Hand weeding is the oldest method of weed control and consists of hand-pulling, handslashing and hoeing of weeds. Smallholder farmers spend 50-70% of their total labor time handweeding (Chikoye *et al.,* 2007a).Women contribute more than 90% of the hand weeding labor for most crops (Ukekje, 2004). 69% of farm children between the ages of 5-14 are forced to leave school and are used in the agricultural sector especially at peak period of weeding (Ishaya *et al.*, 2008b).

In Africa, 80% of the cultivated land is currently prepared by hand and on 16% of the land animal draught power is used while only 4% is prepared with mechanical power (Adolfsson,

1999). Family sizes have, in many traditional African societies, been increased to cope with weeding activities (Adegoroye *et al.*, 1989).

The contrast between research recommendations and farmers' practices is particularly stark in the case of weed management. Researchers have produced clear-cut recommendations for optimal time of weeding. Research on experimental plots has indicated that to produce maximum yields, a large number of hours of handweeding must be undertaken. Groundnuts needs 378 hours/ha, maize 276 hours/ha and sorghum 150 hours/ha (Akobundu, 1987). Weeding of cotton requires 200 - 400 hours per hectare while ricerequires 200 - 418 hours/hectare (Ishaya *et al.*, 2007a; Mavudzi *et al.*, 2001).

A recent study of women in African agriculture confirmed that weeding took up more days in the field than any other operation (IFAD, 1998). Minimum estimates of the days spent weeding were 60. In Uganda, this figure increased to as much as 120 because of the country's two cropping seasons. In Zambia, with a single cropping season, the estimated time spent weeding was in the 90 to 120 day range. Several interviews with specialists confirmed that it was impossible for any woman to keep more than one hectare free of weeds in a typical cropping season (IFAD, 1998). Research has demonstrated the impacts on yield of performing fewer than the optimal number of handweedings. With three properly spaced handweedings, the highest cotton yields (549 kg/ha) were obtained while with two handweedings, the yield was reduced by 27% (401 kg/ha). One handweeding resulted in a loss of 55% (249 kg/ha); and zero handweeding resulted in yields that were 87% lower (73 kg/ha) than the optimal yields (Prentice, 1972). Typically, 30 - 90 hours per hectare are required to remove weeds before planting cotton (Kienzie, 2002).

Generally, two properly spaced hand weedings within eight weeks of planting of maize (at three weeks and six weeks) give yields comparable to keeping the crop weed-free for the first eight weeks after planting (Orr *et al.*, 2002). One week's delay in first weeding may reduce maize yields by one-third, and two week's delay in second weeding may reduce maize yields by one-quarter. A delay of the first weeding in cotton by a week increased the initial weed growth by 600% and doubled the initial labor demand. Delay of the first weeding by two weeks increased the initial weed growth by 2000% and trebled the initial labour demand (Druijff and Kerkhoven, 1970).

Although a lot of energy is expended in removing weeds by hand, crop yields are generally very low due to weed competition, as a result of untimely and ineffective weed control (Chikoye *et al.*, 2004). On most farms, weeding usually competes with other farm activities and is postponed to a later date. Farmers will not weed crops that are sown first until they complete the seedbed preparation and sowing of all other fields. Farmers prefer to go on planting to take advantage of moisture in the soil (Makanganise *et al.*, 1999). This usually results in delayed weeding. Late weeding results in crop losses, especially if it is carried out after the critical period of weed competition. Poor weed management in cassava fields caused an average yield gap of 5t/ha and restricted production in farmers fields in Kenya in 2004 by 11.6t/ha (Fermont *et al.*, 2009).

Several constraints limit the effective use of hand weeding, including limited cash for hiring labor and labor not being available for hire during peak periods (Johnson, 1995). The supply of labour in rural areas has been significantly reduced in many African countries due to HIV/AIDS and migration to urban areas which has led to less weeding of crops (Bisikwa *et al.*, 1997). HIV/AIDS is causing the loss of at least 10% of the agricultural workforce in most countries and,

in at least five countries including South Africa, Nigeria, Zimbabwe, Kenya and Tanzania has more than 20% (Bishop-Sambrook, 2003). The scarcity of labour and the concurrent rise in the cost of handweeding make timely removal of weeds by direct labour difficult and expensive. There is an acute shortage of labour at the beginning of the wet season for land preparation, planting and adequate first weedings. No pool of landless rural labourers can be called upon during periods of peak labour demands (Byerlee and Heisey, 1996).

There is a shortage of male labour for weeding due to competing labour activities such as wage employment, livestock tending and fencing (Shaxson *et al.,* 1993). People usually prefer alternative jobs to hand weeding, if they are available. It is often assumed that family labour is free but labour in all activities has an "opportunity cost." During the peak period, farmers have little rest. Farmers are often too sick or fatigued to complete weeding (Orr *et al.*, 2002). African women pointed out that crop management can be neglected during pregnancy, with tasks requiring hard physical work (such as weeding) particularly affected (Webb and Conroy, 1995). In addition to farming responsibilities, African women farmers have numerous family responsibilities including typically caring for upto six children, elderly parents and sick family members. As a result of these conflicting time demands, weeding is not always carried out in a timely fashion or in the right amounts.

Malaria is also a common problem on farms, reducing the availability of productive labour. The scarcity of labour coupled with early season rains often impede timely removal of weeds. The sowing time of some crops coincides with or just precedes periods of heavy rain, and wet soil conditions do not permit efficient hand weeding or hoeing. For those farmers with heavy soils,

excessively wet conditions do not permit efficient handweeding to be done resulting in long periods of crop-weed competition and yield reduction (Chivinge, 1990).

In sub-Saharan Africa, the once readily available and reliable cheap labour force has disappeared in the face of rapid urbanization, improved living standards, and increased educational opportunities. Landless young people have shifted from agricultural activities to off-farm activities (Vissoh *et al.*, 2004). Labour for handweeding is, therefore, very scarce and when available too expensive for the average farmer to afford (Akobundu, 1979). As a result, it is often impossible to carry out timely weeding by hand. In many instances, labour constraints force farmers to plant their crops after weeds have begun to grow. Such crops are easily smothered by weeds and give an extremely poor yield and in such cases, these fields are abandoned (Ndahi, 1982). Family labour is seriously stretched on farms and has to be deployed continuously for weeding, as the first weeded plots are re-infested by the time the last plots are cleaned. One effect of the large demand for handweeding labour is that a considerable portion of a farmer's fields may be left fallow and not planted to a crop (Tittonell *et al.*, 2007). The area cultivated is often reduced by 50% because of the farmer's assessment that not enough labour would be available to weed the additional fields (Bishop-Sambrook, 2003).

The principal limiting factor to the size of farms in Africa is the number of necessary weedings during the period following planting for various crops (Kent *et al.*, 2001). Eighty per cent of farmers indicated that if weeds were less of a problem, they would increase the area of land under cultivation (Johnson, 1995). African farmers tend to plant as much as they think they will be able to weed. As a result, weeds can be considered as the main constraint on agricultural production.

In Malawi, a nationwide survey data suggested that one-third of the area planted to maize by smallholders is either left unweeded or weeded after the critical six weeks (Orr *et al.*, 2002). Maize is generally the first crop to be planted and weeding becomes necessary at a time when labour is critical for planting cash crops such as groundnuts (Mloza-Banda, 1997). Shortage of labour early in the season results in delayed weeding and subsequent maize yield losses of 15 - 90% due to weed competition (Kibata *et al.*, 2002).

In Nigeria, maize farmers' weeding practice (one weeding) resulted in 42% yield loss in comparison to fields weeded three times (Chikoye *et al.*, 2004). Delayed weed removal is the primary cause of maize yield loss in smallholder agriculture (Rambakudzibga *et al.*, 2002; Chikoye *et al.*, 2005). A survey of farmers in Malawi revealed that a majority weeded groundnuts late (later than 30 days after sowing) because their limited labour resources were used for other crops (Luhana *et al.*, 1994). The yield loss for late weeding groundnuts is up to 40%. Most Nigerian cowpea farmers rarely weed the crop within the first six weeks of growth because of instability in labour supply, cost and demands on their time for other activities, and hence the low yields on most farms (Olofintoye and Adesiyun, 1990).

Time-of-planting trials have shown the vital importance of planting at the start of the rains. In Zimbabwe one third of the maize is planted late because of labour constraints with a yield loss of up to 75% on late-planted fields (Byerlee and Heisey, 1996). In West Africa, yields of upland rice with farmers' weed control were 44% lower than on researcher weeded plots (Johnson, 1995). In a survey of rice farmers in Cote d'Ivoire, fifty three per cent said that their fields were not always weeded (Johnson and Adesina, 1993). A reason given for this by almost two-thirds of these farmers was that weed infestation in the crop may be so severe that weeding was not

always worthwhile; therefore, the field would be effectively abandoned. Other reasons given for not weeding included lack of cash to hire labour, sickness, and lack of labour. In Uganda, eighty seven per cent of the farmers believed that they currently lose yield to weeds, mainly due to late or inadequate weeding (Webb and Conroy, 1995).

In Zimbabwe, twenty one per cent of the cotton farmers abandon more than twenty per cent of their cropped area each year as a result of weed infestation (Mavudzi *et al.*, 2001). Weeds are a major factor in reducing crop yields in Zambia, many farms recording an average 30% yield reduction. Indeed some farmers have lost entire crops due to heavy weed infestation (Masole and Kasalu, 1997). In Africa, yield losses in farmers' fields range from twenty five per cent to total crop failure because farmers are unable to perform the necessary weedings at the optimal times (Vissoh *et al.*, 2004). Weeds are perceived by most smallholder farmers as the greatest yield-limiting constraint (Fofana and Rauber, 1999; Vissoh *et al.*, 2004). In Africa yield losses due to weeds average thirty but losses of fifty percent or more are frequently reported in some parts of sub-Saharan Africa (Sibuga, 1999). Since handweeding demands a relatively wider spacing, there is little chance to optimize cropspacing in favour of higher yields.

An econometric analysis of labour decisions by small scale farmers concluded that farmers are unable to allocate sufficient weeding labour for optimal yields in years when rains are abundant because weed growth is rapid and prolific and labour shortages preclude the availability of sufficient weeding (Fafchamps, 1993). As a consequence of less than optimal weeding, yields do not achieve their full potential even in years of considerable rainfall. Farmers do not undertake overly ambitious production plans since they are likely to lead to weeding manpower constraints.

There is a widespread belief that weeding can be properly performed only if the worker is bent double and armed with a short-handled hoe (IFAD, 1998). It is common that the handle is short to allow the farmer full control of the hoe while he/she works around the plants, leaving the other hand free to pull out weeds and shake the roots free from soil (Adolfsson, 1999). Such hand hoes and other weeding tools are known for high prevalence of back ache among users. Sharp pains result low in the back from the use of the short-handled hoes (Nwuba and Kaul, 1986). Farmers can be seen to be suffering as they often rise and stretch their backs. To weed one hectare of maize a farmer would have traveled a distance of ten kilometers in a stooped position (Mangosho *et al.*, 1999). Handweeding in a stooped position for long periods of time results in permanent spinal deformation (Oyedemi and Olajide, 2002).

1.2.15 Justification

Cotton is an important fibre crop that provides a source of income to farmers and fibre to the textile industries. The seeds provide an important source of oil and feed cake for humans and livestock (Lutrell *et al.*, 1994). However, yields are low mainly due to pests, weeds and diseases. Consequently, a large amount of herbicides and pesticides are applied each year on cotton fields leading to a rise in the cost of cotton production. Chemicals used possibly affect the health of farmers and cause environmental pollution to the soil and water systems. Weeds and pest-resistant cotton varieties could greatly contribute to reduced herbicide and pesticide use and consequently reducing environmental pollution. A large number of weeds are found in association with cotton fields. Weeds are an underestimated crop pest for which government spending in Africa on training, research and education is minimal and appropriate weed management technologies remain

largely unavailable and/or undeveloped (Sibuga, 1997). Crop losses caused by weeds are often "invisible" and are not as spectacular as those caused by other pest organisms (Labrada, 1996). Studies on the effect of *Bt* cotton on aboveground organisms have been done in Kenya (Ngari *et al.,* 2003; Waturu *et al.,* 2006; Kambo *et al.,* 2008). However, the competitive ability of *Bt* cotton with weeds has not been evaluated. This information is necessary for the development of management options and biosafety issues for sustainable transgenic cotton production.

1.2.16 Objectives of the study

The main objective of this study was to determine the effect of weeds on cotton growth and to evaluate the competitive ability of *Bt* cotton with common weeds in Kenya. The specific objectives of the study were:

i). To compare the effect of weeds on growth of *Bt* and non-*Bt* cotton.

ii). To determine weed diversity in the cotton fields.

1.2.17 Hypotheses

This study had the following hypotheses:

i). There is no difference in competitive ability between *Bt* cotton and non-*Bt* cotton with weeds.

ii). Weeds have no significant effect on cotton growth.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Study area

The study was conducted in a confined field trial at Animal Health Training Institute (AHITI) Ndomba Farm, run by KARI, in Mwea Division of Kirinyaga East District in Central Kenya. Mwea Division is located about 100 km northeast of Nairobi, the capital city of Kenya. It lies on the base of Mt Kenya at an altitude of approximately 1200 m above sea level. It has an area of 513 square kilometers. Several perennial rivers flow through the flat terrain of the poorly drained area. These kinds of conditions have formed swamps and wetlands that have lead to the development of Mwea Tebere Rice Irrigation Scheme, which is the largest rice irrigation scheme in Kenya.

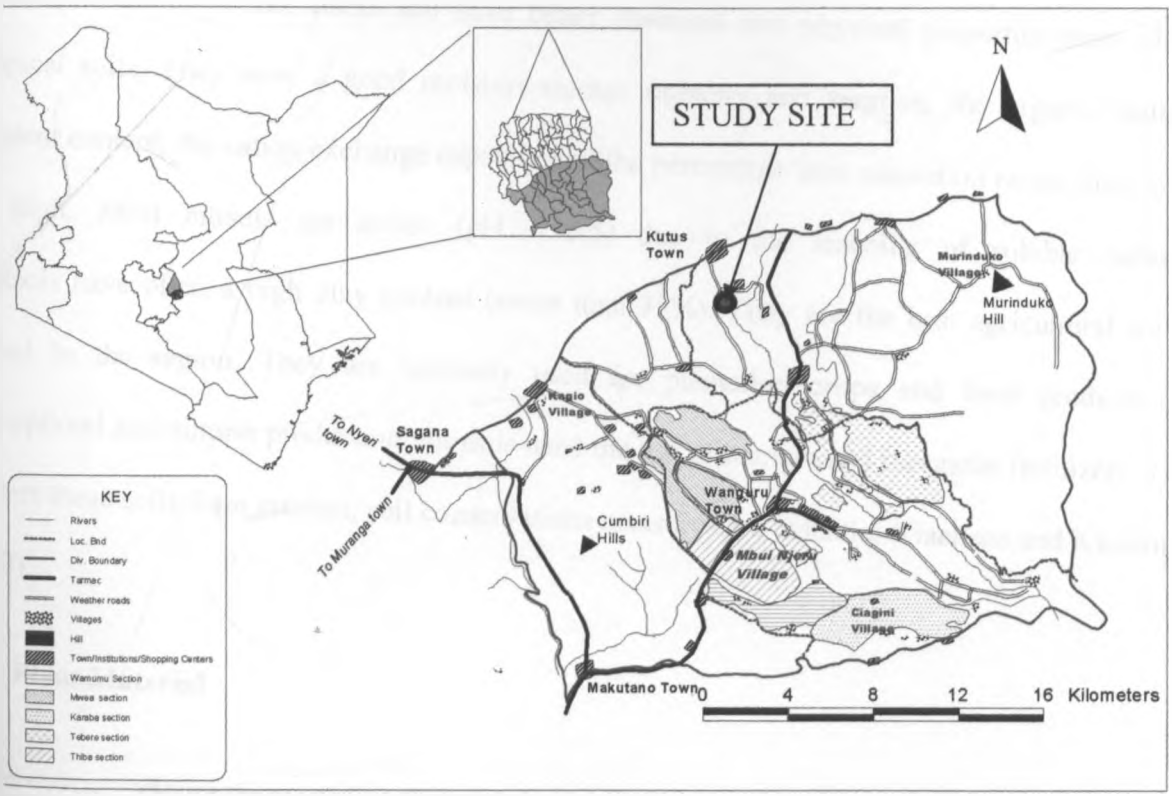

Figure 2.1 Map of Mwea Division in Kirinyaga District

2.1.1 Climate

The mean annual rainfall in this area is in the range of 1200 - 1600 mm per year and varies by the time of year. The long rains usually begin in March and subside in June and the short rains from October to December. Temperatures ranges between 10°C and 30°C, with occasional easterly wind.

2.1.2 Soils

Soils at Mwea are red volcanic soils (Nitisols). Nitisols occur in highlands and on volcanic steep slopes, for example in the central highlands of Kenya and around Mts. Kenya. They are developed from volcanic rocks and have better chemical and physical properties than other tropical soils. They have a good moisture-storage capacity and aeration, the organic matter content content, the cation exchange capacity and the percentage base saturation range from low to high. Most nitisols are acidic (pH < 5.5) due to the leaching of soluble bases. Nitisols have often a high clay content (more than 35%). They are the best agricultural soils found in the region. They are intensely used for plantation crops and food production. For optimal agricultural production, nitosols need the use of manure and inorganic fertilizers. To protect these soils from erosion, soil conservations measures are essential (Gachene and Kimaru, 2003).

2.2 Plant Material

Three cotton varieties were used in this study namely; Bollgard II (06Z604D), Isoline (99M03), and HART89M. Planting was done on 15th December 2009. Bollgard II variety 06Z604D carries *Cry1Ac* and *Cry2Abz* genes responsible for two toxic proteins for control of African bollworm as

described by Benedict *et al.* (1996). HART89M is a local non-*Bt* cotton variety developed for the enviroments south of the Rift Valley in Kenya (Waturu *et al.*, 2008). It is a new variety of cotton that was developed by Kenya Agricultural Research Institute (KARI) at Mwea in Kirinyaga District through multiline crossing.

2.3 Experimental Design

The study used a completely randomized block design. Four experimental plots each measuring 26 × 13 m were set up in the confined field trial (CFT) plot. Each plot was divided into nine subplots each measuring 5x4m, separated by 2m wide pathways (Fig. 2.2). The nine subplots were based on weeding, non-weeding and weed manipulated conditions. The treatments were randomly allocated to the nine plots.

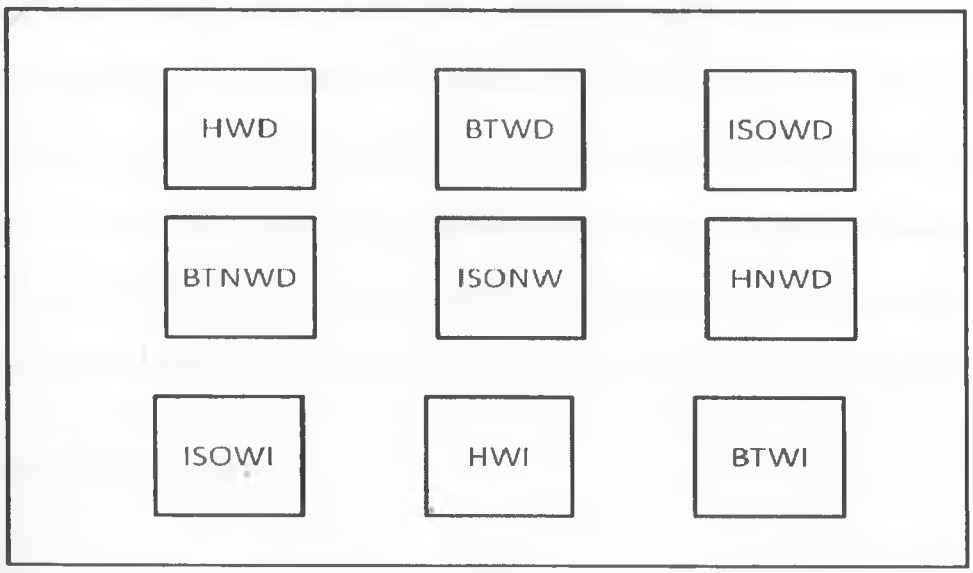

Figure 0.2 Experimental field layout showing arrangement of plots HWD (HART weeded), ISOWD (Isoline weeded), BTWD (*Bt* cotton weeded), ISOWI (Isoline weed increase), HNWD (HART non-weeded), BTNWD (*Bt* cotton non-weeded), ISONWD (Isoline non-weeded), BTWI (*Bt* cotton weed increase), HWI (HART weed increase)

2.4 Planting

Three cotton seeds were planted in rows of 1 × 0.30 m according to Vories *et al.,* (2001) and Williford, (1992). *Bt* cotton planting in the confined field trial were governed by rules and regulations stipulated by the National Biosafety Authority and enforced by Kenya Plant Health Inspectorate (KEPHIS, 2003). The regulations include a 500 m separation from the nearest conventional cotton and a 12 m border/buffer comprising conventional cotton pollen trap around the entire *Bt* cotton trial.

2.5 Determination of effects of weeds on cotton growth

2.5.1 Plant height

In each subplot, twenty cotton plants were randomly selected and marked for use in all subsequent sampling. These seletion was done at seedling stage, two weeks after planting (Oosterhuis and Jernstedt, 1999). Plant height was taken on weekly basis in each of the selected crop plants. For seedlings, at vegetative and reproductive stages, height was measured from the base to the tip of the tallest leaf using a meter rule. However, at maturity stages, height was measured from the base to the tip of the tallest branch as described by Yoshida (1981).

2.5.2 Leaf and boll count

The total number of leaves were counted in the twenty selected plants in each subplot. The number of damaged leaves was determined and recorded. Similary, the number of green bolls (capsules) and open bolls were also determined. Boll counts was done at fertilization and fruit development stage, seven weeks after planting (Oosterhuis and Jernstedt 1999).

2.5.3 Determination of weeds diversity and abundance in cotton fields

Quadrats of 0.5 x 0.5m were randomly located four times in the non-weeded plots. Weed species were identified, counted and recorded. Data on weeds were collected three weeks after planting and the subsequent weed identification and counting were done at intervals of two weeks. Weed species that were not identified in the field were collected, pressed and taken to the University of Nairobi Herbarium for identification.

2.6 Data analysis

Two way analysis of variance (ANOVA) was used to check for significant differences in plant height, leaf and boll counts within and between the treatments using R statistical programme version 2.11.1 (2010-05-31) (R Development Core Team, 1999 - 2010). Tukey's test was used to separate the means when F value was significant. Weed diversity was determined using Renyi's entropy diversity index (T´othm´er´esz, 1995) (Equation1).

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

Where,

H= index of species diversity

α= scale parameter

Pi = proportion of total sample belonging to the ith species

Weed abundance and rank frequency were determined using PC-ORD version 5 (McCune and Mefford, 1997).

CHAPTER THREE: RESULTS

3.1 Plant height

There was significant ($F=4.3554$, $df=2$, $p<0.05$) difference in plant heights among the three cotton varieties (Table 3.2). Similarly the difference in heights between weeded and the non-weeded plots was highly significant ($F=8.9375$, $df=2$, $p<0.005$) (Table 3.2). There was however no significant ($p>0.05$) difference between Isoline and *Bt* cotton variety, and between non-weeded and weed increased plots (Table 3.1). In non-weeded plots, the tallest mean height was recorded in HART89M (96.22±10.76cm), Isoline (74.34±9.51cm) and *Bt* cotton (73.56±7.46cm) while in weeded plots, the mean height of *Bt* cotton (105.33±3.12cm) was higher than Isoline (94.42±4.52cm) (Table 3.3). HART89M variety had high growth rate in plant height compared to *Bt* cotton and Isoline cotton in the weeded plot while Isoline cotton had the least growth rate (Figure 3.1). In non-weeded plots, the growth rate for both *Bt* and non-*Bt* cotton varieties were low as they were suppressed by weeds (Figure 3.2).

Table 3.1: Tukey's multiple comparison of means 95% confidence levels for cotton plant height.

Cotton plant height means comparison				
Tukey's multiple comparison of means 95% confidence levels				
variety				
	diff	Lower limit	Upper limit	p value
H-BT	15.84333	0.159717	31.52695	0.047314
ISO-BT	-0.855	-16.5386	14.82862	0.990127
ISO-H	-16.6983	-32.382	-1.01472	0.035005
Weed Treatments				
Control				
	diff	Lower limit	Upper limit	p value
WD-NWD	22.98083	7.297217	38.66445	0.003006
WI-NWD	-0.6875	-16.3711	14.99612	0.993605
WI-WD	-23.6683	-39.352	-7.98472	0.002253

Table 3.2: ANOVA on plant heights

Plant Height: Analysis of Variance Table						
Response:	Week					
	df	Sum sq	Mean sq	F value	pr (>F)	
Variety	2	2122.3	1061.15	4.3554	0.02153	*
Control	2	4355.1	2177.56	8.9375	0.000862	***
Residuals	31	7552.9	243.64			

Table 3.3: Cotton plant heights means and standard errors

Cotton heights (cm)	Non-weeded	weeded	Weed increased
Bt cotton	73.56±7.46	105.33±3.12	72.54±10.49
HART89M	96.22±10.76	113.32±6.94	89.42±7.51
Isoline	74.34±9.51	94.42±4.52	80.10±9.04

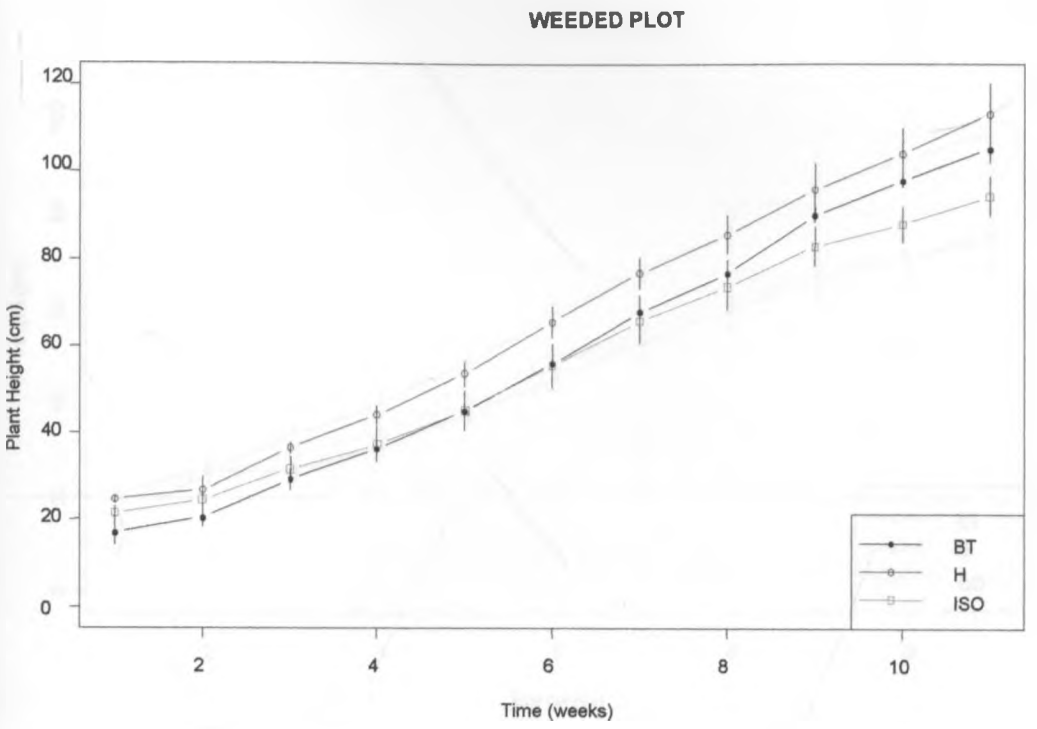

Figure 3.1: Growth curves showing cotton plant heights per week for weeded (WD) plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton.

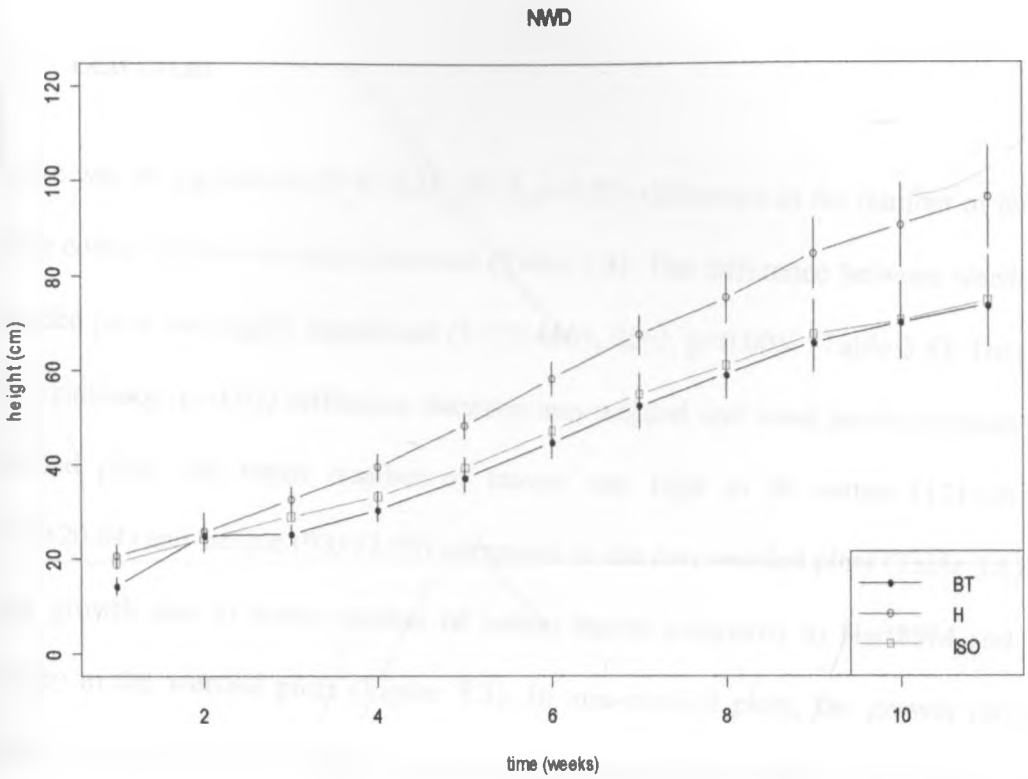

Figure 3.2: Growth curves showing cotton plant heights per week for non weeded (NWD) plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton.

3.2 Leaf count

There was no significant ($F=0.7625$, $df=2$, $p>0.05$) difference in the number of leaves among the three cotton varieties in each treatment (Table 3.4). The difference between weeded and the non-weeded plots was highly significant ($F=22.4463$, $df=2$, $p<0.005$) (Table 3.4). There was however no significant ($p>0.05$) difference between non-weeded and weed increased plots (Table 3.5). In weeded plots, the mean number of leaves was high in *Bt* cotton (121±16.41), Hart89M (112±20.04) and Isoline (93±13.99) compared to the non-weeded plots (Table 3.6). *Bt* cotton had high growth rate in mean number of cotton leaves compared to Hart89M and Isoline cotton variety in the weeded plots (Figure 3.3). In non-weeded plots, the growth rate of number of leaves for both *Bt* and Isoline cotton varieties were low as they were suppressed by weeds (Figure 3.4).

Table 3.4: ANOVA on cotton leaf counts

Leaf counts: Analysis of Variance Table						
Response:	Week					
	df	Sum sq	Mean sq	F value	Pr (>F)	
Variety	2	1018.2	509.1	0.7625	0.4751	
Control	2	29973.2	14986.6	22.4463	9.4E-07	***
Residuals	31	20697.6	667.7			

Table 3.5: Tukey's multiple comparison of means 95% confidence levels for cotton leaf counts

Cotton leaf count comparison				
Tukey's multiple comparison of means 95% confidence levels				
Variety				
	diff	Lower limits	Upper limits	p value
H-BT	4.2625	-21.7001	30.22511	0.914182
ISO-BT	-8.52917	-34.4918	17.43344	0.700614
ISO-H	-12.7917	-38.7543	13.17094	0.45483
				Weed Treatments
Control				
	diff	Lower limit	Upper limit	p value
WD-NWD	62.1	36.13739	88.06261	0.000005
WI-NWD	1.820833	-24.1418	27.78344	0.983717
WI-WD	-60.2792	-86.2418	-34.3166	8.1E-06

Table 3.6: Means number of cotton leaves and standard error per treatment

Leaf Counts	Non-weeded	weeded	Weed increased
Bt cotton	40±7.74	121±16.41	47±13.59
HART89M	58±15.25	112±20.04	50±5.85
Isoline	41±8.80	93±13.99	48±11.97

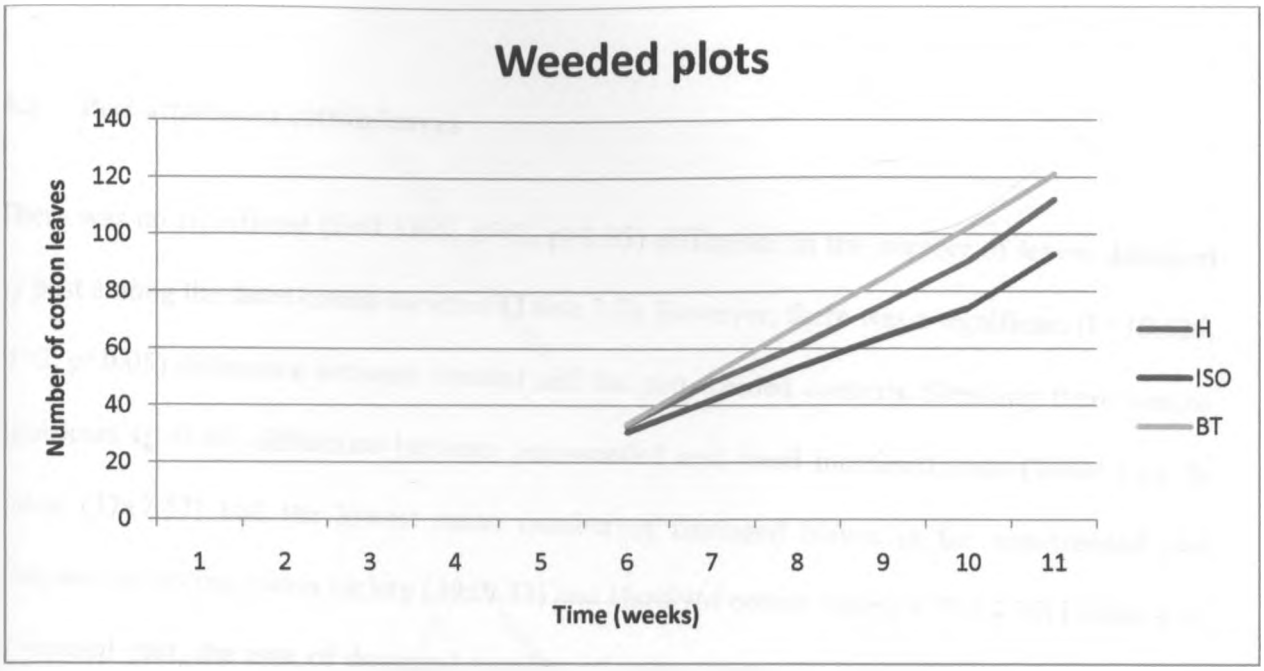

Figure 3.3 Growth curves showing mean number of cotton leaves per week for weeded plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton

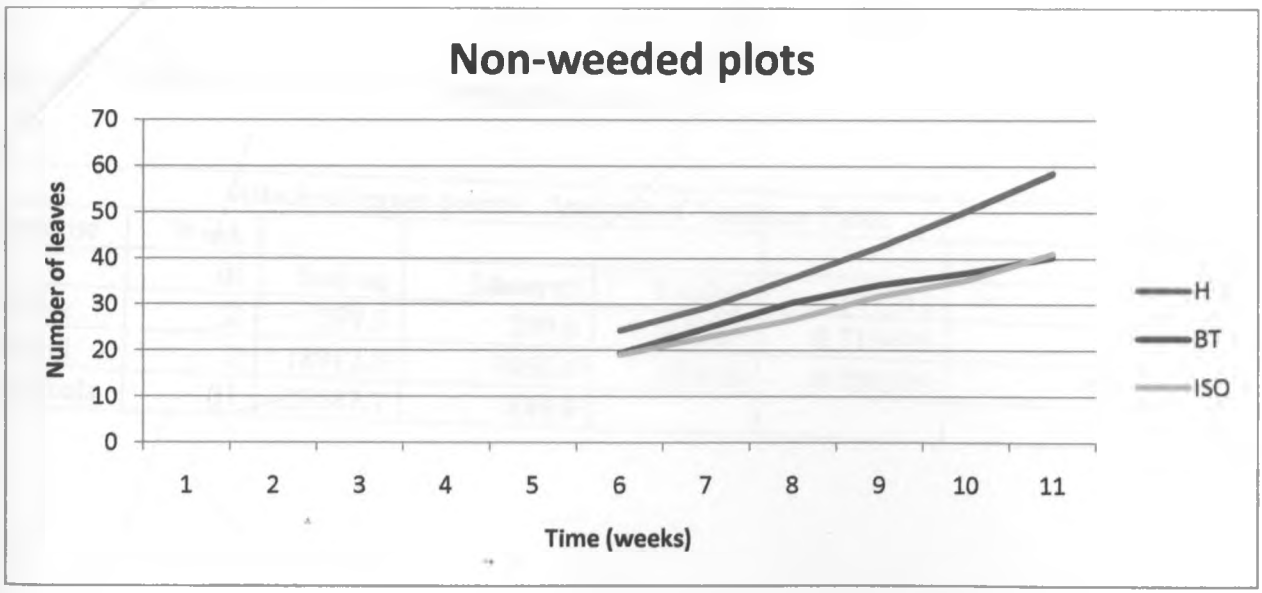

Figure 3.4 Growth curves showing mean number of cotton leaves per week for Non-weeded plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton

3.3 Pest attacks on cotton leaves

There was no significant ($F=0.3367$, $df=2$, $p>0.05$) difference in the number of leaves damaged by pest among the three cotton varieties (Table 3.7). However, there was a significant ($F=10.626$, $df=2$, $p<0.05$) difference between weeded and the non-weeded controls. Similarly there was no significant ($p>0.05$) difference between non-weeded and weed increased plots (Table 3.8). *Bt* cotton (37±7.57) had the lowest mean number of damaged leaves in the non-weeded plot compared to Isoline cotton variety (39±9.33) and Hart89M cotton variety (55±14.94) (Table 3.9). In weeded plot, the rate of damaged number of cotton leaves was high in *Bt* cotton varieties compared to Hart89M and isoline varieties (Figure 3.5). Hart89M cotton variety had the highest rate of damaged number of cotton leaves in the non-weeded plots compared to *Bt* cotton and isoline cotton variety (Figure 3.6).

Table 3.7: ANOVA on number of damaged leaves

Attacked leaves counts: Analysis of Variance Table						
Response	Week					
	df	Sum sq	Mean sq	F value	pr (>F)	
Variety	2	599.3	299.6	0.3367	0.716696	
Control	2	18912.8	9456.4	10.626	0.000306	***
Residuals	31	27587.7	889.9			

Table 3.8: Tukey's multiple comparison of means 95% confidence levels for the number of damaged cotton leaves

Cotton attacked leaves counts					
Tukey's multiple comparison of means 95% confidence levels					
Variety					
	diff	Lower limits	Upper limits	p value	
H-BT	5.4625	-24.5116	35.43662	0.895413	
ISO-BT	-4.51667	-34.4908	25.45745	0.927166	
ISO-H	-9.97917	-39.9533	19.99495	0.693998	
Weed Treatments					
Control					
	diff	Lower limits	Upper limits	p value	
WD-NWD	48.81667	18.84255	78.79078	0.001013	
WI-NWD	0.391667	-29.5825	30.36578	0.99943	
WI-WD	-48.425	-78.3991	-18.4509	0.001107	

Table 3.9: Means and standard errors for the number of damaged cotton leaves per treatment

Attacked leaves	Non-weeded	weeded	Weed increased
Bt cotton	37±7.57	101±19.73	42±13.39
HART89M	56±14.94	96±29.19	45±5.49
Isoline	39±9.33	82±16.41	45±11.34

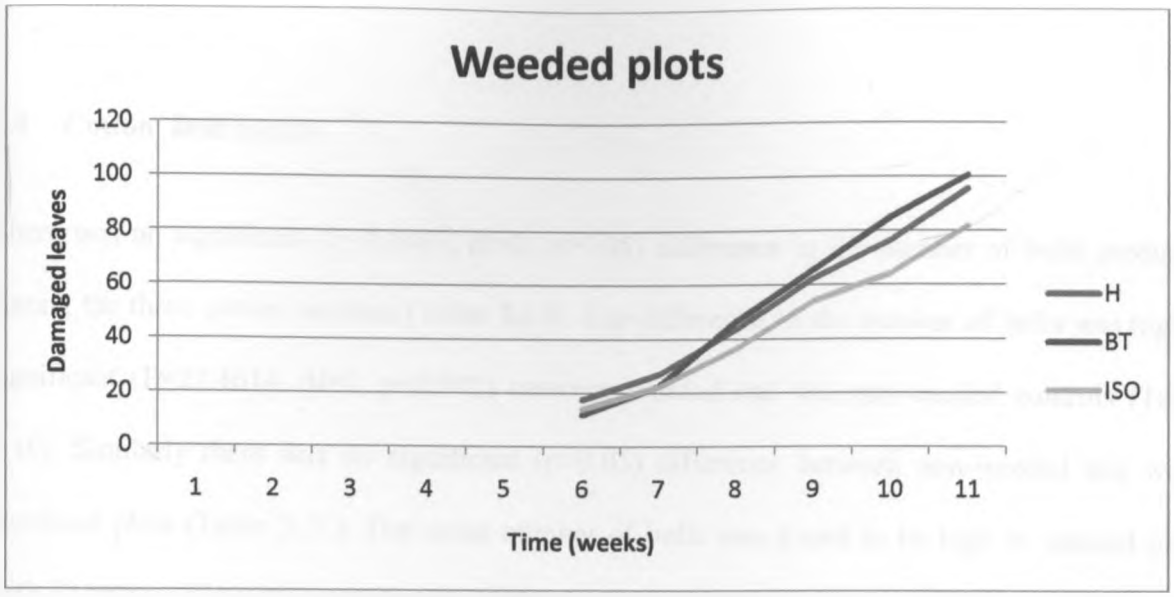

Figure 3.5 Graphs showing mean number of damaged cotton leaves per week in weeded plots; H - HART89M, ISO - Isoline, *Bt*- *Bt* cotton.

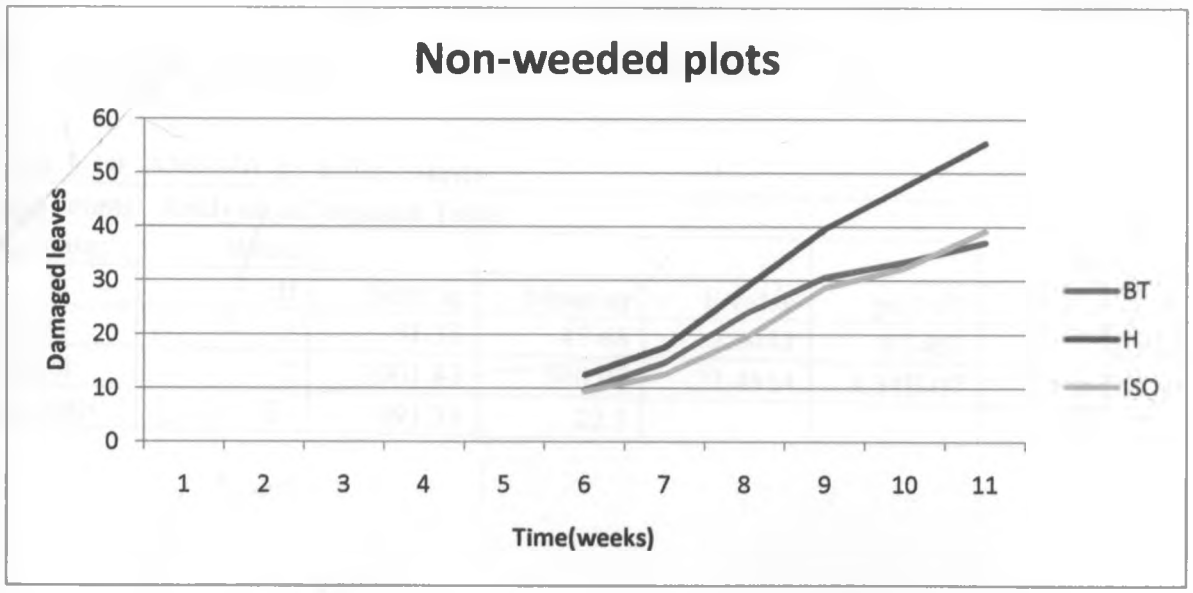

Figure 3.6 Graphs showing mean number of damaged cotton leaves per week in Non-weeded plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton.

3.4 Cotton Boll counts

There was no significant ($F=2.0481$, $df=2$, $p>0.05$) difference in the number of bolls produced among the three cotton varieties (Table 3.10). The difference in the number of bolls was highly significant ($F=22.4614$, $df=2$, $p<0.005$) between weeded and the non-weeded controls (Table 3.10). Similarly there was no significant ($p>0.05$) difference between non-weeded and weed increased plots (Table 3.11). The mean number of bolls was found to be high in weeded plots with *Bt* cotton (27±2.20) recording the highest bolls compared to Isoline (20±2.31) and Hart89M (17±1.65) (Table 3.12). *Bt* cotton variety had the highest rate of boll production compared to Hart89 cotton variety which had the lowest in the weeded plots (Figure 3.7). In the non-weeded plot, isoline cotton variety had the lowest rate of boll production compared to both Hart89M and *Bt* cotton variety (Figure 3.8).

Table 3.10: ANOVA on bolls counts

Bolls counts: Analysis of Variance Table						
Response:	Week					
	df	Sum sq	Mean sq	F value	pr (>F)	
Variety	2	91.35	45.68	2.0481	0.1461	
Control	2	1001.83	500.92	22.4614	9.34E-07	***
Residuals	31	691.34	22.3			

Table 3.11: Tukey's multiple comparison of means 95% confidence levels for cotton boll counts and weed levels

Cotton bolls counts				
Tukey's multiple comparison of means 95% confidence levels				
Variety				
	diff	Lower limits	Upper limits	p value
H-BT	-3.3875	-8.13248	1.357476	0.200737
ISO-BT	-3.37083	-8.11581	1.374143	0.20375
ISO-H	0.016667	-4.72831	4.761643	0.999959
Weed Treatments				
Control				
	diff	Lower limits	Upper limits	p value
WD-NWD	11.675	6.930024	16.41998	3.1E-06
WI-NWD	1.041667	-3.70331	5.786643	0.85213
WI-WD	-10.6333	-15.3783	-5.88836	1.43E-05

Table 3.12: Means and standard error of number of cotton bolls per treatment

Cotton bolls	Non-weeded	weeded	Weed increased
Bt cotton	10±1.73	27±2.20	11±3.47
HART89M	10±2.49	17±1.65	11±1.22
Isoline	9±2.04	20±2.31	10±7.76

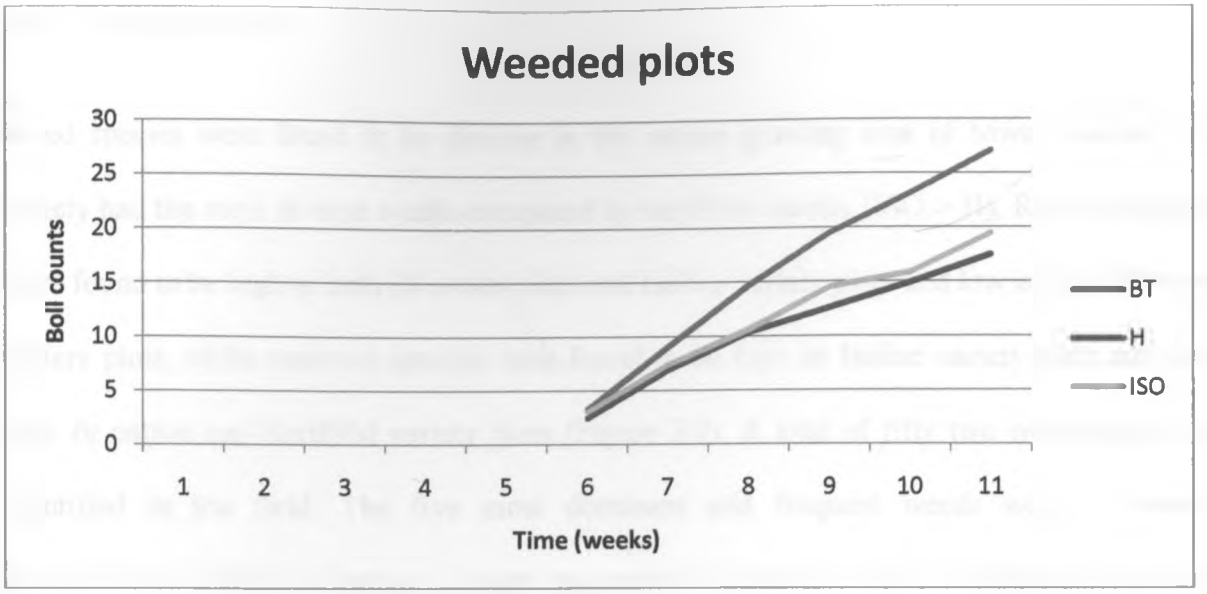

Figure 3.7: Mean number of cotton bolls per week in weeded plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton.

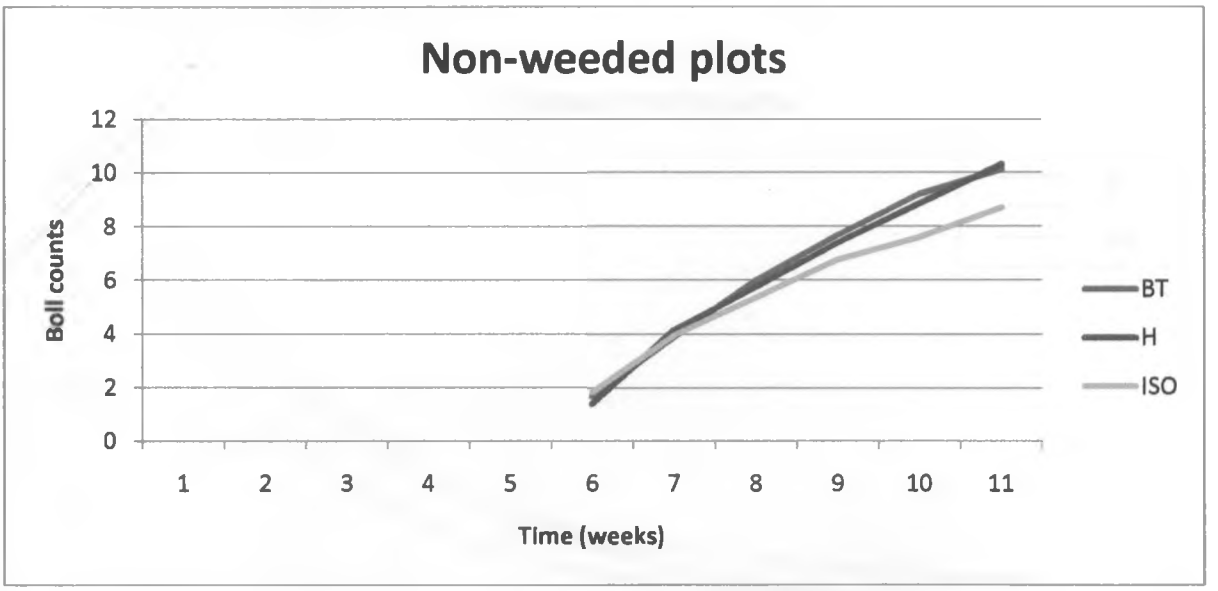

Figure 3.8: Mmean number of cotton bolls per week in Non-weeded plots; H - HART89M, ISO - Isoline, BT - *Bt* cotton.

3.5 Weed diversity

Weed species were found to be diverse in the cotton growing area of Mwea. Isoline cotton variety had the most diverse weeds compared to Hart89M vareity (ISO > H). Rare weed species were found to be high in both *Bt* cotton plots and Isoline variety plots and low in Hart89M cotton variety plots, while common species were found to be high in Isoline variety plots and low in both *Bt* cotton and Hart89M variety plots (Figure 3.9). A total of fifty two weed species were identified in the field. The five most dominant and frequent weeds were *Commelina benghalensis, Waltheria indica, Cleome monophylla, Sida ovata* and *Triumfetta rhomboidea,* while the least abundant species were *Amaranthus hybridus, Crotalaria spinosa, Aristida kenyensis, Corchorus tridens* and *Eleusine indica* (Figure 3.10 and Table 3.13).

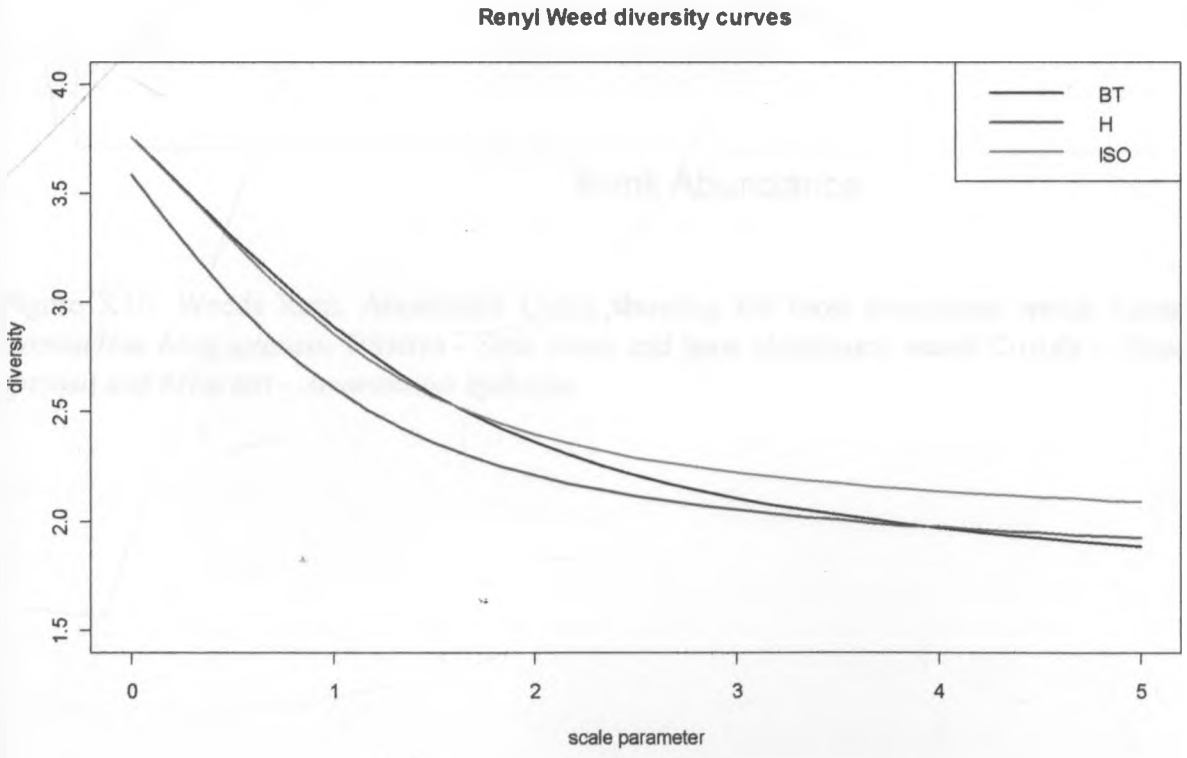

Figure 3.9: Diversity ordering of three non-weed plots in cotton fields using Renyi's index family (BT - *Bt* cotton variety, H - Hart89M variety, ISO - isoline cotton variety).

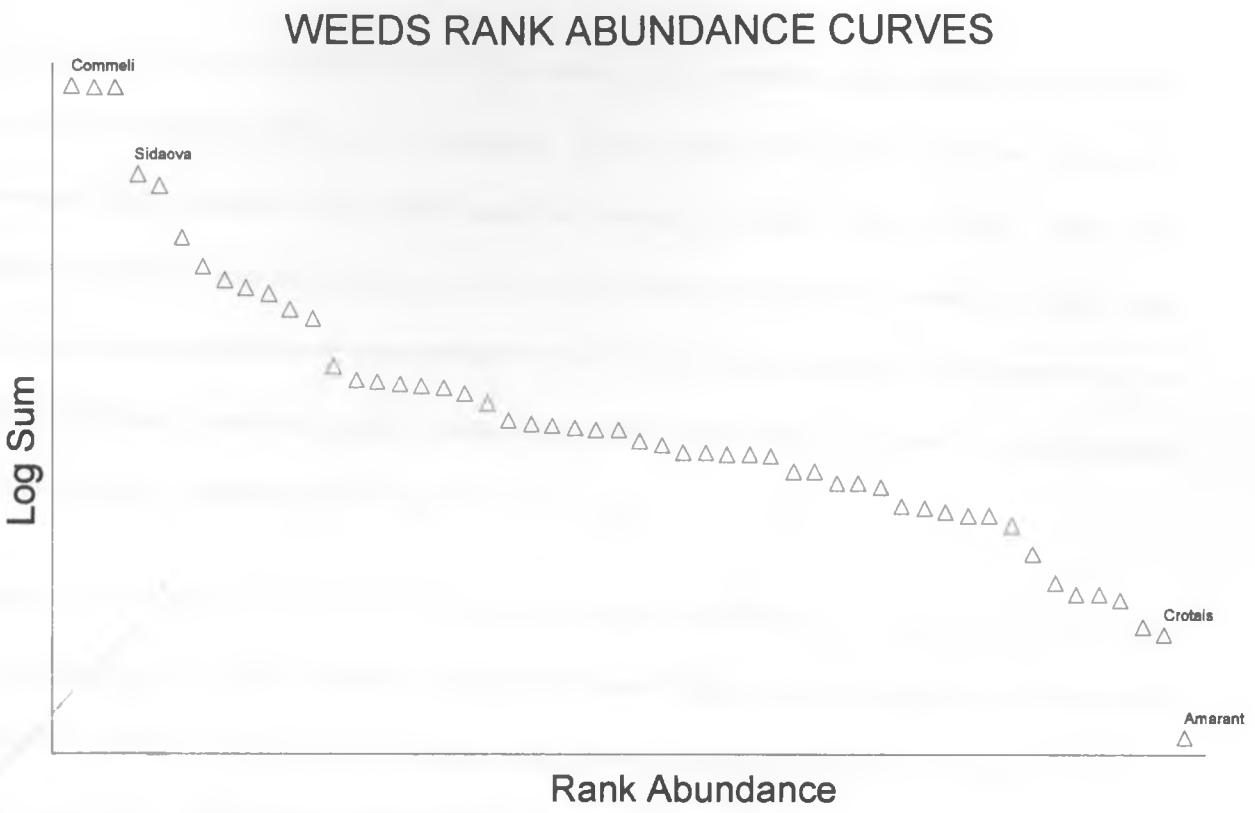

Figure 3.10: Weeds Rank Abundance Curve showing the most abundance weeds Commeli - *Commelina benghalensis*, Sidaova - *Sida ovata* and least abundance weeds Crotals - *Crotalaria spinosa* and Amarant - *Amaranthus hydridus*.

CHAPTER FOUR: DISCUSSION AND CONCLUSIONS

4 EFFECTS OF WEEDS ON COTTON

The results of the study generally show that cotton plants in weeded plots performed better than those in non-weeded plots in all treatments. These results are in line with the findings of Schwerzel and Thomas (1971) who found that weeds consumed three to four times more nitrogen, potassium and magnesium in non-weeded plots as compared to weed free crops. They also noted that weeds removed more moisture from the soil than crop plants. Although there was no data collected on nutrient uptake, it was infered that cotton plants competed for vital nutrients such as nitrogen, potassium and magnesium with weeds.

Nitrogen is an essential nutrient used in relatively large amounts. It is a fundamental part of the chlorophyll molecule and is essential in the formation of amino acids and proteins. The proteins which are formed act primarily to control plant growth processes through enzymatic action. A good supply of N is associated with vigorous growth and a deep green color. Plants deficient in N become stunted and yellow (chlorotic) in appearance. Since plants can remobilize N from older tissue to provide N to younger tissue, chlorosis usually appears on the lower leaves first while the upper leaves remain green (Hodges, n.d). Potassium is required by plants in approximately the same or slightly larger amounts as nitrogen. Uptake of K occurs in the K^+ form. The plant uses K in photosynthesis, in carbohydrate transport, in water regulation, and in protein synthesis. The benefits of proper K nutrition include improved disease resistance, vigorous vegetative growth, increased drought tolerance, improved winter hardiness of forages, and decreased lodging. Plants deficient in potassium are stunted and develop poor root systems. Deficiency symptoms are most obvious on the older, lower leaves since this element is readily

translocated within the plant. Symptoms begin as interveinal chlorosis or "bronzing" near the edges of lower leaves, and develop into a firing or scorch as the deficiency continues. This firing moves inward until the entire leaf dies and is shed. Since K deficiency can result in leaf shedding, it reduces the ability of the plant to produce carbohydrates, and ultimately crop yields. (Hodges, n.d). Magnesium has a role in chlorophyll development and photosynthesis for growth and development. Magnesium also appears to activate a number of enzymes and plays a role in protein synthesis and phosphorus reactions (Hodges, n.d). One other factor that influences the competitive balances between crops and weeds is moisture stress. Weeds compete for water in the soil (Jody *et al.*, 1998).

4.1 Plant Heights

Generally plants in weeded plots had higher plant heights compared to plants in non-weeded plots. Weed-crop competition is one of the major causes of low growth rate and yield loss (Cao *et al.*, 2007) in cotton. In this study HART89M cotton variety was morphologically taller compared to *Bt* cotton variety (06Z604D). This explains why HART89M in non-weeded plants were taller than *Bt* cotton non-weeded plants. Also HART89M in the weeded plots had taller plants compared to weeded *Bt* cotton plants. Weeds prevent cotton plants from growing upright due to competition for light and nutrients, thus causing cotton stalks to grow shorter than the actual height. This confirms the earlier findings by Buchanan *et al.* (1971) that weeds supress the height of cotton. However there was no significant difference in cotton height between *Bt* cotton (06Z604D) and Isoline (99M03). This indicates that the *Bt* gene had no effect on weeds.

4.2 Number of bolls

Bolls were fewer in the non-weeded cotton crops compared to the weeded crops suggesting that weeds affect boll formation in cotton and this is inline with the findings of Abernathy and McWhorter (1992), who found that competition of cotton crop with weeds resulted in fewer and less mature bolls per plant and lower lint quality than in weeded crotton crops. Weeds reduce growth rate and yields by competing with cotton for water, nutreints, light and space. Boll distribution patterns can be used to explain the cause of cotton yield differences and are frequently used for assessing pest damage and effect of weed management in cotton fields (Kerby and Bruxton, 1981). Cotton growth is indeterminate, bolls appear at regular intervals. There was high production of bolls in weeded *Bt* cotton plots than weeded Hart89M cotton plots. These results confirm the earlier findings by Barwale *et al.* (2004) that *Bt* cotton variety had significantly higher yeilds and boll retention compared to non-*Bt* cotton. However, the lack of difference in bolls production between Hart89M, *Bt* and Isoline cotton varieties was possibly due to the effects of weed competition for nutrients. These results indicate that weeds had similar effects on both *Bt* and non-*Bt* cotton varieties.

4.3 Number of leaves attacked by pests

Results showed that there was no significant ($p>0.05$) difference in the number of leaves damaged by pests between *Bt* and non-*Bt* cotton varieties. This is possibly because in this study no pest control was done although *Bt* cotton has been genetically modified to be resistant against bollworm (Novillo *et al.*, 1999), and not other cotton pests. This confirms the findings by (Wu, Li, Feng, Guo, 2002) that secondary pests can become key insects pest in *Bt* cotton fields, and

their damage to cotton could increase further with expansion of *Bt* cotton growing areas if no additional controls are adopted.

4.4 Weed diversity and abundance

It was found out that diversity of weed species and density determined the competitive damage to cotton. The more competitive a species, weed density and the duration in which the weed remained in contact or close proximity with the cotton crop is, the greater the reduction in cotton production. This was also witnessed by Coble and Byrd (1992) in their findings in the USA. Apart from the weeds competing with the cotton crop for nutrients, light, and water, weeds also damage the crop physically by restricting the normal growth habit of cotton plants. Common weeds such as *Commelina benghalensis, Sida ovata,* and *Triumfetta rhomboidea* were found to completely cover cotton plants and prevent normal upright growth of cotton plants. As a result, stalks were bent and twisted with most of the cotton bolls near the soil surface.

4.5 Conclusions

Weed management is an important component of cotton production. Cotton must be kept weed free in order to avoid crop loss. In the presence of weeds, it was found that *Bt* cotton had no significant competitive advantage in terms of growth and boll formation compared to non-*Bt* cotton under the same field conditions. The study showed that growth performance of *Bt* cotton in weeded condition was better than in non-weeded plots. It can also be concluded that *Bt* cotton variety 06Z604D is effective in controlling the pest (African bollworm) in presence of weeds and consequently reducing damage of the leaves and fruiting structures of cotton plants. These results are consistent with the findings of Novillo *et al.* (1999), who confirmed that genetically modified *Bt* cotton was resistant to damage caused by the larvae of *H. armigera*, *Pectinophora gossypiella*

(Saund), *Earias insulana* (Boisd) which are common pests attacking cotton plants. Generally, weeding improves cotton perfomance. Therefore, cotton must be keep weed-free for a period after emergence in order to avoid crop loss. Weeding should be done to realize the full potential of *Bt* cotton.

4.6 Recommendations

From the results of this study the following recommendations were made:

1) *Bt* cotton production should now be assessed in small-scale farming systems under non-optimal conditions of weeds in order to determine whether there would be comparable benefits.
2) Weeding should be done in both *Bt* and non-*Bt* cotton to realize maximum production.

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APPENDICES

Appendix 1: Species list summary showing rank abundance, and families of weeds found in cotton fields in Mwea Division of Kirinyaga District in Kenya.

Weed species name	Family	Rank Abundance	Log (Sum Abundance
Commelina benghalensis	*Commelinaceae*	1	3.08
Waltheria indica	*Malvaceae*	2	3.07
Cleome monophylla	*Capparaceae*	3	3.07
Sida ovata	*Malvaceae*	4	2.78
Triumfetta rhomboidea	*Tiliaceae*	5	2.74
Nicandra physoloides	*Solanaceae*	6	2.56
Panicum maximum	*Poaceae*	7	2.46
Digitaria ciliaris	*Poaceae*	8	2.42
Oxygonum sinuatum	*Polygonaceae*	9	2.39
Portulaca oleracea	*Portulacaceae*	10	2.37
Indigofera arrecta	*Fabaceae*	11	2.32
Glycine wightii	*Fabaceae*	12	2.29
Datura metel	*Solanaceae*	13	2.12
Clotalaria brevidens	*Fabaceae*	14	2.08
Cyperus pectinatus	*Cyperaceae*	15	2.07
Tephrosia purpurea	*Fabaceae*	16	2.07
Tephrosia hildebrandtii	*Fabaceae*	17	2.06
Crotalaria incana	*Fabaceae*	18	2.05
Datura stramonium	*Solanaceae*	19	2.03
Ipomoea acuminata	*Convolvulaceae*	20	2
Spermacoce laevis	*Rubiaceae*	21	1.94
Solanum incanum	*Solanaceae*	22	1.92
Cassia mimosoides	*Fabaceae*	23	1.92
Ipomoea obscura	*Convolvulaceae*	24	1.91
Crotalaria cephalotes	*Fabaceae*	25	1.91
Dactyloctenium aegyptium	*Poaceae*	26	1.9
Indigofera bangweolensis	*Fabaceae*	27	1.87
Hibiscus micranthus	*Malvaceae*	28	1.85
Cucumis dispsaceus	*Cucurbitaceae*	29	1.83
Leucas grandis	*Lamiaceae*	30	1.83
Ipomoea mombassana	*Convolvulaceae*	31	1.82
Euphorbia prostrata	*Euphorbiaceae*	32	1.82

Weed species name	Family	Rank Abundance	Log (Sum Abundance
Setaria pumila	*Poaceae*	33	1.81
Cassia occidentalis	*Fabaceae*	34	1.76
Cucumis aculeatus	*Cucurbitaceae*	35	1.76
Evolvulus nummularius	*Convolvulaceae*	36	1.72
Tagetes minuta	*Compositae*	37	1.72
Boerhavia erecta	*Nyctaginaceae*	38	1.71
Bidens pilosa	*Compositae*	39	1.64
Solanum nigrum	*Solanaceae*	40	1.63
Rhynchosia minima	*Leguminosae*	41	1.62
Monechma debile	*Acanthaceae*	42	1.61
Indigofera ambelancensis	*Fabaceae*	43	1.61
Sphenoclea zeylanica	*Sphenocleaceae*	44	1.58
Digitaria velutina	*Poaceae*	45	1.48
Euphorbia adjurana	*Euphorbiaceae*	46	1.38
Cocculus hirsutus	*Malvaceae*	47	1.34
Eleusine indica	*Poaceae*	48	1.34
Corchorus tridens	*Malvaceae*	49	1.32
Aristida kenyensis	*Graminae*	50	1.23
Crotalaria spinosa	*Fabaceae*	51	1.2
Amaranthus hybridus	*Amaranthaceae*	52	0.85