WAGENINGEN UNIVERSITY

LABORATORY OF ENTOMOLOGY

Performance and behaviour of the leafhopper (*Maiestas banda;* Homoptera: Cicadellidae) vector of the Napier Stunt Disease on Napier Grass (*Pennisetum purpureum*)



Report Number: 014-47
Name: Mabel Chigora
Study Programme: MSc Plant Sciences
Period: 2 – 6
Thesis Code: ENT-80436 MSc Thesis Entomology
1st Examiner: Marcel Dicke
2nd Examiner: Zeyaur Khan

icipe
African Insect Science for Food and Health

PERFORMANCE AND BEHAVIOUR OF THE LEAFHOPPER (*MAIESTAS BANDA*; HOMOPTERA: CICADELLIDAE) VECTOR OF THE NAPIER STUNT DISEASE ON NAPIER GRASS (*PENNISETUM PURPUREUM*)

Mabel Chigora

MSC Thesis: Laboratory of Entomology

ENT-80436

August 2014

Supervisor:	Prof. Dr. Zeyaur Khan Head, Plant Health Department International Centre of Insect Physiology and Ecology, Kenya
Co-supervisors:	Dr. Charles Midega Senior Scientist, Plant Health Department International Centre of Insect Physiology and Ecology, Kenya
Examiner:	Prof.Dr. Marcel Dicke Head, Laboratory of Entomology Wageningen University, The Netherlands

WAGENINGEN UR
For quality of life

icipe
African Insect Science for Food and Health

FOREWORD

This report was written as my major thesis report in fulfilment of the requirements of my Master of Science in Plant Sciences studies under the Laboratory of Entomology at Wageningen University. The research work was conducted in western Kenya at the International Centre of Insect Physiology and Ecology (ICIPE) Thomas Odhiambo Campus, Mbita Field Station. The field work was carried out from March to July 2014 within a project funded by the McKnight Foundation, USA, seeking to develop a management approach for the Napier stunt disease in eastern Africa.

Mabel Chigora

ACKNOWLEDGEMENTS

For this thesis to be a success l owe my appreciation to a number of people. I wish to thank Prof. Marcel Dicke (Wageningen University) for the academic papers, ideas and road map. I also extend my gratitude to Prof. Z. Khan (ICIPE) and Dr. C. Midega for hosting me at their institute and for allowing me to use their premises and resources. I also wish to thank Karen and Frank for helping me edit my document. I also wish to extend my gratitude to E. Kadivai, Hamzah, Raphael and Silas who helped me with hands on setting up of all the experiments from planting up to the end. I also wish to thank Carol for housing me in Mbita and for the girl talk when everything was not taking shape. To everyone at ICIPE who helped in one way or the other thank you.

I also wish thank my family and friends for believing in me and for all the encouragements during the entire study period. Lastly, l thank God for the strength and protection during the entire study period.

DEDICATION

To my late mom who has always been a source of inspiration for each achievement in my life, thank you for sowing the seed of determination, perseverance, hardwork and endurance.

ABSTRACT

Napier grass remains an important strategic grass species in east African mixed crop-livestock systems. However, production of several varieties of the grass remains constrained by phytoplasmic disease, Napier stunt disease (NSD), which is transmitted by the leafhopper, *Maiestas banda.* For smallholder farmers of western Kenya who rely on the grass for livestock feed and pest control in push-pull farming systems, effective control strategies of the disease and the vector remain elusive. With a view to understanding potential host plant resistance mechanisms against the leafhopper, I investigated growth, development and behaviour of the leafhopper on a variety of host plants: Pearl millet and five Napier grass varieties; susceptible Bana, resistant Kitale A, Kitale B, South Africa and Ouma 2. To determine performance and behaviour of *M. banda* on susceptible and resistant varieties, the following experiments were conducted: a) host selection and preference in a choice test using i) live plants and ii) plant volatile organic compounds (VOCs) collected using head space sampling. b) determination of feeding through honeydew excretion c) population development. On host selection based on volatiles and choice tests between Bana and the other varieties there was no preferential selection by *M. banda* to settle or go for susceptible Bana than the resistant varieties. There were no significant differences in honeydew excreted between susceptible and resistant Napier variety-fed insects. Significant differences in honeydew excretion were only found between Bana and pearl millet, both susceptible to the pathogen. Population development was not different between the insects raised on Bana and those raised on Kitale A, B, South Africa and Ouma 2. However, insect numbers were significantly higher on pearl millet. This study therefore did not show any significant differences in performance and behaviour of *M. banda* on resistant and susceptible varieties when the three insect behaviour attributes were studied.

Table of Contents

1 INTRODUCTION

Napier grass (*Pennisetum purpureum*: Schumach) is a grass native to the grasslands of Africa (Farell *et al*, 2002). It is commonly known as Elephant grass or Uganda grass among others. The grass has been successfully cultivated in the past in western Kenya. However, production has been under threat since 1997 (Jones *et al*., 2004) when a deadly disease called Napier Stunt Disease (NSD) was found to attack Napier grass. The disease is caused by a phytoplasma 16SrXI (Jones *et al*., 2007) which is known to be transmitted by the leafhopper, *Maiestas (=Recilia) banda* in Kenya (Obura *et al*., 2009).

1.1 Napier Grass

Napier grass is a robust perennial grass which grows to a height of about 4 meters when mature and has up to 20 nodes (Henderson and Preston, 1977). Generally its seeds have a low genetic stability and viability (Humphreys, 1994) which is the reason why it is mostly vegetatively propagated. The grass has an extensive root system which enables it to forage for nitrogen (FAO database) whilst the deep root system is good adaptation for drought tolerance.

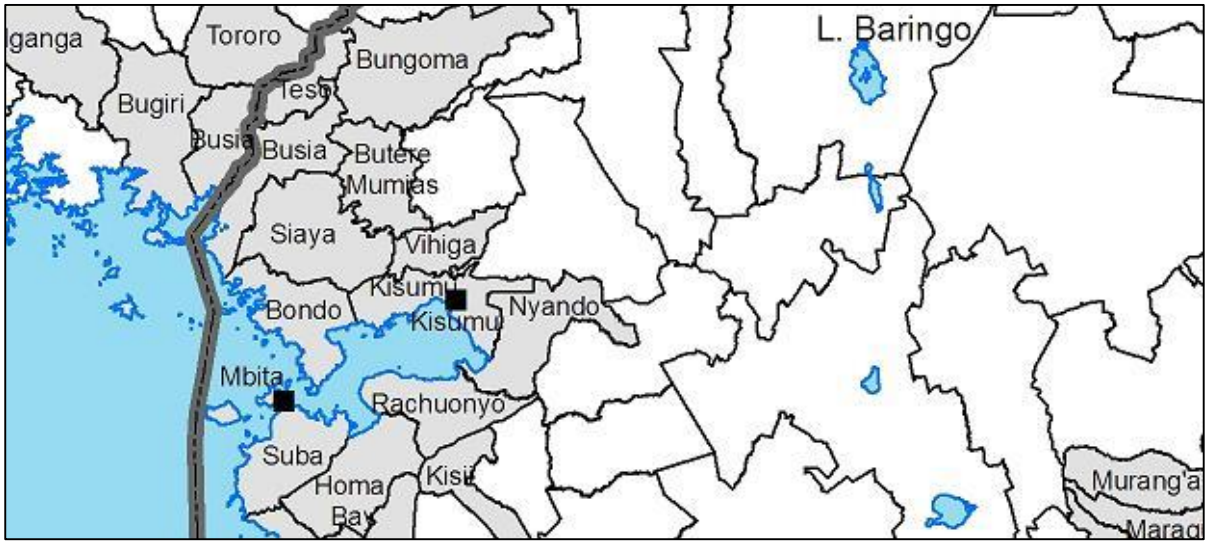

Figure 1a: Map of Western Kenya showing Napier grass production areas

The grass has been mainly adopted as fodder especially for dairy cows in east Africa (Kenya and Uganda but also in Tanzania and Ethiopia) by smallholder livestock farmers. Most of the Napier grass in Kenya is grown in the western part of the country in areas shown on the map (Fig 1a) above and also in the Rift Valley. The ever increasing population in east Africa is making it virtually impossible to raise livestock through open grassland grazing. In Kenya, an average farm holding size is 0.9 -2 ha (Gitau *et al.*, 1994) and 80 % of smallholder dairy farmers have resorted to zero-grazing feeding of their livestock (Staal *et al.*, 1998). Napier grass has made it practically possible for the smallholder farmers to adopt the zero-grazing livestock feeding method because of its biomass yield, perennial nature, high nutritive value and high pest resistance (Bhandari *et al.*, 2006). Napier grass constitutes 40 – 80 % on average of the forage used in east

Africa by smallholder dairy farmers (Arocha *et al*., 2009). Napier also increases the milk production yields as dairy cows are fed on Napier grass produce an average of 7–10 litres of milk compared to less than 6 litres/cow/ day when given natural pastures (ASARECA, 2010).

Napier grass also has other good agronomic qualities when compared to other tropical grasses. Among these are its high yielding capabilities such that it can yield 50-100 tonnes of green matter per hectare per year if recommended agronomic practices are followed (Muyekho *et al*., 2003). When compared with other potential tropical fodder grasses Napier grass is a good yielder in terms of dry matter yields (Humphreys, 1994; Skerman & Riveros, 1990). According to Wouters, 1987, the average dry matter yield of Napier grass from different regions of Kenya is about 16 tonnes/ha/year when no or little fertilizer is used. These yields are much higher when compared to Rhodes grass (*Chloris gayana*), Setaria (*Setaria sphacelata*) and Kikuyu grass (*Pennisetum clandestinum*) which are popular pasture grasses also but yield between 5 to 15 tonnes of DM per year (Boonman, 1993). Napier grass is also preferred agronomically because of its rapid regeneration, easy establishment, drought tolerance and pest resistance (Bhandari *et al*., 2006) among others.

Napier grass besides being the major fodder crop is also used in crop protection of cereals in the 'push-pull' technology (Khan *et al*., 2008). This technology has been adopted by more than 50, 000 smallholder farmers in the western Kenyan region (www.push-pull.net) and the numbers continue to increase as shown in Fig 1b. In this technology which involves crop protection based on non-chemical cereal pest management; Napier grass is used as a trap 'pull' crop that is planted as a border crop and attracts pests especially stem borers out of cereal fields (Fig 1c). Napier grass lures stem borers out of maize fields as the pests prefer the plant for oviposition more than maize. However, the grass produces sticky sap as response to the feeding larvae and about 80 % stem borer larvae do not survive (Cook, 2007).

Other benefits of Napier grass include generating extra revenue as it is sold in village market places, (Abate, 1992), soil conservation (wind breaking) and also as a source of organic manure

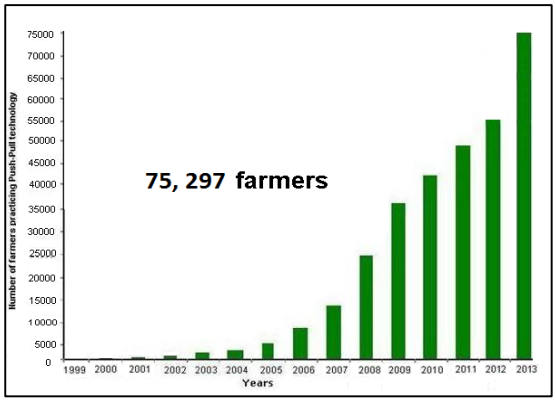

Figure 1b. Number of farmers using the push-pull technology in East Africa as at long rainy season of 2013


Figure 1c. Napier grass' use as a border 'pull' in push-pull technology

1.2 Napier Stunting Disease

Despite the successful cultivation of Napier grass for decades Napier Stunting Disease (NSD) has compromised production since it was first reported in western Kenya in 1997 (Jones *et al.*, 2004). Since then some small holder farmers have recorded yield losses of up to 90 % (Lusweti *et al*., 2004). The disease which becomes visible especially in re-growth after cutting or grazing is caused by a phytoplasma of the 16SrXI group and the pathogen is mainly transmitted by a leafhopper, *Maiestas* (=*Recilia*) *banda* Kramer (Obura *et al*., 2009). The disease can also be transmitted vegetatively through infected Napier grass cuttings. The disease is a big threat to Napier grass production and also food security since Napier grass is the main fodder crop for over 70 % of smallholder livestock farmers in Western Kenya and provides over 40% - 80% of forage used in the Western Kenyan region (Potter, 1987; Staal *et al.*, 1998; Arocha *et al*., 2009). The disease outbreak has been attributed to extensive cultivation of this grass for fodder and crop protection (www.push-pull.net). The biggest threat that the disease poses is being transmitted to food crops. Some artificial transmission experiments done showed that other food crops like finger millet, rice, sugarcane had the ability to acquire the pathogen (Weintraub and Jones, 2010). Therefore if resistant Napier grass varieties are adopted there is a risk of a strong selection pressure which may lead to the emergence of virulent biotypes (Lombaert *et al*., 2009) and spread of the disease to food crops, especially if the material is resistant to the insect vector. Napier Stunting Disease like most plant diseases is as a result of a three way interaction of the vector-phytoplasma-plant relationship. The life cycle is as shown below in Fig 1d.

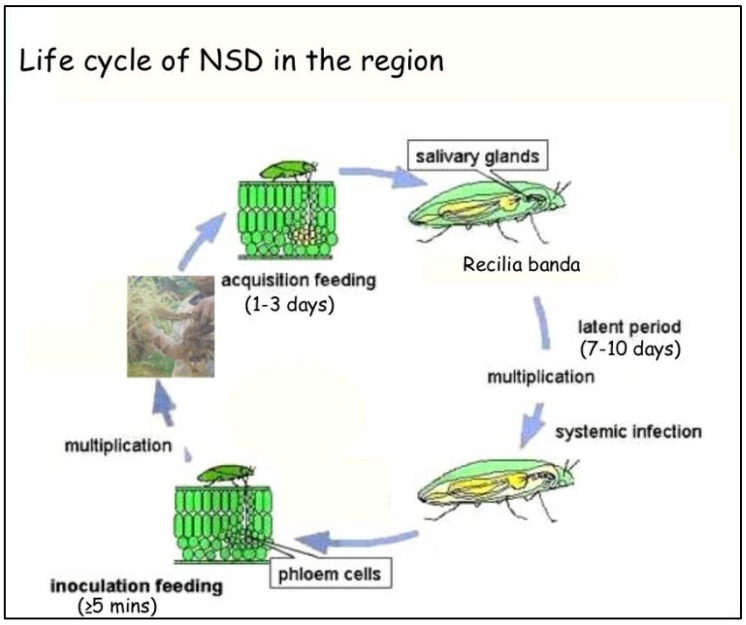

Figure 1d: Lifecycle of Napier Stunting Disease as caused by phytoplama 16 SrXI and transmitted by a leafhopper, *M. banda*

1.3 Symptoms of Napier Stunt Disease (NSD)

The 16 SrXI phytoplasma is a pleomorphic bacteria, which lives in the phloem of Napier grass (IRPCM, 2004). This phytoplasma modifies the carbohydrate translocation in the

plant and when the titre is high causes symptom development. In most grasses, phytoplasma infection results in yellowing and a bushy growing habit. NSD results in development of little leaves and lethal yellowing, profuse tillering and shortening of internodes to the extent that the whole stool is severely stunted (Fig. 1e). There is low-biomass yield due to the bushiness and shortening of internodes.

Figure 1e. Napier Stunt diseased plants showing symptoms

Figure 1f. Healthy Napier grass with long internodes and green leaves

1.4 Transmission by vector

As mentioned before the pathogen 16SrXI causing NSD is transmitted by a leafhopper, *M. banda (*Obura *et al.,* 2009*).* Most phytoplasma disease systems that have been studied show a specific sequence of events for effective transmission of the pathogen to new hosts. As shown in Fig 1d. above an uninfected insect upon feeding on the phloem of an infected plant, obtains amino acids and sugars and in the process ingests phytoplasma particles residing in the phloem. The acquisition access period (AAP) which is the feeding duration that is necessary to acquire a sufficient titre of phytoplasma (AAP), is 1-3 days in *M. banda*. Upon ingestion the phytoplasma particles then penetrate the insect midgut cells and move into the insect haemocoel, and are transported throughout the insect body with haemolymph. When the phytoplasma particles reach the salivary glands, they penetrate the gland cells, where they can multiply. This latent period (period of time that elapses from initial acquisition to ability to transmit) is about 7-10 days with 16SrXI in *M. banda*, the phytoplasma particles invade the insect tissues and multiply. Phytoplasma transmission is by a persistent propagative manner in the vector (Murray and Schleifer, 1994). The insect will now go on to feed on a healthy host and during feeding will release phytoplasma with saliva secreted from the salivary glands when feeding. This inoculation feeding in the vector of concern is ≥ 5 minutes. The phytoplasma then resides in the plant host and begins to multiply, after which the plant begins to show symptoms. When the titre of phytoplasma is sufficiently high, the infected plant can now serve as an acquisition host for any vector feeding on it.

Pathogen transmission by insect vectors is generally dependent on the abundance of the insect vector and inter-plant movements (Irwin and Ruesink, 1986). The abundance and movement are in turn affected by plant traits like age (Atakan, 2011), host plant density (Power, 1987), vectors' gender (Beanland *et al*., 1999) and also by environmental factors such as temperature, precipitation.

1.5 The vector, leafhopper (*M. banda*)

M. banda (Fig 1g) is a leafhopper in the family Cicadellidae, subfamily Deltocephalinae, and tribe Deltocephalini. Previously known as *Recilia banda*, *Maiestas banda* was transferred from the former genus (Webb and Viraktamath, 2009). It is a small leafhopper with triangularly produced vertex (Obura *et al*., 2009). Generally leafhoppers of this tribe are widely distributed and are Gramineae grass feeders feeding on rice, maize, wheat, sorghum, sugarcane and other wild grasses (Satoshi, 1999).

Figure 1g: The leafhopper vector, *M. banda* (photo by *ICIPE*)

It has been found that *M. banda* is the vector of this particular phytoplasma, 16SrXI. In Weintraub and Beanland's (2006) review they pointed out that most phytoplama vectors possess several similar characteristics that make them efficient in transmission:

(i) they are Hemimetabolous; nymphs and adults having similar feeding characteristics, found in the same physical location and can both transmit the phytoplasma.

(ii) their feeding is specific and selectively on certain plant tissues, which makes them efficient in transmitting pathogens residing in those tissues. In most cases feeding is not destructive which promotes successful inoculation without damaging conductive tissues and eliciting defensive host plant responses;

(iii) their relationship with the vector is propagative and persistent and

(iv) they can pass phytoplasmas transovarially to their offspring

1.6 The Phytoplasma, 16SrXI

As mentioned above NSD is caused by a phytoplama 16SrXI 'Candidatus Phytoplasma *oryzae'*. Plant- pathogenic phytoplasmas are a group of obligate, intracellular prokaryotic wall-less bacteria which cannot be cultured in-vitro and belong to the class

Mollicutes (Sears and Kirkpatrick, 1994). 16SrXI like most phytoplasmas reside exclusively in the phloem sieve tube elements, and are therefore transmitted by leafhoppers which are sap-sucking insects feeding from the phloem (Weintraub and Beanland, 2006). Upon entering the phloem sieve tubes the phytoplama spreads throughout the plant systematically by passing through phloem sieve plate pores. Some studies have shown that in phytoplasma infected plants, there is inhibition of transportation of phloem. Changes occur in phloem translocation between the source and sink (Guthrie *et al*., 2001; Maust *et al*., 2003) and other impaired physiological functions, such as reduction of photosynthesis, stomatal conductance and plant hormonal imbalances. These might be a result of phloem dysfunction and could explain the symptoms on infected plants (León *et al*., 1996; *Choi et al*., 2004).
Napier stunting disease is not only posing threat to Napier grass but they are growing fears of the phytoplasma infecting other food crops (Jones *et al*., 2007). According to some phylogenetic analysis of 16S rDNA of Napier stunt phytoplasma in Kenya (Jones *et al*., 2004) and Uganda (Nielsen *et al*., 2007) showed that strains were very closely related to phytoplasmas of Sorghum grassy shoot, sugarcane yellow leaf and rice yellow dwarf among others.

The phytoplasma 16SrXI is maintained by a natural disease cycle just like any other phytoplasma consisting four components: the phytoplasma as the causative agent; Napier grass – the host plant; leafhopper - vector transmitting the pathogen and alternative grasses which are reservoirs of the phytoplasma inoculum.

1.7 Disease Control

Control of phytoplasma diseases has proven difficult because phytoplasmas cannot be cultured in-vitro and also because of their life cycle. Therefore firm data on phytoplasma pathogenicity, phytoplasma–host interactions and the molecular basis of resistance are sparse. Insecticides are less opted for by these smallholder farmers as most of them are expensive and beyond the reach of many (www.push-pull.net). According to Firrao *et al*. (2007), spraying insect vectors with insecticides is not very effective because disease severity intensifies despite extensive use of these chemicals. Also many negative effects of conventional insecticides on the environment have been noted which has resulted in them being used as a last resort.

Farmers in western Kenya are therefore mostly encouraged through training from ICIPE to control the disease through phytosanitary measures since its reproduction is mainly through vegetative propagative means. Other useful recommended cultural practises are rouging and weed control. There has been a lot of work being done of late at ICIPE in trying to come up with resistant genotypes. A lot of resistant genotypes are still under study to understand the plant-vector relationship. As such this research focuses also on biological control with focus on some of these resistant genotypes.

Use of resistant plant varieties has been keenly welcomed as an environmentally friendly insect pest control method. Therefore, the current study sought to explore host

plant resistance by understanding the mechanisms behind host-plant resistance of some resistant cultivars and how this can be used in controlling the Napier stunting disease through the understanding of host-vector relationship. As such, the current research seeks to understand the host-vector relationship, with particular focus on the performance and behaviour of the insect vector on resistant and susceptible Napier grass varieties and Pearl millet. Another aim was to establish the underlying mechanisms of resistance, however, this could not be achieved due to time constraints.

An insect resistant plant can be defined as the one that has received heritable plant qualities that result in its being relatively less damaged than a plant without the qualities upon insect/pathogen attack (Panda and Kush, 1995). The general understanding is that; Insect-resistant crop varieties suppress insect pest abundance or increase the damage tolerance level of the plants. Host plant insect resistance can be broadly classified under three of the following mechanisms:

Antibiosis:

Antibiosis resistance reduces pest abundance by affecting the biology of the insect and therefore subsequently reduces the damage on a resistant variety compared to on a susceptible variety. Antibiosis often results in increased insect mortality, or retarded growth and reduce the insects' reproduction. In some cases, antibiotics may involve morphological, physiological and biochemical features of the host plant (Kogan and Ortman, 1978; Heng-Moss *et al*., 2003).

Antixenosis (Non-preference Resistance):

Antixenosis resistance also known as non-preference in general affects the desirability of the resistant plant to the insect pest. With antixenosis the non-prefered plant provides a stimuli that is unattractive to the pest or completely fail to provide some stimuli that are attractive to the pest. Various plant characteristics or features are associated with antixenosis and these are mainly morphological and chemical features which include: colour, light penetration, hairiness, leaf angle, odour and taste (Kogan and Ortman, 1978; Heng-Moss *et al*., 2003).

Tolerance: is resistance which involves the plants' response to an insect pest. Unlike antibiosis and antixenosis, tolerance differs in how it affects the insect-plant relationship. In this resistance the resistant plant recovers or remains healthy and yields well despite having same levels of insect attack as the susceptible variety. Tolerance of a variety is usually measured in terms of rejuvenation potential, healthy leaf growth, flowering compensation potential and superior plant vigour (Kogan and Ortman, 1978; Heng-Moss *et al*., 2003).

2 PROBLEM STATEMENT

Napier grass had been under cultivation successfully until 1997, when NSD was found to be a threat to production with yield losses of upto 90 % (Lusweti *et al*., 2004). Napier grass is the main fodder for smallholder dairy farmers in East Africa but is also used for many other uses. Some of which include their use in crop protection, its commercial value, soil conversation and many others. Therefore Napier grass offers many advantages to smallholder farmers in Western Kenya. For these reasons farmers' willingness to adopt other fodder crops have generally been negative according to researches done at ICIPE (not published) research. Also most farmers are not willing to downscale their Napier grass plots as it brings them extra revenue and is the major fodder crop they rely on and can be grown perennially. These reasons and Napier grasses' high yields are the reasons why most farmers are not willing to adopt other grasses as fodder.

The continued extensive cultivation of Napier grass will result in continual disease outbreaks unless the disease is controlled. One control measure will be the use of chemicals however, most Western Kenyan farmers are smallholder farmers who have limited access to capital and pesticides. The need to come up with disease control measures that are more environmentally friendly and are less harmful to the environment has probed the need to use NSD resistant cultivars. Also according to Firrao *et al*., (2007), severity of phytoplasma diseases intensify, despite the extensive use of insecticides hence insecticides do not offer the best results for pest control. However, there is fear of the disease severity increasing because of: cultivation intensification, limited diversity of animal feed, climate change, intensification of dairy farming and promotion of zero grazing

Use of resistant or tolerant plant varieties will therefore be especially useful in Western Kenya and other parts of East Africa were Napier grass is mainly grown extensively. Thus the need to understand the host-vector relationship of the resistant and susceptible varieties which are not yet published will be essential. Understanding the host-vector relationship is especially important as tolerant varieties will be more preferable as there are fears that completely resistant plants will result in a strong selection pressure which may lead to development of more virulent biotypes or hoppers opting for food crops.

Knowledge of the performance, behaviour and biology of the vector, *M. banda*, on susceptible and resistant Napier grass varieties is therefore important for further understanding of disease transmission, resistance and susceptibility. The later use and adoption of resistant cultivars underlies this study as we seek to understand the host-vector relationship.

The resistance there of which can be scored through evaluating how much damage the insect has inflicted on the plant in terms of yield, growth, plant vigor and appearance. Resistance can also be assessed through the insect establishment on the plant itself (Saxena, 1969). The degree of insect establishment will be determined through measurement of insect responses. According to Saxena 1969 and Saxena *et al*.,

1974, the most important behavioural and physiological responses important during insect establishment on plants can be grouped into six main categories. (1) orientation and settling, (2) feeding, (3) metabolism of ingested food, (4) growth, (5) adult survival and egg production, and (6) oviposition. A plant will be rendered resistant if there is an interruption of any one or more of these insect responses due to its unfavourable characteristics.

It was therefore important to investigate the different insect responses (orientation, feeding, growth, survival and reproduction and egg hatching) in order to determine resistance or susceptibility of the varieties under investigation. Various interactions between Napier grass infected with the stunted disease and vector are not well known. For this reason the insect responses mentioned above will be looked at under the following research questions.

2.1 General Objective

The general objective of this study was to evaluate performance and behavioural responses of leafhopper vector, *M. banda*, to Napier varieties; resistant and susceptible to Napier Stunt Disease. This information will be important for wide-scale screening for disease resistance in Napier grass varieties.

The purpose of this study was to assess the behaviour and biology of the leafhopper, *M. banda* on resistant and susceptible Napier grass varieties.

2.1.1 Research Questions

Are there any differences in insect behaviour and biology on susceptible and resistant cultivars when the following are considered?

- Host selection and preference based on choice test
- Host selection based on volatiles / odours
- Feeding
- Population Development

A resistant plant is one where the insects' response is attenuated in any one of the above behavioural and biological elements. Therefore the present study seeks to investigate how Napier grass varieties with diverse resistance genes influenced behaviour and biology of *M. banda*.

3 MATERIALS AND METHODS

3.1 Study site

This study was carried out at International Centre of Insect Physiology and Ecology, Mbita Point, in western Kenya. The site lies at (0°25′ S, 34°12′ E) in Suba district. All the plants were grown in insect-free screen houses and the insects were also reared in screen houses at 20–28°C and 65–70% RH.

3.2 Planting Material

Five Napier grass varieties and pearl millet were planted in pots of 20 cm in diameter and in cups of 8cm diameter. Thirty plants were planted every seven days consecutively. For each of the six genotypes there were five replications each week. A total of 60 plants were planted for the reproduction assay (10 replications per variety); 36 plants for the volatile assay (6 plants of each variety); 36 plants for the multiple choice test (6 replications per variety) settling response; 25 plants for the paired choice test (5 replications for each variety) settling response. For the feeding bioassay 36 plants were planted in cups (6 replications per variety). Root splits and stem cuttings were used as the propagative material for the five Napier varieties (Bana, South Africa, Ouma 2, Katali 1 and Katali 2) and seeds were planted for pearl millet (Fig 3a and 3b). The seedlings were kept in the insect free screen house and were watered every two days. The screen house had 65-75% RH and temperatures ranging, 20-28°C.

Figure 3a. Napier grass planting material (cuttings)

Figure 3b. Pearl millet seeds

3.3 Insect Rearing

M. banda used in all the experiments were reared on pearl millet in cages (Fig 3c) in the virus free insect rearing at ICIPE Mbita Point, Kenya. For all the experiments only the adult female hoppers were used.

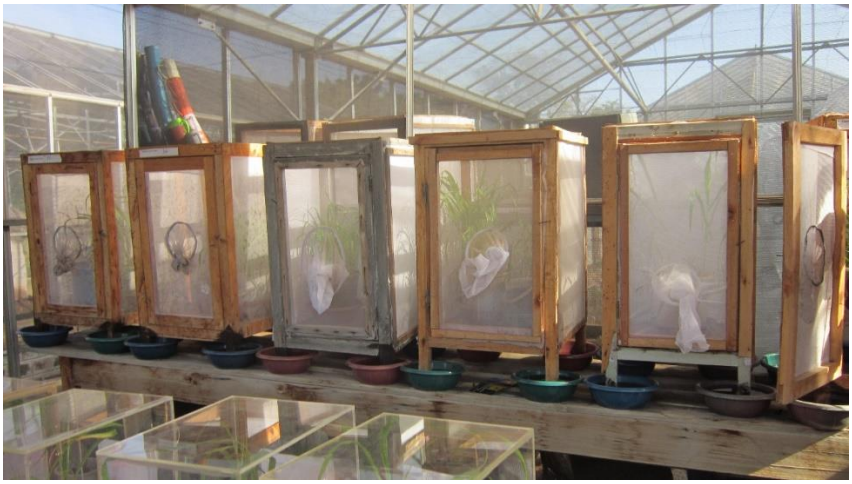

Figure 3c. *M. banda* rearing cage with Pearl millet

3.4 BIOASSAYS

For leafhopper performance and behaviour every plant was considered one replicate. Results were first calculated per plant and means and S.E were calculated over all plants.

3.4.1 Orientational and Settling Responses: Multiple choice

To determine the orientational and settling responses of *M. banda* on Napier grass and pearl millet, Khan and Saxena's, 1985, protocol was followed. The leafhoppers were provided with a choice of the five Napier test varieties and pearl millet. The plants in cups were randomly but equidistantly placed from the centre of a nylon mesh wooden cage with a median hole through which 60 insects were introduced (Fig 3d). Each cage had 6 plants = 1 replication and there were 6 replications. The number of individuals that settled on the different varieties was recorded at 1-, 4-, 8-, 24- and 48 hour time intervals. The percentages of insects settling on the different plants at the different time intervals were calculated (Saxena and Khan, 1985).

Figure 3d. *M. banda* in a multiple choice test.

3.4.2 Orientational and Settling Responses: Two-Choice test

M. banda besides the multiple choice test was also given a two-choice test to settle on either Bana or the other four Napier grass varieties (S. Africa, Kitale A, Kitale B and Ouma 2) and pearl millet. In each cage was Bana and one of the other test varieties

(choice test) (Fig 3e). There were five replications of each varietal combination with Bana. 20 female adult hoppers that had been starved for four hours prior infestation were then put in each cage. The number that settled on either Bana or the other test varieties was recorded at 1-, 4-, 24- and 48h time intervals. The percentage of hoppers settling on each of the plants was then calculated.

Figure 3e. Two-choice tests between Bana and resistant varieties

3.4.3 Volatile collection

Volatile collection and the four-arm olfactometers' procedure followed Tamiru *et al*, 2011, protocol. Volatile compounds from whole Napier grass plants (Fig 3f), for use in subsequent bioassays and chemical analyses. Prior to volatile collection six healthy plants of each variety (P. millet, S. Africa, Ouma 2, Kitale A, Kitale B and Bana) were selected (50-60 days old). The plant volatiles were entrenched for 48 hours (Fig 3f). Leaves of plants were enclosed in polyethyleneterephthalate (PET) bags (volume 3.2 L, ~12.5 mm thickness) heated to 160 ^{0}C for an hour before use and fitted with Swagelock inlet and outlet ports. Charcoal-filtered air was pumped (600mL min^{-}1) through the inlet port. Volatiles were collected on Porapak Q (0.05 g, 60/80 mesh; Supelco) filters inserted in the outlet port through which air was drawn at 400mL min^{-}1. After entrainment, volatiles were eluted with 0.5 mL dichloromethane.

3.4.4 Four-arm olfactometer bioassay

A four-arm olfactometer (Pettersson 1970) (Fig 3.g) was used to investigate *M. bandas'* response to odours drawn directly from whole plants. Responses of hoppers to volatiles were tested in a Perspex four-arm olfactometer (Pettersson 1970). Air was drawn through the four arms towards the centre at 260mL min^{-}1. Volatile samples (10 µL aliquots) were applied, using a micropipette (Drummond 'microcap', Drummond Scientific Co., Broomall, PA, USA), to a piece of filter paper (4 x 25 mm) subsequently placed in an inlet port at the end of each olfactometer arm. Adult female hoppers were transferred individually into the central chamber of the olfactometer (Fig 3g) using a custom-made piece of glass tubing. Time spent in each olfactometer arm was recorded

with 'Olfa' software (F. Nazzi, Udine, Italy) for 12 min. The experiments were replicated at least 12 times. Two tests were carried out. First, was the non-choice test to determine if Napier grass was attracted to the Napier grass varieties odours at all. Second, to determine if hoppers were more attracted to susceptible Bana odour or to any of the resistant varieties odour. On two opposite arms were Bana and either of the resistant varieties. On the other two opposite arms were the solvent.

Figure 3f. Volatile entrenchment kit (2 plants per kit). Entrenchment was 48 hours

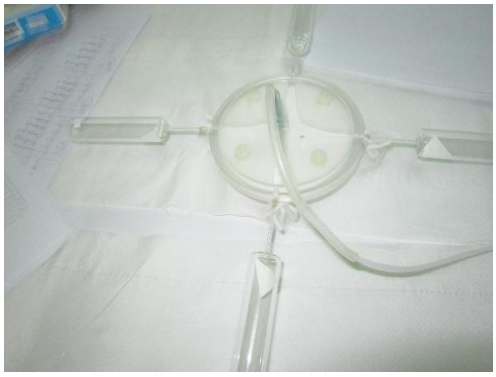

Figure 3g. Four-arm olfactometer. Hopper was released at the centre volatiles eluted on the filter papers

3.4.5 Honeydew excretion (Feeding)

To determine the feeding extent plantlets aged between 20-30 days were used. There was one plantlet in each cup and on each cup an 11cm petri dish was placed at the base of each plantlet as shown in Fig 3h then enclosed in a 1litre plastic bottle. Meanwhile, 60 female adult *M. banda* leafhoppers were collected from the mass rearing cages and starved for 2 hours. Ten hoppers were then released on each plantlet. The honeydew excreted by the hoppers dropped on the filter paper disks and was readily absorbed for 24 hours. After 24 hours the filter paper disks were collected and treated with ninhydrin in acetone solution. The filter papers were oven dried for 30 min at 50°C and the honeydew spots that developed were traced on tracing papers. The area was measured in square millimetres using suitable graph paper.treated with SIGMA ninhydrin solution.

Figure 3h. Honeydew excretion for 24 hours on filter paper

3.4.6 Population Development

Potted 40-50 day-old plants were covered in perforated bottles covered with nylon mesh on two sides for aeration (Fig. 3i). The plants were arranged in a randomized block design on each bench. They were 10 replications of each variety, each pot was infested with six gravid females. The number of nymphs and adults were recorded at 30 and 60 days after infestation.

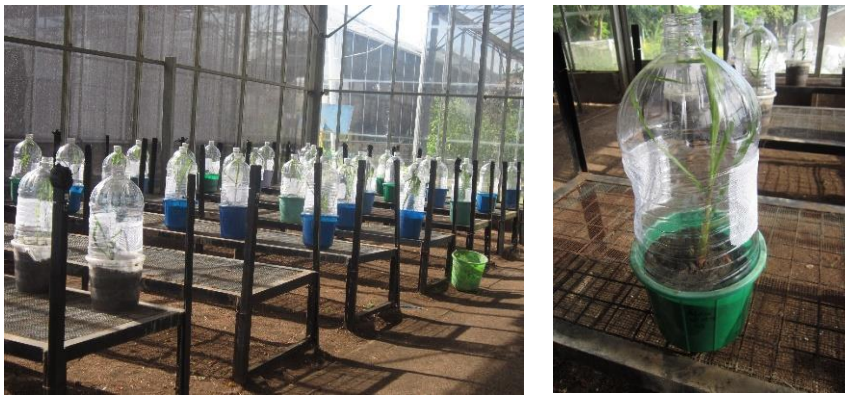

Figure 3i. Population development assay

3.5 Statistical Analysis

To study the effect of genotype on the behaviour and biology of *M. banda*; 4 assays were done and for each of these we will test whether the genotype had an effect on:

- Population development
- Host selection and preference in a settling behaviour bioassay
- Volatile attraction, preference and selection
- Feeding and ultimately honeydew excretion

Analysis was done on both resistant and susceptible varieties. Data was analysed using Genstat version 15.0. The Shapiro-Wilk test for Normality was used to test for normality of data (when data was normal $P>0.05$). The Bartlett's test for homogeneity of variances will be used to check for homogeneity of variances (variances are homogenous when

P>0.05). Data was $\log_{10}$ transformed when it did not satisfy the two tests for Normal distribution. To determine differences within the data was analysed using one-way ANOVA's and when the data did not satisfy conditions for ANOVA after transformations the Kruskal-wallis one-way anova was then used. For pairwise comparisons one-way ANOVA and Mann-Whitney U test were used. For the pairwise comparisons using the four-arm olfactometer data was analysed using SPSS version 20 (paired t-test).

4 RESULTS

In this study pearl millet and 5 Napier grass varieties (Bana, Kitale A, Kitale B, Ouma 2 and South Africa) were used. Kitale A and B and Ouma 2 are local Kenyan varieties whilst South Africa is a South African variety and Bana is a commercial hybrid between the annual babala (*Pennisetum americanum*) and the perennial Napier grass (*Pennisetum purpureum).* The study was seeking to see how the leafhopper, *M. banda* performs and behaves on the six genotypes when a) host selection based on i)orientation and settling on live plants ii) host plant volatile preference b) feeding based on honeydew excretion and c) population development were looked at. In each of the experiments different number of hoppers were used.

4.1 Orientation and Settling Behaviour

The results of orientation and settling behaviour are presented in the graphs and tables below. Data was analysed by doing pairwise comparisons using the Mann-Whitney U test.

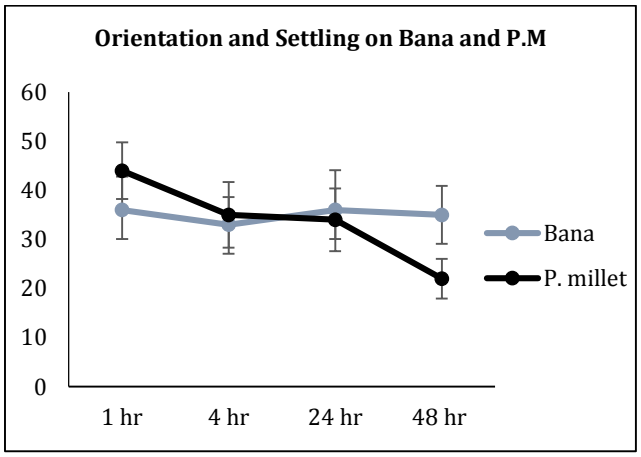

Figure 4.1a: Percentage numbers of *M. banda* settling on Bana and pearl millet at 1-, 4-,24- and 48hrs.

Table 4.1a: Settling response of *M. banda* on Bana (susceptible control) and pearl millet plants 1-, 4-, 24- and 48-hours
after infestation

	Females (%) settled on plants[1]			
Variety	1 hr	4 hr	24 hr	48 hr
Bana	36 ± 6.782	33 ± 5.612	36 ± 8.124	35 ± 5.916
P. millet	44 ± 5.788 ns	35 ± 6.708 ns	34 ± 6.403 ns	22 ± 4.062 ns
P value	0.452	0.778	0.873	0.111

[1] Average of 5 replications, each replication had one healthy plant and 20 female adult hoppers.
ns = not siginificant, ** = significant < 0.05 Mann-Whitney U test

On the first hour after infestation more hoppers settled on pearl millet though not significantly (Figure 4.1a and Table 4.1a). However, at final settling more hoppers preferred Bana although, there were no significant differences in numbers. There were no significant differences in the number of vectors that settled on Bana from those on pearl millet as shown by the p-values in table 4.1a above (Appendix 2) after 1-, 4-, 24- and 48 hours.

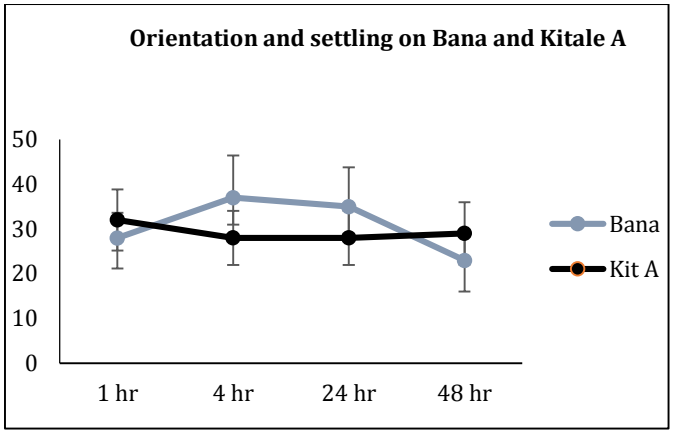

Figure 4.1b: Percentage numbers of *M. banda* settling on Bana and Kitale A at 1-, 4-,24- and 48hrs.

Table 4.1b: Settling response of *M. banda* on Bana (susceptible variety) and Kitale A (resistant variety) plants 1-, 4-, 24-, and 48-hours after infestation

	Females (%) settled on plants[1]			
Variety	1 hr	4 hr	24 hr	48 hr
Bana	28 ± 5.612	37 ± 9.434	35 ± 8.803	23 ± 5.612
Kitale A	32 ± 6.819 ns	28 ± 6.042 ns	28 ± 6.042 ns	29 ± 6.964 ns
P value	0.897	0.460	0.611	0.627

[1] Average of 5 replications, each replication had one healthy plant and 20 female adult hoppers.
ns = not siginificant, ** = significant < 0.005 Mann-Whitney U test

After 1-, 4-, 24- and 48 hours of being given a choice of settling on Bana or Kitale A, there were no significant differences in hoppers that settled on the two genotypes as given in Figure 4.1b and Table 4.1b above; (p values Appendix 2). However, there were more hopper number on Kitale A at 1 hour and at the final settling (48 hours), though the difference was not significant (*P*=0.897 and *P*=0.627 respectively, Mann-Whitney U pairwise comparisons).

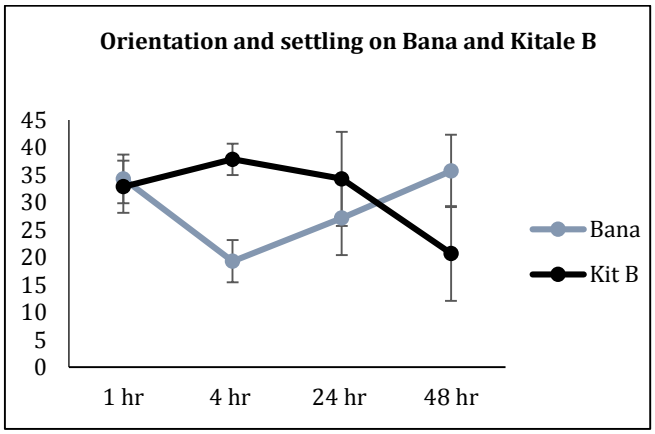

Figure 4.1c: Percentage numbers of *M. banda* settling on Bana and Kitale B at 1-, 4-, 24- and 48hrs.

Table 4.1c: Settling response of *M. banda* on Bana (susceptible variety) and Kitale B (resistant variety) plants 1-, 4-, 24-, and 48-hours after infestation

	Females (%) settled on plants[1]			
Variety	1 hr	4 hr	24 hr	48 hr
Bana	34.29 ± 4.442	19.29 ± 3.847	27.14 ± 6.713	35.71 ± 6.585
Kitale B	32.86 ± 4.738 ns	37.86 ± 2.857**	34.29 ± 9.552 ns	20.71 ± 8.621 ns
P value	0.931	0.012	0.710	0.128

[1] Average of 5 replications, each replication had one healthy plant and 20 female adult hoppers.
ns = not siginificant, ** = significant < 0.005 Mann-Whitney U test

When *M. banda* was given a choice to settle on either Bana or Kitale B there were no significant differences in hopper numbers that settled on the two genotypes at 1-, 4-, 24- and 48 hours (Figure 4.1c Table 4.1c). However, the number that settled on Kitale B was more than that which settled on Bana after 4 hours and 24hours, were the percentage number was higher on Kitale B than on Bana. At final settling (48 hours) more hopper numbers preferred settling on Bana than on Kitale B.

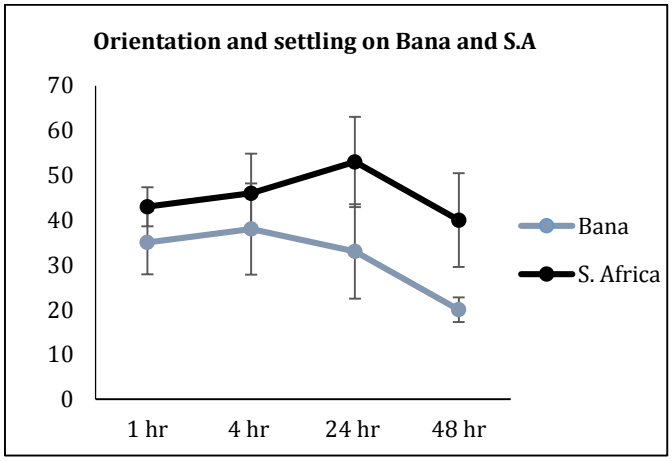

Figure 4.1d: Percentage numbers of *M. banda* settling on Bana and South Africa at 1-, 4-, 24- and 48hrs.

Table 4.1d: Settling response of *M. banda* on Bana (susceptible variety) and S. Africa (resistant variety) plants 1-, 4-, 24-, and 48-hours after infestation

	Females (%) settled on plants[1]			
Variety	1 hr	4 hr	24 hr	48 hr
Bana	35 ± 7.071	38 ± 10.198	33 ± 10.559	20 ± 2.739
S. Africa	43 ± 4.359 ns	46 ± 8.860 ns	53 ± 10.075 ns	40 ± 10.488 ns
P value	0.413	0.548	0.393	0.175

[1] Average of 5 replications, each replication had one healthy plant and 20 female adult hoppers.
ns = not siginificant, ** = significant < 0.005 Mann-Whitney U test

Although there were no significant differences in hopper numbers preferring to settle on South Africa than Bana (Figure 1.d and Table 4.1d). Leafhopper numbers were always higher on South Africa than on Bana at 1-, 4-, 24-, and 48 hours (Figure 4.1d). However, higher hopper numbers on South Africa were highest after 48 hours were there were time two the hopper numbers on Bana (Table 4.1d, Appendix 2).

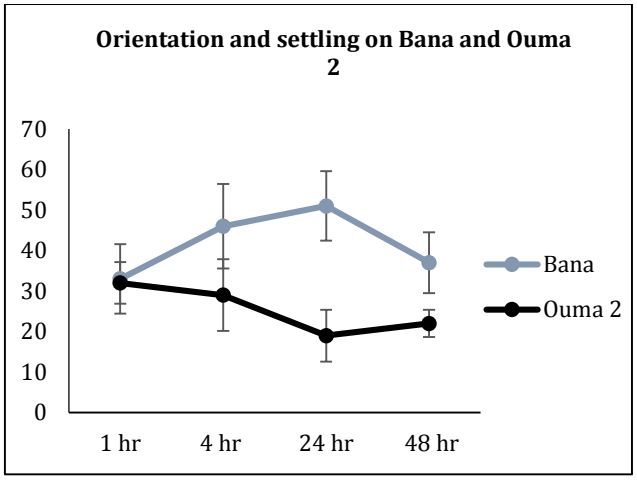

Figure 4.1e: Percentage numbers of *M. banda* settling on Bana and Ouma 2 at 1-, 4-,24- and 48hrs

Table 4.1e: Settling response of *M. banda* on Bana (susceptible variety) and Ouma 2 (resistant variety) plants 1-, 4-, 24-, and 48-hours after infestation

	Females (%) settled on plants[1]			
Variety	1 hr	4 hr	24 hr	48 hr
Bana	33 ± 8.602	46 ± 10.416	51 ± 8.573	37 ± 7.517
Ouma 2	32 ± 5.148 ns	29 ± 8.860 ns	19 ± 6.403**	22 ± 3.391 ns
P value	1.00	0.413	0.024	0.056

[1] Average of 5 replications, each replication had one healthy plant and 20 female adult hoppers.
ns = not siginificant, ** = significant < 0.005 Mann-Whitney U test

When *M. banda* was given a choice between Bana and Ouma 2 there were no significant differences in numbers settling on the two at 1-, 4- and 48 hours (*P*=1.00, 0.413 and 0.056 respectively) although hopper numbers were always higher on Bana at the three time intervals (Figure 4.1e and Table 4.1e, Appendix 2). Hopper numbers settling on Bana were however significantly higher than those that settled on Ouma 2 after 24 hours (*P*=0.024, Table 4.1e, Appendix 2). There were however significant differences in the number that settled on Bana and Ouma 2 after 24 hours. After 24 hours less number of hoppers settled on Ouma 2 than there were on Bana.

4.2 Volatile preference: Non-choice test

In this study we were also interested in finding out if *M. banda* was at all attracted to the Napier grass volatiles. For this test the hopper was subjected to a no-choice test of same odour in two opposite arms and two ams were blank with the solvent. The outcomes of the different experiments were subsequently compared. The assays included volatiles of Bana, Kitale A, Kitale B, Ouma 2 and South Africa. The attraction of the hopper to a certain volatile was given as observations of time spent in each arm pursuing a certain odour and the number of entries the hopper made to that arm.

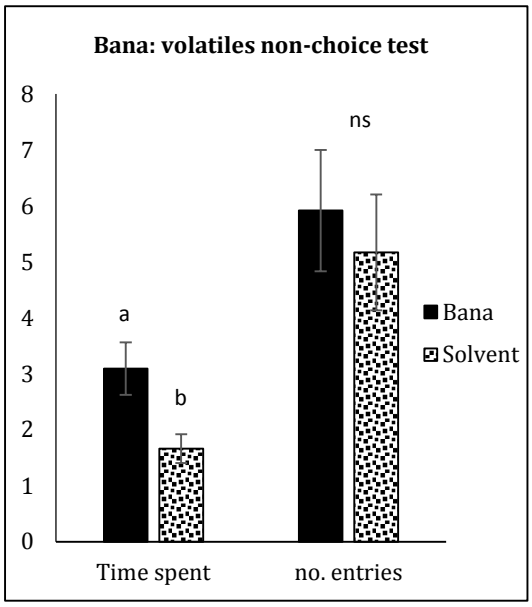

Figure 4.2a Time spent pursuing Kitale B over blank (solvent) and number of entries made to each arm. ns denotes non-significant differences (*P*=0.05).

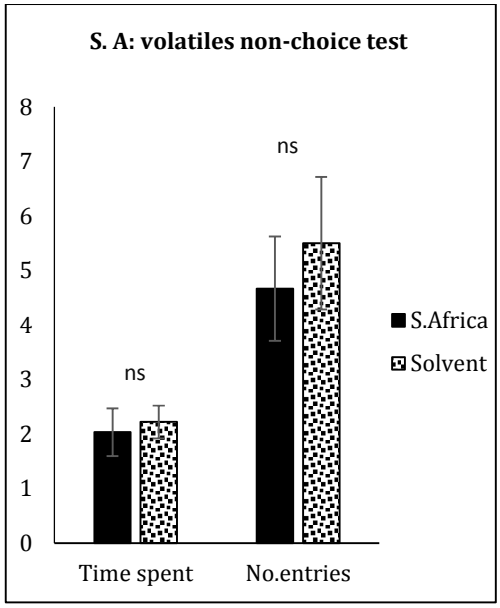

Figure 4.2b Time spent pursuing South Africa over blank (solvent) and number of entries made to each arm. ns denotes non-significant differences (*P*=0.05).

Fig 4.2a above shows the time spent by the hopper on Bana and the solvent and the number of entries made to each arm pursuing a particular volatile. There were significant differences in the time spent pursuing the Bana volatiles than the solvent (*P*=0.014 Mann Whitney-U, Fig 4.2a Appendix 2). The hopper spent much more time pursuing Bana than the solvent. However, there were no significant differences in the number of entries made to Bana and solvent (*P*=0.699, Mann Whitney-U, Appendix 2).
There were no significant differences in the time spent on South Africa than on the solvent (*P*=0.799). The number of times the hopper entered the arm with the solvent was not significantly different from that of South Africa (*P*=0.764) (Fig 4.2b, Appendix 2).

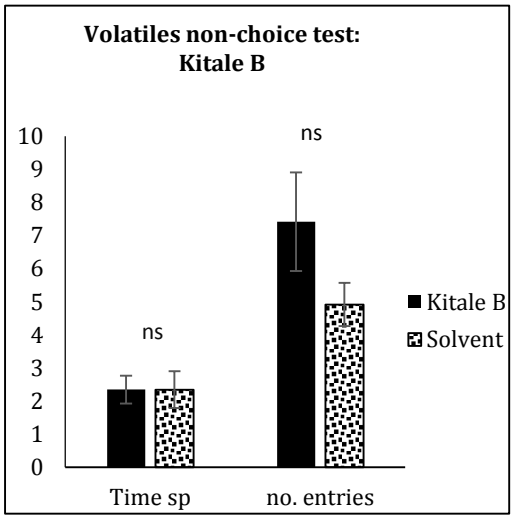

Figure 4.2c Time spent pursuing Kitale B over blank (solvent) and number of entries made to each arm. ns denotes non-significant differences (*P*=0.05).

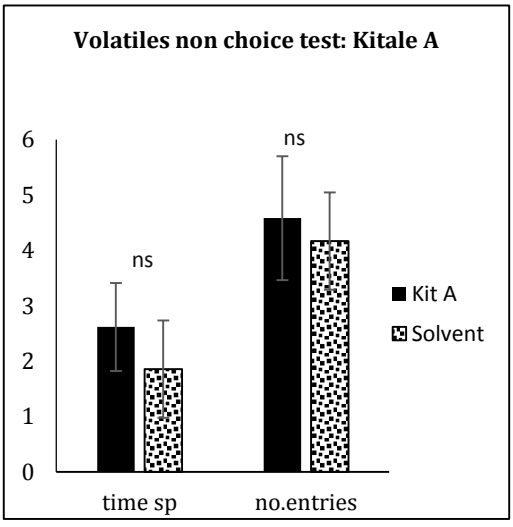

Figure 4.2d Time spent pursuing Kitale A over blank (solvent) and number of entries made to each arm. ns denotes non-significant differences (*P*=0.05).

There were no significant differences in time spent on Kitale B and on the solvent (*P*=0.810) (Fig 4.2c and Appendix 2). The number of times the hopper went for Kitale B was not significantly different from the number of times the hopper went for solvent (*P*=0.366, Fig 4.2c and Appendix 2).

There were also no significant differences in the time spent on Kitale A than on the solvent (*P*=0.713, Fig 4.2d and Appendix 2) and also the number of entries the hopper made to the arm with Kitale A and solvent (P=0.872, Fig 4.2d and Appendix 2).

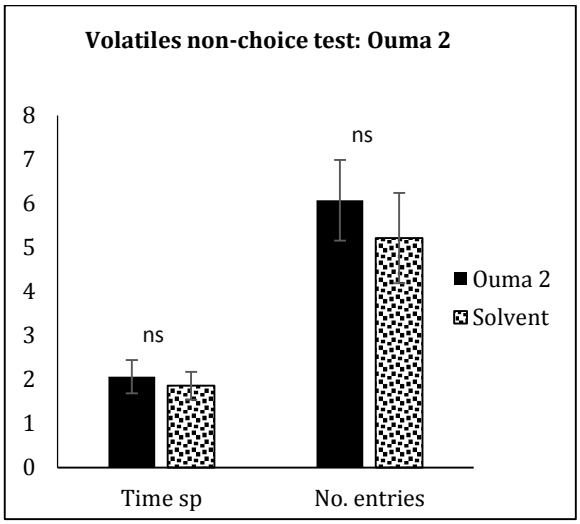

Figure 4.2e Time spent pursuing Kitale B over blank (solvent) and number of entries made to each arm. ns denotes non-significant differences (*P*=0.05).

There were no significant differences in time spent on Ouma2 than on the solvent (*P*=0.777) (Fig 4.2e and Appendix 2). The number of times the hopper went for Ouma 2 was not significantly different from the number of times the hopper went for solvent (*P*=0.331 Mann-Whitney U, Appendix 2).

4.3 Volatiles preference: Choice test

To determine which host the hopper prefers and would select *M. banda* was given a choice between each of the four supposedly resistant Napier grass varieties (South Africa, Kitale A, Kitale B and Ouma 2) and susceptible Bana. A four arm olfactometer was used in the bioassay and the time spent by the leafhopper in each arm was recorded and the number of times it went into a particular arm. More time pursuing an odour was taken to indicate preference for that volatile. The number of times it went into a particular arm was also taken to indicate more preference for that volatile when compared to the other.

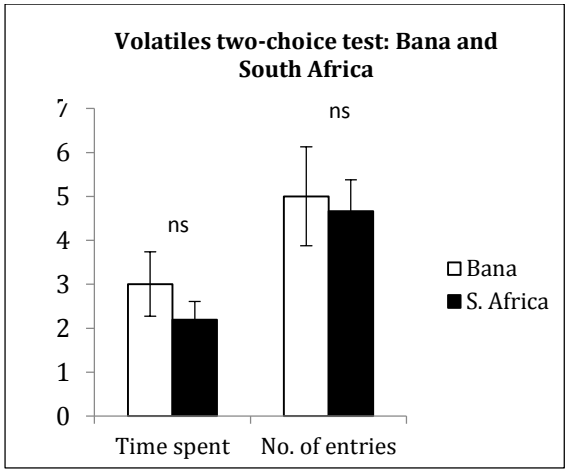

Figure 4.3a Two-choice test between Bana and S. Africa with time spent pursuing each volatile and number of entries made to each volatile. ns denotes non-significant differences (*P*>0.05).

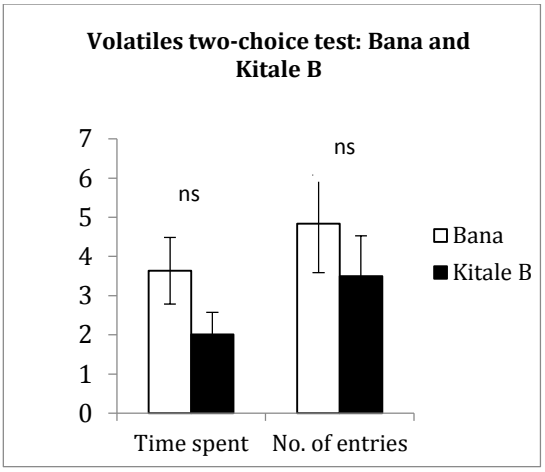

Figure 4.3b Two-choice test between Bana and Kitale B showing time spent (minutes) pursuing each volatile and number of entries made to each volatile. ns denotes non-significant differences (*P*>0.05).

Figure 4.3a above (left) shows the time spent by the hopper pursuing volatiles from Bana or S. Africa. There were no significant differences in time spent pursuing volatiles whether it was Bana or South Africa (*P*=0.345 paired t-test, Appendix 2). The data was averaged over 12 replications of twelve minutes each. The number of entries the hopper made to Bana and South Africa were also not significantly different from each other (*P*=0.732, one-way ANOVA, Figure 4.3a; Appendix 2).

Figure 4.3b above shows the time spent by the hopper in each arm as an indication of its preference for either Bana or Kitale B. There were no significant differences in time spent pursuing each volatile whether it was Bana or Kitale B (*P*=0.235 paired t-test, Table 4.3b, Appendix 2). The data was averaged over six replications of twelve minutes each. The number of entries the hopper made to either Bana or Kitale B were also not significantly different from each other (*P*=0.235, paired t-test, Appendix 2).

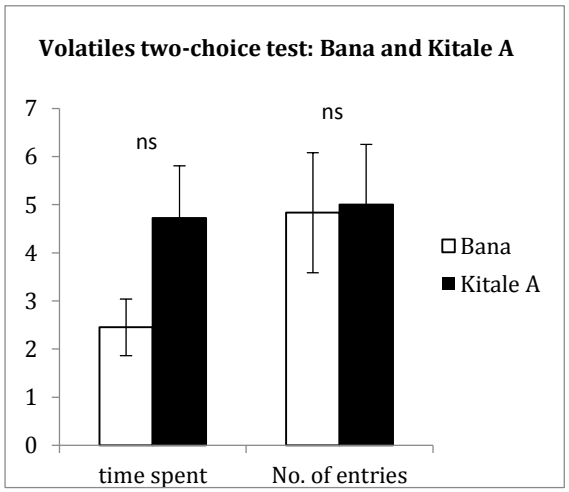

Figure 4.3c Two-choice test between Bana and Kitale A showing time spent (minutes) pursuing each volatile and number of entries made to each volatile. ns denotes non-significant differences (*P*>0.05).

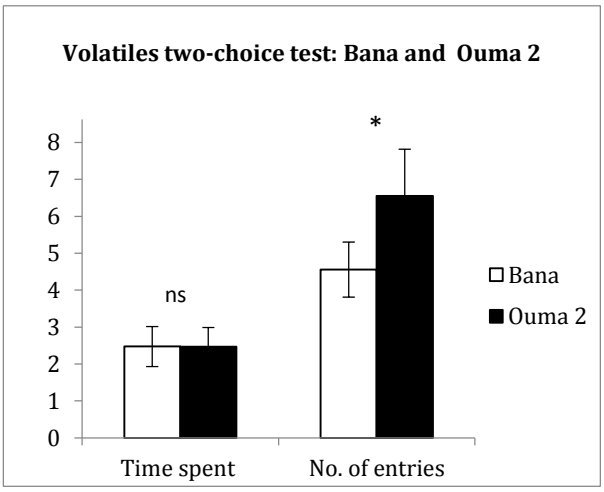

Figure 4.3d Two-choice test between Bana and Ouma 2 showing time spent (minutes) pursuing each volatile and number of entries made to each volatile. An asterisk denotes significant differences (*P*<0.05). ns denotes non-significant differences (*P*>0.05).

Figure 4.3c above (left) shows time spent by the hopper in each arm as preference for a particular volatile. Although the time spent on Kitale A volatiles was higher it was not significantly than the time spent on Bana (*P*=0.219 paired t-test, Table 4.3c Appendix 2). The data was averaged over six replications of twelve minutes each. The number of entries the hopper made to Bana and Kitale A were not significantly different from each other (*P*=0.867, paired t-test, Figure 4.3c Appendix 2).

Figure 4.3d above (right) shows the time spent by the hopper in each arm with Bana or Ouma 2 volatiles. There were no significant differences in time spent pursuing each volatile whether it was Bana or Ouma 2 (*P*=0.995 paired t-test, Table4.3d Appendix 2). However, there were significant differences in the number of entries made to Bana and Ouma 2 (*P*=0.028). Number of entries made to the arm with the Ouma 2 volatiles was higher than to Bana (Figure 4.3d). The data was averaged over six replications of twelve minutes each.

4.4 Honeydew excretion

For the feeding bioassay the extent of feeding was estimated through the honeydew that was excreted by the leafhopper. The estimation was done using the stained area on a graph paper. The area stained by ninhydrin solution after honeydew excretion was averaged per genotype and Fig 4.4a shows the results. Original data was used for analysis since it still did not satisfy the ANOVA conditions after tranformations, the Kruskal Wallis one way ANOVA was used for analysis.

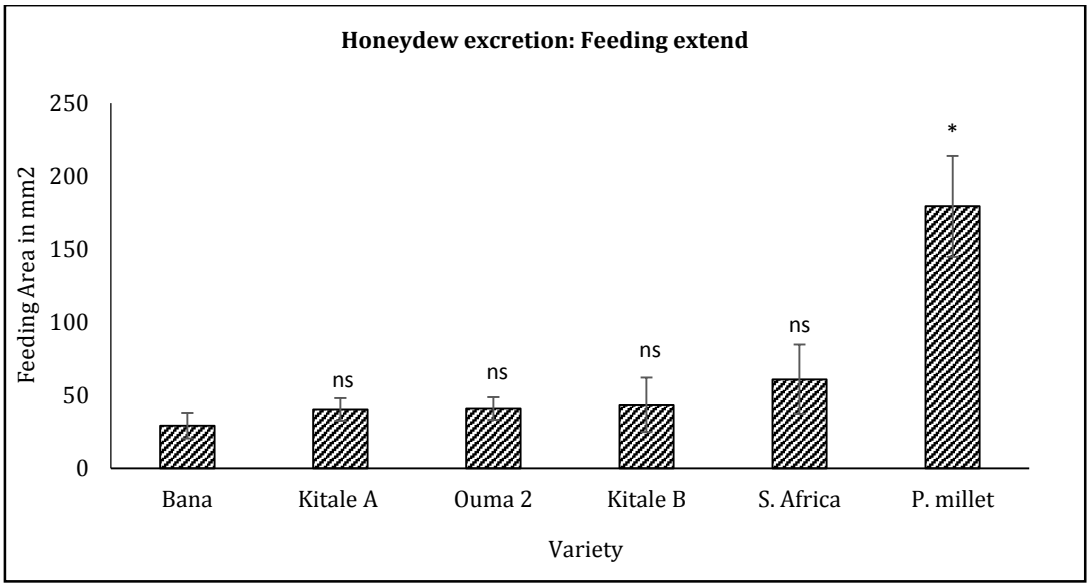

Figure 4.2a: Honeydew excretion area after leafhopper feeding for 24 hours averaged over five replications per genotype per bioassay and five days for all the replications. Data analysed with one way ANOVA. An asterisk signifies significant differences and ns denotes non-significant differences (*P*<0.05).

There were significant differences in the honeydew excreted among the six genotypes (*P*=0.014) (Fig 4.4a and Appendix 2). However, on pairwise comparisons between Bana which was the susceptible control there were significant differences in feeding extent only when Bana was compared to P. M (*P*=0.004, Kruskal Wallis one way ANOVA)

(Appendix 2) and not the other four Napier varieties (Kitale A, Ouma 2, Kitale B and S. Africa) (Fig 4.4a and ANOVAS in Appendix 2).

4.5 Population development

To determine the population development on each of the six genotypes, total numbers of hoppers were counted after sixty days from infestation.

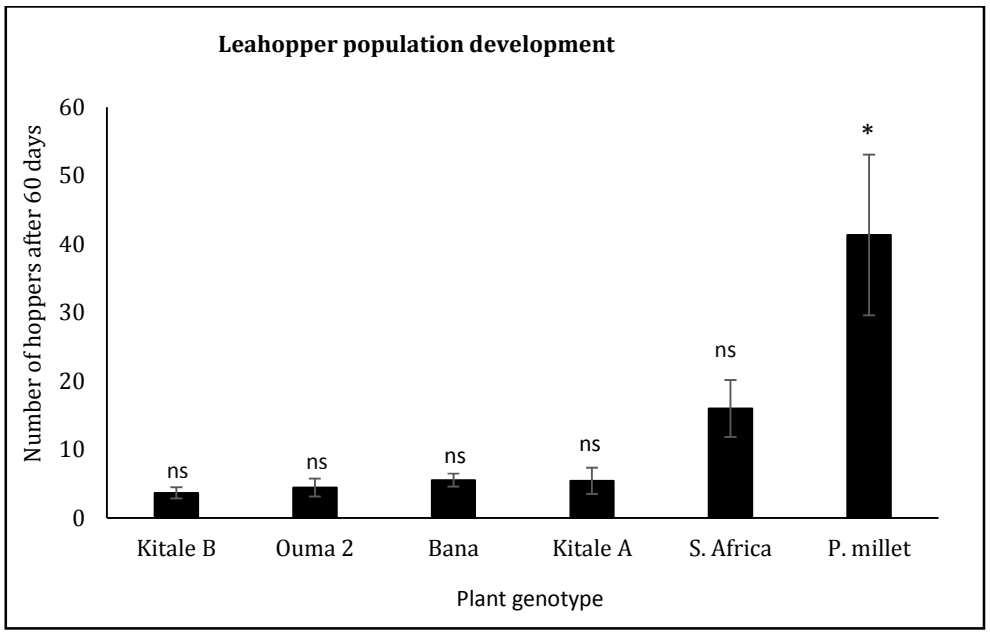

Figure 4.5a: Reproduction of hoppers on the different Napier grass varieties and pearl millet (averaged over sixty days and over ten positions in the screen house) (pairwise comparisons with Mann- Whitney U). An asterisk indicates a significant difference (P<0.05) between the number of hoppers on P. millet and the other four Napier grass varieties and Bana (control). NS denotes no significant difference between the Napier grass varieties and P. millet when compared to Bana.

The number of hoppers on the different plantlines were analysed per group; groupwise comparisons were done over all the six genotypes. The data did not satisfy the normal distribution assumptions. The Kruskal-Wallis one way ANOVA was used to determine the significant differences in hopper numbers on all the plantlines. There were significant differences in hopper numbers on the different plantlines (P=0.006) (Fig 4.5a and Appendix 1).

Pairwise comparisons were done for each of the four Napier grass varieties (Kitale B, Ouma 2, Kitale A and S. Africa) and pearl millet against the susceptible Bana. The Mann-Whitney U test was used for the pairwise comparisons and there were no significant differences in hopper population between Bana and Kitale B (P=0.211), Bana and Ouma 2 (P=0.471), Bana and Kitale A (P=0.529) and Bana and South Africa (P=0.066). Although the differences in hopper population on Bana and South Africa were not significantly different (Fig 4.5a), hopper population was much higher on South Africa than on Bana. On pearl millet hopper population was significantly much higher than on Bana (P=0.011) (Fig 4.5a, Appendix 2).

5 DISCUSSION

5.1 Orientation and Settling

Orientation and settling may give an indication of the genotype that the insect may prefer for subsequent feeding and/or oviposition. However, there was no difference in final settling (after 48 hours) by *M. banda* on susceptible Bana and the resistant Napier grass varieties. Although, orientation and settling of leafhoppers did not differ on susceptible and resistant varieties hopper numbers on Bana were higher than on pearl millet, Kitale B and Ouma 2 at final settling (48 hours). According to Saxena, 1969, an insects' orientation involves visual and volatile chemical stimuli emanating from the plant which the insect perceives from a distance. A negative oriental response will result in repulsiveness whilst a positive orientation response results in possibility of the insect making contact with the plant and possibly settling there. Therefore in this study insects were not repulsed from settling on the resistant varieties suggesting that the resistant Napier grass varieties used do not repulse the insect upon its settling. However, more replications were needed to ascertain this result as leafhopper numbers were very different between individual comparisons.

5.2 Volatile preference

The present study showed that *M. banda* was generally not attracted to Napier grass volatiles compared to the blanks with solvent as they were no significant differences in time spent and number of entries made to the test stimuli (Appendix 2). However,the time spent and number of entries made to the arms with Bana, Kitale A, B, South Africa and Ouma 2 volatiles was higher than made to the solvent though not significantly. As the result did not show a general preference for the test varieties doing more replications to see if the result will be the same would be important. Also no test was done to show that there were enough volatiles in the olfactometer which might have influenced the results if the concentrations were low. This result did not show higher preference for Napier grass odours than solvent, and we don't know if the solvent was dominating the odours or that the odours were in low quantities. Also to determine insect response to Napier grass volatiles/odours it might be useful to compare infested with uninfested plants. This is because according to Sugio *et al*., 2011, some phytoplasmas have the ability to regulate the down synthesis of Jasmonic acid and other plant defence hormones. Phytoplasmas can also alter plant morphology but also longevity, reproduction and behaviour of their insect vectors. Therefore comparisons between infested and uninfested plants might give better indication of insects' response to Napier grass volatiles.

For the two-choice test when *M. banda* was given a choice between susceptible Bana and other varieties it did not show higher preference for susceptible Bana than resistant varieties. The time spent pursuing Bana and the number of entries made to Bana were not significantly different than to other varieties except to Ouma 2. Several studies have demonstrated the importance of plant chemical factors in determining the susceptibility or resistance of rice varieties to insect pests (Khan and Saxena 1985; Saxena and Okech, 1985), although there were no significant differences in volatile preference in this study.

There is need to understand the distribution of these chemicals in Napier grass as the conclusion that insect responses to the volatiles from all tested varieties was the same is premature. Also repeating the experiment by combining visual and odour stimuli might enhance insect response to the odours. According to Patt and Tamou, 2007, with a study done with *Homalodisca coagulata* Say (Homoptera: Cicadellidae by combining odour and visual stimuli tests in an olfactometer nymphs jumped to the colored targets, chemical stimulus seemed to have no significant effect on their target choice. In the second experiment, host odours did not affect orientation and residence in the coloured treatments. High response to brighter color (yellow) was entirely as a result of visual stimulus. However, when the dark color (grey) was combined with a host odour more nymphs jumped to this treatment. Their outcome showed that for insects to respond to certain behavioural parameters chemical cues must be paired with certain visual stimuli.

5.3 Feeding

After feeding for 24 hours there were no significant differences in honeydew excreted by the hoppers between susceptible Bana and the resistant Napier grass varieties (Kitale A, B, South Africa and Ouma 2). However, honeydew excreted by hoppers feeding on P. millet was higher than those that fed on Bana (Fig 4.2a, Appendix 2). Findings of this study therefore show that there was no preferential feeding on susceptible Bana than on the resistant varieties. Although the result should be treated with caution because of overlapping stained areas on the filter paper which indicate that feeding was not different on susceptible and resistant varieties. Furthermore, the results maybe an indication that the plant varieties do not have different nutritional values or if resistance is there it is not based on *non-preference* of feeding on resistant varieties.

Most studies done on feeding using electronically recorded waveforms showed that there was deterred feeding on resistant varieties than on susceptible varieties (*Sogatella furcifera*, in rice) (Khan and Saxena, 1984). Electronically recorded waveforms are useful in Napier grass-leafhopper studies to understand if the resistance is as a result of deterred feeding. Honeydew excretion experiments using filter paper and graph paper are not accurate because of overlaps. Analysis of feeding behaviour will give an indication of resistance by giving information on: duration of phloem feeding and xylem drinking, mean number of probes during feeding on resistant and susceptible plants, duration of salivation and others. Increased probing and reduced phloem feeding on a genotype may indicate that the insect is deterred during feeding and that the genotype is resistant as has been the case with other leafhoppers: *Perkinsiella vitiensis* Kirklady (Chang and Ota, 1978; *Nephotettix virescens*, Khan and Saxena, 1985 and *Graminiella nigrifons* Forbes, Tripplehorn et al., 1984. Feeding can therefore be done adjunct to other bioassays to ascertain insect responses on susceptible and resistant Napier grasses.

5.4 Population Development

Reproduction was not significantly different on all five Napier grass varieties (Fig 4.5a Appendix 2) whether it was susceptible Bana or the other resistant Napier grass varieties Kitale A, B, Ouma 2 and South Africa. Although Bana is susceptible to the phytoplasma causing NSD, it may have some natural defence mechanisms which enables it to call for natural enemies upon herbivory. This is because spiders were found outside all Bana plant pots after damage by *M. banda*. However, repeating the experiment might be necessary to ascertain these results especially in summer season when hoppers have high reproduction rates. Hopper numbers were however, higher on Pearl millet than Bana. The high population on pearl millet can be attributed to the fact that adult females were used for the assay which had already been accustomed to the millet genotype during rearing. Hoppers might have been accustomed to the plant especially, feeding and did not require time to adapt to the old diet. Plants used were between 40 – 55 days, however, high soluble nitrogen is higher in the phloem sap of young or senescent leaves (Dixon, 1985) which supports higher reproduction rates. Testing hopper behaviour on younger and older plants might have led to the differential results.

This study did not show that resistance to the vector was correlated to resistance to the phytoplasma. This is because on Bana which is susceptible to the phytoplasma there was no higher preference of this genotype for settling, feeding, host selection and even reproduction although this was expected to be the case.

6 CONCLUSION AND RECOMMENDATIONS

There is need for serious considerations of the control of NSD and because the causative agent cannot be cultured in-vitro. Further studies on feeding experiments, host selection tests are necessary to further understand and exploit the host-vector relationship. The artificial phytoplasma transmission tests showed that the pathogen could be transmitted to food crops; this research is therefore high priority as it gives indication on the threat that NSD might pose to food security.

Antibiosis resistance may not be a good option in the case of Napier grass as it may result in a strong selection pressure which may result in emergence of more virulent biotypes (Smith, 1989) which can infect food crops. Tolerance and antixenosis maybe good resistance mechanisms. In this study host selection and preference for orientation and settling, olfactory preference and reproduction were not different on susceptible and resistant varieties suggesting that this is not antibiosis resistance. Some molecular tests are still being done to determine resistance of some of the used varieties to the phytoplasma and these might be useful in finding if varieties resistant to both vector and phytoplasma.

From the findings of this research the bioassays used did not seem useful for screening host plant resistance to the vector and transmitted disease because there were no significant differences in host selection and preference, honeydew excretion and even reproduction. It might be useful to do other bioassays like electronically recorded feeding to see how the insect is deterred from feeding and also multiple-choice tests, nymphal growth and survival, and egg hatchability and fecundity. These will assist in showing if insect responses are truly not significantly different on susceptible and resistant cultivars.

The perspex caged system might also not be good enough representation of population development in the field and might attribute to the low hopper numbers on test varieties. Also, NSD is usually seen in Napier grass re-growth therefore the plant age used might have a different reflection of the host-plant repsonses to the vector than when in its re-growth. For testing volatile preference female adults were used however, future studies may use males also and determine their responses. Moreover, it has been shown that in most insect species, males are more sensitive to odours than females and this might also be the case with hoppers (Guering and Visser, 1980; Honda et al., 1986). Insect ages were also not synchronized and some insects used might have been too old or young like in the reproduction assay were gravid females were visually identified. If they were old hoppers they might have died before laying eggs whilst the younger ones might have been immature to lay eggs at the time of infestation.

This study was important as Napier stunt disease is a serious disease limiting production of smallholder dairy farmers main fodder crop in Western Kenya. Elucidation of the host-vector relationship is a step towards understanding the biological processes underlying the host plants' resistance mechanisms against

herbivory. As the phytoplasma causing NSD cannot be cultured in-vitro understanding the host-vector relationship can be a useful tool in breeding for resistance or finding sources of resistance within the already cultivated Napier grass varieties.

REFERENCES

1. Abate (1992). Analysis of the Kenyan Dairy Industry in the Last Decade. Constraints and Options. In: Proceedings of a Workshop on Priority Setting in Dairy Cattle Research, held at the National Agricultural Research Centre (NARC), Muguga, Kenya, 4 - 9 May, 1992.
2. Arocha, Y., Zerfy, T., Abebe, G., Proud, J., Hanson, J., Wilson, M., Jones, P. and Lucas, J. (2009). Identification of potential vectors and alternative plant hosts for the phytoplasma associated with Napier grass stunt disease in Ethiopia. J Phytopathol 157:126–132
3. Atakan, E. (2011). Development of a sampling strategy for the leafhopper complex [*Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* (Paoli)] (Hemiptera: Cicadellidae) in cotton. J Pest Sci 84:143–152
4. ASARECA, (2010). Workshop on mitigating the impact of Napier grass smut and stunt diseases for the smallholder dairy sector. Final Report, June 1-3.
5. Beanland, L., Hoy, C. W., Miller, S.A. and Nault, L.R. (1999). Leafhopper (Homoptera: Cicadellidae) transmission of aster yellows phytoplasma: does gender matter? Environ Entomol 28:1101–1106
6. Bhandari, A.P., Sukanya, D.H. and Ramesh. C.R. (2006). Application of isozyme data in fingerprinting napiergrass (*Pennisetum purpureum* Schum.) for germplasm management. Genet. Resour. Crop Evol. 53:253–264. doi:10.1007/s10722-004-6120-2
7. Boonman, J.G. (1993). East Africa's grasses and fodders: Their ecology and husbandry. Kluwer Academic Publishers, Dortrecht, Nethelands.pp.343.
8. Chang, V.C.S. and Ota, A.K. (1978). Feeding activities of Perkinsiella leafhoppers and Fiji disease resistance of sugarcane. J. Econ. Entomol 71:297-300.
9. Choi, Y.H., Tapias, E.C., Kim, H.K., Lefeber, A.W.M., Erkelens, C., Verhoeven, J.Th.J., Brzin, J., Zel, J. and Verpoorte, R. (2004). Metabolic discrimination of *Catharanthus roseus* leaves infected by phytoplasma using 1H-NMR spectroscopy and multivariate data analysis. Plant Physiology 135:2398–2410.
10. Cook, S.M. (2007). The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52:375-400.
11. Dixon, A.F.G. (1985). Aphid ecology. Blackie, Glasgow
12. http://www.fao.org/ag/agp/AGPC/doc/gbase/data/Pf000301.HTM
13. Farrell, G., Simons, S.A. and Hillocks, R.J. (2002). Pests, diseases, and weeds of Napier grass, *Pennisetum purpureum*: a review. International Journal of Pest Management 48:39-48.
14. Firrao G., M. Garcia-Chapa and C. Marzachì, 2007. Phytoplasmas: genetics, diagnosis and relationships with the plant and insect host. Frontiers Bioscience 12:1352–1375.
15. Guerin, P.M. and Visser, J.H. (1980) Electroantennogram responses of the carrot fly, Psila rosae, to volatile plant components. Physiol. Entomol.,5 , 111-119.
16. Gitau, G.K., McDermott, J.J., Adams, J.E., Lissemore and Walter-Toews,D. (1994). Factors influencing calf growth and daily weight gain on smallholder dairy farms in Kiambu District, Kenya. Prev. Vet. Med. 21:179-190.
17. Guthrie, J.N., Walsh, K.B., Scott, P.T. and Rasmussen, T.S. (2001). The phytopathology of Australian papaya dieback: a proposed role for the phytoplasma. Physiological and Molecular Plant Pathology 58:23–30.
18. Heng-Moss, T., Baxendale, F.P., Riordan, T.P., Young, L. and Lee, K. (2003). Chinch Bug-Resistant Buffalograss: An Investigation of Tolerance, Antixenosis, and Antibiosis. Journal of Economic Entomology 96(6):1942-1951.
19. Humphreys, L.R. (1994). Tropical forages: Their role in sustainable agriculture. Longman, Harlow, UK. pp. 414.

20. IRPCM, 2004. 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonise plant phloem and insects. International Journal of Systematic and Evolutionary Microbiology 54:1243–1255.
21. Irwin, M.E. and Ruesink, W.G. (1986). Vector intensity: a product of propensity and activity. In: McLean, G.D., Garrett, R.G., Ruesink, W.G. (Eds.), Plant Virus epidemics: Monitoring, modelling and Predicting Outbreaks. Academic Press, Sydney, pp. 13–33.
22. Jones, P., Devonshire, B.J., Holman, T.J. and Ajanga, S. (2004). Napier grass stunt: a new disease associated with a 16SrXI group phytoplasma in Kenya. Plant Pathology 53:519
23. Jones, P., Arocha, T., Zerfy, J., Proud, J., Abebe, G. and Hanson, J. (2007). A stunting syndrome of Napier grass in Ethiopia is associated with a 16SrII Group phytoplasma. New Disease Reports 10. http://www.bspp.org. uk/ndr/july2006/2006-19.asp. Accessed on 25th April 2014
24. Khan, Z.R. and Saxena, R.C. (1984). Behavioural and Physiological Responses of *Sogatella furcifera* (Homoptera: Delphacidae) to Selected and Resistant and Susceptible Rice Cultivars. J. Econ. Entomol. 78:1280-1286
25. Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A. and Pickett, J.A. (2008). On-farm evaluation of the 'push-pull' technology for the control of stemborers and striga weed on maize in western Kenya. Field Crops Res 106:224–233
26. Kogan, M. and Ortman, E.F. (1978). Antixenosis–A New Term Proposed to Define Painter's "Nonpreference" Modality of Resistance. Entomological society of America. Volume 24: 175-176
27. León, R., Santamaría, J.M., Alpizar, L., Escamilla, J.A. and Oropeza, C. (1996). Physiological and biochemical changes in shoots of coconut palms affected by lethal yellowing. New Phytologist 134:227–23
28. Lombaert, E., Carletto J., Piotte, C., Fauvergue, X., Lecoq, H., Vanlerberghe-Masutti, F. and Lapchin, L. (2009), Response of the melon aphid, *Aphis gossypii*, to host-plant resistance: evidence for high adaptive potential despite low genetic variability. Entomol Exp Appl 133(1):46–56
29. Maust, B.E., Espadas, F., Talavera, C., Aguilar, M., Santamaría, J.M. and Oropeza, C. (2003) Changes in carbohydrate metabolism in coconut palms infected with the lethal yellowing phytoplasma. Phytopathology 93:976–981.
30. Murray, R.G.E. and Schleifer, K.H. (1994) Taxonomic notes: a proposal for recording the properties of putative taxa of prokaryotes. Int J Syst Bacteriol 44:174–176
31. Muyekho, F.N., Mose, L. and Cheruiyot D.T. (2003). Development and transfer of forage production technologies for smallholder dairying: Case studies of participatory evaluation of species and methods of establishment in western Kenya. Tropical Grasslands 37:251-256.
32. Nielsen, S.L., Ebong, C., Kabirizi, J. and Nicolaisen, M. (2007). First report of a 16SrXI group phytoplasma (*Candidatus* Phytoplasma *oryzae*) associated with Napier grass stunt disease in Uganda. Plant Pathol 56:1039–1039
33. Obura, E., Midega, C.A.O., Masiga, D., Pickett, J.A., Hassan, M., Koji, S. and Khan, Z.R. (2009). *Recilia banda* Kramer (Hemiptera: Cicadellidae), a vector of Napier stunt phytoplasma in Kenya. Naturwissenschaften 96: 1169-1176
34. Panda, N. and Khush, G. S. (1995). Host plant resistance to insects. CABI Publishing, UK. ISBN: 0851989632
35. Patt, J. M. and Tamou, M. (2007). Olfactory and Visual Stimuli Affecting Host Plant Detection in Homalodisca coagulata (Hemiptera: Cicadellidae). Environ. Entomol. 36(1): 142-150
36. Pettersson, J. (1970). An aphid sex attractant 1. Biological studies. Entomol. Scand., 1:63–73.
37. Potter, H.L. 1987. Inventory of feed resources for smallholder farms in Kenya. In: Proceedings of the 2nd PANESA workshop IDRC (International Development Research Centre) Nairobi, Kenya pp. 2-22.

38. Power, A.G. (1987) Plant community diversity, herbivore movement, and an insect-transmitted disease of maize. Ecology 68:1658–1669
39. Rapusas, H.R. and Heinrichs, E.A. 1982. Plant age and levels of resistance to green leafhopper, *Nephotettix virescens* (Distant), and tungro virus in rice varieties. Crop Portection 1:91-98
40. Satoshi, K. (1999) The Phylogeny of the genera in the tribes Deltocephalini, Paralimnini, and their allies (Homoptera, Cicadellidae, Deltocephalinae). Esakia 39:65–108
41. Saxena, K. N. (1969). Patterns of insect-plant relationships determining susceptibility and resistance of different plants to insects. Entomol. Exp. Appl 12:751-766
42. Saxena, K. N. (1985). Behavioural basis of plant resistance or susceptibility to insects. Insect Sci. Application 6:303-313
43. Saxena, K.N. and Khan, Z.R. (1985). Electronically recorded disturbances in feeding behaviour of *Nephotettix virescens* (Homoptera: Cicadellidae) on neem-oil treated rice plants. J. Econo. Entomol 78:222-226
44. Saxena, R.C. and Okech, S.H. (1985). Role of plant volatiles in resistance of selected rice varieties to brown planthopper *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). J. Chem. Ecol. 11:1601-1616.
45. Sears, B.B. and Kirkpatrick, B.C. (1994) Unveiling the evolutionary relationships of plant pathogenic mycoplasma-like organisms. ASM News 60:307–312
46. Silva, R., Furlong, M.J., Wilson, L.J. and Walter, G.H. (2013). How Predictable Are the Behavioral Responses of Insects to Herbivore Induced Changes in Plants? Responses of Two Congeneric Thrips to Induced Cotton Plants. PLoS ONE 8(5): e63611. doi:10.1371/journal.pone.0063611
47. Skerman, P.J. and Riveros, F. (1990). Tropical Grasses. FAO, Rome. pp.832.
48. Staal, S., Chege, L., Kenyanjui, M., Kimari, A., Lukuyu, B., Njubi, D., Owango, M., Tanner, J., Thorp, W. and Wambugu, M. (1998). A cross-sectional survey of Kiambu Distrrict for the identification of target groups of smallholder dairy producers. KARI/ILRI collaborative project research report, Nairobi, Kenya.
49. Sugio, A., Kingdom, H.N., MacLean, A.M., Grieve, V.M. and Hogenhout, S.A. (2011). Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. www.pnas.org/cgi/doi/10.1073/pnas.1105664108
50. Tamiru, A., Bruce, T.J.A., Woodcock, C. M., Caulfield, J.C., Midega, C.A.O., Ogol, C.K.P.O., Mayon, P., Birkett, M. A., Pickett, J.A. and Khan, Z.R. (2011). Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. Ecology Letters 14:1075–1083
51. Triplehorn, B.W., Nault, L. R. and Horn. D. J. (1984). Feeding behavior of *Graminella nigrifons* (Forbes). Ann. Entomol. Soc. Am. 77:102-107.
52. Webb, M.D. and Viraktamath, C.A. (2009). Annotated check-list, generic key and new species of Old World Deltocephalini leafhoppers with nomenclatorial changes in the Deltocephalus group and other Deltocephalinae (Hemiptera, Auchenorrhyncha, Cicadellidae). Zootaxa 2163:1–64
53. Weintraub, P.G and Beanland, L. (2006). Insect vectors of phytoplasmas. Ann Rev Entomol 51:91–111
54. Weintraub, P. G and Jones, P. (2010). Phytoplasmas: Genomes, Plant hosts and Vectors. CABI Publishers. UK.
55. Wouters, A.P. (1987) Dry matter yield and quality of Napier on farm level 1983-1986, Research Report, Ministry of Livestock Development, Nairobi Kenya . pp. 39.
56. www.push-pull.net

APPENDIX 1: RAW DATA

ORIENTATION AND SETTLING: TWO-CHOICE TEST

BANA & OUMA 2

Variety	Replication	1 hour	% 1 hr	4 hours	% 4 hr	24 hours	% 24 hr	48 hours	% 48 hr
Bana	1	11	55	4	20	5	25	5	25
Bana	2	4	20	6	30	8	40	5	25
Bana	3	6	30	11	55	12	60	5	25
Bana	4	2	10	9	45	15	75	12	60
Bana	5	10	50	16	80	11	55	10	50
Ouma 2	1	4	20	11	55	8	40	4	20
Ouma 2	2	7	35	5	25	4	20	4	20
Ouma 2	3	6	30	6	30	4	20	7	35
Ouma 2	4	10	50	7	35	0	0	4	20
Ouma 2	5	5	25	0	0	3	15	3	15

BANA & KITALE A

Variety	Replication	1 hour	% 1hr	4 hours	% 4 hr	24 hours	24 hr	48 hours	48 hr %
Bana	1	5	25	10	50	12	60	6	30
Bana	2	5	25	4	20	3	15	3	15
Bana	3	10	50	12	60	8	40	6	30
Bana	4	4	20	2	10	3	15	1	5
Bana	5	4	20	9	45	9	45	7	35
Kit A	1	6	30	4	20	2	10	5	25
Kit A	2	4	20	8	40	9	45	8	40
Kit A	3	3	15	3	15	4	20	3	15
Kit A	4	10	50	9	45	6	30	10	50
Kit A	5	9	45	4	20	7	35	3	15

BANA & KITALE B

Variety	Replication	1 hour	% 1 hr	4 hours	% 4 hr	% 24 hours	%24 hr	48 hours	% 48 hr
Bana	1	4	20	2	10	7	35	6	30
Bana	2	11	55	6	30	6	30	7	35
Bana	3	6	30	2	10	1	5	2	10
Bana	4	8	40	6	30	11	55	10	50
Bana	5	8	40	3	15	1	5	4	20
Bana	6	5	25	2	10	5	25	9	45
Bana	7	6	30	6	30	7	35	12	60
Kit B	1	7	35	9	45	4	20	2	10
Kit B	2	3	15	6	30	3	15	14	70
Kit B	3	10	50	9	45	13	65	4	20
Kit B	4	4	20	6	30	3	15	0	0
Kit B	5	9	45	8	40	13	65	4	20
Kit B	6	7	35	9	45	8	40	2	10
Kit B	7	6	30	6	30	5	20	3	15

BANA & SOUTH AFRICA

Variety	Replication	1 hour	% 1 hr	4 hours	% 4hr	24 hours	% 24 hr	48 hours	% 48 hr
Bana	1	9	45	11	55	10	50	4	20
Bana	2	6	30	3	15	5	25	4	20
Bana	3	2	10	8	40	13	65	3	15
Bana	4	8	40	3	15	2	10	3	15
Bana	5	10	50	13	65	3	15	6	30
S. Africa	1	6	30	9	45	4	20	6	30
S. Africa	2	10	50	7	35	13	65	13	65
S. Africa	3	10	50	4	20	8	40	1	5
S. Africa	4	7	35	14	70	13	65	11	55
S. Africa	5	10	50	12	60	15	75	9	45

BANA & PEARL MILLET

Variety	Replication	1 hour	% 1 hr	4 hours	% 4 hr	24 hours	% 24 hr	48 hours	% 48 hr
Bana	1	8	40	4	20	4	20	8	40
Bana	2	10	50	9	45	4	20	3	15
Bana	3	10	50	7	35	6	30	8	40
Bana	4	4	20	4	20	12	60	10	50
Bana	5	4	20	9	45	10	50	6	30
P. millet	1	11	55	10	50	9	45	6	30
P. millet	2	7	35	4	20	5	25	3	15
P. millet	3	8	40	7	35	10	50	6	30
P. millet	4	12	60	10	50	7	35	2	10
P. millet	5	6	30	4	20	3	15	5	25

HOST ATTRACTION: NON-CHOICE TEST

BANA

Treatment	Replication	Time spent	No. of entries	1st choice
Bana	1	6.37	10	x
Solvent	1	2.23	10	
Bana	1	2.48	8	
Solvent	1	0.88	4	
Centre	1	1.52		
Bana	2	3.08	13	x
Solvent	2	2.18	11	
Bana	2	4.88	10	
Solvent	2	1.85	11	
Centre	2	1.12		
Bana	3	2.08	4	x
Solvent	3	0.12	2	
Bana	3	2.08	2	
Solvent	3	0.9	2	
Centre	3	7.17		
Bana	4	3.47	2	x
Solvent	4	2.87	3	
Bana	4	2.35	2	
Solvent	4	0.97	1	
Centre	4	6.33		
Bana	5	0.15	2	
Solvent	5	2.52	3	
Bana	5	4.85	7	
Solvent	5	0.73	4	x
Centre	5	3.77		
Bana	6	2.68	6	
Solvent	6	2.3	5	
Bana	6	2.63	5	x
Solvent	6	2.4	6	
Centre	6	4.38		

KITALE A

Treatment	Replication	Time spent	No. of entries	1st choice
Kitale A	1	0.18	1	
Solvent	1	0.1	1	
Kitale A	1	10.03	2	x
Solvent	1	1.6	2	
Centre	1	1.38		
Kitale A	2	2.2	9	
Solvent	2	2.87	8	
Kitale A	2	2.68	14	x

Solvent	2	4.38	11	
Centre	2	0.2		
Kitale A	3	0.93	4	x
Solvent	3	2.95	6	
Kitale A	3	2.08	6	
Solvent	3	0.5	4	
Centre	3	7.48		
Kitale A	4	5.28	6	x
Solvent	4	1.52	4	
Kitale A	4	0.15	1	
Solvent	4	0.42	3	
Centre	4	4.67		
Kitale A	5	2.23	5	
Solvent	5	3.82	5	
Kitale A	5	2.6	4	
Solvent	5	1.8	4	x
Centre	5	5.82		
Kitale A	6	2.82	2	x
Solvent	6	0	1	
Kitale A	6	0.23	1	
Solvent	6	2.32	1	
Centre	6	7.73		

KITALE B

Treatment	Replication	Time spent	No. of entries	1st choice
Solvent	1	2.4	5	
Kitale B	1	3.12	12	x
Solvent	1	1.81	3	
Kitale B	1	2.88	4	
Centre	1			
Solvent	2	0.88	8	x
Kitale B	2	2.4	14	
Solvent	2	0.61	4	
Kitale B	2	4.1	16	
Centre	2			
Solvent	3	0.67	6	
Kitale B	3	4.21	12	
Solvent	3	2.1	8	x
Kitale B	3	3.81	10	
Centre	3			
Solvent	4	1.66	5	
Kitale B	4	1.53	5	
Solvent	4	5.07	7	
Kitale B	4	3.3	6	x
Centre	4	1.57		
Solvent	5	6.35	5	x

Kitale B	5	0.75	3	
Solvent	5	2.6	5	
Kitale B	5	1.63	5	
Centre	5	0.98		
Solvent	6	0	0	
Kitale B	6	0.22	1	
Solvent	6	3.98	3	x
Kitale B	6	0.2	1	
Centre	6	8.6		

OUMA 2

Treatment	Replication	Time spent	No. of entries	1st choice
Ouma 2	1	2.1	5	
Ouma 2	1	1.27	5	x
Solvent	1	0.98	3	
Solvent	1	1.55	2	
Centre	1	6.42		
Ouma 2	2	2.13	14	
Ouma 2	2	1.27	9	
Solvent	2	3.4	12	
Solvent	2	2.42	9	x
Centre	2	3.05		
Ouma 2	3	1.95	7	
Ouma 2	3	0.93	5	x
Solvent	3	1.5	6	
Solvent	3	1.05	3	
Centre	3	6.87		
Ouma 2	4	2.12	5	
Ouma 2	4	3.57	4	x
Solvent	4	0	0	
Solvent	4	1.77	2	
Centre	4	4.73		
Ouma 2	5	1.4	4	
Ouma 2	5	1.05	4	
Solvent	5	3.38	6	x
Solvent	5	0.53	3	
Centre	5	6.2		
Ouma 2	6	0.33	1	
Ouma 2	6	5.48	5	x
Solvent	6	1.05	2	
Solvent	6	1.4	4	
Centre	6	5.05		
Ouma 2	7	4.1	12	
Ouma 2	7	1.2	5	
Solvent	7	3.88	10	x
Solvent	7	3.08	11	

Centre	7	0.5	0	

SOUTH AFRICA

Treatment	Replication	Time spent	No. of entries	1st choice
S. Africa	1	4.37	1	
Solvent	1	3.88	4	x
S. Africa	1	0	0	
Solvent	1	2.4	1	
Centre	1	2.18		
S. Africa	2	1.28	5	
Solvent	2	1.67	4	x
S. Africa	2	0.8	4	
Solvent	2	2.95	4	
Centre	2	7.52		
S. Africa	3	1.98	8	
Solvent	3	2.48	12	
S. Africa	3	1.68	10	
Solvent	3	2.27	14	x
Centre	3	3.88		
S. Africa	4	2.53	7	
Solvent	4	2.68	6	
S. Africa	4	3.03	7	
Solvent	4	2.93	9	x
Centre	4	1.35		
S. Africa	5	4.5	8	
Solvent	5	0.6	5	
S. Africa	5	0.7	3	
Solvent	5	1	5	x
Centre	5	5.57		
S. Africa	6	0.32	1	
Solvent	6	3.2	1	
S. Africa	6	3.24	2	x
Solvent	6	0.63	1	
Centre	6	6.43		

HOST SELECTION: TWO-CHOICE TEST VOLATILE ASSAY

BANA AND SOUTH AFRICA

Treatment	Replication	Time Spent	No. of Entries	1st choice
Bana	1	1.8	9	
S. Africa	1	1.38	7	
Bana	2	3.12	7	
S. Africa	2	3.25	6	x
Bana	3	4.55	4	x
S. Africa	3	1.12	4	
Bana	4	5.12	4	x
S. Africa	4	3.52	2	
Bana	5	0.22	1	
S. Africa	5	2.38	5	x
Bana	6	3.23	5	x
S. Africa	6	1.52	4	

BANA & OUMA 2

Treatment	Replication	Time Spent	No. of Entries	1st choice
Bana	1	1.95	7	
Ouma 2	1	4.35	13	x
Bana	2	0.48	3	
Ouma 2	2	1.47	5	x
Bana	3	1.95	8	
Ouma 2	3	5.6	12	x
Bana	4	1.77	2	
Ouma 2	4	0.47	3	
Bana	5	6.58	3	x
Ouma 2	5	1.77	3	
Bana	6	1.8	3	
Ouma 2	6	2.12	4	
Bana	7	2.18	3	
Ouma 2	7	3.13	7	X
Bana	8	2.45	7	
Ouma 2	8	3.53	8	X
Bana	9	1.38	5	X
Ouma 2	9	1.03	4	
Bana	10	4.2		X
Ouma 2	10	1.22		

BANA AND KITALE A

Treatment	Replication	Time Spent	No. of Entries	1st choice
Bana	1	0.28	1	
Kit. A	1	8.47	4	x
Bana	2	3.57	9	

Kit. A	2	3.23	6	
Bana	3	2.87	4	x
Kit. A	3	1.83	2	
Bana	4	2.68	6	x
Kit. A	4	3.42	8	
Bana	5	1.23	7	
Kit. A	5	7.57	8	
Bana	6	4.08	2	
Kit. A	6	3.8	2	

BANA & KITALE B

Treatment	Replication	Time Spent	No. of Entries	1st choice
Bana	1	4.4	9	
Kit. B	1	1.12	6	
Bana	2	3.23	1	X
Kit. B	2	4.03	3	
Bana	3	1.43	2	X
Kit. B	3	0.88	1	
Bana	4	1.78	6	X
Kit. B	4	3.08	7	
Bana	5	7.2	4	X
Kit. B	5	0.55	1	
Bana	6	3.77	7	
Kit. B	6	2.38	3	

HONEYDEW EXCRETION

variety	replication	Feeding area (mm2)
Ouma 2	1	30
P. Millet	1	301
S. Africa	1	30
Kitale A	1	29
Kitale B	1	13
Bana	1	20
Ouma 2	2	32
P. Millet	2	161
S. Africa	2	40
Kitale A	2	13
Kitale B	2	24
Bana	2	21
Ouma 2	3	65
P. Millet	3	67
S. Africa	3	40
Kitale A	3	43
Kitale B	3	22
Bana	3	9
Ouma 2	4	18
P. Millet	4	233
S. Africa	4	174
Kitale A	4	49
Kitale B	4	16
Bana	4	36
Ouma 2	5	64
P. Millet	5	201
S. Africa	5	13
Kitale A	5	38
Kitale B	5	132
Bana	5	20
Ouma 2	6	37
P. Millet	6	113
S. Africa	6	69
Kitale A	6	70
Kitale B	6	54

Bana	6	69

POPULATION DEVELOPMENT

Genotype	Plant ID	Plant Age	no. of adults	no. of nymphs	total no.	comments
Kitale A	1	54	0	0	0	
Kitale B	1	54	0	3	3	spider
Bana	1	54	1	1	1	spider
Ouma 2	1	47	1	3	4	
S. Africa	1	54	0	9	9	
P. millet	1	47	2	54	56	
Kitale A	2	54	0	1	1	
Kitale B	2	54	0	0	0	open top
Bana	2	47	1	8	9	
Ouma 2	2	47	0	5	5	
S. Africa	2	54	0	35	35	
P. millet	2	54	1	3	4	spider
Kitale A	3	54	0	11	11	
Kitale B	3	54	0	3	3	
Bana	3	47	2	3	5	
Ouma 2	3	47	0	0	0	
S. Africa	3	54	1	27	28	
P. millet	3	54	0	1	1	spider
Kitale A	4	54	0	4	4	
Kitale B	4	47	0	1	1	
Bana	4	47	0	3	3	
Ouma 2	4	54	0	3	3	
S. Africa	4	54	0	11	11	
P. millet	4	47	0	21	21	
Kitale A	5	47	0	3	3	
Kitale B	5	54	2	4	6	
Bana	5	47	1	5	6	spider
Ouma 2	5	47	0	13	13	
S. Africa	5	54	0	6	6	spider
P. millet	5	54	0	49	49	
Kitale A	6	35	2	16	18	
Kitale B	6	35	1	2	3	1 dead
Bana	6	35	3	11	14	spider
Ouma 2	6	35	0	44	44	2 dead + spider
S. Africa	6 -		0	26	26	
P. millet	6	35	3	50	53	
Kitale A	7	35	0	2	2	
Kitale B	7	35	0	0	0	spider
Bana	7	54	0	2	2	
Ouma 2	7	35	1	5	6	
S. Africa	7	35	0	11	11	
P. millet	7	35	0	115	115	
Kitale A	8	54	0	3	3	spider
Kitale B	8	35	0	0	0	ants + spider
Bana	8	35	0	0	0	spider

Ouma 2	8	35	0	0	0	ants + spider
S. Africa	8	49	0	1	1	
P. millet	8	35	0	0	0	
Kitale A	9	35	1	5	6	
Kitale B	9	35	0	0	0	
Bana	9	54	0	3	3	spider
Ouma 2	9	35	2	1	3	
S. Africa	9	49	0	23	23	
P. millet	9	35	0	7	7	
Kitale A	10	35	4	0	4	
Kitale B	10	35	1	1	2	moulds
Bana	10	35	1	0	1	spider
Ouma 2	10	35	0	0	0	
S. Africa	10	35	0	0	0	
P. millet	10	35	1	16	17	

APPENDIX 2: DATA ANALYSIS

ORIENTATION AND SETTLING BEHAVIOUR: TWO CHOICE TEST

BANA AND KITALE A

Shapiro-Wilk test for Normality
Data variate: %1hr
Test statistic W: 0.8343
Probability: 0.038

Bartlett's test for homogeneity of variances
Chi-square 0.13 on 1 degrees of freedom: probability 0.714

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %1hr
Group factor: Variety
Value of U: 11.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.897
(under null hypothesis that group Bana is equal to group Kit A).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %4_hr
Test statistic W: 0.9008
Probability: 0.223

Bartlett's test for homogeneity of variances
Chi-square 0.68 on 1 degrees of freedom: probability 0.408

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %4_hr
Group factor: Variety
Value of U: 8.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.460
(under null hypothesis that group Bana is equal to group Kit A).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %24_hr
Test statistic W: 0.9447
Probability: 0.607

Bartlett's test for homogeneity of variances
Chi-square 0.49 on 1 degrees of freedom: probability 0.483

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %24_hr
Group factor: Variety
Value of U: 9.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.611
(under null hypothesis that group Bana is equal to group Kit A).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %48_hr
Test statistic W: 0.9644
Probability: 0.834

Bartlett's test for homogeneity of variances
Chi-square 0.16 on 1 degrees of freedom: probability 0.685

Mann-Whitney U (Wilcoxon rank-sum) test

Variate: %48_hr
Group factor: Variety
Value of U: 10.0 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.627
(under null hypothesis that group Bana is equal to group Kit A).
Sample sizes: 5, 5.

BANA & KITALE B
Shapiro-Wilk test for Normality
Data variate: %1_hr
Test statistic W: 0.9737
Probability: 0.922

Bartlett's test for homogeneity of variances
Chi-square 0.03 on 1 degrees of freedom: probability 0.871

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %1_hr
Group factor: Variety
Value of U: 23.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.931
(under null hypothesis that group Bana is equal to group Kit B).
Sample sizes: 7, 7.

Shapiro-Wilk test for Normality
Data variate: %4_hr
Test statistic W: 0.8554
Probability: 0.026

Bartlett's test for homogeneity of variances
Chi-square 0.48 on 1 degrees of freedom: probability 0.487

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %4_hr
Group factor: Variety
Value of U: 4.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.012
(under null hypothesis that group Bana is equal to group Kit B).
Sample sizes: 7, 7.

Shapiro-Wilk test for Normality
Data variate: %24_hr
Test statistic W: 0.9211
Probability: 0.228

Bartlett's test for homogeneity of variances
Chi-square 0.32 on 1 degrees of freedom: probability 0.571

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %24_hr
Group factor: Variety
Value of U: 21.0 (second sample has higher rank sum).
Exact probability: 0.710
(under null hypothesis that group Bana is equal to group Kit B).
Sample sizes: 7, 7.

Shapiro-Wilk test for Normality
Data variate: %48_hr
Test statistic W: 0.9223
Probability: 0.237

Bartlett's test for homogeneity of variances
Chi-square 0.40 on 1 degrees of freedom: probability 0.529

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %48_hr
Group factor: Variety
Value of U: 12.0 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.128
(under null hypothesis that group Bana is equal to group Kit B).
Sample sizes: 7, 7.

BANA AND OUMA 2
Shapiro-Wilk test for Normality
Data variate: %_1_hr
Test statistic W: 0.9335
Probability: 0.484

Bartlett's test for homogeneity of variances
Chi-square 0.90 on 1 degrees of freedom: probability 0.343

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %_1_hr
Group factor: Variety
Value of U: 12.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 1.000
(under null hypothesis that group Bana is equal to group Ouma 2).
Sample sizes: 5, 5.

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %_4_hr
Group factor: Variety
Value of U: 8.0 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.413
(under null hypothesis that group Bana is equal to group Ouma 2).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality

Data variate: %_24_hr
Test statistic W: 0.9644
Probability: 0.835

Bartlett's test for homogeneity of variances
Chi-square 0.30 on 1 degrees of freedom: probability 0.585

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %_24_hr
Group factor: Variety
Value of U: 1.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.024
(under null hypothesis that group Bana is equal to group Ouma 2).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %_48_hr
Test statistic W: 0.8122
Probability: 0.020

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %_48_hr
Group factor: Variety
Value of U: 3.0 (first sample has higher rank sum).
Exact probability: 0.056
(under null hypothesis that group Bana is equal to group Ouma 2).
Sample sizes: 5, 5.

Bartlett's test for homogeneity of variances
Chi-square 2.05 on 1 degrees of freedom: probability 0.152

BANA AND PEARL MILLET

Shapiro-Wilk test for Normality
Data variate: %1_hr
Test statistic W: 0.9415
Probability: 0.570

Bartlett's test for homogeneity of variances
Chi-square 0.09 on 1 degrees of freedom: probability 0.765

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %1_hr
Group factor: Variety
Value of U: 8.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.452
(under null hypothesis that group Bana is equal to group P. millet).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %4_hr
Test statistic W: 0.8188
Probability: 0.024

Bartlett's test for homogeneity of variances
Chi-square 0.11 on 1 degrees of freedom: probability 0.737

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %4_hr
Group factor: Variety
Value of U: 10.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.778
(under null hypothesis that group Bana is equal to group P. millet).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %24_hr
Test statistic W: 0.9324
Probability: 0.472

Bartlett's test for homogeneity of variances
Chi-square 0.20 on 1 degrees of freedom: probability 0.655

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %24_hr
Group factor: Variety
Value of U: 11.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.873
(under null hypothesis that group Bana is equal to group P. millet).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %48_hr
Test statistic W: 0.9499
Probability: 0.668

Bartlett's test for homogeneity of variances
Chi-square 0.49 on 1 degrees of freedom: probability 0.483

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %48_hr
Group factor: Variety

Value of U: 4.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.111
(under null hypothesis that group Bana is equal to group P. millet).
Sample sizes: 5, 5.

BANA AND SOUTH AFRICA
Shapiro-Wilk test for Normality
Data variate: %1_hr
Test statistic W: 0.8349
Probability: 0.038

Bartlett's test for homogeneity of variances
Chi-square 0.80 on 1 degrees of freedom: probability 0.371

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %1_hr
Group factor: Variety
Value of U: 8.0 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.413
(under null hypothesis that group Bana is equal to group S. Africa).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %4hr
Test statistic W: 0.9209
Probability: 0.364

Bartlett's test for homogeneity of variances
Chi-square 0.07 on 1 degrees of freedom: probability 0.791

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %4hr
Group factor: Variety
Value of U: 9.0 (second sample has higher rank sum).
Exact probability: 0.548
(under null hypothesis that group Bana is equal to group S. Africa).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %24_hr
Test statistic W: 0.8972
Probability: 0.204

Bartlett's test for homogeneity of variances
Chi-square 0.01 on 1 degrees of freedom: probability 0.929

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: %24_hr
Group factor: Variety
Value of U: 6.0 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.206
(under null hypothesis that group Bana is equal to group S. Africa).
Sample sizes: 5, 5.

Shapiro-Wilk test for Normality
Data variate: %48_hr
Test statistic W: 0.9242
Probability: 0.393

Bartlett's test for homogeneity of variances
Chi-square 5.09 on 1 degrees of freedom: probability 0.024

Mann-Whitney U (Wilcoxon rank-sum) test

Variate: %48_hr
Group factor: Variety
Value of U: 5.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.175
(under null hypothesis that group Bana is equal to group S. Africa).
Sample sizes: 5, 5.

VOLATILES NON-CHOICE TEST

BANA
Shapiro-Wilk test for Normality
Data variate: No_of_entries
Test statistic W: 0.8941
Probability: 0.016

Shapiro-Wilk test for Normality
Data variate: Time_spent
Test statistic W: 0.9164
Probability: 0.049

Shapiro-Wilk test for Normality
Data variate: Time_spent
Test statistic W: 0.9164
Probability: 0.049

Bartlett's test for homogeneity of variances
Chi-square 0.02 on 1 degrees of freedom: probability 0.885

Bartlett's test for homogeneity of variances
Chi-square 3.65 on 1 degrees of freedom: probability 0.056

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: Time_spent
Group factor: Treatment
Value of U: 30.0 (first sample has higher rank sum).
Exact probability: 0.014
(under null hypothesis that group Bana is equal to group Solvent).
Sample sizes: 12, 12.

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: No_of_entries
Group factor: Treatment
Value of U: 65.0 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.699
(under null hypothesis that group Bana is equal to group Solvent).
Sample sizes: 12, 12.

SOUTH AFRICA
Shapiro-Wilk test for Normality
Data variate: No_of_entries
Test statistic W: 0.9370
Probability: 0.140

Shapiro-Wilk test for Normality

Data variate: Time_spent
Test statistic W: 0.9636
Probability: 0.516

Bartlett's test for homogeneity of variances
Chi-square 0.60 on 1 degrees of freedom: probability 0.438

Bartlett's test for homogeneity of variances
Chi-square 1.43 on 1 degrees of freedom: probability 0.232

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: No_of_entries
Group factor: Treatment
Value of U: 66.5 (second sample has higher rank sum).
Exact probability (adjusted for ties): 0.764
(under null hypothesis that group S. Africa is equal to group Solvent).
Sample sizes: 12, 12.

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: Time_spent
Group factor: Treatment
Value of U: 67.0 (second sample has higher rank sum).
Exact probability: 0.799
(under null hypothesis that group S. Africa is equal to group Solvent).
Sample sizes: 12, 12.

KITALE B
Shapiro-Wilk test for Normality
Data variate: Time_spent
Test statistic W: 0.9581
Probability: 0.401

Shapiro-Wilk test for Normality
Data variate: No_of_entries
Test statistic W: 0.9233
Probability: 0.069

Bartlett's test for homogeneity of variances
Chi-square 4.43 on 5 degrees of freedom: probability 0.490

Bartlett's test for homogeneity of variances
Chi-square 13.36 on 5 degrees of freedom: probability 0.020

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: No_of_entries
Group factor: Treatment
Value of U: 56.0 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.366
(under null hypothesis that group Kitale B is equal to group Solvent).
Sample sizes: 12, 12.

Mann-Whitney U (Wilcoxon rank-sum) test

Variate: Time_spent
Group factor: Treatment
Value of U: 67.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.810
(under null hypothesis that group Kitale B is equal to group Solvent).
Sample sizes: 12, 12.

KITALE A
Shapiro-Wilk test for Normality
Data variate: Time_spent
Test statistic W: 0.8083
Probability: <0.001

Shapiro-Wilk test for Normality
Data variate: No_of_entries
Test statistic W: 0.8664
Probability: 0.004

Bartlett's test for homogeneity of variances
Chi-square 4.01 on 1 degrees of freedom: probability 0.045

Bartlett's test for homogeneity of variances
Chi-square 0.61 on 1 degrees of freedom: probability 0.435

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: No_of_entries
Group factor: Treatment
Value of U: 69.0 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.872
(under null hypothesis that group Kitale A is equal to group Solvent).
Sample sizes: 12, 12.

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: Time_spent
Group factor: Treatment
Value of U: 65.0 (first sample has higher rank sum).
Exact probability: 0.713
(under null hypothesis that group Kitale A is equal to group Solvent).
Sample sizes: 12, 12.

OUMA 2
Shapiro-Wilk test for Normality
Data variate: Time_spent
Test statistic W: 0.9109
Probability: 0.024

Shapiro-Wilk test for Normality
Data variate: No_of_entries
Test statistic W: 0.9184
Probability: 0.036

Bartlett's test for homogeneity of variances
Chi-square 0.50 on 1 degrees of freedom: probability 0.479

Bartlett's test for homogeneity of variances
Chi-square 0.28 on 1 degrees of freedom: probability 0.596

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: Time_spent
Group factor: Treatment
Value of U: 80.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.624
(under null hypothesis that group Ouma 2 is equal to group Solvent).
Sample sizes: 14, 13.

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: No_of_entries
Group factor: Treatment
Value of U: 66.5 (first sample has higher rank sum).
Exact probability (adjusted for ties): 0.240
(under null hypothesis that group Ouma 2 is equal to group Solvent).
Sample sizes: 14, 13.

VOLATILE PREFERENCE: TWO-CHOICE TEST

BANA AND SOUTH AFRICA

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	3.0067	6	1.79624	.73331
	time spent s.africa	2.1950	6	1.01768	.41547
Pair 2	number of entries bana	5.0000	6	2.75681	1.12546
	number of entry s.africa	4.6667	6	1.75119	.71492

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent s.africa	6	.173	.744
Pair 2	number of entries bana & number of entry s.africa	6	.580	.228

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent s.africa	.81167	1.90552	.77793	-1.18806	2.81139	1.043	5	.345
Pair 2	number of entries bana - number of entry s.africa	.33333	2.25093	.91894	-2.02887	2.69554	.363	5	.732

BANA AND KITALE A

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	2.4517	6	1.43791	.58702
	time spent kit a	4.7200	6	2.65683	1.08464
Pair 2	number of entries bana	4.8333	6	3.06050	1.24944
	number of entry kit a	5.0000	6	2.75681	1.12546

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent kit A	6	-.853	.031

Pair 2	number of entries bana & number of entry kit A	6	.687	.131

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent kit A	-2.26833	3.95532	1.61475	-6.41919	1.88252	-1.405	5	.219
Pair 2	number of entries bana - number of entry kit A	-.16667	2.31661	.94575	-2.59780	2.26446	-.176	5	.867

BANA AND OUMA 2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	2.4740	10	1.71908	.54362
	time spent ouma 2	2.4690	10	1.63772	.51789
Pair 2	number of entries bana	4.5556	9	2.24227	.74742
	number of entry ouma 2	6.5556	9	3.77859	1.25953

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent ouma 2	10	-.105	.773
Pair 2	number of entries bana & number of entry ouma 2	9	.844	.004

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent ouma 2	.00500	2.49583	.78925	-1.78041	1.79041	.006	9	.995
Pair 2	number of entries bana - number of entry ouma 2	-2.00000	2.23607	.74536	-3.71879	-.28121	-2.683	8	.028

BANA AND KITALE B

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	3.6350	6	2.08709	.85205
	time spent kitB	2.0067	6	1.38295	.56459

Pair 2	number of entries bana	4.8333	6	3.06050	1.24944
	number of entry kit B	3.5000	6	2.50998	1.02470

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent kitB	6	-.426	.400
Pair 2	number of entries bana & number of entry kit B	6	.638	.173

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent kitB	1.62833	2.95421	1.20605	-1.47192	4.72858	1.350	5	.235
Pair 2	number of entries bana - number of entry kit B	1.33333	2.42212	.98883	-1.20853	3.87519	1.348	5	.235

FEEDING

Shapiro-Wilk test for Normality

Data variate: Feeding_area_mm2
Test statistic W: 0.7363
Probability: <0.001

Bartlett's test for homogeneity of variances

Chi-square 17.85 on 5 degrees of freedom: probability 0.003

Kruskal-Wallis one-way analysis of variance

Variate: Feeding_area_mm2
Group factor: variety
Value of H = 14.24
Adjusted for ties = 14.26

Sample	Size	Mean rank
Group Bana	6	11.42
Group Kitale A	6	17.50
Group Kitale B	6	13.83
Group Ouma 2	6	16.75
Group P. Millet	6	32.17
Group S. Africa	6	19.33

Degrees of freedom = 5
Chi-square probability = 0.014

Mann-Whitney U (Wilcoxon rank-sum) test

Variate: Feeding_area_mm2
Group factor: variety
Control: 1
Tests of null hypothesis that each group is equal to control.
Sample size for control: 6

Group: 2
Value of U: 10.0 (rank sum higher than control)
Exact probability: 0.240
Sample size: 6

Group: 3
Value of U: 15.0 (rank sum higher than control)
Exact probability: 0.699
Sample size: 6

Group: 4
Value of U: 12.0 (rank sum higher than control)
Exact probability: 0.394
Sample size: 6

Group: 5
Value of U: 1.0 (rank sum higher than control)
Exact probability: 0.004
Sample size: 6

Group: 6

Value of U: 9.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.199
Sample size: 6

POPULATION DEVELOPMENT

Kruskal-Wallis one-way analysis of variance
Variate: total_no
Group factor: Genotype
Value of H = 14.98
Adjusted for ties = 15.16

Sample	Size	Mean rank
Group Bana	10	26.70
Group Kitale A	10	28.55
Group Kitale B	10	17.05
Group Ouma 2	10	27.50
Group P. millet	10	42.70
Group S. Africa	10	40.50

Degrees of freedom = 5
Chi-square probability = 0.010

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: total_no
Group factor: Genotype
Control: 1
Tests of null hypothesis that each group is equal to control.
Sample size for control: 10

Group: 2
Value of U: 45.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.752
Sample size: 10

Group: 3
Value of U: 30.0 (rank sum lower than control)
Exact probability (adjusted for ties): 0.133
Sample size: 10

Group: 4
Value of U: 49.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.985
Sample size: 10

Group: 5
Value of U: 23.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.045
Sample size: 10

Group: 6
Value of U: 23.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.045
Sample size: 10

VOLATILE PREFERENCE: TWO CHOICE TEST

BANA & SOUTH AFRICA

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	3.0067	6	1.79624	.73331
	time spent s.africa	2.1950	6	1.01768	.41547
Pair 2	number of entries bana	5.0000	6	2.75681	1.12546
	number of entry s.africa	4.6667	6	1.75119	.71492

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent s.africa	6	.173	.744
Pair 2	number of entries bana & number of entry s.africa	6	.580	.228

BANA AND KITALE A

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	2.4517	6	1.43791	.58702

Pair 2	time spent kit a	4.7200	6	2.65683	1.08464
	number of entries bana	4.8333	6	3.06050	1.24944
	number of entry kit a	5.0000	6	2.75681	1.12546

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent kit a	6	-.853	.031
Pair 2	number of entries bana & number of entry kit a	6	.687	.131

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent kit a	-2.26833	3.95532	1.61475	-6.41919	1.88252	-1.405	5	.219
Pair 2	number of entries bana - number of entry kit a	-.16667	2.31661	.94575	-2.59780	2.26446	-.176	5	.867

BANA & OUMA2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	2.4740	10	1.71908	.54362
	time spent ouma 2	2.4690	10	1.63772	.51789
Pair 2	number of entries bana	4.5556	9	2.24227	.74742
	number of entry ouma 2	6.5556	9	3.77859	1.25953

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent ouma 2	10	-.105	.773
Pair 2	number of entries bana & number of entry ouma 2	9	.844	.004

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Time spent bana - time spent ouma 2	.00500	2.49583	.78925	-1.78041	1.79041	.006	9	.995

Pair 2	number of entries bana - number of entry ouma 2	-2.00000	2.23607	.74536	-3.71879	-.28121	-2.683	8	.028

BANA AND KITALE B

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Time spent bana	3.6350	6	2.08709	.85205
	time spent kit B	2.0067	6	1.38295	.56459
Pair 2	number of entries bana	4.8333	6	3.06050	1.24944
	number of entry kit B	3.5000	6	2.50998	1.02470

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Time spent bana & time spent kitB	6	-.426	.400
Pair 2	number of entries bana & number of entry kit B	6	.638	.173

Paired Samples Test

	Paired Differences				t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			

					Lower	Upper			
Pair 1	Time spent bana - time spent kit B	1.62833	2.95421	1.20605	-1.47192	4.72858	1.350	5	.235
Pair 2	number of entries bana - number of entry kit B	1.33333	2.42212	.98883	-1.20853	3.87519	1.348	5	.235

HONEYDEW EXCRETION

Shapiro-Wilk test for Normality
Data variate: Feeding_area_mm2
Test statistic W: 0.7363
Probability: <0.001

Bartlett's test for homogeneity of variances
Chi-square 17.85 on 5 degrees of freedom: probability 0.003

Kruskal-Wallis one-way analysis of variance
Variate: Feeding_area_mm2
Group factor: variety
Value of H = 14.24
Adjusted for ties = 14.26

Sample	Size	Mean rank
Group Bana	6	11.42
Group Kitale A	6	17.50
Group Kitale B	6	13.83
Group Ouma 2	6	16.75
Group P. Millet	6	32.17
Group S. Africa	6	19.33

Degrees of freedom = 5
Chi-square probability = 0.014

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: Feeding_area_mm2
Group factor: variety
Control: 1
Tests of null hypothesis that each group is equal to control.
Sample size for control: 6

Group: 2
Value of U: 10.0 (rank sum higher than control)
Exact probability: 0.240
Sample size: 6

Group: 3
Value of U: 15.0 (rank sum higher than control)
Exact probability: 0.699
Sample size: 6

Group: 4
Value of U: 12.0 (rank sum higher than control)
Exact probability: 0.394
Sample size: 6

Group: 5
Value of U: 1.0 (rank sum higher than control)
Exact probability: 0.004
Sample size: 6

Group: 6
Value of U: 9.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.199
Sample size: 6

REPRODUCTION

Kruskal-Wallis one-way analysis of variance
Variate: total_no
Group factor: Genotype
Value of H = 14.98
Adjusted for ties = 15.16

Sample	Size	Mean rank
Group Bana	10	26.70
Group Kitale A	10	28.55
Group Kitale B	10	17.05
Group Ouma 2	10	27.50
Group P. millet	10	42.70
Group S. Africa	10	40.50

Degrees of freedom = 5
Chi-square probability = 0.010

Mann-Whitney U (Wilcoxon rank-sum) test
Variate: total_no
Group factor: Genotype
Control: 1
Tests of null hypothesis that each group is equal to control.
Sample size for control: 10

Group: 2
Value of U: 45.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.752
Sample size: 10

Group: 3
Value of U: 30.0 (rank sum lower than control)
Exact probability (adjusted for ties): 0.133
Sample size: 10

Group: 4
Value of U: 49.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.985
Sample size: 10

Group: 5
Value of U: 23.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.045
Sample size: 10

Group: 6

Value of U: 23.5 (rank sum higher than control)
Exact probability (adjusted for ties): 0.045
Sample size: 10