Thesis to obtain Joint Doctoral Degree from
Montpellier SupAgro (France) and University of Catania (Italy)

Doctoral School
SIBAGHE (Integrated Systems in Biology, Agronomy, Geoscience, Hydroscience and Environment) Montpellier SupAgro, France and Department of Agro-Food and Environmental Management Systems (Di3A), University of Catania, Italy

Defended in public by:
ANDNET BAYLEYEGN ABTEW

THE BEHAVIOR, ECOLOGY AND CONTROL OF LEGUME FLOWER THRIPS, *Megalurothrips sjostedti* (Trybom) IN COWPEA *Vigna unguiculata* (L.) TOWARDS THE DEVELOPMENT OF AN INTEGRATED PEST MANAGEMENT (IPM) PROGRAM IN KENYA

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This thesis was elaborated within the framework of the European Erasmus Mundus Programme “Agricultural Transformation by Innovation (AGTRAIN)”

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work conducted at the international center of insect physiology and ecology (icipe), Nairobi, Kenya and all references to ideas and work of other researchers have been specifically acknowledged. This thesis is my original work and it has not been presented for any degree in any other University.

Andnet Bayleyegn Abtew

(PhD Candidate)

July 2015

THÈSE EN COTUTELLE

Pour l'obtention du titre de Docteur en Philosophie

Université de Montpellier 2 (France) – University of Catania (Italy)

Champ disciplinaire: Chemical Ecologie

Ecole doctorale: Systèmes Intégrés en Biologie, Agronomie, Géosciences, Hydrosciences, Environnement (SIBAGHE)

Présentée et défendue publiquement par :

Andnet Bayleyegn ABTEW

Le 9 Octobre 2015 à Montpellier SupAgro

COMPORTEMENT, ÉCOLOGIE ET CONTRÔLE DU THRIPS DES FLEURS *Megalurothrips sjostedti* TRYBOM POUR LE DÉVELOPPEMENT D'UNE STRATÉGIE DE PROTECTION INTÉGRÉE DU NIÉBÉ Vigna *unguiculata* (L.) AU KENYA

Composition du Jury:

Dr. Finn Kjellberg (UMR CEFE) (France) - Examiner
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Dr Sevgan Subramanian, ICIPE, Nairobi, Kenya – co-supervisor- Invited persons

DEDICATION

To my beloved family, my wife Eyerusalem Shawel, my son Aklilemariam Andnet, and my Triplet daughters Tamiremariam Andnet, Wudasiemariam Andnet and Tselotemariam Andnet.

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ABSTRACT

The overarching aim of this thesis is to investigate the behaviour and ecology of the legume flower thrips *Megalurothrips sjostedti* Trybom and develop alternative control method. Its specific goals are to: (1) assess pest problems and management methods practiced by grain legume producers, and identifying the cropping systems in the study areas in Kenya; (2) study the impact of climate change on the geographic distribution of legume flower thrips on cowpea growing regions of Sub Saharn African countries; (3) study the behavior and survivorship of the legume flower thrips to develop an alternative control techniques using different essential oils and their derivative compounds. In most cases, development of alternative pest management strategy poorly considers to assess the indigenous knowledge and the perception on pest management practice. All of that can give ideas to be studied, adapted and eventually served as the building blocks in IPM programmes. In order to estimate the status of grain legume pest management practice, a field survey was conducted through household interviews based on a semi-structured questionnaire in eastern Kenya districts in highly representative areas of the main grain legume producing zone. The main results were the following ones: farmers were able to identify key pests of grain legumes using pictorial guide namely the legume flower thrips *Megalurothrips sjostedti*, the cowpea aphid *Aphis craccivora* and the legume pod borer *Maruca vitrata.* In addition to that, the application of chemicals is the main control method practiced with an increasing concentration, chemical alternation, frequent application and mixing of chemicals to ensure pesticide effectiveness. This is an indicator of resistance development and less efficient way of managing pests. In addition, farmers mentioned the erratic rain as the main challenge together with damage caused by pests. These findings help filling the gap in the literature related to grain legume farmer's knowledge on pest management and its practice. This can be a base line for developing alternative control strategy which can be helpful for an easy adaptation of newly developed strategies to the farming system and other realities. Since climate change is also one of the main problems mentioned by 93% of the respondents, an investigation on climate change effect on the geographic distribution of the legume flower thrips *Megalurothrips sjostedti* Trybom and its host crop in Africa has been conducted using 166 presence records of *Megalurothrips sjostedti* and 350 presence records of cowpea in order to model the climate change effects. MAXENT (Maximum entropy) modelling tool is used to predict the effects of climate change for the year 2055. AFRICLIM, a high-resolution (up to 1 km) climate variable was used for projecting current and future potential distribution of this pest and its host in Africa. Rainfall and temperature are the influential environmental variables identified by a "jackknife" analysis. A study of the behavior and survivorship of legume flower thrips *M. sjostedti* was carried out with the intention of developing an alternative thrips controlling strategy; this study on the repellent effect of 24 plant extracts against legume flower thrips *M. sjostedti* was conducted using a visual cue-still air olfactometer in a fume hood. The results revealed that, among the tested 24 plant extracts, 7 showed a good repellency against *M. sjostedti.* The analysis based on this identification of the constituent compounds of the best repellent extracts was done using Gas Chromatography Mass Spectro photometer (GC-MS). Mono- and sesqui terpene hydrocarbon compounds from seven highly repellent extracts were identified. This has led to an investigation of the behavioural effects of the identified compounds on the second instars larvae and the adult female *M. sjostedti.* It has been studied the repellent, toxic and deterrent effect of 16 identified compounds against the adult female legume flower thrips *M. sjostedti* using different bioassay methodologies. The result revealed that the use of repellent extracts could be one of the useful in developing integrated pest management strategies for thrips on legume crops. In addition, the study of specific mode of actions of the identified compounds indicates that the tested compounds are not efficient as alternatives for insecticide application. Thus plant compounds could be used as a safe method of control in integrated pest management for the control of *M. sjostedti* and Thripidae family in combination with the current pest management strategies for grain legumes in Sub-Saharan Africa. However, the plant extracts and our results may be exploited by extending behavioural manipulation to plant compounds released by companion plants through volatile collection and test under laboratory and field condition.

Key words

Farmers knowledge, cowpea, integrated pest management, Eastern Kenya *Megalurothrips sjostedti*, AFRICLIM, climate change, climatic favorability, Sub Saharan Africa, Plant extracts, Repellency, Olfactometer; second instar larvae, Secondary plant compounds, Settlement preference, Toxicity, behaviour.

RESUMEN

L'objectif principal de cette thèse a été de rechercher une technique alternative de protection du niébé *Vigna unguiculata* (L.) contre le thrips des fleurs *Megalurothrips sjostedti* Trybom en se basant d'une part sur les pratiques actuelles des petits producteurs au Kenya et d'autre part sur le comportement et l'écologie de cette espèce à l'égard des signaux olfactifs qui seraient susceptibles de les repousser. Plus spécifiquement les buts à atteindre étaient les suivants: (1) Identifier et recenser les dégâts et l'impact sur la production occasionnés par les ravageurs du niébé dans différents agrosystèmes du Kenya et les méthodes utilisées par les petits producteurs pour les éviter ou les limiter; (2) Estimer l'impact des changements climatiques attendus dans les différentes régions d'Afrique sub-Saharienne sur la distribution du thrips des fleurs du niébé; (3) Etudier l'effet répulsif et insecticide de plusieurs huiles essentielles et de leurs composants majeurs sur les thrips pour proposer des associations avec des plantes potentiellement répulsives dans le cadre de stratégies de protection intégrées. L'idée de départ a été de recenser les pratiques des petits producteurs en matière de protection des cultures de niébé mais aussi identifier leur connaissance et leur perception des différentes techniques utilisées qui pourraient servir au développement d'une stratégie de protection intégrée en alternative à la lutte chimique. Afin de recenser le statut des différents ravageurs et d'identifier les pratiques de lutte utilisées, j'ai réalisé une enquête auprès des familles de petits producteurs, basée sur un questionnaire semi-structuré et ce dans plusieurs districts représentatifs de la région Est du Kenya principale zone de production du niébé. D'après les producteurs, les principaux ravageurs du niébé sont le thrips des fleurs *M. sjostedti*, le puceron *Aphis craccivora* et le foreur des gousses *Maruca vitrata.* La lutte chimique serait la principale, si ce n'est la seule, technique utilisée par les petits producteurs pour la protection du niébé avec une augmentation des fréquences d'application et des doses de pesticides chimiques utilisés en mélange qu'ils doivent pulvériser pour conserver leur efficacité. Ce résultat suggère la sélection de populations de ravageurs devenues résistantes aux pesticides qui ne peut qu'accentuer la baisse d'efficacité de cette lutte chimique sans parler de son impact croissant sur la santé et l'environnement. Ce problème viendrait s'ajouter, toujours aux dires des producteurs, à un régime pluviométrique irrégulier qui constitue avec les dégâts des ravageurs les principales contraintes de cette culture. Ces résultats nous ont permis de confirmer et compléter ceux de la littérature concernant en particulier les ravageurs et le niveau de connaissances des petits producteurs en matière de protection de la culture du niébé et de montrer la nécessité de développer des méthodes de lutte alternatives à la lutte chimique qui pourraient être adaptées aux conditions de cultures et adoptées par les producteurs. Nos résultats d'enquête ayant montré que les aléas climatiques étaient aussi un facteur important de perte de production pour 93% des producteurs, j'ai tenté d'estimer l'impact des changements climatiques potentiels en Afrique sub-Saharienne sur la distribution géographique du thrips *M. sjostedti* en me basant sur sa répartition actuelle (166 références) et la répartition du niébé (350 références). J'ai utilisé pour cela les outils MAXENT (Maximum Entropy) et AFRICLIM pour prédire les effets des changements climatiques en 2055. La pluviométrie et la température ont été les variables environnementales retenues en utilisant une méthode de ré-échantillonnage de type 'jacknife'. J'ai aussi étudié l'effet répulsif et insecticide de 24 huiles essentielles et de leurs composés majeurs sur des adultes de thrips *M. sjostedti* en utilisant un olfactomètre à colonnes placées verticalement sous une hotte. Les résultats ont mis en évidence un fort effet répulsif de 7 extraits de plantes. J'ai effectué des analyses chimiques par chromatographie en phase gazeuse couplée à un spectrophotomètre de masse (GC-MS) pour identifier les composés hydro-carbonés de ces 7 extraits. J'ai pu montrer l'effet répulsif mais pas insecticide de 16 de ces composés sur larves et adultes de *M. sjostedti* en utilisant différents bioessais.

Les résultats de mes études ont montré le potentiel de l'utilisation d'extraits de plantes pour repousser les thrips. L'utilisation d'extraits de plantes répulsives pourrait être intégrée à une nouvelle stratégie de protection du niébé. Cependant aucun des composants testés n'a montré de propriété insecticide. Des plantes productrices de certains de ces composés volatiles répulsifs pourraient aussi être utilisées en association pour mieux protéger les cultures de niébé dans le cadre d'une stratégie de lutte intégrée de type push-pull. Mais ces plantes potentiellement répulsives devront avant tout être validés en laboratoire puis dans des expérimentations en plein champ.

Mots clés Petits producteurs, niébé, *Vigna unguiculata*, lutte intégrée, Kenya, thrips, *Megalurothrips sjostedti*, extraits de plante, huiles essentielles, répulsif, insecticide, comportement, AFRICLIM, changement climatique, Afrique sub-Saharienne.

RIASSUNTO

L'obiettivo generale di questa tesi è quello di studiare il comportamento, l'ecologia e di sviluppare un metodo di controllo alternativo del tripide dei fiori delle leguminose *Megalurothrips sjostedti* Trybom. Gli obiettivi specifici sono i seguenti: (1) valutare i problemi legati al fitofago e i metodi di gestione praticati dai produttori di leguminose da granella, e di identificare i sistemi colturali nelle aree di studio in Kenya; (2) studiare l'impatto del cambiamento climatico sulla distribuzione geografica del tripide dei fiori delle leguminose sul fagiolo dall'occhio coltivato in aree dei Paesi dell'Africa subsahariana; (3) studiare il comportamento e la sopravvivenza del tripide dei fiori delle leguminose per sviluppare una tecnica di controllo alternativa utilizzando diversi oli essenziali e i relativi composti derivati. Nella maggior parte dei casi, lo sviluppo di una strategia alternativa di gestione delle specie nocive considera in scarsa misura la valutazione delle conoscenze locali e la percezione delle pratiche di gestione delle specie nocive. Ciò può fornire idee da studiare, adattare e infine servire come elementi fondamentali dei programmi di gestione integrata. Al fine di valutare lo stato delle pratiche di gestione dei parassiti delle leguminose da granella, è stata condotta un'indagine in campo mediante interviste alle famiglie sulla base di un questionario semi-strutturato nei distretti del Kenya orientale, in aree altamente rappresentative della principale zona di produzione dei legumi da granella. I risultati essenziali sono i seguenti: gli agricoltori sono stati capaci di identificare i parassiti-chiave dei legumi da granella e cioè il tripide dei fiori delle leguminose *M. sjostedti*, l'afide del fagiolo dall'occhio *Aphis craccivora* e la piralide delle leguminose *Maruca vitrata*. Inoltre, l'applicazione di prodotti chimici è il principale metodo di controllo praticato con concentrazioni in aumento, alternanza delle molecole chimiche, l'applicazione ripetuta e la miscelazione di prodotti chimici per garantire l'efficacia pesticida. Ciò è indice di sviluppo di resistenza e di modalità scarsamente efficaci di gestione dei parassiti. Inoltre gli agricoltori hanno riferito che il regime pluviometrico irregolare e i danni provocati dai parassiti possono essere di ostacolo alla crescita della coltura. Questi risultati aiutano a colmare la lacuna nella letteratura relativa alla conoscenza del coltivatore delle leguminose da granella circa la gestione dei parassiti e il suo impiego. Ciò può essere una linea di base per lo sviluppo di strategie di controllo alternative utili per un facile adattamento di nuove tecniche al sistema agricolo e altre realtà. Dal momento che il cambiamento climatico è anche uno dei principali problemi citati dal 93% degli intervistati, una ricerca sugli effetti dei cambiamenti climatici sulla distribuzione

geografica del tripide dei fiori delle leguminose *M. sjostedti* e la sua coltura ospite in Africa è stata condotta con 166 dati di presenza di *M. sjostedti* e 350 dati di presenza del fagiolo dall'occhio allo scopo di costruire un modello relativo agli effetti del cambiamento climatico. Per prevedere gli effetti dei cambiamenti climatici per l'anno 2055 è stato utilizzato il modello MAXENT basato sul principio della massima entropia. La variabile climatica ad alta risoluzione (fino a 1 km), AFRICLIM, è stata utilizzata per la proiezione attuale e la potenziale distribuzione futura di questo parassita e il suo ospite in Africa. Le piogge e le temperature sono delle influenti variabili ambientali identificate mediante il metodo di analisi "Jackknife". Uno studio del comportamento e della sopravvivenza del tripide dei fiori delle leguminose *M. sjostedti* è stato condotto con l'intenzione di sviluppare una strategia alternativa di controllo dei tripidi; questo studio sugli effetti repellenti di 24 estratti di piante nei confronti del tripide dei fiori delle leguminose *M. sjostedti* è stato realizzato utilizzando un olfattometro in aria ferma con segnale visivo posto in una cappa aspirante. I risultati hanno rivelato che, tra i 24 estratti vegetali testati, 7 hanno mostrato una buona repellenza contro *M. sjostedti*. L'analisi basata sull'identificazione dei composti costituenti dei migliori estratti repellenti è stata effettuata mediante gas cromatografia/spettrometria di massa (GC-MS). Sono stati identificati composti di idrocarburi mono- e sesqui terpenici provenienti da sette estratti altamente repellenti. Questo ha portato a un'indagine sugli effetti comportamentali dei composti identificati sul secondo stadio larvale e sugli adulti di sesso femminile di *M. sjostedti*. E' stato studiato l'effetto repellente, tossico e deterrente di 16 composti identificati contro le femmine del tripide dei fiori delle leguminose *M. sjostedti,* utilizzando diversi saggi biologici. Il risultato ha rivelato che l'uso di estratti repellenti potrebbe essere utile nello sviluppo di strategie di difesa integrata contro i tripidi delle leguminose. Inoltre, lo studio della specifica modalità di azione dei composti identificati indica che i composti saggiati non sono efficaci come alternativa agli insetticidi. Pertanto i composti vegetali potrebbero essere usati come un metodo sicuro di controllo nella gestioneintegrata per il controllo di *M. sjostedti* e della famiglia Thripidae, in combinazione con le attuali strategie di lotta per i legumi da granella nell'Africa subsahariana. Inoltre, gli estratti vegetali e i nostri risultati possono essere sfruttati estendendo la manipolazione del comportamento all'utilizzo di composti vegetali rilasciati da piante in consociazione attraverso la raccolta di volatili e il saggio in condizioni di laboratorio e di campo.

Parole chiave

Conoscenza degli agricoltori, fagiolino dall'occhio, gestione integrata dei fitofagi, Kenia Orientale, *Megalurothrips sjostedti*, AFRICLIM, cambiamento climatico, vantaggiosità climatica, Africa subsahariana, estratti vegetali, repellenza, olfattometro, secondo stadio larvale, composti secondari delle piante, valutazione della preferenza, tossicità, comportamento.

CHAPTER ONE - GENERAL INTRODUCTION

1. Introduction

Food and nutritional security are an important requisite for sustainable development in Africa. Diversification of African small-holder production system including both staple food crops and non-staple crops like grain legumes is essential to achieve the objective of food and nutritional security in Africa and as an adaptation to climate change effects (Heidhues et al. 2004). Among the grain legumes, bean (*Phaseolus vulgaris* L.), (cowpea (*Vigna unguiculata* L.) and pigeon pea (*Cajanus cajan* L.) are among the most important food legumes grown extensively in tropical Sub-Saharan Africa for their dry seeds, leaves and green pods and also as livestock feed. Cowpea (*Vigna unguiculata* L. Walp.) is a herbaceous, warm-season annual requiring temperatures of at least 18^{o}C throughout all stages of its development and having an optimal growing temperature of about 28 ^{o}C (Craufurd et al. 1997). It is one of the most important food and forage legumes in the semi-arid tropics. It is grown in 45 countries across the world which includes parts of Africa, Asia, Southern Europe, Southern United States and Central and South America (Singh 2005; Timko et al. 2007).An estimated 14.5 million ha of land is planted to cowpea each year worldwide. The Sub-Saharan Africa (SSA) region accounts for about 84% of area under cowpea production as well as production (Abate et al. 2011). Cowpea production is regarded as an integral part of the traditional cropping system throughout Africa (Isubikalu et al. 2000). The crop provides food for man and feed for livestock and serve as a valuable and dependable revenue-generating commodity for farmers and grain traders (Singh 2002; Langyintuo et al. 2003; Owolade et al.2004).

1.1 Production Constraints

Although potential yields of the crop (3000 kg/ha) have been reported (Rusoke & Rubaihayo 1994), cowpea grain yields average only 200–400 kg/ha in Uganda (Sabiti et al. 1994; Omongo et al. 1997), and 200–300 kg/ha in Nigeria (Alghali 1992). In all of the cowpea growing regions, both abiotic and biotic stresses can result in a significant yield reduction in cowpea. Despite cowpea being more drought tolerant than many other crops, still moisture availability is the major constraints to growth and development, especially during germination and flower stage. Erratic rainfall affects adversely both plant population and flowering ability, resulting into tremendous reduction of grain yield and total biomass in general (Timko & Singh 2008).

Insect pests attack the crop at all stages and are probably the main factor limiting grain legume yields in the tropics (Singh & van Emden 1979; Omongo et al. 1997; Karungi et al. 2000; Ajeigbe & Singh 2006). High pest densities occur at many locations with complete loss of grain yield if no control measures are taken. The crop is attacked by a spectrum of pest species (Isubikalu et al. 2000). Aphids (*Aphis craccivora* Koch) attack cowpea especially in the seedling stage, while flower thrips (*Megalurothrips sjostedti* Trybom) and pod borer (*Maruca vitrata* Fab.) attack the crop at flowering and pod formation. A complex of pod sucking bugs and weevil (*Callosobruchus maculatus*) attack the crop at podding and during seed storage, respectively. Moreover, the legume flower thrips, *M sjostedti* alone can cause 20-100% when control measures are absent (Singh & Allen 1980). Thrips occur every growing season and result in yield losses through premature dropping of flowers (Ekesi et al. 1998).

1.2 Thrips classification

Thrips belong to the class Insecta in the Order Thysanoptera. Thrips are tiny slender insects with fringed wings (Lewis 1997). There are two suborders, Terebrantia with saw like ovipositor for endophytic oviposition and the Tubulifera without saw like ovipositor with exophytic oviposition. Sub-order Terebrantia includes seven families, with the members of the family Thripidae comprising of economically important vegetable and flower crop pests worldwide. Tubulifera includes only one family and few economically important pests, which include Terebrantia and Tubiliferans. The number of thrips species is estimated to 7000 of which 5500 are identified and confirmed (Lewis 1997a). The order Thysanoptera is recognized in nearly 800 genera (Mound 2008). For their accurate identification new tools such as the Lucid dichotomic key which is based on morphological and molecular characteristic of thrips are being developed (Moritz et al. 2001; EPPO 2002).

The family Thripidae is currently interpreted as comprising rather more than 2000 described species, which are grouped into four subfamilies, i.e. Panchaetothripinae, Dendrothripinae, Sericothripinae and Thripinae with 125/35, 95/13, 140/3 and 1700/225 species/genera, respectively (Masumoto 2010). Currently, eight genus-groups are used instead of the formal tribal classification, as follows: *Anaphothrips* genus-group, *Frankliniella* genus-group, *Thrips* genus-group, *Megalurothrips* genus-group, *Taeniothrips* genus-group, *Mycterothrips* genus-group, *Scirtothrips* genus-group and *Trichromothrips* genus-group (Mound & Palmer 1981; Masumoto & Okajima 2007; Mound & Masumoto 2009; Mirab-balou & Chen 2011).

From the eight genus-groups mentioned above, *Megalurothrips* genus-group comprises six genera, i.e. *Ceratothripodes, Ceratothrips*, *Megalurothrips*, *Pezothrips*, *Odontothripiella* and *Odontothrips* (Mound & Palmer 1981).

1.3 The Genus Megalurothrips

The genus *Megalurothrips* Bagnall, 1915 has thirteen species (Mound 2011, Mirab-balou et al. 2011) that all breed in the flowers of Fabaceae and some are pests of cultivated legumes (Masumoto 2010).

Table 1.1 List of species of the Genus *Megalurothrips*

No	Species Name	Reported Location
1	*Megalurothrips sjostedti* Trybom, 1908	Sub saharan Africa countries
2	*Megalurothrips distalis* Karny, 1913	USA, Iran, Korea, India, Fiji, Indonesia, Sri Lanka, Philippines
3	*Megalurothrips usitatus* Bagnall, 1913	Sri Lanka, India, Austaralia
4	*Megalurothrips typicus* Bagnall, 1915	India, Indonesia, Phlippines, Uganda
5	*Megalurothrips peculiaris* Bagnall, 1918	India
6	*Megalurothrips formosae* Moulton, 1928	Taiwan
7	*Megalurothrips mucunae* Priesner, 1938	Fiji
8	*Megalurothrips sinensis* Woo, 1974	Korea
9	*Megalurothrips basisetae* Han & Cui, 1992	China - Sichuan
10	*Megalurothrips grisbrunneus* Feng, Chou & Li, 1995	China - Shaanxi
11	*Megalurothrips equaletae* Feng, Chao & Ma, 1999	China - Shaanxi
12	*Megalurothrips haopingensis* Feng, Chao & Ma, 1999	China - Shaanxi
13	*Megalurothrips guizhouensis* Zhang, Feng & Zhang, 2004	China - Huizhou

1.4 Megalurothrips sjostedti (Trybom)

Among the thirteen species of Megalurothrips, the Legume Flower Thrips (LFT) *Megalurothrips sjostedti* (Trybom) are among the most serious constraints to grain legume production in the humid tropical zones of Eastern and Western Africa (Ekesi et al. 1998). In tropical Africa *M. sjostedti* causes damage and distortion to cowpea through feeding on leaves, fruits or petals. Their raspingsucking mouthparts puncture plant surfaces and suck the cytoplasm. Egg-laying also damages plants. The injury appears in streaks rather than spots. Blossoms become brown, petals are distorted and buds fail to open, floral parts especially the anthers and filaments of cowpea, are malformed and distorted (Singh & Taylor, 1978, Ekesi & Maniania, 2000). The malformation of reproductive parts reduces pollination and seed production, while heavily infested plants produce no flowers (Taylor 1965). Injury to flowers begins before they open when larvae and adults attack the terminal buds and bracts. Infested flowers and buds appear dried, brown and may eventually abort leaving dark red scars (Alghali 1992). Grain yield losses of between 20 and 100% have been reported for *M. sjostedti* on cowpea from different parts of Africa (Ekesi et al. 1998). Cowpea yield have been increased by 30 to 90 percent by controlling this thrips species with insecticides (Bal, 1991).

In addition to cowpea, *M. sjostedti* is a known primary pest of French beans (*Phaseolus vulgaris* L.) in Kenya. It causes 40-60% yield losses at farm level (MOA 2006), mainly through abscission of buds, flower abortion and pod malformation making them unfit for the export market (Seif et al. 2001). Their punching and sucking feeding behaviour also blemishes and causes silvery lesions on pods, resulting in a further 20% loss at harvest sites (Kibata & Anyango 1996; Lohr 1996).

1.5 Farmers Knowledge and pest management practices

One of the major constraints upon establishing an IPM programme is the lack of adequate information about farmers' knowledge, perceptions and practices in pest management (Heong 1985; Teng 1987; Morse & Buhler 1997). If scientists have to work with farmers to improve crop production and crop protection, they should recognize farmers' constraints and their existing technical knowledge (Kenmore 1991; Bentley 1992; Morse & Buhler, 1997). Knowledge of pests varies between farmers working in similar or different agro-ecosystems. In some cases, pest recognition is a major problem, while in others knowledge about pest ecology is the major constraint (van Huis & Meerman, 1997). Generally, farmers have good knowledge about easily observable and important pests (Bentley 1992). However, farmers and scientists may differ in their opinion about the importance of a particular pest.

Farmers know some things that scientists know, some things that scientists do not know, and, also, farmers do not know some things that scientists do know. There are, in addition, a lot of things that neither group knows (Chambers 1991). Much of this epistemological difference can be attributed to different styles of observation. People (including scientists and farmers) perceive more about things that are easy to observe. They pay more attention to things that are culturally and economically important. Sometimes, different styles of observation lead to radically different conclusions. To innovate or develop new, appropriate technologies or devise effective modifications of existing practices it is therefore necessary to study farmer's knowledge and practice.

Pest management practices in traditional legume cropping systems depends on a complex management system (Abate et al. 2000). Thus, grain legumes crop protection practices used by farmers represent a rich resource for entomologists to develop IPM systems that are well adapted to the agroecological, cultural and socio-economic circumstances facing smallholder farmers in the developing world. Integrated pest management has long been proposed as the future for different crop production. However, the concept requires new ideas through knowledge of biological interactions and information on the crop and on the surrounding environment (Adati et al. 2008) and understanding farmers' knowledge and perceptions on the pest constraints and IPM (Nwilene et al. 2008). Understanding grain legume farmers' knowledge and perceptions of pests and management practices is the first step in developing innovative pest management strategies to enhance sustainable grain legume production and to improve grain legumes productivity to improve the livelihood of the smallholder farmers in Sub-Saharan African countries.

There is a wide diversity of research and development projects in agriculture. One type of project is related to IPM programs, which are generally presented as an alternative to the indiscriminate use of pesticides. IPM makes use of various forms of control: biological, ethological, mechanical, physical, genetic, legal and chemical, which generally imply the farmer knows the biology and behavior of pests so he or she can make appropriate management decisions.

1.6 *Megalurothrips sjostedti*, cowpea and climate change

Agriculture is one of the sectors that directly depend on climate factors and thus climate variability and climate change have been implicated to have significant impacts on global and regional food production. Global warming is projected to raise the mean temperature of the earth by 1.5–5.8 ^{0}C by the end of the century. Besides, the earth is warming and leading to unnatural migration, global warming is being blamed for insects becoming populous and larger in size (Chakraborty et al. 2000). On a world-wide average, 18% of the yield losses in agricultural crops are caused by arthropod pests despite the application of pesticides for their control (Oerke 2006) and this climatic change effect is expected to aggravate the already serious challenges to food security and economic development, especially in developing countries where pests cause 30 to 50% of the yield losses in agricultural crops (IPCC, 2001, 2007). Recent predictions forecast, with a high probability (>90%), higher growing season temperatures in the tropics and subtropics that will exceed the most extreme seasonal temperatures recorded in the last century (Battisti and Naylor 2009).

Arthropods are exothermic organisms that cannot internally regulate their own temperature, and their development depends on the temperature to which they are exposed in the environment. Hence, temperature is considered, among climate-change elements, the dominant abiotic factor directly affecting herbivorous insects (Bale et al. 2002). Considerable attention has already been given to the impacts of climate change on insects in temperate regions (Ward and Masters 2007; Battisti et al. 2006; Harrington et al. 2001; Volney & Fleming 2000; Cammell & Knight 1992; Porter et al. 1991).

Thrips populations in crops are determined by the natural growth of population and the influence of weather on activity and rate of multiplication of the insects (Kirk 1997). An understanding of the factors that influence these population changes is essential in predicting thrips population. Weather variables including rainfall, temperature, relative humidity and wind have been reported as important factors that significantly affect thrips numbers (Ananthakrishnan 1993; Kirk 1997; Legutowska 1997). In a climate change scenario, techniques developed to detect areas of climatic favorability, as species distribution models, are considered to represent useful approaches for detecting suitable areas of colonization and establishment of a species (Lo´pez- Darias et al. 2008). Empirical studies have shown in fact that climate change has already caused range shifts in many species (Parmesan & Yohe 2003).

Relatively high temperatures and lack of rainfall have been associated with increase in thrips population, while high relative humidity and rainfall reduce thrips population (Hamdy & Salem 1994). In addition to their effect on thrips activity, temperature and relative humidity further i.nfluence the intrinsic rate of natural increase of the thrips (Murai 2000). A basic understanding of the relationship of these factors with thrips population is important in developing an integrated control strategy for thrips in grain legumes and in determining the potential pest control needs for a given climatic trend.

Among the principal pests of cowpea, the legume flower thrips *M sjostedti* alone can cause 20-100% when control measures are absent (Singh & Allen 1980). Therefore, as part of the study, chapter 3 presents a study on the impact of climate change on the legume flower thrips *M sjostedti* distribution and suitability of the host crop cowpea in Africa. It is necessary to determine the population suitability pattern of thrips in cowpea growing regions of the continent to characterize the associated climate change scenario and thrips population dynamics with the aim of predicting climatic trends that would cause thrips distribution in Africa. I believe that this study is timely and highly relevant given the potential threats to overall cowpea and other legume production habitat in Africa due to this harmful species (Abudulai et al. 2006; Asante et al. 2001; Oparaeke 2005).

1.7 Developing alternative pest management

Integrated Pest Management (IPM) is an effective and environment-friendly pest management system. It is an ecological approach to pest management in which all available effective techniques are deployed in a unified programme so that the pest populations can be managed to avoid economic damage and minimize adverse side effects.

Current control strategies on cowpea production mainly include the use of chemical insecticides. However, the application of various synthetic insecticides has led to a number of problems such as environmental pollution, pesticide residue in food grains, development of insecticide resistance and toxicity to non-target organisms (Jahromi et al. 2012). Additionally, the cost of chemical insecticides is becoming increasingly inaccessible to farmers, particularly in developing countries. Insecticides for thrips control and the exclusive use of resistant crop varieties for management of plant pathogenic viruses do not have long-term sustainability (Gillett et al. 2009). This, together with the demand for contaminant-free food, has fostered the search for alternative methods of control (Ekesi et al.1998). Techniques such as cultural practice, plant sanitation, the use of varietal resistance and intercropping are used to limit thrips population to below economical threshold levels (Waiganjo et al. 2006). Use of colored sticky traps and semiochemicals is also being developed for thrips monitoring, mass-trapping and population control (Teulon & Penman 1992;

Teulon et al. 2010; Berry et al. 2010) and use of entomopathogenic fungi are used in small-scale. A multi-tactic IPM program for thrips management is especially critical due to the potential development of insecticide resistance among thrips populations.

The increasing public concern over pesticide safety and possible damage to the environment has resulted in increasing attention being given to natural products for the control of pests (Jahromi et al. 2012). In this context, many plant products have been evaluated for their toxic properties against different insect pests, especially in the form of essential oils (Regnault-Roger, 1997). In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides. Terrestrial plants are known to contain a rich source of bioactive metabolites which show anti-feedant, repellent and toxic effects in a wide range of insects (Jahromi et al. 2012).

Plant essential oils in general have been recognized as an important natural source of pesticides and essential oil compounds and their derivatives are considered to be an alternative means of controlling insect pests (Tripathi et al. 2009). The use of unattractive plant odors to repel or deter pest insects from crops has resulted in several commercial pest-control products in recent years (Isman 1999, 2000, 2006; van Tol et al. 2006). Essential oils extracted from aromatic plant species are an important source of repellents which is used in food flavoring and the perfume industry (Coppen 1995). These odors have been extensively tested for safety and toxicity and have shown no detrimental impact on beneficial insects, and are therefore considered to be an interesting potential new means of crop protection (Plimmer 1993; Isman 1999, 2006).

Developing a new IPM control technique like a push-pull and kill strategy for *M. sjostedti* using visual attractive insecticide impregnated net in combination with different repellent essential oils could help to control legume thrips. A reliable, cost effective and sustainable IPM strategy for control of *M. sjostedti* on cowpeas would lead to further uptake of IPM and reduce reliance on pesticides.

The introduction of the maximum residue limit on agricultural products and the development of thrips resistance to synthetic chemical pesticides underlie the research for environmentally-friendly alternatives. Among the alternatives, plant extracts and essential oils in general have been recognized as an important natural sources of pesticides because their compounds and their derivatives are considered to be an alternative means of controlling insect pests. However, no research has not been reported on the use of repellent essential oils against *M. sjostedti*. Therefore, there is need to identify repellent essential oils and their derivative compounds for a combined use with other available controlling techniques to formulate new and modify existing thrips control strategy to develop a push pull and kill strategy as a component of IPM. This technique will work jointly with other components such as agrochemicals, entomopathogenic fungus and other lure and kill strategies in thrips controlling to be taken into consideration. Joint implimentation of this strategy would deliver the repellent product with a spatial arrangment as intercroping or border cropping .

1.8 Thesis overview

The overarching aim of this thesis is to investigate the behaviour, ecology and develop alternative control method for legume flower thrips *Megalurothrips sjostedti* Trybom. Its specific goals are to: (1) assess pest problems and management methods practiced by grain legume producers, and identifying the cropping systems in the study areas in Kenya; (2) study the impact of climate change on the geographic distribution of legume flower thrips on cowpea growing regions of sub saharn african countries; (3) study the behavior and survivorship of legume flower thrips to develop an alternative control techniques using different essential oils and their derivative compounds. The thesis consists of five research chapters, which are written as stand-alone co-authored scientific papers, either published, submitted, or in preparation. For this reason, there is a significant overlap in the study and method section of chapters 4, 5 and 6.

In most cases, development of alternative pest management strategy poorly considers to asses indigenous knowledge and perception on pest management practice which can give ideas can be studied, adapted and eventually served as the building blocks in IPM programmes. In order to assess the status of legume cropping system pest management practice in Kenya, a field survey was conducted and assessed by household interviews based on a semi-structured questionnaire in eastern Kenya districts in areas representative of the main grain legume producing zone. The main points were to identify farmer's pest controlling practice and different cropping systems in which alternative controlling techniques are likely to bring more advantages for grain legumes in the study area. Therefore, **in Chapter 2**, I present the results of my field survey study entitled" Farmers' knowledge and perception to manage pest problems on legume cropping system in Eastern Kenya". This chapter aims to help fill the gap in the literature related to grain legume farmer's knowledge on pest management and their practice, which can be a base line for developing alternative control strategy which can be helpful for easy adaptation of newly developed strategy to the farming system and other realities. Hence, grain legume pest problems and management methods practiced by grain legume producers in relation to the legume cropping systems are presented.

In Chapter 3, an investigation of climate change effect on the geographic distribution of the legume flower thrips *Megalurothrips sjostedti* Trybom in Africa is presented in this chapter. I used 166 presence records of *Megalurothrips sjostedti* and 350 presence records of cowpea to model the effect climate change. MAXENT (Maximum entropy) modelling tool is used to predict effect of climate change for the year 2055. AFRICLIM, a high-resolution (up to 1 km) climate variable was used for projecting current and future potential distribution of this pest in Africa. Influential environmental variables are identified using a jackknife analysis.

In Chapter 4, with an intention of developing alternative thrips controlling strategy, I studied the repellent effect of 24 plant extracts against legume flower thrips *Megalurothrips sjostedti* using a visual cue-still air olfactometer in a fume hood. In addition, I have identified the constituent compounds of the best repellent extracts using Gas Chromatography Mass Spectro photometer (GC-MS).

In Chapter 5, Based on these findings of the repellent extracts and component compounds, to investigate the behavioural effects of the identified compounds a study conducted on the second instar larvae and adult female *M. sjostedti.* I investigated the toxic and behavioral effects of these compounds against the second instar larvae using different bioassay techniques. Finally **In Chapter 6**, I summarized the whole Thesis and I gave a summary about the study. The chapter includes, forward, a brief discussion, conclusion and recommendations.

PART I - SOCIOLOGICAL CONSIDERATION

CHAPTER TWO - FARMERS' KNOWLEDGE AND PERCEPTION OF GRAIN LEGUME PESTS AND THEIR MANAGEMENT IN THE EASTERN PROVINCE OF KENYA

Prepared as:

Statement of contribution

AA, HA and SS conceived and designed research. AA and SN conducted experiments. TM contributed reagents. AA, HA analyzed data, AA, SK and GTG and wrote the manuscript. All authors read and approved the manuscript.

Abstract

Grain legume production in Kenya is severely affected by various insect pests, resulting in poor yields and economic losses. A total of 216 farmers were surveyed in eight districts of eastern Kenya to evaluate farmers' knowledge and perceptions of grain legume pests; to examine current pest management practices and to identify other production constraints. Results showed that cowpea *Vigna unguiculata*, common beans *Phaseolus vulgaris* and pigeonpeas *Cajanus cajan* produced by 99.1 %, 88.0 % and 55.6 % of farmers, respectively. Majority of the farmers (94%) were aware of arthropod pests and considered them as the main constraint to grain legume production. Farmers identify pests descriptively using a pictorial guide. The legume flower thrips *Megalurothrips sjostedti*, the cowpea aphid *Aphis craccivora* and the legume pod borer *Maruca vitrata* were identified as key pests affecting the grain legumes. Application of chemicals is the main control method practiced. However, to ensure pesticide effectiveness, practices such as increased concentration, chemical alternation, frequent application and mixing of chemicals are used by 27.8 %, 25.9 %, 22.7 % and 19.9 % of farmers, respectively. The results showed that 89 % of farmers did not receive any extension services on pest management, which indicates the need for capacity building training on pest management. Here we noticed that, there is a lack of viable integrated pest management (IPM) strategy. Hence, there is a need to develop an innovative IPM strategy that could be cost effective with less deleterious effects on the environment through a multidisciplinary and participatory approach.

Keywords Farmers knowledge, grain legumes, integrated pest management, Eastern Kenya

2.1 Introduction

Food and nutritional security through diversification of African small-holder production systems is an important requisite for sustainable development in Africa (Heidhues et al. 2004). Grain legumes complement the nutritional value of cereals and enable the sustainable intensification of farming systems through nitrogen fixation, extending land cover and nutrient utilization by fitting into a wide range of intercropping configurations (CGIAR 2012). In Sub-Saharan Africa (SSA) countries, grain legume cultivation directly benefits women because they are often the primary cultivators of these crops as well as being employed in small-scale processing, preparation and marketing of foods derived from these crops (CGIAR 2012). Common beans *Phaseolus vulgaris* L., cowpea *Vigna unguiculata* L. and pigeonpea *Cajanus cajan* L. are the first three important food grain legumes in Kenya (Mergeai et al. 2001). The production of grain legumes has been known as a strategic sub-sector with the potential to alleviate food and nutrition security. Cowpea and common beans are the most popular sources of protein for many Kenyans, and particularly for poor people who often cannot afford to buy meat (USAID 2010).

The Eastern province of Kenya represents one of the key areas with high potential for grain legume production (Nagarajan et al. 2007). Although Kenya has two growing seasons for grain legumes, a significant number of farmers grow them once a year because of adverse climatic conditions. More than 90% of the total national production of grain legumes, especially cowpea, and approximately 89% of the total planted area is found in the Eastern region of the country (USAID 2010). Annual production for Kenya is estimated at 65,941 tonnes and 436,279 tonnes for cowpea and common beans, respectively (FAO STAT 2012). This production volume however is insufficient to meet demand, particularly for dry grains.

Grain legume production is constrained by several factors among which arthropod pests are considered the most important and pest attack occurs during all stages of growth (Omongo et al. 1997). The diversity of legume pests dictates that a single control strategy is unlikely to produce satisfactory control in a sustainable manner. The goals and values of long-term sustainability must be reflected in combinations of practices and methods consistent with an individual farmer's resource, including technical know-how and farming practices (Ikerd 1993). Unfortunately, small-holder farmers in developing countries are resource-constrained, and this limits their capacity to pursue the goals of sustainability.

Pest management practices in traditional legume cropping systems depends on a complex management system (Abate et al. 2000). However, failure by farmers to correctly diagnose the real problems, a lack of fit between the proposed techniques and the local farming systems and livelihood strategies, and limited availability of and access to external inputs have been reported as factors that hinder adoption (Midega et al. 2012). Thus, grain legumes crop protection practices used by farmers represent a rich resource for entomologists to develop IPM systems that are well adapted to the agroecological, cultural and socio-economic circumstances facing small land holder farmers in the developing world. Integrated pest management (IPM) has long been proposed as the future for different crop production. However, the concept requires new ideas through knowledge of biological interactions and information on the crop and on the surrounding environment (Adati et al. 2008) and understanding farmers' knowledge and perceptions on the pest constraints and IPM (Nwilene et al. 2008).

Objectives

Understanding grain legume farmers' knowledge and perceptions of pests and management practices is the first step in developing innovative pest management strategies to enhance sustainable grain legume production. Therefore, the objectives of the study were: (i) to evaluate farmers' knowledge and perceptions of grain legume pests; (ii) to examine farmers' current pest management practices on grain legumes; and (iii) to identify other grain legume production constraints in order to develop an efficient IPM approach that would contribute to improve grain legumes productivity in eastern Kenya and to improve the livelihood of the small land holder farmers in Sub-Saharan African countries.

2.2 Materials and Methods

Study Sites. The study was conducted between April and June 2013 in the main grain legume growing areas in eight (8) districts of the Eastern province of Kenya. These were Embu- East, Machakos, Makueni, Masinga, Mbeere-North, Mwingi, Mwingi-Central and Nzambani (Fig 1). These sites are located in lower midland, semi-arid agro-ecology zones that ranges between 500 - 1200 m above sea level and is characterized by erratic bimodal rainfall with an average annual rainfall of 640 - 1000 mm (Nagarajan et al. 2007). The main cropping systems comprise cereal crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and pearl millet (*Pennisetum glaucum* L.) that are generally grown in mixed stands and intercropped with a range of legumes, including common beans (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* L.) and pigeonpea (*Cajanus cajan* L.).

Data Collection The surveys were conducted through household interviews using a semi-structured questionnaire methodology adapted from Midega et al. (2012). A total of 216 farmers were interviewed by seven teams of trained enumerators.each team consisted one to two enumerators. Recruitment of enumerators and teams was done in such a way that, at least one of the team members was knowledgeable of the local language and familiar with the targeted study area.

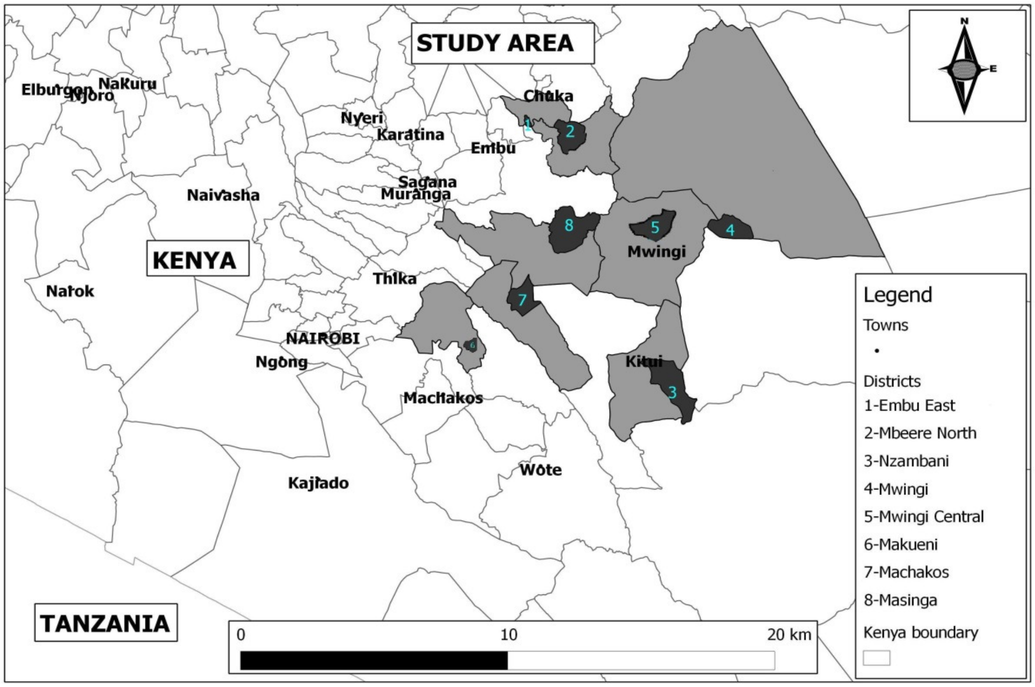

Figure. 1. Study areas in the main grain legume growing districts of eastern Kenya. The areas colored in grey are the districts in eastern province where this study was conducted. The specific study areas within the districts where the questionnaire was conducted are shown in black.

The questionnaire was pretested and interviewers translated the questions into local language but the responses were recorded in English. Farmers' knowledge of grain legume crop pests was scored by displaying a pictorial guide showing the pest and its damage to facilitate pest recognition by the interviewed farmer.

Data Analysis. Survey data were analyzed using descriptive statistics (means, frequencies and percentages). To examine the socio-economic characteristics of the rural households and the differences between districts, gender and education levels with regard to the perception of pests and their management practices Chi-square and one-way analysis of variance (ANOVA) were conducted using JMP, statistical software version 5 (SAS Institute 2002). Significance level was set at 0.05 and means separated by Tukey HSD test. In order to evaluate the knowledge of farmers on grain legume crop pests, we considered (1) the farmers' personal characteristics (age, gender, formal education, legume growing experiance and income) (2) exposure to sources of information about pest management and method of control practiced (3) the perception on the level of pest importance for for different grain legume pests. The variable was in terms of crop attack (i.e. no damage, some damage and significant damage). (4) Farmers' knowledge on legume pests. This variable was measured using a score from 0 to 3 as follows: a farmer who could not mention a legume pest by a name, its description or the type of damage was given a score of zero; (i.e. No knowledge = 0); a farmer who gave the name of only one pest, one feature and one type of damage caused by the pest was given a score of one; (i.e. low knowledge = 1); a farmer who was able to give the name of two pests, describe at least one feature of each pest and at least one type of damage caused by the pests was given a score of two; (i.e. medium knowledge = 2). A farmer who was able to name three or more pests, describe one or more features of each, and identify at least one type of damage caused by each pest was given a score of three (i.e. high knowledge = 3) additionally, all the categorical and ordinal parameters were compared with the reference district(i.e) Nzambani.

The dependent variable knowledge level was categorical and ordinal, thus we used multivariate ordered probit regression analysis to analyse the data. In the context of this model, the dependent variable takes j ordered categories, where j = 0 if the knowledge is low or non-existent, and j = 3 if knowledge is high. The observed ordered responses are assumed to be linked to a latent variable z_i that is normally distributed. This link is represented in the following equation: $z_i = \chi_i\beta + \varepsilon_i$ where x_i is a $n \times k$ matrix of explanatory variables, β is a $k \times 1$ vector of unknown coefficients to be estimated, and ε_i is a normally distributed random error term.

2.3 Results and Discussion

Socio-demographic and household characteristics

The Farmers' age averaged 47.5 years with a minimum of 19 years and maximum of 78 years. More than 44.4% being above 50 years-old, 37.1% being of the middle age category (31-50 years), and only 18.5% being 30 years and below. There was no significant difference in average age across districts (Table 1). Almost all the participants (95%) were the head of their families. There was no significant difference between gender across districts. Fifty one percent (51 %) of cowpea growers were women who grow the crop primarily for household food, and for whom crop sale occurs only in case of yield surplus.

The majority (77.8%) of the respondents had received formal education. Among the respondents with formal education, 23.2 % had ≤ 5 years of basic education, 22.0% had attained 6-10 years of education, 40.5 % had 11-12 years of education, and 14.3% had >12 years of education. Only 22.2% of respondents had no formal education. The overall average household size was 6.3±0.41 individuals. Average household sizes vary significantly across districts (Table 1).

Cultural practices of grain legume farming. Farmers used most of their land for the production of food crops with about 99% of the respondents growing maize *Zea mays* L. as a staple crop. The majority of them (97.2% of the total) was growing grain legumes as main intercrops with maize and other cereals. While only 2.8% of the respondents grew legumes as sole crop. Intercropping and rotation are commonly used especially to maintain soil fertility, space utilization, diversification and in some cases to reduce pest damage. Some studies have reported the reduction of pests and diseases in the intercropping systems (Ampong-Nyarko et al. 1994; Abate et al. 2000; Karungi et al. 2000). Similarly, most of the interviewed farmers (86.6%) practice crop rotation as

Table 2.1 Socio-economic characteristics, grain legume farming experience, yields and incomes of legume farmers in eastern Kenya

Variables	Districts									
	Embu east	Machacos	Makueni	Masinga	Mbeere North	Mwingi	Mwingi central	Nzambani	Mean	F
Age (years)	50.6(3.00)a	42.0(2.54)a	44.9(2.50)a	44.8(2.91)a	43.3(3.11)a	48.5(2.61)a	48.4(2.83)a	46.6(2.55)a	46.1(2.76)	1.02
Gender (1 Male, 0 Female)	0.71(0.12)a	0.50(0.08)a	0.62(0.10)a	0.55(0.11)a	0.33(0.12)a	0.63(0.10)a	0.42(0.10)a	0.42(0.08)a	0.52(0.10)	1.39
F.Education (1 Yes, 0 No)	1.00(0.00)a	0.74(0.07)ab	0.58(0.10)b	0.80(0.09)ab	0.94(0.06)ab	0.71(0.09)ab	0.69(0.09)ab	0.86(0.05)ab	0.79(0.07)	2.66
Pri_Edu (1 Yes, 0 No)	0.53(0.12)a	0.14(0.05)bc	0.0(0.00)c	0.1(0.07)bc	0.44(0.12)ab	0.38(0.10)ab	0.12(0.06)bc	0.05(0.03)c	0.22(0.07)	7.08
Jun_Edu (1 Yes, 0 No)	0.12(0.08)ab	0.12(0.05)ab	0.15(0.07)ab	0.40(0.11)a	0.22(0.10)ab	0.04(0.04)b	0.23(0.08)ab	0.19(0.06)ab	0.18(0.08)	1.77
High_Edu (1 Yes, 0 No)	0.29(0.11)a	0.36(0.07)a	0.38(0.09)a	0.10(0.09)a	0.17(0.09)a	0.25(0.09)a	0.27(0.09)a	0.46(0.08)a	0.28(0.09)	1.79
Colle_Edu (1 Yes, 0 No)	0.06(0.06)a	0.14(0.05)a	0.04(0.04)a	0.20(0.09)a	0.11(0.08)a	0.04(0.04)a	0.08(0.05)a	0.16(0.06)a	0.10(0.06)	0.93
HH_size (head)	5.71(0.21)ab	6.19(0.34)b	6.77(0.43)ab	6.05(0.62)ab	5.94(0.37)ab	6.58(0.38)ab	7.92(0.70)a	5.34(0.26)b	6.31(0.41)	3.58
Pest mgt (1 Yes, 0 No)	0.41(0.12)a	0.05(0.03)c	0.00(0.00)c	0.10(0.07)bc	0.00(0.00)c	0.29(0.09)ab	0.04(0.04)c	0.07(0.04)c	0.12(0.05)	5.79
Legume farming (years)	27.9(2.91)a	10.8(0.98)c	14.4(0.80)bc	10.1(1.43)c	24.8(2.91)abc	17.58(1.95)abc	24.53(2.98)a	20.88(2.43)ab	19.0(10.2)	8.76
Land_ size (Ha)	0.99(0.18)b	0.91(0.07)b	1.09(0.09)b	1.71(0.13)a	1.02(0.18)b	1.92(0.09)a	1.23(0.10)b	0.38(0.04)c	1.15(0.11)	26.6
Cowpea farming (1 Yes, 0 No)	1.00(0.00)a	0.99(0.15)a	1.00(0.00)a	1.00(0.00)a	1.00(0.00)a	1.00(0.00)a	0.96(0.04)a	1.00(0.01)a	0.99(0.44)	0.67
Common beans farming (1 Yes, 0 No)	1.00(0.00)a	0.99(0.02)a	1.00(0.00)a	1.00(0.00)a	1.00(0.00)a	0.29(0.09)c	0.96(0.04)ab	0.81(0.06)b	0.88(0.02)	24.95
Cow pea yield (kg/Ha) least pest attack	154.7(37.1)bc	123.3(26.0)c	393.2(79.4)ab	59.6(11.5)c	325.7(101.0)abc	381.8(87.9)ab	471.4(89.5)a	125.5(17.8)c	254.4(56.2)	7.14
Cow pea (kg/Ha) High pest attack	24.9(4.84)b	37.3(6.7)b	60.9(10.8)b	24.2(4.24)b	85.5(30.04)b	224.9(67.6)a	96.6(34.6)b	0.4(0.35)b	69.3(96.9)	7.28
Common bean (kg/Ha) least pest attack	845.5(208.4)a	170.2(43.2)c	347.5(66.2)bc	64.3(26.5)c	758.0(205.9)ab	78.6(26.5)c	304.9(81.7)c	324.0(56.4)	362.0(423.0)	8.45
Common bean (kg/Ha)High pest attack	182(39.6)a	44.2(8.69)b	58.6(11.27)b	14.0(2.57)b	237.4(72.7)a	48.5(16.6)b	29.3(6.71)b	2.5(1.76)b	77.1(20.0)	12.67
Cowpea annual sales – Leg_Rev (USD)	69.3(12.0)c	49.8(13.2)c	272.7(22.4)a	136.2(19.9)b	46.5(6.73)c	45.2(4.02)c	55.5(25.4)c	0.46(0.07)d	84.5(13.0)	33.19
Common beans sales – Leg_Rev (USD)	305.6(48.7)a	36.6(6.4)c	255.5(19.9)a	228.5(19.9)a	122.4(32.6)b	7.0(2.77)c	32.2(20.2)c	1.00(0.10)c	123.6(18.4)	44.79

Mean (SE) , df = 7, Means within a row followed by different letters are significantly different at $P = 0.05$ (Tukey HSD test).

compared to mono culture (13.4%). Henriette et al. (2012) reported that, crop rotation enhances pest and disease management in a sustainable way such that growers use less pesticides and chemicals against pests, thus decreasing both production costs and environmental impacts. In addition, crop rotation has a number of agronomic, economic and environmental benefits compared to a monoculture one, such as improved soil structure with higher levels of organic matter and water provision, resulting in long term yield increment (Henriette et al. 2012).

In our study area, the most commonly produced grain legumes were cowpea *Vigna unguiculata* (99 %), common beans *Phaseolus vulgaris* (88%), pigeon pea *Cajanus cajan* (52%), green grams *Vigna radiata* (23 %) and dolichoes *Dolichos biflorus* (2%). Most of the farmers grew grain legumes on 1.2 ha but this varied significantly across districts, ranging from 0.4 ha in Nzambani to 1.9 ha in Mwingi (Table 2.1). Similarly, USAID (2010) had reported that the average farm size was 1.6 ha was believed to be declining rapidly with continued sub-division of household farms resulting in uneconomical landholdings. Therefore, enhancing the performance of small land holders through promotion of sustainable farming coupled with provision of quality and timely support services such as exntension is critical to adoption of good farming practices and future agricultural growth.

Majority of the respondents (93.5%) had greater than five years experience in grain legumes cultivation, but the number of years cultivating grain legumes varied widely and significantly across districts, ranging from about 10 years in Masinga and Machacos to 27 years in Embu East (Table 2.1). However, in spite of the relatively long experience of grain legumes farming, yields remained low. Here, we found that interviewed farmers related yield loss to the level of pest attack. In the year 2012, for example, farmers estimated 59.6 to 471.4 kg/ha for low level of pest attack and 0.3 to 224.9 kg/ha for high level of pest attack on cowpea (Table 2.1). For the same year, farmers estimated 64.3 to 845.5 kg/ha for common bean for low pest infestation level and 2.5 to 237.4 kg/ha for high one. The average annual income ranged from a minimum of 0.46 in Nzambani to a maximum of 272 USD/year in Makueni for cowpea and from a minimum of 1 in Nzambani to a maximum of 305.6 USD/year in Embueast for common beans.In both cases, Nzambani district shows higher yield losses by pests. The overall mean annual incomes for the year 2012, obtained from the sales of grain legumes of cowpea and common beans followed a similar trend with an average of USD 84.5 and USD 123.6 respectively. Overall mean annual incomes varied significantly across districts (Table 2.1). Similar results have been reported from different areas in Africa, where modern pest control measures are absent resulting in up to 100% yield losses (Ekesi et al. 1998; Karungi et al. 2000).

A high percentage of the respondents (99.5% of the total) planted local varieties or land races of grain legumes. However, Nagarajan *et al.* (2007) pointed out that improved seeds of cowpea and common beans were sold through small seed packs (SSP) program in eastern Kenya between the years 1998 and 1999. It is probable that farmers in the study area may not have access to such improved seed program due to the short period of the seed distribution program. Thus programs for production and supplying of improved and pest-resistant seed varieties should be developed in order to increase the agro-ecosystem productivity. Early maturing seeds, for example, may help farmers overcome the short rainfall season which was mentioned by a majority of respondents as a constraint in effective grain legume production.

In addition to the practice of intercropping and crop rotation, the majority of the farmers only use hand tools to remove weeds (77.8 %), which is labor intensive. Removal of weeds can positively influence crop yield by enhancing sprouting of crop seeds as well as reducing pest damage (Takim and Uddin 2010). Tijani-Eniola (2001) reported that not managing weeds result in yield losses ranging from 50 to 80 %. Crop losses by weeds could be aggravated by the delay in weeding or the inability to weed throughout the entire crop growth period. Weeds also act as reservoirs or alternate hosts for insects that reduce yields in agricultural systems. Similarly, Takim and Uddin(2010), reported that weeding greatly reduced the population densities of cowpea pests, such as thrips Megalurothrips sjostedti Trybon, the pod borer Maruca vitrata Fabricius, Aspavia armigera Fabricius (Pentatomidae), and Chelomenis spp., while, increasing the cowpea aphid Aphis craccivora Koch population densities.

Determinants of farmers' knowledge of grain legume pests. The results of the ordered probit regression model (Table 2.3) showed that the coefficients for the variables representing gender (gender), household size (hh_size), and farm size (farm_size) had a negative relationship with the level of farmers knowledge. This is likely because the process of grain legume production up to marketing is often handled by women. Therefore, it is likely that women gain more knowledge on pest and other problems affecting the crop. In Eastern Kenya, as in many Sub-Sahara African (SSA) countries, grain legumes are regarded as a "women's crop" (Sariah 2010). In contrary cash crops like cotton is mainly handled by men (Midega et al. 2012).

The variables representing age (age), primary education (pri_edu), junior education (jun_edu), high school education (high_edu), college education (col_edu), pest management training (pest_mgt tr.), years of grain legume farming experience (leg_yrs) and revenue from sales of cowpea and common bean (leg_rev) are likely to influence positively the level of farmers' knowledge. Although the coefficient for the variables representing high school education and college education was positive, the marginal effect (ME) was only significant at high knowledge level with ME = 0.375 and 0.631 respectively.

Table 2.2 Pest management methods practiced by legume farmers in eastern Kenya

Variables	Districts									
	Embu east	Machacos	Makueni	Masinga	Mbeere North	Mwingi	Mwingi central	Nzambani	Mean	F
Experiencing legume pests	1.00(0.07)a	0.95(0.04)a	0.88(0.06)a	0.95(0.06)a	0.89(0.07)a	1.00(0.06)a	0.88(0.06)a	0.98(0.04)a	0.94(0.06)	0.73
Chemical control	0.88(0.08)a	0.93(0.04)a	1.00(0.00)a	0.90(0.07)a	0.89(0.08)a	1.00(0.00)a	0.96(0.04)a	0.98(0.02)a	0.94(0.04)	1.1
Biological control	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)a	0.00(0.00)	5.62
Cultural control	1.00(0.00)a	0.71(0.07)bc	1.00(0.00)a	0.95(0.05)ab	0.94(0.06)abc	1.00(0.00)a	0.65(0.49)c	0.12(0.05)d	0.80(0.04)	32.9
Physical control (weed removal)	0.76(0.11)a	1.10(0.12)a	1.00(0.00)a	0.00(0.00)b	0.78(0.10)a	1.00(0.00)a	0.31(0.09)b	1.00(0.00)a	0.74(0.05)	21.9
Knowledge on Enemy plant	0.29(0.11)a	0.02(0.02)b	0.00(0.00)b	0.00(0.00)b	0.17(0.09)ab	0.00(0.00)b	0.12(0.06)ab	0.00(0.00)b	0.07(0.04)	5.15
Pesticide effectiveness	0.76(0.11)a	0.74(0.07)a	0.15(0.07)b	0.90(0.07)c	0.89(0.08)a	1.00(0.00)a	0.62(0.10)ab	0.95(0.03)a	0.75(0.07)	22.9
Mix pesticides	0.12(0.08)a	0.17(0.06)a	0.15(0.07)a	0.05(0.05)a	0.28(0.11)a	0.42(0.10)a	0.08(0.05)a	0.33(0.07)a	0.20(0.07)	2.69
Increase concentration	0.06(0.06)b	0.74(0.07)a	0.00(0.00)b	0.10(0.07)b	0.06(0.06)b	0.08(0.06)b	0.08(0.05)b	0.65(0.07)a	0.22(0.05)	24.7
Changing chemicals	0.76(0.11)a	0.02(0.02)b	0.00(0.00)b	0.55(0.11)a	0.61(0.12)a	0.00(0.00)b	0.00(0.00)b	0.00(0.00)b	0.24(0.05)	37.2
Frequent spray	0.06(0.06)cd	0.05(0.03)cd	0.85(0.07)a	0.30(0.11)bc	0.06(0.06)cd	0.50(0.10)b	0.85(0.07)a	0.00(0.00)d	0.33(0.06)	35.9

Mean (SE) , df = 7, Means within a row followed by different letters are significantly different at P = 0.05 (Tukey HSD test).

Moreover, farmers with greater than five years of grain legume farming were likely to have medium level of pest knowledge (ME = -0.021). Probably due to the experience that they gained over time as they cultivate the crop. This indicates that education helps farmers to understand and identify different pests on grain legumes. This also indicates the need to provide continuous training and build capacity on various aspects of grain legume farming. Farmers which have been growing grain legumes for more than five years resulted having a medium level of pest knowledge probably due to the experience that they gained over time as they cultivate the crop. Furthermore, the coefficient representing the revenue gained from the sales of grain legumes yield was positive, but its marginal effect was only positive for high level of knowledge. This can be explained by the fact that the higher the revenue from grain legumes, the more effort farmers would put into understanding the process of producing the crop, including addressing the constraints affecting its production such as insect pests or the farmer knows the constraints and how to manage them, which indirectly shows high knowledge.

Although the coefficients for the variable representing Embu East, Makueni, Mbere North and Mwingi district was negative, their marginal effect was only significant at high level of knowledge (ME = -0.344, -0.198, -0.353 and -0.212 respectively). While, in Machakos the marginal effect is only significant at medium level of knowledge (ME = 0.029). Farmers in Embu East and Mbere North were more knowledgeable about grain legume pests compared to Nzambani. i.e the reference district. Proximity of Kenya Agricultural and Livestock Research Institute (KALRI Embu) to Embu East and Mbere North can explain our result. This institution in fact may be a source of information for farmers that can benefit from this service improving their pest knowledge and management.

Table 2.3 Factors determining farmers' level of knowledge of grain legume pests from an ordered probit regression analysis

Variables	Coefficients		Marginal effects for different pest knowledge levels							
			No knowledge		Low knowledge		Medium knowledge		High knowledge	
	Coef.	Std. Err.	dy/dx	Std. Err.	dy/dx	Std. Err.	dy/dx	Std. Err.	dy/dx	Std. Err.
Gender	-0.213	0.174	0.018	0.015	0.029	0.024	0.027	0.024	-0.074	0.060
Age	0.009	0.007	-0.001	0.001	-0.001	0.001	-0.001	0.001	0.003	0.002
Pri_edu	0.384	0.283	-0.026	0.016	-0.047	0.032	-0.067	0.063	0.140	0.107
Jun_edu	0.095	0.278	-0.007	0.020	-0.013	0.036	-0.013	0.043	0.033	0.099
High_edu	1.038***	0.254	-0.067***	0.021	-0.118***	0.030	-0.191***	0.064	0.375***	0.090
College_ edu	1.822***	0.366	-0.057***	0.017	-0.124***	0.025	-0.449***	0.081	0.631***	0.085
hh_size	-0.040	0.039	0.003	0.003	0.005	0.005	0.005	0.005	-0.014	0.014
Land_ size (Ha)	-0.112	0.160	0.009	0.013	0.015	0.022	0.014	0.021	-0.039	0.055
Pest_Mgt_training	0.388	0.305	-0.024	0.015	-0.046	0.032	-0.072	0.074	0.143	0.118
Leg_5yrs	0.290	0.350	-0.030	0.044	-0.042	0.054	-0.021*	0.012	0.093	0.102
Leg_rev	0.0003	0.0006	-0.000	0.000	-0.000	0.000	-0.000	0.000	0.0001	0.0002
Embueast	-2.011***	0.456	0.504***	0.167	0.167***	0.052	-0.326***	0.118	-0.344***	0.041
Machacos	-0.461	0.298	0.049	0.041	0.067	0.046	0.029*	0.017	-0.146	0.085
Makueni	-0.685	0.459	0.089	0.086	0.102	0.071	0.007	0.055	-0.198*	0.105
Masinga	-0.675	0.458	0.089	0.088	0.101	0.071	0.002	0.060	-0.192	0.102
Mbeerenorth	-2.115***	0.384	0.539***	0.138	0.160***	0.051	-0.346***	0.096	-0.353***	0.039
Mwingi	-0.755*	0.413	0.103	0.084	0.113*	0.063	-0.004	0.061	-0.212**	0.089
Mwingicentral	0.006	0.360	-0.000	0.029	-0.001	0.049	-0.001	0.046	0.002	0.124
/cut1	-1.534	0.534								
/cut2	-0.856	0.528								
/cut3	0.780	0.533								
Log likelihood	-201.919									
Number of obs	216									
LR chi2(18)	110.25									
Prob > chi2	0.2145									

Statistically significant level: ***$P < 0.01$; **$P < 0.05$; *$P < 0.1$; (n = 216).

Insect pests as constraints to grain legume production

The majority of the respondents (94.0 %) mentioned insect pests as the main constraint to efficient production of grain legumes on their farms. Responses varied between districts, ranging from 88 % in Makueni and Central Mwingi to 100 % in Embu East (Table 2). Moreover, farmers' were able to identify four important pests of grain legumes by describing them more than by giving the specific names of the pests mainly for cowpea and common beans. Midega et al. (2012) have reported that small-farm holders have difficulties in pest recognition and understanding pest ecology. Pest identification and recognition is crucial for a successful pest management programme. Among the large diversity of pest species, the key pests infesting the grain legumes across the study districts were the legume flower thrips *M sjostedti*, the cowpea aphid *A craccivora*, the legume pod borer *M vitrata* and the *pod-sucking bug C tomentosicollis*. Farmers considered thrips and aphids as the first two important pests among the pest complex. Similarly, these insects are identified as key pests of grain legumes in different parts of the Africa (Alghali 1991; Karungi et al. 2000; Tamo` et al. 2002; Ajeigbe and Singh 2006; Oparaeke 2006; Nyakou et al. 2008; Sariah 2010). The level of importance of these key pests for the two mainly grown legumes cowpea and common beans showed a highly significant difference between the identified pests for cowpea (χ^2=198.98; df=6; $P < 0.0001$) and for common beans (χ^2=200.6; df=6; $P < 0.0001$). However, farmers considered thrips and aphid as the first two important pests among the pest complex.

Farmers estimated the average losses due to insect pests as 74.0 % for cowpea and 76.3 % for common beans, respectively. The average loss ranges from a minimum of 41.1 % in Mwingi to a maximum of 99.8 % in Nzambani for cowpea and from a minimum of 38.3 % in Mwingi to a maximum of 99.2 % in Nzambani for common beans. The result of actual average cowpea yield ranges between 200 to 400kg/ha(Table 1). This indicates that there is higher yield loss due to insect pest and other environmental constraints. USDA (2010) reported that the potential grain yield for these legumes is usually higher, averaging 1500 - 3000 kg/ha. However, we have to consider that most poor farmers in Kenya do not have access to improved seedsas well as to improved farming techniques, thus producing only 400 kg/ha, with a loss of about 70 to 85 % compared to the potential yield. Apart from the insect pests, farmers experience different challenges in growing grain legumes. Among others 93.1 % of the farmers mentioned that erratic rainfall and poor climatic condition adversely affects the grain legume farming. Similarly, USAID (2010) had shown that despite the increase in area planted from around 130,000 in 2007 to about 148,000 hectares in 2008 annual production of cowpea in Kenya had declined from about 83,000 Metric Ton (MT) to about 48,000 MT over the same period, and this is attributed to poor climatic condition. In addition, high labor cost, lack of capital, high cost of chemical, lack of certified seeds and shortage of land were perceived as important challenges by 54.6, 53.7, 47.2, 44.4 and 12% of farmers, respectively.

Pest control methods. Chemical pesticides use was the most common pest control method (96.3 %), with percentage varying across districts from 88 % in Embu East to 100 % in Makueni and Mwingi (Table 2.2). Most farmers (61%) applied synthetic pyrethroids, 14.3% of the farmers used organophosphate while only 5.1% of the farmers applied a mixture of pyrethroids and organophosphate. Among the respondents, 19.9 % of the total did not know the name of chemicals used to spray their grain legumes. The remaining 80.1 % mentioned, a wide range of broad spectrum pesticides. Out of the 80.1 % of the farmers, 38.8 % reported applying KARATE 2.5 water dispersible granule (WDG), (lambda cyhalothrin 25g/kg); 10.2 % applied ALFA - CYPER-M Emulsifiable Concentrate (EC), (alphacypermethrin 100g/L), 8.7 % applied BESTOX 20 EC (alphacypermethrin 20g/L), 7.8 % applied SELECRON 720 EC (profenofos 720g/L), 6.5 %

applied DIMETON 40 EC (dimethoate 400g/L), 5.1 % applied POLYTRIN P 440EC (Profenofos 400g/L + cypermethrin 40g/L), 3.2 % applied TRIGER 5% EC (lambdacyhalothrin 5% EC) and 0.46 % applied AGRINATE 90 water soluble powder (SP), (methomyl 90 %). Most of the farmers (69.9 %) applied pesticides when they saw pest damage symptoms on the leaves, while 19.0 % applied pesticides when they saw the presence of insects. Moreover, among the 95 % of the farmers who applied pesticides, 25 % of them did not experience effective control of the pests due to different challenges. However, pyrethroids may not be effective to control all the pests and particularly the sucking pests such as thrips and aphids which are known to develop resistance against pyrethroids (Ofuya 1997; Jensen 2000; Toda and Morishita 2009). This was evident in our survey as well to ensure effectiveness of the pesticide 94.5% of farmers adopted practices such as increasing concentration (27.8%), alternating chemicals (24%), frequent spraying (22.7%) and mixing pesticides (20%) to enhance their effectiveness. Further, in just one growing season, farmers used two to three different pesticides. Except for pesticide mixture, the percentage of farmers using these practices vary significantly across districts (Table 2.2). Despite of the different approaches adopted to ensure pesticide effectiveness, more than 20% of the interviewed farmers reported ineffectiveness of such approaches (Table 2). Combination of different practices have been shown to be detrimental for pest control, having on one hand a negative impact on the local and beneficial fauna and on the other hand, causing resistance development by the targeted insect pests (Ekesi et al. 1998; Oparaeke 2006).

Except a few farmers in Embu East, the majority of the interviewed farmers were not familiar with biological control methods with a very poor knowledge of natural enemies. Almost all the farmers (99.3%) considered all insects found on their legume crops as harmful, showing a general lack of knowledge of pest natural enemies. Natural enemies for the pest of interest in this study have been widely identified and documented (Tamo` et al. 2002). There was also a general lack of knowledge among the farmers interviewed on IPM as a method to controlinsect pests. This knowledge gapcan be due to a deficit of inappropriate extension services and advices on pest control, including use of pesticides.

With regard to the knowledge of plants with repellent and toxic characteristics, a very low percentage of the interviewed farmers (5.6 %) showed knowledge for plants having insect toxic or repellent properties. Among the farmers with knowledge on botanicals, only 1.4 % tried to use them for insect pest control by planting them around their farming areas (Table 2.2).Among these plants, Mexican marigold *Tagetes minute* (3.24 %), pepper *Capsicum annuum* (1.38 %), neem *Azadirachta indica* (1.38 %), Aloe vera *Aloe secundiflora* (0.92 %), Basil *Ocimum basilicum* (0.46 %) and wild sunflower *Tithonia diversifolia* (0.46 %) are known having repellent effect to the targeted insect pests considered in this study.

The survey revealed that there was limited information available to farmers on pest control methods. About 89.8% of the respondents never received any information on pest management and pesticide use; and none of the respondents received any information on cultural, physical and biological methods of pest control. Those who reported receiving information on the use of pesticides mentioned various sources of information such as input suppliers (57.1 %), government ministry of agriculture staff (38.9 %) and fellow farmers (2.9 %) and other sources e.g. media (mainly radio), farmer organizations and pesticide container labels. There is thus a need to ramp up the capability of the farmers by providing information through appropriate channels on alternative pest control methods and by giving full attention to the poor extension system in order to enhance farmers' knowledge and practice on pest management of grain legumes.

2.4 Conclusions

This study confirmed that insect pests are perceived by the farmers as one of the most serious constraints to efficient production of grain legumes in Eastern Kenya, with thrips and aphid being the most important. The pests are controlled mainly by chemical pesticides, while other minor IPM practices are marginally used. Farmers also perceived erratic rainfall, high labor cost, lack of capital, high cost of chemical, lack of certified seeds and shortage of land as important challenges of grain legumes production. Although farmers benefit from institutions like KALRO, this study confirmed that weak extension service in the area of IPM in the region that needs to address with a participatory approach based extension service through participatory approach to help the resource-poor farmers implement IPM including colored sticky traps for pest monitoring, use of entomopathogenic fungi (Ekesi et al. 1998), botanical pesticides (Oparaeke 2006) and resistant varieties (Abudulai et al. 2006) that fits within their practice of intercropping.. The other possible IPM component could be to involve companion crops with repellent or toxic characteristics to disrupt the host location by the pests and improve abundance and activity of the pests' natural enemies, thereby delivering effective pest control (Khan et al. 2010). However farmers lack knowledge on such IPM strategies in Eastern Kenya. This was attributed to the fact that most farmers have not been exposed to sources of agricultural extension information, especially on pest management. Therefore there is a need to build the capacity of the farmers by giving different extension service on alternative pest control measures as a comprehensive pest management approach for sustainable grain legume production in the region.

PART II - ECOLOGICAL AND PHENOLOGY CONSIDERATION

CHAPTER THREE - ECOLOGICAL NICHE MODELING TO PREDICT EFFECT OF CLIMATE CHANGE ON THE GEOGRAPHIC DISTRIBUTION OF *Megalurothrips sjostedti* TRYBOM AND ITS HOST COWPEA

Prepared as:

Statement of contribution

AA, VM and SS conceived and designed research. AA and VM conducted experiments. TM supplied data. AA GM analyzed data, AA, TM, SK and GTG and wrote the manuscript. All authors read and approved the manuscript.

Abstract

Insect pests, biological invasions and climate change are considered to represent major threats to biodiversity, ecosystem functioning and agriculture. Deriving hypothesis of contemporary and/or future potential distributions of insect pests and invasive species is becoming an important tool for predicting the spatial structure of potential threats. The legume flower thrips (LFT) *Megalurothrips sjostedti* is a pest, infesting especially legumes, such as cowpea, causing up to 100% in terms of yield loss in Africa. It is wide spread throughout Sub Saharan African countries, resulting in economic costs in terms of grain legume yields and farmers' income mainly in west, central and eastern parts of the continents. Until now no studies have been investigating potential areas of invasion for this species. Because of this, our study proposes to estimate the climatically favorable zones for the establishment of the legume flower thrips in Africa. Using present and future climatic data we projected the potential suitable areas in order to estimate the dynamics of invasion risk areas through time. The areas at risk under this scenario were assessed by comparing, using the spatial projections of current and future areas of climatic favorability of the LFT and its host crop cowpea. Spatial hypothesis were generated with respect to the presence records both for present and future (2055). We used an occurrence data for the pest from literature and cowpea georeferenced data from IITA data base to estimate the climatic favorability and geographic distribution of the LFT. We used climatic predictors from AFRICLIM and then used Maximum Entropy (MAXENT) modeling of species geographic distributions for ecological modelling to predict potential distributions for current and future climatic conditions. The results obtained showed a higher suitability for the pest in eastern Africa and West Africa for current and future (2055) respectively. The Maxent model's internal jackknife test of variable importance showed that Rainfall wettest month, Annual temperature range and Rainfall wettest quarter influence the prediction of *M. sjostedti* similarly, Rainfall wettest month and Temperature Seasonality influence the prediction of cowpea suitable areas. Modelling the potential habitat distribution for *M. sjostedti* and the host crop can help in planning pest management around its existing populations, discover other pest populations, identify top-priority survey sites, or set priorities to manage its natural habitat for more effective controlling, moreover, the results suggest that climatic variables can be used to determine the potential thrips control needs in cowpea production.

Keywords *Megalurothrips sjostedti*, AFRICLIM, climate change, climatic favorability, insect pest, Sub Saharan Africa.

3.1 Introduction

Climate change is taking place all over the world (IPCC, 2001; Rosenzweig et al., 2008). Global temperature as predicted by Intergovernmental Panel on Climate Change (IPCC) will increase between 1.1 and 5.4°C by the year 2100 (IPCC, 2001). Many countries in the tropical and subtropical regions are expected to be more vulnerable to climate change because additional warming will affect, among other things, the agricultural sectors (Dinar et al., 2012). This is projected to worsen the already bad food situation in sub-Saharan Africa, where a large percentage of the population depends upon agriculture for their livelihood (Slingo et al., 2005; Hellmuth et al., 2007; IPCC, 2007; McIntyre et al., 2009).

Climate, especially temperature, has a strong and direct effect on insect development, reproduction, survival and colonization success of insect species (Bale et al., 2002; Parmesan and Yohe 2003; Chown and Terblanche 2007; Ladányi & Horváth, 2010). In a climate change scenario, techniques developed to detect areas of climatic favourability, such as those used in species distribution models, are considered to represent useful approaches for detecting suitable areas of colonization and establishment of a species (Lo´pez- Darias et al. 2008). Insect pests may experience variations in population growth rates, increase in the number of generations, extension of the development season, changes in geographical distribution, crop–pest synchrony and interspecies interactions (Porter et al., 1991; Roy et al., 2001; Bale et al., 2002; Hance et al., 2007). Empirical studies have shown in fact that climate change has already caused range shifts in many species (Parmesan and Yohe 2003). These models are particularly useful when applied to invasive and pest species. Thanks to their high plasticity, in fact, pest species usually adapt very easily to new environmental conditions, in most of the cases displacing the original fauna.

Arthropod pests are one of the major constraints to agricultural production in Africa (Jackai and Adalla 1997). Arthropods are exothermic organisms that cannot internally regulate their own temperature, and their development depends on the temperature to which they are exposed in the environment. Hence climatic changes could profoundly affect insect's population on essential crops. A large number of insect pests attack crops during all stages of growth, from seedling to harvesting (Karungi et al. 2000) with high ecologically and economically adverse impacts on agriculture and forestry (Arag´on and Lobo 2012).

Among others, Thrips (order: Thysanoptera) are recognized as one of the most important crop pest in the world (Lewis 1997). In Sub Saharan Africa (SSA), In particular the legume flower thrips, *Megalurothrips sjostedti* (Trybom) is a primary pest of Cowpea (*Vigna unguiculata* Walp). The damage that this pest can cause can go up to 100% in terms of yield loss (Singh & Allen, 1980; Ekesi et al. 1998). Recently this pest was found attacking alternative host than legumes, both of economical and not economical importance (Tamo` et al. 2002). Although *M. sjostedti* is an African native species, reported in more than 20 SSA countries, it has also been reported to occur in Madagascar (Charles et al. 2004),Comoros Islands and Malta (Ekesi 1999), highlighting the capacity of this pest to move and colonize new areas. With the increase of exchanges between countries and the climate change, this pest has potential probability to penetrate in new areas outside its home range.

However, until now no studies have been investigating potential areas of invasion for this species. Because of this, our study proposes to estimate the climatically favorable zones for the establishment of the legume flower thrips in Africa. Using present and future climatic data we projected the potential suitable areas in order to estimate the dynamics of invasion risk areas through time. Compared with temperate regions, tropical countries are more prone to pest problems and outbreaks because of the year-round favorable climatic conditions for pest population growth and food (host) availability. Infestations by all other pests increased in agricultural and horticultural crops. And the farmers' only adaptive strategy to cope was to apply high doses of pesticides every 2–3 days (Cisneros and Mujica, 1999). Early predictions of the potential new distribution and abundance of pests could help to adapt to climate change by developing and supporting farmers with adequate pest management strategies to reduce greater crop and quality losses. In this sense, models are important analytical tools for understanding and predicting the dynamics of insect populations in ecosystems under a variety of environmental conditions and management practices, and are also used in phytosanitary risk assessments (Baker, 1991; Jarvis and Baker, 2001a, 2001b).

Reasonable estimates of climate change impacts require combined use of climate, crops, pests, and economic models. However, climate change models often ignore possible effects on dynamics and infectivity of pests and its host crop. In particular, the objectives of this study were: (1) to determine the environmental factors that most explains the distribution of *M. sjostedti* and its host crop cowpea (2) to show current cowpea growing areas with high potential occurrences of *M. sjostedti* in Africa; and (3) to predict areas with high potential suitability for the establishment of *M. sjostedti* in Africa for future (2055) based on climatic factors.

3.2 Materials and methods

Megalurothrips sjostedti occurrence and Cowpea presence data

A total of 166 known records for locations of known occurrences of *M. sjostedti* in the cowpea growing regions of Sub-Saharan African countries were compiled though comprehensive literature search and IITA cowpea landrace georeferenced data base Figure 1A. The IITA gene bank holds the world's largest and most diverse georeferenced collection of cowpeas, with 15,122 unique samples from 88 countries, representing 70% of African cultivars and nearly half of the global diversity. Distributional data representing 5486 records (i.e. latitude-longitude combinations) for different cowpea landraces from different parts of Sub Sahara African countries were drawn from the IITA cowpea gene bank. Because the original sampling of cowpea distributions was highly biased towards a few well-sampled states (especially to western Africa mainly to Nigeria and Niger), we subsample randomly those densely sampled states to match the sampling density of remaining states from which sample points were available; this procedure left 350 unique sampling points for analysis, that are well-balanced across African geography (Figure 1B).

Environmental variables

Twenty one environmental variables were considered as potential predictors of *M. sjostedti* habitat distribution (Table 1), these bioclimatic variables that are biologically meaningful for defining the eco-physiological tolerances of a species (Graham and Hijmans 2006; Murienne et al. 2009). All variables for the present and future (i.e. 2055) were obtained from the AFRICLIM database which is a high-resolution climate projection which can be used for ecological applications in Africa available at https://webfiles.york.ac.uk/KITE/AfriClim (Platts et al, 2014). We used the bioclimatic variables at a spatial resolution of ~1 km^2. The AFRICLIM data has 21 variables which include ten temperature and eleven moisture/precipitation metrics. These climate surfaces are used in many applications, particularly in environmental, agricultural and biological sciences (Waltari et al. 2007; Jones and Gladkov, 2003). These climate data were derived from monthly temperature and rainfall values and represents biologically meaningful variables for characterizing species distribution (Nix 1986). The spatial resolution of the climate surfaces used in a particular study depends on the needs for that application and on the data available. For many applications, data at a fine (≤1 km^2) spatial resolution are necessary to capture environmental variability that can be partly lost at lower resolutions (Gadsden et al. 2012).

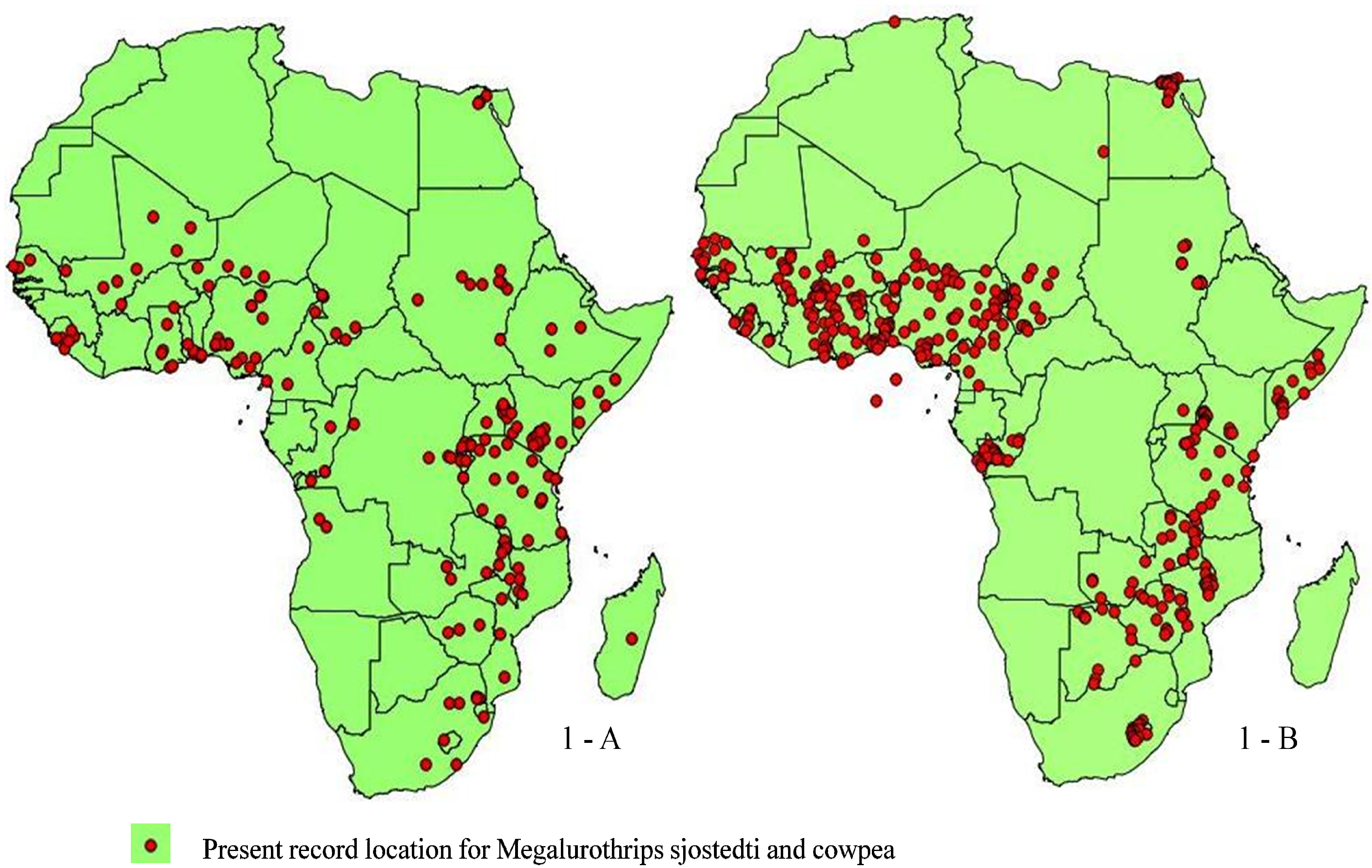

Figure 3.1 Occurrence data A. *Megalurothrips sjostedti* (166 occurrence data) B. Cowpea (350 occurrence data)

Table 3.1 Environmental variables used for the distribution of *Megalurothrips sjostedti* and the host crop cowpea the mean estimative of its relative contributions to the Maxent model

Code	Variables	% contribution	
		M. sjostedti	**Cowpea**
BIO1	Mean annual temperature[1]	2.1	6.4
BIO2	Mean diurnal range in temp [2]	2.0	6.2
BIO3	Isothermality [3]	2.7	5.8
BIO4	Temperature Seasonality [4]	2.0	12.2
BIO5	Max temp warmest month	3.2	0.7
BIO6	Min temp coolest month	7.3	6.4
BIO7	Annual temperature range [5]	13.6	8.6
BIO10	Mean temp warmest quarter [6]	3.6	1.4
BIO11	Mean temp coolest quarter [6]	1.0	5.9
PET	Potential evapotranspiration [7]	10.3	2.6
BIO12	Mean annual rainfall [8]	5.1	2.1
BIO13	Rainfall wettest month	16.7	4.7
BIO14	Rainfall driest month	1.7	0.4
BIO15	Rainfall seasonality [4]	7.5	7.3
BIO16	Rainfall wettest quarter [6]	12.4	16.0
BIO17	Rainfall driest quarter [6]	0.8	0.7
MI	Annual moisture index [9]	0.3	0.4
MIMQ	Moisture index moist quarter [6]	0.8	9.1
MIAQ	Moisture index arid quarter [6]	2.8	1.1
DM	Number of dry months [10]	2.2	1.1
LLDS	Length of longest dry season [11]	2.6	1.0

'BIO' variables correspond to ANUCLIM/WorldClim nomenclature, although derivation for [4] is not identical [1] Mean of monthly means. [2] Mean of monthly (max temp - min temp). [3] 100 × BIO2 / BIO7. [4] Standard deviation over monthly values. [5] BIO5 - BIO6. [6] Any consecutive three-month period. [7] Hargreaves 1985 method. [8] Sum of monthly rainfall. [9] BIO12 / PET. [10] Dry if monthly moisture index < 0.5. [11] Maximum run of consecutive dry months [10,11] Multimodel estimates are given to nearest month. Bimodality in moisture index is given by (DM-LLDS)>1. A quarter is a period of three months (1/4 of the year).

Modeling procedure

In order to model present and predict future distribution of the legume flower thrips *M.sjostedti* and its host cowpea we used Maximum Entropy software (Phillips et al. 2006) as a modeling tool. Previous studies showed that Maxent is more powerful compared to other software when the number of occurrence data is small (Hernandez et al. 2006; Pearson et al 2007). Maxent follows the Maximum Entropy principle (Jaynes ET, 1957) and combines presence-only data and environmental layers to create a gridded model of the potential distribution of the target species. Several studies have shown that Maxent produces better results than comparable methods (Elith et al. 2006; Wisz et al. 2008) and have confirmed its ability to predict a species' distribution outside its known range (Peterson and Vieglais, 2001;Pearson et al. 2007).

Maxent software version 3.3.3 k was used with the following settings: auto features (feature types are automatically selected depending on the training sample size), logistic output format (provides an estimate of presence probability), random seeds, replicates = 15, replicate run type = subsample validate, regularization multiplier = 1, maximum iterations = 5,000, convergence threshold = 10-5 and maximum number of background points = 10,000. The model was developed based on *M. sjostedti* and cowpea occurrences and projected onto Africa to assess the potential geographic distribution of *M. sjostedti*.

We performed subsample validations. Subsample validation is usually done with training points randomly extracted from the data set (Araujo & Pearson, 2005) or spatially stratified subsamples (Peterson et al., 2007). Repeated sub sampling, in which the presence points are repeatedly split into random training and testing subsets. Subsample validation is the most flexible and allows the user to specify the test percentage and number of replicates. It is good to set the number of replicates and the percentage to be withheld from each replicated run. This method is optimal for many models and one can control their number of reps and percentage of withheld test occurrences and it is an option for moderate to many occurrences for their species of interest.

The jackknife approach (Yost et al. 2008; Phillips et al. 2012) was used to assess variable importance. This helps to find the most influential environmental variables for *M. sjostedti* and cowpea distribution. This approach excludes one variable at a time then using it singly when running the model, by training with each environmental variable first omitted and then used singly. In so doing, it provides information on the performance of each variable in the model in terms of how important each variable is at explaining the species distribution and how much unique information each variable provides. The area under the curve (AUC) of the receiver operating characteristics (ROC) was used to test the agreement between observed species presence and projected distribution (Hanley and McNeil 1982; Manel et al. 2001, Philips et al. 2004; Phillips et al. 2006). The ROC plot relates the sensitivity (proportion of observed presences correctly predicted) with 1-specificity (proportion of observed absences/pseudo-absences incorrectly predicted). To develop a ROC plot, a certain percentage of the data is selected for training data; the other portion is used for test data. A good model is defined by a curve that maximizes sensitivity for low values of the false-positive fraction. The significance of this curve is quantified by the AUC and has values that typically range from 0.5 (no better than the expected by random) and 1.0 (perfect fit). Values\<0.5 indicate that a model fits worse than random (Fielding and Bell 1997; Engler et al. 2004; Hernandez et al. 2006; Phillips and Dudik, 2008; Baldwin 2009). Finally, in order to draw a map for present and future distribution of the pest and the host plant, we exported the final model outputs in Quantum GIS 1.8.0-Lisboa (2012).

3.3 Results

Model performance

We performed Maxent modeling on 125 training and 41 testing presence records of megalurothrips and 248 training and 82 testing presence records of cowpea in a fifteen fold subsample run that considered all occurrence points.

The average AUCs for the *Megalurothrips sjostedti* distribution were 0.905 and 0.810 for the training and test data, respectively, and for the cowpea distribution were 0.884 and 0.833 for the training and test data, respectively, all suggesting that the model had high predictive power.

The environmental variables that most influenced the predictions of *M. sjostedti* distribution were 'Rainfall wettest month' (16.7 %), 'Annual temperature range' (13.6 %), 'Rainfall wettest quarter' (12.4 %), and 'Potential evapotranspiration' (10.3 %). Similarly, 'Rainfall wettest month' (16.0 %) and 'Temperature Seasonality' (12.2 %) influenced the predictions of cowpea distribution. The influence of all other variables was less than 10 % (Table 3.1). The values in Table 3.1 are averages over fifteen replicated runs.

For the *Megalurothrips sjostedti* distribution, the environmental variables with the highest gain when used in isolation (Deep blue bars in Figure 3.2) were rainfall wettest quarter (Bio 16) 87%, rainfall seasonality (Bio 15) 6.5% and annual temperature range (Bio 7) 6.5 %. The variables that decreased the gain most when it was omitted (light blue bars in Figure 3.2) were minimum temperature coolest month (Bio 6) 47% and potential evapotranspiration (pet) 27% and mean annual rainfall (Bio 12)13%, Isothermality (Bio 3) 6.5% and moisture index moist quarter (mimq) 6.5 %. Similarly, for the cowpea crop distribution, which is the most suitable host of *Megalurothrips sjostedti*, the environmental variables with the highest gain when used in isolation (Deep blue bars in Figure 3.3) were mean annual rainfall (bio12) 40%, moisture index moist quarter (mimq)13.3%, temperature seasonality (bio4)13.3%, and rainfall seasonality (bio15) 13.3%. The variable that decreased the gain most when it was omitted (blue bars in Figure 3.3) were temperature seasonality (bio 4) 46.7, minimum temperature coolest month (bio 6) 33.3%, mean diurnal range in temp (bio 2) 13.3% and Isothermality (bio3) 7.5%.

Current distribution

Megalurothrips sjostedti and its host crop cowpea

Based on the model output for current distribution in Figure 3.4A, highly suitable niches are in red, medium to low suitable areas are in yellow and green while the unsuitable niches are in blue which indicate that *M. sjostedti is* generally distributed throughout the tropical regions of the continent, however, the Sub Saharan African countries and the western part of southern Africa are unsuitable niches. The model predication showed that higher niche suitability (areas in red color) were in eastern parts of Africa (mainly some parts of Kenya, Uganda and Tanzania), and in West Africa (the coastal areas of Cameroon to Sierra Leone). . In cowpea distribution the eastern part of Africa showed higher niche suitability as compared to western and other parts of Africa a suitable habitat Africa map for cowpea crop, a good host crop for the legume flower thrips *Megalurothrips sjostedti* is presented in Figure 3.4B. The predicted occurrence is in good agreement with the occurrence data. However, the model predictions suggest that there is more suitable habitat than is currently occupied and indicate that *M. sjostedti* may still be in the early stage of invasion in western Africa cowpea growing areas.. According to the potential distribution in Africa, the area suitable for *M. sjostedti* is wider than the area defined thus far by the occurrence points. coastal regions of western African countries which include, Benin, Nigeria, Togo, Ghana, Ivory coast, Liberia and Sierra Leone. Other countries have regions with moderate suitability, such as the Lesotho, Zambia, Tanzania and south Sudan (Figure 3.4B).

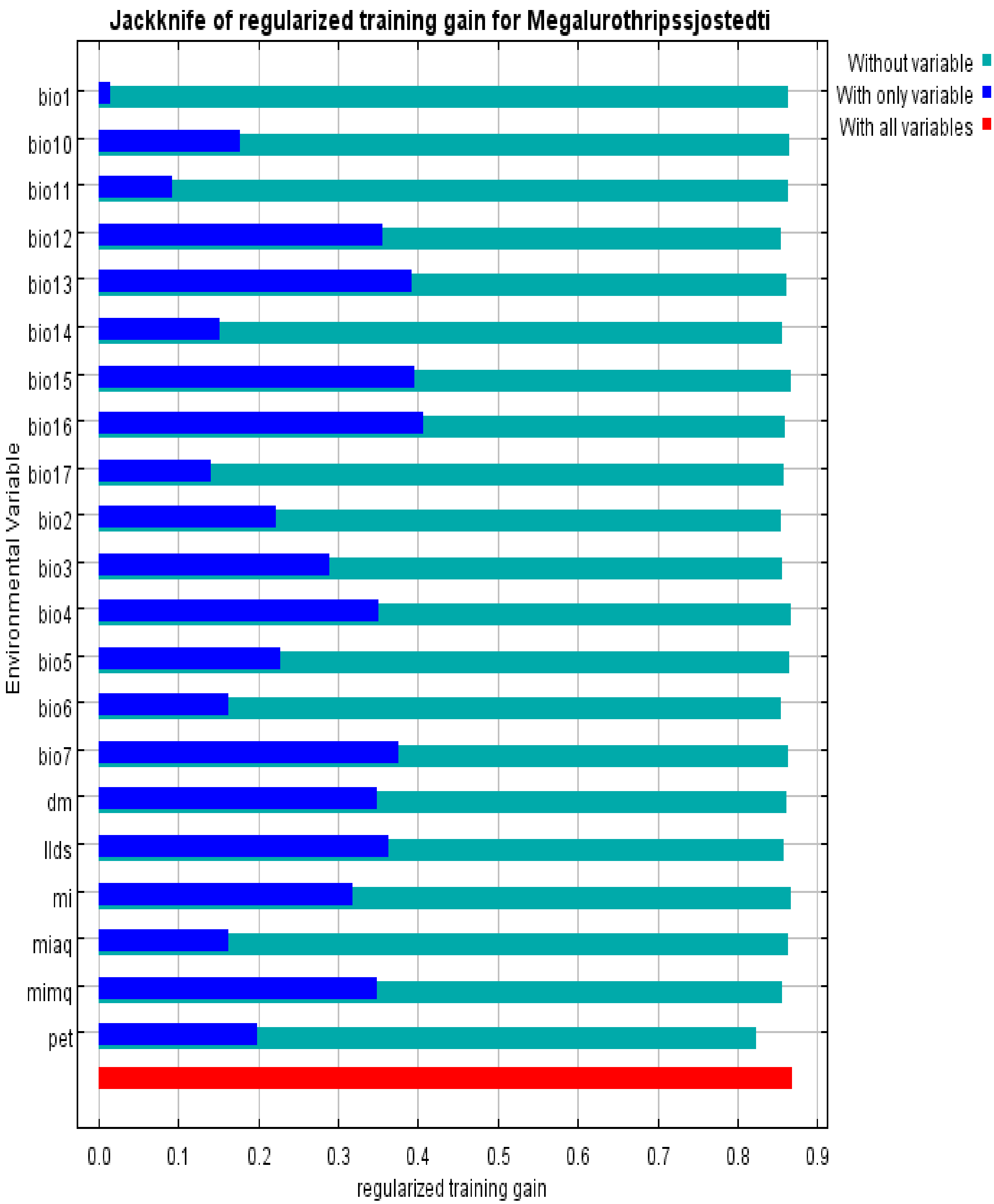

Figure 3.2 Jackknife test of regularized training gain for *Megalurothrips sjostedti* modeling

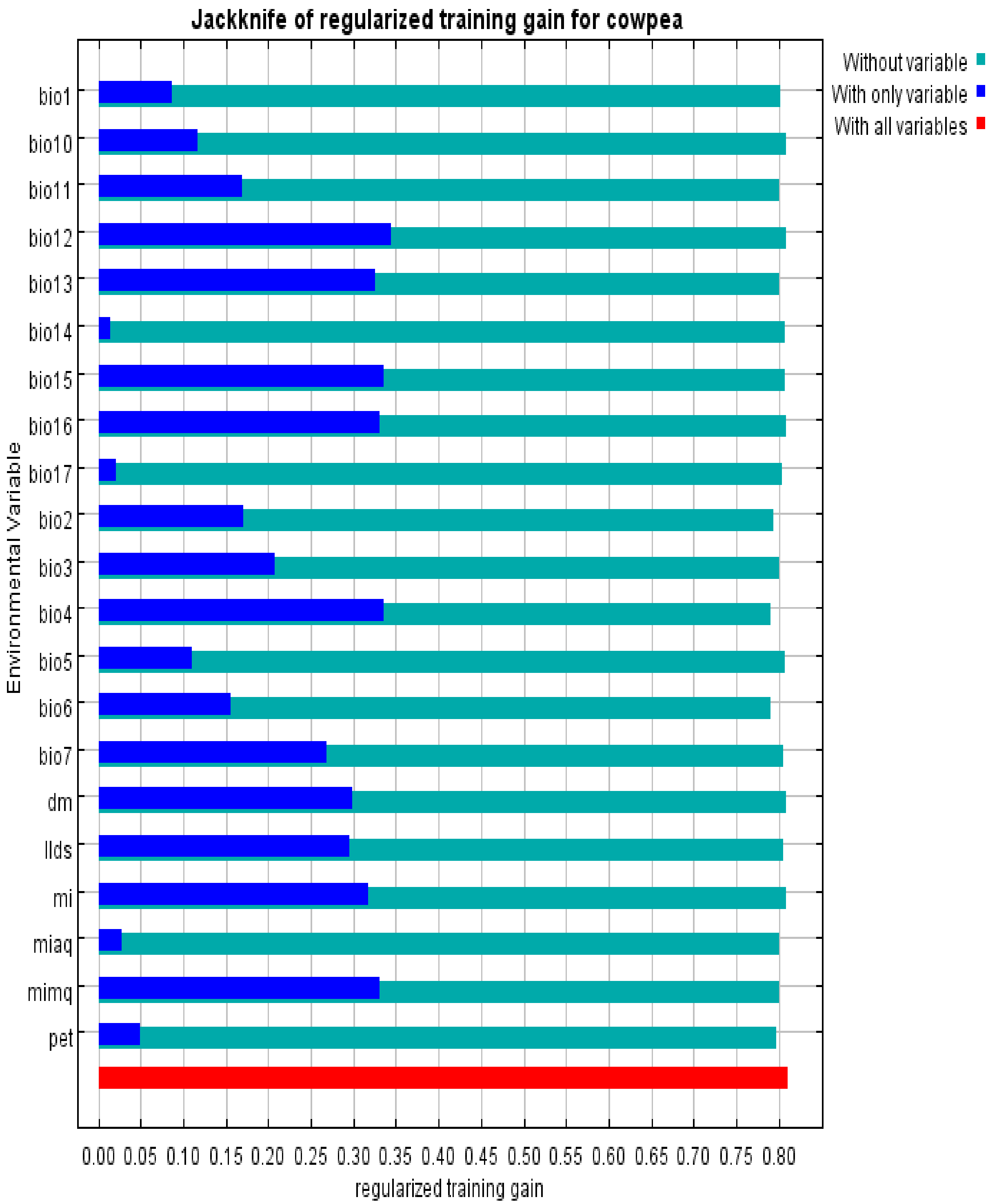

Figure 3.3 Jackknife test of regularized training gain for Cowpea modeling

Future potential distribution

Megalurothrips sjostedti and its host crop cowpea

The model prediction for the future scenario (year 2055) showed that the potential distribution of the legume flower thrips *M. sjostedti* has high niche suitability in areas with red color on the map. These areas are mainly the Coastal regions of Western African countries which include, Cameron, Nigeria, Benin, Togo, Ghana, Ivory coast, Liberia and Sierra Leone while the Eastern part of the continent include, South Sudan, the coastal area of Kenya and Somalia (Figure 5A). The future spatially potential suitable cowpea g rowing areas predicted by the model indicates that cowpea is geographically distributed with a higher probability mainly in western Africa. These areas covers, except the geographic discontinuation from central Africa republic, the area between Mauritania to the south Sudan and the coastal regions of Ivory coast, Ghana, Togo, Benin, Nigeria, Cameroon, Congo and northern coastal region of Angola. The blue color on the map (Figure 5B) is interpreted as unsuitable for cowpea and its pest *M. sjostedti*.

3.4 Discussion

In this study we showed that the habitat distribution patterns for *M. sjostedti* and its host plant cowpea can be modeled using a small number of occurrence records and environmental variables using Maxent. This study provides the first predicted potential habitat distribution map for legume thrips pest species *M. sjostedti* in Africa. Since Maxent is mapping the fundamental niche (different from occupied niche) of the species using bioclimatic variables the suitable habitat for *M. sjostedti* may be over predicted in some areas (Pearson 2007; Murienne et al. 2009).
From the result of the jackknife test of variable importance, precipitation and temperature were the most important influencing factors of *M. sjostedti's* habitat distribution. This prediction is in line with the previously reported studies. Harding 1961 reported that precipitation and mean daily temperature below 10^0C reduced thrips movements, heavy rains reduced thrips while 14.4^0C favored destructive infestation of thrips (Harding 1961) Temperature is the most important single factor influencing take-off and apparent temperature thresholds for take-off ranged from 14°C to 19°C. Take-off was not retarded by temperatures up to 35°C (Lewis 1963). Sunny and warm weather was favorable for thrips flight, and that unstable thundery weather is adverse (Hurst 1964).

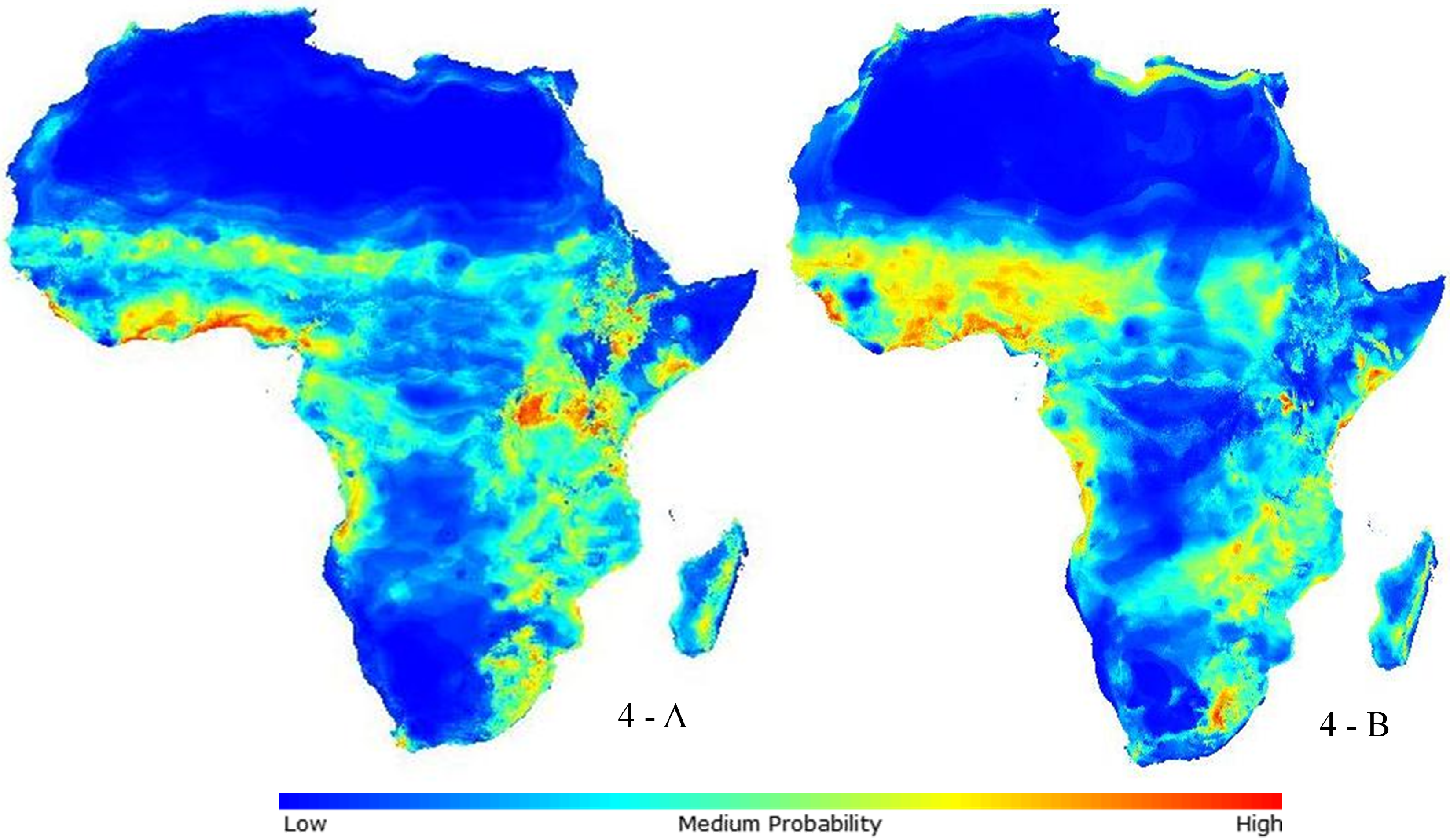

Figure 3.4 Current potential distribution in Africa using Maxent A. *Megalurothrips sjostedti* present distribution B. Cowpea present distribution

A similar pest shared the same characteristics that the prevailing daily maximum temperature, minimum night temperature and daily mean relative humidity were the most important weather factors influencing the density of the pest *T. tabaci* (Shaarawy et al 1975). Moreover, Shaarawy et al 1975 pointed out that, the minimum night temperature appears to be the most important weather factor governing the density of *T. tabaci*. The results indicated that a daily maximum temperature of 30° C, minimum night temperature of 17° C and Relative Humidity (RH) of 54% (combined) are the optimum weather conditions for the development of *T. tabaci* population. These optimum weather conditions resulted in a higher population size, relatively long activity season and more generations. Similarly, Waiganjo et al 2008 reported that dry weather with 30.3 mm rainfall with moderately higher temperatures (15.6-28.2^{o}C) increased seasonal thrips numbers, while wet season up to 391mm rainfall) with moderately high relative humidity was negatively correlated with thrips numbers. Moreover, Ekesi et al 1999 reported that the developmental rate and reproduction of *M. sjostedti* ranged from 33.1 days at 14 ^{o}C to 19.2 days at 26 ^{o}C.

Similar to that of the *M. sjostedti,* the result of the jackknife test of variable importance precipitation and temperature were the most important influencing factors of cowpea habitat distribution. Cowpea performs well in drier agro ecological zones where the rainfall range is between 500 and 1200 mm/year and the optimum temperature for growth and development is around 30 °C (Dugje et al 2009). Varieties of cowpea differ in their response to day length, some being insensitive and flowering within 30 days after sowing when grown at a temperature around 30 °C. Similar to our finding, Kiprotich et al 2015 reported that rainfall variations have a positive and significant effect on cowpeas yields while temperature variations or increase has a negative and significant effect on cowpeas yields. This implies that a continuous increase in the temperatures would lead to decreased productivity of crops and thus translating to reduced crops yields. Moreover, Rainfall is the most important climate parameter which influences the growth characteristics of crops (Bewket 2009; Befekadu & Berhanu, 2000); therefore, inadequacy of water supply hampers efficient crop growth, resulting in low productivity. According to von Braun (1991), for instance, a 10% decrease in seasonal rainfall from the long-term average generally translates into a 4.4% decrease in food production. According to Bryan *et al.* 2012, a range of climate models suggest median temperature increases of between 3^{o}C - 4^{o}C in Africa by the end of the 21st Century, roughly 1.5 times the global mean response.

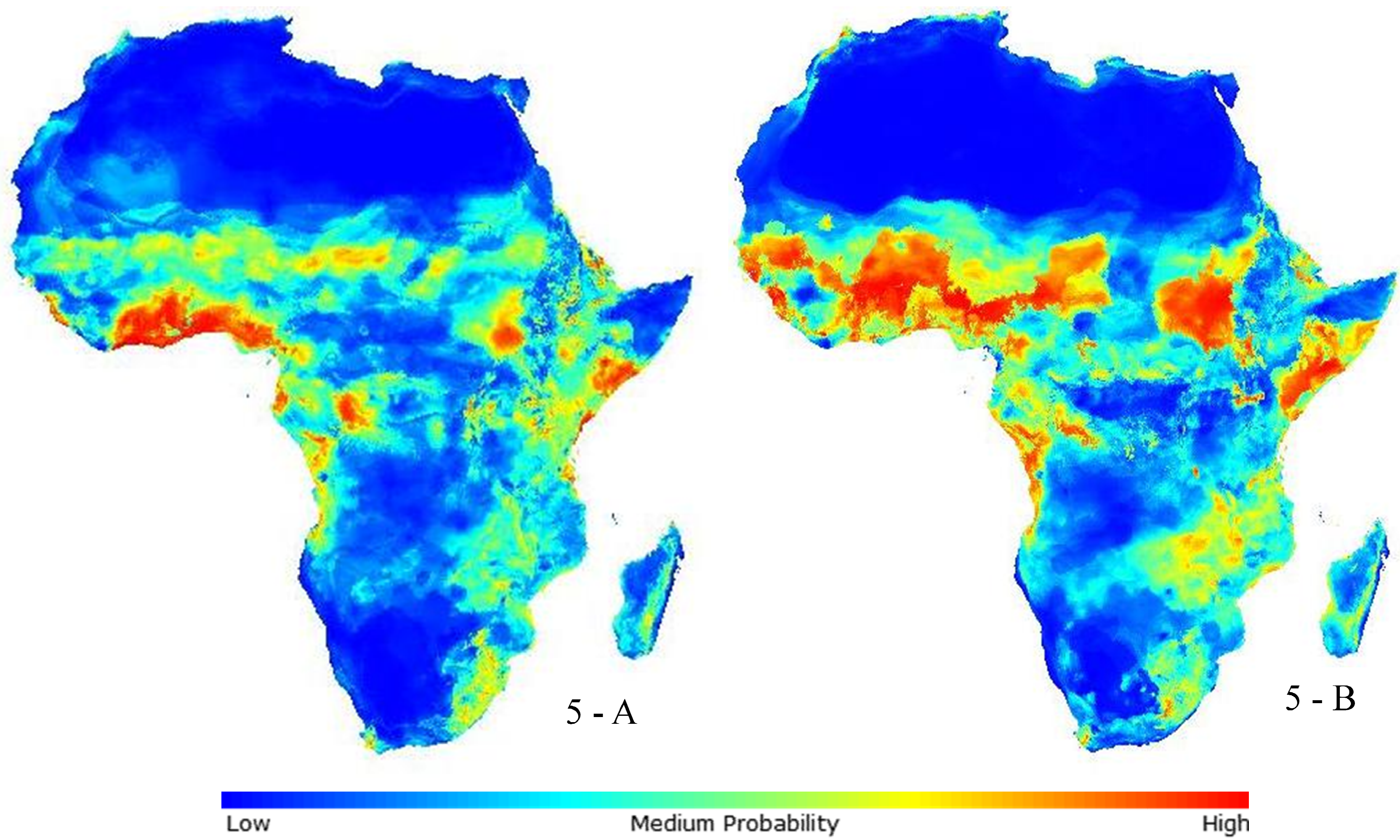

Figure 3.5 Modeled Future (2055) potential distribution in Africa using Maxent

A. *Megalurothrips sjostedti* future distribution B. Cowpea future growing suitable areas

This will likely result in significant yield losses of key staple crops, such as maize, sorghum, millet, groundnut, and cassava, of between 8 % - 22 % by 2050 unless key investments are made to improve agricultural productivity under climate risk (Schlenker and Lobell 2010). Further, rainfall variability and associated droughts have been observed to be major causes of food shortages and famines in sub-Saharan Africa regions, largely practicing smallholder subsistence farming which rely solely on high unpredictable and sporadic seasonal rainfall (Ndamani & Watanabe 2008 and Ifejika Speranza, et al., 2008). Cowpeas utilize soil moisture by limiting growth (especially leaf growth) and reducing leaf area by changing leaf orientation and closing the stomata. Flower and pod abscission during severe moisture stress also serves as a growth-restricting mechanism. (Dugje et al. 2009)

The information produced from this study is therefore timely and highly relevant given the potential threats to overall cowpea and other legume production habitat in Africa due to the harmful invasive species *M. sjostedti* (Abudulai et al. 2006; Asante et al. 2001; Oparaeke 2005). The potential habitat distribution map for *M. sjostedti* can help in planning pest management around its existing populations, discover other pest populations, identify top-priority survey sites, or set priorities to manage its natural habitat mainly the grain legume cropping system specially cowpea for more effective controlling. In addition to that, development of extra-early and early maturing or crop varieties resistant to extreme temperatures and to pest and diseases attack should be developed, this will help to maintain the productivity of cowpea in the drier and Sahel regions of sub-Saharan Africa where the rainfall is less than 500 mm/year. However, more research is needed using more accurate environmental variables with higher spatial resolution to predict and determine more accurate geographic areas to adequately cover suitable habitat for *M. sjostedti*. In addition, the methodology presented here could be used for quantifying pest habitat distribution patterns in other areas and may aid field surveys and allocation of pest management and protection of agro ecosystems efforts.

PART III - BEHAVIORAL AND SURVIVORSHIP

CHAPTER FOUR - REPELLENCY OF PLANT EXTRACTS AGAINST THE LEGUME FLOWER THRIPS *Megalurothrips sjostedti* Trybom (THYSANOPTERA: THRIPIDAE)

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Statement of contribution

AA, TM and SS conceived and designed research. AA and XC conducted experiments. TM contributed reagents. AA analyzed data, AA, SK and GTG and wrote the manuscript. All authors read and approved the manuscript.

Abstract

Megalurothrips sjostedti Trybom is an important pest of cowpea (*Vigna unguiculata* L.) in Africa. To propose an alternative to chemical control, the repellency of 24 plant extracts were evaluated against adult female thrips of *M. sjostedti* in the laboratory. Plant extracts in ethanol were separately applied on a filter paper disk in still air visual cue olfactometer. The results showed highly significant differences in repellency among extract type, concentration and their interactions. We classified the level of repellency into four categories as strong, good, moderate, weak or non- repellent based on hierarchical ascendant classification. We identified *Piper nigrum, Cinnamomum zeylanicum, Cinnamomum cassia* as strong repellents. Five extracts were classified as good, eight as moderate and the remaining eight extracts were weak or non-repellent. Repellency of the extracts increased with the concentration suggesting that the behavioural response of *M. sjostedti* was dose-dependent. Mono- and sesqui terpene hydrocarbon compounds from seven highly repellent extracts were identified by GC/MS. The use of repellent extracts could be one of the useful in developing integrated pest management strategies for thrips on legume crops. In this regard, the specific mode of actions of the identified compounds needs to be investigated to incorporate them in the existing crop protection strategies.

Keywords: Plant extracts; Repellency; *Megalurothrips sjostedti*; Olfactometer; *Piper nigrum*.

4.1 Introduction

The Legume Flower Thrips (LFT), *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), is one of the most serious insect pests of leguminous plants including cowpea in tropical Africa (Singh & Allen 1980; Jackai & Adalla 1997; Ekesi et al. 1998; Karungi et al. 2000). Thrips occur on legumes in every growing season, and their direct feeding cause destruction of buds and flowers as well as malformations of pods (Abudulai et al 2006). Yield losses ranging from 20 to 100 % have been reported on cowpea (*Vigna unguiculata* L.) from different areas of Africa where modern pest control measures are absent (Singh & Allen 1980; Jackai & Adalla 1997; Ekesi et al. 1998; Karungi et al. 2000).

Currently, the control of *M. sjostedti* in Sub-Saharan African countries relies heavily on synthetic insecticide application (Omo-Ikerodah et al. 2009). The indiscriminate use of these chemicals has given rise to problems such as resistance of the legume pests to insecticide (Oparaeke 2006 ; Bediako 2012), accumulation of toxic residues in food, health risks to the user and livestock and environmental contamination (Oparaeke 2006 ; Muthomi et al. 2008; Egho 2011). As a result, there is an urgent need to develop alternatives which are safe, effective, biodegradable and highly selective. Pesticides from plant-based extracts have been suggested as a better alternative to synthetic insecticides (Isman 2008).

Plant extracts contain many secondary metabolites that act as repellents, feeding deterrents, and toxins which have a role in defence against herbivores, pests and pathogens (Maia & Moore 2011). These secondary metabolites are released in the form of plant volatiles. Plant extracts are a complex mixture of general leaf volatiles, found in most plant species with more specific components that are shared by some plant species groups (Van Tol 2006). Essential oils generally consist of several constituents produced as secondary plant metabolites, the majority of which are terpenes hydrocarbons, polyphenolic compounds and alkaloids (Tânia et al. 2012). Essential oils from different plant species are an important source of repellents. These odours have been extensively tested for safety and toxicity and have shown no deleterious effects on beneficial insects, and are therefore considered to be one of the new means of crop protection (Plimmer 1993; Isman 2006). Hence the use of essential oils for pest management is becoming popular and many new applications are under investigation (Van Tol 2006; Regnault-Roger 1997; Koschier 2006; Reitz et al. 2008; Picard et al. 2012).

The release of repellent volatiles into the air by associated plants may disrupt the olfactory orientation of insects such as thrips (den Beider et al. 2001). Integrating naturally occurring repellent volatiles that defend plants by irritating insects to spend a shorter time period in a treated area may help reduce insect damage to crops (Peterson 2001). However, profound knowledge about the behavioural response of the target pest to the specific compound is the precondition for successful utilization of biologically active secondary plant compounds in crop protection strategies (Koschier 2006). Although several repellent plant extracts and essential oils have been identified against onion thrips and western flower thrips (Van Tol 2006; Picard et al. 2012), there is limited information on use of repellents against *M. sjostedti.*

Thus, the aim of our study was to investigate potential repellent plant extracts which can be integrated for the management of thrips in grain legumes and to characterize constituent volatiles released from highly repellent extracts. Integrating repellents that modify pest behaviour in conventional legume flower thrips management strategies might improve efficacy in the management of thrips in the small holder farmers in Sub-Saharan African countries.

4.2. Experimental Section

Insect Culture and Rearing

The initial culture of adult *M. sjostedti* was field-collected from pigeon pea, *Cajanus cajan* (L.) Millsp. in Matuu, Yatta district, Kenya (1°16'N; 37°53'E; 1246 m a.s.l.). The thrips were subsequently reared in ventilated plastic jars provided with French bean (*Phaseolus vulgaris* L.) pods at as described by Ekesi et al. 1998. The insect cultures were maintained in a laboratory at *icipe*, Nairobi, Kenya at 26 ± 2 °C; 60 ± 5 % RH; and 12L: 12D photoperiod. Bean pods containing fresh thrips eggs were transferred to new jars to obtain adult thrips of known age. Adult female thrips (four to five days old) were used in the bioassays. To avoid behavioural bias prior to experimentation, adult thrips were conditioned for half a day without beans in empty jars.

Olfactometer Bioassay

To compare repellent effects of plant extracts against *M. sjostedti,* we adapted a simple tube still-air visual and odour cue olfactometer used by (Deletre et al. 2015a; Deletre et al. 2015b) as detailed in Figure 1. The olfactometer measured 20 cm in length and 0.8 cm internal diameter. The tube was divided into 3 equally partitioned sections, top, middle and bottom. The top section of the tube was covered with a blue colour 3M Vinyl electrical tape (Taiwan Scotch™) with a size of (2.4 cm x 4.5 cm) as a visual cue (Figure 1). The bioassay was carried out in a completely randomized design (CRD) experimental set up at room temperature (26 ± 2 °C) inside a fume hood (Telstar BIO II) illuminated with fluorescent light. All the seven olfactometer tubes were placed vertically on a test tube rack, parallel to each other inside the hood. The still-air visual cue olfactometer used in the study provided a combination of visual (blue colour) and olfactory cues (plant extracts at different concentrations) in the test for repellency. A combination of visual and olfactory cues is involved in responses of thrips to host cues (Teulon et al. 1993; Terry et al. 1997) including *M. sjostedti* (Muvea et al. 2014).

Plant extracts tested in repellency bioassays were sourced from different suppliers as listed in

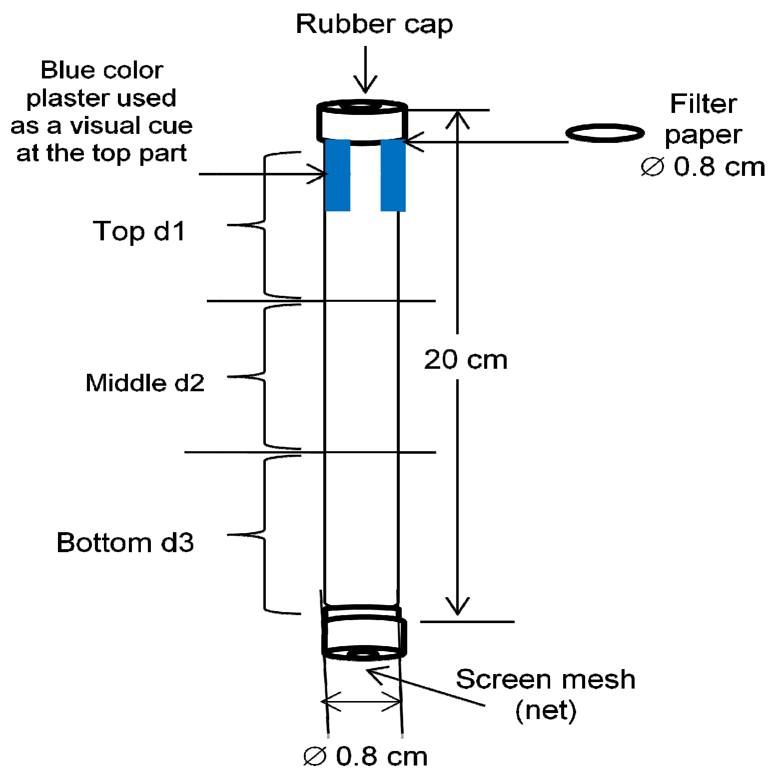

Figure 4.1 Schematic representation of simple tube still-air visual and odour cue olfactometer

Table 4.1 Aliquots (3 µl) of plant extracts at three selected concentrations prepared in absolute ethanol (0.01 %, 0.1 % and 1 %) were applied on a piece of filter paper (∅ 0.8 cm) using a pipette (Finnpipette® 2-20 µl Thermo Labsystems) and placed at the top end part of the tube. Ten female *M. sjostedti* thrips were released at the bottom end of each tube using a small aspirator. The top end of the tube was closed with a rubber cap to prevent the odour escape whereas the other end was closed using a silk micro-screen fine mesh to protect escape of the insects and ensure ventilation. The number of insects in the three parts was counted 5 and 10 min after introduction. Seven replicates were run for each treatment tested which comprised of different concentrations of plant extracts. Absolute ethanol (Scharlad S.L., Spain) which was used as solvent to plant extracts was used as a control in each experiment. The treatments were only different doses of odor cues and the visual cue was the same for all the treatment and control. Since the experiments were repeated at different times, the control solution was run with each experiment. To avoid dose effect, a new cohort of *M. sjostedti* was assayed for each concentration taken from the batch of starved and conditioned adult thrips.

Data Collection

To determine repellency, the number of thrips was recorded for the three sections of the tube 5 and 10 min after odour introduction. Based on the observations, mean repellency was estimated as described below:

$$S = ((mdT*t) + (mdM*m) + (mdB*b)) / n$$

where: *S* is the mean repellency per thrips for each extract or essential oil, mdT is the mean distance for top section = 3.3 cm, mdM is the mean distance of thrips movement for middle section = 10.0 cm, mdB is the mean distance of thrips movement for bottom section = 16.5 cm, t is the mean number of insects count at the top, m is the number of insects count at the middle, b is the number of insects count at the bottom and n is total number of insect per tube (n = t + m + b).

GC-MS Analysis

Aliquots (1 µl) of samples from the top seven repellent plant extracts from the olfactometer assay, prepared at concentration of 1000µg were analysed on GC (Agilent 7890A) capillary column (HP-5, 30 m, 0.25 mm, i.d. 0.25 µm) directly coupled to a mass spectrometer (Agilent, Palo Alto, California, USA) for identification of component compounds of the extracts. Ionisation was performed by electron impact (70 eV, 230 ˚C). The oven temperature was maintained at 35^{o}C for 3 min and then programmed at 10˚C to 285˚C min^{-1}. The carrier gas was helium, at a flow rate of 1.25 ml min^{-1}. Compounds were identified by comparison of retention indices and mass spectra with those of authentic standards. In the absence of corresponding reference compounds, structures were proposed on the basis of MS fragmentation pattern combined with reference spectra in the database (NIST 05, NIST 08, Adams and chemical).

Quantification of Terpenes

GC-MS in full scan mode was used to detect terpenes in the oil and plant extracts. Serial dilutions of Limonene and Caryophyllene oxide (1-100 pg/µl) were analyzed by GC-MS in scan mode to generate linear calibration curves (peak area vs. concentration) with the following equations; Limonene (y = 8E+06x R^2 = 0.9979) for mono-terpenes and Caryophyllene oxide (y = 4E + 06x - 2E + 07; R^2 = 0.9584) for sesqui-terpenes. The two compounds were randomly selected based on their column chemistries in relation to the target class of compounds. Relative percentages of each compound in compositions of essential oils were calculated based on the corresponding areas of the identified compounds.

Table 4.1 Name and source of the plant extracts used for repellency experiment.

No	Common name	Scientific name	Family	Extract type, plant part used	Supplier, country
1	African blue basil	*Ocimum kilimandscharicum*	*Lamiaceae*	Essential oil, leaf	*icipe* - Bioprospecting unit, Kenya
2	Black pepper	*Piper nigrum*	*Piperaceae*	Essential oil, seed	IBMM[1], France
3	Ceylon cinnamomum	*Cinnamomum zeylanicum*	*Lauraceae*	Essential oil, inner bark	Nactis, France
4	Chinese cinnamomum	*Cinnamomum cassia*	*Lauraceae*	Essential oil, bark	Huiles & Sens, France
5	Citronella	*Cymbopogon nardus*	*Poaceae*	Essential oil, leaf	Burgess & Finch, South Africa
6	Conyza	*Conyza newii*	*Asteraceae*	Essential oil, leaf	*icipe* - Bioprospecting unit, Kenya
7	Coriander	*Coriandrum sativum*	*Umbelliferae*	Essential oil, seed	Fabster, France
8	Dill	*Anethum graveolens*	*Apiaceae*	Essential oil, seed	IBMM, France
9	Eucalyptus	*Eucalyptus globulus*	*Myrtaceae*	Essential oil, leaf	Huiles & Sens, France
10	Geranium	*Pelargonium graveolens*	*Geraniaceae*	Essential oil, leaf	IBMM, France
11	Ginger	*Zingiber officinale*	*Zingiberaceae*	Essential oil, root	Burgess & Finch, South Africa
12	Lemon	*Citrus limon*	*Rutaceae*	Essential oil, fruit	Capua, Italy
13	Lemon grass	*Cymbopogon citratus*	*Poaceae*	Essential oil, leaf	Burgess & finch, South Africa
14	Lemon savory	*Satureja biflora*	*Lamiaceae*	Essential oil, leaf	*icipe* - Bioprospecting unit, Kenya
15	Marjoram	*Origanum majorana*	*Labiatae*	Essential oil, leaf	Burgess & Finch, South Africa
16	May chang	*Litsea cubeba*	*Lauraceae*	Essential oil, fruit	IBMM, France
17	Myrrha	*Commiphora myrrha*	*Burseraceae*	Essential oil, oleoresin-gum	Burgess & Finch, South Africa
18	Neem	*Melia azadirachta*	*Meliaceae*	Vegetable oil, seed	Huiles & Sens, France
19	Pennyroyal	*Mentha pulegium*	*Lamiaceae*	Essential oil, leaf	IBMM, France
20	Rosemary	*Rosmalinus officinalis*	*Lamiaceae*	Organic floral water , leaf	Huiles & Sens, France
21	Savory	*Satureja abyssinica*	*Lamiaceae*	Essential oil, leaf	*icipe* - Bioprospecting unit, Kenya
22	Solidage	*Solidago canadensis*	*Asteraceae*	Essential oil, flower	Huiles & Sens, France
23	Thyme (wild)	*Thymus satureioides*	*Lamiaceae*	Essential oil, flower	Huiles & Sens, France
24	Thyme (common)	*Thymus vulgaris*	*Lamiaceae*	Essential oil, leaf	Burgess & Finch, South Africa

[1]IBMM - Institut des Biomole´cules Max Mousseron, Montpellier, France

Data Analysis

The count data of thrips observed at the three sections of the olfactometer were converted to continuous data of mean repellency as detailed earlier. Data analysis was carried out using Analysis of Variance (ANOVA). Since the response to the control did not vary significantly among the different experiment a mean control value was used in the statistical analysis. Where the ANOVA showed significant differences on the interaction between concentration and extract, pairwise comparison of the concentrations of each extract with the control was performed using Student Newman Keuls test. The differences in thrips response among different extracts for each dose were also tested with the control and comparison of means was implemented using Student Newman Keuls test. The pooled interaction effect of plant extracts and concentrations was further explored using principal component analysis (PCA). Then, based on the similarity of their repellent effect, a hierarchical ascendant classification (HAC) on ward's algorithm was used to group the plant extracts using PCA-axes coordinates. This process yielded a binary segmentation tree (dendrogram), reflecting the hierarchy of similarities between *M. sjostedti* responses to plant extracts. The optimal number of classes in the tree was determined by the decrease of the interclass variance (branch height-Appendix 3). The analyses were implemented using R version 3.11 (R core team 2014).

4.3. Results

Olfactometer Bioassays

Among the three observation sections of the olfactometer, 52% of the thrips were observed in the top of the olfactometer (where the visual and olfactory cues were presented), while 34% and 13% of the thrips were observed at the bottom and the middle of olfactometer, respectively when considered irrespective of doses, extracts and time of exposure ($F_{2, 3063}$= 1840, $P < 0.001$). Analysis of the mean repellency indicated that the effect of the interaction between extract type, concentration used and time on mean thrips repellence was not significant ($F_{69, 1152}$= 0.56, $P = 1$). The two ways interaction effect between extract and time and concentration and time were also not significant ($F_{23, 1152}$= 0.88, $P = 0.63$; $F_{3, 1152}$= 1.94, $P = 0.121$, respectively). However, the interaction between extract and concentration was highly significant ($F_{69, 1152}$= 3.43, $P < 0.001$). The main effects of concentration and extracts were highly significant; $F_{3,1152}$=143.3,P<0.001 and $F_{23,1152}$=8.65, P<0.001, respectively) while main effect of time (5min and 10min) after odour introduction was not significant ($F_{1,1152}$=0.43, P=0.51).There was no significant difference in mean repellency between the two ways interaction effects, extract types and time ($F_{23, 1152}$= 0.88, $P = 0.62$), and concentration and time ($F_{3, 1152}$= 1.94, $P = 0.12$) and between the time 5 and 10 min after odour introduction ($F_{1, 1152}$= 0.43, $P =$ 0.51).

In general, the repellency of extracts increased as the concentration of plant extract/essential oils increased from 0.01 to 1 %.. At lower concentration (0.01 %), the oils *P. nigrum, C. zeylanicum, C. cassia, C. citratus* were significantly different over in repelling *M. sjostedti* and the repellency ranged from 9.2 to 10.3 cm away from the odor source ($F_{24,326}$=5.33, P<0.001). At 0.1 % concentration, repellency of *P. nigrum, C. zeylanicum,, C. cassia, E. globulus, C. myrrha Z. officinale*, *T. vulgaris* and *P. graveolens* were significantly different over control and repellency ranged from 9.3 to 11.3 cm ($F_{24,\ 326}$ = 5.15, P<0.001). At 1% concentration, *P. nigrum, C. zeylanicum, C. cassia, E. globulus, C. myrrha, T. vulgaris, M. pulegium, C. citrates, C. nardus, S.biflora, M. azadirachta, A. graveolens, L. cubeba, C. sativum, O. majorana, S.abyssinica* and *P. graveolens* were significantly different over control and repellency ranged from 9.1 to 11.8 cm ($F_{24,\ 326}$ = 5.99, P<0.001). At all three doses, *T. satureioides, O. kilimandscharicum, S. canadensis, C. newii, C. limon* and *R. officinalis* were not significantly different over the control for repellency of *M. sjostedti* (Table 2).

Table 4.2 Response of female *Megalurothrips sjostedti* to the repellent effect of 24 plant extracts at three concentrations (0.01, 0.1 and 1%) of extract solution and control on filter paper

S.N	Extract	Concentration (%)				
		0 (control)	**0.01**	**0. 1**	**1**	**SE of mean**
		6.9				
1	Black pepper		10.3*	11.3*	10.1*	±0.59
2	Ceylon Cinnamomum		9.4*	9.7*	10.5*	±0.35
3	Chinese Cinnamomum		9.5*	9.3*	11.8*	±0.41
4	Myrrh		8.7	9.9*	9.9*	±0.38
5	Lemongrass		9.2*	9.0	9.8*	±0.41
6	Marjoram		8.9	8.9	9.2*	±0.43 NS
7	Eucalyptus		8.6	9.7*	9.5*	±0.46
8	Citronella		8.4	9.2	9.8*	±0.43
9	Pennyroyal		6.8	9.0	11.1*	±0.35
10	Geranium		7.6	9.3*	9.8*	±0.36
11	Thyme (Common)		7.9	10.1*	9.4*	±0.49
12	Lemon savory		8.1	8.9	9.7*	±0.42
13	Neem		7.7	8.8	9.6*	±0.28
14	Dill		7.3	9.0	9.4*	±0.38
15	Litsea		8.5	7.2	9.3*	±0.39
16	Thyme (wild)		6.9	8.9	8.9	±0.43
17	Coriander		8.5	8.3	9.3*	±0.36
18	African blue basil		8.3	9.1	6.6	±0.36
19	Savory		7.7	7.5	9.1*	±0.38
20	Solidago		7.7	7.5	8.3	±0.34 NS
21	Ginger		7.6	9.3*	8.4	±0.37
22	Conyza		7.4	8.6	8.5	±0.39 NS
23	Lemon		8.1	8.4	7.6	±0.45 NS
24	Rosemary		6.6	6.6	7.7	±0.38NS
	SE of mean		±0.43	±0.46	±0.48	±0.59

Within concentrations of 0.01, 0.1 and 1%, asterisks "*" indicate significant difference in thrips repellence of extracts over control. Within row [NS], indicates no significance in thrips repellence across concentrations for the extract, while all other extracts differed significantly for thrips repellence across concentration (Student Newman Keul test, p = 0.05).

Based on the Hierarchical Ascendant Classification (HAC) analysis of repellence effects, the different plant extracts were categorised in to four classes: strong (Class 1), good (Class 2), moderate (Class 3) and weak or non repellent (Class 4). *Piper nigrum, C. zeylanicum* and *C. cassia* extracts were classified as strong repellents (Class 1), while *C. myrrha*, *C. citratus*, *O. majorana*, *E. globulus* and *C. nardus* were found to be good repellents (Class 2). Eight extracts were moderately repellent (Class 3); these were: *M. pulegium*, *P. graveolens*, *T. vulgaris*, *S. biflora, M. azadirachta, A. graveolens*, *L. cubeba,* and *T. satureioides*. As compared to the other extracts, the remaining eight extracts were weak or non repellent (Class 4). These were: *C. sativum, O. kilimandscharicum, S. abyssinica*, *S. canadensis*, *Z. officinale*, *C. newii*, *C. limon* and *R. officinalis* (Figure 2).

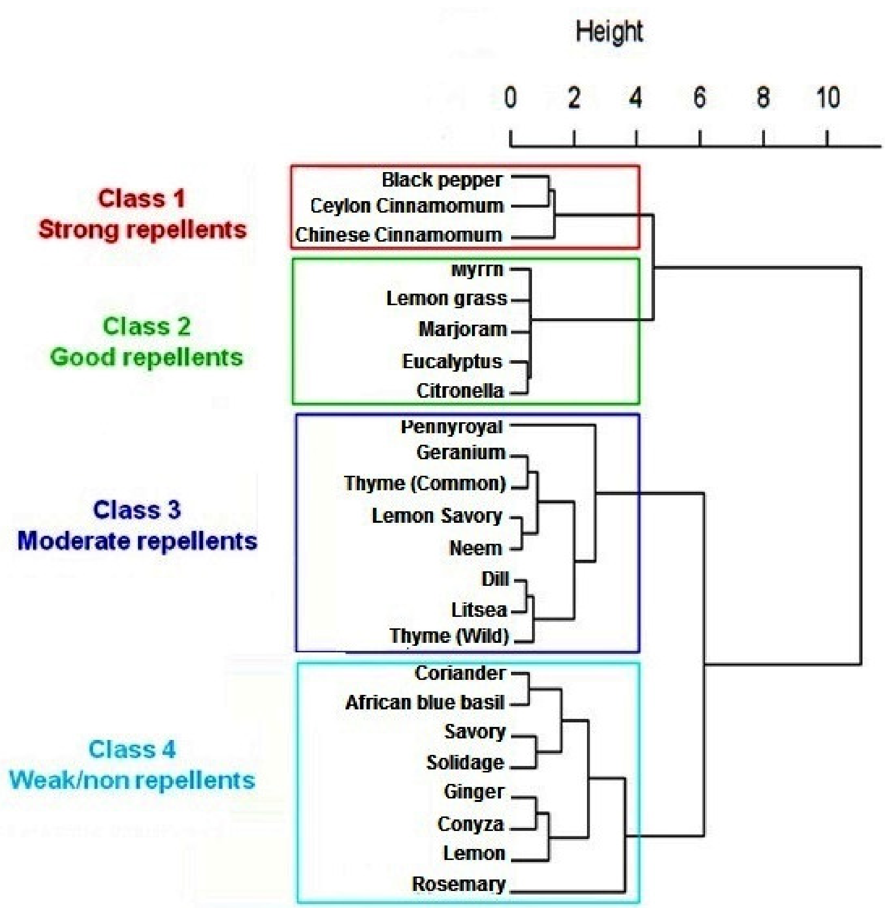

Figure 4.2 Dendrogram representing response of female *M. sjostedti* to the pooled interaction effect of extracts and concentrations

Identification of the chemical constituents from seven highly repellent extracts showed that mono- and sesquiterpenes were the most abundant (Table 3). Fifteen compounds were identified from *P.*

nigrum extracts. The major compounds were: β-caryophyllene (45.9 %), caryophyllene oxide (12.9 %) and α - copaene (12.3 %). Cinnamaldehyde (79.6 %), *trans*-cadina-1(6) and 4-diene (13.24 %) were the most abundant compounds identified from *C. zeylanicum* extract. On the other hand, cinnamaldehyde (76.7 %) was the major constituent followed by (E)-ortho-methoxy cinnamaldehyde (16.1 %) were the most abundant compounds identified from extracts of *C. cassia*.Six compounds were identified from *C. myrrha* extracts with curzerene (75.3 %) and β-elemene (13.5 %) being the two major constituents. Five compounds were identified from extracts of *E. globules* of which 1,8-cineole (93.4 %) was the major compound. The major compounds identified from *C. citrates* extracts were geranial (38.3%) and neral (33.7 %). Seventeen compounds were identified from *O. marjorana* with trans-sabinene hydrate (16 %), terpinene-4-ol (17.9 %) and γ-terpinene (11 %) being the major component compounds. The most frequently detected component compounds were limonene (detected from four different extracts), α-copaene and β-caryophyllene (detected from three different extracts), α-humulene, camphene, δ-3-carene, cinnamaldehyde, β-selinene, α-muurolene and caryophyllene oxide (detected from two different extracts) (Table 4.3).

4.4 Discussion

The still-air visual and odour cue olfactometer used in the present study provided the opportunity to test the repellency response of legume flower thrips (LFT) to a combination of visual and olfactory cues, which is in line with the host response behavior of thrips (Teulon et al. 1993, Terry et al. 1997). However we observed that a significantly higher proportion of LFT preferred to move to the top section of the olfactometer where the visual cue (blue colour) was presented. This could be due to fact that flower/anthophilous thrips are highly attracted to flower colour (Terry et al. 1997; Matteson & Terry 1992; Vernon & Gillespie 1990). Recently LFT was reported to be highly attracted to a combination of flower volatiles and blue colour, followed by blue colour alone compared to yellow colour with or without flower volatiles and flower volatiles alone (Muvea et al. 2014). The role of negative geotaxis behavior as reported in thrips species such as western flower thrips (Ebssa et al 2001) and thrips species infesting cereals (Holtmann 1962) cannot be discounted but needs to be further tested for LFT. Following the top section, thrips preferred to remain in the bottom section of the olfactometer, which is due to the repellency of the extracts tested. Such interaction of colour and volatile based cues in eliciting response of thrips needs to be considered in further refining the still-air visual cue olfactometer used for the first time in this study.

Based on the repellency response of LFT to 24 plant extracts, most of the extracts were repellent to *M. sjostedti*. Black pepper, Ceylon cinnamomum and Chinese cinnamomum were identified as strong repellents, while myrrh, lemon grass, marjoram, eucalyptus and citronella were classified as good repellents. To our knowledge, this is the first study about repellency of essential oils against *M. sjostedti*. Generally, the repellency of most extracts varied with concentration and indicated a dose dependent behavioral response of *M. sjostedti.* Our result corroborate previous observations on repellent effect essential oils and plant volatiles for other thrips species such as the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Picard et al. 2012 ; Pow et al. 1999; Koschier et al. 2000) and the onion thrips, *Thrips tabaci* (Lindeman) (Van Tol 2006; Koschier et al. 2002). Extracts from plant species from the same family did not exhibit similar levels of repellency against *M. sjostedti* for all plant families' evaluated. For example, the repellency among the two *Cinnamomum* species of Lauraceae family was similar and categorized as strong repellent. However, the two species of *Satureja* from the Lamiaceae family showed different levels of repellency where Lemon savory (*S. biflora*) was classified as moderate and savory (*S. abyssinia*) as a weak repellent (Figure 4.2).

Extract from *P. nigrum* was the most repellent out of the 24 plant extracts tested. On a related field study Oparaeke [7] observed that application of 10 and 20% extracts of West African black pepper, *Piper guineense* (Schumacher), caused significant reduction of *Megalurothrips* on flowers as compared to synthetic insecticide treatment and increased pod yield on cowpea. GC-MS analsysis revealed β-caryophyllene, caryophyllene oxide and α-copaene as the major compounds in *P. nigrum* as also observed by Delétré et al. 2013. Apart from *P. nigrum*, plant extracts from the two *Cinnamomum* species also showed a strong repellent effect against *M. sjostedti*. Plant species of the genus *Cinnamomum* are fairly well-known to have a repellent and toxic effect to several insect species as house fly *Musca domestica* (Samarasekera et al. 2006), rice weevil *Sitophilus zeamais* (Ishii et al. 2010), pulse beetle *Callasobruchus maculatus* (Ratnasekera, & Rajapakse 2009) and mosquito, *Culex quinquefasciatus* (Deletre et al. 2013 ; Prajapati et al. 2005; Chang et al. 2006). Results from chemical analysis were comparable to Delétré et al. 2013, who reported cinnamaldehyde at a similar quantity as the major compound of *C. zeylanicum*. Extracts from *C. myrrha,* which were classified as good repellent, had curzerene (75.27 %) and β - elemene (13.52 %) as major constituents. In previous studies, *Commiphora rostrata* Engler (Burseraceae) extracts showed repellency against the maize weevil (Lwande et al. 1992), while extracts of *C. myrrha* and *C. holtziana* showed repellency against the poultry red mite, *Dermanyssus gallinae* (De Geer) (Mesostigmata: Dermanyssidae) (Birkett 2008). The repellency of Lemongrass reported in the present study is also in line with a previous report on repellency activity of essential oil of *C. citratus* against adults of *Culex quinquefasciatus* Say [44]. In terms of compositional analysis, our results

were comparable to previous studies (Deletre et al. 2013; Olivero-Verbel et al. 2010; Franz 2011; Costa 2013). where geranial and neral were identified as the major compounds in *C. citratus*.
Marjoram exhibited good repellent effect against *M. sjostedti.* Similarly, van Tol et al. 2006, reported *O. majorana* as a promising repellent against *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Moreover Yi et al. 2006, observed potent fumigant toxicity of marjoram on the melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae). In our study, Eucalyptus extract with 1-8 cineole as a major constituent was a good repellent against *M. sjostedti*. Oparaeke et al. 2006 reported that the mean number of *M. sjostedti* was significantly reduced on plots sprayed with plant extracts mixed with *Eucalyptus* than on unsprayed plots for two consecutive seasons. Similarly, Koschier & Sedy 2003 reported repellency of 1,8-cineole (eucalyptol), a major constituent in the rosemary oil to female onion thrips. Citronella was also a good repellent against *M. sjostedti*. Similarly, Pinheiro et al 2013 reported that citronella grass, *C. winterianu,* showed enhanced insecticidal activity against the common blossom thrips, *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae) and green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) .
Among the eight moderate repellent extracts, Pennyroyal and Thyme exhibited the highest repellency against *M. sjostedti* at higher concentrations of 1% . Yi et al. 2006, reported a 23.6 fold more toxic effect than organophosphate dichlorvos against adult melon thrips. Essential oil from Thyme was reported to be highly repellent to western flower thrips *F. occidentalis* (Picard et al. 2012).

Eight extracts such as coriander, African blue basil, savory, solidage, ginger, conyza, lemon and rosemary were categorized as less or non-repellent extracts. Similar results from Delétré et al. 2013, showed that, Lemon and Rosemary extracts did not exhibit a significant repellent effect against adults of *Anopheles gambiae* at a concentration of 1.0 %, while Coriander, Ginger and Solidage showed slight repellent effect against *A. gambiae*. Nevertheless, Mayeku [52] reported that Conyza essential oil showed repellency and fumigant toxicity against *A. gambiae*. Rosemary was the least repellent among all the tested extracts. However, at a higher concentration (10%) of Rosemary essential oil repelled female *T. tabaci* significantly in olfactometer bioassays (Matteson & Terry 1992). Moreover, Rosemary essential oil at 0.1% and 1% concentration decreased feeding damage of *T. tabaci* [53]. A possible reason for the varying results could be due to the low concentrations used in the present experiments as well as the composition of the Rosemary oil. For example, the major constituent in the Rosemary oil used in the olfactometer test by Koschier and Sedy 2003 was 1,8-cineole (51%), whereas, the Rosemary extracts used in the current study contained < 1% of 1,8-

cineole. Another potential reason for the varying results could be the different behavioral responses between the two thrips species *M. sjostedti* and *T. tabaci*.

The chemical composition and broad spectrum of biological activity of essential oils, even from the same source, can be inherently variable for many reasons. Such as, plant age, plant tissues used in the distillation process and type of distillation can cause variability of chemical composition of essential oils from a plant species (Pushpanathan et al. 2006). Likewise, variability in behavioral and biological activity can be due to the age of the targeted pest organism (Koschier & Sedy 2003; Chiasson et al. 2001; Choi et al. 2003) . Biological activity could be affected by interactions among structural components in the extracts. Even minor compounds can have a critical function due to coupled effects, additive action between chemical classes and synergy or antagonism (Katerinopoulos et al. 2005).
The knowledge of extent, interaction and mode of inhibition of specific compounds in plant extracts, may contribute to the successful application of pest management. Although several essential oils are repellent to thrips species in the laboratory, due to high volatility the efficacy in the field is usually low (Van Tol 2006Reitz et al. 2008; Picard et al. 2012). Picard et al. 2012 indicated that application method incorporating the oils into polymeric mixture coatings to protect the bioactivity of the active compounds shows a better distribution and maintains high concentrations of active compounds on the surface of the leaves for longer period. Many plant extracts are selective to certain pests, often biodegrade to nontoxic products, and have few or no harmful effects on non target organisms and the environment (Oparaeke et al. 2006; Barton 1999; Isman 2001). They also can be useful to maximize thrips control efficiency and sustainability, while minimizing negative environmental effects. Integrating host plants with repellents (push) as intercropping, row planting or border crop may improve the effectiveness of the formulated repellents in the field. However, all these applications needs further study.

4.5 Conclusions

Our results provide evidence that female *M. sjostedti* are repelled by several plant extracts and *P. nigrum, C. zeylanicum* and *C. cassia* are strong repellents. This indicates that plant extracts or phytochemicals have potential as natural pesticides for thrips control. The repellent effect could be related to presence of different active compounds or a blend of odors, which induce an oriented movement away from the odor source. The biological activity of the major and most abundant compounds of the tested plant extracts should be further investigated under laboratory conditions to identify and evaluate specific behavioural responses of *M. sjostedti. This will help* to identify precisely the main mode of action and levels of bioactivity of different compounds to better integrate them in management strategies for legume flower thrips. Behavioural manipulation using natural products with fewer deleterious effects on non-targeted organisms and the environment, for the management of thrips can be considered as a new approach for pest management in grain legumes.

Table 4.3 Compounds of the top seven plant extracts identified using GC-MS.

No	Name	Retention time (min)	Types of essential oils and relative percentage (%)						
			P.nigrum	*C. zeylanicum*	*C. cassia*	*C. myrrha*	*C. citratus*	*O. marjorana*	*E. globulus*
1[◊]	α-Phellandrene	9.69	-	-	-	-	-	1.34	-
2[*]	α-Pinene	9.83	-	-	-	-	-	0.74	1.71
3[*]	Camphene	10.21	-	-	-	-	1.44	-	0.02
4[◊]	Sabinene	10.68	0.08	-	-	-	-	8.95	-
5[*]	Myrcene	11.04	-	-	-	-	1.15	1.79	-
6[*]	δ-3-Carene	11.40	0.37	-	-	0.94	-	-	-
7[*]	δ-2-Carene	11.53	-	-	-	0.57	-	8.45	-
8[*]	Limonene	11.75	3.43	-	-	2.10	0.72	3.09	3.09
9[*]	β-Pinene	11.76	-	-	-	-	-	4.38	-
10[*]	1,8-Cineole	11.80	-	-	-	-	-	-	93.43
11[*]	(Z)-Ocimene	11.91	-	-	-	-	0.40	-	-
12[◊]	γ-Terpinene	12.31	-	-	-	-	-	12.7	1.75
13[◊]	Sabinene hydrate=cis->	12.47	-	-	-	-	-	4.26	-
14[◊]	Terpinolene	12.83	-	-	-	-	-	3.22	-
15[*]	Linalool	13.01	-	-	-	-	2.06	-	-
16[◊]	Sabinene hydrate=trans->	13.03	-	-	-	-	-	18.59	-
17[◊]	Menth-2-en-1-ol=cis-para->	13.39	-	-	-	-	-	1.58	-
18[◊]	(E)-Isocitral	14.33	-	-	-	-	2.78	-	-
19[*]	Terpinen-4-ol	14.33	-	-	-	-	-	20.79	-
20[*]	α-Terpineol	14.51	-	-	-	-	0.55	4.87	-
21[◊]	Neral	15.29	-	-	-	-	33.66	-	-
22[*]	Linalool acetate	15.41	-	-	-	-	-	2.29	-
23[*]	Geraniol	15.43	-	-	-	-	5.98	-	-

24[◊]	Geranial	15.72	-	-	-	-	38.32	-	-
25[*]	(E)-Cinnamaldehyde	15.79	-	79.6	76.7	-	-	-	-
26[◊]	δ-Elemene	16.64	7.0	-	-	-	-	-	-
27[◊]	α-Cubebene	16.82	1.00	-	-	-	-	-	-
28[◊]	Geranyl propanoate	17.18	-	-	-	-	6.4	-	-
29[◊]	α-Copaene	17.20	12.3	1.49	0.7	-	-	-	-
30[◊]	β-Elemene	17.40	2.5	-	-	13.52	1.16	-	-
31[*]	β-Caryophyllene	17.80	45.9	-	-	-	3.78	3.07	-
32[◊]	Sesquithujene	17.94	1.65	-	-	-	-	-	-
33[◊]	(E)-Cinnamyl acetate	18.05	-	-	0.74	-	-	-	-
34[*]	α-Humulene	18.25	3.05	-	-	-	0.95	-	-
35[*]	allo- Aromadendrene	18.34	-	-	0.55	-	-	-	-
36[◊]	γ-Muurolene	18.50	-	2.80	-	-	-	-	-
37[◊]	β-Selinene	18.68	1.73	-	-	7.60	-	-	-
38[◊]	Curzerene	18.74	-	-	-	75.27	-	-	-
39[◊]	α-Muurolene	18.79	1.76	0.54	-	-	-	-	-
40[◊]	Bicyclogermacrene	19.79	-	-	-	-	-	1.10	-
41[◊]	γ- Cadinene	18.99	-	0.7	-	-	-	-	-
42[◊]	δ- Cadinene	19.08	3.35	-	-	-	-	-	-
43[◊]	*trans*- Cadina-1(6),4-diene	19.08	-	13.24	-	-	-	-	-
44[◊]	(E)-Methoxy cinnamaldehyde	19.21	-	-	16.1	-	-	-	-
45[*]	Caryophyllene oxide	19.86	12.95	-	-	-	-	-	-

[*] Identified by comparison with authentic samples. [◊] Identification by library data

PART III - BEHAVIORAL AND SURVIVORSHIP

CHAPTER FIVE - TOXIC AND BEHAVIORAL EFFECTS OF 16 COMPOUNDS AGAINST THE LEGUME FLOWER THRIPS *Megalurothrips sjostedti* TRYBOM LARVAE (THYSANOPTERA: THRIPIDAE)

Prepared as:

Statement of contribution

AA, TM and SS conceived and designed research. AA and NJ conducted experiments. TM contributed reagents. AA analyzed data, AA, SK and GTG and wrote the manuscript. All authors read and approved the manuscript.

Abstract

Sixteen constituents of different essential oils were tested for their effect on the feeding activity, toxicity and behavioural effects on the second instar larvae of legume flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). The larval stage causes more direct feeding damage. The larvae showed a significant preference $p<0.0001$ for untreated leaf discs in a dual choice cowpea leaf disc assay. The application of most of compounds at 1% concentration significantly deterred the larvae from settling on the treated leaf discs. We observed a dose dependent feeding deterrence of the majority of tested compounds and calculated the mean percentage of deterrence for each compound and concentration. The feeding damage was reduced by the application of the majority of compounds and with an increase concentration from 0.1 to 1 %. The toxicity test performed showed that the maximum mortality rate after 24h was observed on caryophyllene oxide and 1, 8 cineole treated leaf discs 19.1 and 16.2% respectively. This indicates that the tested compounds are not efficient as alternatives for insecticide application. Our results may be exploited by extending behavioural manipulation using plant compounds released by companion plants through volatile collection and test under laboratory and field condition. Thus plant compounds could be used in integrated pest management strategies for the control of *M. sjostedti* and family thripidae in combination with the current pest management strategies for grain legumes in Sub-Saharan Africa.

Key words: *Megalurothrips* sjostedti, second instar larvae, Secondary plant compounds, Settlement preference, Toxicity, behaviour

5.1 Introduction

The legume flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), is one of the most serious insect pests of leguminous plants in Sub Saharan Africa (SSA) (Singh and Allen 1980; Jackai and Adalla 1997; Ekesi et al. 1998; Karungi et al. 2000). The adults and larvae of this insect pest feed on epidermal and sub-epidermal plant tissues, causing destruction of buds, flowers and malformations of pods (Abudulai et al. 2006). Yield losses ranging from 20 to 100 % have been reported on cowpea (*Vigna unguiculata* L.) from different areas of Africa (Singh and Allen 1980; Karungi et al. 2000).

Currently, the control of *M. sjostedti* in SSA countries relies heavily on synthetic insecticide application (Omo-Ikerodah et al. 2009). The indiscriminate use of these chemicals has given rise to problems such as resistance of the legume pests to insecticide (Oparaeke 2006; Bediako 2012), accumulation of toxic residues in food, health risks to the user and livestock and environmental contamination (Oparaeke 2006; Muthomi et al. 2008; Egho 2011). As a result, there is an urgent need to develop alternative management strategies which are safe, effective, biodegradable and highly selective. Pesticides from plant-based extracts have been suggested as an alternative to synthetic insecticides (Isman 2008).

Among possible approaches to thrips control, the integration of secondary plant compounds that disrupt host acceptance behaviour with other control measures into behavioural manipulation strategies is broadly considered to have great potential (Cowles, 2004; Cook et al., 2007; Egger et al., 2014). Plant extracts contain many secondary metabolites that act as insect behaviour-modifying agents as repellents, feeding deterrents, and toxins which have a role in defence against herbivores, pests and pathogens (Renwick, 1999; Kim & Ahn, 2001; (Maia and Moore 2011); Egger et al., 2014). These active compounds in plant extracts may act to inhibit pest populations on crops and are less likely to develop pest resistance problems when used over time (Berenbaum et al. 1991; Chen et al. 1995). Although several secondary compounds and their blend have been tested against the behaviour of other thrips species (Riefler and Koschier 2009; Peneder and Koschier 2011; Egger and Koschier 2014; Egger et al., 2014), there is limited information on the behavioural effects and toxicity against *M. sjostedti.*

Abtew et al 2015 reported that among the 24 extracts tested for their repellence effect on *M. sjostedti*, *Piper nigrum, Cinnamomum zeylanicum* and *Cinnamomum cassia* were classified as strong repellents, while *Commiphora myrrha*, *Cymbopogon citratus*, *Origanum majorana*, *Eucalyptus globulus* and *Cymbopogon nardus* were found to be good repellents and *Thymus vulgaris* was categorised as a moderate repellent. Furthermore, the identification of the chemical constituents from seven highly repellent extracts revealed the presence of mono- and sesquiterpenes compounds. The study also recommended further studies on the effect of the major compounds of the tested plant extracts on behavioural responses of *M. sjostedti* under laboratory conditions. Thus, among the recommended extracts we selected 16 different compounds.

The aim of our study was to investigate the potential deterrence and toxic effects of 16 different compounds to the second instar larvae of *M sjostedti*. Firstly, we observed the settlement preference of the larvae for treated or untreated leaf discs in choice bioassays. Secondly, we determined the percentage deterrency index and for each compound and concentration used. Finally, we assessed and evaluated the feeding damage on the leaf and the toxicity of the compounds on *M sjostedti* second instar larvae after 24 hrs with the test dilutions in a no-choice leaf disc assay.

5.2 Materials and Methods

Insect Culture and Rearing Condition

The initial culture of *M sjostedti* was field-collected from pegeonpea from Matuu, Yatta district, Kenya and reared in the thrips laboratory at *icipe* Duduville Campus, Nairobi, Kenya. A culture of *M. sjostedti* was maintained in ventilated plastic jars provided with French bean (*Phaseolus vulgaris* L.) pods in a controlled environment at 26 ± 2°C and 60 ± 5 RH under a photoperiod of L: D 12:12 as described by (Ekesi et al. 1998). French bean (*Phaseolus vulgaris* L.) pods surface-coated with sugar and honey solutions were used as a food supplement. Four days old female second instar larvae were used for bioassays.

Application of compounds

The test compounds (Table 1) were diluted in absolute ethanol, analytical grade, ACS (Scharlab S.L., Gato Perez, Spain) at a ratio of 1:10 and in a relative quantity of distilled water mixed with a surfactant (0.05 % Triton X-100, Scharlau chemie SA, Sentmenat, Spain) to obtain a range of concentrations (0.01,0.1 and 1 %). The control solution consisted of absolute ethanol, analytical grade, ACS (Scharlab S.L., Gato Perez, Spain) and distilled water with the surfactant (0.05 % Triton X-100, Scharlau chemie SA, Sentmenat, Spain) at a ratio of 1:10. For the leaf disc bioassays, the cowpea leaf discs were painted with the respective solution using a pipette (Finnpipette® 2-20 µl Thermo Labsystems) and the tip of the pipette was used to spread the solution evenly over the excised leaf disc surface. DEET (N,N-Diethyl-meta-toluamide) also called diethyltoluamide, is the most common active ingredient in insect repellents and was used as a positive control. The treated leaf discs were allowed to dry for approximately 10 min before releasing the test insects and subsequently placed on 1 % water agar (Oxoid ltd., Basingstoke, Hampshire, England) droplets in a Petri dish to prevent the leaf discs from wilting. After releasing the test insects, Petri dish arenas were ventilated by cutting 45mm diameter holes into the dish lid and covered with micro silk screen inserts glued into place (Fig. 1). The bioassay units were sealed with a Parafilm tape (*Parafilm* "*M*" *Laboratory Film*, Pechiney Plastic packing, *Chicago*, *IL* 60631).

Settlement preference

Choice experiments were conducted to test for settlement preferences of *M.sjostedti* larvae on treated and untreated leaf discs at 1% compound concentration as described by Egger and Koschier (2014). A treated cowpea leaf discs (1.8-cm diameter each) were placed at a distance of approximately 4 cm to each other on a thin layer of water agar droplets in a glass Petri dish (8 - cm diameter) to prevent the leaf discs from wilting. A cohort of 10 second instar larvae of *M.sjostedti* was released at the centre of the Petri dish. The dish was sealed as described earlier. After 30 min, 1, 2, 3, 4, 5 and 6 h the positions of the larvae on either leaf disc were recorded. Any larvae elsewhere in the Petri dish were not taken into account. Each treatment was replicated 15 times with groups of 10 larvae.

Table 5.1 List of compounds used for the experiment from Sigma aldrech

No	Name of compound	Lot #	Product code
1	β -Caryophyllene	MKBJ4602V	101232171
2	Caryophyllene oxide 95%	MKBG8637V	101270084
3	Trans-Cinnamaldehyde 99%	STBC1360V	101154251
4	2 Methoxy cinnamaldehyde 98%	MKBL5406V	1001447314
5	Cinnamyl acetate	MKBG0257V	1001239666
6	1, 8 - Cineole 99%	-	C80601
7	Citral 95%	STBC5273V	101185723
8	Sabinene natural, 75%	MKBL5214V	1001558218
9	γ-Terpinene	MKBL6917V	1001712938
10	α – terpineol ≥ 96%	MKBL5404V	101359091
11	Terpinolene ≥ 90%	MKBH3144V	101381850
12	Citronellal 95%	BCBG6382V	110137706
13	R + Pulegone ≥ 85%	STBC7590V	101193586
14	Geranyl acetate 96%	STBB9996V	101099957
15	Tymol	SLBB4066	1001315752
16	DEET 97% (Control)	14329 KB-377	-
17	Ethanol (Control)	-	-

Feeding deterrence index

In a choice bioassay, a control and a treated cowpea leaf disc (1.8-cm diameter each) were be placed on a thin layer of water agar droplets in a glass Petri dish (8-cm diameter) at a distance of approximately 4 cm to each other to prevent the leaf discs from wilting. A single second instar larva of *M. sjostedti* was released at the centre of the Petri dish. The Petri dish arenas were sealed as described earlier. After 24 h, the larvae were removed, and the feeding damage was measured using a transparent counting grid (0.25 x 0.25 mm) and a stereo microscope. This procedure was repeated 15 times with three different concentrations (0.1, 0.5 and 1 %) of each test compound for calculating a feeding deterrence index (FDI) using the formula:

$$FDI = 100[(C-T) / (C+T)]$$

Where C and T are the control and treated leaf areas damaged by the larvae (Isman et al. 1990). The bioassay was replicated 15 times per concentration for each compound.

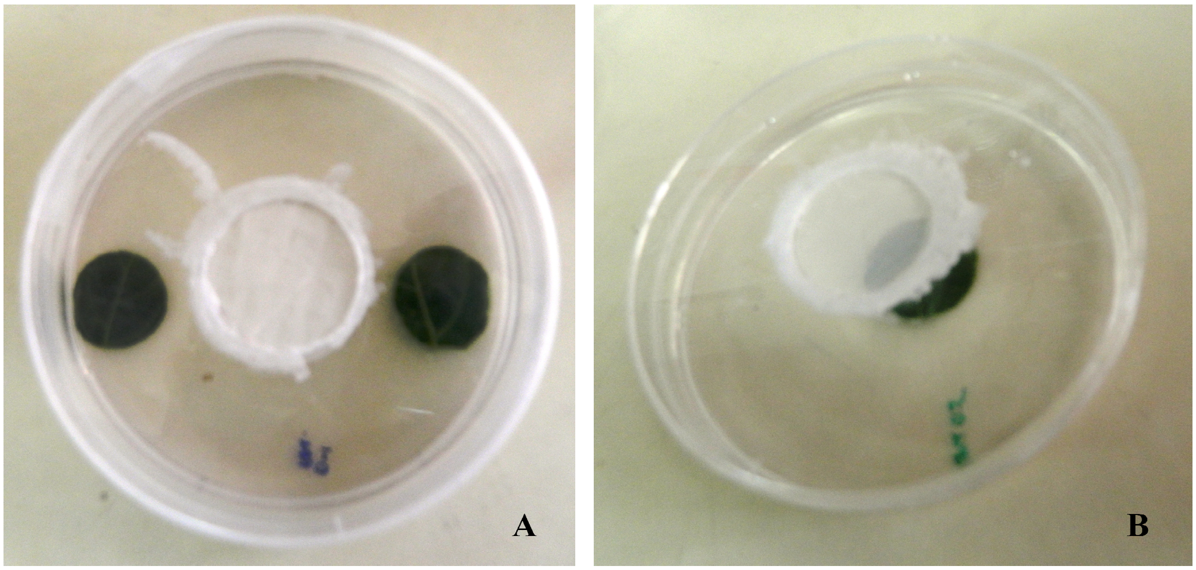

Figure 5.1 Leaf disk bioassay arena with one ventilation hole at the center of the lid. (A) Choice bioassay treated and control leaf disks. (B) No choice bioassay a treated leaf disk alone.

Assessment of feeding damage and Toxicity

For this bioassay, a single leaf disc (2.3-cm diameter) treated with compounds at (0.1, 0.5 and 1 %) concentration or control solutions was placed on a thin layer of water agar droplets in a glass Petri dish (8-cm diameter) to prevent the leaf discs from wilting. A group of 10 adult female *M.sjostedti* second instar larvae was released on the leaf disc and the Petri dish was sealed with a perforated plastic sealing film as described earlier. The Petridishes were placed in the rearing conditions mentioned earlier for 24h, avoiding exposure to direct sunlight for the requisite period. After 24 h, a stereo microscope was used to assess mortality. Paint brush was used to stimulate individual thrips, recording those that are incapacitated or fail to show any signs of movement as 'dead'. Record was done for the total number of thrips and the number of dead thrips per Petri dish. The toxic effect of each product was expressed as the proportion of dead thrips. Simultaneously, the feeding damage was measured using a transparent counting grid (0.25x0.25 mm) and a stereo microscope and subsequently converted into the percentage of damaged area on the leaf discs. The test was replicated 15 times with a group of 10 second instar larvae per treatment.

Statistical analysis

For settlement preferences, t tests were performed to compare the numbers of settled larvae on the treated and control leaf discs. In addition, one way ANOVA was also performed to evaluate the compounds and the number of larvae settled in the treated leaf disk. The FDI was calculated based on the above formula using the feeding damage on the control and at three different concentrations of each compound. ANOVA was used to calculate the FDI. ANOVA was used to compare and analyze the feeding damage of the leaf. Data on toxicity was analyzed using one way ANOVA to evaluate the number of dead larvae. All ANOVA analysis was followed by Tukey's honestly significant difference (HSD) test. We analyzed the mortality data without using Abbott's formula (Abbott 1925) since; the percent mortality of the control was less than 5%. All statistical analyses were performed using the JMP 5 and SAS statistical software (SAS 2002).

5.3 Results

Settlement Preference

The t test analysis revealed that within the 6hr observation, the second instar larvae significantly preferred ($p < 0.0001$) the control leaf disk over the treated leaf disk (Table 2B). Based on the result from t test, to identify the effect of time and compound on settlement preference of we performed ANOVA. The result of the ANOVA reveled that, the mean number of second instar larvae on the treated leaf was significantly lower and influenced by the type of the compound ($p < 0.0001$). But time and the interaction effect between time and type of compounds didn't influence the settlement preference of the larvae (Table 5.2A).

The overall ANOVA revealed that among the tested 16 compound β- caryophyllene, caryophyllene oxide and citral showed the strongest effect with a maximum of one larvae per leaf disk followed by 2 Methoxy cinnamaldehyde and R+Pulegone with two larvae per leaf disk. These compounds showed as good repellent effect than the positive control DEET. Among the least performed compounds, γ-Terpinene, Cinnamyl acetate, Geranyl acetate and Tymol with an average four larvae on the treated leaf disk. All the other compounds are between these two groups (Table 5.2).

Table 5.2 [A] ANOVA of Mean number (±SE) of settled *M. sjostedti* second instar larvae on the treated leaf disc in the bioassay unit. [B] T tests comparison between the numbers of settled larvae on the treated leaf disc with the control.

[A] Compounds	Time (min)							Overall Mean±SE^{a}
	30	60	120	180	240	300	360	
γ-Terpinene	3.5±0.53^{ab}	3.5±0.6^{ab}	4.1±2.3^{ab}	4.3±0.6^{ab}	4.9±0.6^{a}	4.7±0.6^{a}	5.0±0.5^{a}	4.30±0.22^{a}
Cinnamyl acetate	4.2±0.44^{a}	4.2±0.5^{a}	4.0±2.1^{ab}	4.6±0.7^{a}	4.0±0.6^{ab}	4.4±0.7^{ab}	3.9±0.7^{abc}	4.18±0.22^{a}
Geranyl acetate	4.1±0.46^{a}	4.3±0.5^{a}	4.2±2.0^{ab}	4.1±0.6^{ab}	4.1±0.6^{ab}	4.3±0.5^{ab}	4.0±0.5^{ab}	4.16±0.19^{a}
Tymol	3.5±0.43^{ab}	4.0±0.5^{ab}	2.8±2.0^{abc}	3.9±0.5^{ab}	4.3±0.4^{a}	4.1±0.5^{ab}	4.9±0.5^{a}	3.94±0.19^{a}
1, 8 - Cineole	4.0± 0.65^{ab}	4.0±0.7^{ab}	4.3±2.9^{a}	3.5±0.6^{abc}	3.0±0.6^{abcd}	3.1±0.6^{abcd}	4.0±0.6^{ab}	3.70±0.24^{ab}
Citronellal	3.1±0.53^{abc}	2.4±0.7^{abc}	3.9±2.8^{ab}	4.0±0.9^{ab}	3.9±0.8^{abc}	3.6±0.8^{abc}	4.5±0.9^{a}	3.63±0.30^{ab}
Sabinene	2.9±0.55^{abcd}	2.5±0.4^{abc}	2.7±2.4^{abc}	3.3±0.6^{abc}	3.2±0.6^{abcd}	2.7±0.6^{abcd}	2.8±0.6^{abcd}	2.89±0.21^{bc}
Trans-Cinnamaldehyde	3.5±0.77^{ab}	2.6±0.7^{abc}	2.3±2.8^{abc}	2.3±0.6^{abc}	2.5±0.6^{abcd}	2.2±0.7^{abcd}	2.3±0.7^{abcd}	2.51±0.26^{cd}
Terpinolene	2.1±0.57^{abcd}	2.6±0.5^{abc}	2.7±1.9^{abc}	2.3±0.4^{abc}	2.3±0.5^{abcd}	2.3±0.5^{abcd}	2.5±0.5^{abcd}	2.39±0.19^{cd}
α – terpineol	1.0±0.22^{cd}	1.7±0.4^{bc}	2.3±2.2^{abc}	2.0±0.5^{abc}	2.4±0.6^{abcd}	2.9±0.6^{abcd}	3.1±0.7^{abcd}	2.20±0.21^{cd}
DEET	2.1±0.40^{abcd}	2.1±0.4^{abc}	2.1±2.0^{abc}	1.7±0.4^{bc}	2.5±0.5^{abcd}	2.1±0.4^{abcd}	2.3±0.5^{abcd}	2.13±0.17^{cd}
R + Pulegone	0.5±0.24^{d}	1.1±0.5^{c}	1.6±2.4^{bc}	2.3±0.7^{abc}	2.2±0.7^{abcd}	2.3±0.7^{abcd}	3.2±0.8^{abcd}	1.89±0.25^{cde}
2 Methoxy cinnamaldehyde	2.4±0.72^{abcd}	2.0±0.6^{abc}	1.5±1.8^{bc}	1.7±0.6^{bc}	1.3±0.4^{bcd}	1.6±0.5^{bcd}	2.1±0.6^{abcd}	1.81±0.21^{de}
Caryophyllene oxide	1.6±0.41^{bcd}	0.5±0.2^{c}	0.7±1.4^{c}	0.9±0.3^{c}	0.7±0.3^{d}	0.8±0.3^{cd}	1.1±0.4^{cd}	0.89±0.13^{ef}
Citral	1.0±0.29^{abc}	0.5±0.2^{c}	0.5±0.7^{c}	1.1±0.4^{c}	1.2±0.4^{cd}	1.1±0.4^{cd}	1.2±0.3^{bcd}	0.94±0.12^{ef}
β -Caryophyllene	0.8±0.28^{cd}	0.7±0.2^{c}	0.5±0.9^{c}	0.8±0.4^{c}	0.7±0.3^{d}	0.3±0.2^{d}	0.5±0.2^{d}	0.63±0.10^{f}
[B] Treated	2.53±0.14	2.43±0.14	2.51±0.16	2.67±0.16	2.71±0.16	2.67±0.16	2.96±0.17	2.64±0.06
Control	4.37±0.15	4.77±0.16	4.76±0.17	5.12±0.17	5.07±0.18	4.70±0.18	4.76±0.18	4.79±0.06
df	239	239	239	239	239	239	239	1679
t Value	7.1	8.81	7.83	8.32	7.87	6.82	5.76	19.74
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means within the columns followed by the same letter are not significantly different at $p \leq 0.05$. Their significance tested using multiple comparison procedures for ANOVA followed by Tukey's Studentized Range (HSD) Test.

At 30 min settlement preference, the least mean number of insects was observed on R+ Pulegone and β- caryophyllene. Through time the number of insects on RPU treated leaf disk increased gradually from 0.53±0.24 at 30 min to 3.2±0.86 at 6hrs. Whereas, the leaf disk treated with BC shows consistent effect on the mean number of second instar larvae from 0.8±0.24 at 30 min to 0.46±0.24 at 6 hour. In general, the mean number of thrips preferred to settle on the control leaf disk over the treated leaf disk. However, the mean number of second instar larvae at 30 min for cinnamyl acetate, geranyl acetate and 1,8 - cineole showed higher than the leaf disks treated with other compounds and showed consistency through time with 4.2±0.44, 4.1±0.46 and 4.0±0.65 larvae per leaf disk.

The positive control, DEET showed an average 2.13±0.40 insect per leaf disk at 30 min and showed consistency throughout the testing period end up with 2.33±0.51 insect per leaf disk after 6 hours. The other compounds with moderate effect on the second instar larvae are found between these strong and less repellent compounds.

Deterrent activity and feeding damage

The Feeding Deterrence Index (FDI) of *M. sjostedti* larvae varied significantly for different type of compounds ($F_{15, 719}$= 17.21, P<0.0001), concentration ($F_{2, 719}$=6.55, P<0.001) and their interaction ($F_{30, 719}$=2.09, P<0.001) Table 4. Similarly, the percent feeding damage observed on the leaf disk varied significantly for different type of compounds ($F_{16, 764}$= 34.85, P<0.0001), concentration ($F_{2, 764}$=35.97, P<0.0001) and their interaction ($F_{46, 764}$=5.14, P<0.0001). The feeding damage of the legume flower thrips *M. sjostedti* larvae was highly significant. Among the tested compounds, Cinnamyl acetate showed the highest FDI than DEET. In addition, other eight compounds showed greater than 50% deterrency considered as good deterrents. Among these, R+Pulegone and Geranyl acetate shows high deterrent effect against the second instar larvae with the mean Feeding Deterrence Index (FDI) above 50%. Less than 50% FDI was observed on Citral, Sabinene, 1,8 cineole and 2 Methoxy cinnamaldehyde (Table 5). Feeding deterrence was positively related as concentration increased from 0.1 to 1% (Fig 2). The compounds with less FDI percentage showed 50 to 73% feeding damage percentage with Sabinene the highest than the control (Table 5.7). However, leaf disks treated with 1,8 cineole showed lower feeding damage. Conversely to that of the feeding deterrence index, the feeding damage decreased as the concentration increases (Fig 5.3).

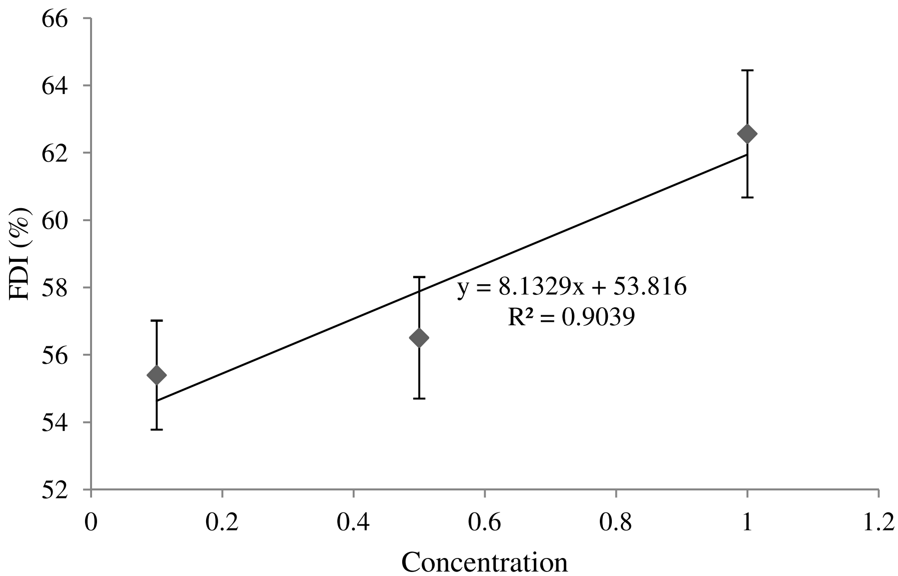

Figure 5.2 Mean feeding deterrence of a compounds used to *M. sjostedti* second instar larvae at increasing concentrations in a choice bioassay

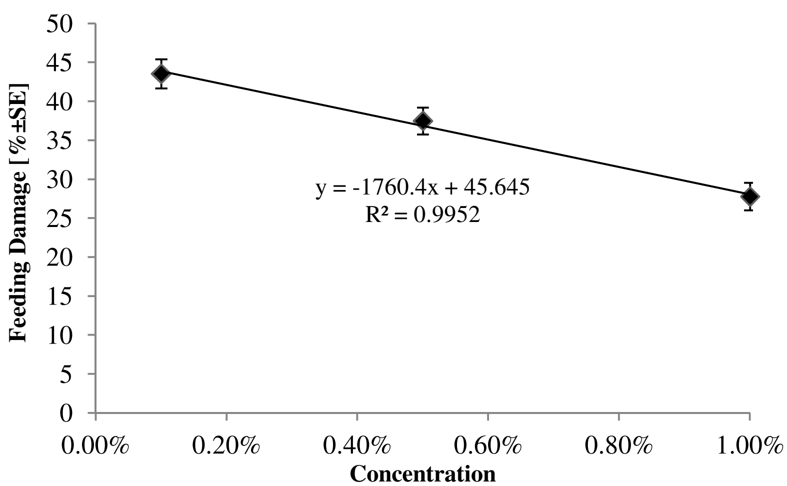

Figure 5.3 The feeding damage on the cowpea leaf disk *M. sjostedti* larvae, was negatively correlated with increasing compound concentrations.

Toxicity

The mean ranks of larval mortality are significantly different between all compounds (Tukey's Studentized Range (HSD) $P < 0.001$) (Table 5.7). The overall mean mortality for five compounds used was significantly different than the control. However, most of the other compounds including DEET didn't show significant difference with the control. In addition, even if there is significant difference ($p<0.001$) between the control and the three concentration used, there is no significant mortality rate differences within the concentrations (Figure 5.4).

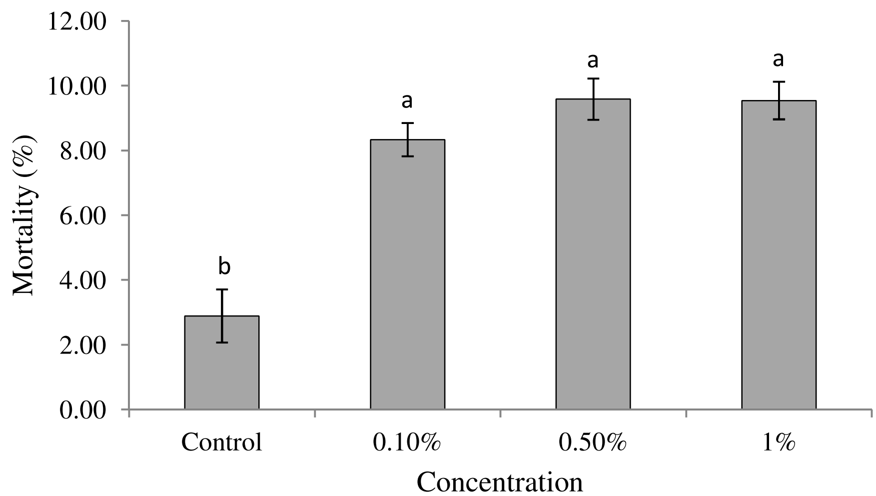

Figure 5.4 The percent mortality of *M. sjostedti* larvae across the concentrations used.

Over all compounds, greater than 10% Mortality rate was observed on five compounds. The compounds were 19.1 % Caryophyllene oxide with a range of 15.5 to 22.7%, 16.2 % 1, 8 -Cineole , 14.9% β – Caryophyllene, 13.78% Trans-Cinnamaldehyde and 13.6 % 2 Methoxy cinnamaldehyde (Table 5.8).

5.4 Discussion

In our study, we investigated the effect of 16 compounds on *M. sjostedti* second instar larvae. The second instar larvae for thrips they are found abundantly in the leguminous plants with specially attacking cowpea yield. These immature stages are responsible for a large part of the direct feeding damage and induced the shedding of all flower buds of a cowpea inflorescence (Tamò et al 1993). Similarly, feeding damage on plants from the second instar of other thrips species also reported (Wiesenborn and Morse 1986; Peneder and Koschier 2011; Egger and Koschier 2014). This study is among a few behavioural studies conducted on the thrips second instar larvae Peneder and Koschier 2011; Egger and Koschier 2014) and the first to report the effect of different compounds such as antifeedent, toxicity and behavioural response on *M. sjostedti* second instar larvae.

Table 5.3 Analysis of variance table for the settlement preference of all thrips

Source	DF	Mean square	F ratio	Pr > F
Compounds	15	161.62	35.55	< 0.0001***
Time	6	7.31	1.61	0.1414
Time*Compounds	90	3.23	0.71	0.981
Error	1568	4.55		

Table 5.4 Analysis of variance table for the percentage of thrips Feeding Deterrence Index

Source	DF	Mean Square	F Value	Pr > F
Compounds	15	9385.71	17.21	<0.0001***
Concentration	2	3571.07	6.55	<0.0001***
Comp*Conc	30	1141.42	2.09	<0.0001***
Error	672	545.24		

Asterisk (*) indicates significance

Table 5.5 The ANOVA result for compounds and interaction of compounds and concentration used for the Feeding Deterrence Index FDI (%)

Compounds	Polled Mean±SE[a]	Concentration [b]		
		0.1	**0.5**	**1**
Cinnamyl acetate	82.79[a]	77.4±6.36	81.8±6.80	89.2±4.94
DEET	80.65[a]	69.5±6.15	83.9±5.56	88.6±6.11
R+Pulegone	69.64[ab]	52.7±5.97	77.9±5.91	78.3±6.91
Geranyl acetate	67.89[ab]	54.2±9.84	66.3±3.46	83.2±6.58
Terpinolene	63.54[bc]	58.3±4.41	63.0±4.72	69.3±3.47
Citronellal	63.23[bc]	55.8±5.32	62.9±7.92	70.9±5.45
α- terpineol	63.11[bc]	59.1±4.25	60.45±8.38	69.8±5.56
β-Caryophyllene	63.04[bc]	56.3±4.29	66.6±5.16	66.2±4.59
Tymol	58.40[bc]	56.1±5.21	57.1±6.55	61.9±4.74
Caryophyllene oxide	57.76[bc]	55.8±5.32	62.9±7.92	58.6±3.27
Trans-Cinnamaldehyde	50.45[cd]	39.6±6.31	53.7±7.70	58.1±6.87
γ-Terpinene	50.18[cd]	40.6±5.23	45.8±6.59	64.1±3.96
Citral	47.89[cd]	40.8±5.15	46.13±8.87	56.8±6.06
Sabinene	46.95[cd]	38.3±7.62	48.9±5.82	53.6±4.55
1,8-Cineole	34.95[de]	23.6±5.83	31.4±6.46	49.9±7.35
2 Methoxy cinnamaldehyde	29.99[e]	23.6±3.71	27.6±6.14	38.8±9.77
F	17.21			
P	<0.0001			

FDI (%) ANOVA followed by Tukey's Studentized Range (HSD) test for [a] test compounds and [b] the interaction effects “Compound x Concentration”. Means within the columns followed by the same letter are not significantly different at $p \leq 0.05$.

In the current study, we used different tests to evaluate the behavior of *M. sjostedti* second instar larvae. The first method used to evaluate the settlement preference of second instar larvae using a treated and control leaf disks. In general, throughout the observation period, 50-55% of the larvae avoided contact with the treated leaf discs. Specifically, more than 90 % of the larvae avoided contact with the treated leaf discs of β –Caryophyllene, Citral and Caryophyllene oxide and 80% of the larvae avoided contact with the treated leaf discs of 2 Methoxy cinnamaldehyde, R+Pulegone and DEET. These are the most important compounds which influence significantly the settlement preference *M. sjostedti* larvae. Similarly, Abtew et al 2015 reported that repellency from the essential oils of *Piper nigrum* and *Cinnamomum* are found to be strong repellents, *Cymbopogon citrates* as good repellent and *Mentha pulegium* as moderate repellent in the olfactometer assay. Furthermore, they reported that β –Caryophyllene, Caryophyllene oxide, 2 Methoxy cinnamaldehyde and Citral found to be the major compounds found during the GC-MS analyses. Similarly, R+Pulegone the major constituent *Mentha pulegium* showed a detterency effect against *M. sjostedti* larvae. Our settlement preference result revealed that, *M. sjostedti* larvae able to respond to deterrent effects of the applied compounds. Similar results were reported for *F. occidentalis* larvae (Egger and Koschier 2014). However, interpretations of the results to explain the behavioural effect of insects for a leaf disk choice bioassay results to use repellency and deterrency interchangeably (Picard et al. 2012; Egger and Koschier 2014).

Table 5.6 GLM Analysis output of the Feeding Damage Assessment (%)

Source	DF	Mean Square	F Value	Pr > F
Compounds	16	14720.31	34.85	<0.0001***
Concentration	2	15194.47	35.97	<0.0001***
Comp*Conc	30	2170.66	5.14	<0.0001***
Error	716	302458.98		

Asterisk (*) indicates significance.

Similarly, to identify the range of effectiveness of the tested compounds, we determined a feeding deterrent index. In general all compounds showed an increasing deterrent effect as the concentration increases and this indicates that thrips larvae showed dose dependent deterrency. This result is in line with other previous studies that the concentration of plant compounds is critical for repellency as well as for deterrence to thrips (Koschier et al. 2000, 2002; Chermenskaya et al. 2001; Koschier and Sedy 2003; Sedy and Koschier 2003; Abtew et al. 2015). Higher Feeding Deterrence Index (FDI) was observed on Cinnamyl acetate, DEET, R+Pulegone and Geranyl acetate with 68%, 70%, 81% and 83% respectively. The least FDI with less than 50% was observed on Citral, 2 Methoxy cinnamaldehyde, 1,8-Cineole and Sabinene. Deterrent compounds applied to their host plants might cause the larvae to leave the treated plant earlier during their development, thus reducing the feeding damage caused by the larvae (Egger and Koschier 2014). Similarly, in our study we found that feeding deterrence and feeding damage are inversely related. As the feeding deterrence index increases the corresponding feeding damage decreases. This indicates that, secondary plant compounds can protect the plant to be damaged thrips. If the release of these active compounds from plant volatiles is effective under field conditions, it can act as a push component which can be used for push pull strategy. The push component will help to push, repel or deter the pest away from the crop by using odor stimuli. This repellent or push component might prevent crop losses due to destruction of buds, flowers and malformations of fruit.

Table 5.7 GLM analysis for the interaction of compounds and concentration for the Feeding Damage Assessment (%)

Compounds	Polled Mean±SE[a]	Concentration [b]		
		0.1	**0.5**	**1**
Sabinene	73.4±3.72 [a]	76.4±5.40	74.8±7.05	68.9±7.03
Control	64.1±3.11 [ab]	-	-	-
γ-Terpinene	61.5±3.32 [abc]	70.5±6.72	64.8±4.84	48.9±4.25
Citral	50.6±4.85 [bcd]	66.0±7.77	50.5±8.71	35.4±7.24
Trans-Cinnamaldehyde	46.0± 4.55[cde]	65.9±7.70	41.3±7.75	30.9±5.53
Citronellal	44.4±3.75 [def]	62.6±5.90	38.2±5.81	32.2±5.21
β-Caryophyllene	42.9 ±4.44[def]	57.9±7.25	51.1±7.77	19.9±3.83
α- terpineol	42.7±3.76 [def]	52.6±6.17	52.1±4.58	23.4±5.88
R+Pulegone	35.6±3.76 [defg]	62.4±6.47	27.9±2.77	16.5±1.65
Caryophyllene oxide	34.6± 4.56[defgh]	45.4±8.84	38.8±7.15	19. 6±6.47
Tymol	31.0±2.36 [defgh]	45.8±4.30	27.6±1.82	19.9±2.43
2 Methoxy cinnamaldehyde	28.9±3.39 [fgh]	35.5±7.06	28.4±4.53	22.9±5.74
Terpinolene	25.6±2.71 [ghi]	33.5±5.30	31.4±3.85	11.8±2.46
Cinnamyl acetate	24.6±1.88 [ghij]	38.4±2.52	20.1±1.00	15.2±2.22
Geranyl acetate	18.1±1.98 [hij]	30.1±2.84	19.9±2.12	4.16±0.36
1,8-Cineole	11.6±2.57 [ij]	22.6±5.91	7.96±3.72	4.20±0.14
DEET	8.6±2.71 [j]	11.8±0.86	9.98±0.91	3.96±0.58
F	34.85	14.54	10.04	18.78
P	<0.0001	<0.0001	<0.0001	<0.0001

[a] The FDA analysis for the compounds. Means within the columns followed by the same letter are not significantly different at $p \leq 0.05$.[b] Analysis for the interaction effects “Compound x Concentration” were compared to the control and their significance tested using multiple comparison procedures for GLM followed by Tukey's Studentized Range (HSD) Test.

Table 5.8 Analysis of Variance (ANOVA) for the Toxicity test

Source	DF	Mean Square	F Value	Pr > F
Compounds	16	1067.53	19.23	<0.0001***
Concentration	3	634.57	11.43	<0.0001***
Comp*Conc	29	77.04	1.39	0.0859
Error	716	39751.11		

Asterisk (*) indicates significance.

Our toxicity study shows that the direct contact toxicity of tested compound against second instar larvae of *M. sjostedti* is relatively low. The highest mortality obtained at a concentration of 1% which is 18% by the application of 1,8 cineole followed by 17.3% caryophyllene oxide. All over concentration, caryophyllene oxide, 1,8 cineole, β- caryophyllene, 2 Methoxy cinnamaldehyde and Trans-Cinnamaldehyde showed higher statistical difference as compared to with the control. There is no statistical difference between all the rests of the tested compounds and DEET with the control. Similar results are reported on 1,8-cineole a major constituent in the rosemary oil (51%) showed 77% repellency against females onion thrips *T. tabaci* (Koschier and Sedy 2003). Similarly, Oparaeke et al. (2006) reported that the mean number of *M. sjostedti* was significantly lower on plots sprayed with plant extracts mixed with *Eucalyptus* than on unsprayed plots for two seasons. Eucalyptus species with main constituents of 1-8 cineole showed insecticidal activities against stored-grain insect pests (Lee et al. 1997; Brito et al. 2006). However, because of the observed mortality did not exceed 20%, all the tested compounds are not effective to control the larvae. Thus the use of tested compounds as a contact toxic product may not give a good result for pest management. Similar low toxicity was reported previously by Peneder and Koschier 2011; in which they found a maximum of 24 % mortality by carvacrol and 7.7% for thymol both at 1%. As that of Peneder and Koschier 2011, in our study thymol showed a mortality of 4.22% this indicates that all the tested compounds do not have a sufficient toxic effect on thrips larvae to be recommended as an insecticidal application in addition, plant compound applications at concentrations higher than 1% do not seem to be practicable.

Table 5.9 Mean mortality of *M. sjostedti* larvae after a 24-hour period on leaf discs treated with compounds.

Compounds	Mortality Mean % ± SE	Confidence Interval 95%
Caryophyllene oxide	19.11 ± 1.79a	[15.5 - 22.7]
1, 8 - Cineole	16.22 ± 1.75a	[12.7 - 19.7]
β -Caryophyllene	14.89 ± 1.22a	[12.4 - 17.4]
Trans-Cinnamaldehyde	13.78 ± 1.24ab	[11.3 - 16.3]
2 Methoxy cinnamaldehyde	13.56 ± 1.01ab	[11.5 - 15.6]
Citral	8.44 ± 0.95bc	[6.53 - 10.4]
Terpinolene	8.22 ± 0.97bc	[6.27 - 10.2]
Geranyl acetate	7.33 ± 1.21c	[4.90 - 9.76]
γ-Terpinene	7.11 ± 0.82c	[5.46 - 8.76]
Cinnamyl acetate	6.89 ± 1.65c	[3.57 - 10.2]
α- terpineol	6.67 ± 1.01c	[4.64 - 8.69]
Sabinene	6.44 ± 0.91c	[4.62 - 8.27]
DEET	5.78 ± 0.93c	[3.91 - 7.64]
R+Pulegone	4.67 ± 0.82c	[3.02 - 6.31]
Tymol	4.22 ± 0.81c	[2.59 - 5.85]
Citronellal	3.11 ± 0.70c	[1.71 - 4.52]
Control	2.89 ± 0.82c	[1.24 - 4.54]

Means within the columns followed by the same letter are not significantly different at $p \leq 0.05$.

Finally, we summarized the result of the study in Table 5.10. We can conclude that deterrents can protect the treated plant part with less damage on the crop. This prospect of plant protection against thrips by means of behavioural manipulation using secondary plant compounds and such as volatiles considered as new approach as a push pull strategy with less effect on non-targeted organisms and the environment. However, further investigation on companion crops that can release plant volatiles under field condition with reasonable release rate of volatiles and the effect on the behavioural responses of adult *M. sjostedti* and other Thysanopterian family are necessary to develop a push, pull and kill strategy for the control of thrips in legume cropping systems in sub Saharan African countries by integrating with the existing control strategies.

Table 5.10 Summary of the behavioural effects and toxicity of the tested compounds against *M. sjostedti* larvae

Name of the extract	Name of compound	Settlement preference	Deterrency	Toxicity
Piper nigrum	β - Caryophyllene	+ + +	+ +	+
	Caryophyllene oxide	+ + +	+ +	+
Cinnamonum zeylanicum	Trans-Cinnamaldehyde	+ +	-	+
Cinnamonum cassia	2 Methoxy cinnamaldehyde	+ + +	-	+
	Cinnamyl acetate	-	+ + +	-
Eucalyptus globules	1, 8 – Cineole	+	-	+
Cymbopogon citrates	Citral 95%	+ + +	+ +	-
Origanum majorana	Sabinene natural	+	-	-
	γ-Terpinene	-	-	-
	α – terpineol	+ +	-	-
	Terpinolene	++	+ +	-
Cymbopogon nardus	Citronellal	+	+ +	-
Mentha Pulegium	R+ Pulegone	+ + +	+ +	-
Pelargonium graveolens	Geranyl acetate	-	+ +	-
Tymus vulgaris	Tymol	-	+ +	-
N,N-Diethyl-meta-toluamide	DEET	+ +	+ +	-

NB: + + + strong effect + + medium effect + less effect - absence effect

CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSION

6.1 Foreword

In order to link my study findings to the overarching aims presented in the first Chapter, the main results will be compiled first in a discussion centered on three parts. The first part of the study was designed to understand farmers' knowledge, perception and pest management practice with respect to grain legume producers in eastern Kenya. The second part of the study of was designed to explore effect of climate change on the geographic distribution of the legume flower thrips. The third set of experiments was designed to develop alternative controlling methods by studying the behavior of the insect to control the legume flower thrips. These contributions increased knowledge on pest management practices in eastern Africa, effect of climate change on the pest distribution in the continent and alternative controlling strategies using natural products by manipulating the behavioural of this pest. In the conclusion, I synthesize the working hypotheses and results within a broader context. Finally, I end with the perspectives emerging from this thesis.

6.2 Discussion

Understanding farmers knowledge and pest management practice

Evaluating farmers' knowledge and perception of pests and natural enemies is especially useful to set research agendas, for planning campaign strategies and developing alternative controlling and messages for communication. Based on the finding from the field survey, this section of the thesis provides significant contribution to understand the current farmer's knowledge, perception and pest management practice in the eastern province of Kenya. From the result, of the survey, majority (96 %) of the farmers use chemical control among these groups, 80% mentioned a wide range of broad spectrum chemicals. The chemicals are catagorised as: 61% synthetic pyrethroids, 14% organophosphate and 5% used a mixture of pyrethroids and organophosphate. These groups of chemicals are responsible for resistance development. In addition, apart from the insect pest farmer's experience, different challenges on growing grain legumes. 93% erratic rain fall (Weather condition) and 47% High cost of chemicals. These results redirect me to study the effect of climate change on the pest distribution and to address pesticide resistance and high cost of chemicals to propose alternative control method, behavioural study was conducted using plant extracts and derived compounds.

Studying effect of climate change on pest distribution and its host crop

Because of the fact that farmer's experience harsh weather condition mainly erratic rain fall as a serious challenge on growing grain legumes, I have studied effect of climate change on the geographic distribution *Megalurothrips sjostedti*, a key pest of cowpea. I investigated the potential areas of invasion in Africa for this species. MAXENT (Maximum entropy) modelling tool is used to predict effect of climate change for the year 2055. AFRICLIM, a high-resolution present and future climate variable was used for projecting current and future potential distribution of the pest and its host in Africa. As it was revealed from the field survey, the ecological niche modelling suggested that Rainfall and temperature were the influential environmental variables identified using a jackknife analysis. I exported the final model outputs in Quantum GIS and drawn a map for present and future distribution of the pest, and the result of the study proposes the potential suitable areas and estimation of the dynamics of invasion risk and an estimation of climatically favorable zones for the establishment of the host crop and legume flower thrips distribution in Africa through time. These results indicate the potential for significant *M. sjostedti* related economic and social impacts in many sub-Saharan African countries, particularly in wider area in Western Africa, because the suitable habitat regions overlap with agricultural areas for *M. sjostedti* host plants such as cowpea.

Behavioural response of M. sjostedti to different plant extracts and derived compounds

Currently, the control of *M. sjostedti* in SSA countries relies heavily on synthetic insecticide application. The indiscriminate use of these chemicals has given rise to problems such as resistance of the legume pests to insecticide and health risks to human and the environment. Farmers in eastern Kenya practice application of high doses (increased concentration), chemical alternation, frequent application and mixture (cocktail) of chemicals to ensure pesticide effectiveness. To propose alternatives for chemical spraying, which are safe, effective, biodegradable and highly selective, I have studied repellent and deterrent effects of plant-based extracts. These extracts have been suggested as an alternative to synthetic insecticides (Isman 2008) with less negative effect on the environment. A study of the behavior and survivorship of legume flower thrips *M. sjostedti* was done with an intention of developing alternative thrips controlling strategy; the result from the study of the repellent effect of 24 plant extracts against legume flower thrips *M. sjostedti* using a visual cue-still air olfactometer. The visual cue olfactometer is a new method developed and used for the first time in my study. The result revealed that, among the tested 24 plant extracts, 7 showed a good repellency against *M. sjostedti.* Based on this identification of the constituent compounds of the best repellent extracts using Gas Chromatography Mass Spectro photometer (GC-MS) was done. Mono- and sesqui terpene hydrocarbon compounds from seven highly repellent extracts were identified. This leads to investigation of the behavioural effects of the identified compounds on the second instars larvae and adult female *M. sjostedti.* The repellent, toxic and deterrent effect of 16 identified compounds against the adult female legume flower thrips *M. sjostedti* using different bioassay methodologies. The result revealed that the use of repellent extracts could be one of the useful in developing integrated pest management strategies for thrips on legume crops. In addition, the study of specific mode of actions of the identified compounds indicates that the tested compounds are not efficient as alternatives for insecticide application. Thus plant compounds could be used as a safe method of control as repellent and deterrent in combination with the current pest management strategies for the control of *M. sjostedti* and family thripidae for grain legumes in Sub-Saharan Africa.

6.3 Conclusion

The development of an Integrated Pest Management (IPM) program is a complicated process that needs to consider all of the relevant information regarding the pest's biology, ecology behavior and the crop response to its presence. In this case, the relationship between cowpea and legume flower thrips was studied. This study indicates that developing an integrated pest management (IPM) strategy for a sustainable crop production needs an inter-disciplinary approach, which compasses biological, socioeconomic, agronomic and ecological concepts.

In light of the results presented in each chapter, various implications are present on how different approaches are used on development of alternative controlling technique. Because behavior and survivorship using chemical ecology techniques shown to enhance performance on pest management behavioural science using different techniques should take advantage of its effectiveness in managing pest infestation. Also, as increasing emphasis is placed on using ecological niche modeling to predict niche suitability in a climatic changing world. Although ecological niche modeling has limitation on considering pests biology, it is necessary to combine with newly developed software's like Insect Life Cycle Modeling (ILCYM) tools to see how effect of temperature and other climatic factors impact its distribution.

Finally, it is necessary to organize all the information in this study that other scholars can apply their knowledge to improve the results and findings acquired during these three years study to develop better techniques which increase the efficiency of pest management in cowpea and other grain legumes.

6.4 Perspectives

This thesis has presented important new knowledge on the the behaviour, ecology and control of legume flower thrips, using sociological, ecological and behavioural ways of study on *Megalurothrips sjostedti* (Trybom) on cowpea *Vigna unguiculata* (L.) todevelop an alternative pest management strategy which can be integrated in the existing pest management methods. Prospect of plant protection using chemical ecology by means of behavioural manipulation using secondary plant compounds and such as volatiles considered as an alternative approach with less effect on non-targeted organisms and the environment. However, further investigation on companion crops that can release constantly repellent plant volatiles under field condition and the effect on the behavioural responses of adult *M. sjostedti* and their natural enemies are necessary. It is because of time limitation that the study ended up at this point. Integrating this with an insecticide impregnated blue colour net (physical barrier) with a repellent volatile which can be companion plant or a product could allow us to develop an IPM strategy for plant health to develop a push, pull and kill strategy for the control of thrips in legume cropping systems in Sub-Saharan African countries by integrating with the existing control strategies (Fig 7.1).

The findings of the thesis also have implications for future research. Specifically future research could explore the use of plant volatile released from repellent plant as intercropping, border cropping or row cropping with legume crops as combined with visual attractive material impregnated with insecticide could reduce the effect of agrochemicals on the human and environment while reducing pesticide resistance.

To continue the current research, proposal writing needs to be done to secure funding from donors to assign a doctoral or post doctoral researcher for further study.

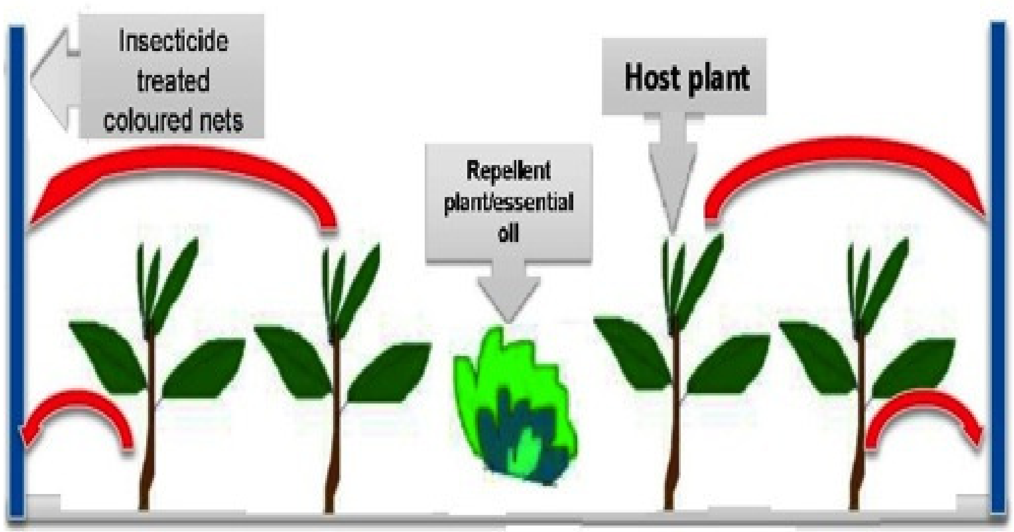

Fig 7.1. Push pull strategy planned to be study.

* Because of time limitation, a study on the toxicity of the chemical impregnated visual attractive material was not conducted.

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APPENDIX

Appendix 1 Questionnaire and pictorial guide used for the field survey

Dear respondent,

Thank you for humbly accepting to answer this Questionnaire. The researcher from icipe is conducting a study to investigate on how thrips pest affect cowpea, dolichos and common beans production and the effect on farmer's income. The information provided will help to develop a better pest controlling strategy to protect cowpea, dolichos and common beans from these pest attacks in order to improve quantity of harvest. Any information provided will be treated as confidential. I Hope you will co-operate.

Farmer Identification:

Farmer's (full) Name: ……………………………………………Age……………… Sex: …………

Interviewer's (full) Name: ………………………………………Time started: ………………..……

Interview date: ……………………… District: ……………………Division: …………………..…

Location: …………………… Sub location………………………… Questionnaire No: ………….

1. Demographic information:

1.1 Status of the respondent: ____________

1. Farmer- husband 2. farmer-wife 3. farmer-son
4. farmer- daughter, 5. other (Specify).____________ 6.☐ farm worker

1.2 Did you take any formal education? 1. ☐ Yes 2. ☐ No

1.3 If yes, what is the highest education level you have completed?

1. ≤5 years (primary schooling) 2. 6-10 years (junior schooling)
3. 11-12 years (high schooling) 4. ≥ 12 years (above high schooling)

1.4 What is your family size? Permanent members of the household? ____________________

Age	Total
0 Years to 14 Years	A
15 Years to 64 Years	B
Greater than 64 years	C

(It will help to calculate dependency ratio) = (A+C)/ (A+B+C)

1.5 How many household members can read and write? __________

1.6 Respondent's language skills: codes 1. Yes 2. No

Language	Listening	Speaking	Reading	Writing
English				
Kswahili				

Other regional language				

1.7 Has the respondent gone for agricultural training? 1. Yes 2. No

1.8 If Yes, when? ___________________________ (year last attended)

In what aspect was he/she trained? *(Tick all that apply)*

1.☐ Crop management 2.☐ Pest Management/IPM 3.☐ Food safety/Quality

4.☐ Marketing 5.☐ others (Specify) ____________________.

1.9 Who was your trainer on pest management?

1. ☐ NGO 2. ☐ Ag. Ministry official's 3. ☐ Farmers 4. ☐ Other (please specify)

1.10 Would you like to have regular training updates on pest management (e.g. every 2-3 years)?

1. ☐ Yes 2. ☐ No

2. Income

2.1 On farm annual income (2012) (Ksh)? _________________________

2.1 Off farm Crops: (2012) (Ksh)? _____________________________

Livestock: (2012) (Ksh)? __________________________

3. Production of grain legume

3.1 Which type of grain legume do you grow? _________________ ____________________

_______________________ _______________________ ____________________________

3.2 For how long you have been growing cowpea, dolichos and common beans? ______ yrs

3.3 Cutivar, cropping system and soil type

Grain legume	**Cultivar**	**Cropping system**	**Spacing (seed rate)**		**Soil type**
			Sole crop	**Inter cropped**	
Cowpea					
Dolichos					
Common beans					

Codes for: Cropping system: 1.☐Sol crop 2.☐ Intercropping 3.☐ both
Soil type: 1.☐sandy 2.☐ loamy 3.☐ clay 4.☐other

3.4 What are the main sources of water/irrigation for your crops?

Grain legumes	**Rain fed / Irrigated**	**If Rain fed- which?**	**Area (Ha/ Acre)**	**If Irrigated – Which?**	**Area (Ha/ Acre)**

Cowpea					
Dolichos					
Common beans					

Codes for water source: 1.□ Rain fed A- Long rain B- Short rain C- both rainy season
2.□ Irrigated A- Manual B- Furrow C - Sprinkler D- Drip

3.5 What is your frequency of irrigation?

1. □ weekly 2. □ daily 3. □ Others (specify)__________

3.6 What are your sources of agricultural information/help including plant protection?

1. □ Agricultural officers 2. □ NGO's 3.□ Input suppliers 4. □ others (specify)

4. Pests and diseases

4.1 What are the key pest infesting your legumes? Use codes to indicate level the importance.

Grain legume	Thrips	Pod borer	Pod bugs	Pollen beetles	Aphids	White flies	Bean fly
Cowpea							
Dolichos							
Common beans							

Codes: 0. No attack 1. □ highly important 2.□ important
3.□ occasionally/ Minor important 4.□ not important

5. Awareness about Thrips

(N.B: This is relevant only if thrips is mentioned as a key pest)

5.1 Have you heard of Thrips before? 1.□ Yes 2.□ No (if No use the **pictorial key** to show the symptoms of the pest)

5.2 If **YES,** which crops/trees were they found on or normally associated with? ________

____

5.3 Do you consider Thrips as beneficial or harmful (pest)?

1. □ Beneficial 2.□ harmful 3.□ none of the above

5.4 If harmful, what kind of damage does it cause? **(use pictorial key)** _______________

____

5.5 If harmful, how do you manage them? ______________________________________

____

6. Thrips damage and loss

6.1 Indicate the time period when thrips pest problem was least and most severe for your legume cropping system?

Legume crops	Months when Least severe	Quantity planted (Kg/Ha)	Quantity harvested (Kg/Ha)	Months when Most severe	Quantity planted	Quantity harvested
Cowpea						
Dolichos						
Common bean						

6.2 Do you use agro chemicals for pest management? 1.□ yes 2.□ No

6.3 From your experience, are pesticides effective in controlling thrips problem?

1.□ yes 2.□ No

6.4 If Yes, Which types of agro-chemical (s) are effective in controlling Thrips? (Please specify)

____

6.5 When do you spray grain legume crop against thrips?

1.□ when I see the symptoms on the leaf 2.□ when I see the flies

3.□ other (specify) ______________________________

6.6 When one pesticide fails to effectively control Thrips, what do you do?

1.□ mix pesticides 2.□ increase concentration 3.□ spray more often

4.□ others (specify_____________)

6.5 Does the method work in controlling the thrips pest? 1.□ Yes 2.□ No

6.6 If No, please indicate what possible controlling method better controls thrips pest?

____

6.7 What is the average labour cost for chemical application per production season? (Ksh?)

____

6.8. Which components of Integrated Pest Management (IPM) system do you use to control pest attacks:

1. □ Chemical control 4. □ Light trap 7. □ Enemy Plants
2. □ Biological control 5. □ Crop rotation 8. □ Do not know
3. □ Smoke 6. □ Manual clearing 9. □ Other (specify)

6.9. Do you know any repellent plants or plants with toxic effect against pest of grain legumes?

1. □ Yes 2. □ No 3. □ I don't know

6.11.1 If **Yes**, please specify ______________________________

6.10 Apart from insects and diseases what are the main challenges you experience in growing grain legumes?

a.) ....

b.) ....

c.) ....

d.) ....

7. Some agronomic practices related to pest management in grain legumes

7.1 How long has the plot been under grain legume production?

Cowpea: ...., Dolichos: ...., Common bean: ....

7.2 Has the plot been under crop rotation? 1=Yes, 0=otherwise

If yes, what is the crop rotation schemes adopted?

1)...

2)...

3)...

7.3 Do you apply fertilizer for your cowpea crop? 1. □ Yes 2. □ No

7.4 What types of fertilizer do you apply for your cowpea crop?

Types ________________ Quantity ____________________ Cost ____________________

Types ________________ Quantity ____________________ Cost ____________________

Types ________________ Quantity ____________________ Cost ____________________

Types ________________ Quantity ____________________ Cost ____________________

7.5 At what time of the planting time usually do you apply fertilizer for your cowpea crop?

1. □ Basal (at planting or before planting) 2. □ Top dressing: (at what interval): ....

8. Harvesting

8.1. Grain legume crops sales in the 2012

	Crops	**Season[1]**	**Annual Income per crop in 2012**
Legumes	Cowpea		
	common beans		
	Dolichos		

[1]Codes for season (1 - short rains, 2 - Long rains)

Time end: …………………..…… **Thank you for your time!**

Pictorial key of thrips infestation

(A guide to help farmer's for easy symptoms of the pest's damage)

Thrips feeding on cowpea flower

Plant severely infested by thrips

Leaves severely infested by thrips

Cowpea infested with thrips rendering the stipules and buds brown.

Legume flower thrips *M sjostedti*

Pictorial key of Aphid infestation

(A guide to help farmer's for easy symptoms of the pest's damage)

Pictorial key of legume pod borer, *Maruca vitrata* infestation

(Used during farmer's survey to easily reminding them about the pest's damage)

Adult female of *Maruca vitrata*. Photo by G. Goergen, IITA.

Bean consumed by Lepidopterous borer

A Maruca vitrata larva. Photo by IITA.

Appendix 2 Major compounds of least repellent plant extracts from different sources.

No	Common name	Botanical name	Major compounds	References
1	Thyme (common)	*T. vulgaris*	Thymol (35%), p-cymene (23%), carvacrol (15%)	[1] IBMM, France
2	Citronella	*Cymbopogon nardus*	Citronellal (35.5%), Geraniol (27.9%), Citronellol (10.7%)	[2] Koba et al.2007
3	Pennyroyal	*Mentha pulegium*	(+)-pulegone (87%)	IBMM, France
4	Satureja biflora	*Satureja biflora*	Linalool (50.60 %), germacrene D (10.63 %)	[3] Matasyoh et al.2007
5	Geranium	*Pelargonium graveolens*	citronellol (41%) – geraniol (18%)	IBMM, France
6	Neem	*Melia azadirachta*	azadirachtin (,1%)	IBMM, France
7	Coriander	*Coriandrum sativum*	(+)-linalool (72%)	IBMM, France
8	Dill	*Anethum graveolens*	(+)-carvone (60%) – limonene (30%)	IBMM, France
9	African blue basil	*Ocimum kilimandscharicum*	Camphor (56.07%), DL-limonene (13.56%)	[5] Narwal et al.2011
10	Ginger	*Zingiber officinale*	Zingiberene (30%)	IBMM, France
11	Litsea	*Litsea cubeba*	Geranial (45%), neral (32%)	IBMM, France
12	Thyme Borneol	*T hymus satureioides*	Borneol (31.2%), camphene (27.4%), α-pinene (17.5%) and linalool (6.3%)	[6] Tantaoui-Elaraki et al 1993
13	Conza newii	*Conza newii*	(S)-(-)-perillyl alcohol, (S)-(-)-perillaldehyde, geraniol, (R)	[4] Mayeku et al. 2013
14	Lemon	*Citrus limon*	Limonene (95%)	IBMM, France
15	Satureja abyssinica	*Satureja abyssinica*	pulegone (43.5%), isomenthone (40.7%)	[7] Tolosa et al. 2007
16	Solidago	*Solidago canadensis*	Germacrene D (32%) - Limonene (13%)	IBMM, France
17	Rose mary	*Rosmalinus officinalis*	1,8-cineole (,1%), camphene (,1%), camphor (,1%)	IBMM, France

References

[1]IBMM - Institut des Biomole´cules Max Mousseron, Montpellier, France.

[2]Koba K, Sanda K, Guyon C, Raynaud C, Chaumont JP, Nicod L (2009) In vitro cytotoxic activity of Cymbopogon citratus L. and Cymbopogon nardus L. essential oils from Togo Bangladesh J Pharmacol 4: 29-34 doi: 10.3329/bjp.v4i1.1040

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[4]Mayeku WP, Omollo NI, Odalo OJ, Hassanali A. (2013) Chemical composition and mosquito repellency of essential oil of *Conyza newii* propagated in different geographical locations of Kenya. Med Vet Entomol, doi: 10.1111/mve.12039

[5]Narwal S, Rana AC, Tiwari V, Gangwani S, Sharma R (2011) Review on Chemical Constituents & Pharmacological Action of *Ocimum kilimandscharicum* . Indo Global J Pharm Sci. 4: 287-293

[6]Tantaoui-Elaraki A, Lattaoui N, Errift A, Benjilali B (1993) Composition and antimicrobial activity of essential oils of Thymus broussonettii, T. zygis, T. satureioides. J. Essent. Oil Res. 5: 45–53

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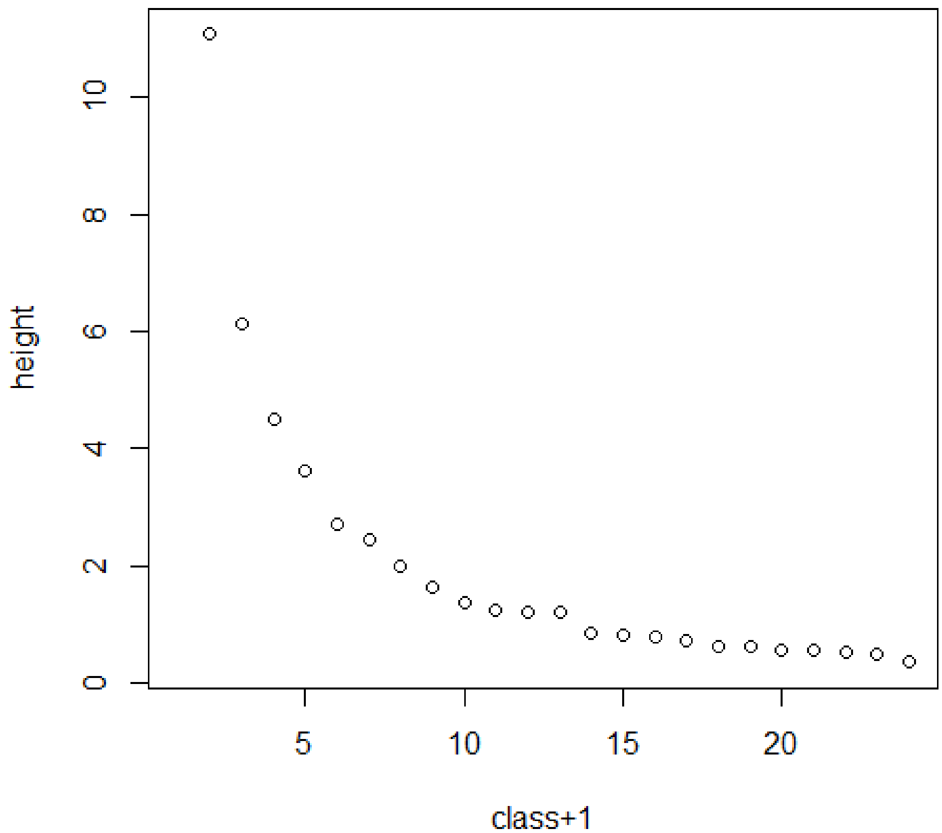

Appendix 3 Dendrogram class determination

Branch height used to get the optimal number of classes in the tree which was determined by the decrease of the interclass variance.

LIST OF PUBLICATIONS

Published Article:

Andnet Abtew, Sevgan Subramanian, Xavier Cheseto , Serge Kreiter, Giovanna Tropea Garzia and Thibaud Martin **Repellency of Plant Extracts against the Legume Flower Thrips *Megalurothrips sjostedti* (Thysanoptera:Thripidae** *Insects* **2015**, *6*, 608-625; doi:10.3390/insects6030608

Planned Articles

Andnet B. Abtew , Hippolyte Affognon ,Thibaud Martin, Saliou Niassy, Serge Kreiter, Giovanna Tropea Garzia and Sevgan Subramanian **Farmers' knowledge and perception of grain legume pests and their management in the Eastern province of Kenya**

Andnet B. Abtew, Thibaud Martin, Valentina Migani, Gladys Mosomtai, Tamo Manuele, Giovanna Tropea Garzia, Serge Kreiter and Sevgan Subramanian **Ecological niche modeling to predict effect of climate change on geographic distribution of *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) and its host Cowpea**

Andnet B. Abtew, Sevgan Subramanian, Johnson O. Nyasani, Serge Kreiter, Giovanna Tropea Garzia and Thibaud Martin **Toxic and behavioral effects of 16 compounds on *Megalurothrips sjostedti* Trybom larvae (Thysanoptera: Thripidae)**

Conferences

Andnet Abtew, Sevgan Subramanian, Valentina Migani, Thibaud Martin, Serge Kreiter and Giovanna Tropea Garzia Ecological niche modeling to predict effect of climate change on the geographic distribution of the legume flower thrips (*Megalurothrips sjostedti*) (Thysanoptera: Thripidae) in Africa; (Abstract). Presented at **OMICS International Symposia on Entomology** Entomol Ornithol Herpetol 2013, 04 September 2013, Holiday Inn Orlando International Airport, Orlando, FL, USA http://dx.doi.org/10.4172/2161-0983.S1.004

Andnet Bayleyegn, Hippolyte Affognon, Sevgan Subramanian, Serge Kreiter, Giovanna Tropea Garzia ,Thibaud Martin**, Farmers' knowledge and practice to manage pest problems on legume cropping system in the eastern province of Kenya**; (Abstract). Presented at 20th biennal meeting of African association of insect Science (AAIS), 27–31 October 2013, Younde, Cameroon

Andnet B Abtew, Sevgan Subramanian, Xavier Cheseto, Serge Kreiter, Giovanna Tropea Garzia, Thibaud Martin, **Repellency of plant extracts against the legume flower thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae)** ; (Abstract/ P003). Presented at Xth European Congress of Entomology (ECE 2014), 3 –8 August 2014, University of York, York, UK

Andnet B Abtew, Thibaud Martin, Valentina Migania, Tamo Manuele, Gladys Mosomatai, Serge Kreiter, Giovanna Tropea Garzia,Sevgan Subramanian, **Ecological niche modelling to predict geographical distribution of the legume flower thrips*Megalurothrips sjostedti* Trybom in Africa (Thysanoptera: Thripidae)** ; (Abstract/ P003). Presented at Xth European Congress of Entomology (ECE 2014), 3 –8 August 2014, University of York, York, UK

Andnet Abtew, Enrique Uribe Leitz, Irene Tamubula, Millicent Oyugi **Factors Influencing Coffee Marketing Strategies among Coffee Farmers in Ggolo Parish, Uganda** (Abstract pp.438). Presented at Tropentag, (Bridging the gap between increasing knowledge and decreasing resources), 17–19 September 2014, Czech University of Life sciences, Prague, Czech Republic