COMBINATION OF 'PUSH-PULL' AND NETTING TECHNOLOGY FOR MANAGEMENT OF COWPEA PESTS (*MEGALUROTHRIPS SJOSTEDTI* TRYBOM AND *APHIS CRACCIVORA* KOCH) IN KENYA

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Combination of 'push-pull' and netting technology for management of cowpea pests (*Megalurothrips sjostedti* Trybom and *Aphis craccivora* Koch) in Kenya

Seydou Diabate

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2019

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature:…………………………………….…Date ……………….…………….

Seydou Diabaté

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

Signature:.…….………………………………….. Date …………………………….

Dr. Lucy Kananu Murungi, PhD

JKUAT, Kenya

Signature: …………………….…….…….……Date…….………………………..

Prof. John Wesonga, PhD

JKUAT, Kenya

Signature:…………………………………..……Date……………………………..

Dr. Thibaud Martin, PhD

Cirad, Cote d'Ivoire

Signature:……….…………………………………Date….………………..……….

Dr. Emilie Délétré, PhD

Cirad, Kenya

DEDICATION

I dedicate this PhD thesis to my family (Diabaté), my wife (Korotoum) and son (Djawé Mourad)

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TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENTS iv
TABLE OF CONTENTS v
LIST OF TABLES xi
LIST OF FIGURES xii
LIST OF APPENDICES...xvi
LIST OF ABBREVIATIONS AND ACRONYMS xviii
ABSTRACT xx
CHAPTER ONE...1
INTRODUCTION 1
  1.1 Background of the study 1
  1.2 Statement of the problem 3
  1.3 Justification of the study 4
  1.4 Null Hypotheses 6
  1.5 Research Objectives 6
    1.5.1 General objective 6
    1.5.2 Specific objectives 6
CHAPTER TWO 7
LITERATURE REVIEW 7
  2.1 Cowpea 7
    2.1.1 Cowpea classification...7
    2.1.2 Cowpea plant 7
    2.1.3 Cowpea economic importance...7

2.2 Key cowpea pests 8
2.2.1 Bean Flower Thrips, *Megalurothrips sjostedti* 8
2.2.2 Black Legume Aphid, *Aphis craccivora* 10
2.3 Management of cowpea pests 11
2.3.1 Chemical control of *M. sjsotedti* and *A. craccivora* 11
2.3.2 Cultural control of *M. sjsotedti* and *A. craccivora* 11
2.3.3 Biological control of *M. sjsotedti* and *A. craccivora* 12
2.3.4 Host Plant Resistance (HPR) 13
2.3.5 Netting technology 14
2.3.6 Companion plant cropping 15
2.3.6.1 Lemongrass (*Cymbopogon citratus)* 16
2.3.6.2 Mexican marigold *(Tagetes minuta)* 17
2.3.7 Use of plant volatiles in pest management strategy 17
2.3.7.1 Monitoring and mass trapping 18
2.3.7.2 Synergistic effect of plant volatiles in pheromone baited- traps 18
2.3.7.3 Plant volatiles as attractants of natural enemies of pests 19
2.3.7.4 Push-pull strategy 19
CHAPTER THREE 21
BEHAVIOURAL RESPONSE OF ALATE *APHIS CRACCIVORA* KOCH (HOMOPTERA: APHIDIDAE) TO VOLATILES FROM DIFFERENT COWPEA CULTIVARS 21
3.1 Abstract 21
3.2 Introduction 22
3.3 Materials and methods 24
3.3.2 Rearing of *A. craccivora* 24
3.3.3 Preference assay 24

3.3.4 Olfactory assay with undamaged cowpea plants 25
3.3.8 Synthetic standards 27
3.4 Data analysis 28
3.5 Results.. 28
3.5.1 Behavioural assays... 28
3.5.2 Analysis of cowpea volatiles 31
3.6 Discussion 37
3.6.1 Behavioural response of *A. craccivora* to cowpea cultivars 37
3.6.2 Volatile compounds in cowpea cultivars.. 38
3.6.3 Effect of blend of compounds on behavioural response of *A. craccivora* 38
3.6.4 Choice of *A. craccivora* in pairwise comparison and preference assays 39
3.7 Conclusion 40
CHAPTER FOUR.. 41
BEHAVIOURAL RESPONSE OF BEAN FLOWER THRIPS, *MEGALUROTHRIPS SJOSTEDTI* (TRYBOM) (THYSANOPTERA: THRIPIDAE) TO VEGETATIVE AND FLORAL VOLATILES FROM DIFFERENT COWPEA CULTIVARS.. 41
4.1 Abstract.. 41
4.2 Introduction... 42
4.3 Materials and methods... 43
4.3.3 Preference assay.. 44
4.3.7 Olfactory assay with synthetic standards 46
4.3.8 Synthetic standards 47
4.4 Statistical analysis 47
4.5 Results.. 47

4.5.1 Behavioural response of *M. sjostedti* males and females to cowpea volatiles at the vegetative and flowering stages 47

4.5.2 Analysis of cowpea volatiles emitted by the cowpea cultivars 55

4.5.3 Behavioural response of *M. sjostedti* female to synthetic compounds that were either abundant or unique to non-attractive cowpea cultivars 58

4.6 Discussion 61

4.6.1 Behavioural response of *M. sjostedti* to cowpea cultivars 61

4.6.2 Influence of cowpea compounds on behavioural response of *M. sjostedti* 61

4.6.3 Choice of *M. sjostedti* in pairwise comparison and preference assays 62

4.7 Conclusion 63

CHAPTER FIVE 64

REPELLENT EFFECT OF *CYMBOPOGON CITRATUS* AND *TAGETES MINUTA* ON THE BEHAVIOUR OF BEAN FLOWER THRIPS, *MEGALUROTHRIPS SJOSTEDTI* 64

5.1 Abstract 64

5.2 Introduction 65

5.3 Methods and material 66

5.3.1 Plants tested 66

5.3.2 Olfactory response of *M. sjostedti* to plant volatiles 67

5.3.3 Collection of volatiles from *C. citratus* and *T. minuta* 68

5.3.4 Olfactory assay with synthetic standards 68

5.3.5 Synthetic standards 69

5.4 Statistical analysis 69

5.5 Results 70

5.5.1 Behavioural response of *M. sjostedti* to *C. citratus* and *T. minuta* 70

5.5.2 Analysis of volatiles 73
5.5.3 Behavioural response of *M. sjostedti* to synthetic standards 80
5.6 Discussion 83
5.6.1 Effect of cut leaves of *C. citratus* and major compounds on *M. sjostedti* 83
5.6.2 Effect of Mexican marigold and their major compounds on *M. sjostedti* 84
5.7 Conclusion 85
CHAPTER SIX 86
EFFICACY OF NETHOUSE TO CONTROL COWPEA PESTS AND TO IMPROVE COWPEA PRODUCTION 86
6.1 Abstract 86
6.2 Introduction 87
6.3 Materials and methods 88
6.3.1 Site and seasons 88
6.3.2 Planting material and land preparation 90
6.3.3 Treatments and experimental design 90
6.3.4 Repellent plants arrangement and net house properties 93
6.3.5 Data collection by counting of insects, flowers, number harvested and weight of pods in the field 93
6.4 Data analysis 94
6.5 Results 94
6.5.1 Season and plant development 94
6.5.2 Effect of seasons on pest populations dynamics 94
6.5.3 Effect of treatments on pest populations in the seasons 96
6.5.4 Effect of treatments on yield in the seasons 104
6.6 Discussion 106
6.6.1 Effect of net house on small insect pests 106

6.6.2 Effect of push-pull strategy in controlling cowpea pests 107

6.6.3 Effect of season on infestation of insect pests 108

6.6.4 Effect of net house on the yield of cowpea 109

6.7 Conclusion 109

CHAPTER SEVEN 111

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS 111

7.1 General discussion 111

7.1.1 Host plant-insect interactions 111

7.1.3 Non-host plant-insect interactions: Repellent effect of non-host plants 113

7.1.4 Pest management strategy 114

7.2 Conclusions 116

7.3 Recommendations 117

7.3.1 Recommendations to the farmers 117

7.3.2 Recommendations for future research 117

7.4 Limitations 117

REFERENCES 118

APPENDICES 143

LIST OF TABLES

Table 3. 1: Volatile compounds emitted by the vegetative parts of four cowpea cultivars: Ex-Luanda, Machakos, Ken Kunde 1, Katumani 80 34

Table 4.1: Estimate amounts of volatile compounds (percentage) emitted by the flowers of the four cowpea cultivars 56

Table 5. 1: Abundance (%) of volatile compounds emitted by one gram (1g) of cut leaves of *C. citratus* at 0 hours and 24 hours 78

Table 5. 2: Abundance (%) of volatile compounds emitted by the vegetative and flowering *Tagetes minuta* 79

Table 6. 1: Mean (± SE) number of insects observed per plant among the treatments in the season 1 (dry)^1 and season 2 (rainy)^2 at KALRO- Mwea (Kenya) 102

Table 6. 2: Mean (± SE) number of insects: *A. craccivora*, *M. sjostedti* and *T. vaporariorum* caught on the sticky traps across the two seasons 103

Table 6. 3: Mean (± SE) of flowers per plant and of the total number and weight of harvested pods and marketable cowpea pods and of number of grains per plot among the treatments 105

Table 6. 4: Estimated yield (t ha^{-1}) of total and marketable pods among the treatments across the two seasons. 106

LIST OF FIGURES

Figure 3. 1: Number (Mean ±SE) of alate *Aphis craccivora* for different cowpea cultivars in the vegetative stage over a period of 24 hours 29

Figure 3. 2: Olfactometer responses of alate *Aphis craccivora* to the odour of undamaged cowpea cultivars in the vegetative stage versus clean air (control) in Y-tube olfactometer. 30

Figure 3. 3: Olfactometer responses of alate *Aphis craccivora* to the odour of undamaged cowpea cultivars in the vegetative stage in pairwise comparison in a Y-tube olfactometer. 31

Figure 3. 4: Representative chromatograms of compounds identified in vegetative stage volatiles of four cowpea cultivars, namely Ex-Luanda (EL), Katumani 80 (K80), Ken Kunde 1 (KK1) and Machakos 66 (M66). 33

Figure 3. 5: Principal component analysis (PCA) of volatile organic compounds in the undamaged vegetative part of four cowpea cultivars 35

Figure 3. 6: Olfactometer responses of alate *Aphis craccivora* to different tests........... 36

Figure 3. 7: Olfactometer responses of alate *Aphis craccivora* to different tests........... 37

Figure 4. 1: Number (Mean ±SE) of *Megalurothrips sjostedti* for different cowpea cultivars a) in the vegetative stage and b) flowering stage over a period of 24 hours 48

Figure 4. 2: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from vegetative stage of cowpea cultivars versus clean air 50

Figure 4. 3: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from vegetative stage of cowpea cultivars in pairwise comparison 51

Figure 4. 4: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from flowering stages of cowpea cultivars versus clean air. ... 53

Figure 4. 5: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from flowers of cowpea cultivars in pairwise comparison 54

Figure 4. 6: Principal component analysis (PCA) of volatiles emitted in the flowers of the four cowpea cultivars 57

Figure 4. 7: Percentage (%) responses of female *Megalurothrips sjostedti* to the compounds hexanal and *(E)*-2-hexenal 59

Figure 4. 8: Percentage (%) responses of female *Megalurothrips sjostedti* to the compounds ocimene and 1-octen-3-ol 60

Figure 5. 1: Percentage (%) responses of a) female and b) male *Megalurothrips sjostedti* to freshly and old cut leaves of *Cymbopogon citratus* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer 71

Figure 5. 2: Poucentage (%) responses of a) female and b) male *Megalurotheips sjostedti* to vegetative and flowering *Tagetes minuta* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer 72

Figure 5. 3: Representative chromatogram of major compounds identified in fresh and old cut leaves of Lemongrass, *Cymbopogon citratus* 74

Figure 5. 4: Principal component analysis (PCA) of volatiles in the fresh (0 h) and old (24 h) cut leaves of *Cymbopogon citratus* 75

Figure 5. 5: Representative chromatogram of major compounds identified in vegetative and flowering stages of Mexican marigold, *Tagetes minuta* 76

Figure 5. 6: Principal component analysis (PCA) of volatiles in vegetative and flowering *Tagetes minuta* 77

Figure 5. 7: Percentage (%) responses of *Megalurothrips sjostedti* female to major compounds of fresh cut leaves of *Cymbopogon citratus*.............................. 81

Figure 5. 8: Percentage (%) responses of female *Megalurothrips sjostedti* to major compounds of vegetative *Tagetes minuta*.. 82

Figure 6. 1: A map of Kenya showing Kalro-Mwea 89

Figure 6. 2: Schematic representation of the experimental field design in the Latin square design. 91

Figure 6. 3: Mean number (±SE) of adult and larval *Aphis craccivora* on cowpea plants per treatment in the dry season (season 1) and the rainy season (season 2) at KALRO- Mwea. 98

Figure 6. 4: Mean number (±SE) of adult and larval *Megalurothrips sjostedti* on cowpea plants per treatment in the dry season (season 1) and the rainy season (season 2) at KALRO- Mwea 100

LIST OF PLATES

Plate 2. 1: Female adults *Megalurothrips sjostedti* 9

Plate 2. 3: Adult alate *Aphis craccivora* 10

Plate 5. 1: Leaves of Lemongrass (1g) cut into 0.5 cm wide strips 67

Plate 5. 2: Collection of volatiles of *Tagetes minuta* 68

Plate 6. 1: Treatments (a = non-treated control; b = push-pull plots; c = nethouse plots; d = nethouse combined with push-pull strategy) in field at Mwea 92

LIST OF APPENDICES

Appendix I: Changes in flowering dates per cowpea plant in the treatments in the dry season and the rainy season 143

Appendix II: Changes in the number of cowpea pods per plant in the treatments in the dry season and in the rainy season 144

Appendix III: Changes in the number of adult and larval *T. vaporariorum* per plant in the treatments in the dry season and rainy season 145

Appendix IV: Changes in the number of adult and larval *Aphis craccivora* per plant in the treatments in the dry season and rainy season 146

Appendix V: Changes in the number of adult and larval *Megalurothrips sjostedti* per plant in the treatments in the dry season and in the rainy season. 147

Appendix VI: Changes in the number of adult and larval *Hydatothrips adolfifriderici* per plant in the treatments in the dry season and in the rainy season 148

Appendix VII: Changes in the number of adult and larval *Empoasca* sp. per plant in the treatments in the dry season and the rainy season 149

Appendix VIII: Changes in the number of adult and larval *Clavigralla tomentosicollis* per plant in the treatments in the dry season and rainy season 150

Appendix IX: Changes in the number of adult and larval *Maruca vitrata* per plant in the treatments dry season and rainy season... 151

Appendix X: Changes in the number of larval *Spodoptera littoralis* per plant in the treatments in the dry season and rainy season 152

Appendix XI: Grains harvested in each treatment in rainy season 153

Appendix XII: List of publications..154

LIST OF ABBREVIATIONS AND ACRONYMS

AFFA	Agriculture, Fisheries and Food Authority
AIVs	African indigenous vegetables
ANOVA	Analysis of variance
ARCU	Arthropod Rearing and Containment Unit
CIRAD	Centre de coopération internationale en recherche agronomique pour le développement
CSA	Climate-Smart Agriculture
eV	Electron volts
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FID	Flame ionisation detection
GC-MS	Gas chromatography–mass spectrometry
GLVs	green leaf volatiles
GMO	Genetically Modified Organisms
HIPVs	Herbivore induced plant volatiles
HP	Hewlett Packard
ICIPE	International Centre of Insect Physiology and Ecology
ICRC	Industrial Crops Research Centre
IPM	Integrated pest management
IS	Internal standard
KALRO	Kenya Agricultural & Livestock Research Organization
KARI	Kenya Agricultural Research Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MSD	Mass Selective Detector
NIST	National Institute of Standards and Technology
OBPs	Odorant Binding Proteins

OECD	Organisation for Economic Co-operation and Development
OR	Olfactory Receptor
PCA	Principal component analysis
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
UN	United Nations
USAID	United States Agency for International Development
USEPA	United States Environmental Protection Agency |
VOCs	Volatile organic compounds

ABSTRACT

Vegetable production is increasing in order to feed the growing urban populations in sub-Saharan Africa. Net houses are one possible solution to increase the quality and yield of cowpea, an important leafy vegetable, and to reduce the use of pesticides. This study tested the hypothesis that net houses do not protect cowpea against the Black Legume Aphid, *Aphis craccivora* or Bean Flower Thrips, *Megalurothrips sjostedti*, two small but major cowpea pests. Thus, a possibility to supplement the physical barrier with repellent volatiles to prevent these pests from getting through the netting was examined. Two sources of repellent volatiles were investigated thus, host-and non-host -plant volatiles. This study focused on four specific objectives that included: (i) to identify cowpea cultivars (vegetative stage) and their bioactive odours which attract or repel alate *A. craccivora*; (ii) to identify cowpea cultivars at different phenelogical stages (vegetative and flowering stages) and their bioactive odours which attract or repel male and female *M. sjostedti*; (iii) to evaluate the repellent effect of Lemongrass, *Cymbopogon citratus* and Mexican marigold, *Tagetes minuta* plants and their major compounds against male and female *M. sjostedti*; and (iv) to evaluate the efficacy of repellent volatiles from Lemongrass and Mexican marigold combined or not with netting to reduce cowpea pests in a ‘push-pull’ system in the field. Behavioural assays were conducted to study host preference and gas chromatography-mass spectrometry (GC/MS) for chemical analysis of volatiles. To this end of both studies the host preference and volatiles emitted by four cowpea cultivars *viz*. Katumani 80, Ex-Luanda, Machakos 66 and Ken Kunde 1 that were tested on *A. craccivora* and *M. sjostedti* using a cage and Y- tube olfactometer respectively. Results of the olfactory tests showed that *A. craccivora* was attracted by the volatiles emitted by Ex-Luanda cultivar but repelled by the cultivar Katumani 80. Machakos 66 and Ken Kunde 1 elicited neutral response. The Bean Flower Thrips, *M. sjostedti* females were repelled by volatiles emitted during the vegetative stage of cowpea cultivars Katumani 80, Machakos 66, Ex-Luanda while Ken Kunde 1 elicited a neutral response. The males were repelled only by the vegetative stage of cultivar Ken Kunde 1. Females were attracted by the flowers of cultivar Ken Kunde 1. The volatiles emitted by the flowers of cultivar Katumani 80 were repellent to female *M. sjostedti* but not to males. Among the 23 compounds identified in the vegetative stage of cowpea cultivars, *(E)*-2-Hexenal tested alone or in combination with attractant cowpea flower (KK1) was repellent to female *M. sjostedti* at a 0.01% concentration but not at 1% concentration. The blend of hexanal and *(E)*-2-hexenal added to cowpea cultivar Ex-Luanda reduced the attractiveness of the cultivar to *A. craccivora* while the addition of 1-octen-3-ol and p-xylene to cowpea cultivar Katumani 80 did not reduce the repellence of the cultivar to *A. craccivora*. *(E)*-β-Ocimene and 1-octen-3-ol were only detected in the volatiles of flower of repellent cultivar Katumani 80 to the bean flower thrips. Tested at concentrations of both 0.01% and 1%, these compounds elicited a neutral response from female *M. sjostedti*. In addition, olfactory tests revealed that both male and female *M. sjostedti* were repelled by fresh cut leaves of *C. citratus*. A combination of fresh cut leaves of *C. citratus* and cowpea flower was less attractive to females than cowpea flowers alone. However, males were not repelled by the combination. Female thrips were more repelled by volatiles emitted by vegetative *T. minuta* and by the combination of either vegetative or flowering *T. minuta* with cowpea flower than by cowpea flowers alone. However, males were not repelled/attracted by the volatiles from *T. minuta*. Citral, a major compound of *C. citratus* and a blend of 4 compounds: dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene and *(Z)*-*β*-ocimene with natural ratio were repellent to female *M. sjostedti*. However, dihydrotagetone alone was an attractant for females *M. sjostedti*. Alone, myrcene was not an attractant but enhanced the attraction of cowpea flowers for female *M. sjostedti*. In the field trials, the net house alone reduced the number of large pests (body length > 5 mm) such as the Brown Pod-Sucking Bug, *Clavigralla tomentosicollis*, Leafhoppers, *Empoasca sp*, Bean Pod Borer *Maruca vitrata* and small pests (body length < 5 mm) such as Bean Flower Thrips, *M. sjostedti*, Greenhouse Whitefly, *Trialeurodes vaporariorum*. Conversely, the population of Black Legume Aphid, *A. craccivora* was significantly higher inside the net house. The populations of *T. vaporariorum* and *A. craccivora* were lower in the push-pull treatment than in either control or net house treatments, respectively. The yield was significantly more abundant and the quality of the pods and grains of cowpea better in the net house than in the open field. This work showed that the volatiles from host or non-host plants can repel *A. craccivora* and *M. sjostedti.* The nethouse was effective in protecting cowpeas against most pests and improved the yield of pods. The repellent compounds identified in this study could be used in the field through dispensers to improve the control of small insects in the net house.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

African indigenous vegetables (AIVs) have the potential to improve the diet diversity of African population and reduce food insecurity (Kansiime *et al.,* 2018). About 45,000 species of AIVs have been identify in sub-Saharan Africa region and 1000 species can be eaten as leafy vegetable, fruit vegetables, roots and grains (Muhanji *et al.,* 2011). Cowpea (*Vigna unguicalata* L. Walp.) (Fabaceae), is one of the AIVs whose leaves are consumed as vegetable as well as grain in Africa and can play a major role in food insecurity and malnutrition. For example, with a total of 65 million tonnes produced in 24,431 hectares, cowpea is the first AIVs in production in Kenya (HCDA, 2014). However, the production is still far below to the demand in Africa (Langyintuo *et al.,* 2003).

In sub-Saharan Africa, insect pests are a serious threat to productivity. The hot wet climatic conditions of the tropics are not only favourable for year-round horticultural production, but also for the proliferation of insects. Chemical pesticides are mainly used by small-scale farmers to control insect pests (de Bon *et al*., 2014). For example, in Kenya, 96% of smallholder farmers apply chemicals to control insects pests in horticulture including cowpea (Abtew, 2015). Excessive application of pesticides is toxic to farmers, consumers, and the environment, and reduces the fauna and flora (de Bon *et al*., 2014). In addition, several pesticides are suspected to be carcinogenic, mutagenic, and endocrine disruptors for humans (Abong'o *et al.*, 2014). Pesticide residues in the leaves and seeds of vegetables are a public health threat. Moreover, several insects including thrips are known to have rapidly developed resistance to pyrethroid and organophosphorus insecticides (Herron & James, 2005; Thalavaisundaram *et al.*, 2008).

In the past three decades, novel types of agriculture including organic agriculture, ecological intensification, permaculture, integrated agriculture, climate-smart agriculture, and agroecology have been proposed and implemented as an alternative to the use of chemical pesticides. Organic agriculture has banned the use of agrochemicals (synthetic fertilisers, pesticides, genetically modified organisms (GMO))(Gomiero *et al.*, 2011a). The goal is to promote a production system that sustains the health of soils, ecosystems and people. Ecological intensification (or "sustainable intensification") is defined in the framework of organic agriculture as the maximisation of primary production per unit area without compromising the ability of the system to sustain its productive capacity (FAO, 2009). Permaculture is also defined within the framework of organic agriculture as the conscious design and maintenance of agriculturally productive ecosystems which have the diversity, stability, and resilience of natural ecosystems (FAO, 2009). Integrated agriculture is a farming method which combines management practices from conventional and organic agriculture, only using chemical pesticides as a last resort (Gomiero *et al.*, 2011b). Climate smart agriculture (CSA) is an approach to boost agricultural production and to reduce the impacts of climate change (FAO, 2013). Agroecology is a promising alternative to conventional agriculture, which makes intensive use of agrochemicals for production. Agroecology can be interpreted as a movement, a science and a practice (Wezel & Silva, 2017). As a science, it is defined as a practice of applying ecological concepts and principles to the study, design and management of the ecological interactions within agricultural systems (FAO, 2009). Theoretical and applied research in agronomy and ecology has been used in combination to control insects without using chemical pesticides. Agroecology is promoted by several international organisations including the UN, FAO, and Cirad, a French research institute for agricultural development (FAO, 2015).

Most agroecological practices include cultivar selection, crop associations, crop rotation, biological pest control, natural pesticides, allelopathic plants, push-pull approaches, intercropping, cover crops or mulch (Wezel & Silva, 2017). The combination of different agroecological practices is a way to improve the biological control of insect pests in horticulture. For example, the combination of autoinoculation device, attractant Lurem-TR (methyl-isonicotinate) and entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorok. was effective to control Bean Flower Thrips, *Megalurothrips sjostedti*

Trybom in field (Mfuti *et al.,* 2016). The exploitation of repellent semiochemical compounds from companion plants combined with physical control, attractant sticky trap and a less attractive cowpea cultivar could improve the control of insect pests without pesticides use. Semiochemicals can be defined as chemicals emitted by living organisms (plants, insects, etc.) inducing a behavioural or a physiological response in other individuals (Heuskin *et al.,* 2011). This communication can be intraspecific (pheromones) or interspecific (allelochemicals). The Allelochemicals include allomones (emitting species benefits), kairomones (receptor species benefits) and synomones (both species benefit). Plant volatile mediate plant-arthropod interactions offer an avenue for novel research in the management of insect pests.

1.2 Statement of the problem

Cowpea production are threatened by several insect pests including Black Legume Aphid, *Aphis craccivora* Koch and Bean Flower Thrips, *M. sjostedti* (Abtew, 2015; OECD, 2015). Several strategies have been developed, such as maize-cowpea intercropping which reduce light intensity in the cowpea canopy, thus the density of *M. sjostedti* on cowpea (Kyamanywa & Ampofo, 1988), blue and yellow sticky traps for effective monitoring (Webb *et al.,* 1994; Muvea *et al.,* 2014), use of biopesticides (Mfuti *et al.,* 2017; Mweke *et al.,* 2018), and autoinoculation devices with entomopathogenic fungus *Metarhizium anisopliae* Metchnikoff (Mfuti *et al.,* 2016), however, the use of repellents volatiles to control *M. sjostedti* and *A. craccivora* are scarce (Abtew, 2015).

Plant volatiles are a promising tools to control the insects (Khan *et al.,* 2016; Midega *et al.,* 2018). Volatiles released by companion plants grown as intercrops may interfere with the ability of insects to locate the host plant, and with the feeding, distribution and mating of insect pests (Parolin *et al.,* 2012; Parker *et al.,* 2013). However, its efficacy can be further reinforced by pull stimuli in the ‘push-pull’ strategy (Khan & Pickett, 2008a; Midega *et al.,* 2018). The push-pull strategy is a crop protection concept which uses attractant and repellent stimuli simultaneously to manipulate the insect’s spatial distribution in order to reduce insect abundance (Cook *et al.,* 2006). Repellent stimuli prevent insects finding or accepting their host. The stimuli include anti-feeding agents, repellent volatiles, alarm pheromones, visual distractions, egg-laying repellents and irritants (Cook *et al.*, 2006; Eigenbrode *et al.*, 2016). Attractant stimuli divert the insect from the crop to be protected by means of taste stimuli, egg-laying stimulants, volatile plant attractants, sexual pheromones, and visual stimulants such as colour sticky traps. The blue and yellow colour sticky traps have been used to monitor *M. sjostedti* and *A. craccivora* but not as “pull” in push pull strategy.

Previous studies revealed differential attractiveness of volatiles emitted by cowpea cultivars to *A. craccivora* and *M. sjostedti* (Givovich *et al.*, 1988; Ekesi *et al.*, 1998a), but the compounds responsible for the differential attractiveness are still unknown. On the other hand, volatiles from Lemongrass, *Cymbopogon citratus* extracts were shown to have a repellent effect on female *M. sjostedti* in the laboratory (Abtew, 2015). The short repellence duration of extracts in the field remain a major problem in the control of these pests. The emission of repellent volatiles from companion plants could increase the efficacy of repellency. The repellent activity of *Cymbopogon* and *Tagetes* genera have been shown against different kind of arthropods worldwide (USEPA 2012; Nerio *et al.*, 2010). Moreover, Mexican marigold *Tagetes minuta* L. and *C. citratus* grow very well in Kenya and growing them is cheaper than using plant extracts or essential oils. However, implementing an effective push-pull strategy requires a lot of work in chemical ecology to control only one insect pests. Thirty years after the conception of push-pull strategy, the success application have been the reduction of population of Stemborer in the maize crop in Africa (Khan *et al*., 1997). The combination of push pull strategy and nethouse could improve the control of pests in field. However, little information is available on efficacy of nethouse and push pull to control cowpea pests.

1.3 Justification of the study

To control the insect pests of cowpea as for many vegetable crops in Africa, farmers mainly use pesticides, which are often obsolete or banned chemical pesticides (Abtew, 2015; Mfuti *et al.*, 2016). The World Health Organization estimates that up to 10 million cases of illnesses and injuries each year are related to the unintentional pesticide poisoning with approximatively 200,000 deaths, primarily in developing countries (WHO, 2017). Pesticide residues have been reported in breast milk, fish, water and air (Sereda *et al.*, 2009; Ministry of Environment water and Natural Ressources of Kenya, 2019). Although Kenya has several legislations and regulatory frameworks addressing chemical management, the implementation of effective control of pesticide applied by the small holders that grow vegetables destined for domestic markets remain difficult (RSA , 2015). The development of sustainable protection of cowpea crop should enhance production, nutrient value and food security.

Intercropping is growing two or more crops in the same field at the same time, but the crops do not necessarily have to be sown and harvested at the same time (Lithourgidis *et al.*, 2011). It is an interesting agroecological practice that could be combined with netting. Companion plants near the main crop can interfere with host selection process of insects infesting the main crop by (i) obscuring the visual profile of the main crop, (ii) as a trap crop, enhancing the attraction of insects to companion plants compared to the main crop, (iii) as a repellent plant, diverting insects away from the main crop, (iv) as a masking plant, that interfere with the chemical signal of host plants preventing host detection (Ninkovic *et al.*, 2013; Ben-Issa *et al.*, 2017). However, numerous failures have been reported on the efficacy of companion plants volatiles to reduce insect pests in the field (Moreau *et al.*, 2006; Webster & Cardé, 2016). Understanding the mechanisms involved in the repellent effect of volatiles from companion plants would improve our knowledge of pest control and the use of such plants.

1.4 Null Hypotheses

The null hypotheses (H_0) are:

1. *Aphis craccivora* (alate) are not attracted or repelled by volatiles emitted by vegetative stage of cowpea cultivars
2. Male and female *M. sjostedti* are not attracted or repelled by volatiles emitted by vegetative and flowering stages of cowpea cultivars
3. Volatiles emitted by herbal plants do not repel male and female *M. sjostedti*
4. The combination of the semio-chemicals and netting do not reduce cowpea pests in the field

1.5 Research Objectives

1.5.1 General objective

The general objective of this study was to investigate the efficacy of 'push-pull' and netting technology for management of *Megalurothrips sjostedti* and *Aphis craccivora*

1.5.2 Specific objectives

1. To identify the vegetative stage of cowpea cultivars and their specific volatiles as attractants or repellents to alate *Aphis craccivora*
2. To identify the vegetative and flowering stages of cowpea cultivars and their specific volatiles as attractants or repellents to male and female *Megalurothrips sjostedti*
3. To evaluate the repellent activity of cut leaves of *Cymbopogon citratus*, intact *Tagetes minuta* and their specific volatiles against male and female *Megalurothrips sjostedti*
4. To evaluate the efficacy of *Cymbopogon citratus* and *Tagetes minuta* volatiles in controlling cowpea pests in a 'push-pull' system combined or not with netting

CHAPTER TWO

LITERATURE REVIEW

2.1 Cowpea

2.1.1 Cowpea classification

Cowpea, *Vigna unguiculata*, belongs to the family Fabaceae and includes 751 genera and 19,500 species (Christenhusz & Byng, 2016).

2.1.2 Cowpea plant

Cowpea is an annual tropical or subtropical herb can grow up to 80 cm tall and up to 2 m for climbing cultivars and the pods may be up to 30 cm long (OECD, 2015). The seeds can measure 2-12 mm long with different colour such as red, black, brown, green and white (Small, 2009; OECD, 2015). The common local name of cowpea around the world include “Niebe,” “Wake,” “Ewa” “Seub”, “Niao”, “Bean” in Western Africa, “Kunde” in Eastern Africa,“Caupi” in Brazil, “Southernpeas,” “Blackeyed peas” in the United States and “Long bean” or “Asparagus bean” in China (Timko *et al.*, 2007; OECD, 2015). Native of tropical Africa, cowpea is an African indigenous vegetables (AIVs) which is a great component of the daily diet of about 200 million people in Africa (Popelka *et al.*, 2006). Cowpea is grown particularly by smallholder farmers for its edible seeds. However, all aerial parts of cowpea are also consumed. The tender green leaves containing important nutritional value are used as green leafy vegetable in many parts of Eastern Africa, immature green pods are consumed in humid regions of Asia and the Caribbean, boiling grains as a fresh vegetable or may be mixed with cereals (for example, rice and beans) in Western Africa (Timko *et al.*, 2007; Muniu, 2017). Cowpea is also used as fodder for livestock (OECD, 2015).

2.1.3 Cowpea economic importance

Cowpea is an economically important vegetable in Africa. For example, the trade of cowpea leaves was valued at KES 812 million in Kenya (HCDA, 2014). In Western and Central Africa, the cowpea crop is a valuable and dependable revenue-generating commodity for farmers and grain traders (Langyintuo *et al.*, 2003). In Nigeria, farmers increase their annual income by selling cowpea fodder during dry season (Dugje *et al.*, 2009). However, the growth and yield of cowpea in Africa are constrained by several abiotic and biotic factors. Among the biotic factors, insect pests are probably the main factor limiting grain legume yields (Abtew, 2015). The four key pests on cowpea are the Bean Flower Thrips *M. sjostedti*, the Black Legume Aphid, *A. craccivora* (Homoptera: Aphididae), the Pod Borer, *M. vitrata* and the Pod sucking Bug, *C. tomentosicollis* (OECD, 2015). However, the Black Legume Aphid and Bean Flower Thrips, two small but severe cowpea pests were the focus of this study.

2.2 Key cowpea pests

2.2.1 Bean Flower Thrips, *Megalurothrips sjostedti*

Bean Flower Thrips (BFT), *M. sjostedti* is a species of thrips belonging to the order Thysanoptera and family Thripidae (Moritz *et al.,* 2013)(Plate 2.1). It is a small insect whose female adult size ranges from 1- 2 mm long and male size is 1 mm (Gonné, 2017; Infonet Biovision, 2018). The body colour is brown to dark brown. The females have the sexual or asexual reproduction and the males are haploid which developed from unfertilized eggs (asexual) and female are diploid from fertilized eggs (sexual)(Moritz *et al.*, 2013; Sani & Umar, 2017)..

Plate 2. 1: Female adults *Megalurothrips sjostedti* (Source: Copeland, icipe, 2018)

The life cycle includes; the egg, two active feeding larval instars, two relatively inactive and non- feeding pre-pupa and pupal stages and adult stage (feeding stage) (Sani & Umar, 2017). A single female lays 0.3- 4.8 eggs per day depending on fluctuations in temperature and photoperiod (Ekesi *et al.,* 1999). The eggs are laid in the buds and calyx of developing flowers. About 2-3 days later, they hatch into 1st instar larvae, which develop to 2nd instar larvae after 2-3 days. Second stage larvae lasts 3-4 days and it searches for shelter to pupate in the soil or under leaf litter or between debris. The pupation period lasts for about 4- 7 days before emerging to an adult. The life cycles is about 12-14 days (Loomans, 2003; Sani & Umar, 2017).

Among the 13 species of the genus *Megalurothrips*, there is only one species from Africa (*M. sjostedti*) and 12 from Southeast Asia (Tyler-Julian *et al.,* 2014). *M. sjostedti* is widespread throughout sub Saharan African countries but the potential dispersion of *M. sjostedti* is unknown. Only adults have wings and should be the dispersal stage.

The Bean Flower Thrips, *M. sjostedti* is a serious pests of legumes in Africa and may cause 20 to 100% yield loss on cowpea without chemical application (Ekesi et *al.,* 1998b;. Abtew, 2015). The damage of larvae and adults *M. sjostedti* is due to direct damage by feeding in the flowers on pollen and other floral tissues. Feeding activity cause abortion and shedding of flowers reducing crop yields. Leaf, buds and bracts/ stipules are also attacked by *M. sjostedti*. To date, no transmittion of viruses to the plants through *M.*

sjostedti have been identified, but possible distribution of phytopathogenic fungi and bacteria has been reported (Moritz *et al*., 2013).

2.2.2 Black Legume Aphid, *Aphis craccivora*

Aphis craccivora belongs to the order: Hemiptera, suborder: Sternorrhyncha, superfamily: Aphidoidea and family: Aphididae (Gullan & Martin, 2009). It is shiny black with legs strikingly white and a black area near apex of femur and tibia (Stoetzel & Miller, 2001). Wingless females have a body length of 1.2-1.9 mm, while winged (alate) ones have a body length of 1.4-2.0 mm (Stoetzel & Miller, 2001) (Plate 2.3).

Plate 2. 2: Adult alate *Aphis craccivora* (Source: Diabate, 2017)

In Africa, mostly females are found and they reproduce parthenogenetically (asexual reproduction in which growth and development of embryos occur without fertilization) (Irwin, 1980). The developmental period has 1st, 2nd, 3rd and 4th instar nymphs during 1-2, 1-2, 1-3 and 1-2 days respectively. The total days of development of the four instar nymphs is about 4-7 days and new born nymphs immediately start feeding by sucking the sap from plant tissues (Saroch, 2000).The high population density in a colony promote the production of winged progeny (Obopile & Ositile, 2010). Adult female longevity varies on average from 2-10 days and females can give birth on average to 64-133 nymphs during its life (Saroch, 2000). *Aphis craccivora* is a cosmopolitan polyphagous which feeds on a large number of different species of plant but affect mostly the leguminosae (Ofuya, 1997; Navas, 2014).

Black Legume Aphid, *A. craccivora* is a major pest of leguminosea including cowpea and it primarily infests seedlings, although large populations may infest flower buds, flowers and pods (Obopile & Ositile, 2010). It causes direct damage to cowpea by sucking out sap from aerial parts and indirectly by transmitting viruses (Stoetzel & Miller, 2001; Borowiak-Sobkowiak *et al.*, 2017). The reduction of the quality of host plant or the increase of the population of the colony may lead to the dispersion of the winged and apterous morphs to other healthy host plants. The winged (alate) morphs can disperse easily to colonize new plants.

2.3 Management of cowpea pests

2.3.1 Chemical control of *M. sjsotedti* and *A. craccivora*

Synthetic and natural insecticides for Bean Flower Thrips, *M. sjostedti* and Black Legume Aphids, *A. craccivora* have been researched and are commonly used by farmers. The synthetic pyrethroids and organophosphate are the major chemicals used to control *M. sjostedti* while neonicotinoids and organophosphate are used to control *A. craccivora* (Abtew, 2015; Choudhary *et al.*, 2017). The plant extract such as Ginger, *Zingiber oficinale* Ros., Grains of paradise, *Aframomum melegueta* Ros., and Neem, *Azadirachta indica* A. Juss. have been reported to have insecticidal properties against Black Legume Aphids (Ofuya, 1997). The extract mixtures of plants Gmelina, *Gmelina arborea* + *Eucalyptus citriodora* tree barks and African marigold *Tagetes erecta* L + *G. arborea* leaves showed insecticidal properties against *M. sjostedti* in the field (Oparaeke, 2006). The biopesticides especially botanicals are highly biodegradable compared to synthetic pesticides and safer to use.

2.3.2 Cultural control of *M. sjsotedti* and *A. craccivora*

In Africa, cowpea is traditionally grown as intercrop with maize, millet, cassava, cotton or sorghum (Jackai & Daoust, 1986). When cowpea is combined with maize, thrips, *M. sjostedti* population is reduced (Kyamanywa & Ampofo, 1988). Navas (2014) also reported that intercropping cowpea and either maize or sorghum reduce aphid infestation. In addition, early sowing in the season reduce outbreaks of *A. craccivora* and *M. sjsostedti* on cowpea (Abudulai *et al.*, 2017; Jackai & Daoust, 1986).

2.3.3 Biological control of *M. sjsotedti* and *A. craccivora*

Several coccinellid species have been reported as natural enemies of *A. craccivora*. In Kenya, the coccinellid predators of aphids such as Sulfurous Lady Beetle, *Cheilomenes sulphurea* Olivier, *Cheilomenes vicina* Mulsant, *Cheilomenes lunata F.*, Adonis Ladybird, *Hippodamia variegate* Goeze, *Coccinella* spp, *Scymnus* sp., and *Exochomus sp,* have been identified in field (Annan *et al.,* 1994; Vaitiaire, 2011). The main predators on *M. sjostedti* are the anthocorid predator, *Orius albidipennis* Reuters, the Staphylinid Beetle *Paederus sabaeuss* Erichson and *Geocoris* sp. (Tamò *et al.*, 2012). The Minute Pirate Bug, *Orius* spp. are natural predators of many thrips species (Deligeorgidis, 2002). *Orius* spp is naturally present in Kenya and it is commercially available as biological control agent and used in greenhouse to control the thrips (Kasina *et al.*, 2006a; Nielsen, 2013).

Parasitoids such as *Ceranisus menes* Walker and *C. femoratus* (Gahan) (Hymenoptera: Eulophidae) are the only two wasps investigated for their potential to control *M. sjsotedti*. *Ceranisus menes* showed a poor parasitim rates (1%) on *M. sjsotedti* while *C. femoratus* showed a relative higher parasitim rates on *M. sjostedti* on important host plants, including cowpea (Adati *et al.*, 2008; Agboton *et al.*, 2011). Discovered in Cameroon, *C. femoratus* has been introduced in Benin, Ghana and Nigeria to control *M. sjsotedti* (Tamò *et al.*, 2012). Black Legume Aphid, *Aphis craccivora* are parasitized by a wide range of parasitoids. For example, *Aphidius colemani* Viereck, *Lysiphlebus fabarum* Marshall, *L. confusus* Tremblay and Eady, *L. testaceipes* Cresson, *Binodoxys acalephae* Marshall, *B. angelicae* Haliday, *Praon volucre* Haliday, and *Ephedrus persicae* Froggatt have been reported as parasitoid of *A. craccivora* (Rakhshani *et al.*, 2005). Vaitiaire, (2011) reported that the wasps *Diaeretiella rapae* (M'Intosh) and *Aphidius colemani* Viereck are indigenous parasitoids of *A. craccivora* in Kenya

The effectivity of entomopathologenic fungi as biocontrol agents against *A. craccivora* and *M. sjsotedti* have been demonstrated by several authors (Ekesi *et al.,* 1998c , 2000; Mfuti *et al.,* 2016; Mweke *et al.*, 2018). *Metarhizium anisopliae* (Metschnikoff) Sorokin isolates ICIPE 62 were found pathogenic to *A. craccivora* in laboratory assays and reduced their population in the greenhouse (Mweke *et al.,* 2018). Whereas, the Bean Flower Thrips population was reduced by *M. anisopliae* isolates ICIPE 69 in the field (Ekesi *et al.*, 1998c). The isolates ICIPE 69 are commercially available as Campaign® by RealIPM in Kenya (Mfuti *et al.,* 2016). However, entomopathologenic fungi conidia applied on foliage are susceptible to UV light, rain, temperature variation reducing its efficiency in field (Mfuti *et al.,* 2016). The combination of entomopathologenic fungi *M. anisopliae*, with autoinoculation device increased persistence of conidia therefore efficacy of *M. anisopliae* to control *M. sjostedti* in the field (Mfuti *et al.,* 2016).

2.3.4 Host Plant Resistance (HPR)

The resistant cultivars is cheapest and effective way to control insect pest (Potarot & Nualsri, 2011). The three mechanisms plants use to resist insects are antibiosis (the ability to the host plants to affect the fitness of insects and their progeny when it uses the plant as food), antixenosis (physical or chemical characteristics of host plants which alter insect behaviour preventing the successful establishment of the insects) and tolerance (the ability of the host plants to grow and produce despite infestation) (Togola *et al.*, 2017). The role of volatiles in antixenosis resistance of cultivars has been demonstrated in the laboratory by many authors (Nottingham *et al.*, 1991; Ekesi *et al.*, 1998a). For example, in olfactory assays, *M. sjostedti* showed the least preference for the volatiles emitted by flowers of the cowpea variety IT84S-2246 compared to three other varieties, indicating an antixenosis resistance in this variety (Ekesi *et al.,* 1998a). Firempong, (1988) showed also the antixenosis resistance in the cultivar IT82D-812 and found both antixenosis and antibiosis in cultivar ICV12 against *A. craccivora*. The moderare resistance of cowpea genotypes TVu-1509, TVu-2870 and TVx-3236 to *M. sjostedti* have been reported by Togola *et al.*

(2017). The same author reported a good resistance in genotypes TVu-36, TVu-408, TVu-410, TVu-801, TVu-2896 and TVu-3000 to *A. craccivora.*

2.3.5 Netting technology

For more than ten years, the Cirad Hortsys Research Unit has been developing netting in Africa as a tool to facilitate the adoption of agroecological practices. Netting has been shown to be efficient in reducing pest infestations and damage to fruit and vegetable crops in experimental stations and in smallholder farms (Martin *et al.*, 2006, 2015). The use of nets has been reported to reduce the use of insecticide sprays on cabbage, green beans and tomato crops by 70% to 100%, to stabilize air temperature and to improves soil moisture (Martin *et al*., 2006; Saidi *et al.,* 2013: Gogo *et al.*, 2014a). In addition, in Benin, netting used by small scale cabbage producers to improve yield reduced the cost of insecticides by 68-95% and labour costs by 3-40% (Vidogbéna *et al.*, 2015). In Kenya, AgroNet® netting provided efficient protection against the Bean Fly, *Ophiomyia* spp. which can cause 90-100% yield losses (Martin *et al.*, 2015). Netting was also efficient in controlling insect pests such as the Diamond Back Moth *Plutella xylostella* L, and the Cabbage Webworm, *Hellula undalis* Fabricius in cabbage crop (Martin e*t al.,* 2006). The nethouse was effective barrier in protecting tomato against Bollworm *Helicoverpa armigera* Hübner, Leafminer, *Lyriomiza spp,* and Greenhouse Whiteflies *T. vaporariorum* (Martin *et al.,* 2015). In green bean crop, nethouse reduced the population of Silverleaf Whitefly *Trialeurodes sp*, Black Bean Aphids *Aphis fabae* Scopoli and thrips *Frankliniella spp*. (Gogo *et al.*, 2014a; Martin *et al.*, 2015).

By contrast, insect damage under nethouses can be sometimes higher than in the open field. For example, the population of the larvae, *Spodoptera littoralis* Boisduval and aphids (*Myzus persicae* Sulzer and *Lipaphis erysimi* Kaltenbach) were significantly higher under nethouse than open field of cabbage (Simon *et al.,* 2014). Indeed, the moth *S. littoralis* may lay its eggs on the net allowing the first larva stage to pass through. The less effectiveness of nethouse to control some pests may be compensated by a combination with repellent plants as an olfactory barrier to prevent the insects to pass through the net.

The challenge to enable small holder farmers to combine this technology with other locally available techniques. These techniques include, namely combining repellent or masking plants which can be placed inside or outside the netting, attracting parasitoids and predators inside the structures, or releasing generalist predatory insects such as Ladybirds, Lacewings, Hoverflies, which would be kept there by the presence of the netting. Indeed, use of netting could help the spread use of biological pesticide such as entomopathogenic fungus among smallholder farmers due to the greater humidity from the confined environment created by the netting.

2.3.6 Companion plant cropping

The companion plant grown near to the main crop which have the potential to increase the main crop plant productivity and/or to protect the main crop against insect pests (Lithourgidis *et al.,* 2011; Parolin *et al.*, 2012). The use of plants belongs to the Fabaceae family as companion plants can contribute to increase the yield of main crop by fixing atmospheric nitrogen on their roots through symbiosis with bacteria. For example, Rao and Mathuva (2000) reported that intercropping maize/cowpea increased maize yields by 17 % than continuous growing of sole maize. For example, the reduction of light intensity in the cowpea/maize mixture reduced the density of Bean Flower Thrips, *M. sjostedti* (Thysanoptera:Thripidae) on cowpea (Kyamanywa & Ampofo, 1988). In intercropping, non-host plant volatile may impede insects to detect their host-plant by emitting repellent volatiles or by masking host plant volatiles (Jactel *et al.*, 2011; Ben-Issa *et al.*, 2017). A repellent phenomenon can be a movement away from an odour source (true repellent) and also an inability to find the host (Deletre *et al.*, 2016). The goal of the repellence in pest management is to create an olfactory barrier to prevent or reduce contact between the insect and the host (Deletre *et al.*, 2016). For example, the repellent plants *Desmodium uncinatum* and/or *Melinis minutiflora* intercropped with maize repel (push) the adult

Stemborer moths out of the field (Khan *et al.*, 1997; Poveda & Kessler, 2012). The odour masking is a constituent of the repellence and may be used to modify the abundance and distribution of insect pests. It is defined as an odour interfering with the host detection/localization or reducing the attractiveness of the host (Deletre *et al.,* 2016). The goal of an odour-masking in pest management is to hide the odour of host from the insect pest (Deletre *et al.*, 2016). For example, the constitutive volatile from Coriander, *Coriandrum sativum* L. alone was found non-repellent to the Silverleaf Whitefly, *Bemisia tabaci* Gennadius but in intercropping with tomato, *Solanum lycopersicum* L., there was reduction of the attractiveness of *B tabaci* on tomato which is due to the masking effect of coriander volatiles (Togni *et al.*, 2010).

2.3.6.1 Lemongrass (*Cymbopogon citratus)*

Cymbopogon citratus (Citronella Grass or Lemongrass) belongs to the order: *Cyperales,* family*: Poaceae*, genus: *Cymbopogon.* The genus *Cymbopogon* includes 144 species and it is widely distributed in the tropical and subtropical regions of Africa, Asia and America (Avoseh *et al.*, 2015). This perennial herb produces aromatic essential oils which is commercially important in cosmetics, pharmaceuticals, and perfumery applications (Khanuja *et al.*, 2005). In the control of insects, the natural product (essential oils, extracts) from Lemongrass, *Cymbopogon spp.* is widely used as natural repellents worldwide (Nerio *et al.*, 2010). For example, essential oil from *C. citratus* was repellent against thrips (Abtew, 2015), whiteflies (Deletre *et al.*, 2015) and toxic for *Frankliniella schultzei* Trybom and aphids *M. persicae* (Costa *et al.,* 2013).The main compounds of the essential oil include citral (geranial + neral), geraniol and myrcene (Nerio *et al.*, 2010). Citral has been reported as a repellent to the *M. sjostedti* larvae (Abtew, 2015) and geraniol repellent of whitefly, *Bemisia tabaci* Gennadius (Deletre *et al.,* 2015). The combination of *C. citratus* with nethouse may prevent *M. sjostedti* adults to pass through the nethouse.

2.3.6.2 Mexican marigold *(Tagetes minuta)*

Tagetes minuta belongs to the order: *Asterales*, family: *Asteraceae*, genus: *Tagetes* (Sadia *et al.,* 2013). The plant *T. minuta* is native to the southern half of South America but nowadays it has been reported in several countries in Europe, Asia, and Africa (Makang'a, 2012). *T. minuta* is an erect annual herb reaching 1 to 2 m and the leaf margins are finely serrate (Makang'a, 2012). Essential oil from *T. minuta* is used worldwide as flavour component in food and perfume industry (Cornelius & Wycliffe, 2016). *Tagetes* oil is also described by the United States Environmental Agency as a biochemical pesticide active ingredient intended for the control of mites, whiteflies, aphids, thrips, mealybugs, scales and pyslla (USEPA, 2012). The repellent effect of *T. minuta* against insects has been demonstrated in the laboratory. For example, the adult Sandflies, *Phlebotomus duboscqi* (Neveu-Lemaire) was repelled by essential oil of *T. minuta* in the laboratory (Kimutai *et al., 2017*). The main compounds of the essential oil include dihydrotagetone, limonene, *β*-ocimene, tagetones, ocimenones, piperitenone, *β*-caryophyllene, bicyclogermacrene (Cornelius & Wycliffe, 2016). The essential oil composition is different according to the different parts of plants and its growth stage. Essential oils from bloomed plant leaves and flowers contain *β*-ocimene and tagetenone whereas non-bloomed plant leaves and flowers mainly have dihydrotagetone (Chamorro *et al.,* 2008). Some of these compounds have been reported in literature as repellent. For example, limonene and *β*-caryophyllene showed a strong repellence against the Cigarette Beetle, *Lasioderma serricorne* Fabricius in the laboratory (Hori, 2003). The repellent properties of *T. minuta* plants could be used to control *M. sjostedti*.

2.3.7 Use of plant volatiles in pest management strategy

The potential of plant volatiles to control insect pests have been demonstrated by several authors (Khan *et al.,* 1997; Parolin *et al.,* 2012; Parker *et al.,* 2013). The volatiles emitted by the plants may be used in management of pests through several approaches such as mass trapping, synergistic effect of plant volatiles in pheromone traps, plant volatiles as attractants of natural enemies of pests, 'push-pull' strategy.

2.3.7.1 Monitoring and mass trapping

The colour traps are important tool to monitor and control the insect pests in the greenhouse and fields. The colour traps are used to provide information on the current status of the pest population (monitoring) or reduce the population of insects through mass trapping. The combination of certain plant volatiles and colour trap have been reported to increase of insects caught by colour traps in previous studies. For example, the yellow sticky traps combined with natural essential oils (Sandalwood oil, Basil oil, and Grapefruit oil) increased attraction of Greenhouse Whitefly, *T. vaporariorum* in greenhouses (Górski, 2004). In the field, the combination of flower volatiles as methyl isonicotinate and blue colour trap increased the attraction of *M. sjostedti* compared to blue traps alone (Muvea *et al.*, 2014). Unfortunately, the colour traps with plant volatile may also capture beneficial insects in the field (Broughton & Harrison, 2012).

2.3.7.2 Synergistic effect of plant volatiles in pheromone baited- traps

The sex pheromone is a tool widely used worldwide to attract the insects for monitoring or mass trapping in horticulture, agriculture, stored products, forests, and gardens. About 20 million pheromone lures are produced for monitoring or mass trapping every year (Witzgall *et al.*, 2010). The sex pheromone can be combined with the plant volatiles to increase the catch of insects. The male bird-cherry aphid, *Rhopalosiphum padi* L was attracted by combining the sex pheromones (nepetalactol) and a major component of the volatiles from its host *Prunus padus* L extract (benzaldehyde) in the field (Hardie *et al.*, 1994). The involvement of plant volatiles in sexual behaviour of the insects have been reported by Bendera *et al.* (2015). The same author showed the compound 1-octen-3-ol from cowpea increased coupling in *Maruca vitrata* Fabricius. By contrast, the attractive effect of sex pheromone may be inhibited by the non-host volatiles. For example, the attraction of male moth *Thaumetopoea pityocampa* Denis and Schiffermuller was reduced by the combination of sex pheromone and volatiles from bark and leaf of non-host plant (Jactel *et al.*, 2011).

2.3.7.3 Plant volatiles as attractants of natural enemies of pests

Thirty years ago, after the first studies on indirect defence of plant by attracting natural enemies of these herbivores (Mumm & Dicke, 2010), many research have been performed on semiochemicals that attract parasitoids and predators (Turlings & Ton, 2006; Mumm & Dicke, 2010; Simpson *et al.,* 2011; Rodriguez-Saona *et al.,* 2012). The field trial performed by James (2003), showed the compounds *(E)*-3-hexenyl acetate attracted the beneficial insects *Deraeocoris brevis* Uhler, *Orius tristicolor* White, and *Stethorus punctum picipes* Casey. Among the herbivore-induced plant volatiles (HIPVs) used as lure to increase the natural enemies in the field, methyl salicylate (MeSA) has received more attention (Rodriguez-Saona *et al.,* 2012). Methyl salicylate was found to attract five species of predatory insects (*Chrysopa nigricornis* Burmeister, *Hemerobius sp.*, *Deraeocoris brevis* Uhler, *Stethorus punctum picipes* Casey, *Orius tristicolor* White) in the vineyard crop (James & Price, 2004). However, the use of HIPV to recruit the beneficial insects can also attract the pests of crops. For example, the spray of HIPV increased the number of thrips *Frankliniella occidentalis* Pergande in the wine grape, sweet corn and broccoli plants (Simpson *et al.*, 2011).

2.3.7.4 Push-pull strategy

The concept of 'push-pull' strategy was described for the first time by Pyke *et al.* (as cited in Khan *et al.*, 2001) to control *Heliothis spp.* in cotton crop. Later, Miller and Cowles (1990) formalized and redefined the concept as 'stimulo-deterrent diversion' strategy where they used cinnamaldehyde and phenethanol as ovipositional deterrents of Onion Flies (*Delia antiqua* Meigen) simultaneously providing deeply planted onion culls on which onion flies prefer to lay eggs. Khan *et al.,* (1997) re-used the term 'push-pull' strategy to manipulate the distribution of Stemborer pests in the maize crop without any chemical deterrents or toxins in Kenya. Push-pull strategy is integrated pest management programs, which uses a combination of behavior-modifying stimuli to manipulate the distribution and abundance of insect pests and/or natural enemies (Khan & Pickett, 2008a). The control of insects with the push-pull strategies have the advantage to avoid insecticide use thus increasing the opportunity for natural enemies to naturally reduce insect pests. By contrast, this strategy needs considerable research in chemical ecology to understand the mechanism of the trap and repellent companion plants before the deployment (Cook *et al.*, 2006; Khan *et al.*, 2016). Success in the application has been the reduction of 52% population of Stemborer in the maize cropping in Western Kenya (Khan *et al.*, 2008b; Khan *et al.*, 2014). Indeed, the local companion plants Desmodium, *Desmodium uncinatum* intercropping with maize repels (push) Stemborer moths, *Chilo partellus* Swinhoe and *Busseola fusca* Fuller and attracts their natural enemies, *Cotesia sesamiae*, while border Napier Grass, *Pennisetum purpureum* (pull) attracts Stemborer moths (Khan *et al.*, 1997, 2001, 2008b). Laboratory studies showed the importance of plant volatiles in the success of the push pull in Kenya. The intact plant *Desmodium* emits ocimene, *(E)*-4,8-dimethyl- 1,3,7-nonatriene (DMNT) and large amounts of α-cedrene (Khan *et al.*, 2016) which repelled Stemborer moths and attracted the pest's natural enemies (Midega *et al.*, 2009) while six active compounds (octanal, nonanal, naphthalene, 4-allylanisole, eugenol, and linalool) were identified as attractive in both maize and Napier grass (Khan *et al.*, 2000, 2016) .

A push-pull strategy was studied in horticulture to control the Western Flower Thrips, *F. occidentalis* on Chrysanthemums. The volatiles of the non-host plant rosemary, *Rosmarinus officinalis* L were used as push stimuli to repel the thrips while a 'lure' or 'trap' Chrysanthemum cultivar as pull stimuli to attract them where the predator bugs, *Orius laevigatus* Fieber can be released. However, the volatiles of the non-host plant rosemary were not only repellent to the thrips, but also to the predatory bug *O. laevigatus* (Bennison *et al.*, 2002). The consideration of effect of both attractants and repellents stimuli affecting herbivores and beneficial insects should be minimized in the management of pests in push-pull or stimulo-deterrent diversion strategy (Cook *et al.*, 2006; Eigenbrode *et al.*, 2016).

CHAPTER THREE

BEHAVIOURAL RESPONSE OF ALATE *APHIS CRACCIVORA* KOCH (HOMOPTERA: APHIDIDAE) TO VOLATILES FROM DIFFERENT COWPEA CULTIVARS

3.1 Abstract

The Black Legume Aphid, *A. craccivora* is a major insect pest of cowpea in Africa. Volatiles mediate plant-arthropod interactions that could be used in the management of insect pests. This study sought to establish the volatile profile involved in the interaction between *A. craccivora* and four cowpea cultivars namely Ex-Luanda, Katumani 80, Machakos 66 and Ken Kunde 1. Whereas host preference was studied using behavioural assays, chemical analyses of volatiles was done using gas chromatography-mass spectrometry (GC/MS). In preference assays, results showed that alate *A. craccivora* had no significant preference for any of the four cowpea cultivars tested. However, in the olfactometer assays, the aphids showed a significant preference for odours from cultivar Ex-Luanda compared to Katumani 80. Machakos 66 and Ken Kunde 1 elicited neutral response. In pairwise comparisons, alate *A. craccivora* did not distinguish between odours of respective cowpea cultivars. GC/MS analysis identified 23 compounds in the volatiles of the four cowpea cultivars. Not all compounds were detected in all cowpea cultivars, and the detected compounds amounts varied in each cultivar. Of these, only four compounds: hexanal, *(E)*-2-hexenal, 1-octen-3-ol and p-xylene were emitted in significantly different quantities in the four cultivars. A blend of hexanal and *(E)*-2-hexenal added to cowpea cultivar Ex-Luanda decreased its attractiveness to *A. craccivora* compared to the control. This work showed differential attractiveness of volatiles of cowpea cultivars to *A. craccivora* suggesting that volatiles could be used in the management of *A. craccivora*.

3.2 Introduction

Volatile released by plants play an important role in plant-herbivore interactions (Dudareva *et al.,* 2006). Plants use volatiles to either attract pollinators or to protect themselves against herbivores (Dudareva *et al.,* 2006). A plant odour can contain more than 200 compounds and insects can detect either specific compounds or specific ratios of compounds (Bruce & Pickett, 2011). The quantitative and qualitative nature of volatiles released by plants belonging to the same family, species and genotypes can differ and consequently influence how attractive they are to phytophagous insects (Kergunteuil *et al.,* 2015).

Many previous studies have investigated herbivore-induced volatiles in leguminous plants (Takabayashi & Dicke, 1997; Kost & Heil, 2006). However, some studies have shown the effect of volatiles from undamaged leguminous plant in attracting insects (Pettersson *et al.,* 1998; Webster *et al.,* 2008). For instance, Webster *et al.,* (2008) showed that the Black Bean Aphid, *Aphis fabae* Scopoli uses a blend of volatiles to recognize its host, the Faba Bean, *Vicia faba* L. In addition, another study reported that both morphs (apterous and alate) of *A. craccivora* responded positively to odours from undamaged cowpea plants (Pettersson *et al.,* 1998).

Aphis craccivora is a major pest of leguminous plants including cowpea, *V. unguiculata* which is mainly grown by smallholder farmers in Africa (Togola *et al., 2017).* Aphids damage plants directly by sucking sap and indirectly by transmitting viruses (Stoetzel & Miller, 2001; Borowiak-Sobkowiak *et al.,* 2017). The alate (winged) morphs can disperse easily and subsequently transmit viruses to new plants. The abundance and damage of *A. craccivora* on cowpea plants is dependent on cultivar differences (Agele *et al.,* 2006). For example, cultivar Katumani 80 was reported as resistant to *A. craccivora* (Kenya Agricultural Research Institute (KARI), 2008) while the cultivars Ex Luanda and Machakos 66 were susceptible (Mbogo, 1985).

Management of *A. craccivora* in cowpea is mainly by use of synthetic chemical pesticides (Reddy *et al.,* 2017) which are costly and have numerous adverse environmental effects.

The potential of host and non-host volatiles in the management of aphids has been demonstrated. For example, the odour differences from the combination of certain barley cultivars reduced the bird cherry-oat aphid, *R. padi* and increased the natural enemies' populations (Glinwood *et al.*, 2009). In addition, volatiles from Lemongrass, *C. citratus*, when intercropped with eggplant reduced the population of aphids (Calumpang *et al.*, 2013).

The push-pull strategy is a crop protection concept which uses attractant and repellent stimuli simultaneously to manipulate the insect's spatial distribution in order to reduce insect abundance (Cook *et al.*, 2006). The attractant or repellent volatiles emitted by plants may be used as stimuli (Cook *et al.*, 2006; Eigenbrode *et al.*, 2016). However, the implementation of an effective push-pull strategy requires a clear understanding of interactions between an insect and its host plant (Cook *et al.*, 2006; Khan *et al.*, 2008b). For instance, understanding the olfactory cues used by *A. craccivora* to locate cowpea plants could improve integrated pest management (IPM) strategies to control this pest by disturbing their orientation or manipulating their distribution. Actually, the role of olfactory cues has been demonstrated in aphids' ability to locate their hosts (Webster *et al.*, 2008; 2010). However, there is limited information of the effect of volatiles from cowpea cultivars on *A. craccivora*'s ability to locate hosts. The aim of the present study was to identify attractant or repellent compounds from cowpea cultivars that could be used to control *A. craccivora*. It is hypothesized that intraspecific variation of cowpea volatiles influences host attractiveness to *A. craccivora*. This variation will enable to identify attractant and repellent cowpea volatiles that could be used in pest management as push pull strategy. The hypothesis was tested by using behavioural assays and chemical analysis.

3.3 Materials and methods

3.3.1 Raising of plants

Seeds of three commercial cowpea cultivars viz. Ken Kunde 1, Machakos 66 and Katumani 80, were purchased from local seed suppliers (Simlaw Seeds, East African Seeds, Kiambu Fertilizers) in Nairobi, Kenya. Seeds of Ex-Luanda, a local cultivar, were obtained from the International Centre of Insect Physiology and Ecology (icipe), Mbita, Kenya (0°25'47.5"S 34°12'24.1"E). To raise seedlings, three seeds of respective cultivars were sowed directly in pots (14 cm diameter x 15 cm depth) in a screen house (27 ± 7 °C temperature, 75 ± 5 relative humidity (RH); 12 L:12 D photoperiod) at icipe, Duduville, Campus Nairobi, Kenya (1°13'17.9"S 36°53'48.1"E). Plants were grown in soil (Nito-rhodic Ferralsols) mixed with cattle manure, free of pesticides sprays and were watered twice weekly. Four weeks after germination, the plants were used either to rear aphids or thinned to a single plant per pot for the experiments.

3.3.2 Rearing of *A. craccivora*

A colony of *A. craccivora* was established in March 2014 and maintained at the icipe insectary on cultivar Ex-Luanda at a temperature of 23 ± 1 °C, 55-65% RH and photoperiod of 12L: 12D. Aphids were reared in plastic cages (61 cm in length x 45 cm in width x 45 cm in height) that had fine nylon mesh on either side to provide ventilation. Four pots, each containing three plants, of 4-week-old cowpea plants were transferred to the aphid rearing cages. Plants in the cage were replaced with fresh ones every two days and were watered three days weekly. Five-day-old alate aphids in the cage were collected with an aspirator for use in the experiments.

3.3.3 Preference assay

An assay was conducted to compare the preference of *A. craccivora* among the four cowpea cultivars. This assay involved olfactory, visual cues and physical contact. Potted single plants of respective cowpea cultivars were placed in a cage (80 cm in length x 50 cm in width x 40 cm in height) in a screenhouse (27 ± 7 °C temperature, 75 ± 5 relative humidity (RH); 12:12L: D photoperiod). In the cage, the four pots were placed 26 cm apart, in a square pattern. A total of 100 five-day-old alate aphids were picked from the colony and starved for 1 h before release in the experimental cage. The aphids were released at the centre of the four potted plants of respective cowpea cultivars. The insects were left in the cage, and those that had settled on respective cowpea cultivars were visually counted 24 h later. The position of each cultivar in the cage was randomly alternated after each replicate and a total of 12 replicates were conducted in one cage.

3.3.4 Olfactory assay with undamaged cowpea plants

The response of *A. craccivora* to four cowpea cultivars was tested using a Y-tube olfactometer (2.8 cm internal diameter, 15.2 cm stem length, 8.9 cm arms' length) in a laboratory (24 ± 1°C temperature; 50 ± 5% RH) (Narayandas & Alyokhin, 2006). Cleaned air was drawn through two flow meters at a rate of 500 ml/min. The bioassays with individual alate aphids were tested with: a) respective cowpea cultivars versus the control (clean air) and; b) the four cultivars tested against each other in pairwise sets. The alate aphids were placed individually in the stem of the Y-tube using a soft camel's hair brush and observed for 7 min. Aphid was observed for 7 min because preliminary results showed that aphid can make a choice over this time. If an aphid did not choose either arm within 7 min, it was considered non-responsive and replaced. Conversely, it was considered to have made a choice after entering the left or right arm beyond the Y-tube intersection. Each aphid tested was a replicate and a treatment was considered complete when 40 aphids made a choice, disregarding the number of no-choice responders (Koschier *et al,*. 2017).

3.3.5 Collection of volatiles

Volatiles were collected from vegetative parts of the four undamaged cowpea cultivars as previously described (Murungi *et al.,* 2016). The polyester oven bags that were used for volatile collection were baked overnight at 100 °C. The Super Q adsorbents (30 mg; Gainesville, FL, USA) were pre-cleaned with 2 ml of hexane (Sigma Aldrich, Gillingham, UK) and 2 ml of dichloromethane (Sigma Aldrich, Gillingham, UK) to remove contaminants by rinsing, and then dried under a gentle stream of white spot nitrogen.

Individual 6-8 -week-old plants were carefully placed in the oven bag and held tightly around the stem with a rubber band. The pots with soil were completely covered with aluminium foil prior to the plants introduction into the oven bags. Air flow into the bag was provided by two Teflon tubes using a pump. One tube pushed air at 300 ml/min into the bag holding the plant while the other pulled the volatiles through the Super Q adsorbent tubes at a flow rate of 200 ml/min for 24 h. The difference in flow rates prevented unfiltered air from entering in the system (Webster *et al.*, 2008). Volatiles were collected from three plants per cowpea cultivar and replicated four times, giving a total of 12 plants per cultivar. After the collection of volatiles, the three Super Q adsorbent tubes from three plants were eluted with 150 µl dichloromethane each in the same vial. The total eluate (450 µl) was placed in a box of ice and was reduced to 50 µl under a gentle stream of white spot nitrogen. Subsequently, 10 ng/µl of 2- hexadecanol (with a different retention time for cowpea volatile compounds) was added as an internal standard to compare the relative amounts of each compound released by respective cowpea cultivars. Samples were either analysed immediately or stored at -80 °C until use.

3.3.6 Analysis of volatiles

The volatiles were analysed using a GC (Agilent Technologies 7890A) equipped with an HP-5 MS ultra-inert column (30 m × 0.25 mm ID × 0.25 µm film thickness) (J&W, Folsom, CA, USA) directly coupled to a 5975C mass spectrometer for identification of the volatile components. The carrier gas was helium, at a flow rate of 1.2 ml/min. The oven temperature was held at 35 °C for 5 min and then programmed at 10 °C steps up to 280 °C/min. The temperature was then maintained at 280 °C for 5.5 min. Volatile compounds were identified by comparing their mass spectral data with library data from the Adams2 terpenoid/natural product library (Adams 1995) and National Institute of Standards and Technology (NIST) (NIST, 2008) (MSD ChemStation F.01.00.1903, MS HP, USA). The retention times of some compounds and mass spectra were compared to those of authentic standards.

3.3.7 Olfactory assay with synthetic standards

In bioassays with known synthetic volatile compounds, Y-tube olfactometer similar to that used with undamaged plants described above were used. Volatile compounds emitted in different quantities from the four cowpea cultivars were tested to evaluate their potential to decrease or enhance the attraction of cultivars to pests. Based on the literature, the blend of compounds seemed more efficient than compound tested singly (Nerio et *al.*, 2010; Bruce & Pickett, 2011), so only blends of compounds were tested. Two synthetic blends were prepared (i) with potential repellent volatiles (blend A) and (ii) with potential attractant volatiles (blend B). The blend (A) was composed of hexanal and *(E)*-2-hexenal and the blend (B) was composed of 1-octen-3-ol and p-xylene. The synthetic blends were prepared in dichloromethane (1 ml). The compounds were diluted at two selected concentrations: 0.1% (v/v) and 1% (v/v) with a ratio 1:1 for both compounds in the synthetic blends. 50 µl aliquots (i.e. 0.5 µl active ingredient (1% dilution) and 0.05 µl active ingredient (0.1% dilution)) of each treatment were applied onto a filter paper and left to dry for 30 seconds at ambient temperature. The filter paper was then placed in an odour source chamber. The respective blends (A and B) were combined with a cowpea cultivar (either A+ attractant cultivar or B + repellent cultivar) and were compared with clean air first and then with the same cowpea cultivar. For each treatment, the odour source was alternated after every five aphids. The plants were replaced after 10 aphids had been tested and the filter paper after every 60 min. A treatment was completed after 40 aphids responding to odour source had been tested. The connecting tubes were cleaned with dichloromethane after each compound tested.

3.3.8 Synthetic standards

The standard compounds, 1-octen-3-ol (purity ≥ 98%), *p*-xylene (purity ≥ 99%), hexanal (purity 98%) and (E)-2-hexenal (purity 98%) were obtained from Sigma-Aldrich, France. Hexane (purity ≥ 95%), dichloromethane, 2-hexadecanol (purity 99%) were purchased from Sigma Aldrich, United Kingdom. Ethanol (purity ≥ 99.8%) was obtained from Sigma-Aldrich, Germany.

3.4 Data analysis

Frequency count data from preference assays conducted in cage experiments and two-choice behavioural assays were subjected to a chi-square (χ^2) test to evaluate the preferences of *A. craccivora* amongst different cowpea cultivars and/ synthetic standards. The null hypothesis was that aphid distribution was 25:25:25:25 on the four cultivars in the preference assay and 50:50 across the two arms of the olfactometer. The non-parametric Kruskal- Wallis one-way analysis and Dunn's method of multiple comparisons were used to differentiate the relative amounts of VOCs among the four cowpea cultivars. Principal component analysis (packages 'ade4': Dray & Dufour 2007) was used to show the relationship between the cowpea cultivars based on the emission of relative amounts of volatile compounds in a graphical approach. All analyses were implemented in R version 3.3.2 (R Core Team, 2016).

3.5 Results

3.5.1 Behavioural assays

Preference of Aphis craccivora to cowpea volatiles: Alate, *A. craccivora,* showed no significant (χ^2 = 5.14, df = 3, P = 0.161) preference for any of the four cowpea cultivars tested (Figure 3.1).

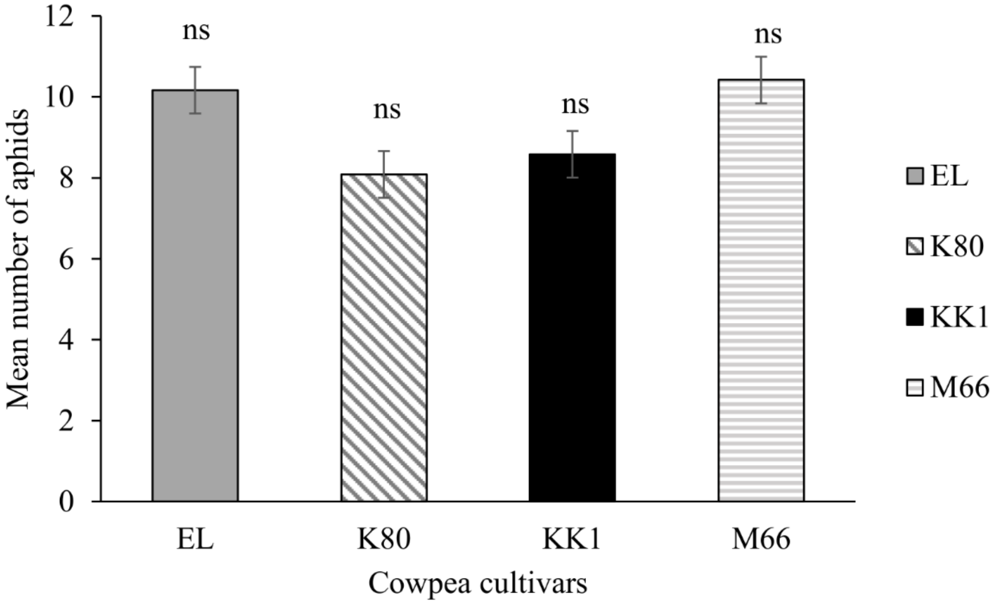

Figure 3. 1: Number (Mean ±SE) of alate *Aphis craccivora* for different cowpea cultivars in the vegetative stage over a period of 24 hours. The total number of insects tested for each replicate was 100 (n=100). EL =Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66. Vertical bars followed by the same letter are not significantly different (chi-square goodness-of-fit test)

Olfactory response of Aphis craccivora to cowpea volatiles: In the olfactometer assays, when cowpea cultivars were compared to clean air, alate *A. craccivora* showed a significant (χ^2 = 4.9, df = 1, P = 0.026) preference for odours from cultivar Ex-Luanda compared to clean air (control). In addition, alate *A. craccivora* showed significant (χ^2 = 10, df = 1, P < 0.01) non-preference for odours of cultivar Katumani 80 compared to clean air (Figure 3.2). When both cultivars Machakos 66 and Ken Kunde 1 were compared to clean air, the response of *A. craccivora* was not significant. In pairwise comparisons, alate *A. craccivora* did not distinguish between odours of: Katumani 80 vs. Machakos 66 (χ^2 = 0.4, df = 1, P = 0.527); Katumani 80 vs. Ken Kunde 1 (χ^2 = 0.9, df = 1, P = 0.342); Katumani 80 vs. Ex-Luanda (χ^2 = 0.9, df = 1, P = 0.342); Ken Kunde 1 vs. Ex-Luanda (χ^2 = 0.1, df = 1, P = 0.751); Machakos 66 vs. Ex-Luanda (χ^2 = 0.4, df = 1, P = 0.527); and Machakos 66 vs. Ken Kunde 1 (χ^2 = 0, df = 1, P = 1) (Figure 3.3).

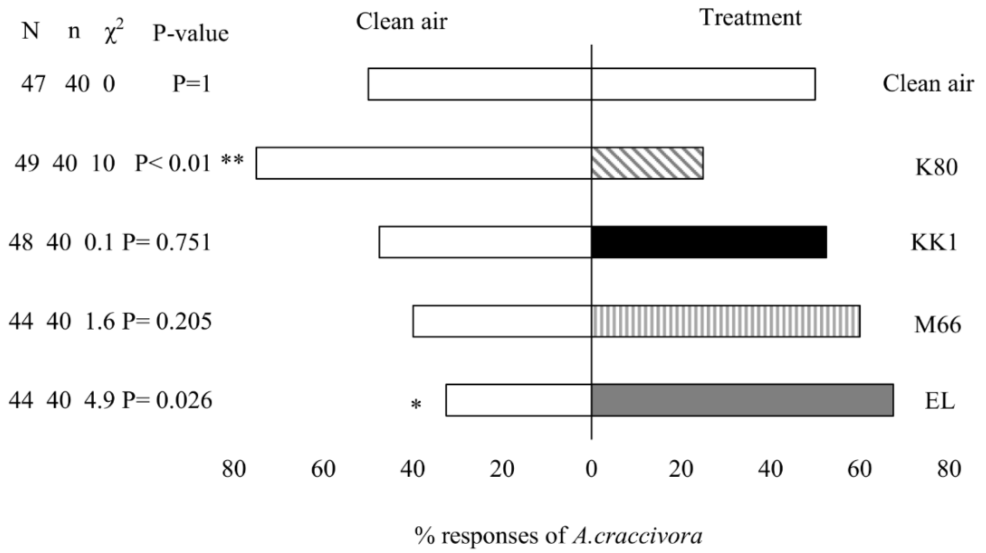

Figure 3. 2: Olfactometer responses of alate *Aphis craccivora* to the odour of undamaged cowpea cultivars in the vegetative stage versus clean air (control) in Y-tube olfactometer. EL =Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66. N= total number of insects tested. n= total number of insects responded for each treatment was 40 (n = 40). χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test)

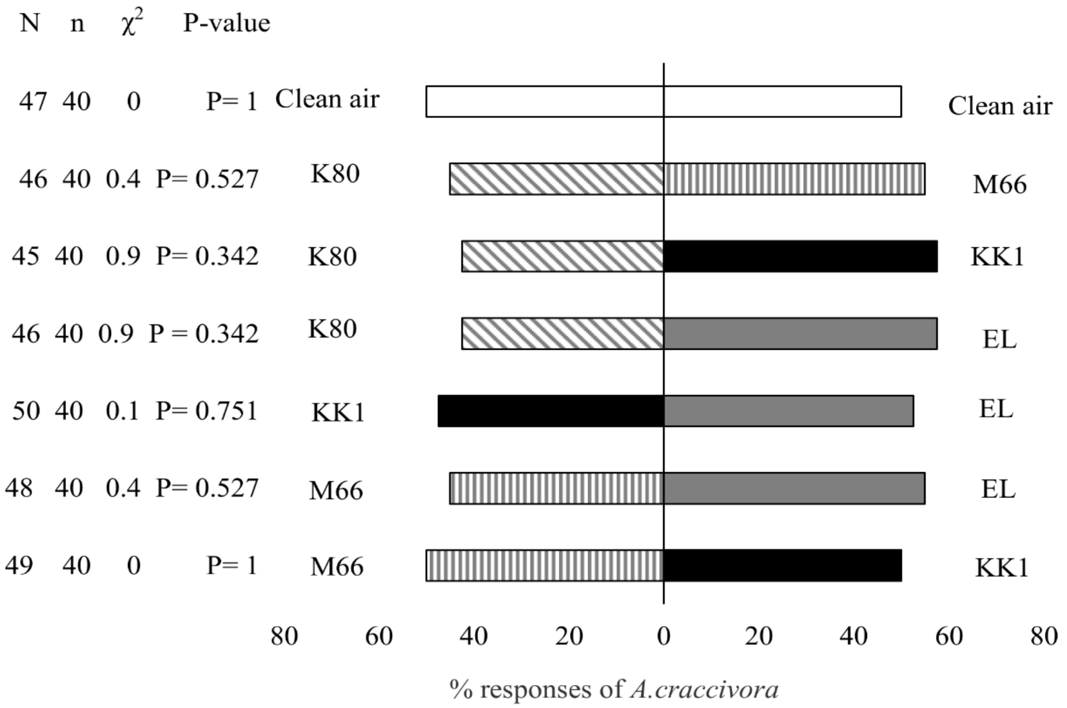

Figure 3. 3: Olfactometer responses of alate *Aphis craccivora* to the odour of undamaged cowpea cultivars in the vegetative stage in pairwise comparison in a Y-tube olfactometer. EL = Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66. N= total number of insects tested. n = total number of insects responded for each treatment was 40 (n = 40). χ^2 tests: * P< 0.05, ** P< 0.01. N = total number of insects tested. n = total number of insects responded for each Treatment were 40 (n=40), (chi-square goodness-of-fit test)

3.5.2 Analysis of cowpea volatiles

Twenty-three compounds belonging to the chemical classes of alcohols, aldehydes, alkanes, benzenoids, esters, ketones, monoterpenoids and sesquiterpenoids were detected in the volatiles of the four cowpea cultivars (Figure 3.4) (Table 3.1). The compounds 1-octen-3-ol and methyl salicylate were absent in the cultivars Machakos 66 and Ken Kunde 1 respectively while*(E)*-*β*-ocimene was only observed in the Ex-Luanda cultivars. Of the

23 compounds, only four were emitted in significantly different amounts among the four cultivars. These included hexanal ($\chi^2 = 8.75$, df = 3, P = 0.030), *(E)*-2-hexenal ($\chi^2 = 9.63$, df = 3, P = 0.020), 1-octen-3-ol ($\chi^2 = 9.06$, df = 3, P = 0.030) and p-xylene ($\chi^2 = 10.87$, df = 3, P = 0.010). The abundance of hexanal in cultivar Katumani 80 was 5, 7 and 8 times more than in cultivars Ex-Luanda, Ken Kunde 1 and Machakos 66 respectively. The abundance of *(E)*-2-hexenal in cultivar Katumani 80 was 9, 30 and 172 times higher than in cultivars Machakos 66, Ex-Luanda and Ken Kunde 1 respectively. The abundance of 1-octen-3-ol in cultivar Ex-Luanda was 3 and 6 times higher than in the cultivars Ken Kunde 1 and Katumani 80 respectively. The abundance of p-xylene in cultivar Ex-Luanda was 2, 4 and 4 times higher in cultivars Ken Kunde 1, Machakos 66, and Katumani 80 respectively. The difference between volatile profiles of cowpea cultivars was explored through a principal component analysis (PCA). The PC1 in the horizontal axis explained 28.60% of the total variance while PC2 in the vertical axis explained about 15.01%. In the score plot, the representation of the four cowpea cultivars based on the emission of volatiles overlapped. The volatile profile of Katumani 80 was close to Ken Kunde 1 but different from Ex-Luanda and Machakos 66 (Figure 3.5 a). In the loading plot, the compounds hexanal and *(E)*-2-hexenal involved in the separation of repellent cultivar Katumani 80 to other cowpea cultivars was correlated (Figure 3.5 b). 1-Octen-3-ol and p-xylene were among the compounds responsible for separating volatiles of attractant cultivar Ex-Luanda to other cowpea cultivars.

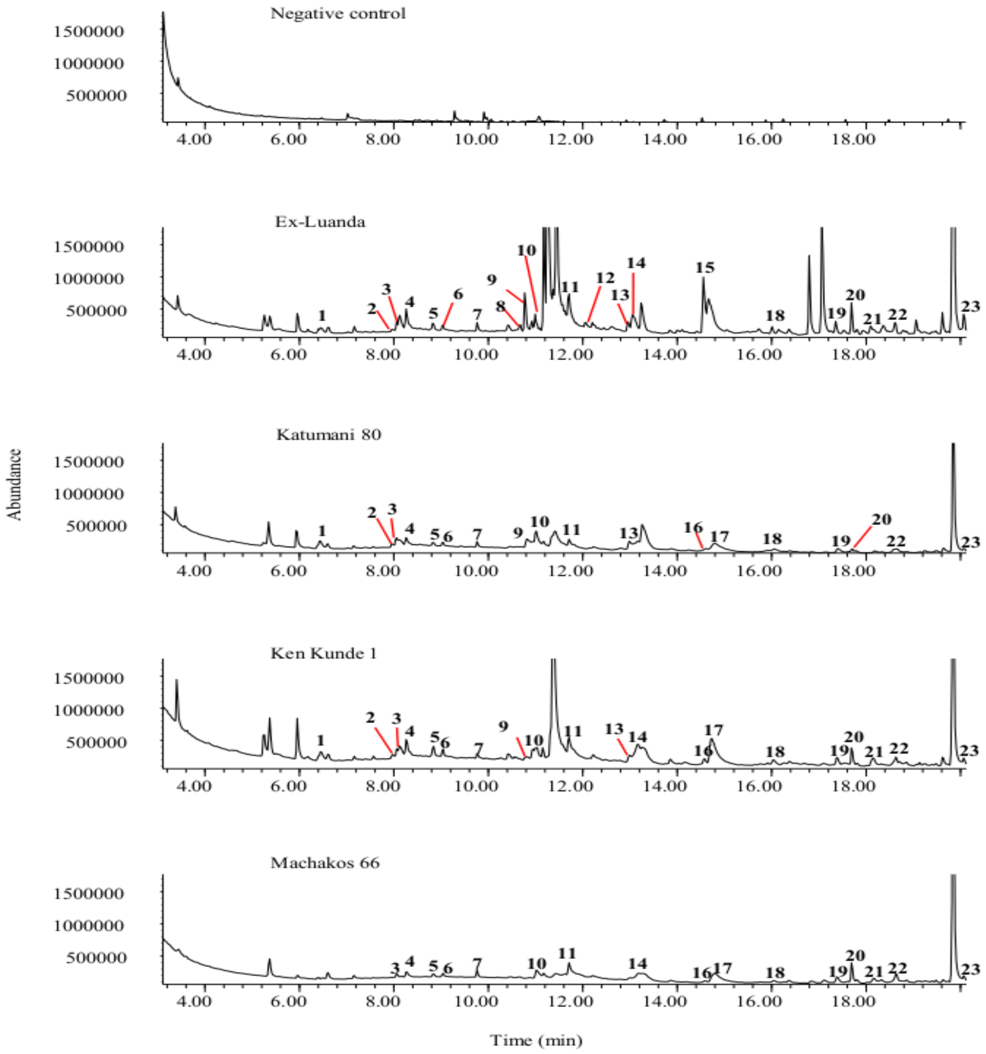

Figure 3. 4: Representative chromatograms of compounds identified in vegetative stage volatiles of four cowpea cultivars, namely Ex-Luanda (EL), Katumani 80 (K80), Ken Kunde 1 (KK1) and Machakos 66 (M66). Peak no: 1, hexanal; 2, *(E)*-2-hexenal; 3, ethylbenzene; 4, p-xylene; 5, o-xylene; 6, nonane; 7, *α*-pinene; 8, *β*-pinene; 9, 1-octen-3-ol; 10, myrcene; 11, limonene; 12, *(E)*-*β*-ocimene; 13, undecane; 14, nonanal; 15, methyl salicylate; 16, dodecane; 17, decanal; 18, tridecane; 19, tetradecane; 20, *α*-cedrene; 21, neryl acetone; 22 , pentadecane; 23, cedrol

Table 3. 1: Volatile compounds emitted by the vegetative parts of four cowpea cultivars: Ex-Luanda (EL), Machakos (M66), Ken Kunde 1 (KK1), Katumani 80 (K80) (Mean ± standard error)

N	Compounds	R. T	Class of compounds	Ex-Luanda, n=4	Machakos 66, n= 4	Ken Kunde 1, n= 4	Katumani 80, n= 4
1	Hexanal[2]	6.46	Aldehyde	2.51 ± 0.91a	1.56 ± 0.74a	1.58 ± 1.16a	11.82 ± 4.56b
2	*(E)*-2-hexenal[2]	7.96	Aldehyde	0.23 ± 0.12a	0.77 ± 0.53ab	0.04 ± 0.04a	6.91 ± 4.57b
3	Ethylbenzene[1]	8.05	Benzenoid	0.92 ± 0.55a	4.36 ± 2.59a	1.54 ± 0.53a	1.83 ± 1.07a
4	p-Xylene[1]	8.26	Benzenoid	11.18 ± 4.1a	2.76 ± 0.94b	6.17 ± 0.59a	2.97 ± 0.79b
5	o-Xylene[1]	8.84	Benzenoid	2.21 ± 0.74a	2.38 ± 0.36a	3.14 ± 0.49a	2.35 ± 0.47a
6	Nonane[2]	9.04	Alkane	4.03 ± 1.83a	1.76 ± 0.68a	2.56 ± 0.17a	3.48 ± 1.28a
7	*α*-Pinene[2]	9.76	Monoterpenoid	2.97 ± 0.58a	5.45 ± 2.15a	2.40 ± 0.54a	3.62 ± 0.69a
8	*β*-Pinene[2]	10.65	Monoterpenoid	0.07 ± 0.07a	3.98 ± 2.94a	0.75 ± 0.46a	0.07 ± 0.07a
9	1-Octen-3-ol[2]	10.79	Alcohol	7.20 ± 3.15a	-	1.18 ± 0.76b	2.49 ± 1.29ab
10	Myrcene[2]	10.99	Monoterpenoid	6.11 ± 1.42a	6.31 ± 2.74a	7.02 ± 2.08a	17.02 ± 6.60a
11	Limonene[2]	11.71	Monoterpenoid	5.31 ± 1.88a	6.86 ± 4.11a	18.88 ± 9.74a	6.97 ± 5.43a
12	*(E)*-*β*-Ocimene[2]	12.06	Monoterpenoid	0.66 ± 0.38a	-	-	-
13	Undecane[2]	12.98	Alkane	0.56 ± 0.56a	0.14 ± 0.13a	0.77 ± 0.47a	0.51 ± 0.51a
14	Nonanal[1]	13.16	Aldehyde	11.90 ± 5.34a	4.44 ± 3.32a	6.65 ± 5.39a	2.76 ± 2.06a
15	Methyl salicylate[1]	14.55	Ester	4.23 ± 4.23a	1.80 ± 1.80a	-	0.41 ± 0.41a
16	Dodecane[2]	14.59	Alkane	0.39 ± 0.23a	0.10 ± 0.10a	1.59 ± 0.71a	0.45 ± 0.28a
17	Decanal[1]	14.75	Aldehyde	11.59 ± 6.72a	6.57 ± 6.48a	18.95 ± 6.78a	8.57 ± 5.92a
18	Tridecane[2]	16.03	Alkane	3.61 ± 1.87a	6.33 ± 2.47a	3.04 ± 1.14a	3.93 ± 1.20a
19	Tetradecane[2]	17.37	Alkane	5.12 ± 1.07a	4.76 ± 1.86a	4.091 ± 1.51a	3.82 ± 1.72a
20	*α*-Cedrene[1]	17.69	Sesquiterpenoid	7.59 ± 3.83a	6.80 ± 2.37a	10.01 ± 2.67a	6.44 ± 3.22a
21	Neryl acetone[1]	18.16	Ketone	3.51 ± 0.42a	2.72 ± 0.91a	1.97 ± 1.02a	3.13 ± 1.31a
22	Pentadecane[2]	18.63	Alkane	6.17 ± 1.80a	10.20 ± 2.09a	5.39 ± 2.30a	8.60 ± 2.59a
23	Cedrol[1]	20.06	Sesquiterpenoid	2.77 ± 1.60a	19.66 ± 16.92a	2.16 ± 1.38a	1.49 ± 0.89a

R. T= retention time (min); - no detected; [1]Identification by mass spectral match; [2]compounds identified by library data and authentic standards. Means followed by the same letter within rows are not significantly different at $p < 0.05$ level, according to the Kruskal-Wallis-test

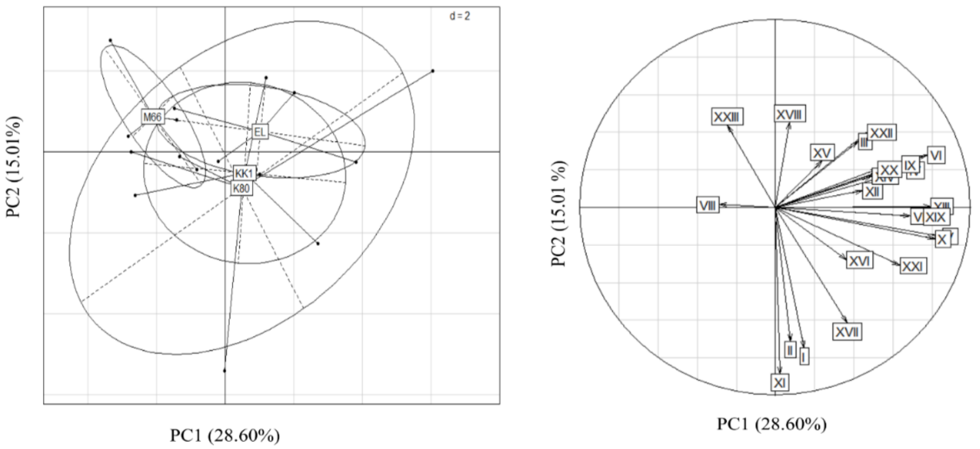

Figure 3. 5: Principal component analysis (PCA) of volatile organic compounds in the undamaged vegetative part of four cowpea cultivars (EL =Ex-Luanda; K80 = Katumani 80; KK1 = Ken Kunde 1; M66 = Machakos 66) – a: score plot with confidence ellipses around each individual, b: Loading plot with arrows showing the direction and intensity of the correlation of variables. Inside the correlation circle, if the arrows are projected in the same direction indicate they are correlated, if they are orthogonal (mean at a 90-degree angle) they are unrelated and if they are pointing in opposite directions, they are negatively correlated. Compound numbers: (I), hexanal; (II), *(E)*-2-hexenal; (III), ethylbenzene; (IV), p-xylene; (V), o-xylene; (VI), nonane; (VII), *α*-pinene; (VIII), *β*-pinene; (IX), 1-octen-3-ol; (X), myrcene; (XI), limonene; (XII), *(E)*-*β*-ocimene; (XIII), undecane; (XIV), nonanal; (XV), methyl salicylate; (XVI), dodecane; (XVII), decanal; (XVIII), tridecane; (XIX), tetradecane; (XX), *α*-cedrene; (XXI), neryl acetone; (XXII), pentadecane; (XXIII), cedrol

Olfactory response of Aphis craccivora to synthetic compounds: No significant differences were found when the treatment combinations (Ex-Luanda + hexanal + *(E)*-2-hexenal) were compared with either the control (clean air) or the cowpea cultivar Ex-Luanda. However, the combination decreased the attractiveness of the insects to the cultivar Ex-Luanda (Figure 3.6). A combination of Katumani 80, 1-octen-3-ol and *p*-xylene showed non-preference ($\chi^2 = 6.4$, df = 1, P = 0.011) by *A. craccivora* compared to clean air (Figure 3.7). In addition, no significant differences were found when the treatment combinations were compared with either the control (clean air) or the cowpea cultivar Katumani 80 (Figure 3.7).

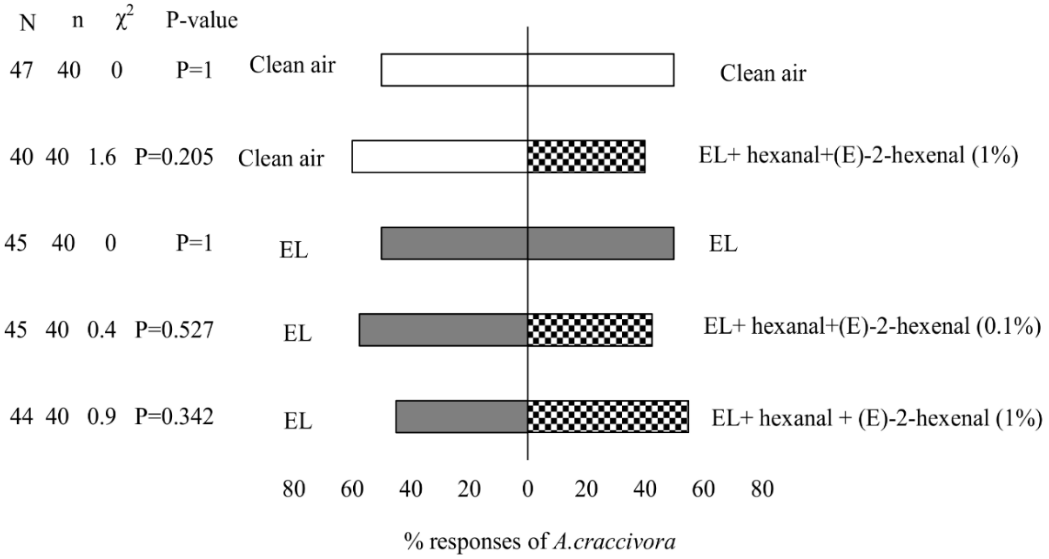

Figure 3. 6: Olfactometer responses of alate *Aphis craccivora* to different tests: Clean air vs. Clean air; Clean air vs. EL + hexanal + *(E)*-2-hexenal (1%); EL vs. EL; EL vs. EL + hexanal + *(E)*-2-hexenal (0.1%); EL vs. EL + hexanal + *(E)*-2-hexenal (1%). EL =Ex-Luanda. N= total number of insects tested. n = total number of insects responded for each treatment was 40 (n = 40). χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test)

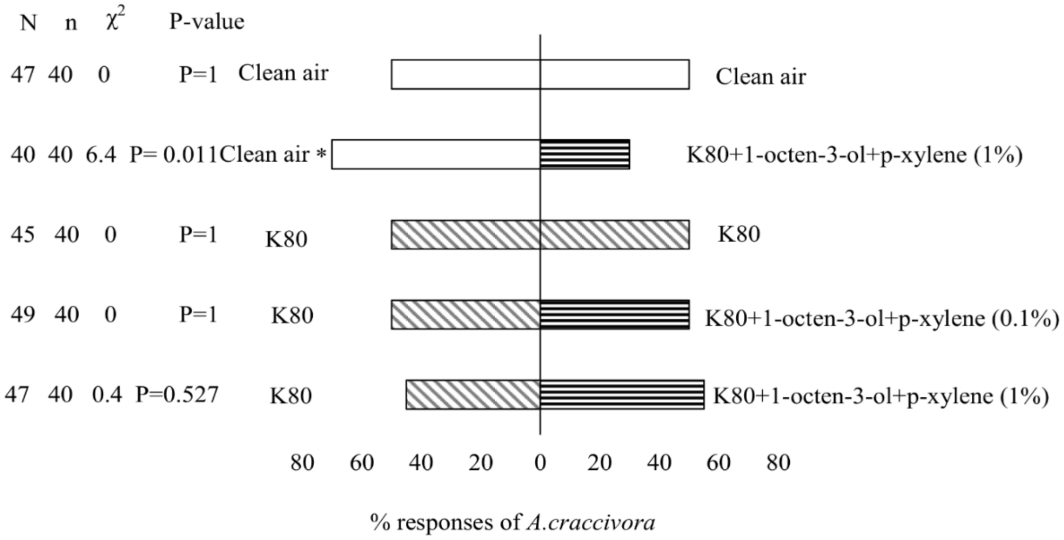

Figure 3. 7: Olfactometer responses of alate *Aphis craccivora* to different tests: Clean air vs. Clean air; Clean air vs. K80 + 1-octen-3-ol + p-xylene (1%); K80 vs. K80; K80 vs. K80+ 1-octen-3-ol + p-xylene (0.1%); K80 vs. K80 + 1-octen-3-ol + p-xylene (1%). K80 = Katumani 80. N= total number of insects tested. n = total number of insects responded for each treatment was 40 (n = 40). χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test)

3.6 Discussion

3.6.1 Behavioural response of *A. craccivora* to cowpea cultivars

In olfactory bioassays, the cultivar Ex-Luanda showed an attractant effect while the cultivar Katumani 80 was repellent to alate *A. craccivora*. The quality of cowpea volatiles and relative amounts of compounds in the volatile profiles were different among the cultivars. Bruce *et al.,* (2005) reported that the insects use the appropriate composition and specific ratio of compounds to recognize their host plant. The attractant cowpea cultivar emits the appropriate composition and specific ratio of compounds used by *A. craccivora* to recognize the host plant while the unattractant cultivar is characterized by the inappropriate composition and ratio of compounds in the volatiles.

3.6.2 Volatile compounds in cowpea cultivars

Through headspace collection and GC-MS analysis, 23 compounds were identified from the four cowpea cultivars. The compounds *(E)*-2-hexenal, *(E)*-*β*-ocimene, *α*-pinene, *β*-pinene, 1-octen-3-ol, myrcene, nonanal, decanal, limonene and *α*-cedrene found in the vegetative stage of cowpea cultivars as have already been reported by Sobhy *et al.,* (2018) and Lwande *et al.,* (1989). In PCA, the overlapping of cultivars volatiles was observed. These overlaps indicated that the volatile profiles of the four cowpea cultivars are quite similar. However, different levels of attraction to *A. craccivora* were observed in the olfactory bioassays. All 23 compounds identified were not present in all cowpea cultivars and their amounts varied in each cultivar. *(E)*-*β*-Ocimene was only observed in the Ex-Luanda cultivars whereas 1-octen-3-ol and methyl salicylate were absent in the cultivars Machakos 66 and Ken Kunde 1 respectively. The quality of volatiles is important in the recognition of host plants by insects. Bruce and Pickett (2011) reported that some compounds in host plant volatiles are more important than others in the recognition of host by insects and the absence of these compounds affect the attraction of insects. On the other hand, the variation in the amounts of compounds in the volatile of cultivars may also explain the differences in the response of *A. craccivora.*

3.6.3 Effect of blend of compounds on behavioural response of *A. craccivora*

In bioassays of standard compounds, the response of *A. craccivora* was neutral when the attractant cowpea cultivar Ex-Luanda was combined with hexanal and *(E)*-2-hexenal relative to clean-air. The reduction in the attractiveness of cowpea cultivar Ex-Luanda volatile could be due to a masking effect by these two compounds. A masking odour interferes with the host detection/localization or decreases the attractiveness of the host by changing the host chemical profile, such compounds are therefore not repellent alone, their effect depends on the context (Schröder & Hilker, 2008; Deletre *et al.*, 2016). Further study needs to be performed for more clarification on both compounds effect in the behavioural response of *A. craccivora.*

Conversely, the attractive cultivar Ex-Luanda was characterised by the compounds 1-octen-3-ol. Despite the presence of the pairs of compounds 1-octen-3-ol and p-xylene with the unpreferred Katumani 80 cultivar, *A. craccivora* was not attracted. Bendera *et al.*, (2015) showed that 1-octen-3-ol was the main compound in undamaged cowpea cultivar Ex-Luanda and was emitted at night. In the study, the collection of volatile of cowpea cultivars was performed for 24 h and perhaps 1-octen-3-ol could have been abundantly emitted within the cycle at night. Aphids are more active in the daytime (Webster *et al.*, 2010) and this could be the reason why 1-octen-3-ol had no effect on the attraction of *A. craccivora* in the study. However, the attractiveness of 1-octen-3-ol has been reported on different insects. Bendera *et al.*, (2015) showed that 1-octen-3-ol emitted by Ex-Luanda cultivar increased attractiveness and coupling in *Maruca vitrata*. Moreover, the synergy between 1-octen-3-ol and carbon dioxide has been reported to be attractive to several mosquito species (Kline, 1994). The compound p-xylene was also abundant in the attractive cowpea cultivar Ex-Luanda. To the best of our knowledge, few studies have reported the attractiveness of p-xylene on the behaviour of aphids. In the study, p-xylene was not attractive to *A. craccivora* in olfactory tests.

3.6.4 Choice of *A. craccivora* in pairwise comparison and preference assays

The pairwise comparison of the cultivars in the Y-tube olfactometer showed an absence of behavioural response of *A. craccivora* to odours of the cowpea. The difference of the ratio of compounds in the volatiles of undamaged cowpea cultivars might be too low or masked by other compounds, making it difficult for *A. craccivora* to detect in pairwise comparisons. Similar results have been observed in the olfactory tests of other pests. For example, tomato Red spider mite *Tetranychus evansi* Baker and Pritchard females did not show a preference for any volatiles from three undamaged African nightshade species in pairwise comparison (Murungi *et al.*, 2016). Aphids also use visual cues in the search for a host plant (Döring, 2014). Aphids can combine visual and olfactory cues to locate their host plants (Döring, 2014). However, in the preference assay involving visual, olfactory and physical contact, *A. craccivora* did not prefer any cowpea cultivars. The short distance between plants might have led to the blend of volatiles of the different cowpea cultivars and consequently prevented/reduced the choice of *A. craccivora*.

3.7 Conclusion

The results of this study show that the attractiveness of phytophagous alate *A. craccivora* differs among cultivars belonging to the same species. The cowpea cultivar Ex-Luanda appeared to be more attractive than the cultivar Katumani 80 according to the plant odour. The presence of hexanal and *(E)*-2-hexenal reduced the attractiveness of Ex-Luanda cultivar in the olfactometer. The negative behavioural response of *A. craccivora* to repellent cultivar Katumani 80 despite the presence of 1-octen-3-ol and p-xylene suggest that *A. craccivora* was not attracted by these compounds. These results suggest that the VOCs from undamaged cowpea cultivars can influence attractiveness of the host plant to *A. craccivora*. The identification of attractant and repellent cowpea cultivars in the present study could be useful in the management of *A. craccivora* through push-pull strategies. Further investigations need to evaluate the application of blend of compounds hexanal and *(E)*-2-hexenal on susceptible cowpea cultivars in order to reduce the attraction of *A. craccivora* in IPM strategies.

CHAPTER FOUR

BEHAVIOURAL RESPONSE OF BEAN FLOWER THRIPS, *MEGALUROTHRIPS SJOSTEDTI* (TRYBOM) (THYSANOPTERA: THRIPIDAE) TO VEGETATIVE AND FLORAL VOLATILES FROM DIFFERENT COWPEA CULTIVARS

4.1 Abstract

Bean Flower Thrips (*Megalurothrips sjostedti)* is a key pest of cowpea (*Vigna unguicalata*) in Africa. To better understand the interaction of *M. sjostedti* to cowpea cultivars to improve management efforts, repellent properties of volatiles of four cowpea cultivars, namely Ex- Luanda, Machakos, Ken Kunde 1 and Katumani 80 at different phenological stages were investgated. Bioassays were conducted to study host preference and gas chromatography-mass spectrometry for chemical analysis of volatiles. The results showed no significant preference of females *M. sjostedti* for any cowpea cultivars tested in preference assays. However, in olfactometer, the volatiles emitted during the vegetative stage of only Ex-Luanda, Machakos and Katumani 80 cultivars were repellent to females, while only Ken Kunde 1 was repellent to males. Volatiles from flowers of Ken Kunde 1 were attractive to females, whereas volatiles from the flowers of Katumani 80 were repellent, respectively. Ex-Luanda and Machakos elicited neutral response. Flowers of Machakos 66 and Ex-Luanda cultivars were repellent to males, while Katumani 80 and Ken Kunde 1 were neutral. The volatile analysis showed that *(E)*-*β*-ocimene and 1-octen-3-ol were unique to the volatile profile of Katumani 80 flowers. Previous study showed *(E)*-2-hexenal and hexanal were only abundant in the vegetative stage of Katumani 80. *(E)*-2-hexenal was repellent to the females at a concentration of 0.01% but not at 1%. Hexanal, *(E)*-*β*-ocimene and 1-octen-3-ol elicited a neutral response from females at 0.01% and 1%. The present study indicates that *(E)*-2-hexenal could be useful in the development of semiochemical-based repellent tools for *M. sjostedti* management.

4.2 Introduction

Bean Flower Thrips (BFT), *Megalurothrips sjostedti*, is widespread throughout sub-Saharan Africa and causes significant yield loss to leguminous crops between 20% to 100% (Mfuti *et al.,* 2017). Bean flower thrips mainly attack the flowering stage of cowpea leading to premature flower drop, hence reduced yield (Abtew, 2015). Synthetic pyrethroids and organophosphates are mostly used to control *M. sjostedti* in Africa (Abtew, 2015). These groups of chemicals are known to be toxic to farmers and consumers, pollute the environment and kill beneficial insects (Abtew, 2015). Further, several thrips species are known to have developed resistance to pyrethroid and organophosphorus insecticides (Herron & James, 2005; Thalavaisundaram *et al.,* 2008). Various alternative methods have been developed for BFT management, such as maize-cowpea intercropping which reduce light intensity in the cowpea canopy, thus the density of *M. sjostedti* on cowpea (Kyamanywa & Ampofo, 1988), blue sticky traps with kairomone attractants for effective monitoring (Muvea *et al.*, 2014), use of biopesticides (Ekesi *et al.,* 1998b; Mfuti *et al.*, 2017), and autoinoculation devices with entomopathogenic fungus *Metarhizium anisopliae* Metchnikoff (Mfuti *et al.*, 2016). More recently aggregation pheromones of BFT have been identified (Niassy *et al.,* 2019), however efforts to identify repellents for BFT are scarce (Abtew, 2015).

Plants emit volatiles to defend themselves, directly by repelling herbivorous insects or indirectly by attracting the natural enemies of insect pests (Dudareva *et al.,* 2006). In crop plants, the composition of the volatiles and magnitude of emission can vary amongst cultivars and within each cultivar depending on the developmental stage and plant part (Wright et *al.*, 2005; Himanen *et al.,* 2017), with consequences for plant defence against insect pests (Schröder *et al.*, 2015). Green leaf volatiles (GLVs) play a significant role in defence of plants against herbivorous insects and pathogens (Scala *et al.*, 2013) and could be used as repellents in pest management (Chen *et al.,* 2015). Green leaf volatiles often consist of compounds with six carbons (C6) as backbone, including aldehydes, alcohols and esters (Matsui, 2006). These compounds are produced either constitutively or by induction after wounding or stress (Shiojiri *et al.,* 2006).

Using olfactory assays, Ekesi *et al*., (1998a) showed that female *M. sjostedti* were not attracted to flowers of cowpea variety IT84S-2246 than to three other varieties (ICV 2, ICV 8, TVx 3236). However, the compounds responsible for the unattractive effect were unidentified. Intact cowpea plants emit GLVs such as hexanal and *(E)*-2-hexanal (Lwande *et al.,* 1989; Sobhy *et al.,* 2018). A repellent phenomenon can be a movement away from an odour source (true repellent) and/or an inability to find the host (Deletre *et al*., 2016). Odour masking is a type of repellent action where an odour that does not have an effect when applied alone can interfere with host detection or reduce the attractiveness of the host (Schröder & Hilker, 2008, Deletre *et al.,* 2016). Hence, the aim was to identify candidate repellent compounds from cowpea cultivars that could be incorporated in the management of *M. sjostedti* by reducing its contact with the host. The use of such repellent host plant volatiles could reduce the use of synthetic chemicals for the management of *M. sjostedti.*

The hypothesis is that volatiles emitted by the vegetative stage and flowers of cowpea cultivars elicit different behavioural responses in *M. sjostedti* adults (male and female) and that specific compounds are responsible for repellence of *M. sjostedti* adults from repellent cowpea cultivars. The hypothesis was tested by (i) evaluating the behavioural response of male and female *M. sjostedti* to four cowpea cultivars at the vegetative stage and flowers in a Y-olfactometer assay; (ii) identifying the volatiles emitted by the cowpea cultivars using GC-MS; and (iii) evaluating the repellent effect of specific compounds that were either abundant or unique to non-attractive cowpea cultivars.

4.3 Materials and methods

4.3.1 Raising of plants

The raising of cowpea was the same described in chapter 3. However, the cowpea plants with open flowers (eight-week-old) were used for the experiments.

4.3.2. Rearing of *M. sjsostedti*

The insects, *M. sjostedti*, were reared at the Arthropod Rearing and Containment Unit (ARCU), icipe, at a temperature of 26 ± 2 °C, with 50–60% relative humidity and a 12 L: 12 D photoperiod. They were reared in ventilated jars with thrips-proof nets and filter paper covers to prevent the insects from escaping. French bean pods, *Phaseolus vulgaris* L., were used for feeding the thrips, as previously described in the rearing of the western flower thrips, *Frankliniella occidentalis* (Nyasani *et al.*, 2013). Adult thrips were allowed to lay eggs on the pods. Two days later, the pods containing eggs were removed and transferred to clean jars. Each jar contained ten bean pods. Freshly emerged males (≈10 days post-oviposition) and females (≈12 days post-oviposition) were used for the experiments. Males and females were identified morphologically based on their robustness and thickness of the abdomen (Sani & Umar, 2017).

4.3.3 Preference assay

Choice assays were conducted in the screenhouse to compare the preference of female thrips among four cowpea cultivars at two phenological stages (vegetative and flowering). Males were not tested, because they are difficult to see and count on the plants. One potted plant for every cowpea cultivar was placed in the same cage (80 cm × 50 cm × 40 cm) randomly in a square pattern with a distance of 26 cm between plants (KK1, M66, K80 and EL). One hundred female thrips were released one hour later at the centre of the cage with the four cultivars. After 24 h, adult thrips were visually inspected amongst the plants and total numbers on each cowpea plant (cultivar) recorded. The position of the cultivars was changed after each replicate (batch of 100 insects) to avoid bias. Twelve replicates were conducted for both the vegetative and flowering stage.

4.3.4 Olfactory assay with undamaged cowpea plants

Bioassays were carried out using a Y-tube olfactometer (internal diameter 0.5 cm, stem length 4 cm, arm length 5.5 cm) to evaluate the behavioural response of male and female *M. sjostedti* to volatiles emitted during the vegetative stage and flowers of the four cowpea cultivars in the laboratory at *icipe* (temperature 25 ± 1 °C; 40–50% relative humidity).

The Y-tube was positioned at an angle of 25° to the horizontal plane (Koschier *et al.*, 2000), covered with a cardboard box (50 cm × 48 cm × 33 cm) that was opened at the top. The olfactometer arena was illuminated from above by a 60 lux light source. Compressed air from a pump (KnF, Laboport, Legallais) was purified by passing it through an activated charcoal filter and humidified by passing it through a conical flask glass containing distilled water. Cleaned air was drawn through two flow meters at a rate of 60 ml/min, then passed through two polyester (Nalophan) bags (38 cm × 25 cm) (cooking bag, Chevalier diffusion-F33890 Pessac sur Dordogne, Belgium) containing the odour sources connected to each arm of the olfactometer. The odour source was either a cowpea plant in vegetative stage placed in the polyester (Nalophan) bag and held tightly around the stem, or a single intact flower placed in the bag and held tightly around the peduncle. The arms of the Y tube were connected to each odour source with PTFE tubing (inner diameter 4 × 6 mm). For the Y-tube choice olfactometer assays, (a) vegetative stage of each cultivar was tested against clean air, (b) an open flower of each cultivar was tested against clean air, (c) the vegetative stages of the cultivars were tested against each other in pairwise sets, and (d) open flowers of the cultivars were tested against each other in pairwise sets. A single insect (male or female) was placed at the stem inlet of the Y-tube using a soft hairbrush and was observed for 3 min. If the insect failed to choose within 3 min, it was considered non-responsive. Conversely, when the insect reached the far end of one arm, it was recorded as a choice. The Y-olfactometer was cleaned with 70% ethanol after testing each insect to remove possible traces of pheromones or contaminants. A one-minute interval was observed between each insect tested to enable the evaporation of ethanol in Y tube. To avoid any bias, the Y-tube was alternated, and the plant materials were connected to the opposite arm after five male and female insects had been tested, while particular plant materials were replaced after ten males and females had been tested. Sixty males and females were tested per treatment. Every day, at the end of bioassays, the Y-tube was cleaned with 70% alcohol, then rinsed with distilled water and dried overnight in an oven at 100 °C. The polyester bags were baked overnight at 100 °C.

4.3.5 Collection of volatiles

Same procedure of collect of volatile as in Chapter 3. Here, volatiles from the flowers were sampled by covering a single flower of an intact plant with an oven bag and held tightly around the peduncle with a rubber band.

4.3.6 Analysis of volatiles

Same procedure of analysis of volatiles as in Chapter 3. However, here, the retention times of compounds and mass spectra were compared to those of authentic standards. The retention indices of compounds were calculated relative to n-alkane standards (C8-30) and compared with library data in the National Institute of Standards and Technology (NIST), corresponding to retention indices in the literature (RIs obtained on HP-5 columns).

4.3.7 Olfactory assay with synthetic standards

The same Y-tube olfactometer was used as described above (Olfactory assays with intact cowpea plants) to determine the repellent effect of compounds emitted either in significantly different quantities in the vegetative stage or unique in the flowers of the single repellent cowpea cultivar. In previous work, the compounds *(E)*-2-hexenal and hexanal were more abundant in the vegetative stage of the repellent cultivar K80 than in other cultivars (M66, EL, KK1) (Diabate *et al.,* 2019a). *(E)-β*-Ocimene and 1-octen-3-ol were unique to the repellent flowers of K80 and thus selected for this experiment. Synthetic compounds identified from vegetative stage and flowers (*(E)*-2-hexenal, hexanal, *(E)-β*-ocimene, 1-octen-3-ol) were tested either alone or added to a bag containing a single cowpea flower. Since volatiles from flowers of cultivar KK1 were attractive to *M. sjostedti* females, these flowers were combined with specific compounds from a single repellent cultivar to evaluate the potential of these compounds to decrease the attractiveness of attractant cowpea flowers to *M. sjostedti.* Each compound or the blend of *(E)*-2-hexenal and hexanal were prepared at concentrations of 0.01% and 1% (v/v) diluted in dichloromethane. The blend of compounds was formulated to simulate the natural ratio occurring in the repellent cultivar. Blend was composed of *(E)*-2-hexenal and hexanal with a ratio (1:2) as observed in vegetative stage of cultivar K80. A 50-µl aliquot was placed on filter paper and left for 30s at ambient temperature (25 ± 1°C). The filter paper was then placed in an odour source chamber. The negative control consisted of 50 µl dichloromethane. Olfactory responses of female *M. sjostedti* were tested on (a) each specific compound versus a negative control, (b) each specific compound + an open cowpea flower var. KK1 versus cowpea flower var. KK1 alone, (c) the blend of *(E)*-2-hexenal and hexanal versus a negative control, and (d) the blend of *(E)-2*-hexenal and hexanal + cowpea flower var. KK1 versus cowpea flower var. KK1 alone. The filter papers were replaced every 60 min. Each female tested represented one replicate and each treatment comprised 60 tested females. The males were not repelled by volatiles of K80, repellent cultivar; hence, they were not used for the tests with synthetic standards.

4.3.8 Synthetic standards

Authentic standards of hexanal (purity 98%), *(E)*-2-hexenal (purity 98%), 1-octen-3-ol (purity ≥ 98%), ocimene (mixture of isomers, stabilized, ≥ 90%) (*(E)-β*-ocimene was not commercially available) were purchased from Sigma-Aldrich, France. Hexane (purity ≥ 95%), 2-hexadecanol (purity 99%) and dichloromethane (purity ≥ 99%) were purchased from Sigma Aldrich, UK. Ethanol (purity ≥ 99.8%) was purchased from Sigma-Aldrich, Germany.

4.4 Statistical analysis

Same procedure of statistical analysis of the behavioural assays and volatiles as in Chapter 3.

4.5 Results

4.5.1 Behavioural response of *M. sjostedti* males and females to cowpea volatiles at the vegetative and flowering stages

Megalurothrips sjostedti females showed no preference for any cultivars at either the vegetative or the flowering stages (vegetative: $\chi^2 = 1.51$, df = 3, P = 0.67, flowering: $\chi^2 = 5.96$, df = 3, P = 0.11) (Figure 4.1).

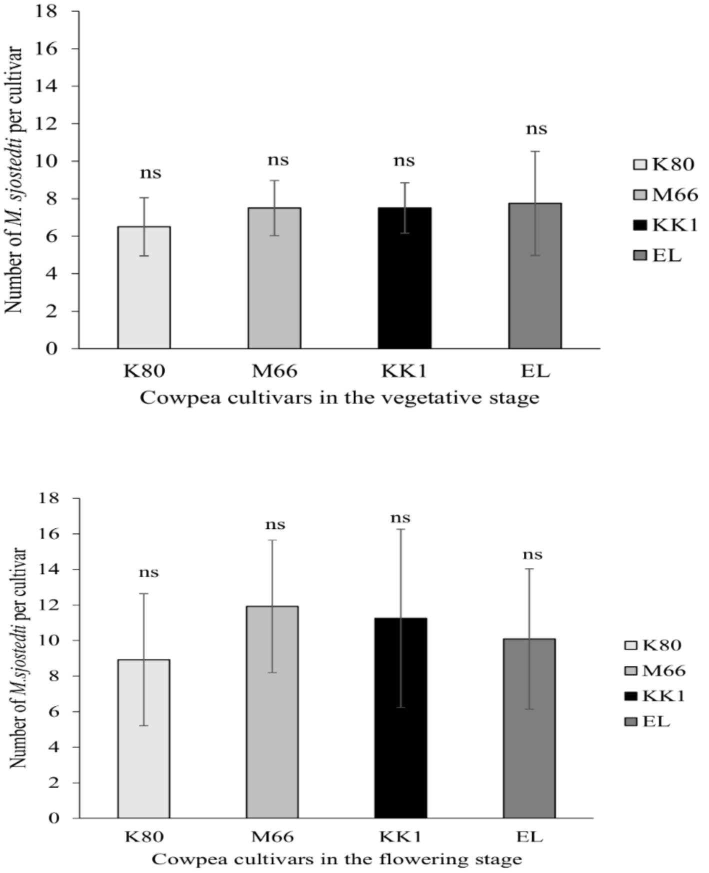

Figure 4. 1: Number (Mean ±SE) of *Megalurothrips sjostedti* for different cowpea cultivars a) in the vegetative stage and b) flowering stage over a period of 24 hours. The total number of insects tested for each replicate was 100 (N=100). EL =Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66. Vertical bars followed by the same letter are not significantly different (chi-square goodness-of-fit test).

Volatiles from vegetative stages of cultivars EL, M66 and K80 were repellent to females compared to clean air (EL: $\chi^2 = 6.66$, df = 1, $P < 0.01$; M66: $\chi^2 = 8.64$, df = 1, $P < 0.01$; K80: $\chi^2 = 9.93$, df = 1, $P < 0.01$), whereas responses to the cultivars KK1 were neutral ($\chi^2 = 0.30$, df = 1, $P = 0.57$). Males were repelled by the cultivar KK1 compared to clean air ($\chi^2 = 6$, df = 1, $P = 0.01$) and showed neutral responses to volatiles of the other cultivars ($P > 0.05$) (Figure 4.2). In pairwise comparisons, male and female *M. sjostedti* failed to distinguish between the odour of cowpea cultivars at the vegetative stage ($P > 0.05$) (Figure 4.3).

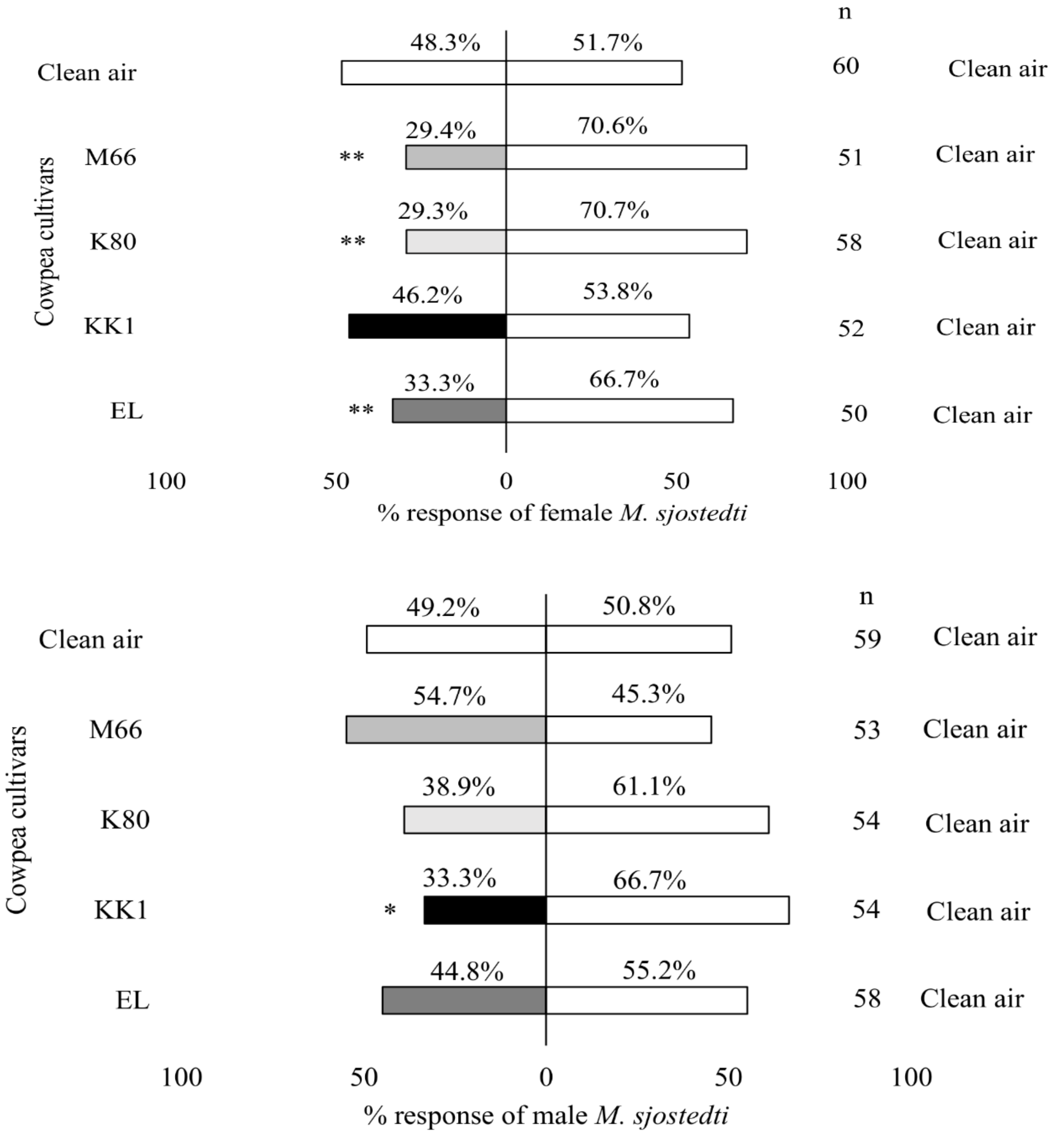

Figure 4. 2: **Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from vegetative stage of cowpea cultivars versus clean air. EL =Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66 ; Flower KK1 = *Vigna unguiculata* var. Ken Kunde 1. Total number of insects tested was 60 (N = 60). n= total number of insects responded for each treatment. χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test).**

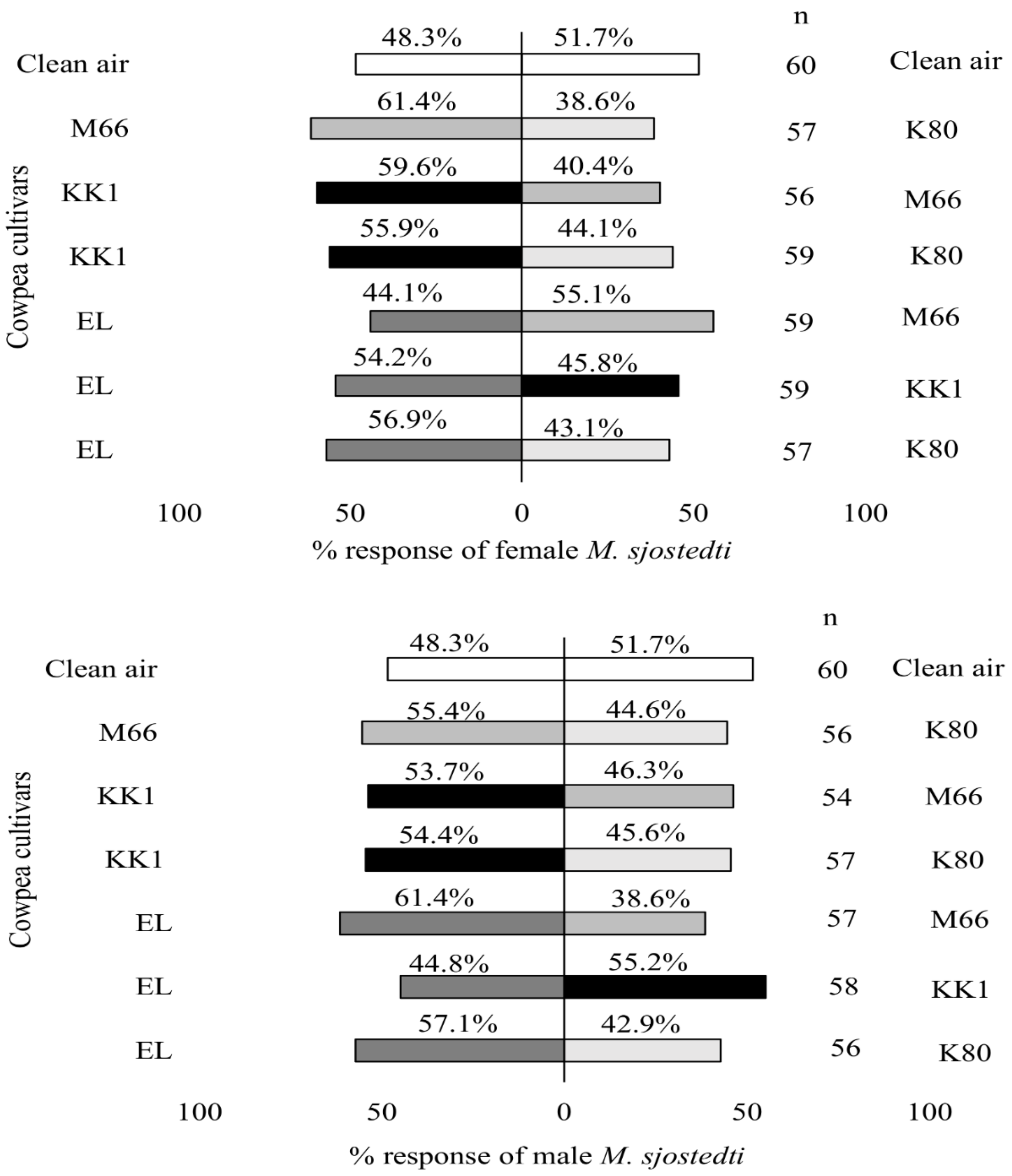

Figure 4. 3: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from vegetative stage of cowpea cultivars in pairwise comparison. EL = Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66 ; Flower KK1 = *Vigna unguiculata* var. Ken Kunde 1. Total number of insects tested was 60 (N = 60). n= total number of insects responded for each treatment. χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test).

Volatiles from flowers of cultivar KK1 and K80 were significantly attractive (χ^2 = 4.41, df = 1, P = 0.03) and repellent (χ^2 = 4.26, df = 1, P = 0.03) to the females, respectively, when, compared to clean air. Males were repelled by the flower volatiles of cultivars EL and M66 compared to clean air (EL, χ^2 = 8.96, df = 1, P = P < 0.01) (M66, χ^2 = 7.14, df = 1, P < 0.01) and showed a neutral response to the flower volatiles of the other cultivars (P > 0.05) (Figure 4.4). Male and female *M. sjostedti* did not show a significant preference for any of the odours emitted by the flowers of the cultivars in pairwise comparisons (P > 0.05) (Figure 4.5).

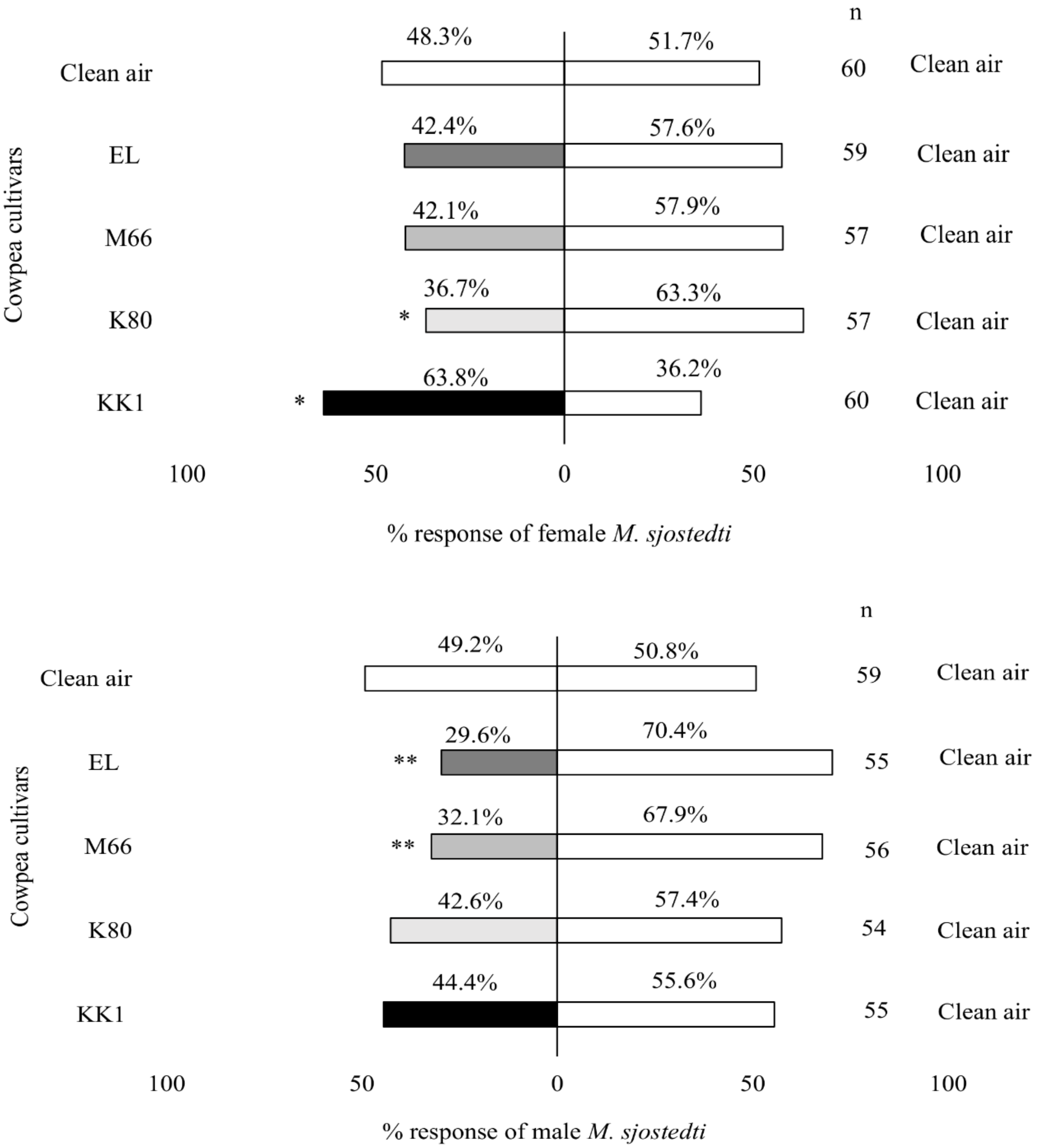

Figure 4. 4: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from flowering stages of cowpea cultivars versus clean air. EL =Ex-Luanda ; K80 = Katumani 80 ; KK1 = Ken Kunde 1 ; M66 = Machakos 66 ; Flower KK1 = *Vigna unguiculata* var. Ken Kunde 1. Total number of insects tested was 60 (N = 60). n= total number of insects responded for each treatment. χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test).

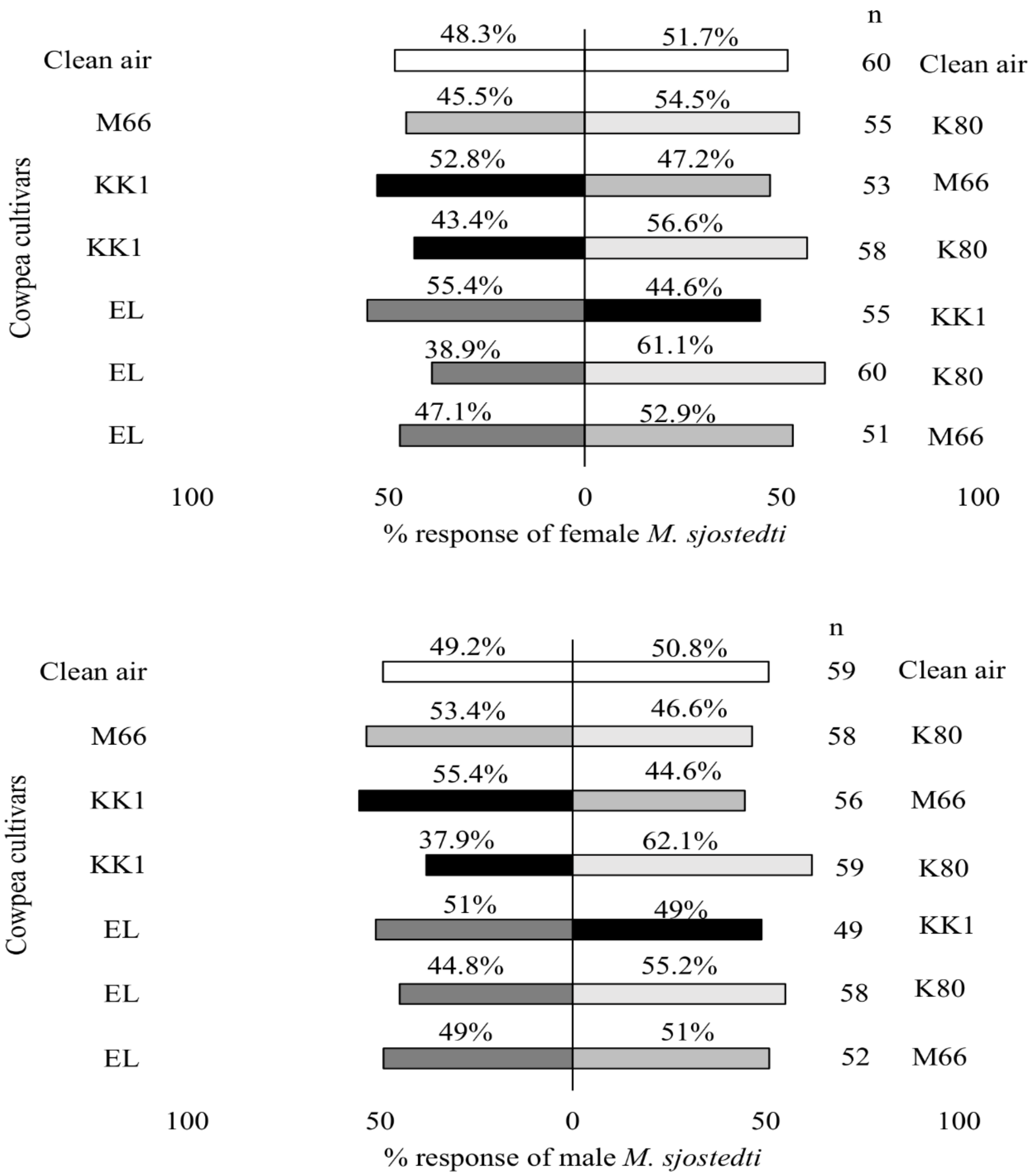

Figure 4. 5: Percentage (%) responses of *Megalurothrips sjostedti* a) female and b) male to the odour from flowers of cowpea cultivars in pairwise comparison. EL = Ex-Luanda; K80 = Katumani 80; KK1 = Ken Kunde 1; M66 = Machakos 66; KK1 flower = *Vigna unguiculata* var. Ken Kunde 1. Total number of insects tested was 60 (N = 60). n= total number of insects responded for each treatment. χ^2 tests: * P< 0.05, ** P< 0.01, (chi-square goodness-of-fit test).

4.5.2 Analysis of cowpea volatiles emitted by the cowpea cultivars

Twenty volatile compounds were identified in the flowers of the four cowpea cultivars (Table 4.1). Eighteen compounds were identified in the flowers of K80 and KK1, whilst 15 compounds were in M66 and EL. These were composed of one alcohol (1-octen-3-ol), three aldehydes (hexanal, nonanal, decanal), six alkanes (nonane, undecane, dodecane, tridecane, tetradecane, pentadecane), three benzenoids (ethylbenzene, p-xylene, o-xylene), one ketone (neryl acetone), four monoterpenoids (*α*-pinene, *β*-pinene, limonene, *(E)*-*β*-ocimene) and two sesquiterpenoids (*α*-cedrene, cedrol). Decanal and cedrol were not detected in the cultivars EL and K80. Undecane was not observed in EL. Pentadecane and neryl acetone were absent in K80 while hexanal was absent in M66. *(E)*-*β*-Ocimene and 1-octen-3-ol were detected in flowers of cultivar K80, but not in other cowpea cultivar flowers. The relative amounts of flower compounds did not significantly differ among the cultivars. In PCA, the volatile profiles of flower of the four cowpea cultivars based on the emission of volatiles overlapped in score plot (Figure 4.6). However, the difference of volatile profiles appeared among the four cultivars. In the loading plot, the compounds *(E)*-*β*-ocimene and 1-octen-3-ol contribute to the separation of repellent cultivar K80 from other cowpea cultivars.

Table 4.1: Estimate amounts of volatile compounds (percentage) emitted by the flowers of the four cowpea cultivars (Mean ± standard error)

R. I	LRI	Compound	Ex-Luanda, n = 4	Machakos 66, n = 4	Ken Kunde 1, n = 4	Katumani 80, n = 4
802	801 a	Hexanal[2]	0.43 ± 0.43	-	0.32 ± 0.32	0.92 ± 0.55
863	857 b	Ethylbenzene[1]	2.77 ± 0.75	5.32 ± 2.23	1.75 ± 1.34	1.61 ± 1.06
870	875.8 c	p-Xylene[1]	7.31 ± 2.11	14.37 ± 5.72	16.97 ± 10.34	14.72 ± 7.45
892	894 d	o-Xylene[1]	3.02 ± 1.12	6.82 ± 2.45	3.05 ± 1.15	3.56 ± 1.34
900	900 b	Nonane[2]	3.00 ± 0.99	5.89 ± 2.67	1.41 ± 1.38	0.71 ± 0.64
934	934 e	*α*-Pinene[2]	4.12 ± 1.02	16.44 ± 8.22	4.83 ± 2.97	19.61 ± 18.40
976	979 d	*β*-Pinene[2]	1.51 ± 0.90	3.82 ± 1.46	1.18 ± 0.65	1.82 ± 1.57
982	981 f	1-Octen-3-ol[2]	-	-	-	0.14 ± 0.14
1030	1029 d	Limonene[2]	12.00 ± 8.06	3.40 ± 1.74	4.81 ± 1.85	0.21 ± 0.21
1049	1048 d	*(E)*-*β*-Ocimene[2]	-	-	-	0.02 ± 0.02
1100	1100 b	Undecane[2]	-	0.08 ± 0.08	1.76 ± 1.60	5.06 ± 3.91
1111	1107 g	Nonanal[2]	17.22±6.47	12.04 ± 7.23	12.95 ± 7.23	7.62 ± 3.15
1200	1200 b	Dodecane[2]	0.04 ± 0.04	1.34 ± 7.23	1.81 ± 1.14	1.05 ± 0.61
1211	1207 f	Decanal[1]	-	-	12.38 ± 11.65	1.07 ± 0.80
1300	1300 b	Tridecane[2]	3.28 ± 3.28	4.97 ± 2.19	15.79 ± 10.00	6.84 ± 5.05
1400	1400 b	Tetradecane[2]	6.84 ± 1.42	7.55 ± 2.71	5.51 ± 2.38	8.69 ± 7.77
1425	1419 f	*α*-Cedrene[1]	13.57 ± 7.46	12.45 ± 2.64	17.50 ± 9.97	14.34 ± 9.21
1463	1445 g	Neryl acetone[1]	0.37 ± 0.37	0.72 ± 0.72	0.39 ± 0.39	-
1500	1500 b	Pentadecane[2]	5.42 ± 5.05	1.11 ± 1.11	3.95 ± 2.72	-
1620	1611 h	Cedrol[1]	-	-	1.52 ± 0.99	2.12 ± 1.01

[1]Identification by mass spectral match; [2]compounds identified by library data and authentic standards; LRI = retention index found in the literature from HP-5MS column: [a]Lopes *et al.*, (2004), [b]Kotowska *et al.*, (2012), [c]Wang and Fingas (1995), [d]Quijano *et al.*, (2007), [e]Benkaci-Ali *et al.*, (2007), [f]Flamini *et al.*, (2006), [g]Saroglou *et al.*, (2006), [h]Radulovic *et al.*, (2010). R. I., Retention indices calculated relative to n-alkanes C8-C30; tr, trace < 0.005; - not detected.

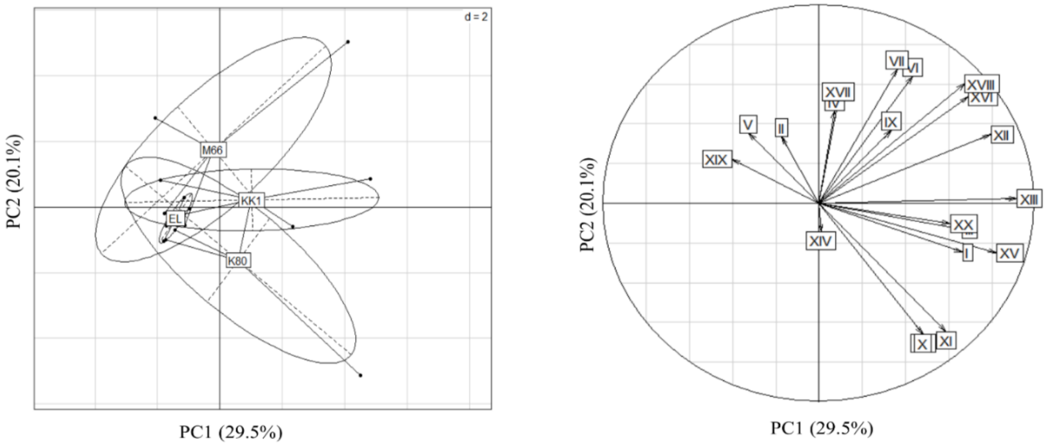

Figure 4. 6: **Principal component analysis (PCA) of volatiles emitted in the flowers of the four cowpea cultivars. Score plot with the cultivars M66, Machakos 66; KK1, Ken Kunde 1; K80, Katumani 80 and EL, Ex-Luanda. Loading plot with arrows showing the direction and intensity of the correlation of 20 compounds. Compound numbers correspond to (I), hexanal; (II), ethylbenzene; (III), p-xylene; (IV), o-xylene; (V), nonane; (VI), *α*-pinene; (VII), *β*-pinene; (VIII), 1-octen-3-ol; (IX), limonene; (X), *(E)-β*-ocimene; (XI), undecane; (XII), nonanal; (XIII), dodecane; (XIV), decanal; (XV), tridecane; (XVI), tetradecane; (XVII), *α*-cedrene; (XVIII), neryl acetone; (XIX), pentadecane; (XX), cedrol**

4.5.3 Behavioural response of *M. sjostedti* female to synthetic compounds that were either abundant or unique to non-attractive cowpea cultivars

The females were significantly repelled by *(E)*-2-hexenal at 0.01% ($\chi^2 = 5.81$, df = 1, P = 0.01), but not at 1% ($\chi^2 = 1.08$, df = 1, P = 0.29), when compared to clean air (Figure 4.7). The females were repelled by the blend of *(E)*-2-hexenal and hexanal compared to clean air and by the combination of cowpea flower var. KK1 + *(E)*-2-hexenal relative to cowpea flower var. KK1 alone at 0.01% (blend: $\chi^2 = 8.96$, df = 1, P < 0.01; combination: $\chi^2 = 10.79$, df = 1, P < 0.01). The same blend and combination elicited a neutral response to the females at 1%. Hexanal, *(E)-β*-ocimene and 1-octen-3-ol failed to affect the thrips' behaviour, even in combination with cowpea flower var. KK1 (Figure 4.8).

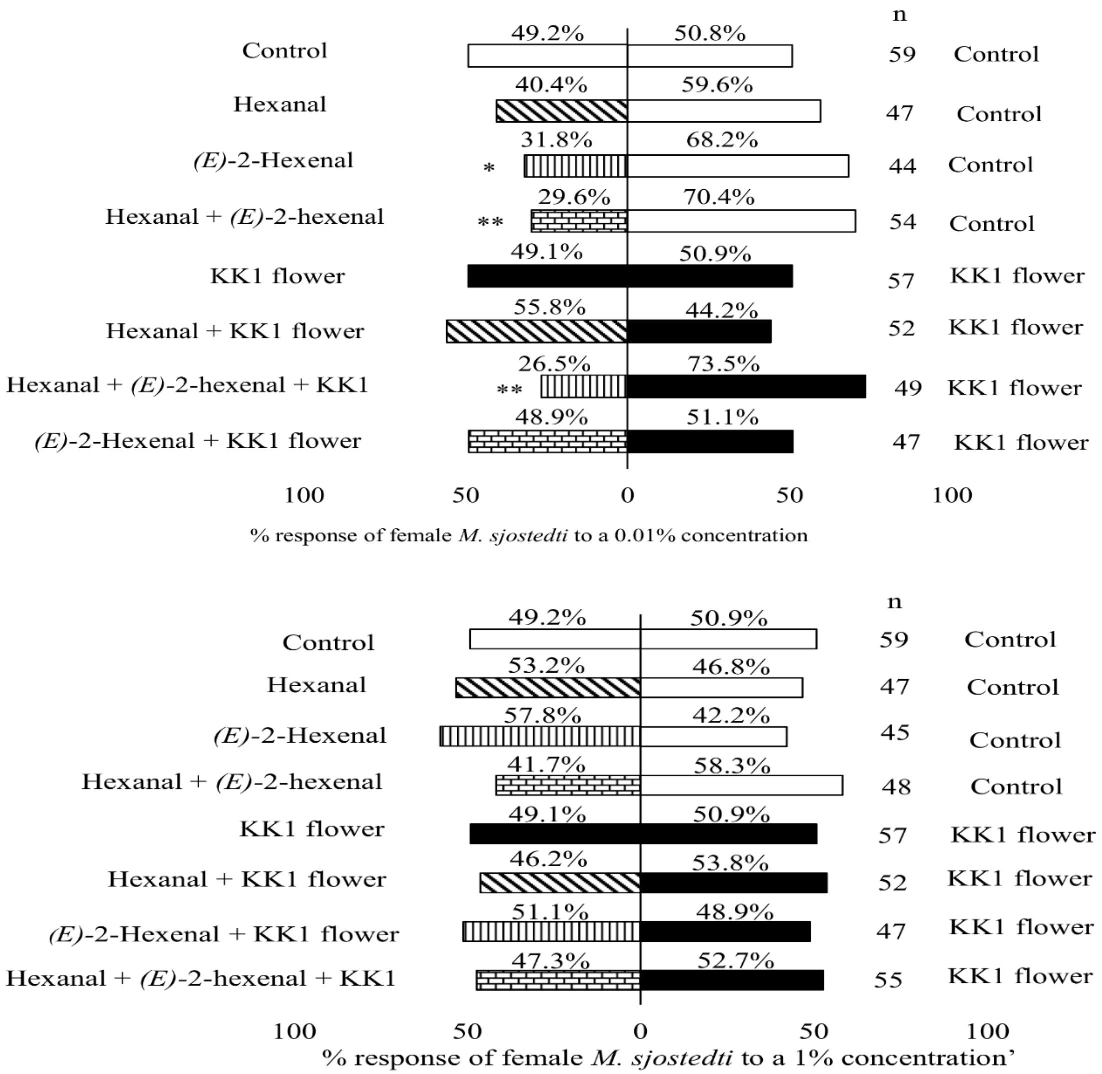

Figure 4. 7: **Percentage (%) responses of female *Megalurothrips sjostedti* to the compounds hexanal and *(E)*-2-hexenal. Each compound or blend of *(E)*-2-hexenal and hexanal versus control (solvent) and each compound or blend of *(E)*-2-hexenal and hexanal + KK1 flower versus KK1 flower as a function of the concentrations a) 0.01% and b) 1%. KK1 flower = *Vigna unguiculata* var. Ken Kunde 1. The total number of insects tested per treatment was 60 (N = 60). n = total number of insects that responded per treatment. Significance levels of χ^2 tests are indicated by * P < 0.05, ** P < 0.01, (chi-square goodness-of-fit test)**

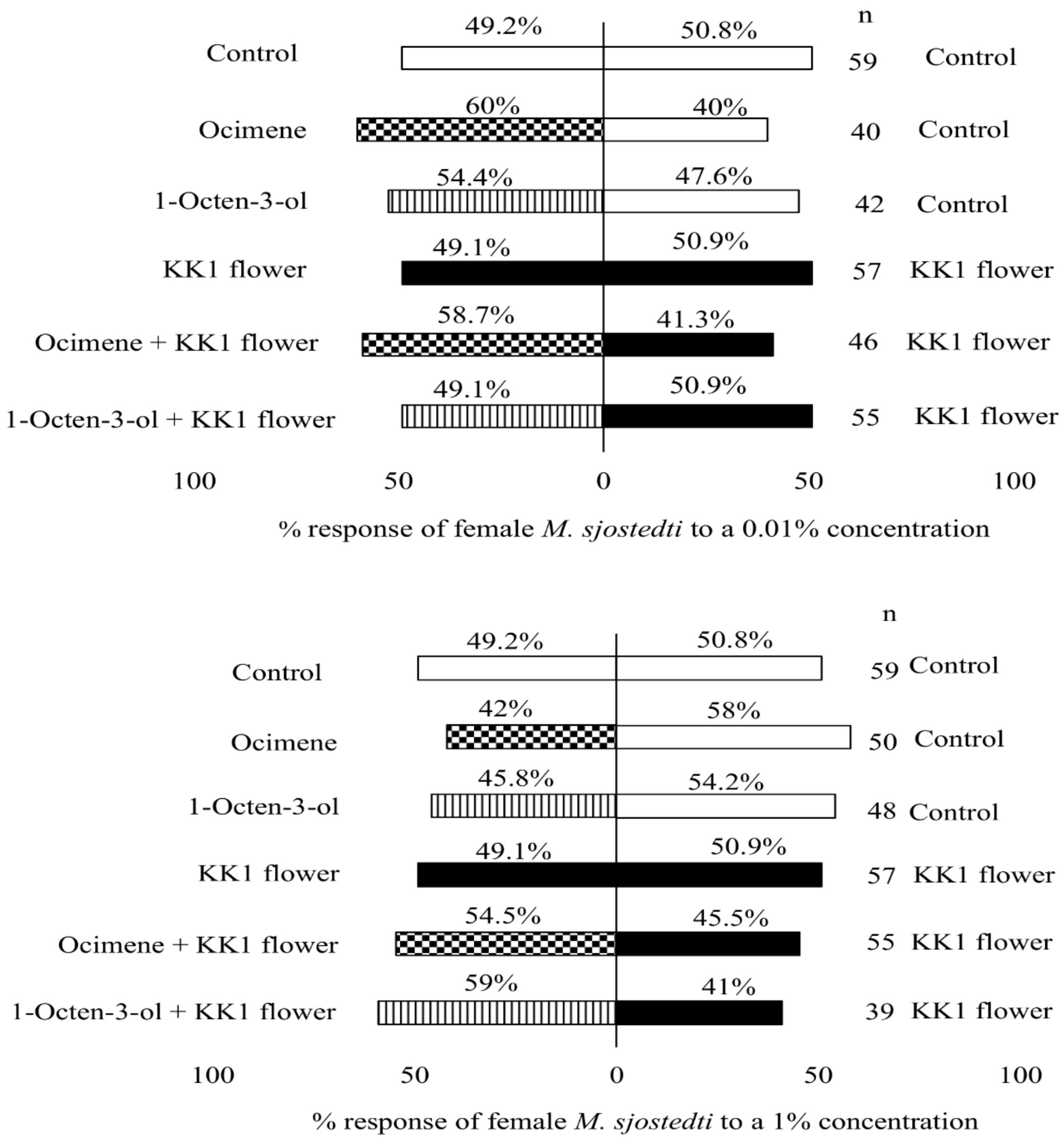

Figure 4. 8: Percentage (%) responses of female *Megalurothrips sjostedti* to the compounds ocimene and 1-octen-3-ol. Each compound versus control (solvent) and each compound + cowpea flower versus cowpea flower as a function of the concentrations a) 0.01% and b) 1%. KK1 flower = *Vigna unguiculata* var. Ken Kunde 1. The total number of insects tested per treatment was 60 (N = 60). n = total number of insects that responded per treatment. Significance levels of χ^2 tests are indicated by * P < 0.05, ** P < 0.01, (chi-square goodness-of-fit test).

4.6 Discussion

Findings of the present study demonstrated that behavioural response of male and female Bean Flower Thrips differed with the phenological stage of cowpea and volatiles of flowers among the cultivars were different. The ability of the females BFT to discriminate the suitable flower volatiles compared to the males is consistent with the field observation of Niassy et *al.*, (2016), who found that only BFT females were observed in the flowers, while BFT males tend to aggregate on leaves.

4.6.1 Behavioural response of *M. sjostedti* to cowpea cultivars

In the present study, the volatile of flowers of cultivar KK1 were attractive to females. Numerous studies reported the preference of female *M. sjostedti* for cowpea flowers (Ngakou *et al.*, 2008; Niassy *et al.*, 2016). By contrast, the volatiles from vegetative stage and flowers of cultivar K80 were repellent to female *M. sjostedti*. Investigators have reported a repellent effect of volatiles emitted by cultivar K80 on other insects. For example, in olfactory tests, *A. craccivora* aphids were repelled by cultivar K80 (Diabate *et al.,* 2019a).

4.6.2 Influence of cowpea compounds on behavioural response of *M. sjostedti*

Through GC-MS analysis, 20 compounds were identified in the flowers of cowpea cultivars. All compounds listed have already been reported in previous studies (Lwande *et al.,* 1989; Ager, 2009; Sobhy *et al.,* 2018). The PCA showed an overlapping of the four cultivars which suggested their volatile profiles were close. However, behavioural response of *M. sjostedti* in olfactory bioassays was observed to be contrasting between the cultivars. The presence /absence of some compounds in volatile profile of cultivars could explain the differential attractiveness of *M. sjostedti*. Decanal, cedrol, pentadecane, neryl acetone, hexanal, undecane, 1-octen-3-ol and *(E)-β*-ocimene were not detected in all cultivars. Bruce and Pickett (2011) reported that certain compounds are essential in the recognition of host plant volatiles by insects and their absence affect the behavioural response of insects. On the other hand, the variation of amounts of compounds among the cultivars can also influence the choice of thrips. *(E)*-2-Hexenal alone or combined with hexanal were repellent to females at lower concentrations, while a neutral response was observed at higher concentrations. A true repellent may repel the insects at low concentration. Deletre *et al.*, (2016) hypothesized that a true repellent volatile might activate a specific olfactory receptor, which may activate a specific glomerulus in the insect brain, thus eliciting a movement away from the odour source. By contrast, the neutral response at higher concentration could be due to the activation of olfactory receptors that are not specific to *(E)*-2-hexenal, thus reducing the repellent effect. It has been shown previously that the increase in the concentration of a compound leads to the activation of additional olfactory receptors (Malnic *et al.,* 1999). However, the mechanism involved in the repellent effect of *M. sjostedti* to different concentrations needs further investigation.

The females *M. sjostedti* were also repelled by the combination cowpea flower and *(E)*-2-hexenal relative to cowpea flower alone. These results suggest that this compound reduces the attractiveness of the cowpea flower to female *M. sjostedti*. *(E)*-2-Hexenal may interfere with host detection by affecting the attractant volatile compounds, binding proteins or blocking olfactory receptors, thus inhibiting the signal to the glomeruli. A repellent volatile can repel the insects from an odour source, but also hide the odour source from the insects (Deletre *et al.*, 2016). The manipulation of olfactory cues may alter the interaction between *M. sjostedti* and cowpea.

Within the flower samples, *(E)*-*β*-ocimene and 1-octen-3-ol only occurred in the repellent cultivar K80. However, in olfactory tests, these compounds failed to repel female *M. sjostedti*. These results suggest that the appropriate composition and specific ratio of compounds may be responsible for the differential attractiveness of *M. sjostedti* to the cowpea flowers. Webster (2012) reported that the insects perceive the appropriate blend based on species-specific ratios of different compounds to recognise a host plant.

4.6.3 Choice of *M. sjostedti* in pairwise comparison and preference assays

The pairwise comparison of cultivars in the Y-tube olfactometer revealed no preference of *M. sjostedti* to odours of the cowpea. The low difference among the ratio of undamaged cowpea cultivars or the masking effect of compounds may prevent the thrips to discriminate the cultivars in pairwise comparison. In intercropping, the volatiles emitted in the mixture of different cultivars can reduce insects host plant acceptance as observed in olfactory assays in the present study (Ninkovic *et al.*, 2002). The absence of choice of any cowpea cultivars by female in preference assays may be due to the short distance among the cowpea cultivars in the cage which could promote the blend of volatiles from different cultivars which could have reduced the choice of *M. sjostedti*. On the other hand, visual cues are also crucial in the orientation of *M. sjostedti* (Muvea *et al.*, 2014). In the field, *M. sjostedti* were more attracted by the combination of flower volatiles and blue colour traps than by blue traps alone (Muvea *et al.*, 2014).

4.7 Conclusion

The results of the present study demonstrate that the attractiveness of cowpea to *M. sjostedti* differs according to cowpea cultivar, phenological stage, and sex of thrips. The flowers of cultivar KK1 were an attractant, but the flowers of cultivar K80 were a repellent to the females. The volatiles from the vegetative stage of four cultivars were neutral or repellent to females and males *M. sjostedti*. *(E)*-2-Hexenal from the vegetative stage of cowpea cultivars was repellent to the females. *(E)*-2-Hexenal could be used in agriculture to manipulate the behaviour of the *M. sjostedti* by repelling the pest from the crop or useful to breeders in selecting non-attractant cowpea cultivars emitting *(E)*-2-hexenal.

CHAPTER FIVE

REPELLENT EFFECT OF *CYMBOPOGON CITRATUS* AND *TAGETES MINUTA* ON THE BEHAVIOUR OF BEAN FLOWER THRIPS, *MEGALUROTHRIPS SJOSTEDTI*

5.1 Abstract

Cowpea is an important source of protein for human beings in Africa. However, the crop suffers major damage and yield losses due to Bean Flower Thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). Although companion plants are known to reduce the damage caused by insect pests, the role of their volatiles in repelling pests from target plants has been the subject of few investigations. In this study, the Y-tube olfactometer experiments was used and chemical analysis to investigate the effect of volatiles from cowpea flowers and two companion plants, Lemongrass, *C. citratus* and Mexican marigold, *T. minuta,* on the olfactory responses of *M. sjostedti.*

Results of the present study revealed that *M. sjostedti* males and females were repelled by the volatiles from fresh cut leaves of *C. citratus*. The combination of freshly cut leaves of *C. citratus* and cowpea flower was repellent to females but not to males. The female thrips but not males, were repelled by the volatiles from the vegetative stage of *T. minuta*. Fifty-four compounds were identified in the volatiles from two herbal plants. Among the major compounds, citral and a 4-component blend comprised of dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene and *(Z)-β*-ocimene repelled females but dihydrotagetone alone attracted females. While myrcene combined with cowpea flower volatiles enhanced the attraction of females *M. sjostedti*, when tested alone was not attractive.

These results highlight the potential of volatiles from *C. citratus* and *T. minuta* to repel *M. sjostedti* females. The use of these plants as companion plants in a cowpea cropping system could reduce *M. sjostedti* infestation.

5.2 Introduction

Cowpea, *V. unguiculata* is an important source of proteins for human nutrition and livestock feed in Africa (Singh *et al.*, 2003; Togola *et al.*, 2017). Annual production of cowpea grains was estimated at 6.7 million metric tons in 2016 (FAOSTAT, 2016). Around 200 million people consume cowpea every day in Africa (Popelka *et al.*, 2006). However, cowpea crops are threatened by several insect pests including, *M. sjostedti* (Abtew, 2015; OECD, 2015). The Bean Flower Thrips, *M. sjostedti* is a major pest of leguminous plants in Africa and can cause between 20% and 100% cowpea pod yield losses without the use of synthetic pesticides (Ekesi *et al.*, 1998b; Abtew, 2015). Their small size, cryptic feeding habit, ability to pupate in the soil and fast development make these thripsdifficult to control with pesticides (Abtew, 2015). Additionally, chemical pesticides are costly for small-scale farmers, have a negative effect on beneficial insects and are harmful to the environment and human health (de Bon *et al.*, 2014; Abtew, 2015). As such, alternative environmentally-friendly methods to reduce pest populations on crops are required. The use of companion plants producing repellent compounds is one possible pesticide-free alternative for the control of *M. sjostedti*. The potential of repellent companion plants to control insect pests has been demonstrated by several authors (Parolin *et al.*, 2012; Parker *et al.*, 2013). For example, *Ocimum basilicum* L. (Basil) has been used to reduce populations of thrips (Parker *et al.*, 2013), aphids (Basedow *et al.*, 2006) and pink bollworm (Schader *et al.*, 2005). However, numerous failures have been reported on the efficacy of non-host volatiles to reduce insect pests in the field (Moreau *et al.*, 2006; Webster & Cardé, 2016). For example, the intercropping of the non-host plant, French marigold with host potato plants did not reduce the population of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, but rather increased the pest attack (Moreau *et al.*, 2006). The identification of one or a blend of repellent plant volatiles could help us to better select companion plants to be used as an olfactory barrier to prevent *M. sjostedti* infestation in cowpea. The *Cymbopogon* and *Tagetes* genera have been shown to have a broad spectrum of activity against many arthropods (Nerio *et al.*, 2010; Singh *et al.*, 2015).

Lemongrass, *C. citratus* extracts have been shown in previous studies to efficiently repel *M. sjostedti* female thrips (Abtew, 2015). However, the short repellence duration of extracts of *C. citratus* remain a major problem in the control of insect pests (Nerio *et al.*, 2010). Whole Lemongrass emits small amounts of volatiles compared to when it is cut or crushed. The repellence duration of volatiles from *C. citratus* on *M. sjostedti* was evaluated by using the freshly cut and old cut leaves of *C. citratus*. Tagetes oil is described by the United States Environmental Agency as a biochemical pesticide that can be used for the control of thrips (USEPA, 2012). However, little is known about the role of volatiles from *C. citratus* and Mexican marigold *T. minuta* in controlling *M. sjostedti*. In addition, the repellent behaviour and its controlling mechanisms are complex (Deletre *et al.*, 2016). Understanding the mechanisms involved in the repellent effect of volatiles from companion plants would improve our knowledge of pest control and the use of such plants. Thus, the aim of the present study was to evaluate the repellent effect of *C. citratus* and *T. minuta* plants and their major compounds against male and female *M. sjostedti*. It was hypothesised that volatiles from *T. minuta* and *C. citratus* plants repel *M. sjostedti*. This hypothesis was tested using behavioural assays and chemical analysis (i) to evaluate the response of *M. sjostedti* to the two plants alone or in combination with cowpea plants, (ii) to identify the volatile compounds from the two plants that mediate the behavioural response of thrips, and (iii) to evaluate the effect of individual compounds or a blend of major volatile compounds from both the plants on *M. sjostedti* behaviour.

5.3 Methods and material

5.3.1 Plants tested

Previous study showed the open flowers of cowpea var. Ken Kunde 1 was an attractant to female *M. sjostedti* (Diabate *et al.*, 2019b), therefore this cultivar was used in the current study. Preliminary results showed that *C. citratus* produces more citral when the leaves are cut or damaged by insects. For this reason, the repellent effect of *C. citratus* was evaluated by cutting leaves into 0.5-cm wide strips in the laboratory (25 ± 1 C temperature;

40-50% RH) (Plate 5.1). The cut leaves of *C. citratus* (1g) were used immediately after cutting (0 h) as fresh leaves and 24 h after cutting as *old* leaves in all the experiments. The cut leaves were kept in a petri dish at ambient temperature (25 ± 1°C). The vegetative and flowering stages of 2 to 3 months old *T. minuta* were used for the experiments.

Plate 5. 1: Leaves of Lemongrass (1g) cut into 0.5 cm wide strips (Source: Diabate, 2017)

5.3.2 Olfactory response of *M. sjostedti* to plant volatiles

Same olfactory test as in Chapter 4. The cut leaves of *C. citratus* or whole plants of *T. minuta* were placed in the polyester (Nalophan) bag and the bag closed tightly. The compounds tested were enclosed alone or with the plant in the bag as an odour source. The olfactory responses of male and female *M. sjostedti* were tested on (a) freshly cut leaves of *C. citratus* (1g) versus clean air; (b) old cut leaves (1 g) of *C. citratus* versus clean air; (c) vegetative *T. minuta* versus clean air; (d) flowering *T. minuta* versus clean air; (e) freshly cut leaves of *C. citratus* + open cowpea flower versus open cowpea flower alone; (f) old cut leaves of *C. citratus* + open cowpea flower versus open cowpea flower alone; (g) vegetative *T. minuta* + open cowpea flower versus open cowpea flower alone and (h) flowering *T. minuta* + open cowpea flower versus open cowpea flower alone.

5.3.3 Collection of volatiles from *C. citratus* and *T. minuta*

Same procedure of collect of volatile as in Chapter 3 and 4. Hovewer, here, individual plant material was enclosed in a Nalophan bag for 4 h (Plate 5.2). After volatile collection, each sample was eluted with 150 µl of dichloromethane.

Plate 5. 2: Collection of volatiles of *Tagetes minuta* (Source: Diabate, 2017)

5.3.4 Olfactory assay with synthetic standards

A Y-tube olfactometer assay (described in the chapter 3 and 4), to evaluate the repellent effect of major compounds identified in the volatiles from *T. minuta* and *C. citratus* was used. The compounds were tested either alone or in blends at a concentration of 1% (v/v) in dichloromethane. The blend was formulated to simulate the ratio corresponding to the natural ratio occurring in the plants. The synthetic blend of *C. citratus* contained citral, myrcene, geraniol and nerol in a ratio of 39:21:8:1, respectively. The blend of vegetative *T. minuta* included dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene and *(Z)-β*-ocimene in a ratio of 4:3:1:1, respectively. A 50 µl aliquot of each compound or blend was deposited on a filter paper and left for 30 s at 25 ± 1 °C to allow the solvent to evaporate. Filter papers were placed in Polyester (Nalophan) bags connected to the olfactometer arms via PTFE tubing. The negative control consisted of 50 µl dichloromethane only. Each compound or blend vs control (solvent) and their combinations vs cowpea flower alone were tested. The 10 treatments tested with compounds from cut leaves of *C. citratus* were:

(a) myrcene vs control; (b) nerol vs control, (c) citral vs control; (d) geraniol vs control; (e) blend vs control; (f) myrcene + cowpea flower vs cowpea flower; (g) nerol + cowpea flower vs cowpea flower; (h) citral + cowpea flower vs cowpea flower; (i) geraniol + cowpea flower vs cowpea flower; (j) blend + cowpea flower vs cowpea flower. The 11 treatments tested with compounds from *T. minuta* were: (a) *(+)(R)*-limonene vs control; (b) *(+)(S)*-limonene vs control; (c) ocimene vs control; (d) *(Z)*-3-hexenyl acetate vs control; (e) dihydrotagetone vs control; (f) blend vs control; (g) limonene + cowpea flower vs cowpea flower; (h) ocimene + cowpea flower vs cowpea flower; (i) *(Z)*-3-hexenyl acetate + cowpea flower vs cowpea flower; (j) dihydrotagetone + cowpea flower vs cowpea flower; (k) blend + cowpea flower vs cowpea flower. The filter papers were replaced every 60 min. Individual female thrips tested represented a replicate and each treatment was complete after 60 females were tested.

5.3.5 Synthetic standards

The synthetic standards including myrcene (purity ≥ 95%), limonene (purity 96%), ocimene mixture (purity ≥ 90%), nerol (purity 98%), geraniol (purity 98%), citral (geranial, neral) (purity 95%) were purchased from Sigma-Aldrich, France; dihydrotagetone was purchased from Santa Cruz Biotechnology, France. Hexane (purity ≥ 95%), dichloromethane (purity ≥ 99%), 2-hexadecanol (purity 99%) were purchased from Sigma Aldrich, UK; ethanol (purity ≥ 99.8%) was purchased from Sigma-Aldrich, Germany.

5.4 Statistical analysis

Same procedure of statistical analysis of the behavioural assays and volatiles as in Chapter 3. In addition, a non-parametric Mann–Whitney–Wilcoxon test was used to analyse differences in the emission of volatiles between vegetative and flowering *T. minuta* plants and fresh and old leaves of *C. citratus*.

5.5 Results

5.5.1 Behavioural response of *M. sjostedti* to *C. citratus* and *T. minuta*

Male and female, *M. sjostedti* were significantly repelled by the volatiles from freshly cut leaves of *C. citratus* relative to clean air (male: $\chi^2 = 14.51$, df = 1, $P < 0.001$; female: $\chi^2 = 11.26$, df = 1, $P < 0.001$) (Figure 5.1 a). However, no repellent effect was observed with old cut leaves ($P > 0.05$). More female *M. sjostedti* were repelled by cowpea flower combined with freshly cut leaves of *C. citratus* compared to cowpea flower alone ($\chi^2 = 6.81$, df = 1, $P < 0.01$). For males, no significant difference in effect was observed between cowpea flower combined with fresh cut leaves versus cowpea flower alone ($\chi^2 = 2.28$, df = 1, $P = 0.13$) (Figure 5.1 b).

Vegetative *T. minuta* repelled more females relative to clean air (female: $\chi^2 = 4.26$, df = 1, $P = 0.03$) but the males were not repelled ($\chi^2 = 1.72$, df = 1, $P = 0.18$) (Figure 5.2 a). Flowering *T. minuta* had no significant attractive/repellent effect on either females or males. More female *M. sjostedti* were repelled from cowpea flower combined either with vegetative *T. minuta* ($\chi^2 = 7.69$, df = 1, $P < 0.01$) or flowering *T. minuta* ($\chi^2 =7.07$, df = 1, $P < 0.01$) compared to cowpea flower alone. A combination of cowpea flower and marigold either at vegetative or flowering stage had no significant effect on males (vegetative: $\chi^2 = 1.61$, df = 1, $P = 0.20$, flowering: $\chi^2 = 1.10$, df = 1, $P = 0.29$) (Figure 5.2 b).

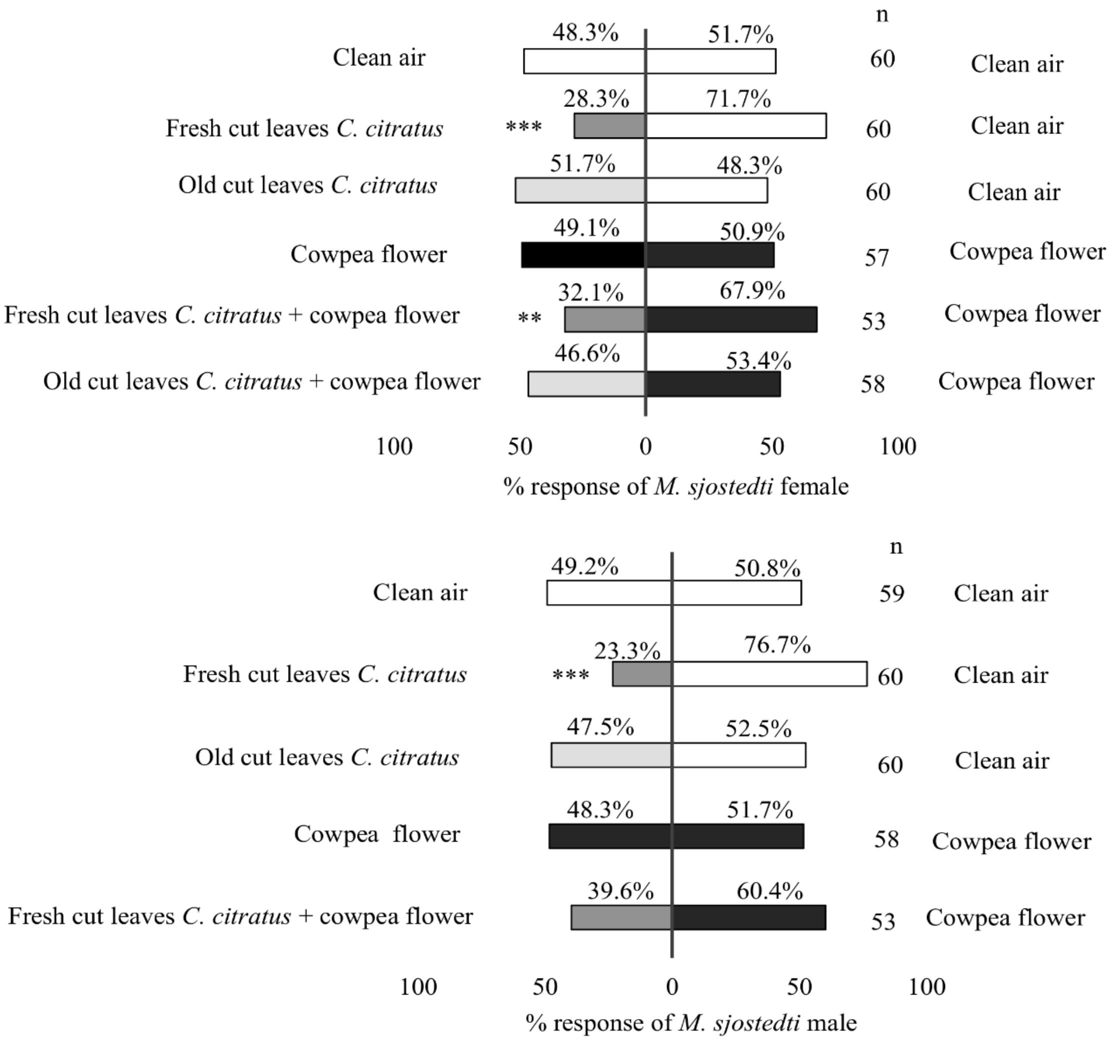

Figure 5. 1: Percentage (%) responses of a) female and b) male *Megalurothrips sjostedti* to freshly and old cut leaves of *Cymbopogon citratus* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer. One gram (1g) of cut leaves of *Cymbopogon citratus* was used in the olfactory tests. Freshly = 0 h, old = 24 h, cowpea flower = *Vigna unguiculata* var. Ken Kunde 1. The total number of insects tested per treatment was 60. n = total number of insects which responded per treatment was 60. n = total number of insects which responded per treatment. Significance levels of χ^2 tests are indicated by * P < 0.05, ** P < 0.01, ***P < 0.001, (chi-square goodness-of-fit test).

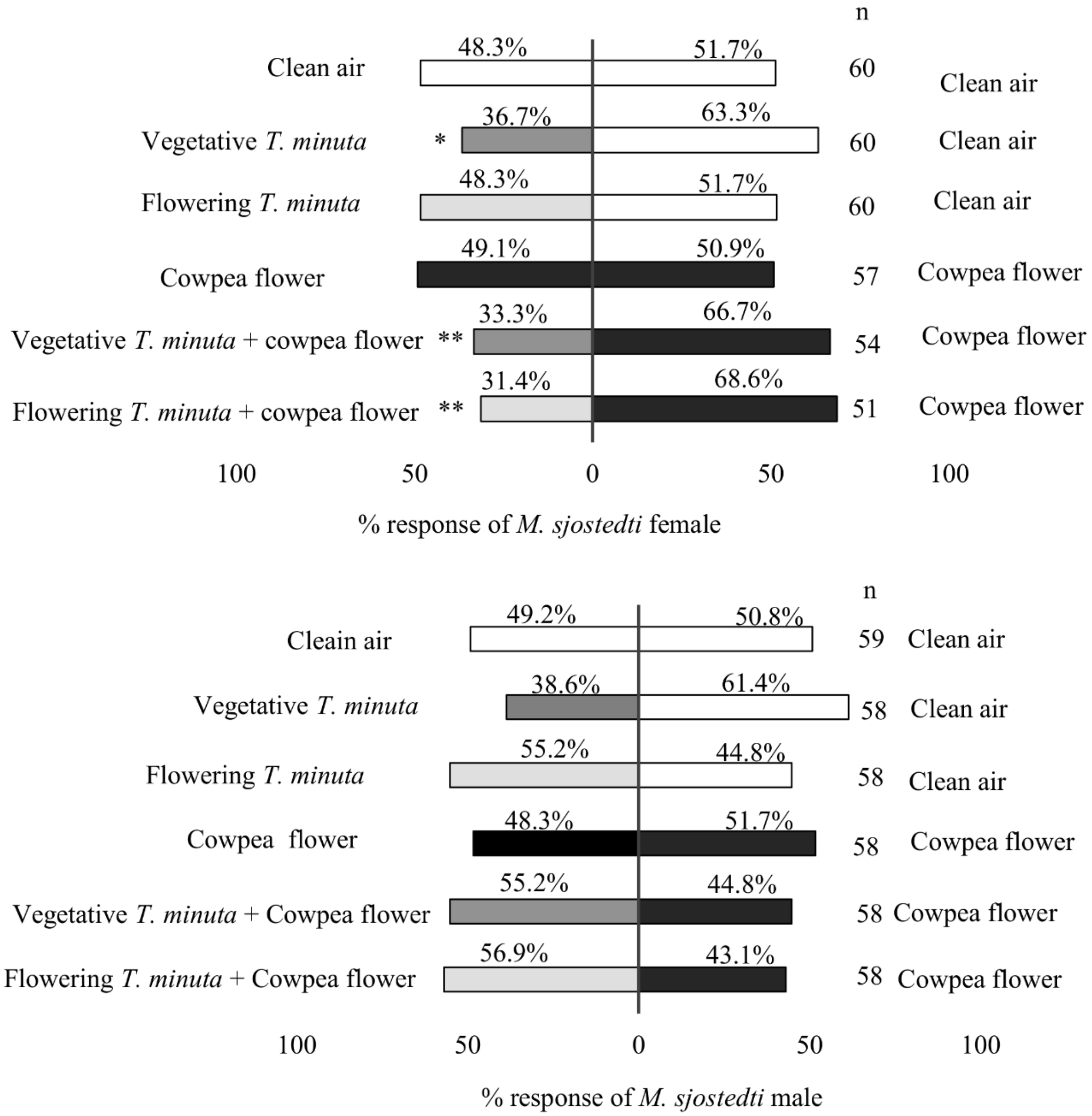

Figure 5. 2: Poucentage (%) responses of a) female and b) male *Megalurotheips sjostedti* to vegetative and flowering *Tagetes minuta* versus clean air or in combination versus cowpea flower alone in a Y-tube olfactometer. Freshly = 0 h, old = 24 h, cowpea flower = *Vigna unguiculata* var. Ken Kunde 1. Total number of insects tested per treatment was 60. n = total number of insects which responded per treatment. Significance levels of χ^2 tests are indicated by * P < 0.05, ** P < 0.01, (chi-square goodness-of-fit test).

5.5.2 Analysis of volatiles

Chemical analysis of volatiles collected from the cut leaves of *C. citratus* and *T. minuta* plant revealed 54 compounds (Table 5.1 and 5.2). A total of 30 compounds were identified in fresh and old (24 h old after cutting) cut leaves of *C. citratus*: 16 monoterpenoids, 5 sesquiterpenoids, 3 aldehydes, 2 esters, 2 ketones, 1 benzenoids and 1 alcohol (Table 5.1). Among the compounds, the abundance of *(Z)*-3-hexenal, *(Z)*-3-hexenol, *(Z)*-*β*-ocimene, *(E)*-*β*-ocimene, *(E)*-isocitral, neral, geraniol and geranial in *C. citratus* was significantly higher in freshly cut leaves than in old cut leaves (W = 16, P = 0.028) (Figure 5.3). Conversely, the myrcene was higher in old cut leaves than in fresh cut leaves (W = 16, P = 0.028). Some minor compounds such as *(E)*-2-hexenal, heptanal, 6,7-epoxymyrcene, *(Z)*-isocitral, geranyl acetate, butylated hydroxytoluene and *β*-sesquiphellandrene were not detected in old leaves. The PCA explained 85.1% of the total variance: 67.4% of the explanation on the horizontal axis (PC1) and 17.7% on the vertical axis (PC2). In the score plot, the emission of volatiles overlapped, but a difference between fresh and old *C. citratus* was distinct (Figure 5.4 a). The loading plot shows that *(Z)*-3-hexenal, *(Z)*-3-hexenol, *(Z)*-*β*-ocimene, *(E)*-*β*-ocimene, *(E)*-isocitral, neral, geraniol, geranial and myrcene were among the compounds responsible for separating volatiles in fresh and old cut leaves of *C. citratus* (Figure 5.4 b).

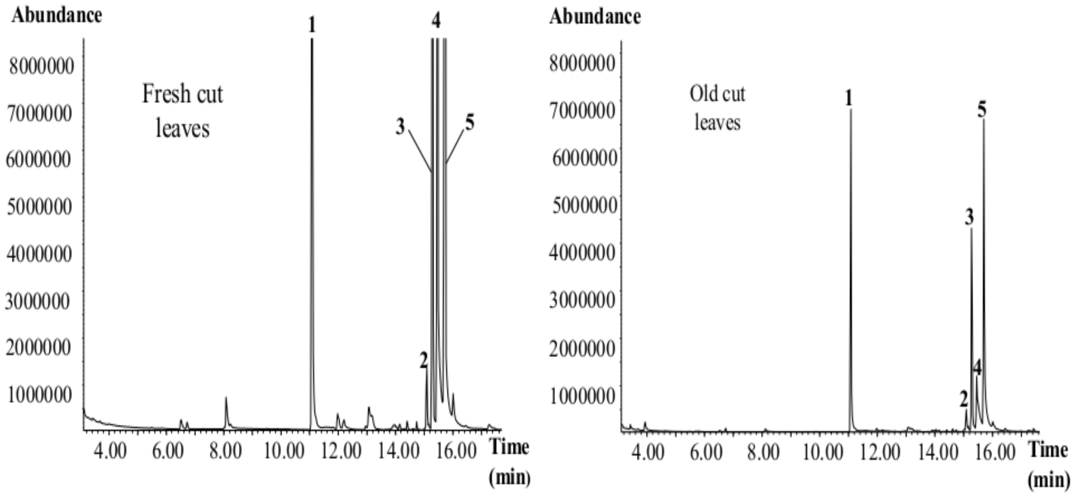

Figure 5. 3: Representative chromatogram of major compounds identified in fresh and old cut leaves of Lemongrass, *Cymbopogon citratus.* Peak no: 1 = Myrcene; 2 = Nerol; 3 = Neral; 4 = Geraniol; 5 = Geranial

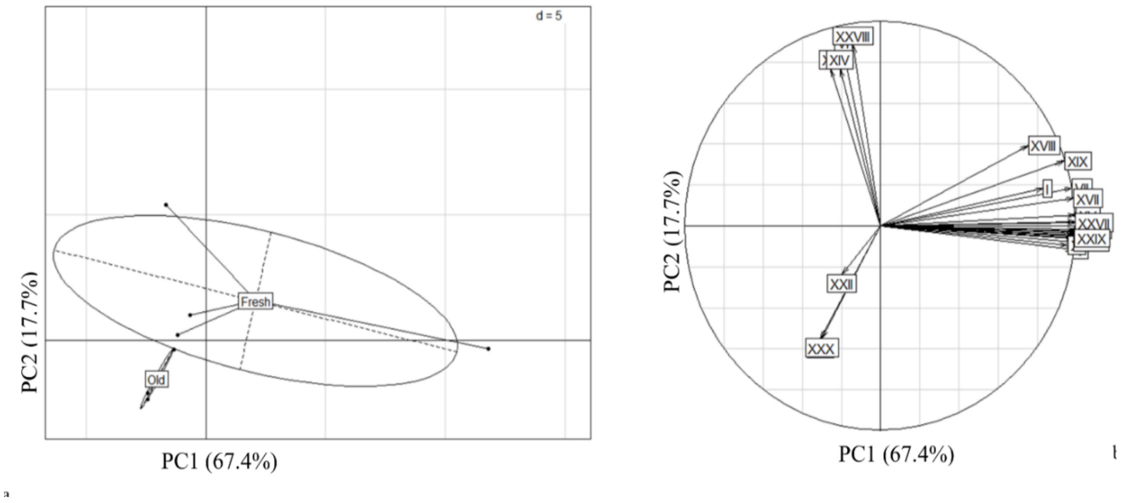

Figure 5. 4: Principal component analysis (PCA) of volatiles in the fresh (0 h) and old (24 h) cut leaves of *Cymbopogon citratus.* a: score plot with the old cut leaves (Old) and the fresh cut leaves (Fresh), b: loading plot with arrows showing the direction and intensity of the correlation of variables. Compound numbers: (I) *(Z)*-3-Hexenal, (II) *(E)*-2-Hexenal, (III) *(Z)*-3-Hexenol, (IV) Heptanal, (V) *α*-Pinene, (VI) *β*-Pinene, (VII) Myrcene, (VIII) Limonene, (IX) *(Z)*-*β*-Ocimene, (X) *(E)*-*β*-Ocimene, (XI) *(Z)*-Linalool oxide, (XII) 6,7-Epoxymyrcene, (XIII) Linalool, (XIV) *(Z)*-Isocitral, (XV) *(E)*-Isocitral, (XVI) Nerol, (XVII) Neral, (XVIII) Geraniol, (XIX) Geranial, (XX) Geranyl acetate, (XXI) 2-Undecanone, (XXII) Carvacrol, (XXIII) Neryl acetate, (XXIV) (*E*)-Caryophyllene, (XXV) *(Z)*-*β*-Farnesene, (XXVI) *α*-Humulene, (XXVII) 2-Tridecanone, (XXVIII) Butylated hydroxytoluene, (XXIX) *β*-Sesquiphellandrene, (XXX) Epi-Cedrol

A total of 24 compounds were identified in the vegetative and flowering *T. minuta*: 9 monoterpenoids, 5 sesquiterpenoids, 5 ketones, 3 esters, 1 aldehyde and 1 alcohol (Table 6.2). The five most abundant compounds were dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene, *(Z)-β*-ocimene and *(Z)*-tagetone at both phenological stages of *T. minuta* (Figure 6.5). *(Z)*-3-Hexenyl acetate was significantly more abundant in the vegetative *T. minuta* than in *T. minuta* with flowers (W = 1, P = 0.057). Conversely, the emission of *(Z)-β*-ocimene (W = 16, P = 0.028), *(E)*-tagetone (W = 12, P = 0.043), ethyl 2-methylbutanoate (W = 15, P = 0.059) and bicyclogermacrene (W = 15, P = 0.057) were significantly higher in flowering than in vegetative *T. minuta* (W = 16, P = 0.028). The PC1 on the horizontal axis explained 66.7% of the total variance while PC2 on the vertical axis explained 13.9%. The separation between vegetative and flowering *T. minuta* is visible in the score plot (Figure 5.6 a). The loading plot indicated *(Z)*-3-hexenyl acetate, *(Z)-β*-ocimene, *(E)*-tagetone, ethyl 2-methylbutanoate and bicyclogermacrene contribute to the separation between vegetative and flowering *T. minuta* (Figure 5.6 b).

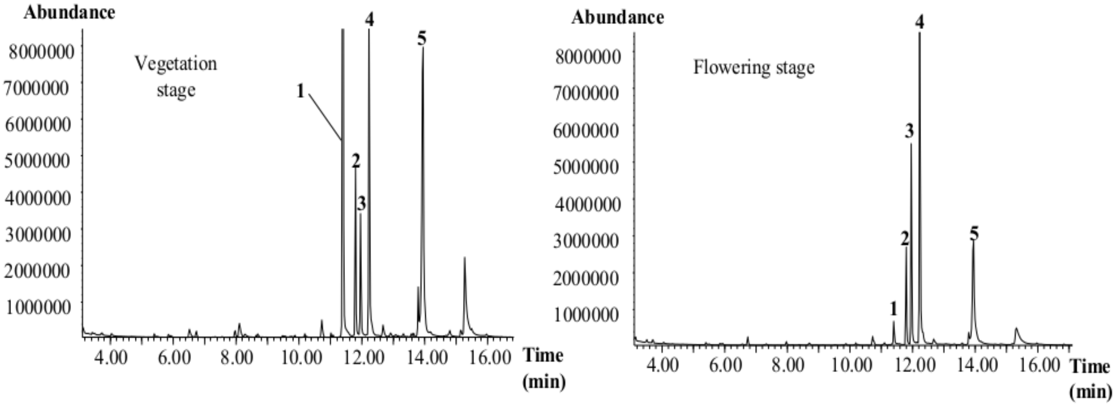

Figure 5. 5: Representative chromatogram of major compounds identified in vegetative and flowering stages of Mexican marigold, *Tagetes minuta*. Peak no: 1 = *(Z)*-3-Hexenyl acetate; 2 = Limonene; 3 = *(Z)-β*-Ocimene; 4 = Dihydrotagetone; 5 = *(Z)*-Tagetone

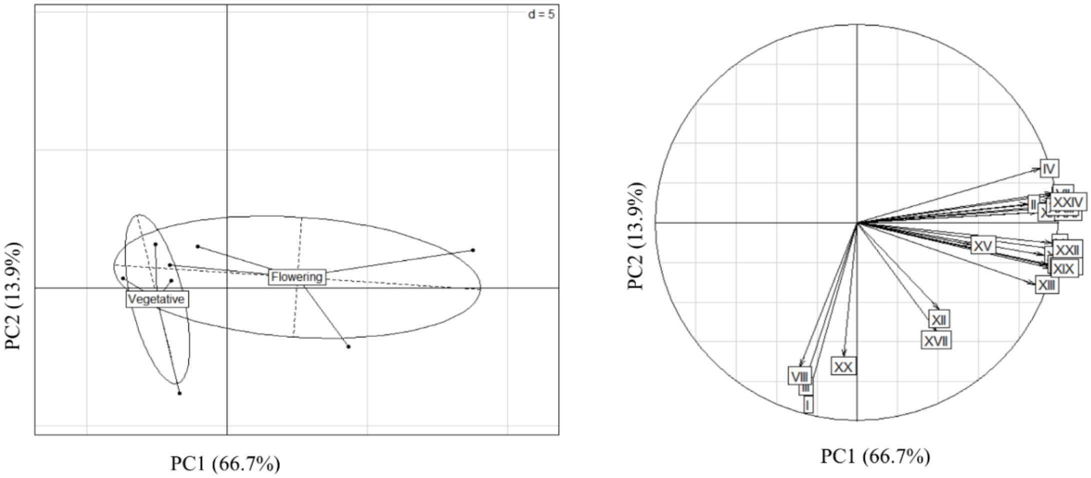

Figure 5. 6: Principal component analysis (PCA) of volatiles in vegetative and flowering *Tagetes minuta.* a: score plot with the vegetative stage (Vegetative) and the flowering stage (Flowering), b: loading plot with arrows showing the direction and intensity of the correlation of variables. Compound numbers: (I) *(Z)*-3-Hexenal, (II) Ethyl 2-methylbutanoate, (III) *(Z)*-3-Hexenol, (IV) *α*-Pinene, (V) Camphene, (VI) Sabinene, (VII) Myrcene, (VIII) *(Z)*-3-Hexenyl acetate, (IX) Limonene, (X) *(Z)-β*-Ocimene, (XI) Dihydrotagetone, (XII) 6,7-Epoxymyrcene, (XIII) Linalool, (XIV) *(Z)*-Epoxy-ocimene, (XV) *(E)*-Tagetone , (XVI) *(Z)*-Tagetone, (XVII) Methyl salicylate, (XVIII) *(Z)*-Ocimenone, (XIX) Car-3-en-2-one, (XX) *α*-Copaene, (XXI) *(E)*-Caryophyllene, (XXII) *α*-Humulene, (XXIII) Germacrene D, (XXIV) Bicyclogermacrene

Table 5. 1: Abundance (%) of volatile compounds emitted by one gram (1g) of cut leaves of *C. citratus* at 0 hours and 24 hours (Mean ± standard error)

R. T	**Compound**	**Class of compound**	**R. I**	**Freshly cut leaf, n= 4**	**Old cut leaf, n= 4**	**P-value**
6.52	*(Z)*-3-Hexenal	Aldehyde	805	0.25 ± 0.07	0.09 ± 0.06	0.029
8.00	*(E)*-2-Hexenal	Aldehyde	861	0.00 ± 0.00	-	
8.11	*(Z)*-3-Hexenol	Alcohol	866	1.20 ± 1.01	0.19 ± 0.11	0.029
9.20	Heptanal	Aldehyde	909	tr	-	
9.87	*α*-Pinene*	Monoterpenoid	941	0.01 ± 0.00[a]	0.10 ± 0.05	0.771
10.78	*β*-Pinene	Monoterpenoid	984	0.00 ± 0.00	0.01 ± 0.01	0.868
11.09	Myrcene	Monoterpenoid	999	28.68 ± 1.92	41.60 ± 9.06	0.028
11.82	Limonene*	Monoterpenoid	1040	0.03 ± 0.03	0.01 ± 0.01	1
11.97	*(Z)*-*β*-Ocimene*	Monoterpenoid	1048	1.11 ± 0.44	0.54 ± 0.08	0.028
12.16	*(E)*-*β*-Ocimene*	Monoterpenoid	1059	0.70 ± 0.26	0.50 ± 0.17	0.028
12.61	*(Z)*-Linalool oxide	Monoterpenoid	1084	0.00 ± 0.00	0.02 ± 0.01	0.868
12.95	6,7-Epoxymyrcene	Monoterpenoid	1103	0.22 ± 0.20	-	
13.08	Linalool*	Monoterpenoid	1111	0.29 ± 0.11	0.26 ± 0.18	0.300
14.13	*(Z)*-Isocitral	Monoterpenoid	1177	0.07 ± 0.02	-	
14.40	*(E)*-Isocitral	Monoterpenoid	1194	0.24 ± 0.05	0.09 ± 0.05	0.029
15.09	Nerol*	Monoterpenoid	1227	1.34 ± 0.48	1.93 ± 1.06	0.628
15.29	Neral*	Monoterpenoid	1235	21.62 ± 1.32	15.03 ± 2.17	0.028
15.46	Geraniol*	Monoterpenoid	1249	10.99 ± 2.53	7.28 ± 3.80	0.028
15.72	Geranial*	Monoterpenoid	1264	31.08 ± 2.63	27.77 ± 3.28	0.028
16.01	2-Undecanone	Ketone	1293	0.65 ± 0.48	0.20 ± 0.13	0.300
16.27	Carvacrol	Monoterpenoid	1298	-	0.43 ± 0.26	
16.99	Neryl acetate	Ester	1359	-	0.01 ± 0.01	
17.26	Geranyl acetate	Ester	1379	0.01 ± 0.01	-	
17.86	*(E)*-Caryophyllene	Sesquiterpenoid	1444	0.34 ± 0.11	1.04 ± 0.31	0.114
18.22	*(Z)*-*β*-Farnesene	Sesquiterpenoid	1472	0.04 ± 0.07	0.04 ± 0.04	0.877
18.29	*α*-Humulene	Sesquiterpenoid	1478	0.03 ± 0.02	0.08 ± 0.06	0.644
18.71	2-Tridecanone	Ketone	1511	0.50 ± 0.20	0.32 ± 0.32	0.183
18.94	Butylated hydroxytoluene	Benzenoid	1531	tr	-	
19.10	*β*-Sesquiphellandrene	Sesquiterpenoid	1544	0.00 ± 0.00	-	
20.17	Epi-Cedrol	Sesquiterpenoid	1635	-	0.06 ± 0.04	

*****compounds identified by library data and authentic standards, R.T = retention time, R.I = retention indices calculated relative to n-alkanes C8-C30 on HP-5MS column; tr = trace < 0.005; - not detected; h = hours. Fresh cut leaf of *C. citratus* at 0 h means the leaves were immediately tested after leaves were cut (olfactory tests were performed between 0 and 1 hours). Old cut leaf of *C. citratus* at 24 h means the leaves were tested 24 hours after the leaves were cut. P-value of comparison of volatile compounds between fresh and old cut leaves of *C. citratus* according to the Mann-Whitney -Wilcoxon test

Table 5. 2: Abundance (%) of volatile compounds emitted by the vegetative and flowering *Tagetes minuta* (Mean ± standard error).

R. T	Compound name	Class of compound	R. I	Vegetative *T. minuta,* n= 4	Flowering *T. minuta,* n= 4	P-value
6.52	*(Z)*-3-Hexenal	Aldehyde	805	0.65 ± 0.39	0.10 ± 0.06	0.300
7.98	Ethyl 2-methylbutanoate	Ester	861	0.28 ± 0.05	0.51 ± 0.14	0.059
8.11	*(Z)*-3-Hexenol	Alcohol	866	0.47 ± 0.45	-	
9.87	*α*-Pinene*	Monoterpenoid	941	0.15 ± 0.09	0.21 ± 0.06	0.114
10.19	Camphene	Monoterpenoid	956	0.18 ± 0.06	0.23 ± 0.04	0.485
10.72	Sabinene	Monoterpenoid	981	1.60 ± 0.64	2.39 ± 0.67	0.200
11.09	Myrcene	Monoterpenoid	999	0.07 ± 0.04	0.37 ± 0.03	0.110
11.4	*(Z)*-3-Hexenyl acetate*	Ester	1016	25.56 ± 4.96	1.95 ± 0.60	0.057
11.82	Limonene*	Monoterpenoid	1040	10.36 ± 3.64	14.74 ± 3.44	0.200
11.97	*(Z)*-*β*-Ocimene*	Monoterpenoid	1048	7.73 ± 2.03	16.32 ± 0.53	0.028
12.23	Dihydrotagetone*	Ketone	1063	34.63 ± 8.34	30.91 ± 1.07	0.200
12.95	6,7-Epoxymyrcene	Monoterpenoid	1103	-	0.01 ± 0.01	
13.08	Linalool*	Monoterpenoid	1111	0.05 ± 0.03	0.03 ± 0.01	0.644
13.62	*(Z)*-Epoxy-ocimene	Monoterpenoid	1145	0.03 ± 0.02	0.20 ± 0.05	0.110
13.79	*(E)*-Tagetone	Ketone	1156	0.08 ± 0.08	0.90 ± 0.57	0.043
13.92	*(Z)*-Tagetone	Ketone	1164	14.25 ± 4.48	18.52 ± 3.19	0.342
14.66	Methyl salicylate	Ester	1212	tr	0.00 ± 0.00	1
15.13	*(Z)*-Ocimenone	Ketone	1244	0.07 ± 0.07	0.18 ± 0.10	0.408
15.26	Car-3-en-2-one	Ketone	1253	1.66 ± 1.66	7.65 ± 0.68	0.103
17.26	*α*-Copaene	Sesquiterpenoid	1397	tr	0.00 ± 0.00	0.538
17.86	*(E)*-Caryophyllene	Sesquiterpenoid	1444	0.62 ± 0.10	1.35 ± 0.13	0.200
18.29	*α*-Humulene	Sesquiterpenoid	1478	0.04 ± 0.03	0.15 ± 0.03	0.110
18.63	Germacrene D	Sesquiterpenoid	1505	0.13±0.05	0.60 ± 0.21	0.342
18.83	Bicyclogermacrene	Sesquiterpenoid	1521	0.50 ± 0.20	2.30 ± 0.43	0.057

*compounds identified by library data and authentic standards, R.T = retention time; R.I = retention indices calculated relative to n-alkanes C8-C30 on HP-5MS column; tr = trace < 0.005; - not detected. P-value of comparison of volatile compounds between vegetative and flowering stages of *T. minuta*, according to the Mann-Whitney -Wilcoxon test.

5.5.3 Behavioural response of *M. sjostedti* to synthetic standards

Among the five major monoterpenoids (geranial, myrcene, neral, geraniol and nerol) identified in the cut leaves of *C. citratus*, only citral showed a repellent effect on female *M. sjostedti* relative to control (χ^2 = 7.36, df= 1, $P < 0.01$) (Figure 5.7 a). The blend of these five monoterpenoids was not repellent (χ^2 = 0.69, df = 1, P = 0.40). Interestingly, the combination of myrcene and cowpea flower was preferred by female *M. sjostedti* to cowpea flower alone (χ^2 = 5.89, df= 1, P = 0.01). Female *M. sjostedti* could not distinguish cowpea flower combined with either nerol, citral, geraniol or a blend of these compounds from cowpea flower alone (Figure 5.7 b).

Among the four major compounds (dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene, *(Z)*-*β*-ocimene) of *T. minuta* tested, dihydrotagetone was the only one that elicited significant behavioural response. Surprisingly, dihydrotagetone was an attractant to female *M. sjostedti* relative to control (χ^2 = 7.36, df= 1, $P < 0.01$) (Figure 5.8 a). However, female *M. sjostedti* were repelled by the blend of the four compounds relative to clean air (χ^2 = 6.75, df = 1, $P < 0.01$) or repelled by the combination of blend + cowpea flower relative to cowpea flower alone (χ^2= 6.56, df = 1, P = 0.01) (Figure 5.8 b).

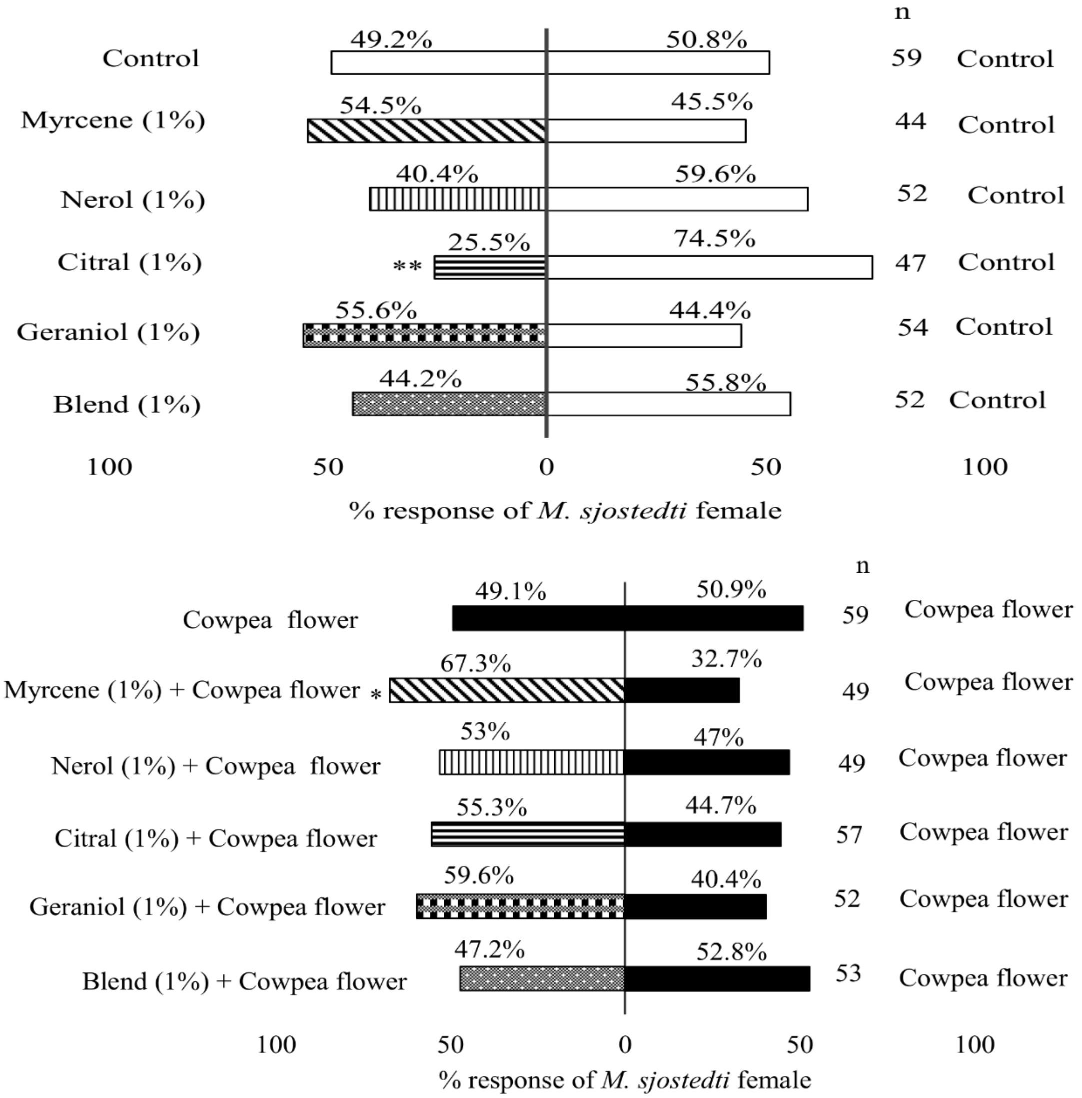

Figure 5. 7: Percentage (%) responses of *Megalurothrips sjostedti* female to major compounds of fresh cut leaves of *Cymbopogon citratus*. a) Each compound or blend versus control (solvent) and b) each compound or blend + cowpea flower versus cowpea flower alone. The compounds tested in the Y-tube olfactometer were diluted in dichloromethane at a concentration of 1%. The total number of insects tested per treatment was 60. n = total number of insects which responded per treatment. Significance levels of χ^2 tests are indicated by * $P < 0.05$, ** $P < 0.01$, (chi-square goodness-of-fit test).

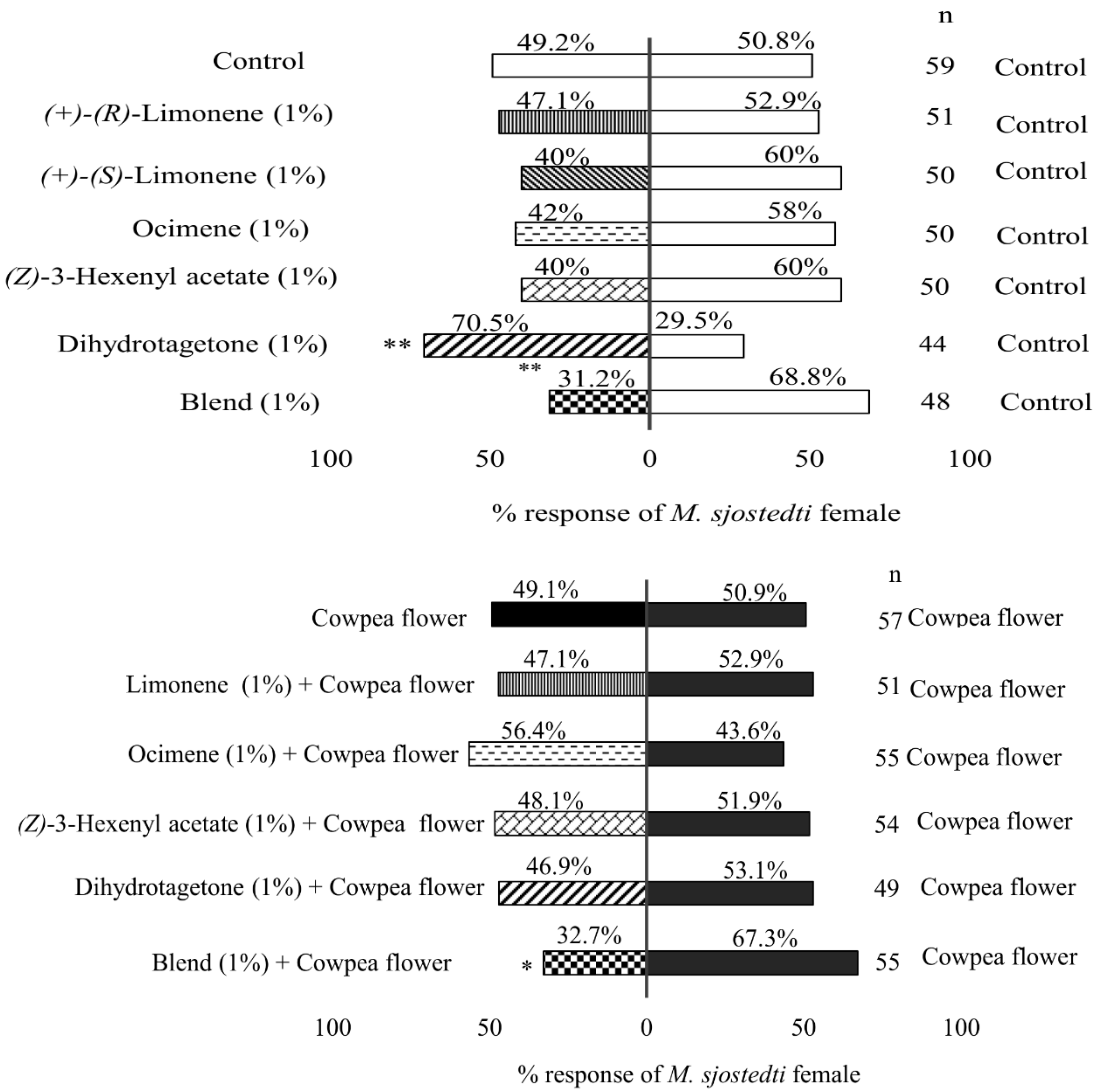

Figure 5. 8: Percentage (%) responses of female *Megalurothrips sjostedti* to major compounds of vegetative *Tagetes minuta*. a) Each compound or blend versus control (solvent) and b) each compound or blend + cowpea flower versus cowpea flower alone. The compounds tested in the Y-tube olfactometer were diluted in dichloromethane at a concentration of 1%. The total number of insects tested per treatment was 60. n = total number of insects which responded per treatment. Significance levels of χ^2 tests are indicated by * P < 0.05, ** P < 0.01, (chi-square goodness-of-fit test).

5.6 Discussion

Results from the current study show that volatiles from *T. minuta* and from freshly cut leaves of *C. citratus* were repellent to female *M. sjostedti*. Evidence of the repellent effect of extracts and essential oils from both plants on several insects including thrips has been reported in previous work (Abtew, 2015; Cornelius & Wycliffe, 2016).

5.6.1 Effect of cut leaves of *C. citratus* and major compounds on *M. sjostedti*

In this study, male and female thrips were repelled by the volatiles from freshly cut leaves, but old cut leaves were not repelled to either males or females. Female *M. sjostedti* were repelled by cowpea flower combined with freshly cut leaves of *C. citratus* relative to the cowpea flower alone. These results suggest that the volatile emitted by fresh cut leaves of *C. citratus* reduces the attractiveness of the cowpea flower to female *M. sjostedti*. The efficacity of volatiles from *C. citratus* in controlling insect pests has been demonstrated in the field. For example, the volatiles from *C. citratus* intercropped with eggplant, *Solanum melongena* L. reduced the infestation of moth, *Leucinodes orbonalis* Guenee in eggplant plants (Calumpang *et al.,* 2013).

PCA analysis indicated a difference in the profiles of volatile emissions between the fresh and old cut leaves of *C. citratus*. The absence of response in both male and female *M. sjostedti* to the old cut leaves could be due to the decrease in the abundance of *(Z)*-3-hexenal, *(Z)*-3-hexenol, *(Z)*-*β*-ocimene, *(E)*-*β*-ocimene, *(E)*-isocitral, neral, geraniol andgeranial, and/or the absence of certain volatile compounds such as *(E)*-2-hexenal, heptanal, 6,7-epoxymyrcene, *(Z)* isocitral, geranyl acetate, butylated hydroxytoluene and *β*- sesquiphellandrene in the leaves. In the olfactory tests, citral (neral + geranial) was repellent to females compared to clean air. These results suggest that citral, and particularly neral, is involved in the repellence of *C. citratus* leaves to *M. sjostedti*. Abtew (2015), reported that citral was a good repellent for *M. sjostedti* larvae. However, in combination with cowpea flower var. Ken Kunde 1, citral was not repellent. The background plant volatiles can affect the behavioural response of thrips to the compound (Koschier *et al.,* 2017). On the other hand, the abundance of myrcene in the old cut leaves may explain the decline in repellence of *M. sjostedti*. Additionally, myrcene appears to enhance the attraction of cowpea flowers for female *M. sjostedti*.

5.6.2 Effect of Mexican marigold and their major compounds on *M. sjostedti*

In this study, females were repelled by vegetative *T. minuta* but not the flowering *T. minuta* while males were not attracted/repelled by *T. minuta* volatiles. The differential responses of male and female *Ceratitis capitata* Wiedemann insects to the volatiles from *T. minuta* has also been reported by López *et al.,* (2011): in a Y-tube olfactometer, *T. minuta* oil was attractive to *C. capitata* males but females avoided the oil. In this study, female *M. sjostedti* seemed to be more susceptible to the plant volatiles than the males. PCA showed that the volatile profile of *T. minuta* differed in the vegetative and flowering stages. The compounds *(Z)-β*-ocimene, *(E)*-Tagetone, Ethyl 2-methylbutanoate, and bicyclogermacrene were abundant during flowering while *(Z)*-3-hexenyl acetate was abundant in the vegetative stage of *T. minuta*. *(Z)-β*-Ocimene and *(Z)*-3-hexenyl acetate were two of the major compounds of *T. minuta* and their individual assays in a Y-tube olfactometer did not elicit a behavioural response from the female *M. sjsotedti* compared to clean air. However, when the four major compounds (*(Z)-β*-ocimene, *(Z)*-3-hexenyl acetate, dihydrotagetone and limonene) were combined in the same ratio of volatiles released by the vegetative stage of *T. minuta,* females *M. sjostedti* were repelled by the synthetic blend. The repellent effect of the vegetative *T. minuta* may be due to the synergistic effect of four major compounds. However, *(Z)*-Tagetone was not commercially available, so it was not included in the bioassays. Further investigation needs to be performed for more clarification of synergistic effects (binary/ ternary/quaternary and other combinations) of the compounds

The combination of flowering *T. minuta* with open cowpea flowers was repellent to female *M. sjostedti*. This suggests that flowering *T. minuta* volatiles interfere with *M. sjostedti* orientation towards cowpea flowers.

Tested individually, dihydrotagetone elicited an attractive response from female *M. sjostedti*. These finding can assume that the attraction of dihydrotagetone was masked by the other major compounds in the vegetative stage. Numerous studies have documented the attraction of thrips to several compounds from floral scents (Koschier, 2006). In-depth investigations of the mechanism involved in the repellent effect of *M. sjostedti* in different concentrations could advance the understanding of thrip behaviour.

5.7 Conclusion

This study has shown that volatiles from freshly cut leaves of *C. citratus* repelled males and females of *M. sjostedti* but old cut leaves did not. The freshly cut leaves of *C. citratus* combined with cowpea flower repelled female *M. sjostedti*. Female *M. sjostedti* were repelled by the volatiles from the vegetative stage of *T. minuta* and repelled by the cowpea flower combined either with vegetative or flowering *T. minuta*. Conversely, the behavioural response of males was not affected by any odour from the two phenological stages of *T. minuta*. Citral, a major compound of *C. citratus* and the blend of major compounds (dihydrotagetone, *(Z)*-3-hexenyl acetate, limonene and *(Z)*-*β*-ocimene) from vegetative *T. minuta* were repellent to females. Dihydrotagetone alone and myrcene combined with cowpea flower attracted the female *M. sjostedti*. Finally, this study identified and characterised two repellent companion plants for *M. sjostedti* that can be used in pest management. After further investigation, the blend of major compounds of *T. minuta* could be used as a repellent with a diffuser in the field. *T. minuta* plant and the freshly cut leaves of *C. citratus* could be used to control *M. sjostedti* in the field. Further studies are needed to investigate the repellent effect of both plants and their arrangement in intercropping. The exact repellence duration of the cut leaves on *M. sjostedti* should be investigated in a further study, because this effect may pose a major obstacle to the practical application of *C. citratus.*

CHAPTER SIX

EFFICACY OF NETHOUSE TO CONTROL COWPEA PESTS AND TO IMPROVE COWPEA PRODUCTION

6.1 Abstract

The use of net houses is one possible way to increase the yield and quality of vegetable production and reduce the use of pesticides. To prevent outbreaks of cowpea pests in the net house, it is investigated a combination of ‘push-pull’ strategy and net house. Whereas the ‘push’ stimuli comprised of two plants: *C. citratus* and *T. minuta*, the ‘pull’ stimuli comprised of visual cues from blue and yellow sticky traps. Field experiments were conducted for two seasons between October, 2017-May, 2018. Four treatments that comprised a control (open field), net house, push-pull strategy (open field) and net house + push-pull strategy were evaluated. The treatments were replicated four times in a Latin square design. The insects were counted on 10 plants per plot.

Results showed that insect pests such as *C. tomentosicollis*, *Empoasca* sp, *M. vitrata*, *M. sjostedti* and *T. vaporariorum* were significantly lower in the net house than in the open field. Conversely, the population of *A. craccivora* was significantly higher in the net house than in the open field. The populations of *T. vaporariorum* and *A. craccivora* were lower in the push-pull treatment than in either control or net house treatments, respectively. The yield and the quality of the pods and grains were higher in the net house than in the open field.

The study indicates that push pull strategy was effective against certain small pests. The use of net house was very effective to reduce the number of some major pests of cowpea and improved the yield of pods irrespective of the season.

6.2 Introduction

The use of netting has already been shown to be effective in protecting different vegetable crops against a range of pests by reducing damage, increasing yields and the quality of products in sub-Saharan Africa (Martin *et al.,* 2006) and more generally in the tropics (Nordey *et al.*, 2017). In Benin, the use of nets reduced insecticide spraying by 70% to 100% on cabbage (Licciardi *et al.*, 2007; Martin *et al.*, 2006). The research in Kenya showed, green beans, cabbages and tomatoes were produced with no or very few chemical applications compared to in open fields, with higher yields and better quality produce (Saidi *et al.*, 2013; Gogo *et al.*, 2014b). Netting is a physical barrier which prevents the pests from passing through. Thirty and forty mesh size netting (0.9 mm, & 0.4 mm diameter respectively) have been shown to be effective as a barrier against pests relative large (body length > 5 mm) such as lepidopteran and dipteran in horticultural crops in Benin and Kenya (Martin *et al.*, 2015). It is hypothesized that nets could effectively protect a cowpea crop against the large insect pests (body length > 5 mm) including *M. vitrata* and *C. tomentosicollis*. However, net houses have been shown to be less efficient in controlling small insects which can pass through the mesh. For example, Simon *et al.,* (2014) showed that a netting with a mesh size of 0.4 mm (30 mesh) was ineffective for a cabbage crop against mustard aphid, *Lipaphis erysimi* Kaltenbach. It is hypothesized that nets (0.4 mm diameter) could not provide efficient protection against the small insects such as thrips, aphids and whitefly.

The bean flower thrips *M. sjostedti* in particular is the main small pest of cowpea and has the most negative impact on yield. Plus netting is also a barrier against the thrips natural enemies, which are really useful for the control of small pests, as these are usually resistant to pesticide thanks to their rapid life cycle (Martin *et al.,* 2013). On the other hand, net houses stabilize air temperature and improve soil moisture (Gogo *et al.,*2012; Saidi *et al*., 2013; Gogo e*t al.*, 2014b; Simon e*t al.*, 2014). Thus, cowpea yield and quality would be improved by control of damaging insect pests and as a result of a better micro climate inside the net houses.

To prevent outbreaks of small pests including the bean flower thrips in the net house, an alternative crop protection strategy needs to be combined with the use of netting. The 'push-pull' strategy is a crop protection concept which uses attractant and repellent stimuli simultaneously to control the spatial distribution of the insects, in order to reduce insect abundance on the target crop (Cook *et al.,* 2006). This strategy could be combined with net houses to control bean flower thrips. *M. sjostedti* are attracted by the colour blue (Muvea *et al.,* 2014), thus blue sticky traps were used as a pull stimulus in the present study. The previous experiment using olfactory tests showed the vegetative stage of Mexican marigold, *T. minuta* and lemomgrass, *C. citratus* plants was repellent to female *M. sjostedti* (Diabate *et al.,* 2019c). Lemongrass produces a compound (citral) which is repellent to *M. sjostedti* when the leaves are freshly cut (Diabate *et al.,* 2019c). These repellent plants were combined with a net house as push stimuli. Controlling insects using the push-pull strategy has the advantage of avoiding the use of insecticides thereby increasing the opportunity for natural enemies to reduce the insect pest populations (Khan e*t al.,* 1997).

The aim of the present study was to evaluate the effectiveness of a control strategy against cowpea pests combining (a) a physical barrier in the form of an insect proof net house, (b) an olfactory barrier based on repellent companion plants and (c) a visual trap in the form of coloured sticky strips as an attractant for pests, to increase the yield and quality of the cowpea grains.

6.3 Materials and methods

6.3.1 Site and seasons

The field trial was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO), Industrial Crops Research Centre (ICRC) Mwea (0°37'09.0"S 37°22'09.4"E) in Kirinyaga County, Central Kenya (Figure 6.1). Mwea is one of the main areas in Kenya where vegetables including cowpea are grown (Musebe *et al.,* 2005). The four seasons in Kenya are characterized by a short rain season (October - November), a hot dry season (January - March), a long rain season (March -June), and a cold dry season (July - August)(Hassan, 1998; Foba *et al.*, 2015). The experiment was conducted in two seasons: in the dry season from 3rd October 2017 to 16th January 2018, and in the rainy season from 30th January 2018 to 22nd May 2018. The dry season was characterized by one rainy month (10th October - 7th November 2017) and three dry months (14th November to 9th January 2018), with a total of 358.8 mm of rain and a mean temperature of 22.71 °C. The rainy period was characterized by one dry month (30th January 2018 - 27th February 2018) followed by three rainy months (27th February -15th May 2018), with a total rainfall of 679.5 mm and mean temperature of 22.50°C. Rainfall and temperature data were provided by KALRO ICRC Mwea Kirogo research experimental farm.

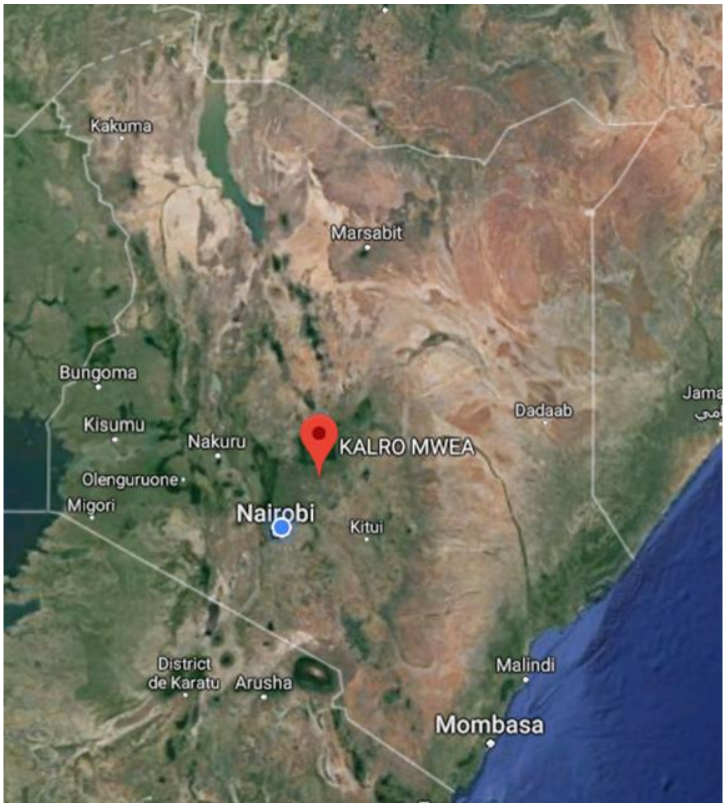

Figure 6. 1: A map of Kenya showing Kalro-Mwea (Source: Google map, 2019)

6.3.2 Planting material and land preparation

Seeds of cowpea, *V. unguiculata* var. Machakos 66 (M66) were purchased from Dryland Seeds Company Limited, and Lemongrass seedlings from Simlaw Seeds Company Limited, in Nairobi, Kenya. Mexican marigold seeds were field collected in KALRO-ICRC Mwea. About 40 kgs of manure (cattle) was spread per plot (10 m x 5m) which was applied two weeks before the cowpea seeds were sown. Two cowpea seeds were sown in two hills on each side of the micro-irrigation emitters at a distance of 25 cm with 1 m inter-row spacing. Each experimental plot measured 5 m x 10 m and 60 cowpea plants were planted per line, giving a total of 300 plants (60 plants x 5 lines) per plot. A total of sixteen plots were prepared including 4 plots per treatments (untreated control, push-pull plots, net house plots, and net house combined with push-pull plots). No insecticide was sprayed in the field experiment. To avoid the destruction of plants by the overwhelming of *A. craccivora* population, a mixture of soap liquid (≈5 ml) and powder pepper (≈5 g) in water (20 litres) was sprayed in the treatments with net houses once a week from the 14th to the 28th of November, 2017 to reduce outbreaks of aphids. The mixture of soap and pepper is a safe method to regulate the population of aphids (Pahla *et al.*, 2014). The solution was applied one time per day in the net house treatments because *A. craccivora* was low or absent in open field treatments.

6.3.3 Treatments and experimental design

Four treatments which included (i) untreated plots in the open field (control), (ii), push-pull plots in the open field (iii) net house plots, and (iv) net house combined with push-pull plots were laid out in a Latin square design in four replicates (Figure 6.2). The push-pull plots included two repellent plants, Lemongrass and Mexican marigold as “push” and coloured sticky traps (blue and yellow) as “pull stimuli” (Plate 6.1). The two blue sticky traps were used to catch *M. sjostedti* and the two yellow ones for other insects including whiteflies and aphids. Four strips of sticky tape (15 cm x 10 m) per push-pull plot were placed at intervals of one metre between the rows at a height of 1 m from the soil. Cowpea was grown for four months and the traps were removed and replaced with new ones at monthly intervals. The old traps were taken to the laboratory where the insects on 10 cm x 10 cm samples of each trap were counted.

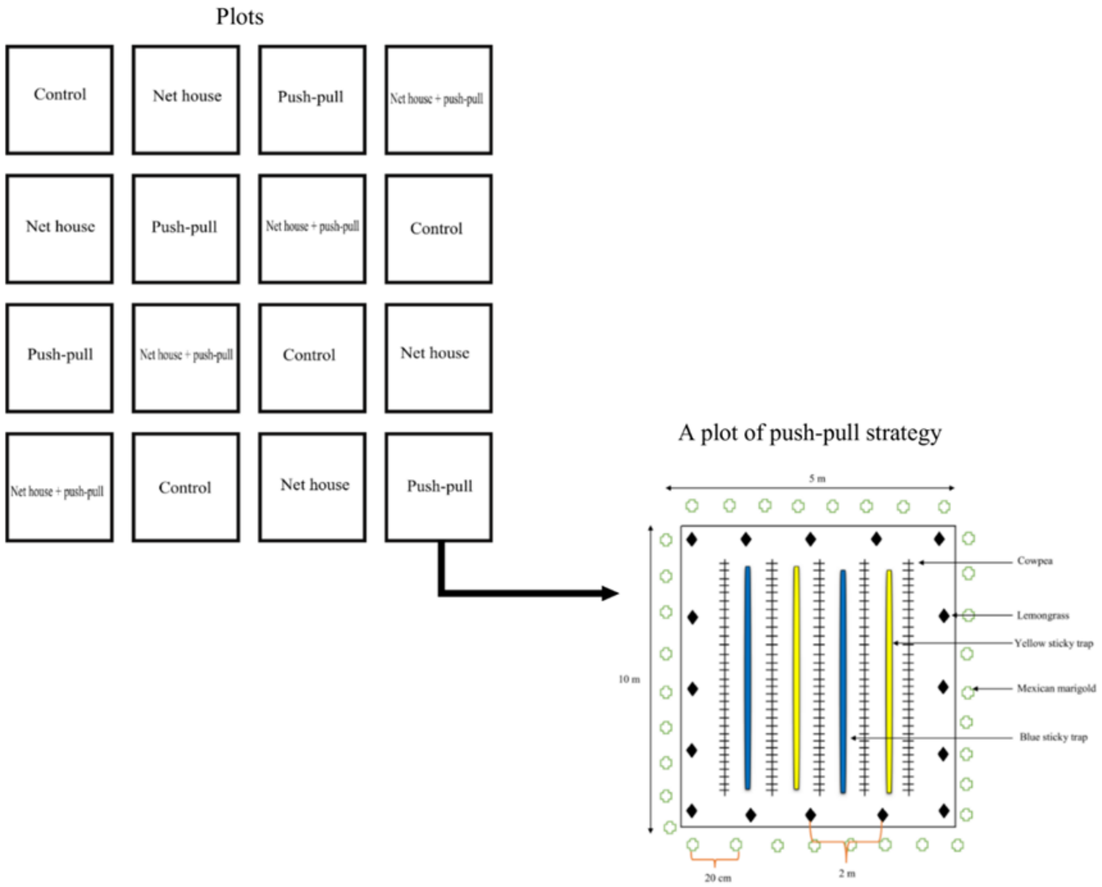

Figure 6. 2: Schematic representation of the experimental field design in the Latin square design, each treatment was repeated once in each row and column.

Plate 6. 1: Treatments (a = non-treated control; b = push-pull plots; c = nethouse plots; d = nethouse combined with push-pull strategy) in field at Mwea (Source: Diabate, 2018).

6.3.4 Repellent plants arrangement and net house properties

Mexican marigold was planted at 20 cm intervals around the plots. Lemongrass was planted at 2 m intervals around the plots. Mexican marigold is a repellent plant of *M. sjostedti* which continuously releases repellent volatiles (Diabate *et al.*, 2019c). Fresh cut leaves of Lemongrass (about 5 cm of each leaf cut with a pair of scissors once a week) were applied as an organic mulch to repel *M. sjostedti*. In the plots with a net house combined with the push-pull strategy. The experimental plots were separated from each other by 2-m buffer strips of bare soil.

The net house was a locally manufactured high tunnel covered by a transparent knitted polyethylene net AgroNet 0.4 (A to Z Textile Mills, Arusha, Tanzania) of 40 mesh size (0.4 mm diameter hole size) under an iron frame structure. The high tunnel was 5 m in width, 10 m in length and 2 m in height (flat on top) in the middle and 2 m in height at the sides with a double door. The rain was able to penetrate through the top of net house.

6.3.5 Data collection by counting of insects, flowers, number harvested and weight of pods in the field

The insects were counted starting two weeks after sowing and thereafter on weekly basis for 14 and 15 weeks in season 1 and 2 respectively. Two different rows were randomly selected each week for observations. The first and last rows were not used to avoid a border effect. Ten individual plants were observed per plot. The plants located on the left facing to the door between the micro-irrigation holes were always selected for observation. If the plants on the left did not germinate, then the plant on the right was selected instead. The number of adult insects and larvae (large and small) on each plant were counted on different parts of the cowpea plant during growth: (i) the whole plant was used to count large insect pests such as Leafhoppers, *Empoasca* sp, Brown Pod-sucking Bug, *C. tomentosicollis*, cotton leafworm, *S. littoralis* and the caterpillar of Bean Pod Borer, *M. vitrata*. Small insects like Bean Flower Thrips, *M. sjostedti*, thrips, *Hydatothrips adolfifriderici* Karny, Black Legume Aphid, *A. craccivora* and Greenhouse Whitefly, *T.*

vaporariorum were counted (ii) on one old and one young leaf, (iii) on a 5 cm portion of the plant stem (iv) on two picked open flowers, and (v) on two pods per plant. The number of flowers per plant were also counted. For the harvest, undamaged pods or pods damaged by insects were counted and weighed to evaluate the quantity of marketable pods per plot. The grains harvested on each plot were placed in bags and weighed.

6.4 Data analysis

The insects, flowers and harvested pods counted in the treatments were systemarically log transformed (log (1+ x)) before analysis using analysis of variance (ANOVA). When there was a significantly difference in the mean among four treatments, a multiple comparison of means was performed using a Tukey's HSD test. For each treatment, the means between seasons were compared using Student's t tests. All analyses were implemented in R version 3.3.2 (R Core Team, 2016).

6.5 Results

6.5.1 Season and plant development

Cowpea seedlings emerged one week after sowing in both seasons. In the season 1, flowering started seven weeks after sowing in both net house plots, and flowering peaked 10 weeks after sowing in the push-pull plots. In the season 1, the first flowers appeared eight weeks after sowing in both the net house plots and in the push-pull plots. Cowpea pods were observed eight weeks after sowing in the both seasons. The first pods were harvested 12 weeks after sowing in the season 1 and 14 weeks after sowing in the season 2. Pods were harvested twice a week.

6.5.2 Effect of seasons on pest populations dynamics

The populations of the major cowpea pests including the bean flower thrips *M. sjostedti*, the Black Legume Aphid *A. craccivora*, the Pod Borer *M. vitrata* and the Greenhouse

Whitefly *T. vaporariorum* were higher in the season 1 than in the season 2, whereas the opposite was true for Leafhopper *Empoasca* sp. The populations of secondary pests such as *S. littoralis, C. tomentosicollis* and *H. adolfifriderici* were quite low and were almost the same amount in the two seasons.

During the season 1, the colonisation of cowpea by insects with piercing-sucking mouthparts like *T. vaporariorum* and *A. craccivora* started two and three weeks after sowing respectively. *T. vaporariorum* was observed on the leaves while *A. craccivora* started by infesting the leaves and stems then moved to the flowers and pods. Peak infestation of *T. vaporariorum* was observed six weeks after sowing in untreated control plots, whereas outbreaks of *A. craccivora* reached their peaks eight and nine weeks after sowing in net house plots during the flowering and podding stages. *M. sjostedti* infestation started with the flowering stage of cowpea, eight weeks after sowing in both open field plots. Peak infestation occurred two weeks later during the flowering and podding stages. *H. adolfifriderici* and *Empoasca sp.* populations were smaller, infestation started two and four weeks after sowing in the net house and push-pull plots respectively. Peak *H. adolfifriderici* infestation occurred 11 weeks after sowing in untreated control plots while peak *Empoasca sp* infestation occurred 13 weeks after sowing in untreated control plots. Infestation of *C. tomentosicollis* started nine weeks after sowing during the podding stage in both open field plots. The first peak was observed 12 weeks after sowing in the push-pull plots and the second peak was observed 13 weeks after sowing in the untreated control plots. The population of larvae of *S. littoralis* and *M. vitrata* chewing mouthpart insects was smaller than that of piercing-sucking mouthpart insects. *S. littoralis* infestation started four weeks after sowing in net house plots and the first and second peaks occurred five and 13 weeks after sowing in net house plots. The infestation of *M. vitrata* started 10 weeks after sowing in untreated control plots during the flowering and podding stages. *M. vitrata* populations reached peak densities 12 weeks after sowing in untreated control plots.

In the season 2, infestations of *T. vaporariorum, A. craccivora, M. sjostedti, C. tomentosicollis, H. adolfifriderici* and *M. vitrata* began in similar periods. Peak *T. vaporariorum* infestation was observed six weeks after sowing in untreated control plots while *A. craccivora* peak infestation occurred seven weeks after sowing in untreated control plots. Three *M. sjostedti* infestation peaks were observed 13, 14 and 15 weeks after sowing in net house plots, push-pull plots and untreated control plots, respectively. Peak *H. adolfifriderici* infestation occurred three weeks after sowing in push-pull plots. *Empoasca* sp populations appeared six weeks after sowing in untreated control plots and the peak was observed four weeks later. Peak *C. tomentosicollis* infestation occurred 16 weeks after sowing in net house combined with push-pull plots. Peak *M. vitrata* infestation was observed 12 and 14 weeks after sowing in push-pull plots. *S. littoralis* infestation was observed 11 weeks after sowing and the peak was reached five weeks later.

6.5.3 Effect of treatments on pest populations in the seasons

The population of the Greenhouse Whitefly *T. vaporariorum* was higher in untreated control plots than in plots in a net house combined or not with the push-pull strategy in the season 1 ($P < 0.001$) (Table 6.1). In the season 2, the number of *T. vaporariorum* was higher in untreated control plots than under all treatments ($P < 0.001$). In addition, the number of *T. vaporariorum* in net house combined with push-pull plots was lower than in push-pull plots in the season 2 ($P < 0.001$). The infestation of *T. vaporariorum* was higher in untreated control plots and push-pull plots in the season 1 than in the season 2 (untreated control: $t = 4.91$, df = 6, $P = 0.002$, push-pull: $t = 5.90$, df = 6, $P = 0.001$). The yellow sticky traps caught significantly higher number of *T. vaporariorum* in push-pull strategy than in the net house combined with push-pull in both seasons ($t = 2.506$, df = 14, $P = 0.025$) (Table 6.2). On blue sticky trap, no different was observed between push-pull strategy and the net house combined with push-pull.

In the season 1, *A. craccivora* were more abundant on cowpea leaves in all net house plots than in push-pull plots and untreated control plots ($F = 9.703$; df = 3; $P = 0.001$) (Figure

6.3). In the season 2, there were no differences. The number of aphids was higher in the net house combined with push-pull plots in the season 1 than in the season 2 ($t = 4.728$, df = 6, P = 0.003). In contrast, the number of aphids on the leaves was higher in the untreated control plots and push-pull strategy plots in the season 2 than in the season 1 (untreated control: $t = -3.0412$, df = 6, P = 0.022; push-pull: $t = -3.566$, df = 6, P = 0.011). *A. craccivora* were only present on flowers and pods in the net house plots combined or not with the push-pull strategy, there was no difference between the two treatments. In addition, more *A. craccivora* were observed on flowers in the plots with net houses combined or not with the push-pull strategy in the season 1 than in the season 2 (net house: $t = 3.325$, df = 6, P = 0.015; net house + push-pull: $t = 5.320$, df = 6, P = 0.001). The number of *A. craccivora* caught on yellow and blue sticky traps were not significantly different between the push-pull strategy plots and net house combined with push-pull strategy in both seasons (P > 0.05).

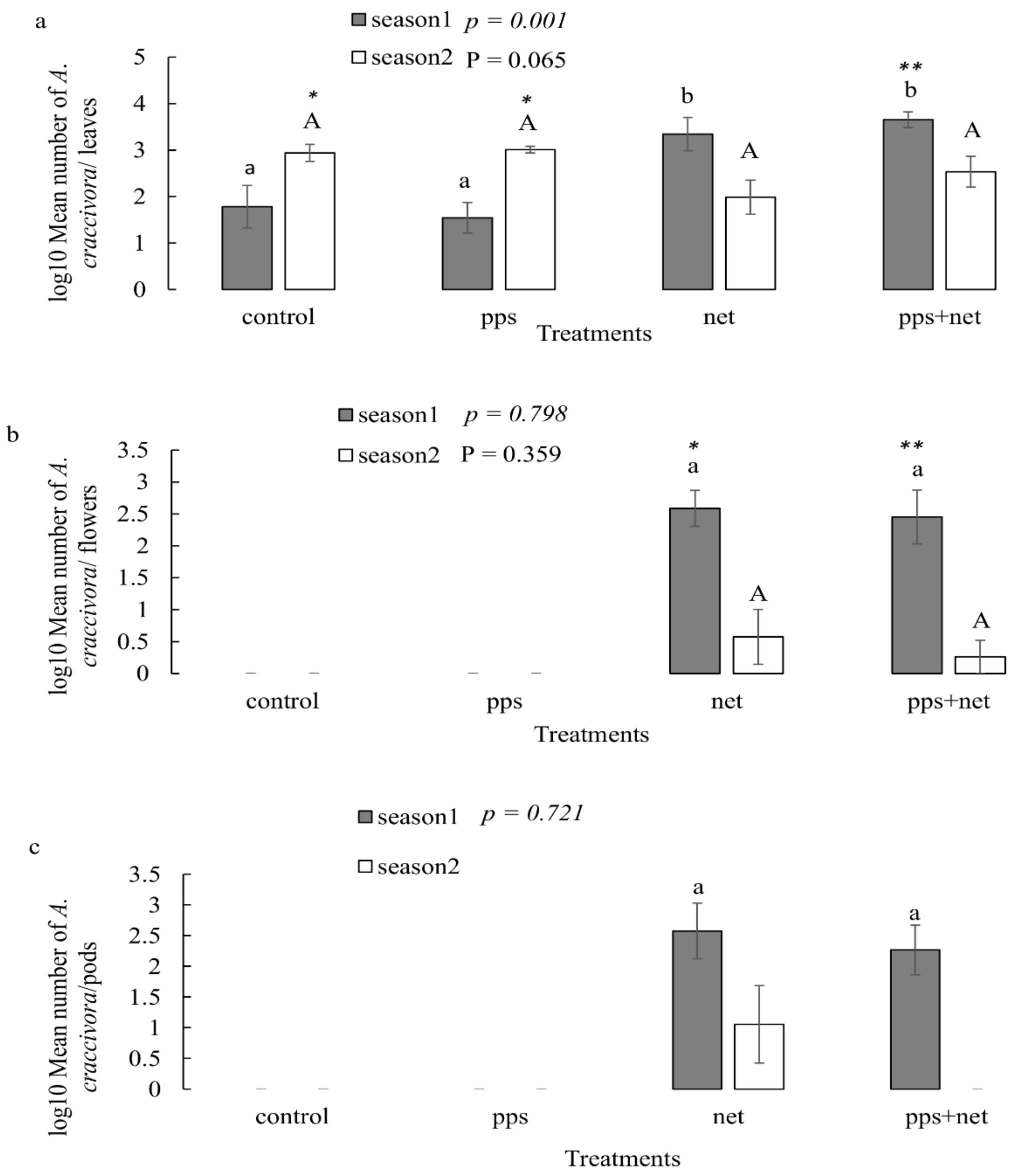

Figure 6. 3: Mean number (±SE) of adult and larval *Aphis craccivora* on cowpea plants per treatment in the dry season (season 1) and the rainy season (season 2) at KALRO- Mwea (Kenya). a) leaves and stem, b) flowers, c) podsThe same letter means no significant difference (ANOVA). Asterisks (*) mean significant difference in the means of the same treatments between seasons (*P < 0.05, **P < 0.01; ***P < 0.001) (Student's t test).

The Bean Flower Thrips, *M. sjostedti* population on the cowpea leaves was no different in both seasons 1 and 2 (Figure 6.4). In addition, more *M. sjostedti* were observed in untreated control plots in the season 1 than in the season 2 (leaves: $t = 3.364$, df = 6, P = 0.015). In the season 1, the population of *M. sjostedti* was more abundant on the flowers in the untreated control plots and push-pull strategy plots than in net house combined or not with push-pull plots ($P < 0.001$). In the season 2, fewer thrips were found in net houses combined with push-pull plots than in plots with push-pull alone (F = 3.916; df = 3; P = 0.036). The number of thrips in the net house plots was higher in the season 2 than in the season 1 ($t = -4.9626$, df = 6, P = 0.002). No difference was observed in the number of thrips on the pods under any of the treatments in either season. The population of *M. sjostedti* was more abundant in the untreated control plots, push-pull plots and net house combined with push-pull plots in the season 1 than in the season 2 (untreated control: $t = 2.566$, df = 6, P = 0.042; push-pull: $P < 0.001$; net house + push-pull: $t = 5.181$, df = 6, P = 0.002). The blue sticky traps caught more *M. sjostedti* in push-pull plots than in net houses combined with push-pull plots ($t = 3.59$, df = 14, P = 0.002). No difference of number of *M. sjostedti* caught on the yellow sticky traps between push-pull and push pull combined with net house plots.

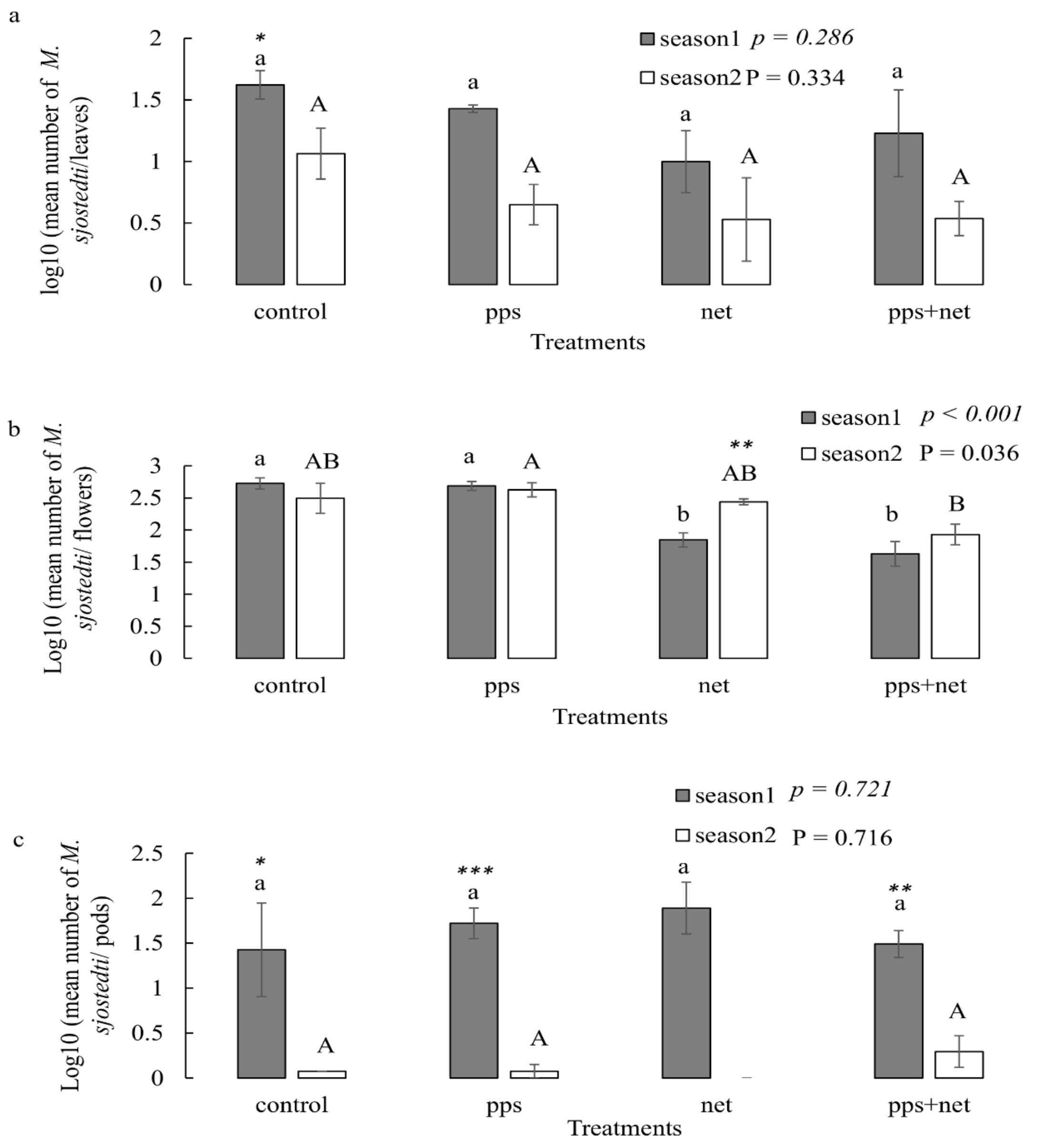

Figure 6. 4: Mean number (±SE) of adult and larval *Megalurothrips sjostedti* on cowpea plants per treatment in the dry season (season 1) and the rainy season (season 2) at KALRO- Mwea (Kenya). a) leaves, b) flowers, c) pods. The same letter means no significant differences (ANOVA). Asterisks (*) mean significant differences in the means of two same treatments between seasons (*P < 0.05, **P < 0.01; ***P < 0.001) (Student's test).

The populations of *H. adolfifriderici* and *S. littoralis* were very low and no significantly differences were found among the plots. *H. adolfifriderici* were more abundant in the net house plots in the season 1 than in the season 2 (*t* = 3.10, df = 6, P = 0.02).

Populations of the Leafhopper *Empoasca* sp. were smaller in net house plots and in net houses combined with push-pull plots than in untreated control plots and push-pull plots in both seasons (s1: F = 6.284; df = 3; P = 0.008; s2: P < 0.001). The number of *Empoasca* sp was higher in untreated plots and push-pull plots in the season 2 than in the season 1 (untreated: *t* = -5.53, df = 6, P = 0.001; push-pull: *t* = -5.31, df = 6, P = 0.001).

Clavigralla. tomentosicollis populations were smaller in net house plots combined or not with the push pull plots than in untreated control plots and push-pull plots in the season 1 (P < 0.001). No difference was found among the treatments in the season 2. The number of *C. tomentosicollis* was higher in the nethouse plots in the season 2 than in the season 1 (*t* = -4.21, df = 6, P = 0.005).

No *Maruca vitrata* were found in the net house plots in either season. *M. vitrata* infestation was significantly lower in net houses combined with push-pull plots than in untreated control plots in the season 1 (F = 7.183; df = 3; P = 0.005).

In general, net house plots contained smaller populations of *M. sjostedti*, *T. vaporariorum*, *Empoasca* sp. *C. tomentosicollis* and *M. vitrata* than open field plots. Conversely, *A. craccivora* infestations were higher in the net house. The push-pull strategy reduced populations of *T. vaporariorum* compared to controls and reduced populations of *Aphis craccivora* compared to net houses alone.

Table 6. 1: Mean (± SE) number of insects observed per plant among the treatments in the season 1 (dry)[1] and season 2 (rainy)[2] at KALRO- Mwea (Kenya).

		Season 1				Season 2			
Insect pests	**Plant parts**	**Control**	**Push-pull**	**net**	**Push-pull + net**	**Control**	**Push-pull**	**net**	**Push-pull + net**
Trialeurodes vaporariorum	Leaves	196.2 ± 26.7a*	78.2 ± 20.5ab*	53.2 ± 46.1bc	2.1 ± 1.4c	54.7 ± 15.1A*	13.0 ± 1.8B*	4 ± 0.8BC	2.0 ± 1.0C
Aphis craccivora	Leaves	343.7 ± 320.7a*	71.5 ± 41.8a*	4037.82 ± 1694.3b	5563.5 ± 1928.2b*	1518.7 ± 132.7A*	637.5 ±116.0A*	902.7 ± 640.6A	187.2 ± 82.8A*
	Flowers	0a	0a	644.75 ± 311.4b*	968.5 ± 760.3b*	0A	0A	18.7 ± 15.5B*	0 ± 0.5B*
	Pods	0aa	0a	1008.8 ± 519.22b	731.2 ± 644.1b*	0A	0A	93.2 ± 77.4B	0A*
Megalurothrips sjostedti	Leaves	46.2 ± 14.7a*	26 ± 1.8a	13.5 ± 5.4a	48.5 ± 40.5a	8.2 ± 4.0A*	10.2 ± 4.5A	3.5 ± 1.2A	6.7 ± 6.0A
	Flowers	564.2 ± 110.4a	505.7 ± 91.3a	77.2 ± 23.4b*	54.7 ± 21.1b	462.0 ± 211.0AB	458.5 ± 79.4A	279.0 ± 28.0AB*	99.2 ± 26.1B
	Pods	103.5 ± 58.0a*	64.2 ± 22.5a*	136.75 ± 78.03a*	35.5 ± 11.2a*	0 ± 0.2A*	0 ± 0.2A*	0A*	1.5 ± 0.9A*
Hydatothrips adolfifriderici	Leaves	35.5 ± 3.9a	28.7 ± 5.9a	24.5 ± 2.1a*	23.2 ± 2.3a	25.5 ± 2.1A	29.0 ± 6.5A	14.7 ± 2.1A*	16.7 ± 3.6a
Spodoptera littoralis	Plants	0a	0 ± 0.2a	1.7 ± 0.8a	0 ± 0.4a	1.5 ± 1.9A	0 ± 0.2A	4.2 ± 2.3A	1.25 ± 0.9A
***Empoasca* sp.**	plants	6.0 ± 1.7a*	4.0 ± 1.0a*	0 ± 0.2b	0 ± 0.2b	119.5 ± 24.7A*	46.5 ± 18.5A*	1.0 ± 0.7B	0B
Clavigralla tomentosicollis	Plants	104.5 ± 48.1a	78.7 ± 24.0a	3.2 ± 3.2b*	0b*	39.0 ± 13.91A	63.0 ± 26.2A	36.0 ± 19.7A*	150.0 ± 25.8A*
Maruca vitrata	Plants	4.7 ± 1.7a*	2.5 ± 0.8ab	0b	0 ± 0.2b	0A*	0 ± 0.4A	0A	0A

[1] Dry season from 3rd October 2017 to 9th January 2018 /[2] Rainy season from 30th January 2018 to 15th May 2018. SE = Standard error. Small letters corresponding to the comparison of treatments in the dry season (season1) and capital letters comparing the treatments in the rainy season (season 2) using analysis of variance (ANOVA). *indicate significant differences between seasons with the Student's t test. The same letter in the same row means not significantly different.

Table 6. 2: Mean (± SE) number of insects: *A. craccivora, M. sjostedti* and *T. vaporariorum* caught on the coloured sticky traps across the two seasons. Dry season (season 1) from 3rd October 2017 to 9th January 2018. Rain season (season 2) from 30th January 2018 to 15th May 2018.

Insects		**Blue trap**	**Yellow trap**
T. vaporariorum	Push-pull	10.6 ± 12.8 a	2315.3 ± 1771.5 a
	Net house + push-pull	1.0 ± 2.0 a	91.2 ± 100.0 b
	t-test	1.488	2.506
	df	14	14
	P value	1.158	0.025
A. craccivora	Push pull	0 a	64.6 ± 99.9 a
	Net house + push-pull	0.25 ± 0.32 a	93.9 ± 88.6 a
	t-test	-1.527	-1.438
	df	14	14
	P value	0.148	0.668
M. sjostedti	Push pull	265.4 ± 131.7 a	4.4 ± 4.5 a
	Net house + push-pull	27.0 ± 14.2 b	2.1 ± 1.4 a
	t-test	3.599	0.958
	df	14	14
	P value	0.002	0.354

SE = Standard error. Small letters compare the two treatments per insect in each column using Student's t test at $P < 0.05$. The same letter means not significantly different

6.5.4 Effect of treatments on yield in the seasons

There was no significant difference in the number of open flowers among the four treatments or between the same treatments in the two seasons ($P > 0.05$) (Table 6.3). In the season 1, there was no significant difference in the total number and weight of harvested pods among the treatments. However, in the season 2, the total number and weight of harvested pods was higher in the net house and net house combined with push-pull plots than in untreated control plots and plots with push-pull alone (number: $P < 0.001$, weight: $P < 0.001$). The number of pods was higher in the untreated control plots and push-pull strategy plots in the season 1 than in the season 2 (untreated control: $P < 0.001$; push-pull: $t = 5.086$, df = 6, $P = 0.002$). The weight of pods harvested in the untreated control plots and push-pull plots was higher in the season 1 than in the season 1 (untreated control: $P < 0.001$; push-pull: $t = 5.385$, df = 6, $P = 0.001$).

The number of marketable pods and their weight were higher in the net house plots than in untreated control plots in the season 1 (number: $F = 5.241$; df = 3; $P = 0.015$; weight: $F = 5.67$; df = 3; $P = 0.011$) (Table 6.3). In the season 2, the number of marketable pods and their weight were higher in net house plots and net houses combined with push-pull plots than in untreated control and push-pull plots (number: $P < 0.001$; weight: $P < 0.001$). The number and weight of marketable pods in push-pull plots were higher in the season 1 than in the season 2 (number: $P < 0.001$; weight: $P < 0.001$).

Grains were significantly more abundant in net house plots and net house combined with push-pull plots than in untreated control plots and push-pull plots in the rainy season ($P < 0.001$) (Table 6.3).

The total yield across the two periods was more abundant in net house compared to open field treatments ($F = 5.14$, df = 3, $P < 0.01$) (Table 6.4). However, no difference was observed between net house combined with push-pull and open field treatments. The marketable yield was significantly higher in the net house and net house combined with push-pull than untreated control and push-pull strategy ($F = 9.35$, df = 3, $P < 0.001$).

Table 6. 3: Mean (± SE) of flowers per plant and of the total number and weight of harvested pods and marketable cowpea pods and of number of grains per plot among the treatments in the season 1 (dry) [1] and season 2 (rainy) [2] at KALRO- Mwea (Kenya).

	Season 1				**Season 2**			
	Control	Push-pull	net	Push-pull + net	Control	Push-pull	net	Push-pull + net
Flowers	41.2 ± 5.1a	81.0 ± 25.6a	52.2 ± 9.07a	45.5 ± 9.6a	26.2 ± 10.5a	38.7 ± 9.0a	49.2 ± 9.6a	47.7 ± 4.6a
Total harvested pods	1544.2 ± 272.1a*	1690.7 ± 53.9a*	2447.5 ± 479.7a	2092.2 ± 174.9a	7.0 ± 7.0A*	32.7 ± 32.7A*	1745.5 ± 381.3B	1415.0 ± 253.1B
Total weight of pods (g)	3953.0 ± 752.4a*	4045.0 ± 182.0a*	6294.2 ± 1157.7a	5654.0 ± 377.2a	20.0 ± 20.0A*	45.0 ± 45.0A*	4186.7 ± 1006.7B	3277.5 ± 581.7B
Marketable harvested pods	988.2 ± 232.3a*	1055.0 ± 50.4ab*	2103.7 ± 397.7b	1740.7 ± 152.2ab	0A*	9.7 ± 9.7A*	1687.0 ± 373.1B	1273.5 ± 230.5B
Marketable weight pod (g)	2775.0 ± 608.7a*	2815.0 ± 57.3ab*	5640.0 ± 987.6b	4885.2 ± 342.5ab	0A*	5.0 ± 5.0A*	4071.7 ± 987.6B	3112.5 ± 569.5B
Grains					10.0 ± 10.0A	12.5 ± 12.5A	3457.5 ± 868.8B	2390.0 ± 424.9B

[1] From 3rd October 2017 to 9th January 2018 i.e. the dry season (season 1)/ [2]From 30th January 2018 to 15th May 2018, i.e. the rainy season (season 2). Small letters refer to the comparison of treatments in the season 1 and capital letters to comparison of the treatments in the season 2 using analysis of variance (ANOVA). *indicate significant differences between seasons with the Student's t test. The same letter in the same row means not significantly different.

Table 6. 4: Estimated yield (t ha^{-1}) of total and marketable pods among the treatments across the two seasons. Dry season (season 1) from 3rd October 2017 to 9th January 2018. Rain season (season 2) from 30th January 2018 to 15th May 2018

Treatments	Mean (± SE) of total yield (t ha^{-1})	Mean (± SE) of total marketable yield (t ha^{-1})
Control	0.4 ± 0.2 a	0.3 ± 0.1 a
Push-pull	0.4 ± 0.2 a	0.3 ± 0.1 a
Net house	1.0 ± 0.2 b	1.0 ± 0.1 b
Net house + push-pull	0.9 ± 0.1 ab	0.8 ± 0.1 b

Letters compare the treatments per yield in each column using analysis of variance (ANOVA). The same letter means not significantly different

6.6 Discussion

Netting effectively protected the cowpea crop against the main big pests: the Bean Pod Borer *M. vitrata*, the Leafhopper *Empoasca* sp, and the Brown Pod-sucking Bug *C. tomentosicollis*. Despite the large number of *Empoasca* sp outside during the season 2, the net house prevented this pest infesting cowpea in the season 2. In the season 1, there were fewer Bean Pod Borers and Brown Pod-sucking Bugs in net house plots than in open field plots, and we observed no difference in the size of the populations in the season 2. The reduction in the number of big pests through the use of net houses in horticulture usually has a major impact on yield. *C. tomentosicollis* and *M. vitrata* can cause 80% yield losses in cowpea in Africa (Ekesi *et al.*, 2002; OECD, 2015). The reduction in the number of big pests in the net houses could thus explain part of the increase in the number of the marketable cowpea pods.

6.6.1 Effect of net house on small insect pests

The study showed that populations of the thrips *M. sjostedti* and the whitefly *T. vaporariorum* on cowpea in the net house were always lower than the populations that increased very rapidly in open field plots, particularly in the season 1. The net house could work as a visual barrier which disturbs the signals thrips and witefly use to locate their host plants. In addition, the populations of *M. sjostedti* and *T. vaporariorum* were low inside the net house. The net house could reduce the UV light which may interfere with insect vision and their dispersion (Raviv *et al.*, 2004; Ben-Yakir *et al.*, 2014).

By contrast, the population of *A. craccivora* was higher in net house plots than in open field plots particularly in the season 1. The outbreaks of aphids in the net house in the season 1 suggest that the temperature and humidity in the net house were very suitable for aphids. A typical life cycle involves flightless females giving birth to female nymphs without the involvement of males (Irwin, 1980). Females mature rapidly and breed profusely, hence the number of these insects rapidly multiplies (Saroch, 2000). In addition, the absence of natural enemies to regulate the aphid population in the net house may also explain the abundance of *A. craccivora*. Martin *et al.*, (2013) reported the small mesh size drastically reduces the presence of aphid parasitoids and predators in net houses.

6.6.2 Effect of push-pull strategy in controlling cowpea pests

Lemongrass and Mexican marigold were used to repel insects from the cowpea crop and sticky traps were used as attractants to catch the flying insects. But the push-pull strategy either alone or combined with net houses did not improve the control of *M. sjostedti* in season 1 and 2. In laboratory assays, *M. sjostedti* was shown to be repelled by fresh cut leaves of Lemongrass but not by old cut leaves (Diabate *et al.,* 2019c). The repellence time of cut leaves of Lemongrass was perhaps too short to repel sufficient *M. sjostedti.* The vegetative stage of Mexican marigold was also shown to repel female *M. sjostedti* in the laboratory (Diabate *et al.,* 2019c) . In the field, the greater abundance of cowpea plants in relation to that of companion plants could also alter their repellent effect. The selection of companion plants which emit larger quantities of repellent volatiles or the use of repellent essential oil through dispensers or in the irrigation system could improve the efficacity of repellent volatiles in the field. In the present study, the repellent plants were planted around the cowpea crop. The spatial arrangement of plants in intercropping may also influence the control of pests in the field. Kasina *et al.,* (2006b) reported that *Coriandrum sativum* L, *Zea mays* L and *Tagetes erecta* L interplanted in French beans reduced the population of thrips. Further investigation of the arrangement of repellent plants in intercropping systems is thus required.

On the contrary, the present study showed that populations of *T. vaporariorum* and *Aphis craccivora* were lower in the push-pull plots than in either control plots or net house plots, respectively. In fact, many greenhouse whiteflies were caught on the yellow sticky traps. The attraction of *T. vaporariorum* to yellow has been demonstrated by many authors (Mutwiwa & Tantau, 2005) The yellow sticky traps may thus have helped reduce the numbers of this insect in the push-pull plots. In addition, the repellent effect of essential oil from Lemongrass has been demonstrated on the whitefly, *Bemisia tabaci*, in the laboratory (Deletre *et al.*, 2015). Lemongrass could be involved in reducing the number of *T. vaporariorum* in push-pull plots. Conversely, Mexican marigold could act as a trap plant diverting *T. vaporariorum* from cowpea. In fact, large number of whiteflies on the marigold plants was observed during this study. In the season 1, the population of *A. craccivora* was also smaller in push-pull plots than in net house plots. Zavaleta Mejía and Gómez (1995) reported that intercropping marigold, *Tagetes erecta* L. and tomato, *Lycopersicon esculentum* Mill reduced the population of alate aphids. Mexican marigold may also disturb the establishment of *A. craccivora* on cowpea. Silveira *et al.*, (2009) reported that planting marigold, *T. erecta* around onion fields increased the number of insect species in the onion fields. Marigold plants surrounding cowpea could thus increase the diversity of the natural enemies of this pest. The repellent effect of Lemongrass volatiles against aphids was reported by Calumpang and Ohsawa (2015) in the field. Lemongrass could thus help reduce populations of *A. craccivora*.

6.6.3 Effect of season on infestation of insect pests

Cowpea production varied with the season. The reduction in cowpea yield may be due to too much rain, as already shown by Parwada (2016). The populations of the major cowpea pests such as *M. sjostedti*, *T. vaporariorum*, *A. craccivora* and *M. vitrata* were much lower in the season 2 than in the season 1. In Kenya, the heavy rainfall between March and June, 2018, may be responsible for the reduction of insect populations by destroying eggs and killing some of the larvae. Nyasani *et al.*, (2013b) reported that heavy rain killed the larvae of western flower thrips, *Frankliniella occidentalis* in the field and hence reduced their population density. The population of *A. craccivora* was reduced in the net houses in the season 2. Indeed, rainfall is considered to regulate population densities of aphids (Kaakeh & Dutcher, 1993). Despite the fact the rainy season was longer than usual in the present study, cowpea production was stable in the net house. The net technology may thus be an efficient tool in unpredictable weather conditions

6.6.4 Effect of net house on the yield of cowpea

The number and weight of marketable pods produced in the net houses were higher than in control plots in both seasons but the combination with push-pull did not increase cowpea yield. Although the number of flowers was the same among the treatments and in the two seasons, the total number and weight of pods produced in the net houses with or without push-pull were higher than in both open field plots in the season 2. In the present study, several data explain the increase in yield in net house plots. Saidi *et al.,* (2013) reported that the constant high temperature and soil moisture in net house improved plant growth and yield. It is also noticed that the quality of the grains produced in the both net house plots was higher. In Kenya, Gogo *et al.,* (2014a) also reported faster development, higher pod yield, and better quality green beans, another leguminous plant growing under low tunnels covered with netting compared with uncovered plants.

6.7 Conclusion

The study showed the total number and weight of pods were the same among the treatments in the season 1 but the marketable number of pods was significantly lower in the open field than in net houses. Net houses considerably reduced infestations of pest on cowpea and consequently the damage they cause, mainly damage by the bigger pest such as *Empoasca sp, M. vitrata* and *C. tomentosicollis* but also by smaller pests such as *M. sjostedti* and *T. vaporariorum*. Conversely, cowpea grow in a net house can be highly infested by *A. craccivora.* Aphids has become the major pest of cowpea in the net house and needs to be carefully surveyed but outbreaks can be reduced by spraying a mixture of soap and pepper. The combination of net house and repellent plants did not improve the control of *T. vaporariorum, A. craccivora* and *M. sjostedti*. However, the push-pull strategy reduced the infestation of *T. vaporariorum* and *A. craccivora*. The study also showed that, in the rainy season, cowpea production was less successful in the open field than in net houses. Cowpea plants were strongly affected by heavy rains, diseases and fungi in both open field plots but not in the net houses. The rainy season is definitely not the best season to grow cowpea in the open fields despite the low insect pest pressure, because of fungi and diseases. But in a net house the yield and the quality of production was still high confirming the interest of netting to produce off-season vegetable crops when the price may be higher. More generally, this study confirms that netting is an efficient tool to protect vegetable crops against insect pests and to improve crop yield.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

7.1 General discussion

7.1.1 Host plant-insect interactions

The results of olfactory tests showed that the vegetative stage of cowpea cultivar K80 was repellent while the cultivar EL was attractant to *A. craccivora*. The identification of compounds showed that not all the compounds were present in all cowpea cultivars and their amounts varied in each cultivar. Principal component analysis (PCA), which was used to show the relationship between the cowpea cultivars based on the emission of relative amounts of volatile compounds, revealed overlapping of cultivar volatiles. The overlaps showed that the volatile profiles of four cowpea cultivars were quite similar. However, the olfactory bioassays revealed different levels of attraction of *A. craccivora*. In this study, the ratio-specific odour recognition hypothesis applies, i.e. the different amounts of certain compounds in the volatiles emitted by the cowpea cultivars may be responsible for differential attractiveness to *A. craccivora*. The repellent cultivar K80 emitted the most hexanal and *(E)*-2-hexenal, and the cultivar EL emitted the most 1-octen-3-ol and p-xylene. It is hypothesised that the compounds identified in attractant and repellent cowpea volatiles could be used to manage pests in a push or pull effect strategy. The blend of hexanal and *(E)*-2-hexenal added to cowpea cultivar Ex-Luanda reduced the attractiveness to *A. craccivora* compared to the control. This result suggests the ratio-specific hypothesis applies. The blend of hexanal and *(E)*-2-hexenal may act as a masking odour to the cultivar EL. By contrast, the addition of 1-octen-3-ol and p-xylene to cowpea cultivar K80 did not reduce the repellence of this cultivar to *A. craccivora*. Bendera *et al.* (2015) reported that 1-octen-3-ol was the main compound in intact cowpea cultivar EL and was emitted at night. In this study, volatiles emitted by cowpea cultivars were collected over a period of 24 h and 1-octen-3-ol could easily have been emitted within the night cycle. Aphids are more active in the day (Webster *et al.*, 2010) and this could be the reason why 1-octen-3-ol had no effect on the attraction of *A. craccivora* in this study. The compound p-xylene was also abundant in the attractive cowpea cultivar Ex-Luanda. To the best of our knowledge, few studies have been conducted on the attractive effect of p-xylene on the behaviour of aphids. In this study, p-xylene was not attractive to *A. craccivora* in olfactory tests.

This study showed a repellent effect of *(E)*-2-hexenal on *M. sjostedti* female at a concentration of 0.01% but not at a concentration of 1%. *(E)*-2-hexenal could activate a specific olfactory receptor in female sensilla which elicits repellent behaviour. Deletre *et al.* (2016) hypothesised that the true repellent volatile may activate specific olfactory receptors, which in turn, activate a specific glomerulus in the insect brain leading to movement away from the odour source. By contrast, the neutral response at higher concentration could be due to the activation of olfactory receptors that are not specific to *(E)*-2-hexenal, thus reducing the repellent effect. It has been shown previously that the increase in the concentration of a compound leads to the activation of additional olfactory receptors (Malnic *et al.*, 2000).

This study revealed that the behavioural response of *M. sjostedti* male and female differed depending on the volatiles emitted by the cowpea cultivar. Male and female antennae may have a different morphology, type of antennae and sensilla located on the antennae (Usha & Nakamuta, 2001). The shape, abundance and structure of the sensilla can differ among insect species or between sexes in the same species. For example, more olfactory sensilla were counted on the antenna of female beetle, *Trogossita japonica* Reitter than on those of males (Usha & Nakamuta, 2001). The same authors suggested that the larger number of sensilla in the female may help them to detect the plant volatile for oviposition sites. Like female herbivore insects, female thrips may use plant volatiles to select oviposition sites and for this reason, females may respond more to plant volatiles than males (Szendrei & Rodriguez-Saona, 2009).

This study showed that the volatiles from cowpea flowers of cultivar KK1 were attractants for female *M. sjostedti*. The Bean Flower Thrips, *M. sjostedti* is a flower thrips which feeds on floral tissues (Moritz *et al.*, 2013). This result confirmed that flower thrips use floral scents for host location, as suggested by Koschier (2006). By contrast, the present study showed that the volatile emitted by the flower of cultivar K80 was repellent to *M. sjostedti* females. The volatiles emitted by cowpea flowers can thus protect them against *M. sjostedti.* Investigators have reported a repellent effect of volatiles emitted by cultivar K80 on other insects. For example, in olfactory tests, *A. craccivora* aphids were repelled by cultivar K80 (Diabate *et al.,* 2019a). Analysis of the compounds showed that *(E)-β-*ocimene and 1-octen-3-ol were only present in the volatiles of K80 flowers. However, tested alone or combined with the cowpea flowers at different concentrations, these compounds did not repel female *M. sjostedti*. This result suggests the ratio-specific odour hypothesis is validated here. This hypothesis could finally be responsible for the repellent effect of cultivar K80.

Finally, the results of this study could help breeders select non-attractant cowpea cultivars emitting *(E)*-2-hexenal. The concentration of specific compounds or the balance between ratios could be used to reduce the attractiveness to insects. Another possible solution is diffusion of *(E)*-2-hexenal by a companion plant around cowpea plants during the flowering stage to manipulate the behaviour of *M. sjostedti* by repelling the pest.

7.1.3 Non-host plant-insect interactions: Repellent effect of non-host plants

This study showed that the vegetative stage of *T. minuta* is repellent to female *M. sjostedti* in the laboratory but that the flowering stage was not. *(Z)-β-*Ocimene, *(Z)*-3-hexenyl acetate, dihydrotagetone, and limonene were the main compounds in the vegetative and flowering stages. Tested alone, these compounds were not repellent to *M. sjostedti* female but at the vegetative stage, a mixture of the compounds at the same ratio, was repellent. Different concentration of compounds between the vegetative and flowering stage may explain the differential response by female *M. sjostedti*. The behavioural response of insects does not only depend on the concentration of compounds in the volatile but also on their ratio (Deletre *et al.,* 2016). The specific ratio in the appropriate blend is important in the recognition of host plant by insects (Bruce *et al.,* 2005). It is hypothesised that a specific ratio of compounds produced by a companion plant could help repel the thrips.

The present study showed that fresh cut leaves of *C. citratus* were repellent to *M. sjostedti*. In this study, the freshly cut leaves of *C. citratus* released a high quantity of citral (geranial + neral) which was repellent to female *M. sjostedti*. Abtew (2015) showed that the citral was repellent to *M. sjostedti* larvae. In the study, the freshly cut leaves of *C. citratus* were repellent to *M. sjostedti* female, but a blend of the major compounds did not significantly repel the thrips. This result suggests minor compounds may influence the repellent effect of *C. citratus* on female *M. sjostedti*. Hummelbrunner and Isman (2001) reported that minor compounds may act as synergists thereby enhancing the effectiveness of the major compounds.

7.1.4 Pest management strategy

The net house itself reduced the infestation of *M. sjostedti* during the flowering stage compared to cowpea flowers outside the net house. The net house seems to work as a screen which disturbs the visual cues used by these insects to locate their host plants. Gogo *et al.,* (2014a) suggested that the bright white colour of the nets could act as a visual barrier.

By contrast, the poor efficiency of net houses in the control of certain insect pests including aphids was reported by Simon *et al.,* (2014). In this study, infestations of aphids *A. craccivora* on cowpea increased considerably inside the net house compared with in the open field. In Africa, *A. craccivora* only use parthenogenesis (asexual reproduction in which the growth and development of embryos occur without fertilization) to multiply (Irwin, 1980). Consequently, if one aphid succeeds in getting through the net, this could trigger an outbreak. Their mode of multiplication by parthenogenesis and the absence of natural enemies to regulate the population may explain the large population of *A. craccivora* inside the net house. Spraying liquid soap with pepper was very effective in reducing the population. Pinnock *et al.,* (1974) reported that a formulated soap spray with a 0.1% concentration was sufficient to remove *Aphis gossypii* Glover and *Aphis spiraecola* Patch from Pyracantha. However, releasing specific predators or parasitoids is an alternative way to reduce the population of *A. craccivora* inside the net house. Aphid predators such as *Cheilomenes sulphurea* , *C. vicina*, *C. lunata*, *Hippodamia variegate*, *Coccinella* spp, *Scymnus* sp., *Exochomus sp*, have been reported in Kenya (Annan *et al*., 1994; Vaitiaire, 2011).

Both repellent plants *C. citratus* and *T. minuta* have been used as companion plants in the field to protect cowpea crops. Earlier study showed that *C. citratus* produces a compound (citral) that is repellent to *M. sjostedti* when the leaves are freshly cut. It was used as a temporary repellent plant to reduce the population of *M. sjostedti*. The leaves were cut once a week and spread around the cowpea plants. *T. minuta* continuously releases repellent volatiles and was thus used to continuously repel *M. sjostedti* by preventing it from locating the cowpea plants. In the field study, the push-pull design included *C. citratus* and *T. minuta* as the push stimuli and the coloured (blue, yellow) sticky traps as pull stimuli to reduce the population of *T. vaporariorum* and *A. craccivora* on cowpea plants. The additive effect of yellow sticky traps and repellent plants in the push-pull strategy could explain the reduction of these insect populations.

The push-pull strategy either alone or combined with a net house did not improve the control of *M. sjostedti*. Whereas, interestingly, a net house alone did reduce the level of *M. sjostedti*. The low densities of repellent plants could explain why the push-pull strategy used in this study was not effective. In fact, the abundance of repellent companion plants can increase the amounts of volatile emitted hence increasing their effectiveness in controlling insect pests. Little information is available in the literature on the number of companion plants required to control insects in intercropping. On the other hand, the spatial arrangement of plants in intercropping may influence the control of pests in the field. In the present study, the repellent plants were planted to surround the cowpea crop and the coloured sticky traps were placed between the rows of cowpeas.

During the season 2, the yield of cowpea pods was significantly higher and the seeds were of better quality in the net house compared to cowpeas grown in the plots in the open field. In Kenya, stabilising cowpea production using net houses during the heavy rains from March to June 2018 may have enabled these African smallholders to produce cowpeas in the off-season and in this way, to increase their income. Netting may thus be an efficient tool in the unpredictable weather conditions usually attributed to “climate change”. This study is the first step in combining net houses and a push pull strategy.

7.2 Conclusions

1. The vegetative stage of cowpea cultivars attracted and repelled alate *Aphis craccivora*. The major compounds from repellent cultivar (Katumani 80) added to attractive cowpea cultivar (Ex-Luanda) decreased its attractiveness to *A. craccivora*

2. The attractiveness of cowpea to *M. sjostedti* differs according to cowpea cultivar, phenological stage, and sex of thrips. *(E)*-2-Hexenal from the vegetative stage of cowpea cultivars was repellent to the females.

3. Freshly cut leaves of *C. citratus* repelled male and female while intact *T. minuta* repelled only the female *M. sjostedti*. A major compound of *C. citratus* and the blend of 4-major compounds of *T. minuta* repelled females. Dihydrotagetone and the combination myrcene + cowpea flower attracted female *M. sjsotedti*.

4. Net house reduced infestation of major pests of cowpea except black legume aphid, *Aphis craccivora*. The combination net house + push pull strategy did not improve the control of small insects.

7.3 Recommendations

7.3.1 Recommendations to the farmers

1. The small-scale farmers should integrate the cowpea cultivar Katumani (K80) in the management of *A. craccivora* and *M. sjostedti*

2. The small-scale farmers should use the net house that will protect cowpea crop against most pests and increasing cowpea yield

7.3.2 Recommendations for future research

1. Further investigations need to evaluate the application of repellent compounds in dispensers to control *A. craccivora* and *M. sjostedti* in the field

2. The spatial arrangement of repellent plants in field trial should be considered in the future work

3. Explore the release of natural enemies of *A. craccivora* in the net house

7.4 Limitations

1. Raising of cowpea cultivars in screen house without fungus attack

2. The behavioural assays and collection of cowpea flowers volatiles on the plant required careful handling

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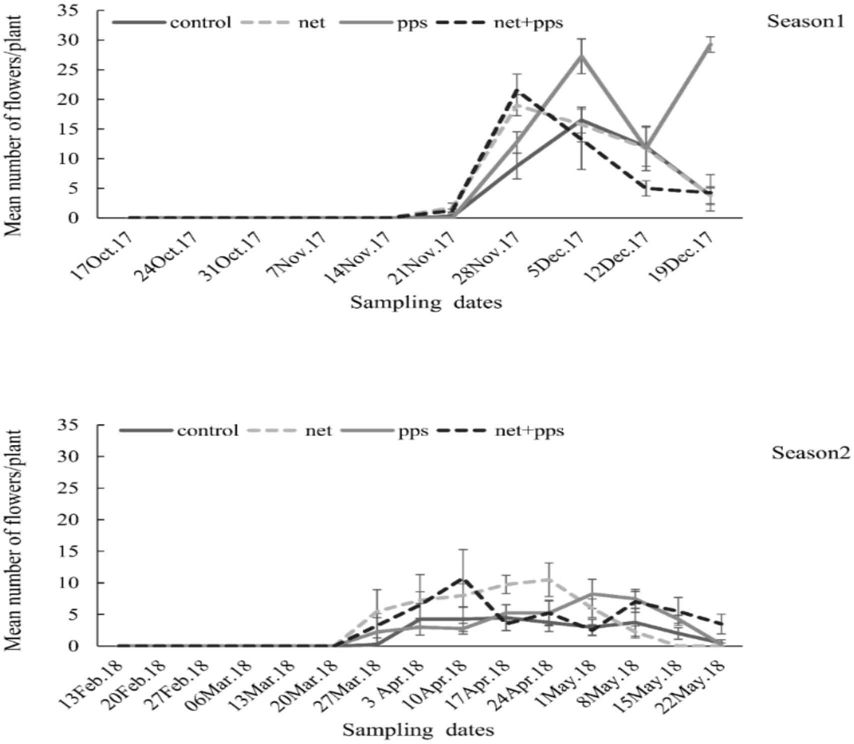
APPENDICES

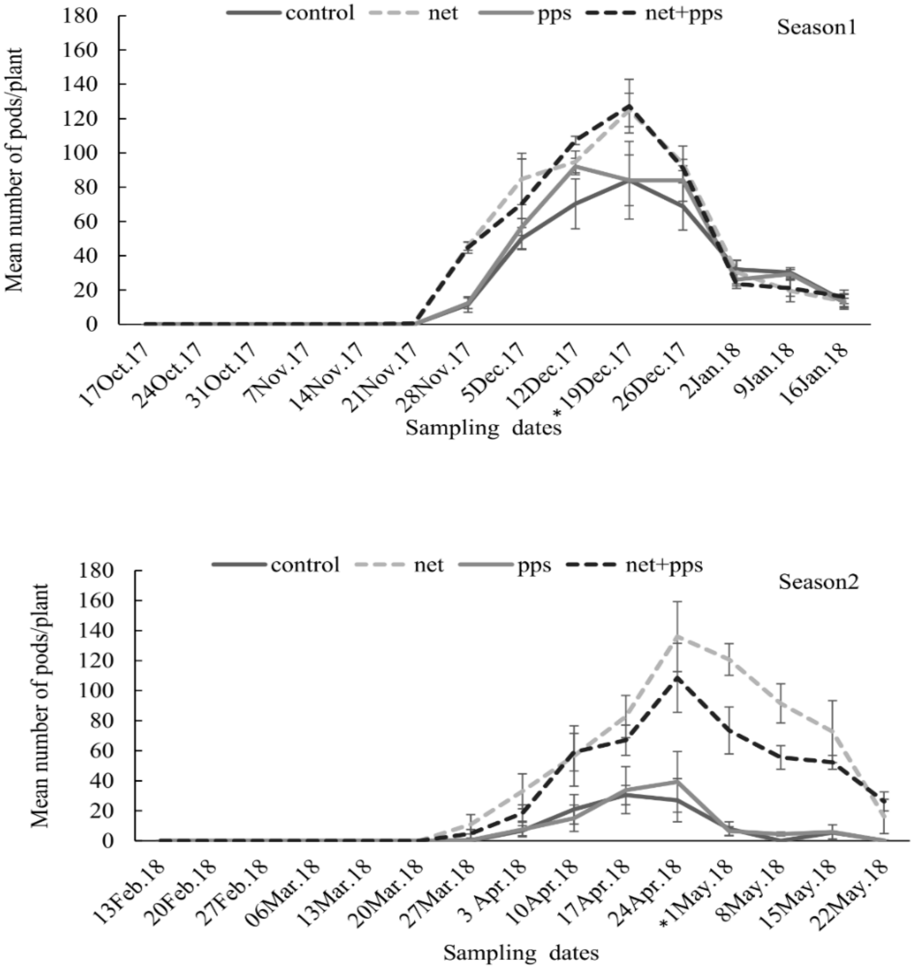


Appendix I: **Changes in flowering dates per cowpea plant in the treatments in the dry season (season 1) and the rainy season (season 2) at KALRO-Mwea (Kenya).**

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)



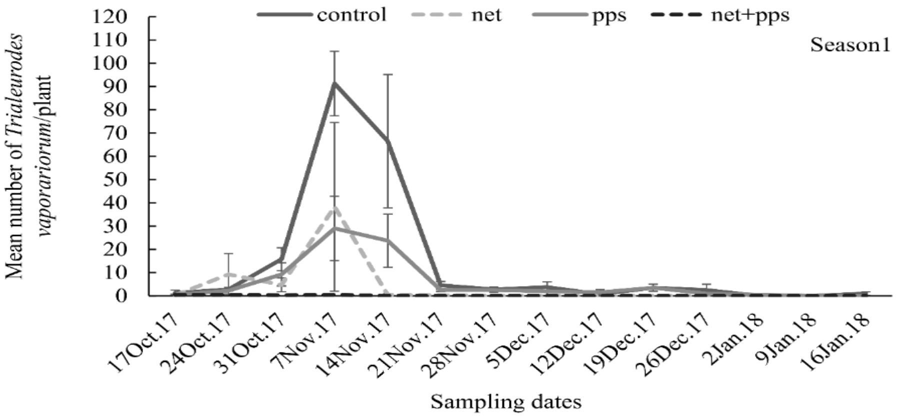
Appendix II: **Changes in the number of cowpea pods per plant in the treatments in the dry season (season 1) and in the rainy season (season 2) at KALRO-Mwea (Kenya).**

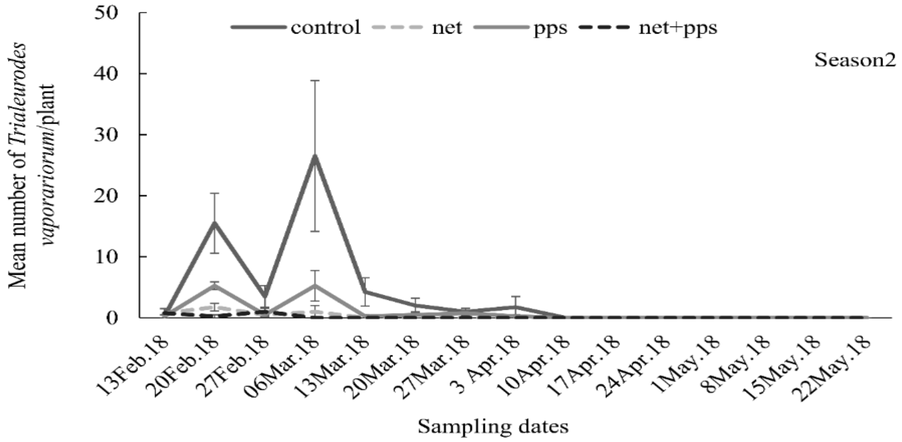
Season 1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)

* = Dates of beginning of pod harvests



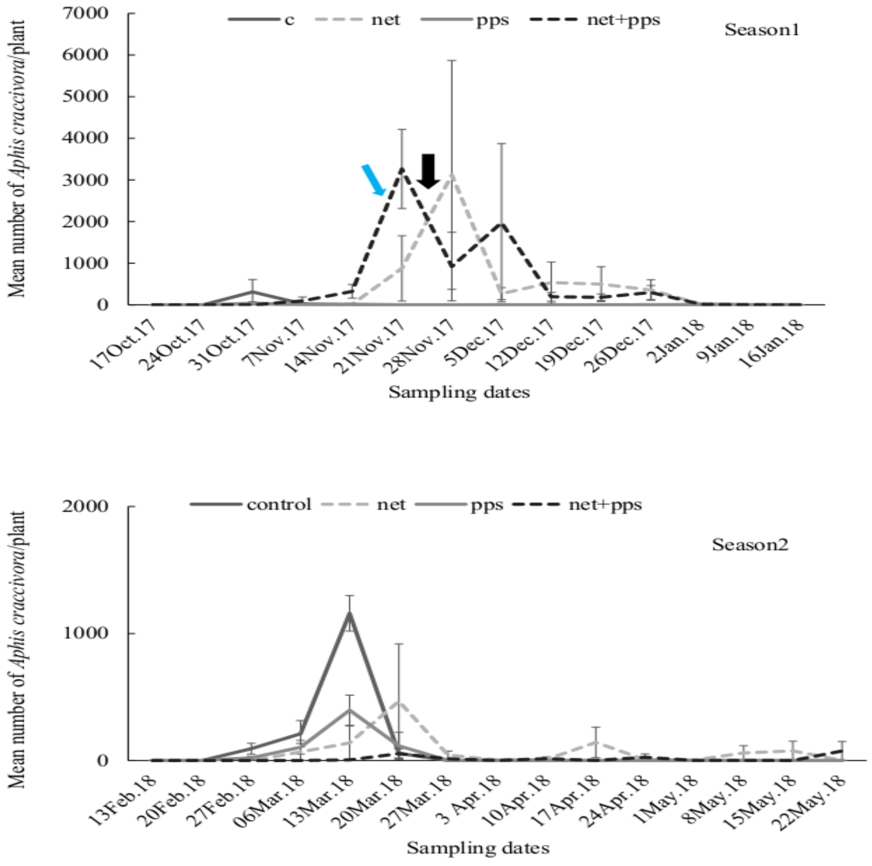


Appendix III: Changes in the number of adult and larval *T. vaporariorum* per plant in the treatments in the dry season (season 1) and rainy season (season 2) at KALRO-Mwea (Kenya).

Season1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

Season2: from 30th January 2018 to 15th May 2018 at KALRO- Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)



Appendix IV: Changes in the number of adult and larval *Aphis craccivora* per plant in the treatments in the dry season (season 1) and rainy season (season 2) at KALRO-Mwea (Kenya).

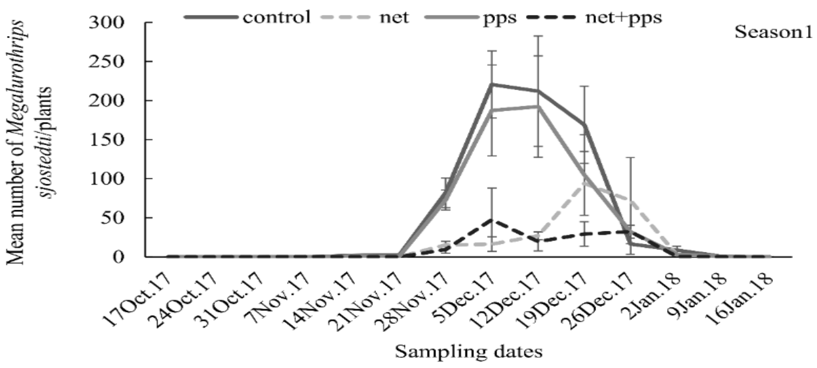
Season1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

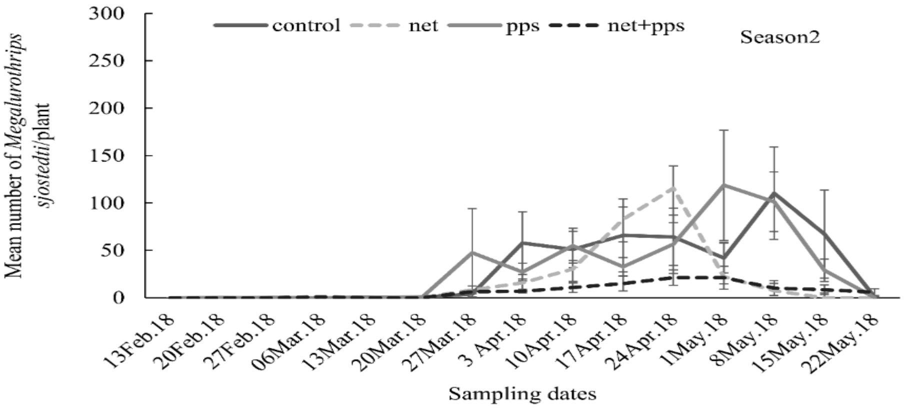
Season2: from 30th January 2018 to 15th May 2018 at KALRO- Mwea (Kenya).

➡ Spray of mixture of pepper and soap on net + pps plot

➡ Spray of mixture of pepper and soap on both net + pps and net plots

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)



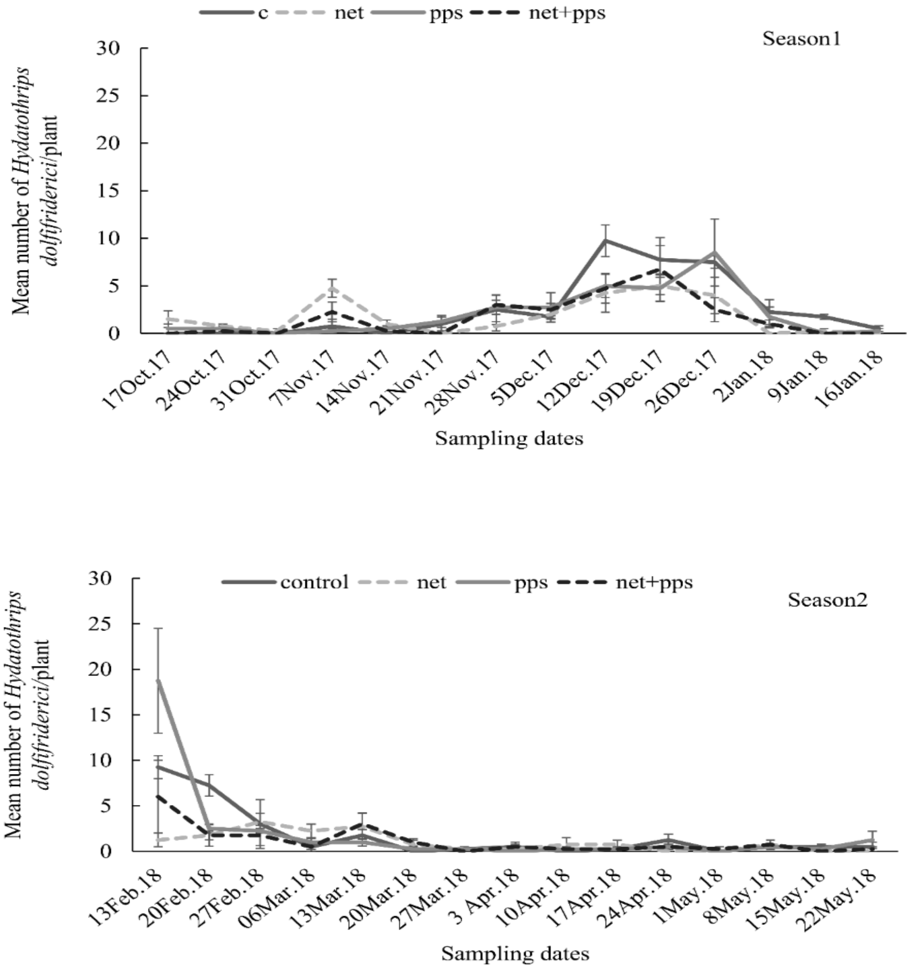


Appendix V: Changes in the number of adult and larval *Megalurothrips sjostedti* per plant in the treatments in the dry season (season 1) and in the rainy season (season 2) in Mwea (Kenya).

Season1: from 3rd October 2017 to 9th January 2018 at KALRO-Mwea (Kenya).

Season2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = ne thouse combined with push-pull)

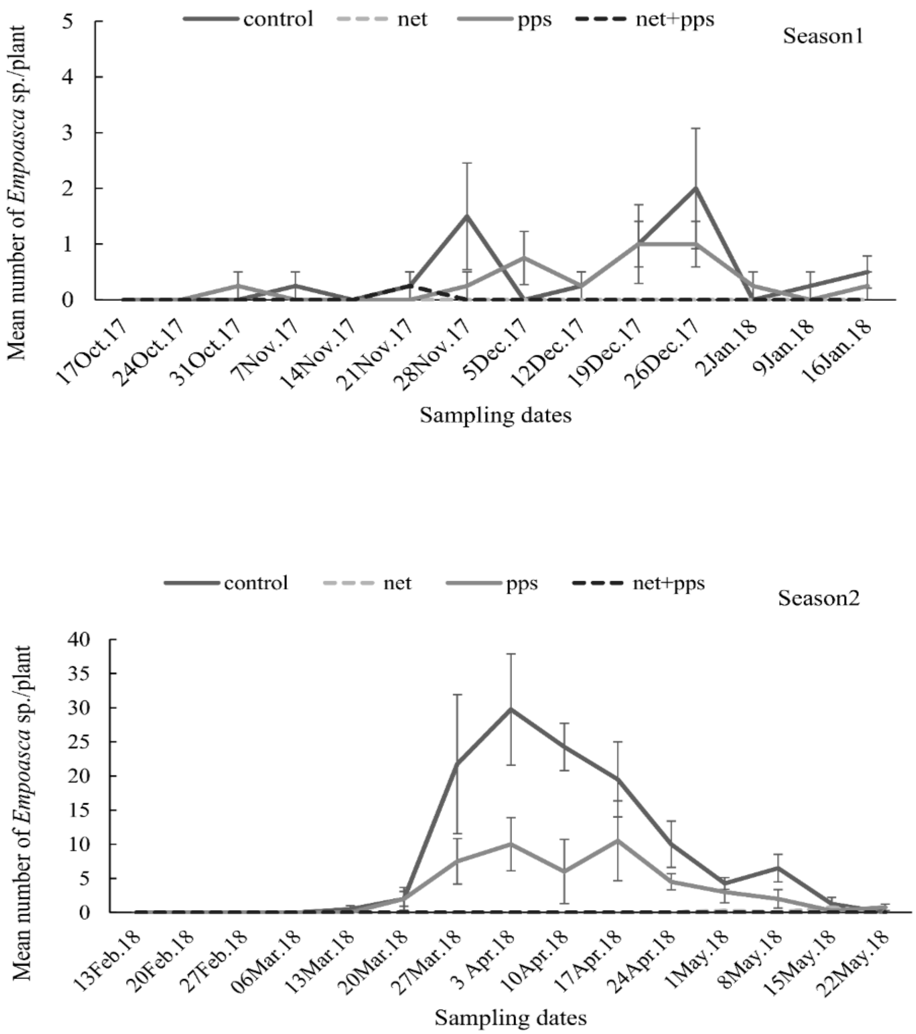


Appendix VI: Changes in the number of adult and larval *Hydatothrips adolfifriderici* per plant in the treatments in the dry season (season 1) and in the rainy season (season 2) at KALRO-Mwea (Kenya).

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO-Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull strategy)

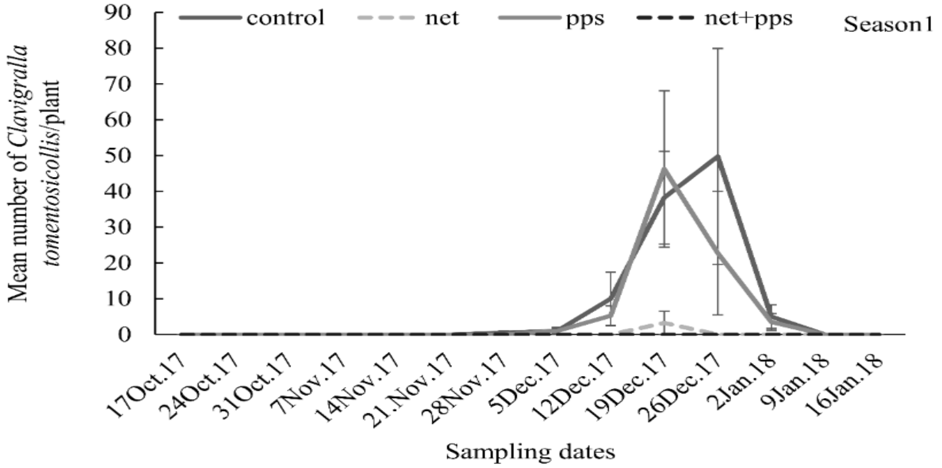


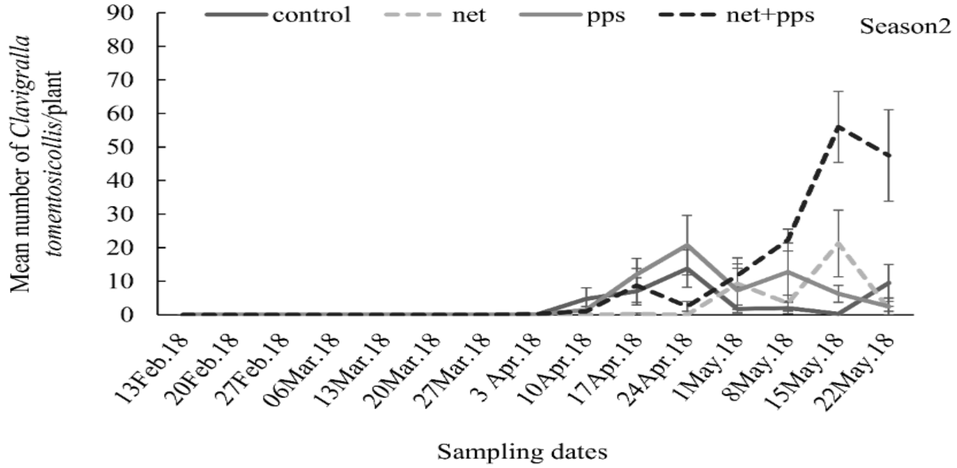
Appendix VII: Changes in the number of adult and larval *Empoasca* sp. per plant in the treatments in the dry season (season 1) and the rainy season (season 2) at KALRO-Mwea (Kenya).

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO-Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)



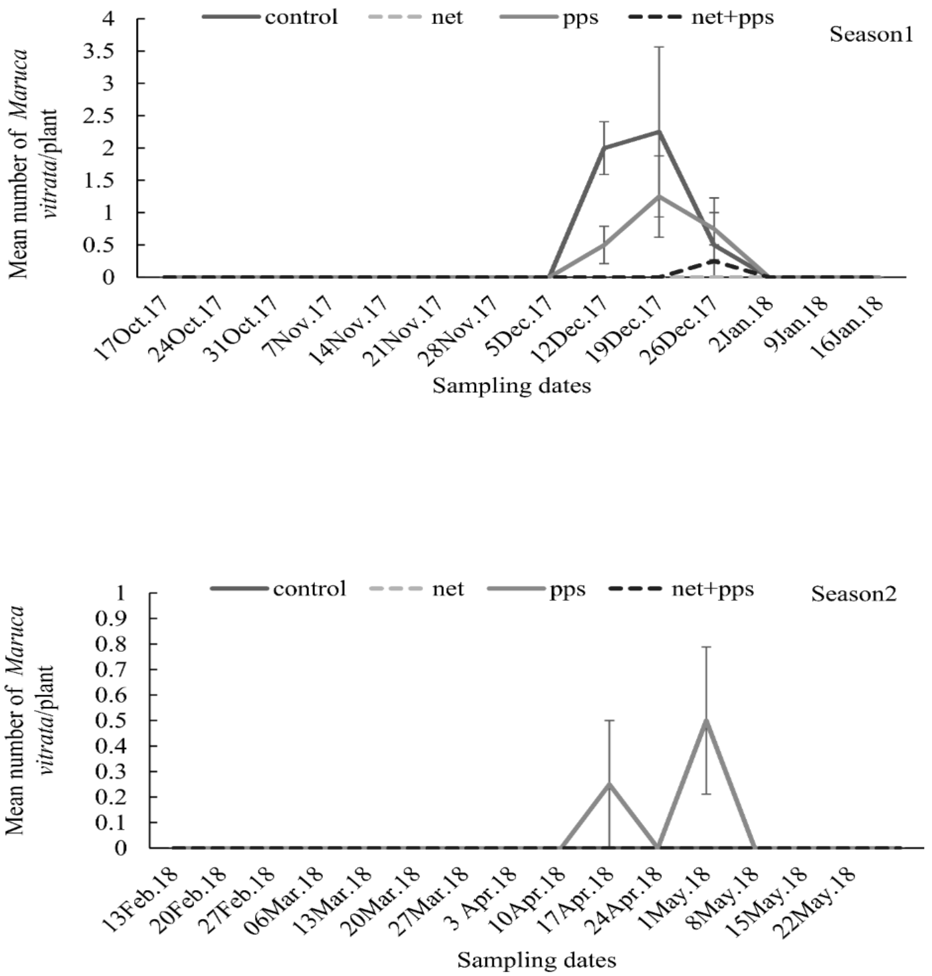


Appendix VIII: Changes in the number of adult and larval *Clavigralla tomentosicollis* per plant in the treatments in the dry season (season 1) and rainy season (season 2) at KALRO-Mwea (Kenya).

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)

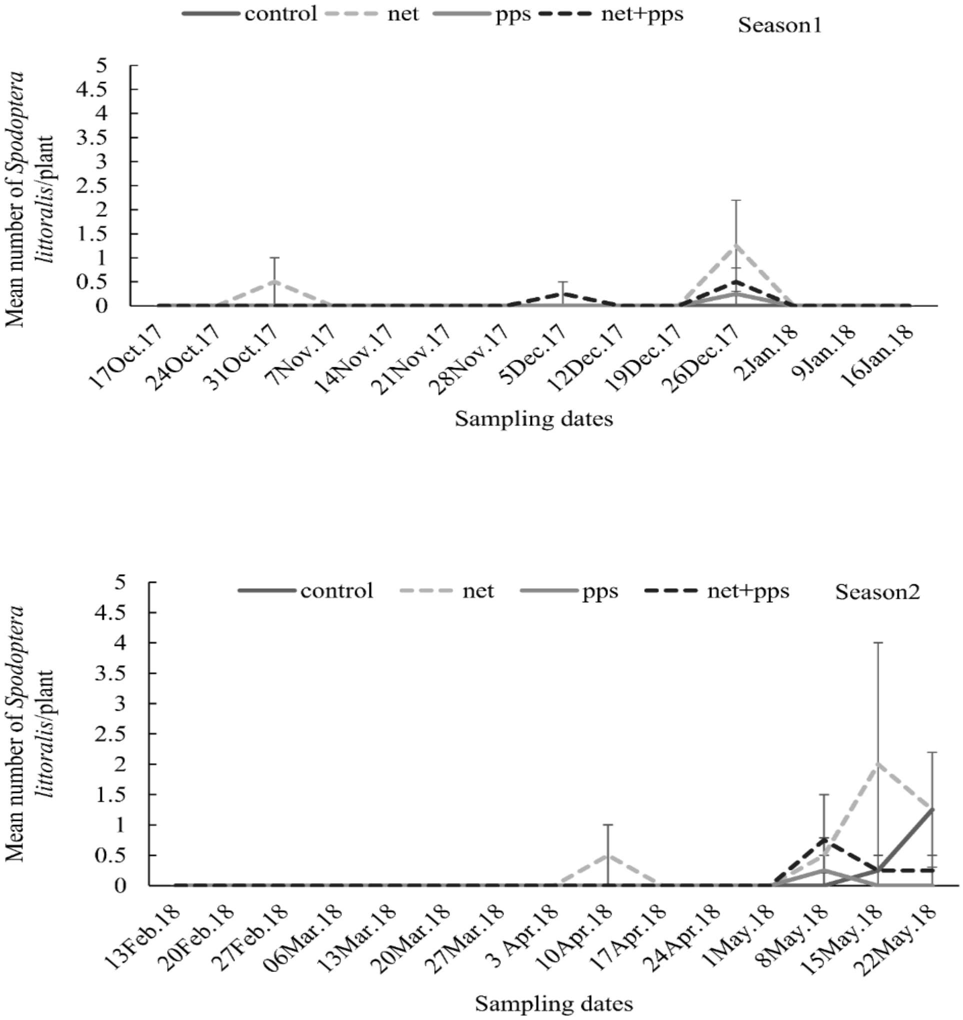


Appendix IX: Changes in the number of adult and larval *Maruca vitrata* per plant in the treatments dry season (season 1) and rainy season (season 2) at KALRO-Mwea (Kenya).

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO-Mwea (Kenya).

Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)

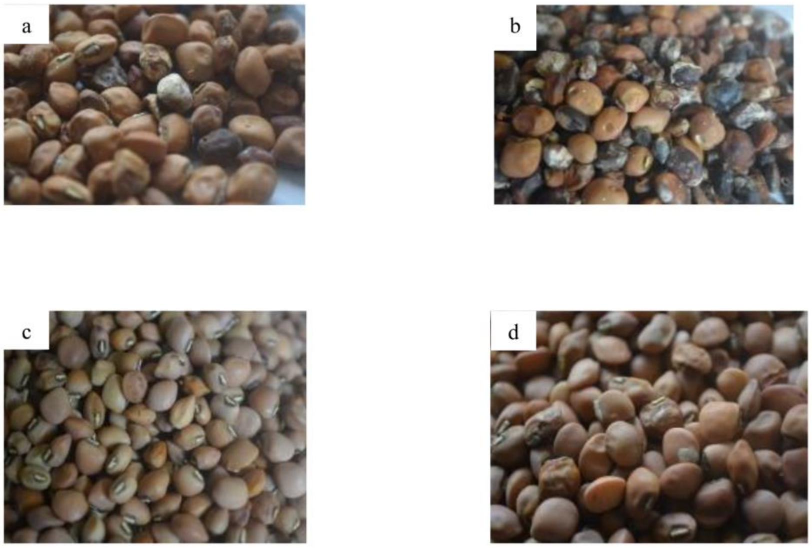


Appendix X: Changes in the number of larval *Spodoptera littoralis* per plant in the treatments in the dry season (season 1) and rainy season (season 2) at KALRO-Mwea (Kenya).

Season 1: from 3rd October 2017 to 9th January 2018 at KALRO- Mwea (Kenya).

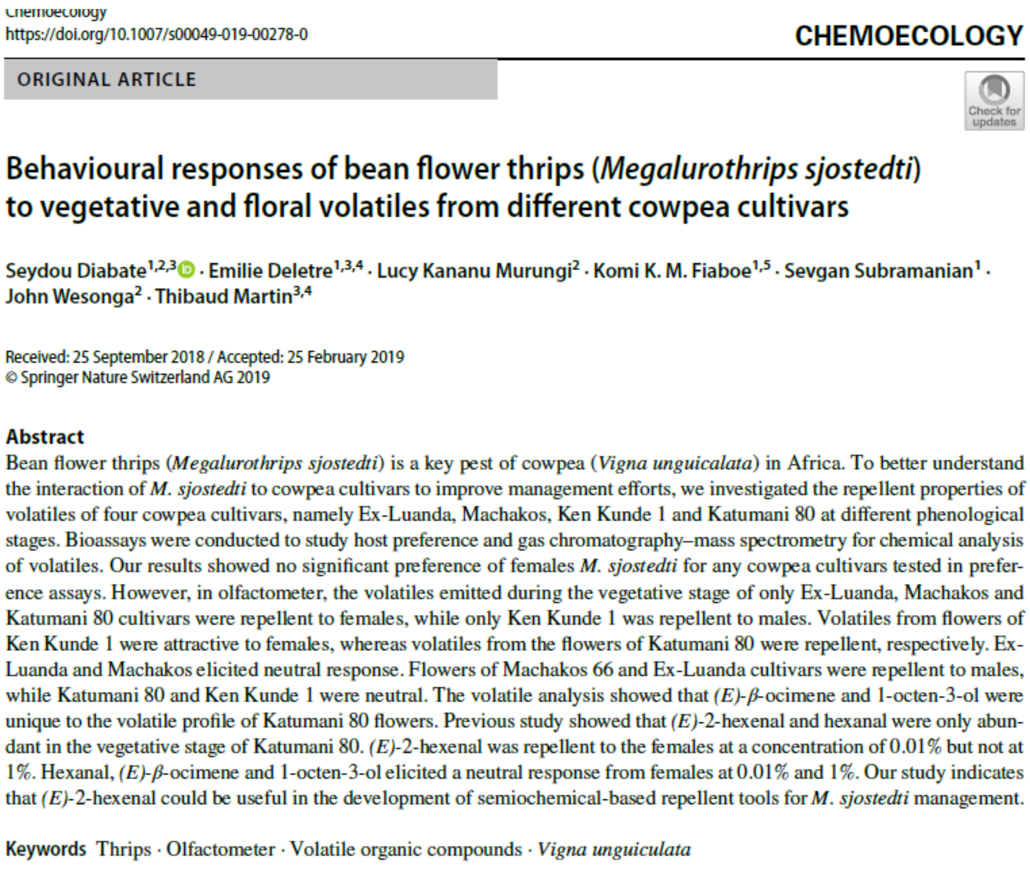
Season 2: from 30th January 2018 to 15th May 2018 at KALRO-Mwea (Kenya).

Four treatments (control = untreated plots; pps = push-pull plots; net = net house plots; pps + net = net house combined with push-pull)



Appendix XI: Grains harvested in each treatment (a = untreated control; b = push-pull plots; c = net house plots; d = net house combined with push-pull strategy) in rainy season. The quality of grains from open fields (control and push-pull plots) were poor compared to the treatments with net house (Source: Diabate, 2018).

Appendix XII: List of publications



Chemoecology
https://doi.org/10.1007/s00049-019-00278-0

CHEMOECOLOGY

ORIGINAL ARTICLE

Behavioural responses of bean flower thrips (*Megalurothrips sjostedti*) to vegetative and floral volatiles from different cowpea cultivars

Seydou Diabate[1,2,3] · Emilie Deletre[1,3,4] · Lucy Kananu Murungi[2] · Komi K. M. Fiaboe[1,5] · Sevgan Subramanian[1] · John Wesonga[2] · Thibaud Martin[3,4]

Received: 25 September 2018 / Accepted: 25 February 2019

Abstract

Bean flower thrips (*Megalurothrips sjostedti*) is a key pest of cowpea (*Vigna unguicalata*) in Africa. To better understand the interaction of *M. sjostedti* to cowpea cultivars to improve management efforts, we investigated the repellent properties of volatiles of four cowpea cultivars, namely Ex-Luanda, Machakos, Ken Kunde 1 and Katumani 80 at different phenological stages. Bioassays were conducted to study host preference and gas chromatography–mass spectrometry for chemical analysis of volatiles. Our results showed no significant preference of females *M. sjostedti* for any cowpea cultivars tested in preference assays. However, in olfactometer, the volatiles emitted during the vegetative stage of only Ex-Luanda, Machakos and Katumani 80 cultivars were repellent to females, while only Ken Kunde 1 was repellent to males. Volatiles from flowers of Ken Kunde 1 were attractive to females, whereas volatiles from the flowers of Katumani 80 were repellent, respectively. Ex-Luanda and Machakos elicited neutral response. Flowers of Machakos 66 and Ex-Luanda cultivars were repellent to males, while Katumani 80 and Ken Kunde 1 were neutral. The volatile analysis showed that *(E)*-*β*-ocimene and 1-octen-3-ol were unique to the volatile profile of Katumani 80 flowers. Previous study showed that *(E)*-2-hexenal and hexanal were only abundant in the vegetative stage of Katumani 80. *(E)*-2-hexenal was repellent to the females at a concentration of 0.01% but not at 1%. Hexanal, *(E)*-*β*-ocimene and 1-octen-3-ol elicited a neutral response from females at 0.01% and 1%. Our study indicates that *(E)*-2-hexenal could be useful in the development of semiochemical-based repellent tools for *M. sjostedti* management.

Keywords Thrips · Olfactometer · Volatile organic compounds · *Vigna unguiculata*

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DOI: 10.1111/jen.12633

ORIGINAL CONTRIBUTION

WILEY JOURNAL OF APPLIED ENTOMOLOGY

Behavioural response of alate *Aphis craccivora* Koch (Homoptera: Aphididae) to volatiles from different cowpea cultivars

Seydou Diabate[1,2,3] | Emilie Deletre[1,3,4] | Lucy Kananu Murungi[2] |
Komi K.M. Fiaboe[1,5] | John Wesonga[2] | Thibaud Martin[1,3,4]

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya
[2]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[3]Cirad UR Hortsys, Montpellier, France
[4]Université Montpellier, Montpellier, France
[5]IITA-Cameroon, Yaoundé, Cameroon

Correspondence
Seydou Diabate, International Centre of Insect Physiology and Ecology, Nairobi, Kenya.
Emails: seydoudiabate01@gmail.com; sdiabate@icipe.org

Funding information
Deutscher Akademischer Austauschdienst, Grant/Award Number: 91602505; Kenyan Government; Federal Ministry for Economic Cooperation and Development; Swedish International Development Cooperation Agency; Swiss Agency for Development and Cooperation

Abstract

The cowpea aphid, *Aphis craccivora*, is a major insect pest of cowpea in Africa. Volatile organic compounds (VOCs) mediate plant–arthropod interactions that could be used in the management of insect pests. In this study, we established the VOC profile involved in the interaction between *A. craccivora* and four cowpea cultivars, namely Ex-Luanda, Katumani 80, Machakos 66 and Ken Kunde 1. Behavioural assays were conducted to study host preference and gas chromatography-mass spectrometry (GC/MS) for chemical analysis of volatiles. In preference assays, alate *A. craccivora* had no significant preference for any of the four cowpea cultivars tested. However, in the olfactometer assays, the aphids showed a significant preference for odours from cultivar Ex-Luanda compared to Katumani 80. Machakos 66 and Ken Kunde 1 elicited neutral responses. In pairwise comparisons, alate *A. craccivora* did not distinguish between odours of respective cowpea cultivars. GC/MS analysis identified 23 compounds in the volatiles of the four cowpea cultivars. Not all compounds were detected in all cowpea cultivars, and the detected compounds amounts varied in each cultivar. Of these, only four compounds (hexanal, *(E)*-2-hexenal, 1-octen-3-ol and p-xylene) were emitted in significantly different quantities in the four cultivars. A blend of hexanal and *(E)*-2-hexenal added to cowpea cultivar Ex-Luanda decreased its attractiveness to *A. craccivora* compared to the control. Our findings showed differential attractiveness of VOCs of cowpea cultivars to *A. craccivora*, suggesting that VOCs could be used in the management of *A. craccivora*.

KEYWORDS
Aphids, behavioural assays, olfactometer, *Vigna unguiculata*, volatile organic compounds

Received: 16 February 2019 | Revised: 2 April 2019 | Accepted: 23 April 2019

DOI: 10.1111/jen.12651

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*

Seydou Diabate[1,2,3] | Thibaud Martin[1,2,4] | Lucy Kananu Murungi[3] | Komi K. M. Fiaboe[1,5] | Sevgan Subramanian[1] | John Wesonga[3] | Emilie Deletre[1,2,4]

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya

[2]CIRAD, UPR Hortsys, Université de Montpellier, Montpellier, France

[3]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[4]Université Montpellier, Montpellier, France

[5]IITA-Cameroon, Yaoundé, Cameroon

Correspondence

Seydou Diabate, International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya.

Emails: seydoudiabate01@gmail.com; sdiabate@icipe.org

Funding information

Deutscher Akademischer Austauschdienst, Grant/Award Number: 91602505; Federal Ministry for Economic Cooperation and Development; Swedish International Development Cooperation Agency; Swiss Agency for Development and Cooperation; German Academic Exchange Service

Abstract

Cowpea is an important source of protein for people in Africa. However, the crop suffers major damage and yield losses due to bean flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). Although companion plants are known to reduce the damage caused by insect pests, the role of their volatiles in repelling pests from target plants has been the subject of few investigations. Here, we used the Y-tube olfactometer experiments and chemical analyses to investigate the effect of volatiles from cowpea flowers and two companion plants; lemongrass, *Cymbopogon citratus* and Mexican marigold, *Tagetes minuta* on the olfactory responses of *M. sjostedti*. The results revealed that *M. sjostedti* males and females were repelled by the volatiles from freshly cut leaves of *C. citratus*. The combination of freshly cut leaves of *C. citratus* and cowpea flower was repellent to females but not to males. The female thrips, but not males, were repelled by the volatiles from the vegetative stage of *T. minuta*. Fifty-four compounds were identified in the volatiles from two herbal plants. Among the major compounds, citral and a 4-component blend comprised of dihydrotagetone, (*Z*)-3-hexenyl acetate, limonene and (*Z*)-β-ocimene repelled females but dihydrotagetone alone attracted females. While myrcene combined with cowpea flower volatiles enhanced the attraction of females *M. sjostedti*, when tested alone was not attractive. These results highlight the potential of volatiles from *C. citratus* and *T. minuta* to repel *M. sjostedti* females. The use of these plants as companion plants in a cowpea cropping system could reduce *M. sjostedti* infestation.

KEYWORDS

companion plant, cowpea, lemongrass, marigold, thrips