Understanding agronomic and phytochemical properties of Brachiaria for management of cereal stemborers in East Africa

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DECLARATION BY THE CANDIDATE

I, DUNCAN CHERUIYOT, declare that the work presented in this PhD thesis is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been acknowledged by complete reference.

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DECLARATION AND APPROVAL BY SUPERVISORS

We declare that the work reported in this thesis was carried out by the candidate under our supervision and approve this submission.

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DEDICATION

I dedicate this work to my son Dylan, who always made me smile even when it was hard to.

This work is also dedicated to my wife Judith, my mother Rachel and my siblings (Evans, Collins and Mercy) for the constant source of love, encouragement and support. I owe it all to you, many thanks!

PREFACE

This work was conducted by D. Cheruiyot under supervision of Prof. J. Van den Berg, IPM-program, Unit for Environmental Sciences and Management, of North-West University and Prof. Z. Khan and Dr. C. Midega of the Department of Plant Health, International Centre of Insect Physiology and Ecology. The thesis is submitted for a degree of *Doctor of Philosophy in Environmental Science* at the North-West University.

The style of the thesis followed an article format. Chapter 1 presents the introduction followed by literature review in Chapter 2. Chapters 3 to 6 report the objectives of the study, presented in the format of manuscripts for publication. Lastly, general discussion, conclusions and recommendations are provided in Chapter 7.

The data chapters yielded three publications with D. Cheruiyot as the first and corresponding author in all the publications. D. Cheruiyot designed and conducted the experiments, collected and analyzed data, and wrote the manuscripts. The publications are as follows:

i. Cheruiyot D, Midega CAO, Van den Berg J, Pickett JA & Khan ZR (2018). Genotypic responses of Brachiaria (*Brachiaria* spp.) genotypes to drought stress. Journal of Agronomy 17:136–146.

ii. Cheruiyot D, Midega CAO, Ueckermann AE, Van den Berg J., Pickett JA & Khan ZR (2018). Genotypic response of brachiaria (*Brachiaria* spp.) to spider mite (*Oligonychus trichardti*) (Acari: Tetranychidae) and adaptability to different environments. Field Crops Research 225:163–169.

iii. Cheruiyot D, Midega CAO, Van den Berg J, Pickett JA. & Khan ZR (2018). Suitability of brachiaria grass (*Brachiaria* spp.) as a trap crop for management of *Chilo partellus*. Entomologia Experimentalis et Applicata 166:139–148.

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It has been an intensive period of learning, not only in the scientific arena but also on a personal level. As I put the finishing touches of my thesis by writing this note, I would like to reflect on the people who have supported and helped me so much throughout this period. First, I want to thank my *icipe* research supervisors Dr. Charles Midega and Prof. Zeyaur Khan for unfailing research facilitation, academic guidance and mentorship. It was a privilege working with the Push-Pull project team of *icipe* under your leadership. Special mention goes to Prof. Johnnie Van Den Berg, my academic supervisor for his patience, motivation, enthusiasm and immense knowledge. You were there to guide me in every step of the way, conveyed a spirit of adventure in this research. Thank you very much for your academic guidance and critical reviews of the manuscripts and this dissertation. Despite of your busy schedule, you worked tirelessly, and your timely responses played a major role towards finishing this study in good time. I sincerely enjoyed your supervision and I couldn't have imagined a better PhD advisor and a mentor, I am forever indebted to you.

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ACRONYMS AND ABBREVIATIONS

CCR	Chlorophyll Content Reduction
CIAT	International Centre for Tropical Agriculture
CRD	Completely Randomized Design
DMNT	(*E*)-4,8-dimethyl-1,3,7-nonatrine
DSI	Drought Stress Index
HIPV	Herbivore Induced Plant Volatiles
ICIPE	International Centre of Insect Physiology and Ecology
ILRI	International Livestock Research Institute
ITOC	ICIPE-Thomas Odhiambo Campus
PCA	Principal Component Analysis
pH	Potential of Hydrogen
R.H	Relative Humidity
SCMR	SPAD Chlorophyll Meter Readings
SPAD	Soil Plant Analysis Development
WAP	Weeks After Planting

ABSTRACT

Grasses in the genus *Brachiaria*, commonly known as brachiaria are grown as a fodder crop in sub-Saharan Africa, with some genotypes being used in management of the spotted stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) through a habitat management strategy. Stemborer is a major insect pest of sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.) in Africa. However, utilization of brachiaria in cereal-livestock based farming systems in the region faces several biotic and abiotic challenges. Increasing drought conditions limit productivity of this grass species as fodder and its value in pest management. Further, spider mite, *Oligonychus trichardti* Meyer (Acari: Tetranychidae), has recently been reported as a major pest of *Brachiaria* spp. in the region. The study aimed at evaluation and identification of drought tolerant, spider mite resistant and adaptable brachiaria genotypes. Potential candidates were further tested for their suitability for use as trap plants in management of *C. partellus*and their roles in tritrophic interactions with the pest's natural enemies. Morphological and physiological characters of 18 brachiaria genotypes were studied under simulated drought conditions, well-watered (control) plants were watered every 48 h to 100% field capacity while treatments were allocated by suspending watering for 14 and 28 days, representing moderate and severe drought, respectively. Shoot length, leaf length and width (leaf area), number of tillers, leaf relative water content, chlorophyll content, and above ground biomass were studied. Based on the drought stress index (DSI) values for the measured parameters and PCA (Principal Component of Analysis) biplots, Xaraes, Piata, Marandu, CIAT 679, Mulato II, and Mulato displayed tolerance to severe drought conditions. The same genotypes were further tested for resistance to *O. trichardti* under controlled conditions in a screenhouse while adaptability to different environments and field resistance to mites was evaluated in three locations for two cropping seasons in 2016 and 2017 under farmers' conditions. The parameters evaluated as indicators of resistance to pest damage included leaf damage, chlorophyll content reduction, plant height, leaf area, number of tillers and shoot biomass. Significant correlations between parameters were only observed between leaf damage and yield (r = -0.50, $P < 0.05$), and leaf damage and chlorophyll loss (r = 0.84, $P < 0.01$). The cultivar superiority index (*Pi*) ranked Xaraes, Piata, ILRI 12991 and ILRI 13810 as reliable genotypes that combined moderate resistance to the mite ($Pi \leq 48.0$) and high biomass yield ($Pi \leq 8.0$). Seven putative candidates of the studied genotypes were assessed for oviposition preference by *C. partellus* moths and subsequent larval performance. In two-choice tests with an open-pollinated maize variety (cv. Nyamula), significantly higher numbers of eggs were deposited on brachiaria genotypes Marandu, Piata, and Xaraes than on maize, whereas fewer eggs were recorded on plants of Mulato II, Mulato, and Cayman. There was a significant and negative correlation between the trichome density on plant leaves and *C. partellus* oviposition preference for the different brachiaria varieties. First instar larvae did not consume leaf tissues of brachiaria plants but consumed those of maize, which also suffered more stem damage than brachiaria plants. No larvae survived on brachiaria plant tissue for longer than five days, whereas 79.2% of the larvae survived on maize. Higher percentages of eggs were laid on previously oviposited plants of Piata and Xaraes varieties ($P < 0.05$), while non-oviposited plants of Mulato II was significantly ($P < 0.05$) preferred to previously oviposited plants. Female *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) spent significantly more time attending to volatiles from previously oviposited than non-oviposited plants of all varieties except Marandu. This study proposes brachiaria genotypes that could be of value in improvement of cereal livestock-based livestock productivity in sub-Saharan Africa in the current scenarios of increasing aridification and attacks by spider mites. Among the proposed genotypes in each category, Xaraes and Piata combined drought tolerance, spider mite resistance and adaptability to different environments. Furthermore, they are both suitable for use as "dead-end" trap plants to *C. partellus* while their head space volatiles are attractive to the parasitoid *C. sesamiae*. Attractiveness of headspace volatiles, both oviposition induced and constitutive, from most of the genotypes highlights the value of these grasses in stemborer management strategies that exploits tritrophic interactions with the pest's natural enemies.

Key words: biomass yield, *Brachiaria* spp., cereal stemborers, drought stress, *Oligonychus trichardti*, parasitoids, trap plants, volatiles

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Brachiaria are perennial C4 plants that are native to Africa (Renvoize *et al*., 1996). Brachiaria species form natural constituents of grasslands in eastern, central and southern Africa where they are adapted to low soil fertility (Boonman, 1993; Maass *et al*., 2015). They play an important role in cultivated pastures in tropical America (Keller-Grein *et al*., 1996), South-East Asia (Phaikaew *et al*., 1997; Hare *et al*., 2015), and East Africa (Maass *et al*., 2015). Brachiaria species such as *Brachiaria brizantha* (Palisade grass), *B. ruziziensis* (ruzi grass), *B. decumbens* (signal grass), and *B. humidicola* (koronivia grass) have been exploited as forage crops in these regions since they sustain animal production by providing high quality forage especially when fertilized and well managed.

In addition to its use as a forage crop, *B. brizantha* cv Mulato II, henceforth referred to as Mulato II, possesses unique phytochemical properties resulting in it being preferred to maize for oviposition by gravid *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) moths (Midega *et al*., 2011; Khan *et al*., 2014). Studies have also shown that Mulato II exhibits highly sophisticated responses to stemborer herbivory that involve multitrophic interactions with certain stemborer natural enemies. Bruce *et al*. (2010) reported an increase in attractiveness of this cultivar to stemborer natural enemy *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) as a result of stemborer oviposition on the grasses (Bruce *et al*., 2010). Agronomic studies on brachiaria reported Mulato II to be tolerant to extended periods of drought and high temperatures (>30 ^{o}C) (Pickett *et al.,* 2014). Mulato II has therefore gained the largest uptake in East Africa where the grass has also been incorporated as a trap plant in the push-pull system (Midega *et al*., 2011; Khan *et al*., 2014). This system was developed for management of cereal stemborers by exploiting behaviour-modifying stimuli to manipulate the distribution and abundance of the pests and their natural enemies (Cook *et al*., 2007; Bruce *et al*., 2010; Khan *et al*., 2010; Midega *et al*., 2011). Despite the benefits of brachiaria observed thus far, its production is limited by several biotic and abiotic challenges.

Drought is a major limiting factor affecting plant growth development and yield, especially in arid and semi-arid regions (Wang *et al*., 2003). Responses to drought stress are multiple and interconnected and are manifested through reduced leaf water potential causing reduced turgor, stomatal closure and a decline in carbon assimilation rates (Baruch, 1994; Yang *et al*., 2006). Consequently, numerous metabolic and physiological processes in plants are impaired (Yang *et al*., 2006). This leads to reduced growth rates, reduction in chlorophyll and water content and changes in fluorescence parameters (Mafakheri *et al*., 2010; Zhang *et al*., 2011; Ajithkumar & Panneerselvam, 2013).

Plants have evolved mechanisms to escape, avoid and tolerate soil water deficits, thereby enabling survival of the adapted plants (Wilson *et al*., 1980; Baruch, 1994; Yue *et al*., 2006; Manavalan *et al*., 2009; Luo, 2010; Jones, 2013; Tardieu, 2014). Notably, plants with a C4 photosynthetic pathway often possess greater competitive ability than C3 species under dry and high irradiance conditions such as those in tropical grasslands and savannas (Edwards *et al*., 2010; Taylor *et al*., 2011, 2014). This competitive advantage is ascribed to the ability of C4 species to maintain greater photosynthetic rates per unit of water loss than C3 species (Sage & Kubien, 2003; Taylor *et al*., 2014). Nevertheless, water availability still dictates the maximum yield achieved by C4 plants such as brachiaria. Previous studies on brachiaria have shown that most species are able to adjust growth and biomass allocation in response to induced mild drought conditions, leaving total plant yield relatively unaffected (Guenni *et al*., 2002). While leaf expansion is reduced by the mild drought conditions, it could quickly resume after rewatering of C4 plants. Studies by Guenni *et al*. (2004) on temporal trends in leaf water potential, relative water content, stomatal conductance and net photosynthesis showed that these were adversely influenced by drought, while osmotic potential at full turgor was significantly adjusted in drought stressed plants as compared to well-irrigated plants. Cardoso *et al*. (2015) reported that Mulato II plants have large root systems which enable them to effectively extract water from drying soils, and that plants could restrict water loss by early stomatal closure.

Insect pests are among the most important biotic constraints responsible for reduced crop plant productivity (Metcalf, 1996) and economic losses (Oliveira *et al*., 2014). Conversely, plants have developed intrinsic or direct antixenosis and antibiosis mechanisms against herbivorous insect pests (Painter, 1951; Kogan & Ortman, 1978; Kennedy *et al*., 1987). Natural enemies are also considered to be a component of plants extrinsic defence mechanisms (Turlings *et al*., 1990; Khan & Pickett, 2004). These mechanisms are based on chemical, physical and semiochemical plant traits that plants use against herbivores and to exploit their natural enemies (Pettersson *et al*., 1987; Turlings *et al*., 1990, 1995; Bruce *et al*., 2005; Khan *et al*., 2010).

Plants of *Brachiaria brizantha* have been observed to emit volatile compounds in response to *C. partellus* oviposition, which then increases attraction of the parasitoid *C. Sesamiae* (Bruce *et al*., 2010). Contribution of antibiosis and tolerance to spittlebug resistance in brachiaria grasses have also been elucidated in previous studies (Ferrufino & Lapointe, 1989; Lapointe *et al*., 1992; Cardona *et al*., 1999; Parsa *et al*., 2011). With the current and expected increase in climate change effects, insects continue to be more abundant as a result of host range extension and phonological changes (Bale *et al*., 2002). Red spider mites (*Tetranychus evansi*) (Baker & Pritchard) (Acari: Tetranychidae) has emerged as a new threat to brachiaria grass production in drier agro-ecologies of sub-Saharan Africa (Miles *et al*., 2004; *Personal communication,* Zeyaur Khan and Charles Midega, *icipe*, Kenya). However, no studies have been conducted on the interaction of this arthropod species with brachiaria grasses.

The push-pull system uses grasses like napier grass (Khan *et al.*, 2007) while vetiver grass and other wild grasses have also been indicated as possible pull crops to *Chilo* spp. (Van den Berg *et al.*, 2001, 2003, 2006; Khan *et al.*, 2007). However, none of these species are adapted to a wide range of environmental conditions (especially arid/semi-arid) and most of them do not provide their intended benefits as forage crop. This therefore highlights the need to identify more grasses, especially drought tolerant and *Chilo* spp. attractive varieties, for use in the push-pull system which is expanding across the east African region. The study seeks to provide understanding of the agronomic and phytochemical properties of brachiaria to enable selection and use of appropriate varieties in the management of cereal stemborers and to improve fodder production under different climate change scenarios.

1.2 Problem statement

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench), are the most important food and cash crops for millions of rural farm families in the predominantly mixed crop-livestock farming systems of sub-Saharan Africa (Romney *et al*., 2003). However, crop yield continues to be severely hampered mainly by lepidopteran stemborers in the families Noctuidae and Crambidae, parasitic weeds in the genus *Striga* (Orobanchaceae) and poor soil fertility. A conservation agriculture technology, climate-smart push–pull that utilizes companion cropping was developed to effectively address these constraints in the face of current climate change scenarios. This climate-smart push–pull system, involves intercropping of maize or sorghum with a forage legume, silver leaf desmodium (*Desmodium uncinatum* (Jacq.) DC.), and planting *Brachiaria brizantha* cv Mulato II as a border crop. Desmodium repels stemborer moths (push), and attracts their natural enemies, while brachiaria grass attracts the moths (pull). Desmodium is also very effective in suppressing *Striga* while improving soil fertility through nitrogen fixation and improved organic matter content. Both companion plant species provide high-value animal fodder, facilitating milk production and diversifying farmers' sources of income. The technology has since been adopted by about 125,000 farmers in eastern Africa where it has effectively addressed the major production constraints, significantly increasing maize yields. The system is economical as it is based on locally available plants and do not require expensive external inputs. Despite the remarkable benefits, its potential is threatened by direct effects of changing climate, *e.g.* aridification, as well as indirect threats such as emerging arthropod pests. For instance, use of Mulato II as a 'pull' crop is adversely limited by attacks by red spider mites. This therefore necessitates further research into the genetic variation in brachiaria and to select cultivars with improved resistance to threatening biotic and abiotic stresses. Selection of cultivars with multiple beneficial traits such as drought and pest tolerance as well as the ability to release kairomones for management of stemborers in the push-pull system is also crucial.

1.3 Justification of study

Increasing global uncertainty about food security, increasing needs for animal protein, intensifying extremity effects of climate change, and growing demands on the world's supply of fresh water all drive the need for forage crops that require less water to maintain productivity and that tolerate episodes of drought and pests. Brachiaria grasses have promising benefits and potential for increased and diverse uses, if more research on these grasses is conducted. As a C4 grass, it has a competitive advantage over their C3 counterparts under drought conditions. This makes it an important tropical forage crop. In the recent past, brachiaria variety Mulato II was incorporated in a push-pull strategy, a chemical ecology based Integrated Pest and Weed management technology in cereal–livestock farming systems in east Africa (Khan *et al*., 2014, 2016).The phytochemical properties of brachiaria render it attractive to oviposition by *Chilo* sp. (Midega *et al*., 2011) as well as some of their natural enemies (Bruce *et al*., 2010). Mulato II also supports minimal survival of stemborer larvae (Midega *et al*., 2011).

In this study, a systematic evaluation of different genotypes of brachiaria was done, based on the agronomic and phytochemical properties. This will provide insights into understanding the associated chemical ecology of plant–insect and plant-plant, as well as plant-environment interactions regarding brachiaria grasses. From this study, varieties with both improved tolerance to drought stress and the ability to release kairomones for management of stemborers were selected. These varieties will be employed to optimize push-pull technology in semi-arid areas and to improve income generation and human nutrition in areas where the projected increase in climate change effects are expected. This will in a broader sense contribute to a real Green Revolution in Africa without causing any ecological and social harm.

1.4 Objectives

1.4.1 General objective

The general objective was to contribute to improved cereal-livestock productivity in Africa through management of stemborers using suitable and adaptable brachiaria grasses.

1.4.2 Specific objectives

i. to evaluate the morphological and physiological performance of 18 genotypes of *Brachiaria* spp. under simulated drought conditions

ii. to determine the levels of resistance of different genotypes of *Brachiaria* spp. to *Oligonychus trichardti* and yield performance across different agro-ecological zones in east-Africa
iii. to determine the suitability of different *Brachiaria* spp. as a trap for *Chilo partellus*
iv. to determine the effects of head space volatiles from different genotypes of *Brachiaria* spp. on behavior of *C. partellus* moths and the parasitic wasp, *Cotesia sesamiae.*

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brachiaria

2.1.1 History and evolution

Brachiaria is a genus in the Poaceae family and commonly occurs in extensive pasture lands of tropical Latin America (Miles *et al.*, 2004). *Brachiaria* species originated primarily from eastern, central and southern Africa where they form natural constituents of grasslands (Boonman, 1993; Miles *et al.,* 1996). It belongs to a small group that includes *Urochloa*, *Eriochloa* and *Panicum*. There is great similarity between brachiaria and these other mentioned genera, making it difficult to separate between them (Renvoize *et al.,* 1996). There are over 100 species of brachiaria, mostly from Africa (do Valle *et al.,* 2009). However, a few species such as; *Brachiaria brizantha*, *B. ruziziensis*, *B. decumbens* and *B. humidicola* have been commercially exploited and are planted as forage crops in Africa and Latin America (Miles *et al.,* 2004). *B. brizantha* is found throughout tropical Africa, while the other three species are found mostly around the Equator in eastern Africa (Keller-Grein *et al.,* 1996). The first brachiaria varieties, all collected from Africa, were introduced in tropical parts of Australia during the early 1960s and subsequently into tropical South America, beginning with Brazil in the early 1970s (Parsons, 1972; Sendulsky, 1978). Currently, in Brazil alone, an area of approximately 99 million hectares of pasture is planted to brachiaria varieties.

2.1.2 Biology and agronomy

There are more than 100 species of species of brachiaria. Generally, all species have the PEP-CK (Phosphoenol pyruvate-caboxykinase) type of C4 photosynthetic pathway (Clayton & Renvoize, 1986). This allows them to tolerate drier conditions and longer exposure to light than many other plant species (Gonzalez & Morton, 2005). A few of the species that have been exploited for commercial production as forage crops include *B. brizantha* (A. Rich) Stapf (palisade grass), *B.*

ruziziensis Germain & Evrard (ruzi grass); *B. decumbens* Stapf (signalgrass); and *B. humidicola* (Rendle) Schweick (Koronivia grass) (Miles *et al.*, 2004).

Signal grass (*B. decumbens*) is a vigorous rhizomatous and stoloniferous, medium-lived (<5 yrs) perennial grass. It has a dense root-system with many bunched, fastgrowing roots that go as deep as 2 m into the soil layers (Husson *et al.*, 2008). Signal grass has a prostrate or decumbent habit and grows up to 60 cm high. Its flowering stems can however be up to 100 cm in height, arising from the stolons (Loch, 1977). The leaves are short, hairy and bright green in colour (Bogda, 1977). Leaf blades are lanceolate, 10 – 14 cm long x 8 – 10 mm wide while the inflorescence is a panicle with 2 – 7 slightly curved racemes which range from 2 – 5 cm long. The racemes are almost at right-angles to the 10 – 20 cm long axis. The spikelets are hairy, 4 – 5 mm long and borne in 2 rows along the rachis (Cook *et al.*, 2005; Husson *et al.*, 2008). This grass species occurs naturally in open grasslands or in partially shaded areas between 27 °N and 27 °S from sea level to an altitude of up to 1750 m. It grows in frost-free areas with temperatures above 19 °C. Optimal growth occurs between 30 – 35°C (FAO, 2016) and in places where average annual rainfall is over 1500 mm. Signal grass has a deep root system which effectively extracts P and N from the soil. This makes it tolerant to low soil fertility and drought. Signal grass is relatively pests and disease free (Loch, 1977). However, it does not do well on heavy clay soils subject to waterlogging and can tolerate a dry season of 4 - 5 months (FAO, 2016).

Palisade grass is a tufted perennial grass, usually 60 – 120 cm high with deep roots and short rhizomes (Renvoize *et al.*, 1996). It has stout, erect or slightly decumbent culms and bright green leaves (Cook *et al.*, 2005). The inflorescence is a typical panicle consisting of 2 – 16 racemes which are 4 – 20 cm long. Spikelets appear as a single row, are elliptical and 4 – 6 mm long with a sub-apical fringe of long purplish hairs. This grass is very variable in growth habit, leafiness, hairiness and yield. It is similar to signal grass, though a little more tufted and with slightly different spikelets and shorter roots (Cook *et al.*, 2005; Husson *et al.*, 2008; FAO, 2010). It is a warm-season grass that can be found from the lowlands up to an altitude of 2000 m in the tropics. Optimum temperature for growth of palisade grass is about 30 – 35 °C and it grows best with 1500 – 3500 mm average annual rainfall, though it tolerates less than 1000 mm rainfall and can withstand dry seasons of 3 – 6 months during which it remains green (FAO, 2010). It can grow on light to heavy textured soils which may vary in fertility and pH.

Ruzi grass also known as Congo grass a short-lived perennial grass that is semi-prostrate with dense leafy cover. (Cook *et al.*, 2005; Husson *et al.*, 2008). It has a dense system of bunched, fastgrowing roots going as deep as 1.8 m into the soil layer (Husson *et al.*, 2008). Culms of ruzi grass grow also from the nodes of the rhizomes and may reach 1.5 m high when flowering (Cook *et al.*, 2005). The leaves are soft but hairy on both sides, lanceolate in shape and up to 25 cm long × 1.0 – 1.5 cm wide. Inflorescences consist of 3 – 9 relatively long racemes (4 – 10 cm), bearing spikelets in one or two rows on one side of a broad, flattened and winged rachis (Cook *et al.*, 2005). The spikelets are hairy and 5 mm long. Ruzi grass can occur from the lowlands up to 2000 m in the humid tropics and does well under temperatures between 28 and 33 °C, and mean annula rainfall of around 1000 mm (Rattay, 1973).

Koronivia grass is a leafy, procumbent and stoloniferous perennial grass. It has a creeping habit different from those of other *Brachiaria* spp. including *Brachiaria dictyoneura* that is often mistaken for it (Cook *et al.*, 2005; Miles *et al.*, 1996). Koronivia grass forms dense sods and its culms remain prostrate sometimes forming roots from the lower nodes. The leaves are flat, lanceolate blades and bright green measuring 4 – 20 cm long and 3 – 10 mm wide. The inflorescences bear 2 – 4 racemes with hairy and bright green spikes measuring 3 – 4 mm long (Clayton *et al.*, 1986; Cook *et al.*, 2005; FAO, 2010). This grass is found in areas from sea level to an altitude of 2400 m in East and South-East Africa and does well with annual rainfall ranging from 600 - 2800 mm and can tolerate average daily temperatures of up to 35 °C (Cook *et al.*, 2005; Schultze-Kraft *et al.*, 1992). It can also withstand drought periods (3 – 4 months), but grows slower when dry periods last longer than 6 months (Tergas, 1981; Urriola *et al.*, 1988). *Brachiaria humidicola* and *B. dictyoneura* are better adapted to longer dry periods whereas *B. brizantha*, *B. decumbens* and to a lesser extent *Brachiaria mutica* are better adapted to short dry periods (Guenni *et al.*, 2002).

2.1.3 Importance of brachiaria

Brachiaria is the single most important forage grass in the tropics. It has impacted the economy in these regions since it grows well in low-fertility acidic soils and is able to produce highly nutritious forage. Over the past 30 years brachiaria cultivation and export has become a major component of sown pastures. In South America, Brazil represents the leading user and producer of brachiaria seeds (Jank *et al*., 2014). In the recent past, brachiaria, particularly *B. brizantha* cv Mulato II, became increasingly known for its use in the push-pull system, a conservation agriculture technology initially developed for management of cereal stemborers. This is due to its ability to produce kairomones that attract *C. partellus* moths (Khan *et al.,* 2014; Midega *et al.*, 2015).

2.2 Drought and water stress responses in plants

Drought continues to be one of the most limiting environmental factors to plant productivity in many regions of the world, especially the arid and semi-arid areas (Fischlin *et al.,* 2007). Drought stress impairs numerous metabolic and physiological processes (Levitt, 1980) leading to reduction in plant growth, reduction in chlorophyll and water content, and changes in fluorescence (Souza *et al.,* 2004, Li *et al.,* 2006, Yang *et al.,* 2006). Under drought conditions, uptake of mineral nutrients from the soil is limited due to a lack of root activity as well as slow ion diffusion and water movement rates (Dubey & Pessarakli, 2001). Furthermore, mineralization processes are affected as they depend on micro-organisms and enzyme activity, which may be affected by drought (Prasertsak & Fukai, 1997).

Drought severely limits productivity of forage grasses (Sheaffer, 1992; Baruch, 1994; Knapp *et al.,* 2001). With global warming, this situation will be aggravated by an increase in frequency and intensity of drought resulting up to 30% increase in land area under extreme drought by the year 2100 (Fischlin *et al.*, 2007; Dai, 2013). In response to drought, plants have evolved complex drought-adaptive strategies from genetic molecular expressions, biochemical metabolism through individual plant physiological processes to ecosystem levels (Osakabe *et al.,* 2014;

Yoshida *et al.,* 2014; Fang & Xiong, 2015; Todaka *et al.*, 2015). Generally, drought resistance strategies in plants involve i) drought escape through short life cycles or developmental plasticity, *e.g.* early flowering in some annual species before the onset of severe drought (Manavalan *et al.*, 2009), ii) drought avoidance by enhancing capacity of accessing water and reducing water loss, *e.g*. developing root systems or conserving water through reduction of stomata and leaf area/canopy cover (Schulze, 1986, Jackson *et al.,* 2000; Luo, 2010, Tardieu, 2013), and iii) drought tolerance through osmotic adjustment, antioxidant capacity, and desiccation tolerance (Morgan 1984; Yue *et al.*, 2006; Luo 2010).

2.3 Stemborers

There are 21 economically important lepidopterous stemborers of cultivated grasses in Africa. They comprise of seven noctuids, two pyralids, and 12 crambids. *Busseola fusca* (Noctuidae) and *Chilo partellus* (Crambidae) are considered economically important pests of maize and sorghum in East Africa (Harris, 1990; Harris & Nwanze, 1992). However, *C. partellus* has proven to be a very efficient colonizer, and it seems to be displacing the indigenous *B. fusca* in East Africa (Kfir 1997*).* The moths lay eggs on maize plants, and the emerging larvae feed on leaves for 2 – 3 days before burrowing inside the stems. *Chilo partellus* lays its eggs on the plant surface in the form of egg batches (Van den Berg 1991). Larvae feed inside the stems for 2 – 3 weeks causing stem damage. The fully-grown larvae pupate and remain inside the stem for 7 – 14 days before they emerge as adults (Fig. 2.1).

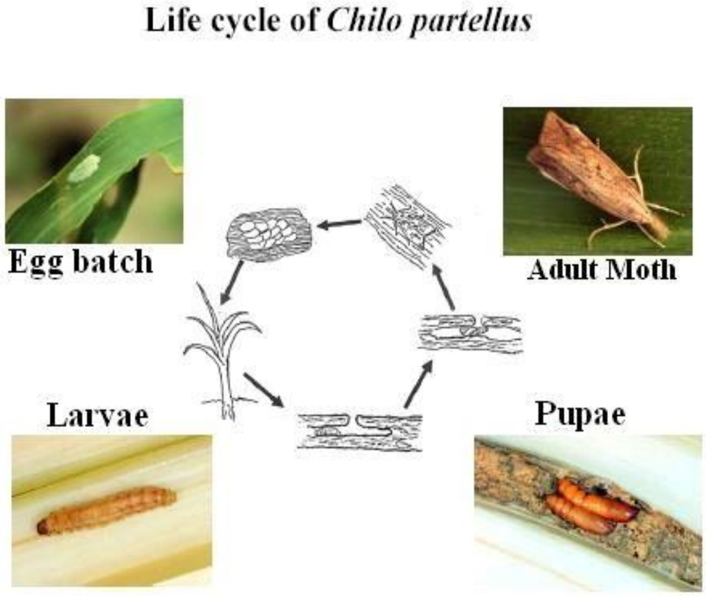


Figure 2.1 Life cycle of *Chilo partellus*
(Source: http://push-pull.net/striga/stemborer.html)

2.4 The red spider mites

Spider mites are major pests of commercial crops in Africa (Sibanda *et al.,* 2000; Gerson *et al.,* 2003). They have developed resistance to most pesticides and is difficult to control (Cranham & Helle, 1985; Picanco *et al.,* 2007; Van Leeuwen *et al.,* 2010). There are over 256 species of phytophagous mites in Africa, and in sub-Saharan Africa, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) and the tomato red spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) are the predominant and closely related species. The striking difference between the two species is in the colour of the soma (thorax, plus abdomen): green or whitish for *T. urticae* (Fig. 2.3 a) and red for *T. evansi*. (Fig. 2.3 b). The colours come from the pigment in the haemolymph and internal tissues (Sato *et al.,* 2014). *Tetranychus urticae* is a worldwide pest of tomatos, beans, maize, soybean, apples, grapes and cucurbit crops (Jepson *et al.,* 1975). *Tetranychus evansi* was recently introduced into Europe and Africa from South America and it attacks host plants such as nightshade, tomato, eggplant and potato (Moraes & McMurrty, 1985; Boubou *et al.,* 2012; Navajas *et al.,* 2013). Recently, spider mites have been observed to be an endemic pest of brachiaria in sub-Saharan Africa (Miles *et al.*, 2004) (Plate 2.1). Arguably, no studies of spider mites on brachiaria have been documented.

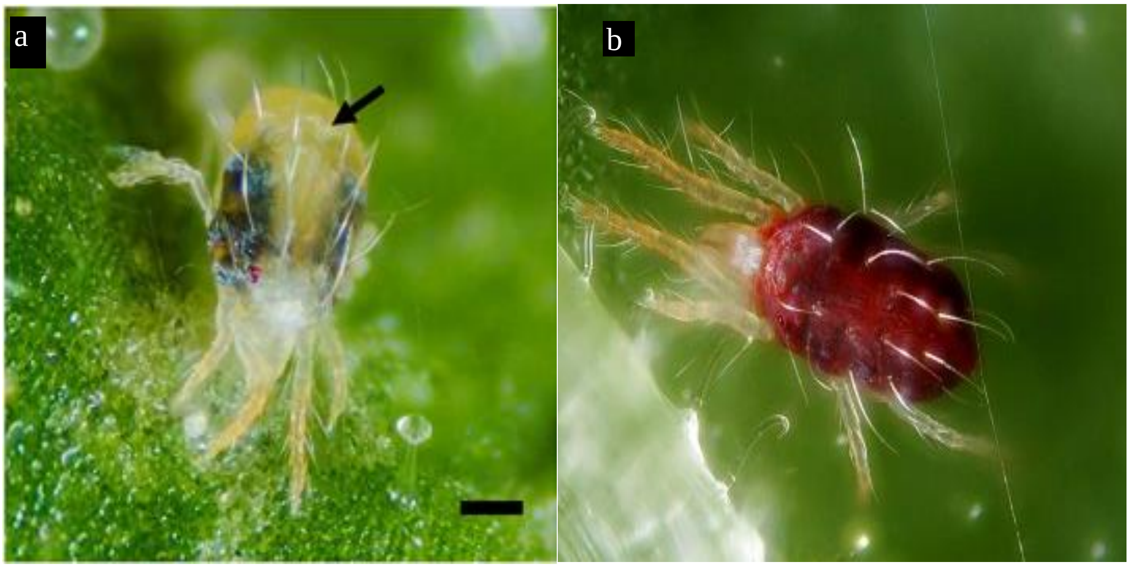


Figure 2.3 Two species of spider mites, (a) *Tetranychus urticae* and (b) *Tetranychus evansi* (Source: Sato *et al.,* 2014).

Spider mites feed by puncturing of leaf epidermal cells, which leads to whitening or yellowing of leaves, followed by desiccation, defoliation and eventually death in severe cases (Fig. 2.4). Webbing can be seen on the underside of leaves in cases of high levels of infestation (Knapp *et al.,* 2003). For *T. evansi*, adult females are 0.5 mm long, oval, orange-red with and indistinct dark blotch on each side of the body. They can lay up to 200 eggs. Males are smaller (0.3 mm) and have a light orange colour. The life cycle consist of eggs, larvae, two nymphal stages and adults (Migeon, 2005) (Fig. 2.5). At 25 °C, its life cycle is completed in 13.5 days, but this is shortened under hot and dry conditions (minimum temperature 10 °C, optimum temperature 34 °C) (Knapp *et al.,* 2003; Migeon 2005).

2.5 Strategies for pest management

2.5.1 Chemical control

Synthetic pesticides have been used as a primary remedy for pest attacks. However, their use has been linked to serious negative impacts including non-target effects on humans and beneficial

Plate 2.1 Symptoms of attack by red spider mites on *Brachiaria brizantha* cv Mulato II in the field. (Photo taken by D Cheruiyot on 10th August 2017).

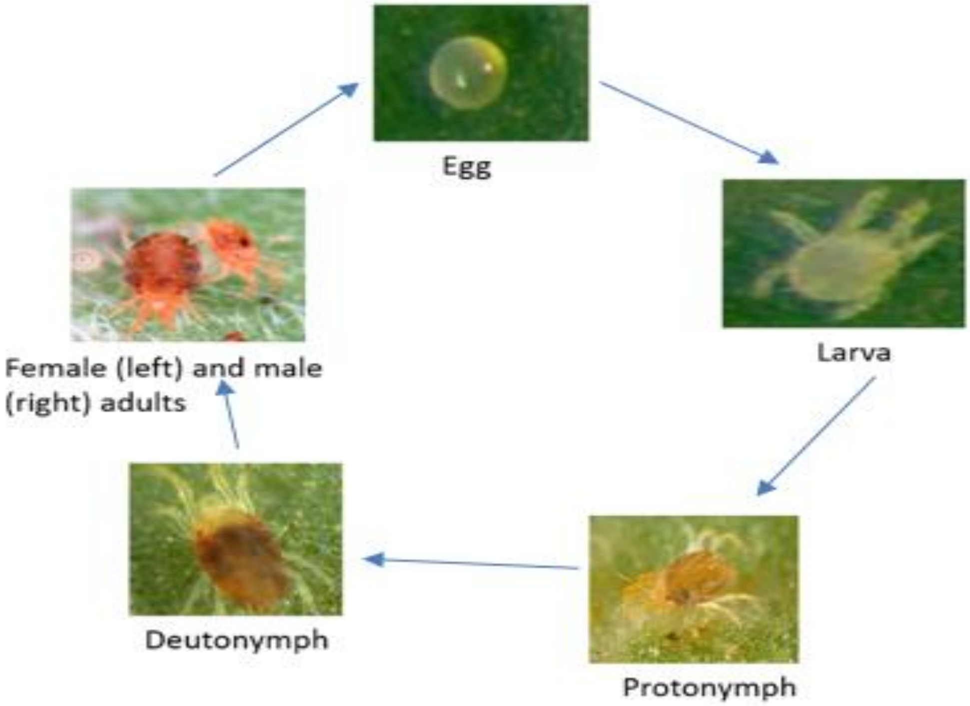


Figure 2.3 Life cycle of *Tetranychus evansi*.
Source: https://www.agric.wa.gov.au/citrus/mites-citrus?nopaging=1

organisms, pest resurgence and emergence of secondary pests, resistance in target pests (Ekström & Ekbom, 2011; Mengistie *et al.,* 2015) and high cost, especially to small scale farmers in sub-Saharan Africa (Ngowi *et al.,* 2007; Macharia *et al.,* 2013; Mengistie *et al.,* 2015). To address the challenges arising from effects of pesticides on non-targeted organisms, integrated pest management programs encourage the use of bio-pesticides, which are efficacious against the target pest but are less detrimental to natural enemies (Schuster *et al.*, 2007).

2.5.2 Biological control

Biological control has a long history of use in pest management and has received renewed interest. In a broad context, the term "biological control" has been used to encompass a full spectrum of biological organisms and biological based products that include; pheromones, autocidal techniques (Lewis *et al.,* 1997), bio-pesticides (Schuster *et al.,* 2007) and natural enemies. Bacteria-based spray formulations that contain *Bacillus thuringiensis* (Berliner) (Cross *et al.,* 1999), virus-based agents such granulovirusses (Tanada, 1964) and even yeast-based agents (Knight & Witzgall, 2013) have been used for control of pests. Natural enemies of insects are usually predators that quickly kill and also consume insects and parasitoids (Capinera, 2010). While arhropod predators have free living larval stages that require several host individuals as food source to complete their life cycle, parasitoids develop as larvae on the host arthropod's tissues, eventually killing it (Hassel & Waage 1984).

2.5.3 Host plant resistance

According to Painter (1951), resistance is the relative amount of qualities that can be inherited and possessed by the plant, which influence the degree of damage caused by insects. He divided resistance resulting from complex plant-arthropod interactions into three mechanisms: antibiosis, non-preference and tolerance. Painter's category of non-preference has since been replaced by 'antixenosis' (Kogan & Ortman, 1978).

Antibiosis

Antibiosis includes adverse effects that develop in an insect life history after it consumes tissue of the host plant (Painter, 1951). This is mediated by various chemical and morphological properties of the host plant. Some antibiotic effects, ranging from mild to lethal, include death of early instars, reduced size or low weight, reduced adult longevity or fecundity, or abnormal wandering behaviour (Painter, 1951; Kant *et al.,* 2015). To detect *antibiosis*, investigators measure the growth, survival, and reproduction of individuals or populations in caged no-choice tests in the field, greenhouse, or laboratory. *In vitro* assays that chart insect growth and development relative to food intake and excretion can be particularly useful in characterizing antibiotic effects (Eickhoff *et al.*, 2008; Parsa *et al.,* 2011; Goggin *et al.,* 2015) (Fig.2.6).

Antixenosis

Antixenosis (deterrence) is the inability of a plant to serve as suitable host to an insect pest, forcing the insect to select an alternate host (Fig. 2.6). There are morphological and chemical plant factors involved in this phenomenon (Painter, 1951; Kogan & Ortman, 1978). Antixenosis traits are usually constitutively expressed and can emanate from colours, odours or textures (such as hairs) that demotivate herbivores from feeding on the plant, or from the absence of feeding stimuli that otherwise would stimulate feeding by herbivores (Kant *et al.,* 2015). To detect *antixenosis*, insect behaviour is monitored in choice tests in response to intact plants, detached plant parts such as leaf discs, or plant-derived cues such as volatiles presented through olfactometers (Eickhoff *et al.,* 2008; Goggin *et al.,* 2015) (Fig. 2.6). The behaviours most commonly monitored in these assays include directed flight, walking, feeding, and oviposition (Khan *et al.,* 1989; Eickhoff *et al.,* 2008; Sarao & Bentur, 2016). Alternatively, for insects that leave quantifiable signs of feeding or oviposition on their hosts, the incidence or magnitude of this damage can be measured (Khan & Saxena, 1985; Khan *et al.,* 1989).

Tolerance

Tolerance is the ability of a plant to withstand or to recover from damage caused by herbivores. The plant can grow and reproduce or repair injury to a marked degree despite supporting a significant level of insect pest (Painter, 1951). Tolerance can be evaluated by measuring the impact of the insect on plant health or productivity (Goggin *et al.,* 2015; Sarao & Bentur, 2016) (Fig. 2.6). Unlike antixenosis and antibiosis, tolerance is determined by the inherent genetic ability of a plant and it involves plant characteristics such as plant stand and production of biomass or yield (Smith *et al.,* 1994). Plant productivity is most readily quantified under field conditions, but in some cases, tolerance can be measured in greenhouse or laboratory assays, particularly if early indicators of damage such as chlorophyll loss can be used as predictors of potential yield loss (Goggin *et al.,* 2015) (Fig. 2.6). Notably, tolerance usually occurs in combination with antixenosis and antibiosis and therefore, the relationship between insect pressure and insect damage can be compared among different plant genotypes (Khan *et al.,* 1989; Eickhoff*et al.,* 2008; Goggin *et al.,* 2015).

2.5.4 Semiochemicals in pest management

Semiochemicals

Semiochemicals are chemicals that plants emit, and which affect behaviour of other organisms (Ridgway *et al.,* 1990). Semiochemicals are subdivided into those that are significant to individuals of a species different from the source species (allelochemicals) and those that are released by one member of a species to cause a specific interaction with another member of the same species (pheromones) (Arthur, 1981). Allelochemicals are further subdivided into several groups depending on whether the response of the receiver is adaptively favourable to the emitter but not the receiver (allomones), is favourable to the receiver but not the emitter (kairomones) or is favourable to both emitter and receiver (synomones). Pheromones may be further classified on the basis of the interaction mediated, such as alarm, aggregation or sex pheromone. It is the sex pheromones of insects that are of interest to agricultural integrated pest management (IPM) practitioners (Dent, 1993). Generally, semiochemicals can be referred to as arrestants, attractants,

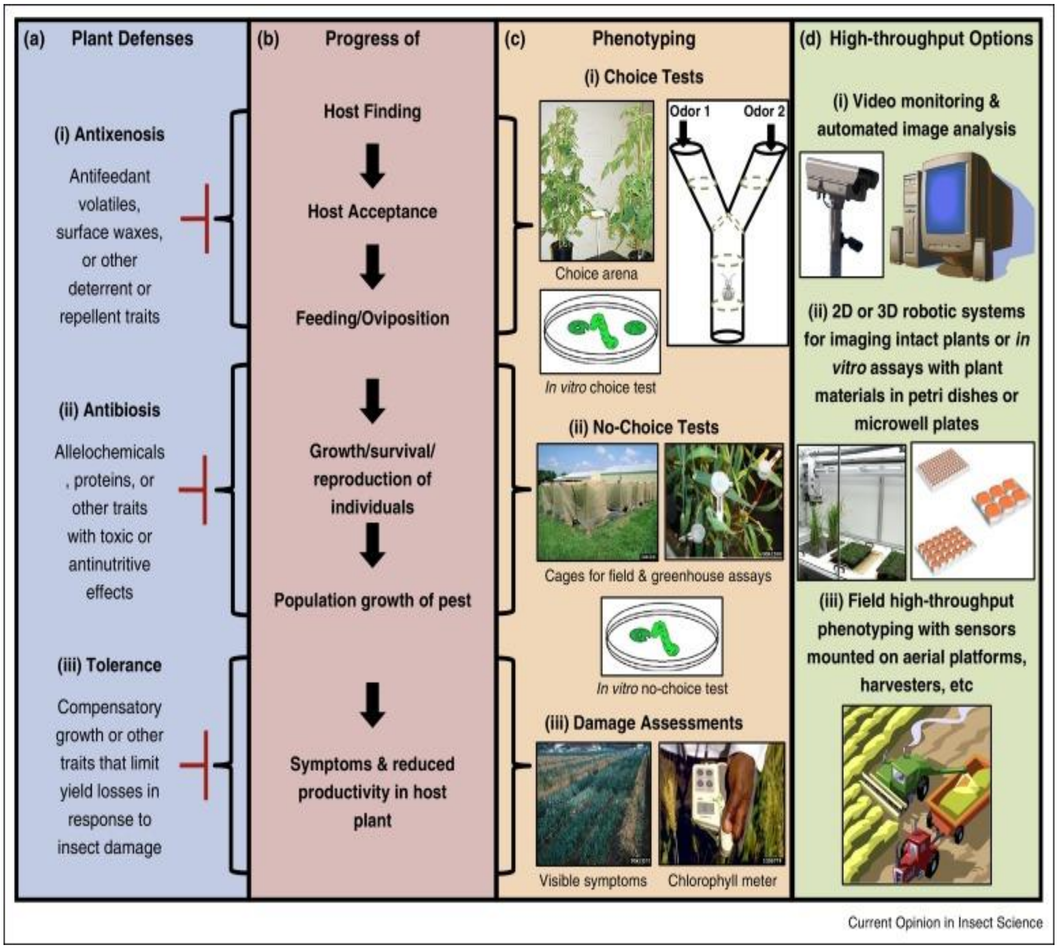


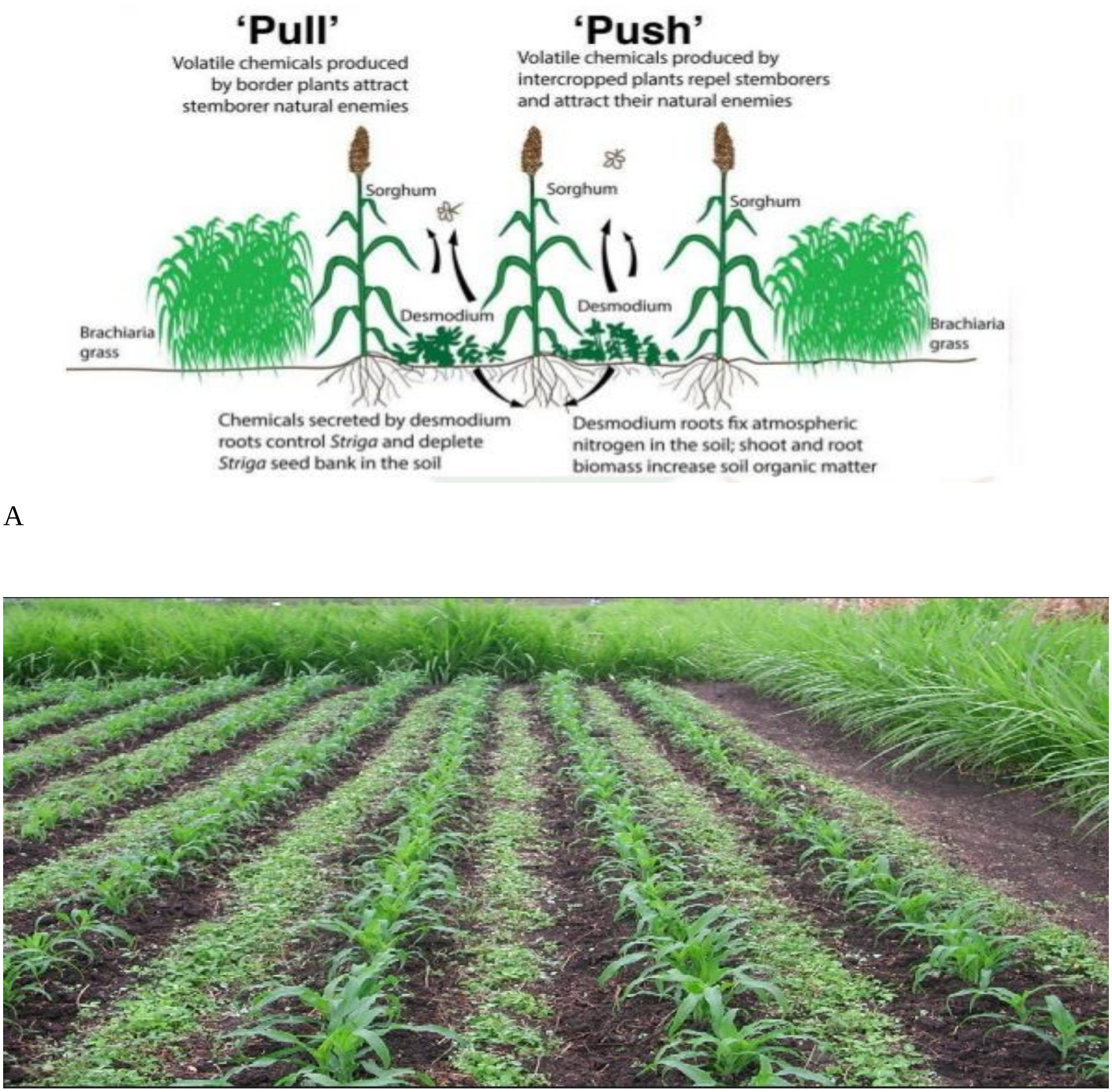
Figure 2.4 Phenotyping host-plant resistance (Source: Goggin *et al.*, 2015).

repellents, deterrents, stimulants or other descriptive terms. These terms can indicate what behaviour is involved in the response such as a feeding stimulant or flight arrestant. When a plant is under attack by a pathogen, herbivorous animal or other biotic factors it emits volatile compounds, either constitutively or as a result of biotic infestation/physical damage. These compounds can affect pathogen development and the behavior of insect herbivores searching for a food source (Agrawal, 1998). Constitutively produced plant volatiles play a role in attracting pollinators and seed-dispersing animals. Additionally, they can repel a wide range of potential herbivores and attract a smaller number of pest species that have evolved to take advantage of these chemicals in finding food. Plant volatiles that are induced upon damage repel the attacking insect and may also act as an indirect plant defense mechanism by attracting other insects that prey on or parasitize the herbivores (Agrawal, 1998; Bruce & Pickett, 2007). Such compounds may also act as signals between plants, whereby defense mechanisms are induced in undamaged plants in response to volatiles produced by neighbouring infested plants, and specific volatiles. Methyl salicylate and methyl jasmonate, have been implicated as such volatiles (Thaller *et al.*, 1996; Boland *et al.*, 1998). A six-carbon atom compound, (*E*)-2-hexenal, which are rapidly emitted from damaged or wounded plant tissue have also been shown to induce the expression of defence-related genes in intact plants (Bate & Rothstein, 1998).

Push-Pull (Stimulo-deterrent diversionary) strategy

Push–pull technology is a cropping system developed by the International Centre of Insect Physiology and Ecology (*icipe*) in collaboration with Rothamsted Research (UK), Kenya Agricultural Research Institute (KARI) and other national partners for integrated pest, weed and soil management in cereal livestock-based farming systems. Initially, the system involved attracting stemborers with either napier grass (*Pennisetum purpureum*), planted on the border of the field as a trap plant (pull), while repelling them from the main crop using a repellent intercrop (push) such as desmodium forage legumes (*Desmodium* spp.) (Khan *et al.,* 1997; Cook *et al.*, 2007) (Fig. 2.6). The companion crop plant releases behaviour-modifying stimuli (semiochemicals) that manipulate the distribution and abundance of stemborers and beneficial insects for management of the pests (Hassanali *et al.,* 2008). The Napier grass trap crop produces significantly higher levels of green leaf volatile cues (chemicals), used by gravid stemborer females to locate host plants other than maize or sorghum (Birkett *et al.*, 2006).

Chemicals released by desmodium roots cause abortive germination of the parasitic striga weed, providing effective control of this noxious weed (Khan *et al.,* 2000). The companion plants provide high value animal fodder, facilitating milk production and diversifying farmers' income sources. Furthermore, soil fertility is improved, and soil degradation prevented (Khan *et al.*, 1997). Chemical analysis of volatile compounds from the companion plants (Sudan and Napier grasses) revealed the presence of octanal, nonanal, naphthalene, 4-allylanisole, eugenol, and linalool (Khan *et al.*,2010) (Fig. 2.7). Electrophysiological and behavioural studies with molasses grass (*Melinis minutiflora* P. Beauv.) revealed the role of semiochemicals in repelling *C. partellus* moths. These compounds comprise of (*E*)-ocimene, (*E*)-4,8-dimethyl- 1,3,7-nonatriene (DMNT), (*β*)-caryophyllene, humulene, and *α*-terpinolene. In addition to playing a role in direct defences, volatiles released play a role in indirect defence, (*E*)- ocimene and DMNT belong to a group of semiochemicals referred to as herbivore-induced plant volatiles (HIPVs) since they are produced when plants are damaged by herbivorous insects (Turlings *et al.,* 1990) (Fig. 2.7.). The DMNT causes increased parasitoid foraging in intercropped plots (Khan *et al.,* 1997), indicating that intact plants such as molasses grass with inherent ability to release such stimuli could be used in development of new crop protection strategies. These previous studies have revealed positive activity in behavioural tests that investigated oviposition onto an artificial substrate treated with the individual compounds (Khan *et al.,* 2000). According to Birkett *et al.* (2006), trap plants produced significantly higher amounts of attractive compounds than maize and sorghum. *Melinis minutiflora* constitutively emits (*E*)-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, *β*-caryophyllene, humulene, and *α*-terpinolene (Khan *et al.,* 1997, 2000; Pickett *et al.,* 2006) (Fig. 2.7). These volatiles are emitted by trap plants, and they repel female *Chilo spp.* while attracting parasitoids, principally foraging female *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) (Khan *et al.,* 1997).



A

B

Figure 2.6 (A) Schematic diagram showing the mechanistic basic of the push-pull system and (B) picture of a typical push-pull plot at vegetative phase of both maize and desmodium
Source: http://www.push-pull.net/components.shtml

With ever increasing climate change effects such as aridification, the rising uncertainties of rain-fed agriculture for farmers in warmer, drier agro-ecosystems of Africa, *icipe* scientists have developed a climate smart push-pull. Drought tolerant companion plants have been incorporated into the technology. *Brachiaria brizantha* cv Mulato II, developed by CIAT and grown locally, can tolerate long droughts of up to two months with no water and temperatures above 30 ^{o}C (Pickett *et al.,* 2014). Mulato II plants were also preferred to maize and sorghum by *C. partellus* moths for oviposition (Midega *et al.,* 2011) and are also used by smallholder farmers as animal fodder (Khan *et al.,* 2014). Similarly, Greenleaf desmodium *Desmodium intortum* (Mill.) Urb. was found to tolerate higher temperatures and with the ability to survive under drier conditions. Mulato II was therefore incorporated into the push–pull technology as the border crop while greenleaf desmodium became the intercrop in the climate-adapted push–pull technology.

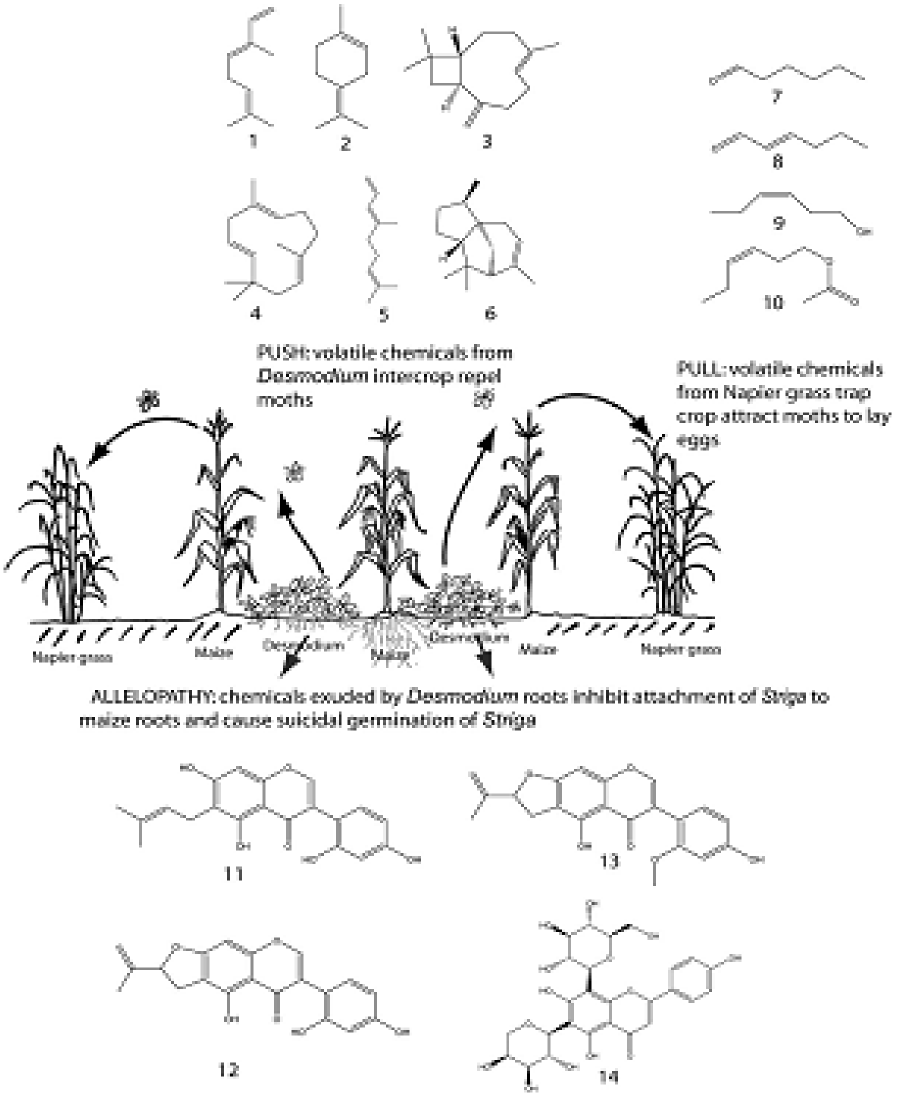


Figure 2.7 Diagrammatic illustration of push–pull systems: stemborer moths are repelled by intercrop volatiles while attracted to trap crop volatiles. Root exudates from the *Desmodium uncinatum* intercrop cause suicidal germination of Striga and inhibit attachment to maize roots. 1, (*E*)-β-ocimene; 2, α-terpinolene; 3, β-caryophyllene; 4, humulene; 5, (*E*)-4,8-dimethyl-1,3,7-nonatriene; 6, α-cedrene; 7, hexanal; 8, (*E*)-2-hexenal; 9, (*Z*)-3-hexen-1-ol; 10, (*Z*)-3-hexen-1-yl acetate; 11, 5,7,2',4'-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone (uncinanone A); 12, 4",5"-dihydro-5,2',4'-trihydroxy-5"-isopropenylfurano-(2",3";7,6)-isoflavanone (uncinanone B); 13, 4",5"-dihydro-2'- methoxy-5,4'-dihydroxy-5"-isopropenylfurano-(2",3";7,6)-isoflavanone (uncinanone C); and 14, di-C-glycosylflavone 6-C-a-L-arabinopyranosyl-8-C-b-D-glucopyranosylapigenin (Source: Khan *et al.*, 2010).

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CHAPTER THREE

3.0 GENOTYPIC RESPONSES OF BRACHIARIA GRASS (*Brachiaria* spp.) GENOTYPES TO DROUGHT STRESS

3.1 Abstract

Brachiaria, a warm season C4 grass, is rapidly gaining popularity as fodder crop in Africa where it is also used as a component of a habitat management strategy for maize stem borers. However, in many parts of Africa, increasing drought conditions limit productivity of this grass species as fodder and its value in pest management. We evaluated the morphological and physiological performance of 18 apomictic genotypes of brachiaria in simulated drought conditions in a screen house. Plants were exposed to different watering regimes. Well-watered (control) plants were watered every 48 h to 100% field capacity while drought was simulated by suspending watering for 14 and 28 days, representing moderate and severe drought conditions, respectively. Shoot length, leaf length and width, number of tillers, leaf relative water content, chlorophyll content, and above ground biomass were measured to determine the level of tolerance to drought. Results showed that water stress had negative effects on the morphological and physiological traits, with the effects being more pronounced under severe drought stress. Based on the drought stress index (DSI) values for the measured parameters as well as PCA biplots, the following genotypes were least affected under severe drought stress: Xaraes, Piata, Marandu, CIAT 679, Mulato II, and Mulato. Under severe drought, DSI of biomass yield produced the largest projection from the biplot origin, suggesting that this trait can be used as an accurate predictor of drought tolerance of the genotypes. Piata and Xaraes combined both drought tolerance and biomass yield. Our study proposes these two genotypes as of value in improvement of the sustainability of cereal-livestock farming systems under conditions of increasing aridification.

Keywords: Brachiaria; drought stress index; drought tolerance; genotypic variation.

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3.2 Introduction

Drought poses one of the most important environmental constraints to plant growth and productivity (Boyer, 1982; Jones, 2013). Plants primarily respond to drought by arresting growth. This reduces metabolic demands and mobilizes metabolites for the synthesis of protective compounds (Sharp & Davies, 1985; Hsiao & Xu, 2000). In some plants, exposure to drought stress leads to changes in carbon partitioning between the source and the sink, resulting in reduced photosynthesis and an associated decrease in chlorophyll content (Roitsch, 1999; Souza *et al.*, 2004; Yang *et al.*, 2006). Numerous metabolic and physiological processes within the plant are also affected (Levitt, 1972). For cultivated plants, tolerance to drought is generally considered as the potential of a species or variety to yield more in comparison to others under limited water conditions (Jones, 2013). Drought tolerance is a highly complex trait that involves multiple genetic, morphological, physiological and biochemical mechanisms (Cushman & Bohnert, 2000; Mattana *et al.*, 2005).

Brachiaria spp. are perennial C4 plants, native to Africa (Renvoize *et al.*, 1996). There are more than 100 species in the world, mostly tetraploid (2n = 4x = 36) and apomictic. Their progenies are uniform, produce high dry matter and persist on poor acid soils (do Valle *et al.*, 1989; Vigna *et al.,*2011). They form natural constituents of grasslands in eastern, central and southern Africa (Boonman, 1993; Maass *et al.*, 2015). These grasses also play an important role in cultivated pastures in tropical America (Keller-Grein *et al.*, 1996), South-east Asia (Hare *et al.*, 2015) and East Africa (Ndikumana & de Leeuw, 1996; Maass *et al.*, 2015). In addition to its use as a forage crop, *B. brizantha* cv Mulato II, henceforth referred to as Mulato II, has gained large acceptance in East Africa where the grass has been incorporated as a trap plant in the ‘push-pull’ pest management system (Khan *et al.*, 2014; Midega *et al.*, 2015). This system was developed for management of cereal stemborers by exploiting behaviour-modifying stimuli to manipulate pest and natural enemy behavior and reduce pest infestations (Cook *et al.*, 2007; Bruce *et al.*, 2010; Khan *et al.*, 2010; Midega *et al.*, 2011).

According to Guenni *et al*. (2002), most *Brachiaria* spp. respond to induced mild drought conditions through adjusted growth and biomass allocation, leaving the total plant yield relatively unaffected. In previous studies, Mulato II has been observed to tolerate extended periods of drought of up to three months with limited water availability and temperatures of 30 ^{o}C and higher (Pickett *et al.,* 2014). Their apomictic nature enables brachiaria to produce seeds which are true to type and which can colonize a wide range of habitats (Dall'Agnol & Schiffino-Wittmann, 2005). This phenomenon preserves the vigour of plants across environments. Such C4 plants possess greater competitive ability than their C3 counterparts under dry and high irradiance conditions such as those that are common in tropical grasslands and savannas (Edwards *et al.,* 2010; Taylor *et al.,* 2011, 2014). This competitive advantage is brought about by the ability of C4 species to maintain greater photosynthetic rates per unit of water loss than C3 species (Sage & Kubien, 2003; Taylor *et al.,* 2014). Nevertheless, water availability still dictates the maximum yields achieved by C4 plants such as brachiaria.

The purpose of this study was to provide an understanding of the morphological and physiological responses of different brachiaria genotypes to different drought stress regimes. We seek to identify putative drought tolerant brachiaria genotypes for utilization in specifically improving cereal-livestock productivity through management of stem borers amid the increasing threat of climate change.

3.3 Materials and methods

3.3.1 Plant materials

Seeds of brachiaria plants were sourced from International Centre for Tropical Agriculture (CIAT), Columbia and International Livestock Research Institute (ILRI), Ethiopia. These genotypes were grown in an on-station nursery at the International Centre of Insect Physiology and Ecology-Thomas Odhiambo Campus (ITOC), Mbita Point (0º25′ S, 34º12′ E; 1200 m above sea level) in western Kenya for observation and pre-selection of candidate genotypes. Eighteen genotypes were selected for further evaluation based on desirable agronomic performance (Table 3.1). The commercial hybrid, Mulato II was included as control treatment due to its previous use as a trap crop in a push-pull system and because it produces comparatively high fodder yield under conditions of drought stress (Midega *et al.,* 2011; Khan *et al.,* 2014).

3.3.2 Experimental site and procedure

The experiment was conducted in a screenhouse at ITOC. Over the period during which the experiment was conducted, mean minimum and maximum daily temperatures in the screenhouse were 18 ºC and 35 ºC, respectively. The soil used in the experiment was well drained alluvial and sandy loam classified as Chronic Vertisols (Jaetzold & Schmidt, 1983). Before planting, field capacity of potting soils was determined as described by Somasegaran and Hoben (1985).

The different genotypes were planted in plastic bags measuring 60 cm deep and 26 cm wide with holes at the bottom. Bags were filled with fine air-dried soil leaving a space of 5 cm from the top. The bags were then placed on 30 cm high benches covered with metallic mesh. The plastic bags provided a plant biomass to pot volume ratio of less than 2 kg m^{-3} as recommended by Poorter *et al*. (2012) (Plate 3.1). This is crucial in minimizing both the risks of having reduced plant growth which may influence the relative differences between treatments. The experimental setup followed a complete randomized design (CRD) in a factorial arrangement (3 × 18) with three replicates.

Five seeds were planted in each bag and later thinned to two plants per pot when most of the seedlings had four expanded leaves. Watering was done by adding 244 ml of water to all plant bags every 48 h, to restore the soil moisture to 100% field capacity until the commencement of the water restriction period. At three weeks after planting (WAP), the plants were top dressed with 60 kg N^{-ha} in the form of Calcium Ammonium Nitrate (CAN). Leaves of plants were also trimmed to standardize their heights at 10 cm. Application of the different watering treatments commenced 25 days after trimming. There were three treatments: well-watered control, moderate drought stress and severe drought stress. The bags in the control group (without water restriction) continued to receive water to 100% field capacity, every 48 h throughout the experiment, while watering was not done for a period of 14 and 28 days for the moderate and severely stressed treatments respectively.

3.3.3 Data collection and analysis

Data were collected 14 and 28 days after water restriction commenced to represent moderate and severe stress regimes. At each sampling period, one plant was randomly sampled from both stressed and control plants. The numbers of tillers per pot were counted. Shoot length (SL) was measured from the surface of the soil to the tip of the youngest fully expanded leaf. Leaf area was determined by multiplying the length of the second fully expanded leaf measured from the tip to the junction of the petiole, by its width (at its widest part). Leaf chlorophyll content was measured by means of a SPAD chlorophyll meter (SPAD-502 Plus; Konica Minolta Sensing Inc., Japan) and presented as SCMR index values. Ten measurements were done on the second fully emerged leaf of each plant. Leaf relative water content was estimated following the procedure used by Chen *et al*. (2015). The youngest fully expanded leaf was removed and weighed immediately to determine fresh weight (FW). Turgid weight (TW) was determined after leaf segments were immersed in distilled water for 6 h, and dry weight (DW) was measured after leaf segments were dried at 70 ºC in an oven for 24 h. Each treatment was replicated three times. The relative water content (RWC) was calculated as follows:

$$\mathrm{RWC} = \frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{TW}-\mathrm{DW}} \times 100$$

The above ground dry biomass (BM) was determined after harvesting all the shoots per plant and drying it at 65 °C for 48 h.

Data were checked for homogeneity of variance between treatments using Bartlett’s test. A two-way ANOVA was used to test for treatment differences, genotypic effects and interactions for each stress regime. Significance of differences between the genotypes was tested by F-test, while the treatment means were compared by least significant differences (LSD) at P = 0.05. Simple correlation coefficients among traits were determined using mean trait values for genotypes, following Pearson’s correlation method. These analyses were performed using R software (Version 3.3.1) (R Core Team, 2016). Drought Stress Index (DSI) values were used to compare the responses between individual genotypes, based on the difference between stress treatments and the control plants. The values were calculated as follows:

$$\text{DSI} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under control condition}} \times 100$$

According to Wójcik-Jagła *et al*. (2013), this equation removes the effect of germplasm variation from the drought stress treatment and can therefore be used to assess a large collection of germplasm simultaneously. Biplots of principle components derived from DSI values of each trait were used to comprehensively identify stress tolerant genotypes, *i.e.* those that were least affected by the stress treatments. This was computed by use of Microsoft XLSTAT software (Addinsoft, 2010).

3.4 Results

3.4.1 Analysis of variance

Our results show that under moderate drought conditions, effects of soil moisture regimes were significant ($P \leq 0.05$) for all the traits except shoot length and tiller numbers. Genotypic effects were significant for all the traits, while interactions between soil moisture and genotype was only significant ($P \leq 0.05$) for relative water content and plant biomass. There was a general decrease in mean values of traits between the control and stressed plants for both stress regimes (Table 3.2). Moderate drought stress resulted in a decrease of 3.8% in shoot length, 13.7% in leaf area, 1.0% in tiller number, 6.2% in leaf chlorophyll content, 5.1% in relative water content and 8.6% in biomass. The highest reduction was observed in the leaf surface area (13.7%) whereas the lowest reduction was observed in the number of tillers per plant (1.1%). Severe drought stress resulted in significant ($P \leq 0.05$) decrease of 14.4% in shoot length, 20.9% in leaf area, 38.0% in number of tillers per plant 13.7% in chlorophyll content, 55.8% in relative water content and 37.8% in biomass. Genotypic effects were also more pronounced except for chlorophyll content, while genotype x treatment effects were only significant ($P \leq 0.05$) for number of tillers and relative water content. Exposure to severe stress resulted in the highest percentage reduction in relative water content (55.8%) and the lowest percentage reduction in SCMR index values (13.7%).

3.4.2 Morphological and physiological characteristics of brachiaria genotypes

Mean values for each measured trait of the different genotypes under the different stress treatments are presented in Table 3.3. Under moderate stress, the shortest shoots were recorded in Mulato II (42.8 cm), Mulato (50.9 cm), and Cayman (56.5 cm), whereas CIAT 679 recorded the longest shoots with 111.9 cm, followed by ILRI 11553 (92.7 cm) and Xaraes (90.6 cm). The lowest ranking genotypes under severe stress conditions were ILRI 13648 (56.2) and Mulato II, while CIAT 679 maintained its rank having the longest shoots (160.7 cm). Although there was a general decrease in shoot length due to drought stress in comparison with control plants, Basilisk, CIAT 679, and ILRI 13545 continued to grow despite the moderate drought conditions.

Leaf area of all the genotypes was reduced under conditions of both moderate and severe stress (Table 3.3). There was a clear variation in leaf area with Xaraes having the largest leaves (100.2 cm²) followed by Piata (75.2 cm²) and ILRI 13810 (71.3 cm²). The smallest leaf areas were recorded for CIAT 679 (14.5 cm²) and ILRI 14807 (34.6 cm²). Under severe stress, Xaraes maintained its rank with a leaf area size of 123.3 cm² followed by Mulato II (74.7 cm²) and Piata (72.7 cm²). The smallest leaves were observed in ILRI 13344 (29.4 cm²) and CIAT 679 (30.7 cm²).

The highest number of tillers per plant under moderate stress conditions was recorded in ILRI 13545 (16.0) (Table 3.3). CIAT 679 and Marandu had the lowest numbers of 5.0 and 6.0 tillers, respectively. Basilisk, Piata, Mulato II, ILRI 11553, ILRI 12995, ILRI 13648, CIAT 679 and ILRI 13497 produced more tillers when exposed to moderate stress than to severe stress. However, growth under severe drought conditions for 28 days resulted in reduced tillering in all genotypes. ILRI 13497 (5.0) and CIAT 679 (6.0) had the lowest number of tillers while the high-ranking counterparts were ILRI 13368 (17.7), ILRI 13545 (16.3) and Mulato II (14.7).

Stressed plants under both moisture stress regimes generally recorded lower values of estimated chlorophyll content (SCMR index) than control plants (Table 3.3). At moderate stress levels, the highest SCMR index values were recorded in Mulato (48.7). CIAT 679 had the lowest SCMR values of 28.3 under moderate stress conditions. Genotypes that recorded the highest SCMR values under severe water stress were ILRI 14807 (45.6), Piata (41.4) and Mulato (41.4), whereas ILRI 13545 (28.3) had the lowest.

Generally, lower values were recorded for relative water content in moisture stressed plants than in control plants under both moisture stress regimes (Table 3.3) of the leaves, but the levels of reduction varied with the genotypes. Among the genotypes that recorded high relative water content under moderate stress were ILRI 13344 (86.0), ILRI 13648 (85.9) and ILRI 13545 (85.5). On the other hand, ILRI 14807 and Basilisk were among those that recorded low relative water content values of 68.6 and 68.9, respectively.

Severe drought generally caused low relative water content in all the genotypes, with ILRI 13648 (57.8), ILRI 13497 (49.9), ILRI 13368 (21.5) and Mulato II (22.7) recording the lowest relative water content (Table 3.3). Among the high biomass yielders at moderate stress levels were ILRI 13368 (18.1 g), ILRI 12995 (18.0 g) and Piata (17.9 g). Under severe stress conditions, all the tested genotypes recorded lower biomass yield compared to their counterparts under no water stress. Nonetheless, outstanding genotypes with regard to biomass yield under severe drought stress were Piata (30.7 g) and Xaraes (30.0 g), closely followed by ILRI 13368 (26.7 g).

3.4.3 Correlation analysis between traits

Correlation coefficients indicating the relationships between the measured variables of the different genotypes exposed to stress conditions are presented in Table 3.4. Under moderate stress, the only significant correlation was a negative relationship between SCMR index values (chlorophyll content) and shoot length (r = -0.6, $P < 0.05$). Under severe stress, there was a significantly positive correlation between biomass yield and leaf area (r = 0.5, $P < 0.05$) and a significant negative correlation between relative water content and the number of tillers per plant (r = -0.6, $P < 0.05$). Correlations between other traits under both stress regimes were however not significant.

3.4.4 PCA analysis based on drought tolerance indices (DSI) values

Principal component analysis (PCA) based on DSI values of the traits were plotted in a Biplot to better understand the relationships among the drought stress indices and the levels of drought tolerance exhibited by different brachiaria genotypes (Fig. 3.1). The PCA converted the traits into six different factors and Eigen values. Under moderate stress, factor 1 accounted for 33.80% of the variation and showed the largest loading values, followed by factor 2 with 23.58%. Under severe drought stress, factor 1 accounted for 35.86% of the variation while factor 2 accounted for 25.09%.

The relationships between indices are illustrated by the axis. The cosine of the angle between the vectors of two indices approximates the correlation coefficients between them, which reflects on the interrelationships among the morpho-physiological indices. An acute angle depicts a positive correlation, while obtuse angle shows a negative correlation. The projection of the traits from the biplot origin shows the impact of that trait on separation of the genotypes. The biplot of DSI traits under moderate stress (Fig.3.1a) shows no outstanding trait that separated the genotypes. However, under severe stress (Fig.3.1b), biomass yield made the largest contribution since it had the largest projection. With regard to genotypic performance under drought conditions, the 18 brachiaria grasses were categorized into three groups. The best performers are indicated in group I (Fig.3.1b; upper right quadrant), intermediate performers in group II (Fig.3.1b; lower right quadrant), while the poor performers (group III) grouped in the upper and lower left quadrants. Under moderate stress conditions, ILRI 13648, ILRI 11553 and Mulato emerged as the best performers, while the performance of CIAT 679, Piata, Xaraes, Mulato II, ILRI 13497 and Basilisk can be described as intermediate. Under severe drought stress, the best performers were Xaraes, Piata, CIAT 679, Marandu, Mulato II, and Mulato while the intermediate performers were ILRI 14807, Basilisk, ILRI 12995, ILRI 13344 and Cayman.

3.5 Discussion

Drought stress is one of the most important factors that limit plant growth and reproduction. Although C4 grasses such as brachiaria show great adaptability to water stress conditions, water availability is still critical in determining the productivity of such grasses and wide variability has been found in their response to prolonged periods of drought (Wedin, 2004). The results of our study showed that drought stress had marked effects on morphological (shoot length, leaf area, number of tillers and biomass yield) and physiological (relative water content and chlorophyll content) traits of brachiaria grasses. Under moderate drought stress, the observed lower values in shoot length and number of tillers were not significant, which implies that even under conditions of low stress, it is still possible to select drought tolerant genotypes based on all traits except shoot length and tillering.

Drought stress resulted in lower values of leaf area, though under moderate drought stress, the lower sizes could be largely ascribed to leaf rolling. Leaf rolling is a common symptom of drought stress and is an expression of leaf turgor and plant water content (Blum, 2011). Stomatal opening and closure responses to evaporative demand (usually higher at noon) and soil water content, lead to changes in leaf turgor (Martínez-Vilalta & Garcia-Forner, 2017). Results of this study which indicate smaller leaves as a result of drought stress are similar to those reported by Santos *et al*. (2013) for other brachiaria genotypes. Leaf expansion generally depends on leaf turgor, temperature, and assimilating supply for growth. Drought also suppresses leaf expansion by reducing photosynthesis (Rucker *et al.*, 1995). Chlorophyll content is a sensitive and easily measurable trait that could be used to screen for stress tolerance among genotypes (O’Neill *et al.*,2006). In our study, reduction in chlorophyll content due to water stress was evident. Studies on barley showed that chlorophyll content was significantly recued in plants exposed to drought stress (Zhao *et al.*, 2010). Even though crucial plant processes such as cell division and cell expansion are the earliest to be affected by water deficit (Dale, 1988), degradation of chlorophyll may arise due to sustained photo-inhibition and photo-bleaching (Long *et al.*, 1994; Yang *et al.*, 2006). There were no effects of the moderate drought on shoot length but under severe drought, the reduction was significant. Reduction in shoot growth due to drought stress was also reported in another brachiaria genotype, Marandu (de Araujo *et al.*, 2011). This reduction may be attributed to progressive water stress that result in reduced plant height which is attributed to a decline in the cell enlargement (Manivannan *et al.,* 2007) and other processes such as cell division and cell expansion (Dale, 1988). Leaf relative water content in drought stressed brachiaria plants declined significantly compared to values recorded in control plants. Similar findings from studies with brachiaria have also been reported by Guenni *et al*. (2002). Maintenance of relative water content is essential in provision of turgor for cell enlargement and growth in plants (Hsiao & Xu, 2000). Therefore, leaf relative water potential may serve as an indicator of plant water status, as well as the ability of a plant to maintain adequate water status which improves drought adaptability by enhancing drought tolerance (Altinkut *et al.,* 2001; Keles & Öncel, 2004).

Moreover, emphasis has been put on responsiveness of relative water content to drought stress and its reliability in distinguishing drought tolerant and susceptible genotypes (O'Neill *et al.*, 2006). Moderate drought stress was not sufficient to affect tillering since there was no significant difference between the numbers of tillers of plants under moderate stress and those of the control. Following severe stress, the number of tillers were significantly reduced. These results confirm those of El-Rawy & Hassan (2014) who observed a reduced number of tillers in wheat (*Triticum aestivum*L. subsp. *aestivum*) in response to drought. According to de Barros Lima *et al*. (2011), reduced tillering in plants exposed to water deficit conditions mainly occurs due to the low immediate availability of nutrients for growth, because the nutrients are taken up by plants through the soil water solution. Plants subjected to drought stress, on the other hand, showed a significant decrease in above-ground biomass accumulation. Evidently, the severity of the adverse effects of drought stress on growth varied among the genotypes. The notable effect of drought stress on biomass production of brachiaria has been reported in previous studies which largely indicate that drought stress reduces plant growth in brachiaria genotypes (Guenni *et al.*, 2002; de Araujo *et al.*, 2011; Santos *et al.*, 2013; Cardoso *et al.*, 2015).

Because of the large genotypic variability in the studied traits of brachiaria genotypes in both non-stressed (control) and drought stressed plants, it is often difficult to assess drought tolerance of large germplasm collections, based only on data collected from drought stressed experiments. The DSI has therefore been used for example to evaluate the effect of drought stress on individual germplasm genotypes based on the difference between drought stress treatments and control plants of switchgrass (*Panicum virgatum* L. Switch grass) (Liu *et al*., 2015) and common bean (*Phaseolus vulgaris* L. (Fabaceae) (Darkwa *et al*., 2016). This approach removes the effect of germplasm variation from the drought stress evaluation and can therefore be used to assess a large collection of germplasm simultaneously (Wójcik-Jagła *et al.,* 2013; Liu *et al.,* 2015).

The PCA biplots based on DSI values for each parameter grouped the genotypes and showed the relative contribution of different parameters in separating the genotypes based on the projection of the traits from the biplot origin. This was sufficient to evaluate the genotypes taking into consideration all the traits that were evaluated. This study showed that biomass yield is a sensitive indicator of drought tolerance under severe drought stress since it produced the largest projection. Under moderate stress conditions, ILRI 13648, ILRI 11553 and Mulato emerged as the best performers, while intermediate performers were CIAT 679, Piata, Xaraes, Mulato II, ILRI 13497 and Basilisk. Under severe drought stress, the best performers were Xaraes, Piata, CIAT 679, Marandu, Mulato II, and Mulato, signifying that these genotypes are more tolerant to drought conditions. Biomass production can also be used as an accurate discriminator between drought tolerant and susceptible genotypes, with the two genotypes, Piata and Xaraes, ranking the highest with 30.7 and 30.0 g biomass, respectively, under severe stress conditions. Despite a very low biomass yield of only 11.0 g, CIAT 679 was less affected by drought conditions. This is ascribed to its slow growing nature and high water-use efficiency that allows it to survive longer under conditions of prolonged drought (Kalopos *et al.*, 1996; Guenni *et al.*, 2002).

3.6 Conclusion

There was a wide variation in drought tolerance of the brachiaria genotypes examined in this study. Based on DSI values for the morphological and physiological parameters and PCA biplots, we conclude that genotypes Xaraes, Piata, CIAT 679, Marandu, Mulato II, and Mulato were similarly and more drought tolerant under severe drought stress. Piata and Xaraes produced the highest biomass yield and outcompeted the popular commercial variety Mulato II. We therefore highlight the fact that these genotypes can be regarded as comparatively drought tolerant and that they would suffer lower yield penalties in arid and semi-arid areas that experience frequent and severe drought conditions. Their apomictic nature enables that true to type seeds are produced and utilized by farmers without losing the vigor of the plant. Utilization of these genotypes would renew confidence in cereal-livestock productivity through management of stem borers in smallholder farming systems in sub-Saharan Africa amid the increasing threat of climate change.

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Plate 3.1 Brachiaria genotypes grown under controlled conditions in a screenhouse

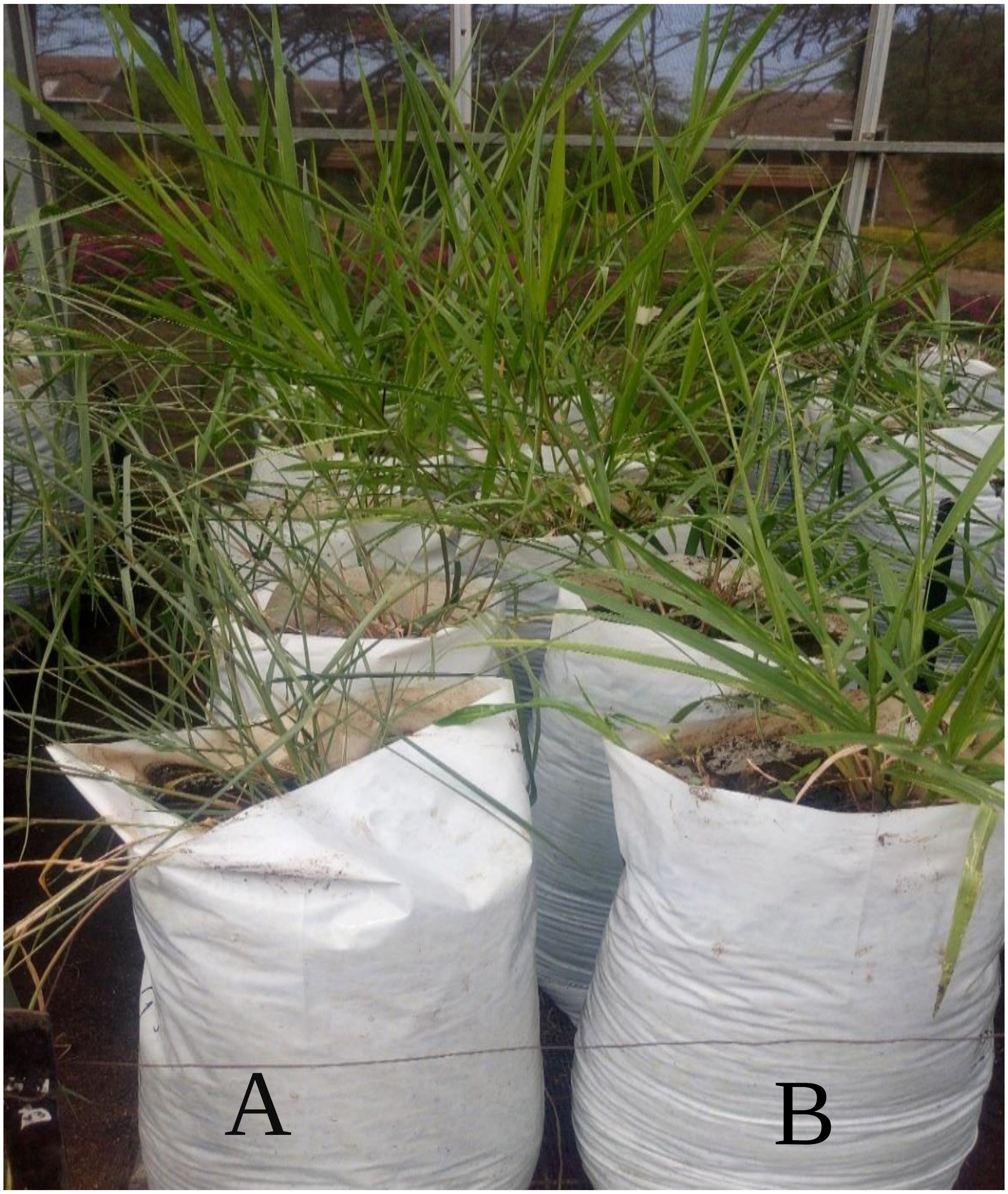

Plate 3.2 Stressed (A) and well-watered (B) plants under a moderate drought stress treatment

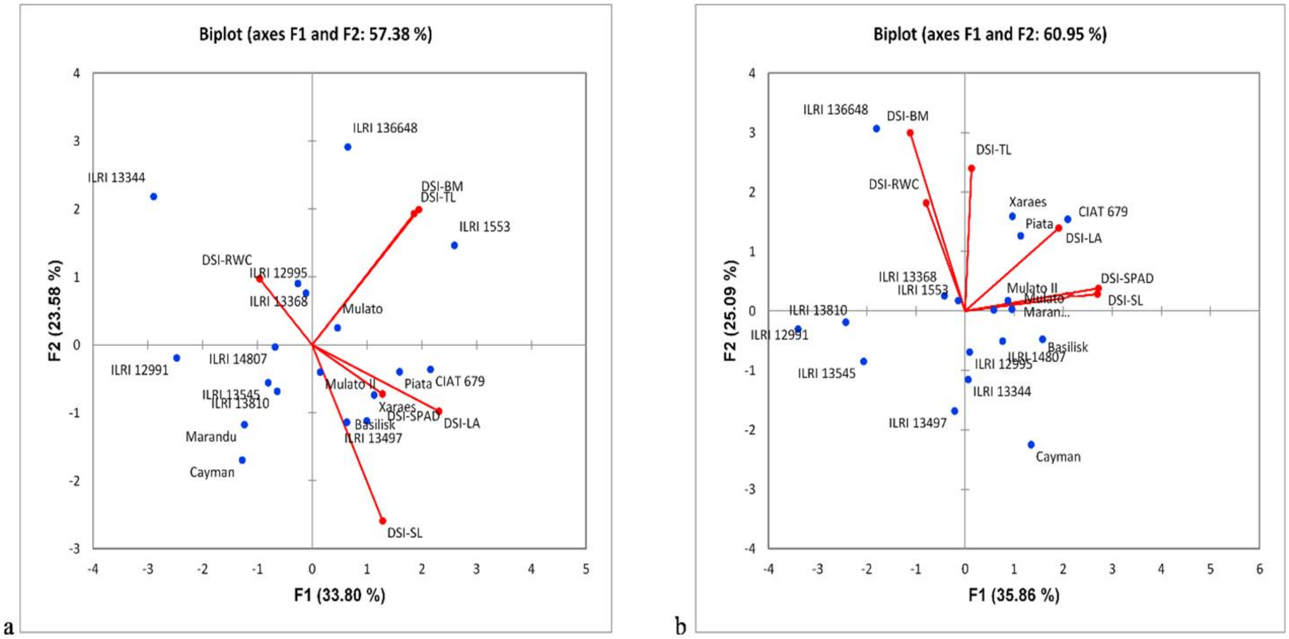

Figure 3.1 Principle component analysis biplot of the DSI of five physiological and morphological traits of 18 brachiaria genotypes under a) well-watered (control) vs moderate (14 days) drought stress and b) well-watered vs severe (28 days) drought stress. The axes represent the traits with various length based on the impact of each trait on the discrimination between genotypes. The 18 brachiaria grasses were categorized into three groups. Based on the DSI values, the best performers are in group I (upper right) and intermediates are in group II (lower right) while the poor performers (group III) are in both the upper and lower left. DSI, drought stress index; BM, biomass; LA, leaf area; SCMR, SPAD chlorophyll meter index; SL, shoot length; TL, tillers.

Table 3.1 Brachiaria genotypes that were evaluated for their response to moisture stress conditions in a screenhouse

Entry	Source	Genotype no.	Genotype	Variety name
1	CIAT	606	*B. decumbens*	Basilisk
2	CIAT	1752	*B. ruziziensis x B. decumbens x B. brizantha*	Cayman
3	CIAT	6294	*B. ruziziensis x B. decumbens x B. brizantha*	Marandu
4	CIAT	16125	*B. brizantha*	Piata
5	CIAT	26110	*B. brizantha*	Xaraes
6	CIAT	36087	*B. brizantha*	Mulato II
7	ILRI	11553	*B. brizantha*	
9	ILRI	12991	*B. brizantha*	
10	ILRI	12995	*B. brizantha*	
11	ILRI	13344	*B. brizantha*	
12	ILRI	13368	*B. brizantha*	
13	CIAT	679	*B. humidicola*	
14	ILRI	13497	*B. brizantha*	
15	ILRI	13810	*B. brizantha*	
16	ILRI	13545	*B. brizantha*	
17	ILRI	14807	*B. brizantha*	
18	CIAT	36061	*B. brizantha x B. ruziziensis*	Mulato

Table 3.2 Significance of treatment, genotype and genotype-treatment effects for traits in 18 brachiaria genotypes grown under moderate and severe drought stress conditions in screenhouse

	Treatment (T)	Genotype (G)	G X T	CV		Average		Minimum		Maximum		
Trait	(df=1)	(df=17)	(df=17)	(%)	R^2	Stress	Control	Stress	Control	Stress	Control	Reduction (%)
Moderate stress												
Shoot length (cm)	ns	**	ns	12.6	83	77.5^{a}	80.6^{a}	35.6	39.9	125.6	136.5	3.8
Leaf surface area (cm^2)	**	**	ns	18.3	75	55.3^{a}	64.0^{b}	14.5	15.0	100.2	106.5	13.7
Tiller number	ns	**	ns	20.5	76	9.2^{a}	9.3^{a}	3.0	4.0	17.0	20.0	1.1
SPAD readings	**	**	ns	11.8	61	36.0^{a}	38.5^{b}	23.5	27.3	51.0	50.9	6.2
Relative water content	**	**	**	8.4	58	77.0^{a}	81.7^{b}	50.7	61.6	93.8	97.6	5.1
Biomass (g)	*	**	**	20.9	86	10.6^{a}	11.6^{a}	2.5	3.1	20.2	25.3	8.6
Severe stress												
Shoot length (cm)	**	**	ns	11.6	88	83.9^{a}	98.1^{b}	56.2	66.7	154.4	160.7	14.4
Leaf surface area (cm^2)	**	**	ns	25.1	74	48.7^{a}	61.5^{b}	29.4	35.5	123.2	128.2	20.9
Tiller number	**	**	*	23.0	83	10.6^{a}	17.2^{b}	5.0	7.3	17.7	30.7	38.0
SPAD readings	**	ns	ns	19.1	44	38.6^{a}	44.7^{b}	28.3	34.1	45.6	58.8	13.7
Relative water content	**	**	*	17.0	89	34.8^{a}	78.6^{b}	21.6	63.8	57.8	89.3	55.8
Biomass (g)	**	**	ns	23.0	79	18.5^{a}	29.7^{b}	9.7	14.7	30.7	41.6	37.8

*Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$, Abbreviations: ns, non-significant. Means followed by the same letters within a row are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 3.3 Means of traits in control and drought stressed brachiaria genotypes grown under moderate drought stress and severe drought stress under screenhouse conditions

	Shoot length (cm)		Leaf area (cm^2)		Number of tillers		SCMR index		Relative water content		Biomass (g)	
Accession	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Moderate drought stress												
Basilisk	59.1	61.0	73.4	66.0	9.3	10.7	36.7	35.5	73.7	68.9	7.5	5.3
Cayman	57.0	56.5	78.6	65.1	7.3	4.3	44.1	40.8	87.5	78.0	12.7	7.3
Marandu	64.0	61.8	78.7	69.2	10.0	6.0	40.3	38.1	77.2	76.4	21.2	12.3
Piata	80.7	79.8	80.3	75.2	7.67	8.0	39.4	39.3	77.8	75.0	14.2	17.9
Xaraes	91.9	90.6	106.5	100.2	10.0	10.0	34.4	33.6	87.3	82.7	14.1	13.8
Mulato II	43.9	42.8	66.6	57.8	10.7	11.0	35.1	33.6	88.2	83.3	16.4	13.0
ILRI 11553	96.5	92.7	53.9	50.7	8.7	11.3	33.4	32.9	81.6	80.2	7.3	15.0
ILRI 12991	92.8	90.2	61.9	44.0	10.7	9.3	37.3	34.3	88.0	85.5	17.6	7.0
ILRI 12995	86.1	82.9	48.9	38.0	9.3	10.3	35.7	33.9	77.1	74.4	14.1	18.0
ILRI 13344	111.8	87.4	72.9	56.9	12.0	10.0	39.1	35.3	86.9	86.0	19.4	14.5
ILRI 13368	86.7	84.5	59.2	50.0	12.0	10.7	36.6	33.0	75.2	73.4	10.9	18.1
ILRI 13648	93.4	82.2	64.2	53.2	6.3	10.0	39.3	35.2	93.6	85.9	6.7	9.9
CIAT 679	109.4	111.9	15.0	14.5	4.3	5.0	31.6	28.3	81.5	70.5	4.0	6.2
ILRI 13497	90.0	78.3	61.3	53.0	6.3	7.0	36.2	34.5	87.0	70.9	6.6	3.7
ILRI 13810	89.2	88.6	80.7	71.3	8.3	8.0	42.0	37.6	80.6	77.6	9.4	5.1
ILRI 13545	68.6	69.6	42.1	37.2	17.3	16.0	39.7	34.6	85.7	85.5	11.2	7.8
ILRI 14807	87.4	83.4	41.3	34.6	8.0	7.7	41.7	40.2	68.6	68.6	9.8	7.1
Mulato I	52.4	50.9	67.0	57.9	10.0	9.7	50.1	48.7	73.3	72.2	6.4	8.7
LSD (5%)	17.5	14.8	20.0	16.3	3.1	3.3	6.9	7.5	10.1	13.2	4.4	3.0
CV (%)	13.1	11.5	18.8	21.6	20.0	21.6	10.8	12.5	7.4	10.2	22.7	17.0
Severe drought stress												
Basilisk	97.2	87.5	61.6	49.1	20.3	17.0	38.8	38.8	81.9	30.4	31.3	12.7
Cayman	77.9	67.5	70.1	56.7	18.0	7.0	39.8	39.7	73.9	25.1	27.3	9.7
Marandu	78.8	68.7	61.2	49.4	13.0	9.0	40.6	37.6	77.9	38.9	25.0	13.3
Piata	102.1	94.5	80.8	72.7	10.0	7.0	44.1	41.4	88.7	35.5	38.3	30.7
Xaraes	99.1	89.2	128.2	123.2	17.7	13.7	40.4	36.8	89.3	46.0	41.7	30.0
Mulato II	66.7	57.0	46.9	40.2	19.3	14.7	40.9	39.4	63.8	22.7	33.3	19.7
ILRI 1553	111.2	87.8	50.5	41.0	16.3	12.7	40.0	36.9	77.9	29.6	36.3	22.0
ILRI 12991	108.1	77.9	83.4	36.0	13.3	8.3	54.7	39.9	76.8	37.1	29.7	23.0
ILRI 12995	99.0	86.2	50.0	38.2	23.0	9.0	45.6	40.9	72.2	40.3	25.0	14.3
ILRI 13344	100.6	91.4	46.9	29.4	22.7	14.3	49.0	40.0	83.0	23.0	33.7	19.3
ILRI 13368	116.0	100.1	50.9	34.3	24.7	17.7	44.3	37.9	75.0	21.5	33.7	26.7
ILRI 13648	71.0	56.2	68.7	59.5	9.3	7.0	45.0	34.9	68.4	57.8	16.7	15.3
CIAT 679	160.7	154.4	35.5	30.7	7.3	6.0	34.1	37.0	84.8	36.3	14.7	11.0
ILRI 13497	106.3	93.9	50.5	36.0	17.0	5.0	49.3	39.6	86.1	49.9	33.3	15.0
ILRI 13810	109.1	83.3	60.7	36.5	15.0	11.7	58.7	38.5	86.2	34.9	17.7	11.7
ILRI 13545	77.2	57.2	40.0	35.3	30.7	16.3	51.7	28.3	82.4	30.2	36.3	21.3
ILRI 14807	114.0	97.3	39.5	33.4	17.0	6.0	44.6	45.6	76.0	36.0	30.7	19.7
Mulato I	70.7	60.3	81.7	74.7	14.7	9.3	43.1	41.4	73.6	30.3	30.0	17.3
LSD (5%)	16.7	16.3	27.3	14.9	6.4	4.4	13.1	13.6	13.2	18.9	11.1	7.6
CV (%)	10.3	11.7	27.1	18.5	22.5	25.1	17.7	21.1	10.1	32.7	22.4	24.9

Table 3.4 Simple correlation coefficients between morphological and physiological traits of 18 brachiaria grass genotypes evaluated under moderate stress (upper diagonal) and severe stress (lower diagonal) conditions

		Moderate stress					
		Shoot length	Leaf area	Tillers	SCMR index	Relative water content	Biomass
Severe stress	Shoot length		-0.303	-0.120	-0.571*	0.004	-0.319
	Leaf area	-0.195		-0.037	0.303	0.200	0.207
	Tiller numbers	-0.203	-0.001		-0.162	0.447	0.264
	SCMR index	0.211	-0.023	-0.420		-0.216	-0.132
	Relative water content	0.003	0.320	-0.558*	-0.098		0.212
	Biomass	-0.011	0.519*	0.302	-0.025	-0.070	

*Significant at $P \leq 0.05$

CHAPTER FOUR

4.0 GENOTYPIC RESPONSE OF BRACHIARIA (*Brachiaria* spp.) TO SPIDER MITE (*Oligonychus trichardti*) (Acari: Tetranychidae) AND ADAPTABILITY TO DIFFERENT ENVIRONMENTS

4.1 Abstract

Grasses in the genus *Brachiaria* (*Urochloa*), commonly known as brachiaria, are grown as forage crops in sub-Saharan Africa, with some genotypes being used in management of insect pests. However, spider mite, *Oligonychus trichardti* Meyer (Acari: Tetranychidae), has recently been reported as a major pest of *Brachiaria* spp. in the region. We evaluated 18 brachiaria genotypes to identify sources of resistance to *O. trichardti*, and to determine their adaptability to different environments in western Kenya. Response to artificial infestation with *O. trichardti* was evaluated under controlled conditions in a screenhouse while adaptability to different environments and field resistance to mites was evaluated in three locations for two cropping seasons in 2016 and 2017 under farmers' conditions. The parameters evaluated as indicators of resistance to pest damage included leaf damage, chlorophyll content reduction, plant height, leaf area, number of tillers and shoot biomass. Rainfall reduced mite infestation and increased biomass yield of the genotypes. Significant correlations between parameters were only observed between leaf damage and yield (r = -0.50, $P < 0.05$), and leaf damage and chlorophyll loss (r = 0.84, $P < 0.01$). The cultivar superiority index (*Pi*) ranked Xaraes, Piata, ILRI 12991 and ILRI 13810 as reliable genotypes that combined moderate resistance to the mite ($Pi \leq 48.0$) and high biomass yield ($Pi \leq 8.0$). Since this is the first documentation of interactions between *O. trichardti* and different brachiaria genotypes, we propose these genotypes as potential candidates for improved forage yields in areas prone to *O. trichardti* infestation in Africa.

Key words: adaptability, biomass yield, damage, host resistance, multi-locations

Publication

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4.2 Introduction

Brachiaria (*Urochloa*) genotypes (Poacea, commonly referred to as brachiaria) are common forage crops native to Africa (Renvoize *et al*., 1996), and are extensively grown in tropical Latin America, Africa and South Asia (Phaikaew *et al*., 1997; Hare *et al*., 2015). There are over 100 species in this genus but only a few, such as *Brachiaria brizantha* (A. Rich.) Stapf (palisade grass), *B. ruziziensis* (R. Germ. & C.M. Evrad) (ruzi grass), *B. decumbens* Stapf (signal grass), and *B. humidicola* (Rendle) Schweick (koronivia grass), have been commercially exploited as forage crops (Miles *et al*., 2004). In addition to its use as a pasture crop, *B. brizantha* cv. Mulato II, has been adopted in combination with greenleaf desmodium, *Desmodium intortum* (Mill.) Urb., in a climate-smart push-pull strategy for management of cereal stemborers, including *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), the main pests of maize, *Zea mays* L., in eastern Africa (Pickett *et al*., 2014; Khan *et al*., 2016). The technology involves intercropping maize with drought tolerant greenleaf desmodium, and planting Mulato II as a trap crop around this intercrop (Midega *et al*., 2015a,b). Greenleaf desmodium emits semiochemicals that are repugnant to the moths (push) while Mulato II emits attractive volatile organic compounds (pull). The pest is thus repelled from the maize crop and is subsequently attracted to the trap plant using a stimulo-deterrent strategy (Miller & Cowles, 1990; Midega *et al*., 2011; Khan *et al*., 2014). Additionally, brachiaria exhibits highly sophisticated responses to *C. partellus* herbivory that involves multitrophic interactions with some of its natural enemies (Bruce *et al*., 2010). The climate-adapted push-pull strategy thus effectively reduces infestations of stemborers, and in combination with other benefits such as suppression of the parasitic *Striga* weeds and improvements in soil fertility, result in significant increases in crop yields (Khan *et al*., 2014, 2016; Pickett *et al*., 2014; Midega *et al*., 2015b). The benefits of brachiaria as trap plant for *C. partellus* and forage crop are however limited by biotic and abiotic challenges associated with climate change (Maass *et al*., 2015).

Tetranychid mites are responsible for significant yield losses in many economically important crops. The most common species of spider mites in Kenya are *Tetranychus evansi* Baker and Pritchard, *Tetranychus urticae* Koch which attack solanaceous crops such as tomato (*Lycopersicon esculentum*), aubergine (*Solanum melongena*), potato (*S. tuberosum*) and tobacco

(*Nicotiana tabacum*) while rapidly expanding its host and geographic range (Tsagkarakou *et al*., 2007; Boubou *et al*., 2010; Toroitich 2011; Ferragut *et al*., 2013). Depending on the species, spider mites have a wide range of alternate hosts including wild grasses and broad-leafed plants (Meyer, 1987) where they can survive and infest the next crop. There is also a possibility of the mites surviving on remaining parts of cut stems, and plant residues in the field. Under extreme conditions, they diapause in the soil (Wilson, 1995). Phytopagous mites feed by piercing the leaf surface with their stylets and sucking out the cell contents (Tomczyk & Kropczynska, 1985) with a subsequent reduction in chlorophyll content and net photosynthetic rates of leaves (Park & Lee, 2005). High infestation levels mostly occur in hot and dry agro-ecological zones which is characteristic of most parts of sub-Saharan Africa. In these regions crop losses of up to 90% have been documented (Saunyama & Knapp, 2004). In sub-Saharan Africa, spider mites have recently been observed in brachiaria grasses and were reported as the main pest of these grasses (Maass *et al*., 2015; Njarui *et al*., 2016). Damage caused by the tetranychid mites on this forage crop is expected to increase in response to increasing climate change effects such as increasing temperatures and drought.

Pesticides are one of the control methods against these mites (Toroitich *et al*., 2014). However, their use, especially in small farming systems, may have human health implications and may lead to adverse environmental impacts which are often ascribed to incorrect and inappropriate use of pesticides, a common scenario in Africa (Mbakaya *et al*., 1994; Van den Berg & Nur, 1998; Ngowi *et al*., 2007; Azandémè-Hounmalon *et al*., 2015). The application of pesticides onto non-cash crops such as maize is also not common practice in Africa (Van den Berg & Nur, 1998; Orr & Ritchie 2004). Application of pesticides on grasses that are used as forage and in an eco-friendly management strategy for stalk borers, would therefore defy the general aims of integrated pest management (IPM) and specifically the push-pull strategy (Khan *et al*., 2016). Significant suppression of mite populations has been achieved through biological control approaches including the use of natural enemies such as *Phytoseiulus longipes* Evans (Ferrero *et al*., 2007,2011; Bugeme *et al*., 2015). Because of the challenges associated with their control and economic impact thereof, there is much interest in host-plant resistance as a management strategy since it is also compatible with other IPM strategies.

Host plant resistance to arthropod pests is influenced by the environmental conditions which further complicates testing and selection of superior genotypes. According to Eberhart & Russell (1966), a desirable genotype is one which has the highest yield across a broad range of environments. This principle is important in achieving good crop yields across an array of environments (Faris *et al*., 1979). The cultivar superiority index (*Pi*) (Lin & Binns, 1988) has been employed to evaluate genotypes for such adaptability to different environments. Regression analyses serve as a useful tool for measuring genotypic stability of resistance traits under conditions of varying biotic/abiotic stresses (Finlay &Wilkinson, 1963). Although the spider mite is known to be an important constraint to brachiaria cultivation, especially in sub-Saharan Africa, no study of interactions between *O. trichardti* and different brachiaria genotypes have been documented. Therefore, the present study was undertaken to identify potential sources of resistance to *O. trichardti* among brachiaria genotypes and to select candidate genotypes that are resistant to the mite and adaptable across different environments.

4.3 Materials and methods

4.3.1 Experimental plants

Seeds of brachiaria cultivars used in this study were sourced from International Center for Tropical Agriculture (CIAT), Cali, Columbia and the International Livestock Research Institute (ILRI), Ethiopia. The genotypes were grown in an on-station nursery at the International Center of Insect Physiology and Ecology - Thomas Odhiambo Campus (ITOC), Mbita Point (0º25′ S, 34º12′ E; 1200 m above sea level) in Kenya for preliminary observation and selection based on agronomic performance.

The 18 genotypes that were evaluated in this study are listed in Table 4.1. A commercial and locally adapted hybrid, Mulato II, was included as a control variety. This variety is preferred by smallholder farmers in sub-Sahara African as animal fodder (Khan *et al*., 2014). Additionally, Mulato II plays a major role in the 'push-pull' habitat management strategy due to its phytochemical properties that make it highly attractive to stemborer oviposition (Midega *et al*., 2011;2015a). Mulato II was however observed to be highly susceptible to spider mites especially in hot and drier ecologies (Maass *et al*., 2015). Spider mite samples were collected from a susceptible genotype Mulato II grown in field experiments in ITOC-Mbita, Siaya and Homabay. The samples were identified as *Oligonychus trichardti* Meyer at the Arachnology unit (Biosystematics Division), Plant Protection Research Institute, South Africa.

4.3.2 Screenhouse experiments

Susceptibility of brachiaria genotypes to *O. trichardti* was evaluated by artificially infesting plants under screenhouse conditions (25 °C, 65% r.h., and L12:D12) at ITOC. Propagules of 18 brachiaria genotypes were planted individually in plastic pots filled with soil and placed on 30 cm high benches covered with metal mesh. One plant was grown per pot. Phosphorus was applied at planting as di-ammonium phosphate (DAP) (60 kg ha^{-1}), while nitrogen was applied in the form of calcium ammonium nitrate (CAN) (60 kg ha^{-1}), two weeks later. The arrangement followed a complete randomized design (CRD) with three replicates. Plants were grown following standard agronomic practices and were artificially infested with mites two weeks after planting. Mites were obtained from the susceptible brachiaria variety Mulato II maintained in an on-station nursery at ITOC.

Infestation with *O. trichardti* was done by placing two fully infested leaves of Mulato II on the adaxial surface of the experimental plants. One on a youngest fully expanded and the other on second young fully expanded leaf of the plant. The damage on leaves was visually estimated 14 days after infestation using a modification of a rating score used by Hussey and Parr (1963), as described by Murungi et al. (2014). According to the 0-5 damage score, 0 = no damage, 1 = 1-19%, 2 = 20-39%, 3 = 40-59%, 4 = 60-79% and 5 = 80-100% of leaf surface exhibiting damage *i.e.*, the total plant leaf area showing chlorotic stippling or death caused by mite feeding. To assess chlorophyll content of plants, an average of 10 SPAD chlorophyll meter readings (SCMR) taken at regular intervals from the base to the tip of a second young fully expanded leaf was recorded. This was done by means of a portable chlorophyll meter SPAD-502 Plus (Konica Minolta Sensing Inc., Japan).

4.3.3 Field experiments

Agronomic performance of brachiaria genotypes under natural infestation of *O. trichardti* was assessed in three agro-ecological zones in western Kenya: Siaya (lower midland 2), Mbita (lower midland 5) and Homabay (lower midland 3), over two cropping seasons (Table 2). These are arid and semi-arid areas suitable for maize (*Zea mays*) and forage production but vary in rainfall distribution and soil characteristics. The sites are also relatively dry with extended periods of drought (Khan *et al*., 2014) and mite infestation.

For the first season at each site, propagules of uniform size, taken from mature plants were planted in plots of 1.5 x 1.5 m. This followed an alpha lattice design (6 rows x 3 columns) with three replications, at an inter row and inter plant spacing of 50 cm (16 plants per plot). Rows, columns and replicates were separated by a 1.5 m wide path. To serve as a source of mite infestation, three rows of Mulato II were planted around the experimental plot as spreader and guard rows. Phosphorus was applied as a basal application in form of di-ammonium phosphate (DAP) (60 kg^{-ha}) and nitrogen applied as top dresser two months after planting in the form of calcium ammonium nitrate (CAN) (60 kg^{-ha}). Recommended agronomic practices except pesticide application were followed to ensure good crop stand and growth. At three months after planting, four plants were randomly selected per plot and tagged for observations. The numbers of tillers (TL) per plant were counted. Plant height was determined by measuring the length of the tiller shoot from the soil surface to the tip of the youngest fully expanded leaf. Leaf area (LA) of the second fully expanded leaf was calculated by measuring its length from the tip to the junction of the petioles and the width at its widest part. Leaf damage was assessed on four plants in the middle rows of each plot by visual estimation of percentage of the total plant leaf area showing chlorotic stippling or death caused by mite feeding. To assess chlorophyll content reduction (CCR) due to leaf damage, an average of 10 SPAD chlorophyll meter readings (SCMR) taken at regular intervals from the base to the tip of a second young fully expanded injured leaf (IL) and non-injured leaf (NIL) were recorded. The percentage reduction in chlorophyll content was calculated as follows:

$$CCR(\%) = \frac{(NIL \times IL)}{NIL} \times 100$$

The above ground parts of the tagged plants were harvested and air-dried to between 12 and 14% moisture content which is recommended for making grass hay (Muck & Shinners, 2001). The biomass yield was then determined and expressed as per hectare values.

4.3.4 Data analysis

Analysis of variance (ANOVA) was performed on leaf damage score (0-5) to compare the resistance levels of the genotypes that were artificial infested with mites in the screenhouse. Field data were combined for genotypes, locations and cropping seasons using restricted maximum likelihood (REML) procedure and factor effects were tested using Wald chi-square tests REML. Genotypes, locations and cropping seasons were considered fixed terms whereas replications, rows and columns were considered random terms. Data on leaf damage percentage were arcsine transformed prior to analysis. Untransformed means are presented in the results. Treatment means were compared by means of least significant differences (LSD). Simple correlations were determined between plant traits using the combined means. Finlay & Wilkinson (1963) regression analysis and the cultivar-superiority measure (*Pi*) described by Lin & Binns (1988) were used to assess genotypic stability and overall reliability across environments (locations and seasons) as follows:

$$P_i = \sum_{j=1}^{n} (X_{ij} - M_j)^2/(2n),$$

where X_{ij} is the response of the *i*th genotype grown in the *j*th location; *M*j is the maximum response among all the genotypes in the *j*th location; *n* is the number of the environment.

Bi-plots were used to explore relationships between genotypes and/or environments. All analyses were done using the GENSTAT 14th edition statistical software programme.

4.4 Results

4.4.1 Responses of brachiaria genotypes to mite infestation under screenhouse conditions

There were significant differences ($P \leq 0.01$) between the degrees of damage observed on genotypes in the screenhouse (Table 4.3). Mean damage scores ranged between 0 (CIAT 679) and 4.7 on the susceptible check (Mulato II) (Table 4.4). Genotypes Piata, Xaraes, ILRI 13344 and ILRI 13810 showed low levels of damage (damage score = 1). Significant ($P \leq 0.05$) variation in chlorophyll content of damaged leaves was observed with ILRI 12991, CIAT 679, ILRI 13344, ILRI 13497 and Piata having higher values of SCMR (>36). A simple regression analysis revealed a significant ($P \leq 0.01$) linear and inverse relationship between leaf damage scores and SCMR (Y= -5.35 + 40.3, s.e = 1.25, R^2=24.7).

4.4.2 Agronomic performance of brachiaria genotypes under natural infestation of spider mites

Significant main effects ($P \leq 0.05$) of genotypes, locations and seasons were observed in all the traits evaluated. The two-way interaction between genotypes (G) and seasons (S) (GS) and genotypes by location (L) (GL) were also significant ($P \leq 0.05$). Means of the measured parameters across seasons and locations are provided in Table 4.4. The total damaged leaf area ranged between 0 (CIAT 679) to 17.5% (Mulato) while the susceptible check, Mulato II recorded a mean of 11.9%. Infestation by *O. trichardti* caused the highest chlorophyll content reduction (CCR) of 20.1% in leaves of Mulato II, while minimal effects of mite feeding were observed on chlorophyll content of leaves of Xaraes (1.6% CCR) and Piata (1.8% CCR). Plant height ranged between 89.4 cm (Mulato) and 141.6 cm (Piata), which was closely followed by ILRI 13368 (141.4 cm), ILRI 12991 (140.9 cm), Xaraes (140.7 cm), ILRI 1553 (135.5 cm) and ILRI 13497 (133.4 cm). The leaf area ranged between 11.7 cm^2 (CIAT 679) and 133.9 cm^2 (Xaraes), while the number of tillers varied between 67.1 (ILRI 13497) and 113.5 (Basilisk). High dry biomass yield under mite infestation were recorded in Piata (8.0 t ha^{-1}), Xaraes (6.2 t ha^{-1}), ILRI 14807 (6.8 t ha^{-1}), ILRI 12991 (7.0 t ha^{-1}) and Mulato II (6.1 t ha^{-1}).

A simple correlation analysis showed a significant negative correlation between percentage leaf damage ($P \leq 0.05$) and CCR ($P \leq 0.01$). Correlations between other traits were not significant at either of the *p* values (Table 4.5). Positive correlation was also observed between rainfall amount and biomass yield ($r = 0.82$; $P = 0.04$), while rainfall was negatively correlated with leaf damage ($r = - 0.76$; $P = 0.07$).

4.4.3 Stability analysis for spider mite resistance and yield

The significant ($P \leq 0.01$) three-way interaction indicated that plant biomass was highly dependent on genetic and environmental factors, including, but not limited to location, season and *O. trichardti* infestation. Means of the different genotypes in each environment (location and season) are presented in Table 4.6. Based on stability analysis of area of leaf damaged (%) ILRI 12991, Cayman, ILRI 13810, ILRI 12995 and ILRI 1553 were stable across locations and seasons (*b*-values close to 1). The highly sensitive genotypes to environmental variations were CIAT 679, Marandu, and ILRI 13368 which recorded lowest *b* values, and ILRI 13648 and Mulato which recorded highest *b* values (>1) (Table 4.7). Genotypes CIAT 679, Piata, Xaraes, ILRI 12991 and Marandu exhibited low cultivar superiority index values (*Pi*) ($Pi \leq 35.03$) for leaf damage. Stable genotypes (*b* values close to 1.0) in regard to dry biomass yield were CIAT 679, ILRI 13810 and Mulato II. The lowest values of *Pi* for biomass yield were observed in Piata, ILRI 12991, ILRI 14807 and Mulato II (*Pi* 0 to 5.42).

The relationships between mean dry biomass yield and area of leaf damaged (%) across environments are illustrated in a scatterplot (Fig. 4.1). Genotypes Piata, Xaraes, ILRI 12991, ILRI 14807, ILRI 13810, Marandu and Cayman had high biomass yields (>5.7 t ha^{-1}) and lower leaf damage (<8.4%). CIAT 679 was among the most stable genotypes across environments, however, it recorded low dry biomass yield (4.8 t ha^{-1}). The GE biplot presentation of average biomass yield of the different genotypes under natural infestation of mites across the six environments (cropping seasons and locations) is presented in Fig. 4.2. The first and the second principal components accounted for 67.6% of the total variation. Both Seasons 1 and 2 at Siaya (SY_SN1; SY_SN2), and both Seasons in Homabay (HB_SN1; HB_SN2) were separated by acute angles indicating the expression of these environments to be similar. All the genotypes recorded lower mean yields in these environments (Siaya and Homabay) (Table 4.6). Obtuse angles between season 1 in Mbita (MB_SN1) and all other environments except Mbita season 2 (MB_SN2) indicate negative correlations demonstrating that the genotypes ranked differently at

Mbita during season 2 (MB_SN1). In general, both SY_SN2 and MB_SN2 displayed the largest projections from the biplot origin, which implies that they played major roles in discriminating between the genotypes. Genotypes that performed comparatively better in specific environments, based on their proximity to the environments, were ILRI 13344 (MB_SN1), and ILRI 14807 (MB_SN2) (Fig. 4.2).

4.5 Discussion

Several brachiaria genotypes with considerable level of resistance to *O. trichardti* damage were identified in this study. Notably, genotypes responded differently in different environments which make it possible to select and recommend cultivation of specific genotypes for different agro ecological zones. The use of resistant genotypes is therefore a viable option for the management of *O. trichardti* in western Kenya, with possibility of use in other areas with similar agro-ecological conditions and farming systems in east Africa. Host plant resistance to arthropod pests has been reported as one of the most effective, economical and environment friendly strategies for pest management (Van den Berg & Nur, 1998; Sharma *et al*., 2015). Other than morphological characteristics, plants have sophisticated defense systems that make use of toxic or anti-feedant secondary metabolites. However, the defense systems vary between and within plant species (Franceschi *et al*., 2005; Mithoefer & Boland, 2012). This explains the variation of resistance to *O. trichardti* in our study with hybrid Mulato II being the most susceptible. Past studies have highlighted success in host-resistance of grasses to mites (Quisenberry, 1990). High to moderate resistant genotypes of bermuda grass (*Cynodon* spp.) to bermuda grass mite *Eriophyes cynodoniensis* Sayed (Acari: Eriophyidae), were identified by Johnson (1975). Similarly, a variety of zoysia grass (*Zoysia tenuifolia*) with high level of resistance to bank grass mite *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) was identified by Busey *et al*. (1982). Host plant resistance to mites has also been identified in maize (Kamali *et al*., 1989; Bynum *et al*., 2004).

To exploit host plant resistance as a management tool, exposure to pests and evaluation of plants of candidate crop varieties in endemic areas is a prerequisite, it contributes to identification of superior crop varieties. In our study, the importance of *O. trichardti* was evident. Regression analysis of damage scores and chlorophyll index of plants grown under screenhouse conditions revealed a negative correlation. Similar effects of spider mite damage were observed in cotton (*Gossypium hirsutum* L.) (Bondada *et al*., 1995). Notably, chlorophyll content of brachiaria is highly and positively correlated with crude protein content, an important nutritional quality of forage crops (Hughes *et al*., 2014). This shows that mite infestations could lower the nutritional value of plant biomass intended for animal feed. Of all the plants that were exposed to artificial infestation in the screenhouse, only one genotype (CIAT 679) was completely resistant to the mites while Piata, Xaraes, ILRI 13344 and ILRI 13810 recorded significantly lower levels of infestation with a damage score of 1 (10-19% of leaf damaged). This highlights the genetic variation in brachiaria and more so the existence of sources of resistance to mite pests.

Results of the GxE interaction (genotype by location and season) indicate genetic variation among brachiaria genotypes which could be exploited through selection based on genotypic resistance to *O. trichardti*, biomass yield and yield related traits (plant height, leaf area and the number of tillers). Leaf damage was notably lower in the field than in the screenhouse. This may be due to effects of the weather patterns on biology of the mites as previously reported for *Oligonychus coffeae*Nietner (Acarina: Tetranychidae) (Ahmed *et al*., 2012). Under hot and dry conditions, mites have a short life cycle and high reproductive potential (Ahmed *et al*., 2012). For example, the life cycle of *T. evansi* is completed in 13.5 days at 25 °C (Knapp *et al*., 2003). Re-infestation usually begins as soon as the crop regenerates and spreads faster within the plant. However, this is hampered when there is precipitation as the rain washes them off the leaves and creates unfavourable humid conditions. In the current study, at all locations, lower mite damage recorded during the previous season. Notably, average rainfall recorded over the entire cropping season was higher in season 2, than in season 1. The negative correlation between leaf damage and the amount of rainfall received, though non-significant, indicates that precipitation probably played a role in reducing mite severity. With increasing aridification due to climate change (Jones & Thornton, 2003; Fischer *et al*., 2005; Burke *et al*., 2009), the pest status of phytophagous mites may increase in future. A similar trend of variation in loss of chlorophyll due to mite feeding was observed in this study. Regression analysis showed that *O. trichardti* played a role in reducing biomass yield. Although correlation analysis showed that biomass yield also depended on the amount of rainfall, this was not always the case. For example, higher biomass yield was recorded at Mbita despite this locality receiving lower rainfall than Siaya. This highlights the role of environmental factors including soil fertility in crop growth and yield. In general, a few genotypes (Piata, ILRI 12991, Xaraes, ILRI 14807, Marandu and ILRI 13810) combined both resistance to *O. trichardti* (≤8.4% of leaf damaged) and high biomass yield (≥5.7 t ha^{-1}) across all environments (Fig. 4.1). Moreover, these genotypes recorded low cultivar superiority index values for leaf damage (%) (≤48.86) and biomass yield (≤8.21) (Table 4.7), indicating that they are reliable across diverse environments. Such genotypes are useful to farmers since they would provide comparative yield advantages under mite infestation in drier conditions which are common to arid and semi-arid environments. Despite of the potential of brachiaria in improving cereal-livestock based productivity in Kenya, it is constrained by high cost and limited availability of seed. This arises from inability of most flowers to form seed coupled with less effective harvesting methods. Phaikaew et al. (1997) reported that seed production in the humid lowland tropics near the equator is usually difficult. However, studies in Kenyan highlands have shown that high yielding brachiaria varieties, for example Xaraes, do produce seed although poorly (Gitari & Njarui, 2016; Kamidi *et al*., 2016).

4.6 Conclusions

In summary, results of our study highlight a wide variation in the levels of resistance to the *O. trichardti* and biomass yield potential of brachiaria genotypes evaluated in different environments. From this multi-trial screenings, genotypes Piata, ILRI 12991, Xaraes, Marandu and ILRI 13810 emerged as candidate genotypes for utilization by African farmers in different agro-ecologies where frequent outbreaks of *O. trichardti* are experienced. The apomictic nature of these genotypes presents an advantage to farmers since they can propagate the grasses without losing their vigour. To fully evaluate the value of such genetic materials, we propose that the candidate genotypes be evaluated in a farmer participatory approach. There is also a need to evaluate seed production of the candidate genotypes in diverse highland conditions and for more seasons to determine their actual potential.

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Plates

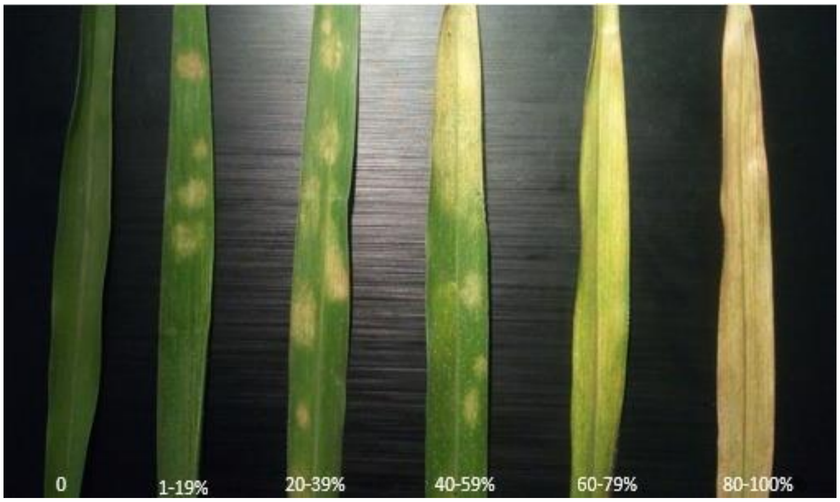

Plate 4.1 Leaf damage rating scale for damage caused by *Oligonychus trichardti* to *Brachiaria brizantha* cv Mulato II leaves

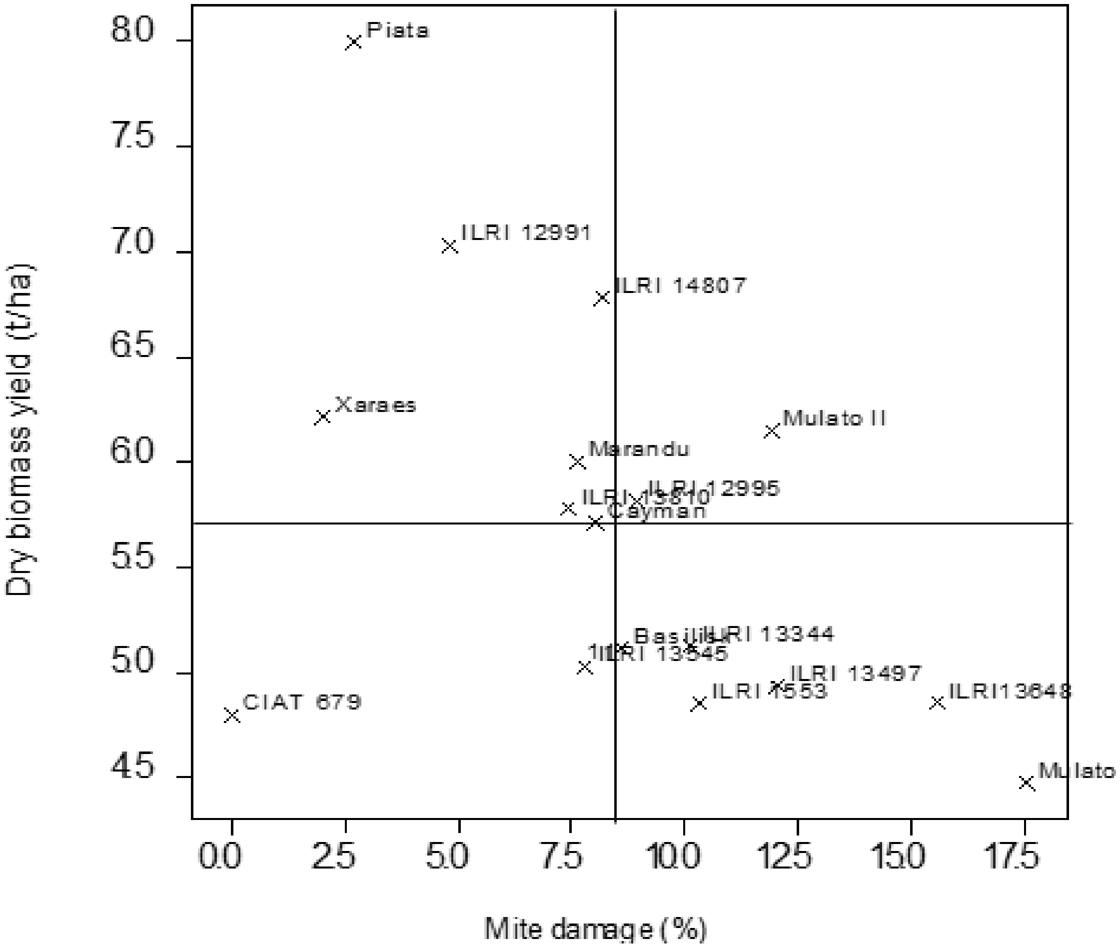

Figure 4.1 Expression of field resistance to the red spider mite *Oligonychus trichardti* in 18 brachiaria genotypes and their dry biomass yield potential over two seasons under natural infestation of *O. trichardti* at Mbita, Homabay and Siaya, Kenya

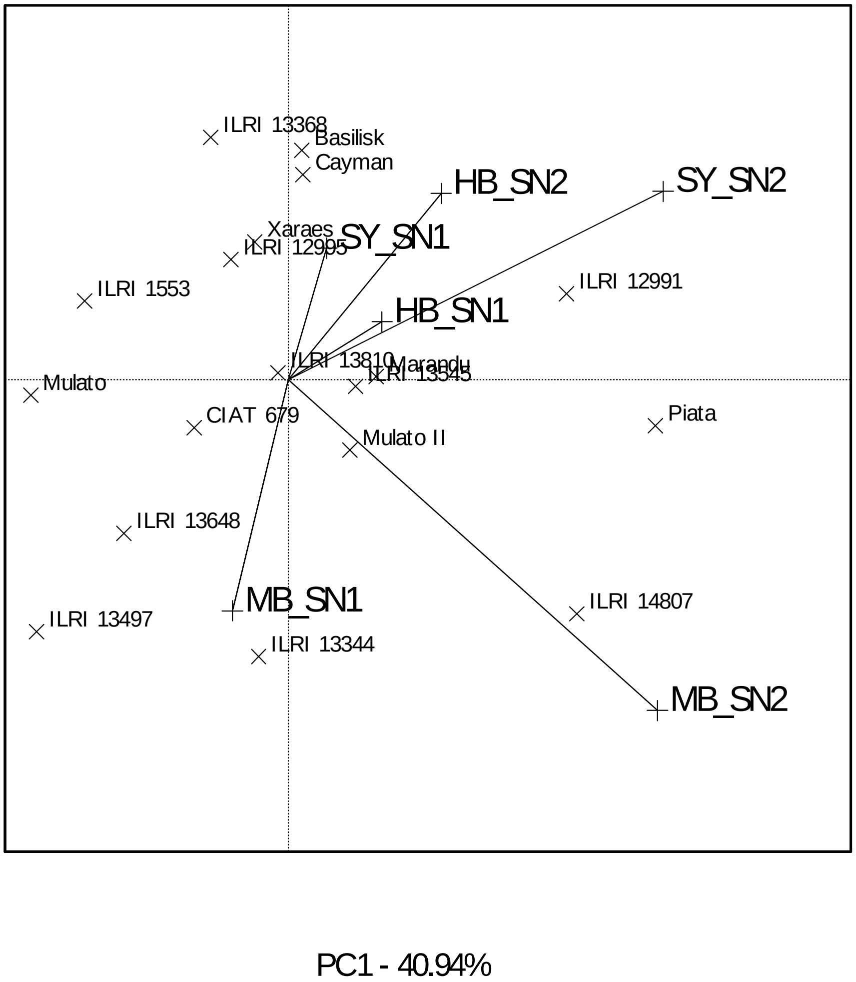

Figure 4.2 Principle component biplot of dry biomass yield for 18 genotypes of brachiaria grown over two seasons under natural infestation of *Oligonychus trichardti* at Mbita, Homabay and Siaya, Kenya. Genotypes are indicated by numbers and the treatments by vectors (HB = Homabay, SY = Siaya, MB= Mbita; SN=season).

Table 4.1 Brachiaria genotypes that were evaluated over two cropping seasons in three locations in Kenya.

Entry	Source	Genotype no.	Genotype	Variety name
1	CIAT	606	*B. decumbens*	Basilisk
2	CIAT	1752	*B. ruziziensis x B. decumbens x B. brizantha*	Cayman
3	CIAT	6294	*B. ruziziensis x B. decumbens x B. brizantha*	Marandu
4	CIAT	16125	*B. brizantha*	Piata
5	CIAT	26110	*B. brizantha*	Xaraes
6	CIAT	36087	*B. brizantha*	Mulato II
7	ILRI	11553	*B. brizantha*	
9	ILRI	12991	*B. brizantha*	
10	ILRI	12995	*B. brizantha*	
11	ILRI	13344	*B. brizantha*	
12	ILRI	13368	*B. brizantha*	
13	CIAT	679	*B. humidicola*	
14	ILRI	13497	*B. brizantha*	
15	ILRI	13810	*B. brizantha*	
16	ILRI	13545	*B. brizantha*	
17	ILRI	14807	*B. brizantha*	
18	CIAT	36061	*B. brizantha x B. ruziziensis*	Mulato

Table 4.2 Agro-ecological zones, coordinates, elevation and cumulative rainfall of three locations in Kenya at which 18 genotypes of brachiaria were evaluated over two cropping seasons.

Location	Agro-ecological zone	Coordinates	Elevation (m a.s.l.)	Season	Total rainfall (mm) during experiment period
Mbita	Lower midland 5	0°25'S, 34°12'E	1200	Season 1/2016	410.5
				Season 2/2017	1455.6
Homabay	Lower midland 3	0°52'S, 34°26'E	1302	Season 1/2016/17	127.1
				Season 2/2017	383.1
Siaya	Lower midland 2	0°23'N, 34°17'E	1319	Season 1/2017	565.0
				Season 2/2017	1039.0

Table 4.3 Anova table for chlorophyll damage on 18 genotypes of brachiaria evaluated under screenhouse conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates	2	218.1	109	0.57	
Genotypes	17	6507.6	382.8	1.99	0.043
Residual	34	6531.5	192.1		
Total	53	13257.2			

Table 4.4 Means of agronomic traits of 18 brachiaria genotypes evaluated in a screenhouse over two seasons under natural infestation of *Oligonychus trichardti* at Mbita, Homabay and Siaya, Kenya.

	Screenhouse		Field experiment					
Genotype	Damage score[a]	SCMR	Leaf damage (%)	CCR (%)	Plant height (cm)	Leaf area (cm^2)	Tillers	Dry biomass (t ha^{-1})
Basilisk	4.3g	14.6	8.6	6.4	111	44.1	113.5	5.12
Cayman	4.3g	26	8	7.2	110.4	69.1	116.8	5.71
Marandu	3.3ef	23	7.6	7.7	103.7	88.5	84.8	6
Piata	1.0b	35.3	2.7	1.8	141.6	121.9	81.6	8
Xaraes	1.0b	27.7	2	1.6	140.7	133.9	88.7	6.22
Mulato II	4.7g	4.2	11.9	20.1	97.4	75.4	106.2	6.15
ILRI 11553	2.3cd	27.2	10.3	13.5	135.5	68.1	81.2	4.85
ILRI 13648	4.0fg	20.9	15.6	11.5	123.6	84	77.8	4.86
ILRI 12991	1.7bc	46.5	4.8	9.3	140.9	54	90.8	7.03
ILRI 12995	3.0de	13.5	8.9	12.3	127.3	47.2	85	5.82
ILRI 13344	1.0b	44.9	10.1	9.9	115.5	67.7	83.6	5.13
ILRI 13368	3.0de	26.4	7.9	12.2	141.4	51.4	75.7	5.04
CIAT 679	0.0a	45.3	0	0	97.3	11.7	78.8	4.8
ILRI 13497	1.3b	36.3	12	15.2	133.4	62.4	67.1	4.94
ILRI 13810	1.0b	27	7.4	12.8	122.6	70.5	70.5	5.78
ILRI 13545	1.3b	29	7.8	9.1	107.4	50.2	87.4	5.03
ILRI 14807	1.3b	22.5	8.2	11.2	124.5	40.6	96.8	6.78
Mulato	4.3g	20.3	17.5	19	89.4	77.6	103.2	4.48
Mean	2.4	27.2	8.4	10	120.2	67.7	88.3	5.65
SE(±)	0.4	11.3	1.5	1.6	9.53	5.3	8.4	0.84
LSD	0.7	23.1	2	2.8	11.05	9.5	14	1.28

SCMR, SPAD chlorophyll meter readings; CCR, chlorophyll content reduction.

[a] Damage score 1 to 5 where, 0 = no damage, 1 = 1-19%, 2 = 20-39%, 3 = 40-59%, 4 = 60-79% and 5 = 80-100% of leaf area damaged

Means within columns followed by the same letter do not differ significantly at $P < 0.05$ (LSD).

Table 4.5 Correlation coefficients between measured parameters of brachiaria genotypes evaluated over two seasons under natural infestation of *Oligonychus trichardti* at Mbita, Homabay and Siaya, Kenya

	Leaf damage	Dry biomass	Plant height	Leaf area	CCR
Leaf damage					
Dry biomass	-0.498*	-			
Plant height	-0.3106	0.4221	-		
Leaf area	-0.004	0.4085	0.306	-	
CCR	0.8354**	-0.3168	-0.209	-0.1229	-
Number of tillers	0.1447	0.0965	-0.464	-0.0345	0.057

CCR, chlorophyll content reduction.
* significance $P < 0.05$, ** significance at $P < 0.01$

Table 4.6 Means of measured parameters of 18 brachiaria genotypes measured in six environments in Kenya

	Mbita season 1	Mbita season 2	Homabay season 1	Homabay season 2	Siaya season 1	Siaya season 2
Leaf damage (%)	14.7	5.1	12.5	9.0	6.7	2.6
CCR	19.4	6.2	16.0	10.4	5.8	2.4
Plant height (cm)	116.4	115.8	77.9	165.3	113.1	132.9
Leaf area (cm^2)	69.5	69.7	44.2	71.3	80.7	70.7
Number of tillers	32.7	130.6	23.7	95.8	59.8	187.2
Dry biomass ($t\ ha^{-1}$)	5.94	10.16	1.96	5.90	1.90	8.06

CCR, chlorophyll content reduction

Table 4.7 Genotypic means and stability for leaf damage and dry biomass yield of 18 brachiaria genotypes evaluated over two seasons under natural infestation of *Oligonychus trichardti* at Mbita, Homabay and Siaya, Kenya.

	Leaf damage			Dry biomass yield			
Genotype	Mean damage (%)	Regression equation	*Pi*	Mean yield (t ha^{-1})	Regression equation	*Pi*	Rank
Basilisk	8.6	Y = 0.35X + 31.82	48.84	5.12	Y = 0.88X + 4.22	11.83	14
Cayman	8.0	Y = 0.85X + 20.13	45.8	5.71	Y = 0.87X + 3.37	8.65	8
Marandu	7.6	Y = 0.19X + 17.12	35.03	6.00	Y = 1.11X + 0.58	6.08	5
Piata	2.7	Y = 0.35X + 3.77	6.04	8.00	Y = 1.37X + 0.80	0.94	1
Xaraes	2.0	Y = 0.34X + 5.90	5.07	6.22	Y = 0.66X + 2.44	8.21	7
Mulato II	11.9	Y = -0.34X + 1.82	72.72	6.15	Y = 1.09X + 0.56	5.42	4
ILRI 11553	10.3	Y = 0.86X + 28.71	69.92	4.85	Y = 0.72X + 2.08	12.55	16
ILRI 13648	15.6	Y = 3.92X + 19.81	272.69	4.86	Y = 0.92X + 3.16	11.47	13
ILRI 12991	4.8	Y = 0.91X + 3.93	20.78	7.03	Y = 1.26X + 2.42	3.71	2
ILRI 12995	8.9	Y = 1.13X + 19.65	58.57	5.84	Y = 0.69X + 1.25	9.06	10
ILRI 13344	10.1	Y = 0.75X + 30.15	66.66	5.13	Y = 1.27X + 3.25	9.01	9
ILRI 13368	7.9	Y = 0.16X + 19.18	37.51	5.04	Y = 0.64X + 3.64	12.78	17
CIAT 679	0.0	0	0.00	4.80	Y = 1.01X + 2.00	11.02	12
ILRI 13497	12.0	Y = 0.23X + 66.61	95.09	4.94	Y = 0.89X + 7.85	12.42	15
ILRI 13810	7.4	Y = 1.13X + 27.38	48.86	5.78	Y = 1.04X + 1.84	7.10	6
ILRI 13545	7.8	Y = 0.88X + 62.94	58.49	5.03	Y = 1.22X + 1.93	9.70	11
ILRI 14807	8.2	Y = 1.25X + 10.12	51.54	6.78	Y = 1.56X + 2.88	3.88	3
Mulato	17.5	Y = 3.04X + 42.44	255.17	4.48	Y = 0.77X + 1.98	13.71	18

Pi, Cultivar superiority index

CHAPTER FIVE

5.0 SUITABILITY OF BRACHIARIA GRASS (*Brachiaria* spp.) AS A TRAP CROP FOR MANAGEMENT OF *Chilo partellus*

5.1 Abstract

The spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is a major insect pest of sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.) in Africa. Trap cropping systems have been shown to be a valuable tool in management of this pest. To optimize trap cropping strategies, an understanding of host-plant preference for moth oviposition and host suitability for larval survival on potential trap plants is a prerequisite. Therefore, seven brachiaria genotypes were assessed for preference by *C. partellus* moths and subsequent larval performance. In two-choice tests with a local open pollinated maize (cv. Nyamula) variety, significantly higher numbers of eggs were oviposited on brachiaria genotypes Marandu, Piata and Xaraes than on maize, while fewer eggs were recorded on plants of Mulato II, Mulato and Cayman. There was a significant and negative correlation between the trichome density on plant leaves and *C. partellus* oviposition preference for brachiaria. In addition to poor larval performance on brachiaria, there was no clear ranking in the genotypes regarding larval orientation, settling, arrest and food ingestion and assimilation. First instar larvae did not consume leaf tissues of brachiaria plants but consumed those of maize, which also suffered more stem damage than brachiaria plants. While no larvae survived on brachiaria plant tissue for longer than five days, 79.2% of the larvae survived on maize. This study highlights the preferential oviposition of *C. partellus* on brachiaria plants over maize and the negative effects that these genotypes have on subsequent larval survival and development. Our findings support the use of brachiaria as a trap crop for management of *C. partellus* through a push-pull technology.

Key words: adult preference, larval performance, leaf trichomes, lepidopterous, trap cropping

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5.2 Introduction

Plants exhibit extensive variation in their suitability as hosts for insect herbivores (Journet, 1980; Service & Lenski, 1982; Weis & Abrahamson, 1986) and evolved a diverse array of adaptations to reduce the degree of damage caused by herbivores. These adaptations include direct defense mechanisms such as production of toxic secondary metabolites that kill or arrest development of herbivores and a number of structural defense systems (hairs, spines and thorns) (Dicke & van Poecke, 2002; Gouinguené & Turlings, 2002; Roda & Baldwin, 2003; Hanley *et al.*, 2007; Bukovinszky *et al.*, 2008; Poelman *et al.*, 2009). Variation in these adaptations can either be intraspecific (Degen *et al.*, 2004; Broekgaarden *et al.*, 2010) or interspecific (Ratnadass *et al.*, 2012). Nevertheless, these adaptations depend on the direction of interaction in the trophic level e.g. “bottom-up” effects of plant traits on higher trophic levels (herbivores and their natural enemies) and “top-down” effects of natural enemies on herbivores (Ratnadass *et al.*, 2012).

Insects, on the other hand, recognize and respond to host cues to find plant species on which they can feed and reproduce (Bruce *et al.*, 2005). Typically, host plant selection by insects is a multifaceted process involving visual and semiochemical stimuli (Atkins, 1980; Calatayud *et al.*, 2008; Finch & Collier, 2012). A number of theories explain the host selection process in herbivores, the most common being ‘mother knows best principle’ also known as the optimal oviposition theory (Thompson, 1988; Scheirs *et al.*, 2000; Johnson *et al.*, 2006) and classically as the preference–performance hypothesis (Jaenike, 1978). The theory is based on the concept that juvenile life stages have little opportunity to change their developmental location and therefore it is the mother’s duty to find a suitable host for their survival and development (Mayhew, 2016). On the other hand, females may select hosts based on factors influencing her own survival rather than that of the juvenile offspring (Nanthagopal & Uthamasamy, 1989). For both scenarios, studies are usually done to encompass ‘preference traits' that determine willingness to accept a host plant and 'performance traits' that encompass the ability of a juvenile to feed, grow, survive, and develop on the host plant.

Some plants emit secondary metabolites that directly mask the specific chemical cues that another's herbivores use to find their hosts, or harbour particularly effective natural enemies of one another's herbivores (Finch & Collier, 2012). This is one of the key concepts that has enabled utilization of such plants as trap crops in management of crop pests (Shelton & Badenes-Perez, 2006). Trap cropping was a common method of pest management in several cropping systems prior to introduction of modern synthetic pesticides (Thurston, 1984; Hokkanen, 1991). In Africa, intercropping and trap cropping systems for management of the cereal stemborers, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is gaining wide adoption (Khan *et al*., 2000, 2016, Midega *et al*., 2011, 2015; Pickett *et al*., 2014). Vetiver grass (*Chrysopogon zizanioides* (L.) Roberty=*Vetiveria zizanioides* (L.) Nash), napier grass (*Pennisetum purpureum*) and sudan grass (*Sorghum vulgare* Pers. var. *sudanense*) have also been shown to be effective trap crops in the management of stemborers (Khan *et al*., 2001; Haile & Hofsvang, 2002; Van den Berg, 2006a,b; Van den Berg & Van Hamburg, 2015).

Cereal stemborers are the most important pest constraints to sorghum and maize production in Africa, causing yield losses ranging between 10 and 88% (Kfir *et al*., 2002). Stemborer moths locate and oviposit on suitable hosts and their offspring feed inside the whorls of plants, damaging the leaves before entering the stem (Slabbert & Van den Berg, 2009). In east Africa, the estimated average annual yield loss due to stemborers is estimated at 13.5% (De Groote *et al*., 2011). Due to the cryptic and nocturnal habits of the adult moths and the burrowing behavior of larvae into the host's stem, effective control of the pest has proved difficult (Ampofo *et al*., 1986). Chemical control is one of the strategies employed to control borers and reduce damage, however, it is largely uneconomical and impractical for resource-poor farmers in sub-Saharan Africa (SSA), besides posing environmental and health hazards if not used carefully or without proper safety measures (Van den Berg & Nur, 1998). A "stimulo-deterrent diversion" or push-pull strategy, based on a combination of a trap crop (pull component) with a repellent intercrop (push component) (push-pull) has allowed farmers to successfully suppress stem borer numbers on maize and sorghum in SSA (Khan *et al*., 2000, 2001, 2012; Midega *et al*., 2015). The trap plant releases semiochemicals that attract stem borer moths while volatiles from the push crop further enhance the effectiveness of the trap crop by repelling moths away from the main crop (Cook *et al*., 2007; Khan *et al*., 2010).

The genus *Brachiaria* is an extensively grown grass pasture crop in tropical Latin America and Africa (Keller-Grein *et al*., 1996; Cardona *et al*., 2004). There are over 100 species in this genus but only a few have been commercially exploited (Miles *et al*., 2004). In addition to use as a pasture crop, *Brachiaria brizantha* cv. Mulato II has been adopted in combination with greenleaf desmodium in a climate smart push-pull strategy (Pickett *et al*., 2014; Khan *et al*., 2016). This combination of crops displayed beneficial effects in reducing infestations of stemborers as well as that of the parasitic weed, *Striga hermonthica*, resulting in a net increase in crop yield (Khan *et al*., 2014, 2016; Pickett *et al*., 2014; Midega *et al*., 2015).

Research has shown that signal grass, *B. brizantha*, is preferred to maize for egg laying by *C. partellus* moths (Midega *et al*., 2011). Additionally, the cultivar Mulato II supports minimal feeding and survival of *C. partellus* larvae (Midega *et al*., 2011). Moreover, this variety exhibits a sophisticated response to stemborer herbivory that involves volatile mediated multi-trophic interactions with natural enemies (Bruce *et al*., 2010). Intra-specific genetic variation in volatile composition as well as release rates of these volatiles do however exist in cereal crops, for example in rice (Rapusas *et al*., 1996) maize (Tamiru *et al*., 2012; Mutyambai *et* *al*., 2016) and wheat (Weaver *et al*., 2009). This variation in volatile blend influences host selection and preference by insects for diferent plant species. In view of this, this study aimed at determining the preference of adults and larvae of *C. partellus* for various brachiaria genotypes and to identify those that could better suffice as trap crop for *C. partellus*. This information will contribute to the understanding and exploitation of brachiaria as a trap crop for *C. partellus* in an innovative push-pull system.

5.3 Materials and methods

5.3.1 Study site

This study was carried out at Thomas Odhiambo Campus, Mbita Point (0° 25'S, 34° 12'E, 1200 m above sea level), a field station of the International Centre of Insect Physiology and Ecology (*icipe*) located on the shores of Lake Victoria in western Kenya. The area receives an average annual precipitation of 900 mm. Temperature in the screenhouse ranged between 25 and 30 ^{o}C and relative humidity was 50 - 65%. Mean temperatures inside the laboratory were 25.5 ^{o}C by day and 23.5 ^{o}C at night, with 70±5% relative humidity and natural light conditions of approximately L12:D12. The area is considered a 'hot-spot' for cereal stemborers with the vegetation type mainly comprising of savannah grassland with mixed combretum and acacia trees to the north and papyrus along the shores of the lake.

5.3.2 Study plants and insects

Planting material of seven different brachiaria genotypes used in the study were sourced from the International Center for Tropical Agriculture (CIAT), Columbia. These genotypes were *B. brizantha* cv. Mulato II, *B. brizantha* cv. Marandu, *B. decumbens* cv. Basilisk, *B. brizantha* cv. Piata, B. *brizantha* cv. Mulato, *B. Brizantha* cv. Cayman and *B. brizantha* cv. Xaraes.

Mulato II is a commercial hybrid which has previously been incorporated into a push-pull strategy as a trap crop mainly due to its attractiveness to the cereal stemborer moths and its benefits as a fodder crop (Midega *et al*., 2011; Khan *et al*., 2014). An open pollinated landrace maize variety Nyamula, a farmer preferred but stemborer susceptible maize variety (Midega *et al*., 2015; Tamiru *et al*., 2015) was included as a control. All plants were grown in pots. The different brachiaria varieties were grown from root splits while maize was grown from seeds. Plants were 3–4 weeks old old when they were used in the experiments and all experiments commenced in the morning.

To build up a sufficient insect culture for the study, original populations of *C. partellus* larvae were collected from sorghum fields and reared on an artificial diet to obtain moths, as described by (Onyango & Ochieng'-Odero, 1994). Rearing was done at ITOC under laboratory conditions of 24±3 ºC, 70±5% relative humidity and L12:D12. The insects used in the experiments were of the second generation of the founder colony and infestations were done early in the morning.

5.3.3 Adult selection of host plants

Two-choice oviposition test. Oviposition tests with *C. partellus* moths were conducted following a procedure adapted from Khan *et al*. (2007) and Midega *et al*. (2011). Two-choice tests were carried out in oviposition cages (80 × 40 × 40 cm) covered with fine wire mesh netting. Two potted 3–4 weeks old plants representing each brachiaria variety and maize, were placed in opposite corners of each cage (Plate 5.1). A 10-cm diameter wad of cotton wool was moistened with water and introduced into the cage for the moths to feed on. Five gravid naïve moths were introduced in the cage and allowed to oviposit for 48 h under natural light conditions of L12:D12. Afterwards, the plants were removed, and the number of eggs counted under a light microscope at 6.5× magnification (Plate 5.1). ‘Preference’ in this context was taken as significant differential oviposition on a plant when the moth was given a choice between two plants of different species.

5.3.4 Trichome assessment

The possible effect of leaf architecture on oviposition was evaluated by determining the trichome density on leaves and correlating that with egg numbers per plant variety. The last five fully emerged leaves representing five replicates from each test plant were obtained and the numbers of trichomes determined on a 0.5 × 0.5 cm area on the adaxial surface of each leaf using a binocular light microscope. Samples were taken from the intermediate position between the leaf margin and the midrib on the leaf lamina and half-way between the proximal end and base of the leaf.

5.3.5 Larval performanceon brachiaria varieties and maize

Larval orientation and settlement. Host-plant preference of *C. partellus* larvae was assessed in a two-choice test between each of the seven brachiaria varieties and maize following a modification of the procedure described by Khan *et al*. (2007). Experiments were conducted inside 15 cm diameter petri dishes lined with moist filter paper discs. Four 3-cm long leaf cuts of brachiaria varieties and maize were laid alternately and radially, two for each plant, with their adaxial sides facing up (Plate 5.2). At the center of each petri dish, 10 first instars of *C. partellus* were introduced. The petri dishes were then placed in a dark room. Larvae were allowed to orientate and settle on their preferred leaf tissue. The larvae on/underneath each leaf cutting were counted after 1 h and 24 h to determine orientation and settling preference, respectively. This experiment was replicated 10 times.

Arrest and dispersal of first instars. This experiment was conducted in a dark room. Leaf cuts of brachiaria and maize plants were placed individually, with their adaxial side facing upwards, in the center of a 9-cm petri dish lined with moist filter paper (Plate 5.2). A moist cotton wad was placed at either end of a 6-cm-long leaf cutting of each of the plants. Ten first instars of *C. partellus* were then introduced on top of each leaf cutting. The larvae remaining on the leaf tissue were counted after 1 h and 24 h of release. The experiment was replicated 10 times.

Leaf and stem feeding and food assimilation. This study was conducted to assess feeding of *C. partellus* larvae on leaves and stem cuttings of brachiaria and maize plants. Pieces of the second-youngest leaf (2.5 x 2.5 cm) of 3-week old plants were placed in a 6-cm diameter petri dish lined with wet filter paper to limit desiccation. Each piece of leaf was placed in a different petri dish. Five newly hatched and unfed larvae were placed on each leaf cutting. The petri dishes were covered and sealed with parafilm to prevent larvae from escaping and kept in a dark room. The leaf area (mm^2) consumed by the larvae was measured after 24 h using a graph paper (Mohamed *et al.*, 2007). The surface area removed or damaged after feeding indicates feeding levels of the larvae on the leaf tissue. This experiment was replicated 10 times.

To determine the degree of stem feeding by *C. partellus* larvae, 4-cm long stem segments of each of the 3-week old potted brachiaria and maize plants were obtained. Each segment was weighed (S1) and then placed in a glass vial (4.1 x 1 cm). A third-instar larva, previously starved for 3 h under high humidity conditions, was also weighed (W1) on a microbalance (Mettler PM460; Mettler Instrument, Greifensee, Zurich, Switzerland) and put on the piece of stem inside the vial. The vials were then covered with cotton wool plugs and kept in a dark room for 24 h. Unconsumed parts of the stem segments were weighed again (S2) after discarding the larvae and excreta. To determine weight loss due to evaporation, 10 stem segments (4 cm long) of each treatment were weighed (CE1), kept in similar vials alongside the experimental ones, and weighed again after 24 h (CE2). The difference between the initial weight (S1) and the final weight (S2) of the stem tissue after adjustment for weight loss due to evaporation represents the degree of feeding on the plant by the larvae (Khan & Saxena, 1985). Each treatment was replicated 10 times. To determine the amount of food assimilated, each larva was weighed again (W2). To determine larval weight loss due to metabolism, ten larvae was weighed (C1), kept alongside the experiment in similar vials without stem pieces and weighed again after 24 h (C2). The amount of food metabolized by each larva was determined using the equation from Khan & Saxena (1985). The following equation was used to calculate food accumulation:

Assimilation of food = $W1 \times (C1-C2)/C1 + W2-W1$

where W1 = initial weight of larva, W2 = final weight of larva, C1 = initial weight of control larva, and C2 = final weight of control larva.

Larval development and survival on stem tissue under laboratory conditions. To evaluate development and survival of *C. partellus* larvae on the test plants, a study was carried out in a laboratory setting. This was done in a room with cooler temperatures during the day due to shading (mean of 26.5°C). Relative humidity was maintained at 65%. Five sections of stems of 3-week old potted plants grown in a screenhouse were placed in screw-top glass jars (20 cm-high and 8 cm-wide) (Plate 5.2). Each of these sections measured between 0.5 - 1.5 cm diameter at the base, were approximately 15 cm long and consisted of stem, leaf, and sheath regions for the larvae to feed on (Khan *et al*., 2007). Twenty-five newly hatched larvae were introduced into each jar using a fine camel hair paint brush. Two replicates were established for each of the plants. The lids of the jars were tightly closed, and paper towels were used to tighten the seal and prevent the larvae from escaping (Khan *et al*., 2007). The larvae were then allowed to feed for five days, the least number of days taken between instars. Afterwards, the plants were removed and carefully dissected to recover and determine the number of live larvae per plant.

5.3.6 Data analysis

All analyses were performed by R software (Version 3.3.1) (R Core Team, 2016). Unpaired two-sample Student t-test was used to analyze differences between maize and each of *Brachiaria* spp. with regard to the number of eggs oviposited and larval orientation and settling on the different varieties. Analysis of variance (ANOVA) was done to determine if the density of trichomes differed significantly between the different *Brachiaria* spp. Prior to analysis, data on oviposition, larval orientation and settlement were log (x+1) transformed to satisfy assumptions of t-tests as indicated by normality tests.

A special correlation (Polyserial) analysis was computed using the ‘polycor’ package for trichome density and preference for oviposition, which was separated into two categories and allocated dummy values of 1 (preferred) and 0 (non-preferred). Similarly, data on arrest and dispersal, leaf feeding, food ingestion and assimilation of stem tissue, and larval survival were subjected to one-way ANOVA using the generalized linear model to test for any significant differences among the test plants. Before analysis, the data on arrest and dispersal, and leaf feeding were log transformed (log x+1). Student-Newman-Keuls test (*SNK*) was used to separate the means at $P < 0.05$. Means of non-transformed data are presented in figures and tables.

5.4 Results

5.4.1 Adult selection of host

Two-choice oviposition test. There were statistically significant differences (P < 0.05) between the number of eggs laid on maize and each of the brachiaria grass varieties except for Basilisk (Fig. 5.1). Significantly higher (P < 0.05) numbers of eggs were oviposited on Marandu, Piata and Xaraes than on maize. Basilisk also recorded higher numbers of eggs, but the difference was not significant (P < 0.05). On the other hand, Mulato I, Mulato II, and Cayman recorded lower numbers of eggs than maize (Fig. 5.1).

5.4.2 Trichome assessment

There were statistically significant differences between trichome numbers per 0.25-m^2 of leaf area of the different treatments ($F_{7,32}$ = 14.98, P<0.001; Table 5.1) (Plate 5.3). Trichome densities were statistically similar among Mulato, Cayman, Mulato II, Basilisk and maize, but significantly (P<0.001) lower on Xaraes, Marandu and Piata. The latter three varieties of brachiaria recorded significantly (P <0.05) higher numbers of eggs than those deposited on maize. Polyserial correlation displayed a strong and negative correlation between the trichome density and *C. partellus* preference of brachiaria for oviposition (-0.86, P < 0.05).

5.4.3 Larval performance

Larval orientation and settlement. First instar larva did not show any significant (P < 0.05) preference in orientation when subjected to a choice between leaf cuttings of maize and brachiaria after 1 h of release. There was however a tendency that more larvae orientated towards maize than they did to brachiaria (Table 5.2). Similarly, after 24 h of release, significantly higher ($P < 0.05$) numbers of larvae settled on maize than on any of the brachiaria plants, with the differences being significant for all treatments (Table 5.2).

Larval arrest and dispersal. The mean number of *C. partellus* larvae arrested on leaf cuts from all the treatments after 1 h of release was statistically similar ($F_{7,72}$=1.9, $P = 0.08$; Table 5.3). Leaf cuttings of maize did however record the highest number of larvae compared to all brachiaria grasses. The number of larvae remaining on leaf cuts after 24 h of release was significantly higher ($F_{7,72}$=2.8, $P = 0.01$; Table 5.3) for maize than on any of the brachiaria plants tested.

Larval feeding and food assimilation (stem). First instar *C. partellus* larvae consumed significantly larger areas ($F_{7,72}$=347.5, $P < 0.05$) of maize leaves than any of the brachiaria genotypes (Table 5.4). Remarkably, the larvae consumed some material from the stem segments of brachiaria plants although significantly heavier ($P < 0.05$) larvae were recovered from maize than brachiaria (Table 4). Xaraes ranked second to maize in terms of larval mass recovered and significantly higher ($F_{7,72}$=175.6, $P < 0.001$) than its counterparts. Similarly, the amount of food assimilated by larvae fed on the stem segments were significantly higher ($F_{7,72}$=26.8, $P < 0.001$; Table 5.4) in maize than in the other test plants.

Larval survival and development under laboratory conditions. By the fifth day of the experiment, no live larva was recovered on any of the brachiaria genotypes while 79.2% survived on maize (Table 5.5). This implies that larvae did not survive beyond the first instar on brachiaria plant tissue.

5.5 Discussion

There is a wide range of grass species in Africa that support insect species including the maize stemborers (Le Ru *et al*., 2006; Moolman *et al*., 2014). Since attractive wild host plants may act as a sink for crop pests, they could be exploited as trap crops (Shelton & Nault, 2004). Host plant recognition by lepidopterous species is a complex process. Location and subsequent selection of suitable hosts for oviposition by adults and for feeding by larvae is strongly influenced by chemical, physical and visual characteristics of the host plants (Van den Berg, 2006b; Calatayud *et al*., 2008, 2014; Dicke *et al*., 2009; Bruce *et al*., 2010; Hare, 2011; Bruce, 2015; Mutyambai *et al*., 2016; Pickett & Khan, 2016).

Our results show that female *C. partellus* moths, under two-choice test conditions discriminated between the maize and brachiaria varieties tested. Marandu, Piata and Xaraes were more preferred to maize. Preference for maize to Mulato and Mulato II is in agreement with previous studies where Mulato ranked lower than maize in multiple preference tests (Khan *et al*., 2007; Midega *et al*., 2011). Nevertheless, Mulato II is currently being exploited as a trap crop for management of stemborers in maize and sorghum in a push-pull habitat management system in which its efficiency can be enhanced especially when combined with an intercropped push crop of *Desmodium* spp. (Khan *et al*., 2001, 2016). According to Cook *et al*. (2007) and Khan *et al*. (2010), effectiveness of a trap crop is further enhanced by volatiles released from the push crop, which repels insects away from the main crop. Furthermore, Mulato II is a highly nutritious and drought resistant fodder crop which is gaining rapid adoption in Africa (Maass *et al*., 2015). Notably, the three brachiaria varieties Marandu, Piata and Xaraes which are significantly preferred to maize for oviposition have higher densities of trichomes than maize. Correlation analysis revealed a strong and negative relationship between trichome density and preference for oviposition, suggesting that trichomes on the leaf surface may have, in part, influenced oviposition preference of the insect. Influence of leaf texture on oviposition by stemborer females has been observed on maize (Van den Berg, 2006a; Rebe *et al*., 2007; Calatayud *et al*., 2008) and on Napier grass (Van den Berg, 2006a). According to Myers (1991), the presence of trichomes provide structural or chemical resistance, or both as for glandular trichomes, which can repel the attacking insects or arrest their movement. Studies with *B. fusca* shows that oviposition is adversely affected by rough and pubescent surfaces, making it difficult for moths to sweep and insert their ovipositors (Calatayud *et al*., 2006).

After host selection and oviposition by an adult moth, plant suitability for larval feeding and development is the next step in the host colonization process. For phytophagous lepidopterous insects, the larva, which is the damaging stage, typically has minimum mobility. Results from two-choice tests clearly showed that more *C. partellus* larvae settled on leaf cuts of maize than on brachiaria within the first 1 h and the difference was significant after 24 h. A similar trend was observed regarding larval arrest in a no-choice test after 1 h and 24 h. The results conform to those of Khan *et al*. (2007) and Mohamed *et al*. (2007) which assert *C. partellus* larvae prefer maize leaf cuts over those of wild grasses for settling and arrest. Other workers (Berger, 1992; Päts, 1992) have also reported dispersal behaviors caused by lepidopterous larvae on non-preferred hosts. The results of the current study suggest that larval non-preference for brachiaria may be due to the presence of plant chemicals and/or physical characters and/or poor nutrient quality, which stimulated the larvae of *C. partellus* to disperse away from the test plant.

First instar *C. partellus* larvae did not consume leaf parts of any plants except maize. Third instar larvae however ingested some tissue from stems of all test plants with significantly more feeding by the larvae on stems of maize, followed by Xaraes. Larvae that fed on maize assimilated significantly higher amounts of food than those feeding on brachiaria. There was no clear difference in the amount of food ingested and assimilated by larvae feeding on brachiaria. First instar larvae of *C. partellus* introduced to stem, leaf and sheath tissue of brachiaria were all dead by the fifth day of the laboratory experiment. Contrary to this, there was 79% survival on maize sections. Other studies conducted with plant parts of napier grass showed similar detrimental effects to *C. partellus* larvae, and the pattern was observed when entire plants were used (Van den Berg *et al*., 2006a; Khan *et al*., 2007). This could validate the use of plant parts in *C. partellus* larval performance studies testing the potential of trap plants. Our observations on larval performance is corroborated by the "optimal bad motherhood" principle (Mayhew, 1997). In this case, adults sometimes spend more time, consequently laying eggs on the host plant that enhances their own long-term fitness, even if the consequence of this behavior is a reduction of offspring survival.

In other studies, Van den Berg & Van der Westhuizen (1997) and Khan *et al*. (2007) observed high levels of oviposition on sorghum varieties that were not suitable for larval development due to high levels of larval antibiosis. Moreover, Van den Berg (2006a) observed that napier grass varieties were preferred to sorghum for oviposition but supported minimal larval survival. As is the case in behavior of the adults, the larval mortality on the host may also be alluded to various morphological, biochemical and molecular mechanisms that the host counter or otherwise offset the effects of herbivore attack (Howe & Jander, 2008; Verhage *et al*., 2010; Hare, 2011). In his study with *C. partellus*, Van den Berg (2006a) observed that migrating larvae were arrested by trichomes of napier grass and that upward migration to the whorl was severely hampered. Khan *et al*. (2000) ascribed the high mortality of stemborer larvae on Napier grass to sticky sap that is produced by the grass in response to penetration by first and second instar larvae. This may have been the case in the current study. More surprising is the fact that even brachiaria varieties that have lower trichome densities nonetheless did not support feeding and survival of larvae. This suggests that there are also other important factors than just trichomes that affect larval survival. The current study highlights discrepancies between adult preference for oviposition and larval survival. Some brachiaria varieties were more preferred to maize for oviposition but subsequently did not support juvenile survival. Mortality of the stemborer larvae observed on brachiaria was very high and could be of value, under field conditions, in reduction of pest populations without acting as a 'nursery' crop on which stemborers could multiply and invade the main crop (Midega *et al*., 2015). We propose that, this is a prerequisite for trap crops in a 'dead end' trap cropping system (Shelton & Nault, 2004).

This study indicated that all brachiaria genotypes tested attracted stemborer oviposition but supported minimal larval survival. These results support the use of brachiaria as a "dead end" trap crop for management of stemborers through a push-pull technology. Of value, will be Marandu, Piata, Xaraes and Basilisk varieties, all which were preferred to maize for oviposition. The differential preference of moths for brachiaria varieties for oviposition and variation in trichome density presents an aspect of genetic variation which could be further exploited in brachiaria and another grass species. A highly interesting study of Magara *et al*. (2015) indicated that *B. brizantha* exposed to *C. partellus* oviposition signalled the maize open pollinated varieties Nyamula and Jowi and the land race Cuba 91 causing these plants to release volatile signals that attract the parasitoid *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae). This, coupled with the observations from our study necessitates further investigation and exploitation of genetic diversity in brachiaria for induction of defense mechanisms against stemborers in cereals through plant-plant signalling.

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Plates

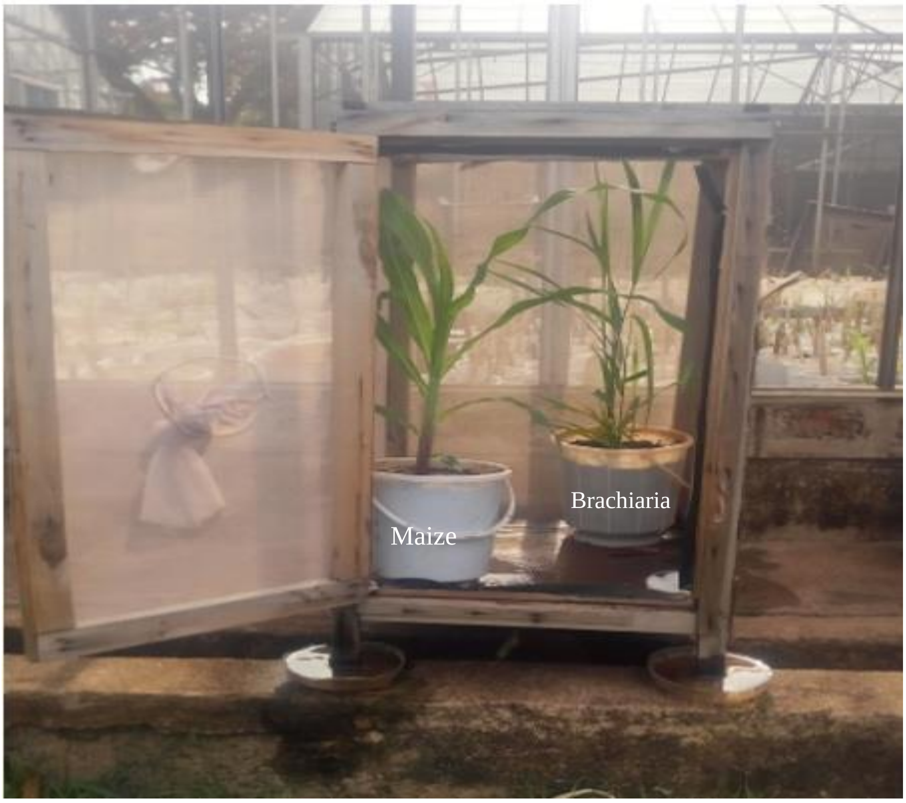
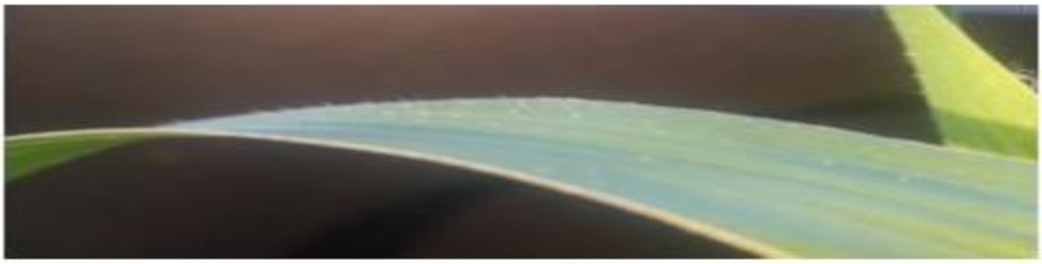


Plate 5.1 Set-up of the two-choice oviposition bioassay to deteremine moth preference for different brachiaria varieties and maize

Plate 5.2 Photos illustrating techniques used to study larval performance on leaf and stem tissue of different brachiaria genotypes.

Mulato II

Nyamula



Piata

Plate 5.3 Brachiaria leaves of different varieties showing different densities of trichomes on upper surfaces of the leaves.

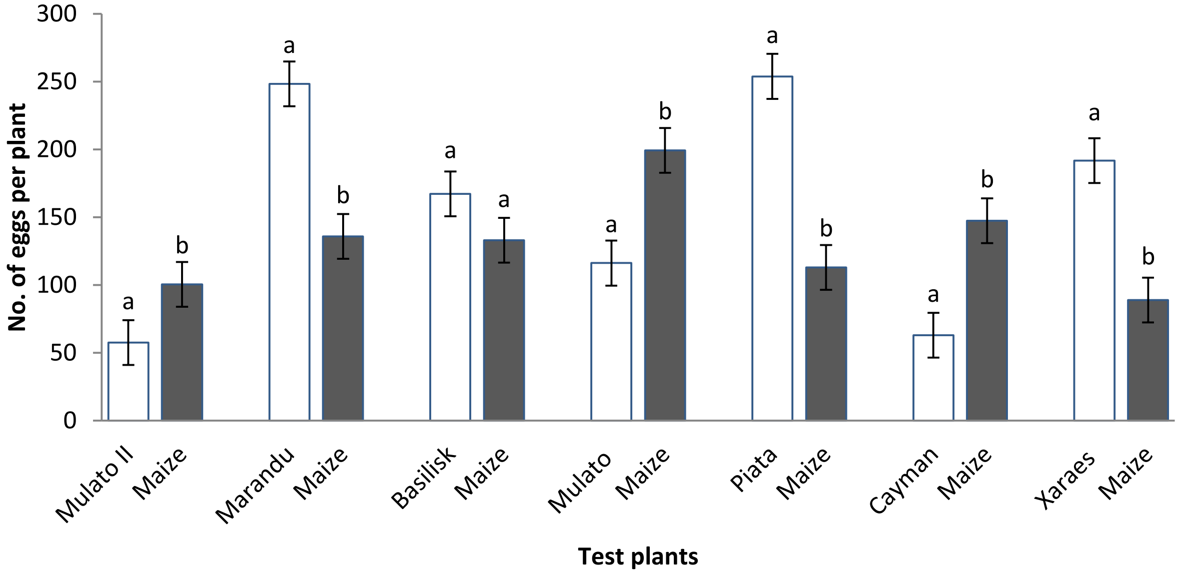


Figure 5.1 Mean (±SEM) number of eggs laid by *Chilo partellus* on maize and different brachiaria genotypes in two-choice tests. Different letters above the bars indicate significant differences between the means (Student t-test: $P < 0.05$).

Table 5.1 Mean (±SEM) trichome number on a 0.25 mm^2 adaxial surface of leaves of 3 to 4 months-old brachiaria genotypes and maize plants, and preference for oviposition by *Chilo partellus* moths in a two-choice tests between a brachiaria genotype vs. maize

Test Plant	Number of trichomes (Mean ± SEM)	Preference for oviposition compared to maize
Mulato	66.2 ± 3.2a	No
Cayman	65.6 ± 2.3a	No
Mulato II	59.2 ± 2.9a	No
Basilisk	50.8 ± 1.5b	No
Maize	21.6 ± 2.5c	-
Xaraes	11.6 ± 3.7d	Yes
Marandu	7.2 ± 1.6d	Yes
Piata	6.8 ± 3.1d	Yes

Means followed by the same letter are not significantly different (SNK test: $P > 0.05$).

Table 5.2 Average (±SEM) number of *Chilo partellus* larvae oriented and settled on leaf cuts of test plants after 1 h and 24 h, respectively

Test plant combination	Orientation (±SEM) 1 h	t-value	P-value	Settlement (±SEM) 24 h	t-value	P-value
Mulato II vs maize	1.7 ± 0.5a vs 3.7 ± 0.8a	-2.09	0.05	0.7 ± 0.2a vs 4.4 ± 0.8b	-4.59	<0.001
Marndu vs Maize	2.5 ± 0.3a vs 2.1 ± 0.6a	0.58	0.56	0.5 ± 0.3a vs 4.8 ± 0.7b	-4.65	<0.001
Basilisk vs Maize	2.6 ± 0.7a vs 3.5 ± 0.7a	-0.9	0.37	0.5 ± 0.3a vs 4.8 ± 1.0b	-4.0	0.002
Piata vs Maize	2.3 ± 0.7a vs 4.2 ± 0.6a	2.09	0.05	0.5 ± 0.3a vs 5.7 ± 0.7b	7.3	<0.001
Mulato vs Maize	2.2 ± 0.6a vs 3.7 ± 0.6a	-2.1	0.05	0.7 ± 0.2a vs 4.4 ± 0.6b	-4.5	<0.001
Cayman vs Maize	2.2 ± 0.4a vs 3.9 ± 0.7a	-2.1	0.06	0.3 ± 0.2a vs 5.7 ± 0.8b	-6.2	<0.001
Xaraes vs Maize	2.5 ± 0.5a vs 4.0 ± 0.7a	1.7	0.39	1.2 ± 0.3a vs 5.2 ± 0.5b	6.7	<0.001

Means followed by same letters within a column are not significantly different from each other at $P > 0.05$; *Student-Newman-Keuls* (SNK).

Table 5.3 Average (±SEM) number of *Chilo partellus* larvae arrested on leaf cuts of test plants after 1 h and 24 h

	Mean (±SEM) number of larva arrested on leaf cuts of each test plant after 1 h and 24 h	
Test plant	1 h	24 h
Mulato II	3.7 ± 0.7a	2.0 ± 0.5a
Marandu	2.6 ± 0.7a	2.4 ± 0.5a
Basilisk	3.3 ± 0.6a	2.3 ± 0.6a
Piata	2.4 ± 0.5a	1.9 ± 0.6a
Mulato	2.7 ± 0.7a	2.0 ± 0.4a
Cayman	2.0 ± 0.5a	1.8 ± 0.5a
Xaraes	2.9 ± 0.6a	2.6 ± 0.5a
Maize	5.0 ± 0.4a	5.9 ± 0.9b

Means within a column followed by the same letter are not significantly different (SNK test: $P > 0.05$).

Table 5.4 Mean (±SEM) feeding and food assimilation parameters by *Chilo partellus* after 5 days on leaves and stems of various brachiaria varieties and maize

Variety	Leaf area (cm^2) consumed by five first instars	Stem weight (mg) consumed by a third instar larva	Stem weight (mg) assimilated by a third instar larva
Mulato II	0 ± 0a	5.80 ± 4.28a	0.14 ± 0.1a
Marandu	0 ± 0a	17.49 ± 8.9a	2.23 ± 0.8a
Basilisk	0 ± 0a	10.87 ± 2.0a	1.42 ± 0.6a
Piata	0 ± 0a	11.51 ± 4.3a	0.98 ± 0.5a
Mulato	0 ± 0a	14.02 ± 6.7a	0.98 ± 0.5a
Cayman	0 ± 0a	7.75 ± 2.9a	2.24 ± 1.1a
Xaraes	0 ± 0a	52.90 ± 8.9b	1.56 ± 0.6a
Maize	8.4 ± 1.3b	293.87 ± 14.5c	15.44 ± 1.8b

Means within a column followed by the same letter are not significantly different (SNK test: $P > 0.05$).

Table 5.5 Mean survival of 25 *Chilo partellus* larvae on brachiaria varieties and maize tissues after 5 days under laboratory conditions

Variety	Mean number of larvae recovered per plant
Mulato II	0 ± 0.0a
Marandu	0 ± 0.0a
Basilisk	0 ± 0.0a
Piata	0 ± 0.0a
Mulato	0 ± 0.0a
Cayman	0 ± 0.0a
Xaraes	0 ± 0.0a
Maize	19.8 ±1.7b

Means followed by different letters are significantly different (SNK test: $P < 0.001$).

CHAPTER SIX

6.0 BEHAVIOURAL RESPONSE OF *Chilo partellus* AND ITS PARASITOID TO OVIPOSITION-INDUCED VOLATILES OF BRACHIARIA

6.1 Abstract

Some genotypes of *Brachiaria* spp. are used in management of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), an economically important pest of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) in sub-Saharan Africa. In a chemical ecology-based companion cropping system, *Desmodium intortum* (Mill.) Urb. emits semiochemicals that are repellent to moths (push) while *Brachiaria brizantha* cv Mulato II emits attractive compounds (pull). This study assesses the presence and effects of oviposition induced plant volatiles (OIPV) of brachiaria on *C. partellus* and its natural enemy *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae). Four brachiaria genotypes were evaluated to determine the moths' preference for either previously oviposited or non-oviposited plants in a two-choice test. Response of *C. sesamiae* to head space volatile samples from oviposited and non-oviposited (control) plants were compared in four arm olfactometer bioassays. In two choice oviposition tests, higher percentages of eggs were laid on previously oviposited plants of Piata and Xaraes varieties ($P < 0.05$), while non-oviposited plants of Mulato II were significantly ($P < 0.05$) preferred to previously oviposited plants. Female *C. sesamiae* spent significantly more time in arms with volatiles from previously oviposited and non-oviposited plants of all varieties except Marandu. Increased ovipositional preference on previously oviposited plants of some brachiaria varieties offers an advantage to their use as trap crops for the pest. Attractiveness of headspace volatiles, both oviposition induced and constitutive, from most of the genotypes highlights the value of these grasses in stemborer management strategy that applies tritrophic interaction with the pest's natural enemies.

Key words: *Brachiaria* spp., olfactometer bioassays, oviposition, trap plants, tritrophic interactions

6.2 Introduction

Release of volatile chemical cues is one of the defense mechanisms that plants employ against insect herbivores (Hare, 2011; Agrawal, 2011). Following an attack, the herbivore induced plant volatiles (HIPV) are emitted as a result of mechanical injury (Schmelz *et al*., 2001) or oral secretions of the herbivore (Turlings *et al*., 1990; Funk, 2001). The HIPV which consist of hundreds of compounds, such as terpenoids, green leaf volatiles and benzenoids negatively affect the physiology or behaviour of the herbivore, either as toxins, digestibility reducers or deterrents (Dicke *et al*., 1990; Roda & Baldwin, 2003; Dicke & Baldwin, 2010; Mumm & Dicke, 2010). They also communicate to the natural enemies of the attacking insects (Turlings *et al*., 1995; De Moraes *et al*., 1998; Tamiru *et al*., 2011a; Mutyambai *et al*., 2015) and warn the neighboring undamaged plants of the forthcoming danger (Frost *et al*., 2008; Heil, 2008; Mutyambai *et al*., 2016).

In some plants, release of HIPV is triggered before the herbivore attacks, when the insect merely lays the eggs thus causing early recruitment of the predators in readiness for impending attack (Colazza, 2004; Hilker & Meiners, 2006; Bruce *et al*., 2010; Khan *et al*., 2011; Tamiru *et al*., 2011b; Mutyambai *et al*., 2015, 2016). Egg deposition may also elicit direct defense through production of neoplasm that elevates the eggs from plant surface causing them to fall off (Doss *et al*., 1995) or by producing ovicidal substances that kill the eggs (Yamasaki *et al*., 2003). Furthermore, direct defense mechanisms that affect egg deposition render the oviposited plants less attractive to gravid females (Blaakmeer *et al*., 1994). Conversely, egg deposition can reduce the constitutive emission of volatiles and suppress the typical burst of inducible volatiles (Peñaflor *et al*., 2011).

The spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) is one of the most destructive pests that, if uncontrolled, can cause up to 80% grain yield loss in sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.) in sub-Saharan Africa (Kfir *et al*., 2002). These are the most important food and cash crops for millions of rural small-holder farmers who account for 80% of the farming communities in sub-Saharan Africa (SSA) (Romney *et al*., 2003). Although synthetic pesticides can be used as a primary remedy, their use has been associated with adverse environmental impacts which arise from incorrect and inappropriate use of pesticides, a common scenario in Africa (Van den Berg & Nur, 1998). Furthermore, the high cost of chemicals makes it non-affordable especially for small-holder farmers (Mbakaya *et al*., 1994; Ngowi *et al*., 2007; Macharia *et al*., 2013; Mengistie *et al*., 2016). A chemical ecology-based companion cropping IPM strategy, push-pull, is a low input and effective technology that can significantly reduce the losses caused by the cereal stem borers (Khan & Pickett, 2004; Khan *et al*., 2010, 2016; Pickett *et al*., 2014; Midega *et al*., 2015; Pickett & Khan, 2016).

In push-pull technology, the main crop is intercropped with drought-tolerant greenleaf desmodium, *Desmodium intortum* (Mill.) Urb., while *Brachiaria* cv Mulato II is planted as the border crop. The intercrop releases the pest repellent semiochemicals (push) while the border crop releases pest attractant semiochemicals (pull). Mulato II, a grass species in the genus brachiaria was incorporated in the technology due to its unique properties that makes them attractive for stemborer oviposition while being detrimental to its larvae (Midega *et al*., 2011; Cheruiyot *et al*., 2018). Furthermore, the high value fodder crop exhibits highly sophisticated responses to attacks by *C. Partellus* that involves multitrophic interactions with some of its natural enemies (Bruce *et al*., 2010; Magara *et al*., 2015). By end of the year 2017, this technology which is adaptable to drier agroecologies and resilient to climate change had been adopted by approximately 150,000 cereal-livestock based small holder farmers in eastern Africa (http://www.push-pull.net/adoption.shtml). However, in the current and foreseeable scenarios of climate change, the technology is being “cushioned” by identification and utilization of climate change resilient and adaptable companion plants that provide additional protection against the lepidopterous pets. To address this, the current study aimed at evaluating more brachiaria genotypes for early-herbivore induced traits that involve production of oviposition induced plant volatiles and attraction of natural enemies in response to plant attack by *C. partellus*.

6.3 Materials and methods

6.3.1 Experimental plants and insects

Brachiaria varieties used in this study were selected from germplasm sourced from International Livestock Research Institute (ILRI) forage gene bank (Ethiopia) and International Centre for Tropical Agriculture (spanish acronym CIAT), Columbia and evaluated at the International Centre of Insect Physiology and Ecology-Thomas Odhiambo Campus, Kenya (*icipe*-TOC) for desirable agronomic properties and their potential in management of *Chilo partellus* (Cheruiyot *et al*., 2018). These varieties were *B. brizantha* cv. Mulato II, *B. brizantha* cv. Marandu, *B. brizantha* cv. Piata and *B. brizantha* cv. Xaraes. Mulato II is a commercial hybrid used as a trap crop mainly due to its attractiveness to cereal stemborer moths (Midega *et al*., 2011) and its tritrophic interaction with the pest’s natural enemies (Bruce *et al*., 2010). Propagules were grown individually in pots filled with fertilized soil under screenhouse conditions (25 °C, 65%RH; 12L: 12D) at International Centre of Insect Physiology and Ecology-Thomas Odhiambo Campus (ITOC) in western Kenya (0 °25 ′S, 34 °12′E; 1200 m above sea level). Seedlings were used in the experiments when they were 3 to 4 weeks old.

Original populations of *C. partellus* larvae were collected from sorghum fields and reared on an artificial diet to obtain moths, as described by Onyango & Ochieng'-Odero (1994). Rearing was done at ITOC under laboratory conditions of 24±3 ºC, 70±5% relative humidity and L12:D12. The insects used in the experiments were of the second generation of the founder colony. Field collected larval parasitoids, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), were reared on stemborer larvae using methodologies described by Overholt *et al*. (1994).

6.3.2 Experimental procedure

Oviposition preference

A two-choice test was conducted following a modification of methodology of Khan *et al*. (2007) as described by Mutyambai *et al*. (2014). Five gravid and naive *C. partellus* moths were introduced into each cage (measuring 80 x 40 x 40 cm) containing one potted seedling for each brachiaria variety to oviposit for 24 h under natural light conditions of L12:D12. The cages were covered by fine mesh netting and a wad of wool moistened in water was provided as food to the insects. The positions of egg batches on leaves were then marked and another brachiaria plant of the same species and age but without prior exposure to oviposition was introduced in each of the cages adjacent to previously exposed plants. The potted plants were placed in opposites sides of the cage. Five gravid *C. partellus* moths were introduced into the cage and allowed to oviposit for 48 h under natural light conditions. The plants were then removed and the number of eggs on each plant counted under a light binocular microscope at magnification of 6.5. This aimed at assessment of the moths' preference for either previously oviposited or non-oviposited plants. In this case 'preference' was taken to be differential oviposition of the insect on the same variety of plants with different treatments (Mutyambai *et al*., 2015). Data collected were expressed as the mean proportion (percentage) of total number of eggs oviposited during the second oviposition period on plants in the two-choice test. The experiment was replicated 10 times.

Collection of headspace plant volatiles

Headspace volatiles were collected from whole plants of brachiaria varieties with or without stemborer oviposition (Agelopoulos *et al*., 2000). Prior to volatile collection, plants for oviposition were placed inside oviposition cages (80 × 40 × 40 cm) into which five gravid female stemborer moths were introduced and allowed to oviposit overnight. A wad of cotton wool (10 cm diameter) moistened with water was placed into the cage for moths to feed on. The cages stood on lids containing clean water to prevent predator arthropods from attacking the moths and the eggs. Control plants were kept inside similar cages, but without stemborer moths. Volatiles were collected on the following day, starting at the last two hours of the photo-phase, for 48 h as described by Mutyambai *et al*.(2015).

Olfactometer bioassay

A choice-test was conducted to compare response of parasitoid *C. sesamiae* to volatile samples from oviposited and control brachiaria plants. The test was conducted in a Perspex four arm olfactometer (Fig. 6.1). The test stimuli (10 µL aliquots of head space samples) were introduced in two opposing arms while the solvent control was put in the remaining arms. Samples were applied using a micropipette (Drummond ‘microcap’, Drummond Scientific Co., Broomall, PA, USA), on a piece of filter paper (4 × 25 mm) placed in an inlet port at the end of each arm of the olfactometer. Air was drawn through the four arms towards the centre at 260 ml min^{-1}. One-day old female parasitoids acclimatized to room temperature for 1 hr and without prior exposure to any plant or host were introduced individually to the central chamber of the olfactometer. During the test, the olfactometer was rotated after every 4 min. The time spent by parasitoids in each arm of the olfactometer was recorded with support of the ‘Olfa’ software (F. Nazzi, Udine, Italy) for 12 min. The experiment was repeated 10 times with thorough cleaning and sterilizing of the glassware between repeats, and random reallocation of positions of the treatments.

6.3.3 Statistical analyses

Time spent in the four arm olfactometer assay for each plant volatile sample were converted into proportions of the total time the insect could make its choice (12 min), followed by a log ratio transformation to allow analysis of compositional data (Aitchison 1981; Tamiru *et al.* 2011; Mutyambai *et al.,* 2014). The proportions were then compared by analysis of variance (ANOVA). Means were separated using the Student-Newman-Keuls (*SNK*) methods (Sokal & Rohlf, 1981) at P = 0.05. The two-sample (unpaired) student’s t-test was used to test the differences between the number of eggs and egg batches laid on plants either exposed or non-exposed to oviposition. All analyses were computed using *R*-software (Version 3.2.2).

6.4 Results

Oviposition preference of *C. partellus* on previously oviposited and non-oviposited brachiaria plants

In two-choice tests, higher percentages of eggs were laid on previously oviposited plants of Piata and Xaraes varieties ($P < 0.05$) than on non-oviposited plants (Table 1) . There was no significant difference ($P > 0.05$) in oviposition between previously oviposited and non-oviposietd plants of Marandu. In contrast, previously non-oviposited Mulato II was significantly ($P < 0.05$) preferred to previously oviposited plants of the same variety. Furthermore, there was also no significant difference ($P < 0.05$) between the numbers of egg batches on the different varieties except for Mulato II which recorded more egg batches on previously non-oviposted plants than previously oviposited plants. Previously oviposited plants of Mulato II recorded lower number of eggs that non-oviposited plants. The same trend was observed regarding the number of eggs per batch, with the difference being significant ($P < 0.05$) in Xaraes.

Behavioural response of *C. sesamiae* to head space samples of volatiles from brachiaria plants with and without eggs

There was statistically significant variation in *C. sesamiae* reponse to volatiles from oviposited, non-oviposited and solvent control in Piata ($F_{2,27}$=13.58, $P < 0.001$), Xaraes ($F_{2,27}$=6.16, $P < 0.01$) and Mulato II ($F_{2,27}$=11.54, $P < 0.001$) (Fig. 6.2). However, the time spent by the parasitoid on volatiles from oviposited and those from non-oviposited Piata variety did not differ significantly ($P > 0.05$). In Xaraes, time spent in volatiles from non-oviposited plants did not different significantly ($P > 0.05$) from times spent in both solvent control and volatiles from oviposited plants. However, significantly ($P < 0.05$) more time was spent on volatiles from oviposited plants as compared to the solvent control. Significant ($P < 0.05$) more time was spent on volatiles from oviposited plants of Mulato II, followed by volatiles from non-oviposited plants while solvent control was least preferred. In contrast, volatiles collected from both oviposited and non-oviposited Marandu were not attractive to *C. sesamiae* ($F_{2,27}$=1.00, $P < 0.371$).

6.5 Discussion

It was observed that female *C. partellus* moths prefer to oviposit on brachiaria grasses that were previously oviposited on, except for Mulato II grass. This proved that plant and insect response to oviposition is more complex than anticipated. In most cases, when plants are attacked by insect herbivores, they emit volatile chemicals that offer protection by attracting natural enemies and/or repelling further herbivore colonization (Bruce *et al*., 2010; Tamiru *et al*., 2011b; Turlings *et al*., 2012; Mutyambai *et al*., 2015). Previous studies with maize indicate that plants without eggs were significantly preferred for subsequent oviposition by *C. partellus* moths compared to plants with prior oviposition (Tamiru *et al*., 2012; Mutyambai *et al*., 2015). Similarly, in previous studies with signal grass *Brachiaria brizantha*, herbivore colonization was reduced on plants after oviposition by *C. partellus* (Bruce *et al*., 2010). Our observations on *Brachiaria brizantha* cv. Mulato II conforms to this finding. However, there exist intraspecific variation in volatile chemical quality and quantity in plants (Gouinguené *et al*., 2001; Degen *et al*., 2004; Mutyambai *et al*., 2015). This variation affects the behaviour of the herbivore insect and can be seen in scenarios where the insect exhibit variation in oviposition preference for previously oviposited and non-oviposited plants as was observed in our study. Oviposited plants of Piata and Xaraes were significantly preferred to non-oviposited plants, suggesting that oviposition on these plants increases their oviposition stimulating capacity. Our findings are supported by a previous report of increase in *C. partellus* oviposition preference on infested maize plants (Kumar, 1986). This phenomenon is advantageous for a trap crop as previously infested plants increasingly becomes more attractive to the herbivore; thus, the herbivore is less likely to be repelled back to the main crop.

In behavioural bioassays with *C. sesamiae*, volatiles from plants exposed to egg deposition were more attractive than the solvent controls in Piata, Xaraes and Mulato II. Headspace volatiles from oviposited Mulato II plants were more attractive than those from non-oviposited plants. This implies that these materials possess enhanced attraction to the natural enemies of the herbivore. In Mulato II, the attraction to the wasps was enhanced by herbivore oviposition. The role of oviposition-induced plant volatiles (OIPV) in attracting carnivorous enemies of herbivores, aboveground or belowground has been extensively studied (Hilker & Meiners, 2006; Turlings *et al*., 2012). Absence of significant differences in attractiveness by volatiles from previously oviposited and non-oviposited plants suggests that production of predator attractant volatiles from brachiaria can also be constitutive as is proposed by Ali *et al.*(2011). Moreover, in absence of herbivore induced plant volatile (HIPV), parasitoids can also respond to general cues from undamaged plants (Gohole *et al*., 2005; Moraes *et al*., 2008). In herbivore induced scenarios, the female parasitoids sting and inject their eggs inside the herbivore larvae. As the wasp’s eggs hatch and a new generation of the wasp is produced, larvae usually cease feeding and die after a few days. This is considered as an indirect defense mechanism also offered by trap plants through tritrophic interactions with natural enemies (Turlings *et al*., 1995; Bruce *et al*., 2010). Head space volatiles from Marandu, however did not elicit this response.

The focus of this research was particularly on interactions between brachiaria plants and a single herbivore, *C. partellus*. We also assessed the response of the parasitoids, *C. sesamiae* towards headspace volatiles of previously oviposited and non-oviposited plants in comparison with solvent controls. The study demonstrates intraspecific variation in attractiveness of the plants to oviposition by *C. partellus* moths. Piata and Xaraes became increasingly attractive for subsequent oviposition, while previously oviposited plants of Mulato II became less attractive. This warrants future studies to elucidate the mechanistic basis of this phenomenon, as well as its value in trap cropping systems. Attractiveness of the volatiles to the natural enemy, *C. sesamiae* also differed among brachiaria genotypes. Oviposited plants of all test varieties except Marandu released volatiles that attracted the parasitoids more than the solvent controls. The roles of OIPV in Mulato II were clearly demonstrated in the study. Interestingly, in Piata and Xaraes, attractiveness of volatiles from oviposited plants and non-oviposited plants were not statistically different. Questions however arise regarding to the additional roles of constitutive or general cues in host location by the parasitoid. There is thus a need for identification of volatile chemicals responsible and mechanisms underlying these observations.

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Plate

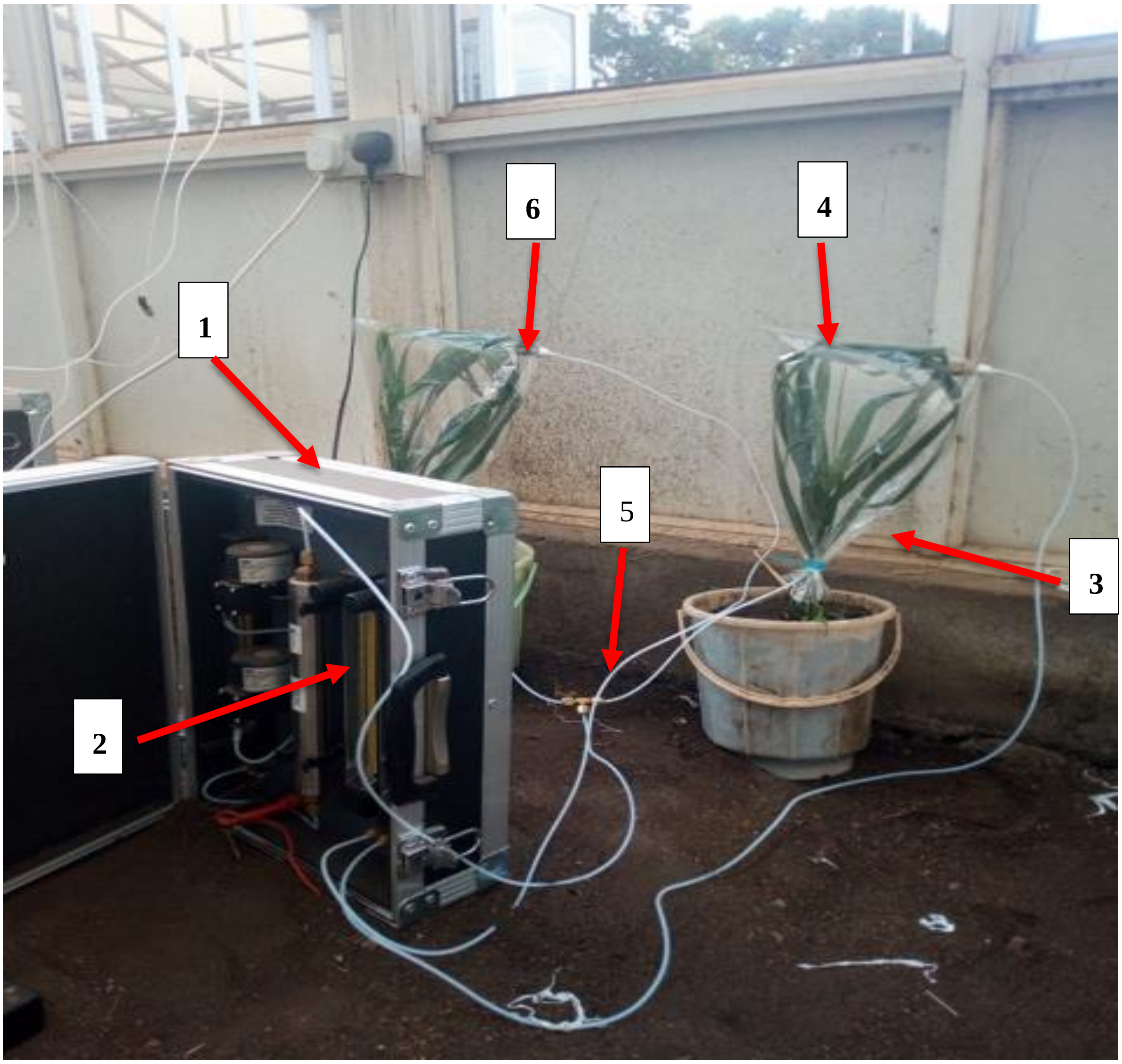

Plate 6.1 Headspace sampling set-up for volatile collection from oviposited and non-oviposited brachiaria genotypes. (1) Entrainment kit, (2) Flow-metre controlling air flow rate, (3) brachiaria seedling being entrained, (4) Polyethyleneterephthalate bag enclosing the plants (5) Ethylene terephthalate tubes transporting air to/from the pump, (6) Porapak Q tubes trapping volatiles

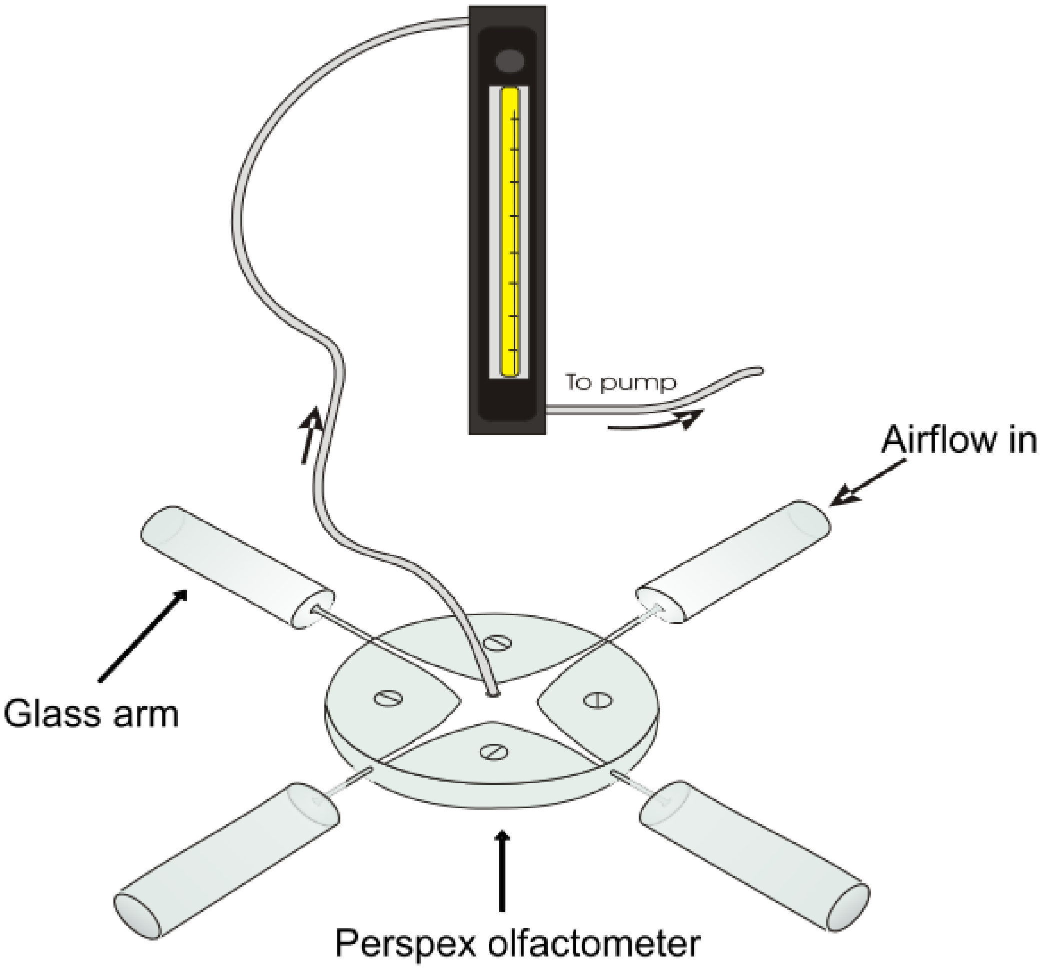

Figure 6.1 Diagram of the four-arm olfactometer that was used to assay for behavioural responses of *Cotesia sesamiae*.

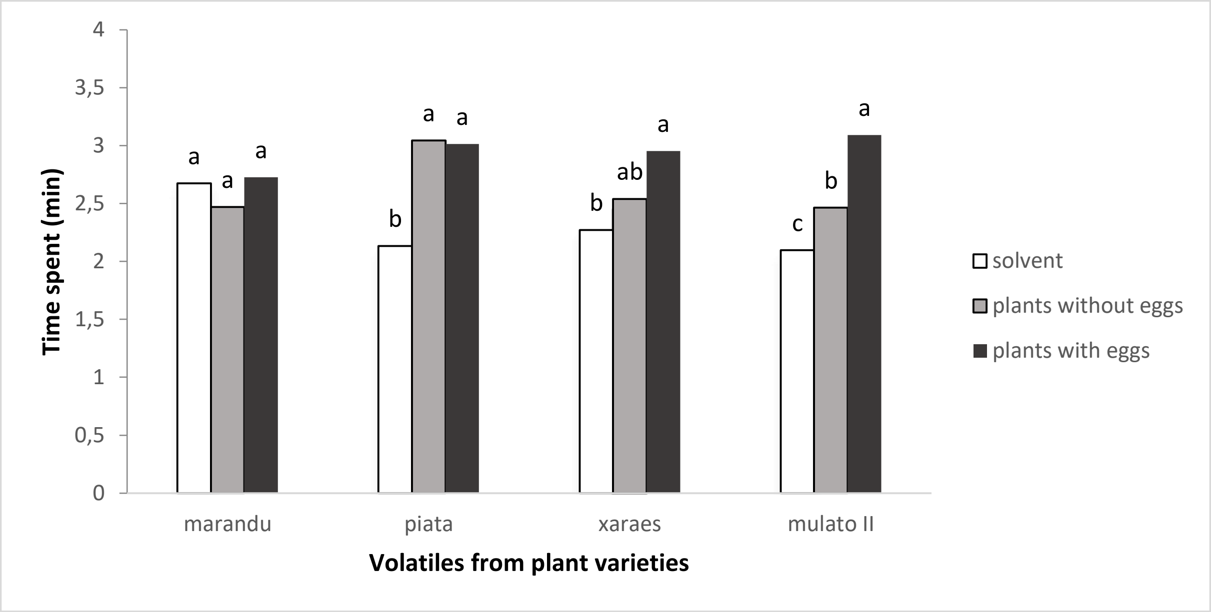

Figure 6.2 Behavioral response of female parasitoid *Cotesia sesamiae* to solvent control and volatiles collected from brachiaria plants with *Chilo partellus* eggs and those without the eggs in a four-arm olfactometer bioassay. Each female parasitoid was observed for 12 min (*N*=10).

Table 6.1 Percentages of eggs laid per plant (±SEM), number of egg batches per plant (±SEM), and number of eggs per batch (±SEM) on four brachiaria varieties previously oviposited and non-oviposited by *Chilo partellus* moths

Choice combination	Mean % of eggs	Number of egg batches	Number of eggs eggs/batch
Marandu (T)	59.96 ± 8.39a	6.50 ± 1.47a	35.96 ± 6.59a
Marandu (C)	40.04 ± 8.39a	3.60 ± 0.74a	31.81 ± 6.75a
Piata (T)	60.62 ± 6.49a	6.50 ± 3.32a	32.56 ± 6.46a
Piata (C)	39.38 ± 6.49b	4.30 ± 1.22a	32.64 ± 4.89a
Xaraes (T)	68.36 ± 7.58a	7.60 ± 2.42a	55.70 ± 7.18a
Xaraes (C)	31.64 ± 7.58b	6.50 ± 2.42a	27.44 ± 5.36b
Mulato II (T)	33.0 ± 5.67a	5.9 ± 1.45a	31.5 ± 7.38a
Mulato II (C)	67.0 ± 5.67b	8.0 ± 1.77a	50.5 ± 11.47a

T, treated plant (previously oviposited); C, control plants (non-oviposited). Means followed by the same letters within a row are not significantly different (Student-Newman-Keuls (*SNK*), $P \leq 0.05$).

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion and conclusion

Habitat management is one the most environmental friendly and economically viable pest management solutions that can be employed by small-holder farmers in sub-Saharan Africa. Push-pull technology, a habitat management strategy exploits behaviour-modifying stimuli to manipulate the distribution and abundance of pests, was originally developed for management of cereal stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and parasitic weed *Striga hermonthica* Benth. (Orobanchaceae), commonly known as Striga. It has displayed additional benefits including improved soil fertility, fodder and milk production. Thus, the technology has improved the livelihoods of more than 150,000 African smallholder farmers and rural families that have adopted the system while improving the environment. Nevertheless, like other cropping systems, push-pull is constrained by several climate change related biotic and abiotic factors. Drought has severely constrained agricultural production in sub-Saharan Africa resulting in significant negative effects on food production and on higher-order, social impact such as food insecurity. Furthermore, climate change has driven host and geographical distribution range expansion of insect pests making them the most important threat to agricultural production in most of the areas in Africa. Brachiaria, a grass in the genus *Brachiaria* is a commonly grown fodder crop in east Asia, Southern America and sub-Saharan Africa. In addition to its use as a fodder crop, some varieties such as *Brachiaria brizantha* cv. Mulato II are employed as trap plants in push-pull due to its attractiveness for oviposition by the stemborers, and their tritrophic interactions with the pest's natural enemies. Recently, the spider mite (*Oligonychus trichardti*) (Acari: Tetranychidae) was reported as a major pest of this crop especially in low altitude areas that experience extended periods of dry and hot conditions.

In view of the challenges observed thus far, this study aimed at optimizing push-pull technology through exploitation of agronomic and phytochemical properties of brachiaria that makes them useful for use in management of cereal stemborers in different climate change scenarios in East Africa. The overall objective was to contribute to improved cereal-livestock productivity in Africa through management of stemborers using suitable and adaptable brachiaria grasses. To achieve this, this study was divided into four parts. The first study was to evaluate the morphological and physiological performance of 18 genotypes of *Brachiaria* spp. under simulated drought conditions. The second part of the study was to determine the levels of resistance in different genotypes of *Brachiaria* spp. to *O. Trichardti* and their yield potential across different environments in East Africa. Thirdly, the study aimed at assessing suitability of selected brachiaria genotypes as trap crops for stemborer *C. partellus*. Lastly, the study determined the effects of head space volatiles from different genotypes of *Brachiaria* spp. on behavior of *C. partellus* moths and its natural enemy, the parasitic wasp, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae).

The morphological and physiological responses of 18 brachiaria genotypes to drought were studied under simulated drought conditions in a screenhouse. The different levels of drought regimes administered to the plants were: well-watered (control) plants which were watered every 48 h to 100% field capacity and drought stressed plants in which watering was suspended for 14 and 28 days, representing moderate and severe drought conditions, respectively. The key findings of the study were as follows:

- Moderate drought conditions significantly and negatively affected ($P \leq 0.05$) brachiaria grasses' leaf length and width (area), leaf relative water content, chlorophyll content, and above ground biomass but did not affect shoot length and the number of tillers. Under severe stress, all the traits measured were significantly and negatively affected. Interactions between treatments and genotypes were only significant ($P \leq$

0.05) for relative water content and plant biomass at moderate drought stress conditions. Under severe drought stress, genotypic effects were more pronounced except for chlorophyll content, while genotype x treatment effects were only significant ($P \leq 0.05$) for number of tillers and relative water content

- Based on the PCA analysis, the best performers under moderate stress conditions were ILRI 13648, ILRI 11553 and Mulato while under severe drought stress, the best performers were Xaraes, Piata, CIAT 679, Marandu, Mulato II, and Mulato.
- Piata and Xaraes produced the highest biomass yield and outcompeted the popular commercial variety Mulato II.
- In conclusion, Piata and Xaraes are also comparatively drought tolerant and would suffer lower yield penalties in arid and semi-arid areas that experience frequent and severe drought conditions. Thus, they are of value in improvement of the sustainability of cereal-livestock farming systems under conditions of increasing aridification.

These genotypes were further evaluated to identify sources of resistance to *O. trichardti*, and to determine their adaptability to different environments in East Africa. Response to artificial infestation with *O. trichardti* was evaluated under controlled conditions in a screenhouse while adaptability to different environments and field resistance to mites was evaluated at three localities for two cropping seasons (2016 and 2017) under farmers' conditions. Leaf damage, chlorophyll content reduction, plant height, leaf area, number of tillers and shoot biomass were assessed as indicators of resistance to pest damage. The study highlights the following main points:

- In the screenhouse, significant differences ($p \leq 0.01$) in the degrees of spider mite damage to different genotypes were observed. This highlights the genetic variation that exists in brachiaria and the potential of exploiting it for host resistance IPM strategies. In a mean damage rating scale of 0-5, CIAT 679 ranked the lowest (0) while the highest score was in the susceptible check Mulato II (4.7). Piata, Xaraes, ILRI 13344 and ILRI 13810 showed low levels of damage (damage score = 1).

- Under natural infestation of spider mites in different environments, significant main effects ($P \leq 0.05$) of genotypes, localities and seasons were observed for all the traits evaluated. The two-way interaction between genotypes (G) and seasons (S) (GS) and genotypes by location (L) (GL) were also significant ($P \leq 0.05$).
- A simple correlation analysis showed that biomass yield was positively correlated with all the traits except the percentage leaf area damage and chlorophyll content reduction. However, a significant negative correlation was observed between percentage leaf damage ($P \leq 0.05$) and dry biomass yield ($P \leq 0.01$) indicating that the damaged caused by the mites on plants leaves translated into decreased biomass yield.
- Based on stability analysis of percentage of area of leaf damaged, ILRI 12991, Cayman, ILRI 13810, ILRI 12995 and ILRI 1553 were stable across locations and seasons (*b*-values close to 1). Genotypes CIAT 679, Piata, Xaraes, ILRI 12991 and Marandu exhibited low cultivar superiority index values (*Pi*) ($Pi \leq 35.03$) for leaf damage. Stable genotypes (*b*-values close to 1.0) regarding dry biomass yield were CIAT 679, ILRI 13810 and Mulato II. The lowest values of *Pi* for biomass yield were observed in Piata, ILRI 12991, ILRI 14807 and Mulato II (*Pi* 0 to 5.42).
- The conclusion of this study is based on the cultivar superiority index values and the scatter plots of leaf damage caused by spider mites and biomass yield. These ranked Xaraes, Piata, ILRI 12991 and ILRI 13810 as reliable genotypes that combined moderate resistance to the mite ($Pi \leq 48.0$) and high biomass yield ($Pi \leq 8.0$). These are potential candidates for improved forage yields in areas prone to *O. trichardti* infestation in Africa.

Plant species selected as potential trap crop must be preferred by the target pest to the high value crop being protected. The best trap crops are those which are attractive for oviposition but do not support development of the immature stages of the pest. Seven selected brachiaria genotypes were tested for preference by *C. partellus* moths and subsequent larval performance. The study revealed the following points:

- It was observed in two-choice tests with a local open-pollinated maize variety (cv. Nyamula) that significantly higher numbers of eggs were deposited on brachiaria genotypes Marandu, Piata, and Xaraes than on maize, whereas fewer eggs were recorded on plants of Mulato II, Mulato, and Cayman.
- The negative effects of leaf trichomes on preference of moths for oviposition was also revealed in the study.
- First instar larvae did not consume leaf tissues of brachiaria plants but consumed those of maize, which also suffered more stem damage than brachiaria plants. No larvae survived on brachiaria plant tissue for longer than 5 days, whereas 79.2% of the larvae survived on maize.

Lastly, the presence and effects of oviposition induced plant volatiles of brachiaria on *C. partellus* and its natural enemy *C. sesamiae* was determined. Four brachiaria genotypes were evaluated to determine the moths' preference for either previously oviposited or non-oviposited plants in two-choice tests. Response of *C. sesamiae* to head space volatile samples from oviposited and non-oviposited (control) plants were compared in four arm olfactometer bioassays. The main highlights were as follows:

- In a two-choice oviposition tests, higher percentages of eggs were laid on previously oviposited plants of Piata and Xaraes varieties, while non-oviposited plants of Mulato II were significantly preferred to previously oviposited plants.
- Female *C. sesamiae* spent significantly more time on volatiles from previously oviposited and non-oviposited plants of all varieties except Marandu

- Attractiveness of headspace volatiles, both oviposition-induced and constitutive, from most of the genotypes highlights the value of these grasses in stemborer management strategy that applies tritrophic interaction with the pest's natural enemies.

7.2 Recommendations and future research needs

Some of brachiaria varieties that were identified in this study could be exploited in small-holder cereal livestock-based farming systems in different agro-ecologies that experience frequent and severe drought conditions and increased pest attacks. To further exploit and optimize the potential of these grasses the following research needs are highlighted:

- To fully evaluate the value of such selected genetic materials, consumer-preference should be an integral part in crop evaluation. Therefore, we recommend evaluation of the candidate genotypes in a farmer participatory approach.
- Genotypes, for instance CIAT 679, that display complete resistance to the spider mites but performed dismally regarding yield could be of importance as parental breeding material for combination of yield and resistance to *O. trichardti*. This underscores the need for researchers to work closely together across multiple disciplines such as entomology and plant breeding to develop better hybrids.
- The role of the host plant's leaf trichomes on oviposition preference by stemborer on brachiaria grasses was postulated in the study. There is, therefore a need to investigate the potential of leaf trichomes as a first line of plant defense mechanism that maize could employ against the stemborers.
- There is a need for identification of volatile chemicals responsible and mechanisms underlying observations made from behavioural analysis of *C. partellus* and *C. sesamiae* with the volatile chemicals from brachiaria plants.
- Furthermore, additional roles of constitutive or general cues in host location by the parasitoid need to be elucidated.