MANAGEMENT OF *Megalurothrips sjostedti* TRYBOM ON COWPEA (*Vigna unguiculata* [L.] Walp.) WITH VOLATILE ORGANIC COMPOUNDS

By

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CERTIFICATION

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DEDICATION

I dedicate this work to my Family, for their immense support throughout the duration of my study.

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ABSTRACT

Cowpea is a vital plant protein in African diet. *Megalurothrips sjostedti* (Ms), is an insect pest of cowpea, which causes severe yield loss to the crop. Farmers manage Ms with conventional insecticides however, they are hazardous to the environment. Volatile Organic Compounds (VOC) developed into attract and kill mechanisms are safer alternatives but have been scarcely documented in cowpea insect pest management. Therefore, Headspace Volatiles (HsV) of cowpea cultivars and VOC were investigated for their attractant and repellant properties for Ms management.

Response of Ms (n=60) to clean air and HsV from five Resistant Cultivars (RC): Moussa Local, Sewe, TVu1509, Sanzibanili, IT90K-277-2 and two Susceptible Cultivars (SC) - Ife brown and Vita7 at the flowering stage, was investigated in the laboratory with a Y-tube olfactometer. Choices of Ms (% response) were determined. The VOC of RC and SC were collected at Wageningen, The Netherlands, and Ibadan, Nigeria, using dynamic HsV collection method and profiled with a Gas Chromatography-Mass Spectrometry. Olfactory response of Ms (n=60) to eleven VOC: α-terpinene, γ-terpinene, (R)-(+)–limonene, tetradecane, sabienene, methyl saliscylate, hexadecane, dodecane, 1-tetradecene, nonanal and undecane were evaluated following standard procedures. Thrips responses (%) to VOC were determined. Three VOC baits: methyl salicylate, hexadecane and tetradecane, unbaited traps (Ut), lambda-cyhalothrin and untreated plot (control) were evaluated on cowpea plots (30x40 m^2) in a Derived Savanna (Ibadan) and Guinea Savanna (Abomey-Calavi) Agro-Ecologies (AgE), established with Ife brown and Kpodiguegue cowpea cultivars, respectively. Fields were laid out in a randomised complete block design (r=3). Number of Ms and orders of insects in cowpea flowers and Sticky traps were recorded, respectively; Grain Yield (kg/ha) and Yield Losses (%YL) were determined. Data were analysed using descriptive statistics, Chi-square and ANOVA at $\alpha_{0.05.}$

Attraction of Ms to HsV of cowpea cultivars relative to clean air was significantly higher in all the cultivars, ranging from 93.3% in Vita7 ($\chi2=45.07$) to 76% in Sewe ($\chi2=17.07$). The VOC identified in Wageningen and Ibadan were 68 and 29, respectively, belonging to 22 different classes of compounds. Attraction of Ms to VOC relative to clean air was significantly higher in the order: 80.0% in 1-tetradecene ($\chi2=16.20$), 75.0% in methyl salicylate ($\chi2=15.00$), 66.7% in γ-terpinene ($\chi2=6.67$), 63.3 % in tetradecane ($\chi2=4.27$) and 61.7% ($\chi2=3.27$) in hexadecane, while nonanal with 9.0% ($\chi2=30.42$) repelled Ms. In Abomey-Calavi, Ms was highest in tetradecane plot (212.2±93.33) and lowest in methyl salicylate (152.3±55.90). Also in Ibadan, tetradecane plot had the highest Ms (619.7±127.27), while lambda-cyhalothrin (198.9±127.85) had the lowest. Eight insect orders: Thysanoptera, Hemiptera, Hymenoptera, Diptera, Odonata, Coleoptera, Lepidoptera and Orthoptera, were identified on sticky traps. Grain yield ranged from 21,927.7 (Ut) to 15,163.6 (untreated) in Ibadan and 723.6 (lambda-cyhalothrin) to 432.8 (Ut) in Abomey-Calavi. Hexadecane elicited the lowest YL in Abomey-Calavi (15.9±14.2%) and Ibadan (3.8±4.4%).

Resistant and susceptible cowpea cultivars were attractive to *Megalurothrip ssjostedti*. Methyl salicylate, 1-tetradecene, tetradecane and gamma terpenene attracted *Megalurothrips sjostedti*, while nonanal repelled it. Hexadecane minimised yield loss of cowpea in derived savanna and Guinea savanna agro-ecologies.

Keywords: Cowpea headspace volatiles, 1-tetradecene, Insect sticky traps, Methyl salicylate, Nonanal

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CHAPTER ONE
INTRODUCTION

Vigna unguiculata (L.) Walpers (Fabales: Fabaceae), commonly known as cowpea, is a leguminous crop that thrives in arid environments. It is a staple food crop in Nigeria, mostly grown by small-holder farmers in the dry savannah region for its edible seeds. The seeds have about 25% high quality protein with a good balance of lysine and tryptophan, and micronutrients such as iron and zinc (Alidu *et al*., 2020; Horn and Shimelis, 2020). Also, animal feed is made from its stems and leaves, making it an important source of plant protein, vitamins for humans and livestock, as well as a significant means of livelihood for farmers.

Cowpea is grown under irrigation or rain-fed conditions, with rainfall ranging from 500 to 1200 mm per annum. It is a hardy crop that grows well in a variety of soils and droughts (Dugje *et al*., 2009; Gomes *et al*., 2020). Cowpea is occasionally grown as a stand-alone crop in the field, but it is more commonly grown as an intercrop so that its decaying leaf litter and roots help to replenish soil nitrogen in addition to nitrogen fixation by the root nodules. It is therefore important in most cereal-legume intercrops, reducing nitrogen fertilizer demand for the crop (Asiwe, 2009; Odireleng*et al*., 2016).

Cowpea output worldwide is estimated to be at 8.9 million tons per year on approximately 14.4 million hectares (FAOSTAT, 2017), Nigeria is the highest producer and user, followed by the Niger Republic and Burkina Faso. Africa accounts for over 95 percent of global output, particularly in sub-Saharan Africa. For over twenty years, total cultivated land area, yield, and population have grown by 4.3%, 1.5%, and 5.8% respectively (Abate *et al*., 2011). Low yields of around 350 kg per hectare characterized production in Nigeria’s dry savanna region (Akah *et al*., 2021) increased production is realized only when cultivated land area is increased rather than improved yield per hectare leading in a 500,000-tonne cowpea demand gap (NBDA, 2019).

Insect pest infestations are a recurrent occurrence in cowpea production, resulting in lower yields. Various insect pests attack the crop at various stages of development. Aphids are common pests that affect cowpea in the field. *Aphis craccivora* Koch feeds on plant juice from the leaves and stems of young cowpea plants, and transmits the cowpea mosaic virus, which reduces seed yield by 13 to 87% (Bashir *et al*., 2002; Togola *et al*., 2017). *Maruca vitrata* Fabricious burrows inside pods and eats the seeds, buds, flowers, and leaves, or webs them together (Ogunwolu, 1990; Msm *et al*., 2016). The pod-sucking bug complex includes *Clavigralla tomentosicollis* Stal., *Anoplocnemis curvipes* Fabricius, *Riptortus dentipes* Fabricius, *Nezara viridula* Linnaeus, *Mirperus jaculus* Thunberg, and *Asparvia armigera* Fabricius. They suck juice from the cowpea pods, causing the pods to shrivel, and thereafter premature pod drying and abscission (Dabire-Binso*et al*., 2010; Mansaray *et al*., 2020).

Megalurothrips sjostedti Trybom, the cowpea flower bud thrips, is the first pest of flowering cowpeas (Alabi *et al*., 2003). They are cryptic insects that lurk inside cowpea flowers, causing irregular pod and seed production, and inducing yield losses of 20–100% (Nabirye *et al*., 2003; Sani and Umar, 2017). *Megalurothrips sjostedti* can be found throughout the growing season, although they are most common during the dry season, when cowpeas are mature. They use their mouth part to scrap plant tissues and suck on the juices, causing flowers to drop prematurely (Adati *et al*., 2007). According to Salifu (1992), three to five adults per raceme on cowpeas was enough to cause economic harm. Adults and nymphal stages of *Megalurothrips sjostedti* suck on the juice of cowpea flowers (Sani and Umar, 2017).

Several ways to control these insect pests have been reported, with conventional insecticides being the most common (Oyewale and Bamaiyi, 2013; Abtew, 2015; Akande *et al.*, 2020). To reduce cowpea pest infestation and boost yields, farmers typically use broad-spectrum insecticides such as pyrethroids and organophosphates (Egho, 2011). The use of *Megalurothrips sjostedti*-resistant cowpea cultivars is a promising strategy towards thrips population management because it is both inexpensive and ecologically friendly. It easily combines with other pest management methods such as modification of habitat and biological control to create a sustainable Integrated Pest Management system (Tamo *et al*., 2002). Host plant resistance has been used in conjunction with biocontrol agents such as *Ceranisus femoratus* Gahan and *Orius albidipennis* Reuter to control *Megalurothrips sjostedti* in cowpea fields (Madadi *et al*., 2008; Tamo *et al*., 2013).

An essential reaction of plants to herbivory is the immediate stimulation of volatile emission. Plant volatiles are airborne signals that insects employ as long-range cues, allowing them to stay in a good habitat (Eigenbrode *et al*., 2002), avoid a bad habitat (Choh and Takabayashi, 2007), and aggregate (Dickens, 2006). Through the attraction of predators, the role of indirect defense is significant in pest control (Rasmann and Agrawal, 2009; Pappas *et al*., 2017; Furlong *et al*., 2018). Plant volatiles have been employed successfully as attractants or repellants in the population monitoring and management of several insect pest species. During a severe whitefly infestation, limonene, a volatile organic compound, was successfully employed to deter whitefly from the infested crop, increasing fruit output by 32%. Another volatile organic compound, methyl salicylate, was found to bring down the population of whitefly and enhance yield by 11% when applied to uninfested tomato plants (Conboy *et al*., 2020).

The introduction of machinery, advances in genetics, adoption of improved crop cultivars, and the heavy use of agro-chemicals such as fertilizers and pesticides, have all contributed to increased crop yields over time (Brilli *et al*., 2019). However, according to FAO (2017), approximately 70% increase in food production is required to meet food demand in the future since the world population is estimated to climb from 7.3 billion to 9.7 billion by the year 2050. Food poisoning, non-target organism loss, and environmental contamination have all been linked to the widespread use of pesticides in agricultural production. New sustainable ways to boost agricultural production while addressing existing environmental challenges are urgently needed. As a result, there is a need to investigate volatile organic Compounds given off by plants as an environmentally friendly technique for insect pest population management to improve crop protection and production.

Insect behaviour in reaction to plant volatiles could have significant implication for crop infestation and production loss. As a result, the attractant, and repellent qualities of headspace volatile organic compounds from selected cowpea cultivars were examined, as well as their appropriateness as lures in an Attract and Kill device, for the management of *Megalurothrips sjostetdi* populations.

The specific objectives of this study were to:

1. Study the olfactory response of adult female *Megalurothrips sjostedti* to headspace odours from two susceptible cowpea cultivars (Vita7 and Ife Brown) and five resistant cowpea cultivars (IT90k-277-2, Moussa Local, Sanzibanili, Sewe, TVu1509).
2. Identify headspace volatile compounds from the seven selected cowpea cultivars grown in Ibadan and Netherlands using chromatographic apparatus.
3. Investigate the olfactory response of adult female *Megalurothrips sjostedti* to synthetic standards of selected cowpea headspace volatile compounds from objective two (2).
4. Determine the efficacy of volatile organic compounds selected from objective 3 as attractive lures for trapping *Megalurothrips sjostedti* on cowpea fields at Ibadan and Abomey-Calavi.

CHAPTER TWO
LITERATURE REVIEW

2.1. Cowpea origin and distribution

Vigna unguiculata (L.) Walpers, is one of about 80 *Vigna* species, but only ten of them are being domesticated (Tomooka *et al*., 2011). Two of the domesticated species are of African ancestry, while others are Asiatic. Green gram/mung bean (*Vigna radiata* (L.) Wilczeck), black gram/urd bean (*Vigna mungo* (L.) Hepper), moth bean (*Vigna aconitifolia* (Jacq) Marechal), adzuki bean (*Vigna angularis* (Willd) Ohwiet Ohashi), and rice bean (*Vigna umbellate* (thumb) Ohwiet Ohashi) has Asia ancestry, while Bambara ground nut (*Vigna subterranean* (L.) Verdc) and cowpea (*Vigna unguiculata* (L.) Walpers) are two African species (Gepts, 2010). It is thought that the northern section of the Republic of South Africa was the core of *Vigna unguiculata* diversification. This is because most of the primitive wild variations and species travelled from the north to Mozambique and Tanzania, where the pubescence sub-species formed (Gomes, 2020). Europe Africa, the United States, Asia, Central and South America have all benefited from cowpea production. The largest land area planted with cowpea is in Central and West Africa (Kebede and Bekeko, 2020).

2.2. Botanical description of cowpea

Cowpeas are also referred to as black-eyed pea, southern pea, crowder pea, or field pea. They are annual herbaceous plants that have a strong tap root system with several lateral roots (Figure 2.1), and can grow as upright, prostrate or as a trailing vine. It has a ribbed and slightly glabrous stems with alternate arrangement of trifoliate compound leaves; the leaflets are smooth and ovate in shape. At the tips of a grown stem, the flowers are held on peduncles in pairs or clusters of three to five. Cowpea flowers are white, purple, or light yellow, depending on the cultivar (Zannou *et al*., 2015), they are hermaphrodites, arranged in indeterminate inflorescences. After pollination, flower buds develop into long and

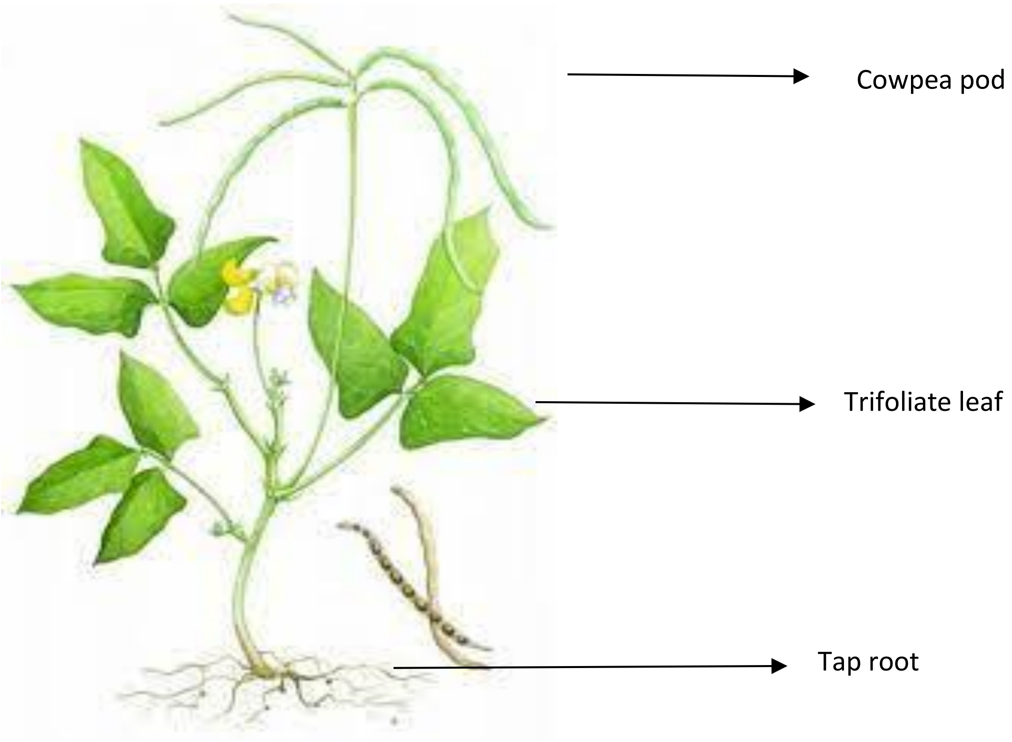

Figure 2.1: Diagramatic illustration of cowpea plant

Source: https://www.flickr.com/photos/iita-media-library/7064543759 cylindrical pods that can grow up to a length of 15–30 cm, bearing seeds within (Edematie *et al*., 2021). The plant produces 2-4 seed pods per peduncle in addition to clusters of flowers at the end of each one. The pods are smooth, cylindrical, sometimes curved. They are mostly green in colour but gradually turn brown as they mature. The seeds have the "eye", and seed coat in varying colours such as white, cream, green, red, brown, or black, depending on the variety (Gaafar *et al*., 2016). Mature cowpea plants can grow to a height of 80 cm or more.

2.3. Cowpea propagation and management

Cowpea grows well in hot and humid climates; it can withstand heat and drought (Hall, 2004). Cowpeas seeds are sown directly on the field when soil temperature has reached a minimum of 18.3°C, to prevent seed decay in the ground. In the Guinea savanna agro-ecology, cowpea sown between August and September were reported to have higher percentage germination than those sown in June and July, when rainfall is at its peak (Olakojo *et al*., 2012). Seeds sown at an inter row spacing of 60 cm spacing and depth of 2.5–5.0 cm have been reported to deliver higher grain yield than 75 cm or 90 cm inter row spacing. (Jakuso *et al*., 2013) Seeds germinate quickly, and after around 60 days, most plants would start producing pods. The majority of cowpea produced in Africa is inter planted with sorghum *(Sorghum bicolor* Linnaeus) or pearl millet (*Pennisetum glaucum* (Linnaeus) Brown), and occasionally with other crops like maize (*Zea mays* Linnaeus), cassava (*Manihot esculenta* Crantz), or cotton (*Gossypium* spp.). The crop is often inter planted across young stands of the component cereal or other crops at a wider spacing of 1 meter (OECD, 2016). The study reported that cowpea is often planted after cereal crop establishment, at low density and without inputs. Cowpeas may be grown in a variety of soil types, from neutral to acidic (up to pH 4), if they drain effectively. However, the plants are not well suited to alkaline soil, they perform optimally when planted in full sun and sandy loam soil with a pH between 5.5 and 6.5 (Baligar and Fageria, 2007). The crop does not require the addition of nitrogen fertilisers and may thrive on poor soil. This is because the plants develop taproots that can reach lengths of over 2.4 Meters, therefore enabling them to obtain moisture and nutrients in the soil that is far below the soil surface.

Because of this, cowpea is exceptionally drought-resistant and requires little to no watering after planting (Shaheen *et al*., 2016).

2.4. Cowpea production statistics

Cowpea is grown in 65 countries in Asia, Southern Europe, Africa, the southern United States, the Middle East, Central and South America (Adati *et al*., 2007; Singh and Ajeigbe, 2002). The crop is grown in about 14.5 million hectares of land every year around the world, with more than 8 million hectares in West and Central Africa and 4 million hectares in Nigeria alone, making it the largest producer (FAOSTAT, 2017). Niger, Mali, Senegal and Burkina Faso are also major producers in Africa. The number of households that grow cowpea is estimated at 38 million households (194 million people) in sub–Saharan Africa, with around 21,000 hectares of cowpea farmed every year commercially for dry grain and over 30,000 hectares of cowpea planted in home gardens mostly for the green peas. Ojiewo *et al.* (2018) projected production in sub–Saharan Africa would grow from about 6.2 million MT in 2010 to nearly 8.4 million MT by 2020.

The United States is the sole significant producer and exporter of cowpea among developed countries. Production is also considerable in Brazil, West India, Myanmar, Sri Lanka, Australia, and Bosnia (Owade *et al*., 2020; Gómez, 2004). Cowpea is plagued by a pest complex that attacks the crop from seed to harvest (Alabi *et al*., 2006; Egho, 2011). Even though the crop is drought tolerant, and well suited to sandy and poor soils (Dugje*et al*., 2009), insect pressure reduces output to nearly nothing (Boukar *et al*., 2015). Insect pests attacking flowers and pods are some of the most damaging pests of cowpea plant (Karungi *et al*., 2000; Alabi *et al*., 2003).

2.5. Cowpea production constraints

Several biotic and abiotic variables limit cowpea productivity (Singh and Ajeigbe, 2002; Timkoet *et al*., 2007). Poor soil fertility, soil acidity, drought, heat, and stress from intercropping with cereals are some of the abiotic variables (Singh and Ajeigbe, 2002). Some biotic factors that reduce production include insect pests, viruses, fungal, bacterial diseases, parasitic plants (Amatobi, 1995; Emechebe and Lagoke, 2002; Singhand Ajeigbe, 2002).

Insect pests have been highlighted as one of the most significant restrictions to cowpea production in Africa (Jackai, 1995; Alabi *et al*., 2003). The low yield of cowpea in Nigeria is due to the usage of native land races with low yields and high susceptibility to pests and diseases and poor cropping pattern (Singh and Ajeigbe, 2002; Tazerouni *et al*., 2019; Mohammed *et al*., 2020).

2.6. *Megalurothrips sjostedti* Trybom, The Flower bud Thrips

2.6.1. Taxonomic classification of *Megalurothrips sjostedti*

Kingdom: Animalia; Bean flower thrips are multicelluar organisms that do not possess chlorophyll in their cells; hence, they depend on other organisms for food which makes them heterotrophic (Mirab-Balou *et al*., 2014).

Phylum: Athropoda; Thrips have a segmented body that divides into the head, thorax and abdomen, and jointed appendages for locomotion. They are also characterised with the organ system level of organization and posses a coelomic cavity filled with blood.

Class: Insecta; the presence of a chitinous exoskeleton and three pairs of jointed appendages or legs, and the absence of a backbone in thrips make it a fit within this group. Members of this class also have two compound eyes and a pair of antennae on the head as seen in the beans flower thrips (Tyler-Julian *et al*., 2014).

Order: Thysanoptera; Insects within these groups are generally referred to as thrips. They are small-sized insect with tapered body and rasp-sucking mouth part. Their metamorphosis is gradual, thereby emerging through a pre-pupae stage before the pupae stage. Some of them have two pairs of fringed slender wings while some are wingless.

Sub-order: Terebrantia; the difference between the two sub-oders of thrips is in the shape of the last abdominal segment and the formation of the ovipositor. Members of the sub-order Terebrantia have almost rounded last abdominal segment, and a developed, saw-like ovipositor (Tyler-Julian *et al*., 2014).

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Family: Thripidae; the bean flower thrips as a member of the family have saw-like ovipositor that curves downwards, their wings have two veins, and antennae have six to ten segments called antennomeres. They have a characteristic ‘Y’ shaped sense cones on antennal segments III and IV (Mirab-Balou *et al*., 2014).

Sub-family: Thripinae; this is the largest of four subfamilies. As seen in the bean flower thrips, most members feed on flowers of higher plants, while some feed on leaves. They are mostly pests of crops, a few species are predatory and a very few have relationship with mosses or ferns. The families of thrips are differentiated by the number of antennae, segments and shape of the sensoria on the third and fourth segments of the antennae

Genus: Megalurothrips; This group mostly breeds in the flowers of crops and trees in the family fabaceae. Females are similar to males, but bigger than the male. They have eight antennae segments, among other features (Mirab-Balou *et al*., 2014).

Megalurothrips sjostedti is the only species from Africa while 12 other species from Southeast Asia are found in the *Megalurothrips* genus (Tyler-Julian *et al*., 2014).

2.6.2 Biology of *Megalurothrips sjostedti*

Thrips are haplodiploid, which means that males have half the number of chromosomes as females (Loomans, 2003). Females can reproduce sexually or asexually, resulting in diploid females or haploid males (Moritz *et al*., 2013; Sani and Umar, 2017). Thrips are holometabolous, which means they go through complete metamorphosis (Figure 2.2). Adult female thrips lay fertilized eggs which develop into female, or non-fertilized eggs which develop into male thrips. Thrips use their saw-like ovipositor to lay eggs on the epidermal layer of various plant parts, particularly in the bud calyx of growing flowers. The eggs hatch into the first active feeding larval instar, which develops into the second active feeding larval instar after three to four days. The first and second instar larvae are white or translucent in colour, but turn yellow after 2–3 days. The yellow form lasts 2–3 days before changing to an orange form that lasts 3–4 days until pupation. After two to three days, a relatively passive and non-feeding pre-pupa stage arises from the second instar larva lasting another three to four days before the pupal stage emerges. The pupa stage is a non-feeding stage that occurs in the earth under fallen plant parts in preparation for adult emergence. Their life cycle may last 14 – 18 days.

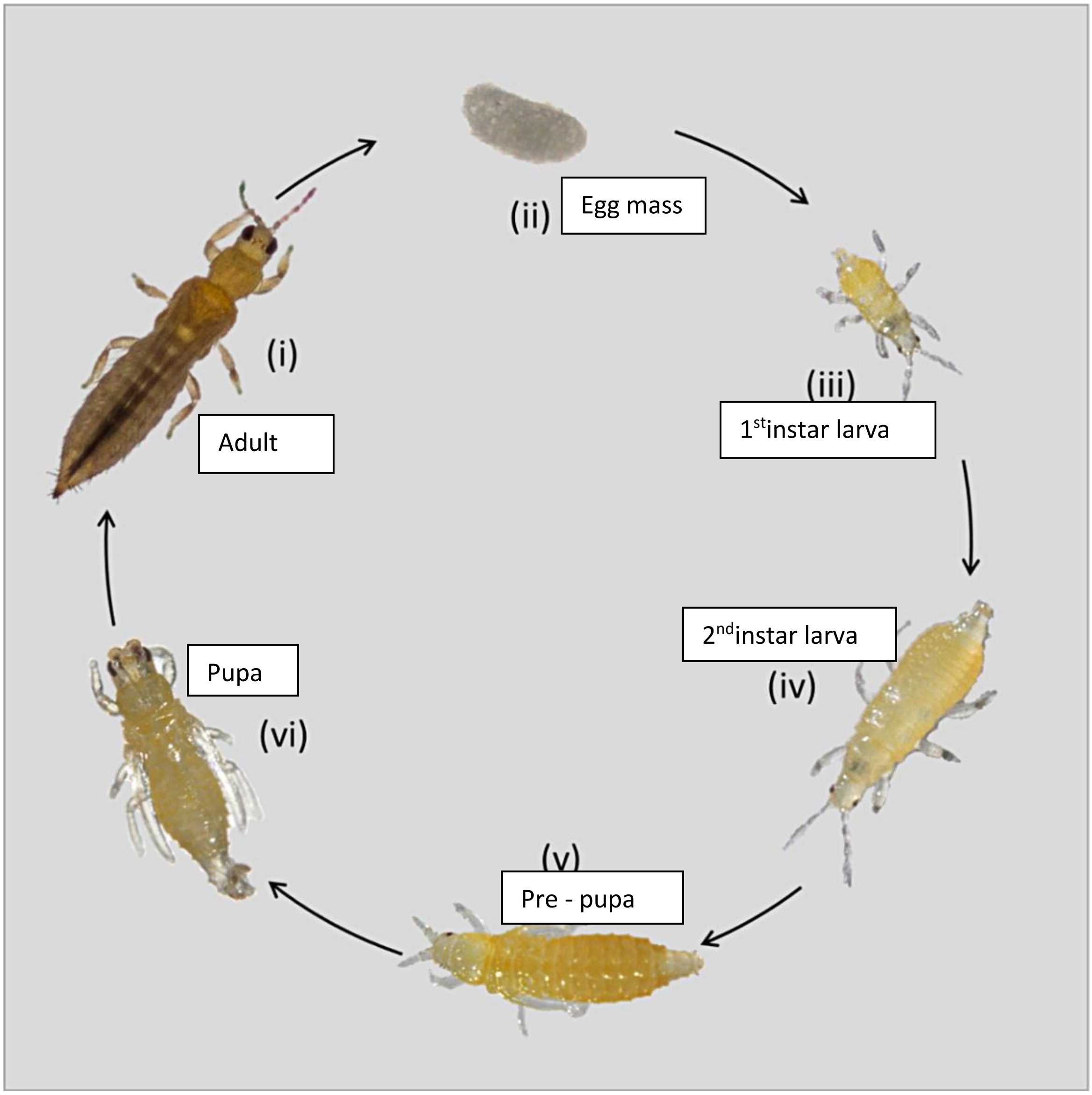


Figure 2.2: Life cycle of *Megalurothips sjostedti*

Source: Steenbergen *et al*., 2020

Adult *Megalurothrips sjostedti* is tiny, shiny black insect with sizes ranging from 1- 2 mm for females (Plate. 2.1) and 1 mm for males Gonne, (2017). The male is slender and paler than the female. Mouthparts are piercing-sucking with only a left mandible. Antennae are short, about four to nine segments. The adults are the only ones with wings, allowing them to move to neighbouring fields or host plants nearby. This is accomplished by wriggling their abdomens, flying their wings, and leaping with their hind legs (Lewis, 1997).

2.6.3 Ecology, distribution and host range of *Megalurothrips sjostedti*

Thrips populations are generally lower during heavy rains, but increase under dry conditions associated with the later stages of crop growth. They live in the flowers and racemes of cowpea (Plate 2.2) and other leguminous plants including *Glycine max* (soya bean), *Pueraria phaseoloides, Lonchocarpus rugosus* Benth, and are widely dispersed in the tropical regions (Jones, 2005; Zhao and Rosa, 2020).

2.6.4 Economic impact of infestation of *Megalurothrips sjostedti*

Megalurothrips sjostedti is a significant legume pest in Africa that can reduce cowpea yields by 20 to 100 percent without the use of chemicals (Abtew *et al.*, 2015; Oyewale and Bamaiyi, 2013). Thrips feed from the plant by piercing the epidermal, mesophyll, and parenchymal cells of the plant with their piercing and sucking mouth portion (stylet) and drinking the juices. Thrips may damage the terminal leaf bud during the pre-flowering stage, causing it to be deformed, with mottled yellow colour (Oparaeke, 2010). The attack starts before the flower blooms, causing the flower bud to dry out and turn brown. They feed on pollen throughout the flowering stage, resulting in flower abortion and reduced yield (Childers and Achor, 1995). An infested cowpea flower is presented in Plate 2.2. During the off-season, cowpea flower thrips can be found on legumes such as *Centrosema pubescence* and *Pueraria phaseoloides* (Tamo *et al*., 2002; Birithia *et al*., 2018), also

Plate 2.1: **Adult female *Megalurothrips sjostedti***

Source: https://plantwiseplusknowledgebank.org/doi/10.1079/PWKB.Species.52634

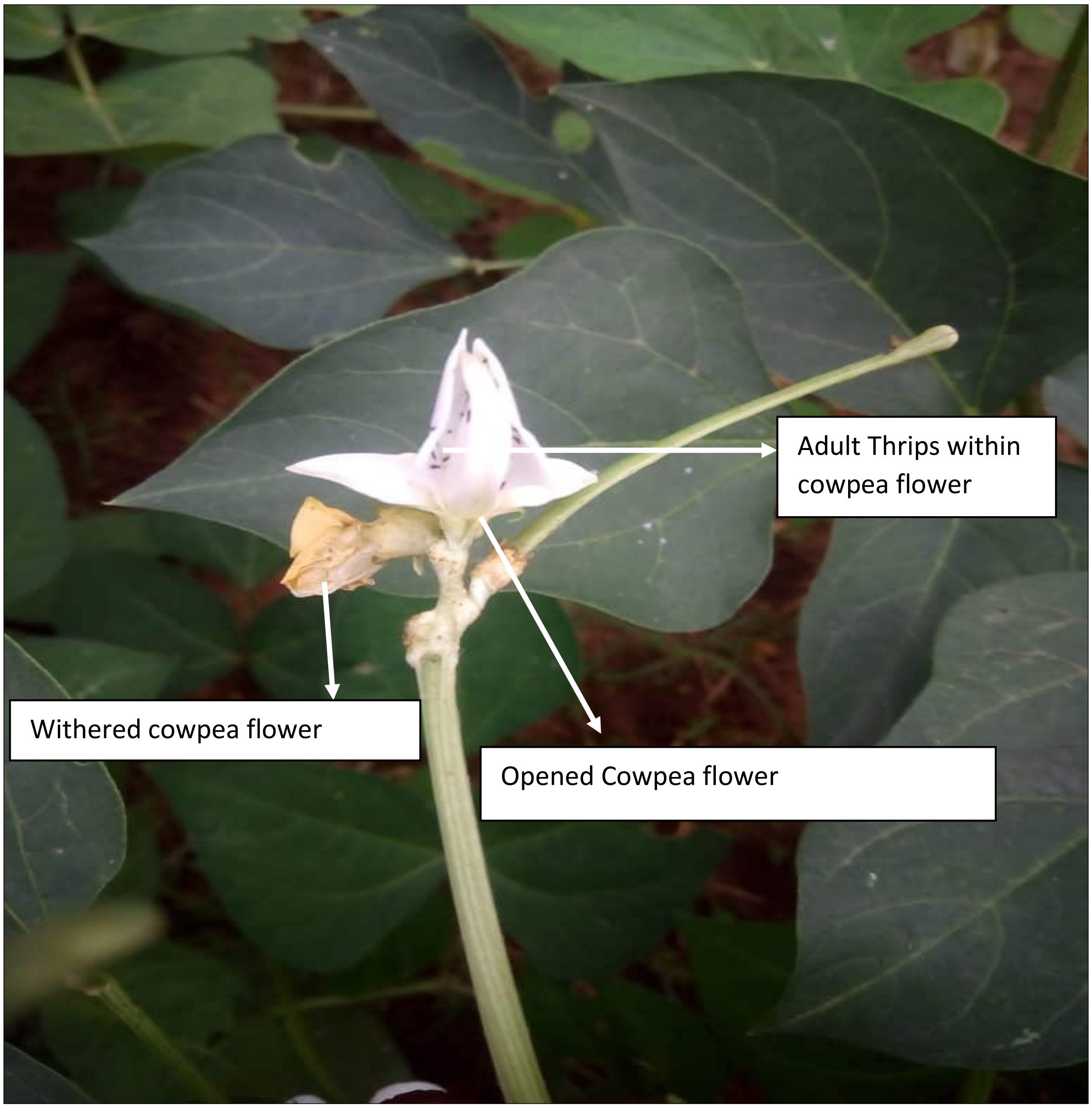


Plate 2.2: Cowpea flower infested by *Megalurothrips sjostedti*

Lonchocarpus rugosus tree. During the dry season, flower thrips populations are higher, favoring rapid thrips multiplication (Nyasani *et al*., 2013). Open flowers become distorted and discolored when there is a high population of thrips. Flowers fall off quickly, and pods are not developed. (Kanteh *et al*., 2013; Knowledge Bank, 2014).

2.7. Population control of *Megalurothrips sjostedti*

2.7.1. Chemical control of *Megalurothrips sjsotedti*

Thrips population density is largely controlled with synthetic insecticides. The most common groups of these insecticides are pyrethroids and organophosphate. Cypemethrin is a pyrethroid. It is a synthetic derivation of pyrethrin which is a substance from the flowers of chrysanthemum plant. The formulation type is emulsifiable concentrate (EC) and its mode of action on insect is both contact and stomach poison (Shilpakar, 2020). It disrupts the correct functioning of ion channel and alters the membrane potential and sodium ion permeability of the cell membrane resulting in the death of the insects. Lambdacyhalothrin is another pyrethoid insecticide used for contolling *Megalurothrips sjostedti* on infested cowpea fields. It is formulated as an emulsifiable concentrate, also a contact and stomach poison. It disrupts the insects' nervous system resulting in paralysis.

Dimethoate is an organophosphate insecticide and acetylcholinesterase inhibitor. Mode of action is by inhalation, ingestion, or contact. It has also been reported that the mixture of cypermethrin and dimethoate substantially reduced the population densities of *Megalurothrips sjostedti* Trybom than each insecticide applied singly. Cypermethrin and organophosphate pesticides were developed to replace organochlorines, which were derivatives of chlorinated hydrocarbons. Dichlorodiphenyltrichloroethane (DDT) was the most used organochlorine in insect pest management but was banned when they were discovered to be highly toxic and persistent in the environment (Egho, 2011).

Monocrotophos is a widespread and effective organophosphate pesticide that has been used to control cowpea pests, however it has been shown to have considerable mammalian toxicity, which is why Pyrethroids are preferred (Dina and Medaiyedu, 1976; Kaur and Goyal, 2019). Other examples of organophosphate insecticides include malathion, parathion, diazinon, among others. In Nigeria, cowpea producers use pyrethroids such as cypermethrin, dimethoate, and lambda-cyhalothrin to control pests of the crop although they leave some residue in the soil (Oladapo *et al*., 2021). Extracts from the leaves of *Gmelina arborea* Candahar, *Eucalyptus citriodora* Hook tree barks and *Tagetes erecta* L. brought down the population of *Megalurothrips sjostedti* in the field (Oparaeke, 2010). The botanicals are safer for use than the synthetic pesticide because they degrade easily.

2.7.2. Cultural control of *Megalurothrips sjsotedti*

Cultural methods of pest management are typically based on the principle of avoidance. The traditional methods involved in raising the crops are tweaked to either avoid the peak of infestation or interrupt free access to the crop (Walgenbach, 2018). Some of these practices include intercropping, crop rotation, adjustment of planting date. Farmers typically sow cowpea with cereals such as millet and maize as intercrops in Africa (Mohammed *et al.,* 2021). When cowpea and maize are intercropped, thrips infestation level reduces (Albert *et al*., 2008; Akhiwu, 2020) and helps to lessen aphid infestations (Navas, 2014). In addition, sowing early in the season decreases outbreak of *M. sjostedti* on cowpea farms (Abudulai *et al*., 2017; Ezeaku *et al*., 2015)

2.7.3. Biological control of *Megalurothrips sjsotedti*

Different research and attempts have been made to manage the infestation of *Megalurothrips sjostedti* and some other thrips species using predators such as the anthocorid bug *Orius albidipennis* Reuters, the Staphylinid Beetle *Paederus sabaeuss* Erichson, and *Geocoris* spp, but they have not been very successful. (Deligeorgidis, 2003; Tamo *et al.,* 2013; Ahmed *et al.,* 2021). Contrarily, the wasp, *Ceranisus femoratus* Ghan is a parasitoid that attacks the larval stage of thrips (Jamali *et al*., 2019). It has shown promising parasitic rate on *Megalurothrips sjostedti* in cowpea fields and other host plants (Adati *et al*., 2007; Tamo *et al*., 2013). The wasp was discovered in Cameroon, and its potential as a biological control agent for thrips have been has been studied in Nigeria, Ghana, and Benin Republic (Tamo *et al*., 2013).

Entomopathogenic fungi are fungi that dwell in the soil and attack insects and other arthropods by penetrating their cuticle (Mantzoukas *et al*., 2022). There has been report of the pathogenic effect of the fungi, *Metarhizium anisopliae* on thrips in Africa, and has been established that strains of *Metarhizium anisopliae* can significantly alter feeding, fecundity, fertility and longevity of *Megalurothrips sjostedti*. Isolates of *M. anisopliae* ICIPE-69 has been used to reduce thrips population densities in cowpea flower (Ekesi *et al*., 1998). They are packaged and sold as Campaign® by RealIPM in Kenya (Mfuti *et al*., 2016). When conidia of the fungus are applied to crop foliage on the field, its efficiency is reduced because of inconsistent environmental factors (Mfuti *et al*., 2016). However, when applied with an autoinoculation device, its persistence on the crop increases, resulting in its effective control of *Megalurothrips sjostedti* in cowpea fields (Mfuti *et al*., 2016).

2.7.4. Host Plant Resistance (HPR)

The ability of plants to escape from pest attack, either partially or completely, is referred to as plant resistance. This helps to minimize the harm that the plant sustains (Mitchell *et al*., 2016). The use of resistant cultivars is an efficient and cost-effective control strategy for insect pests (Potarot, 2012). Plants employ either of three different mechanisms to express resistance to a particular pest: antibiosis, antixenosis and tolerance (Togola *et al*., 2017). Antibiosis occurs when plants alter the state of wellness of insects as they feed on the plant. With antixenosis, the chemical or physical traits of plants alters the behaviour of the insect infesting the plant, therefore, are unable to successfully establish on the plant, and tolerance is exhibited when infested plant can perform optimally despite infestation.

According to a laboratory study by Ekesi *et al.,* (1998), antixenosis resistance is conferred on a cultivar by the type of volatile organic compounds it produces (Ekesi *et al*., 1998). Some cowpea genotypes with resistance to thrips are TVu 1509, IT90K-277-2, TVu 2870, Moussa Local, Sanzibanili, Sewe, TVx 3236 and KVx 404-8-1 (Alabi *et al*., 2004; Togola *et al*., 2017; Sidibe *et al*., 2018). Some cowpea genotypes with resistance to thrips are

TVu1509, IT90K-277-2, TVu 2870, and TVx 3236 (Alabi *et al*., 2004; Togola *et al*., 2017).

2.8 Non-conventional Methods of Control

2.8.1 Floral volatiles

Floral volatiles are chemicals that give each plant its own odour and flavour (Surburg *et al*., 1993; Mostafa, 2022). They are classed as aromatics, terpenes, nitrogen, and sulphur-containing chemicals because they are complex combinations of acyclic, aromatic, and heterocyclic molecules. Volatiles are crucial indicators that assist insects discover flowers and convey the availability of food or mates as pollinator attractants (Knudsen *et al*., 1993; Mostafa, 2022). Floral volatiles attract pollinators that are particular to each species. Terpenoids, simple aromatics, amines, and hydrocarbons make up most floral fragrance compounds. Monoterpenes are the most frequent flower scent compounds (Williams and Whi, 1983). Most of the volatiles in cowpea flower fall into the following categories: monoterpenes, sesquiterpenes, norisoprenoids, aromatics, aliphatic compounds, and other miscellaneous compounds (Ager, 2009; Feng *et al.*, 2017).

Plant volatiles can be utilized to manage insect pests in a variety of ways, including mass trapping (Górski 2004; Osei-Owusu, 2020), the synergistic action of plant volatiles in pheromone traps (Hardie *et al*., 1994), plant volatiles as a lure for biocontrol agents of insect pests, and the 'push-pull' technique (Rodriguez-Saona *et al*., 2012).

2.8.2 Plant volatiles for pest control

Plant volatiles are formed as a by-product of plant processes. Insect pest control is mostly based on the alteration of normal behaviour of insect pests (Foster and Harris, 1997; Gould, 1991; Mostafa, 2022), either through chemical (volatiles or non-volatile compounds, feeding deterrents), visual, or auditory signals. Plant species produce volatiles to defend themselves, either directly against herbivores by producing semio-chemicals or toxins, or indirectly by producing chemicals that attract pests' natural enemies (Pare and Tumlinson, 1999; Gershenzon and Ullah, 2022). Induced, direct, and indirect defenses are used by plants in response to herbivore attacks. The chemicals released by the plant directly target the herbivore in direct defense (Lou and Baldwin, 2003), whereas chemicals in indirect defense increase herbivore mortality by attracting natural enemies (Kessler and Baldwin, 2001; Lou and Baldwin, 2003). The importance of indirect impacts is currently garnering considerable attention, as there are more reports of natural enemies utilizing plant volatiles while locating their prey (Dicke and Baldwin, 2010).

Methyl salicylate is a phenolic compound and common among most plant volatile compounds; it is used by plants to protect themselves from diseases and herbivores (Park *et al*., 2007; Vlot *et. al*., 2008). Natural enemies can be attracted to methyl salicylate before herbivore outbreaks. Early entrance of natural enemies into an agro-ecosystem is thought to be a significant factor in effective herbivore biological control (Khan *et al*., 2008; Tamiru and Khan, 2017). Traps set next to methyl salicylate lures in treated soyabean plots caught considerably more natural enemies than traps in untreated plots with no lures (Mallinger *et al*., 2011). Another aspect that could skew indirect effects is the fact that some bio control agents are omnivorous. In their search for prey, omnivorous predatory mites, and ladybirds, which also feed on numerous plant products, respond to plant volatiles, according to recent research. Omnivores are expected to respond to both constitutive and herbivore-induced plant volatiles, as they can sometimes survive on plant food alone in the absence of prey (Stenberg *et al*., 2010). To use an herbivore's natural enemies in a biological control programme, it is vital to understand how natural enemies react to the volatiles released by a certain plant species or cultivar. To reduce the need for chemical control, concerted efforts are being made to create insect-resistant cowpea types. A land race Sanzisabinli from Ghana (Omo-Ikerodah *et al*., 2009) and TVu1509 from Obafemi Awolowo University, Ile - Ife, have both been identified as having flower thrips resistance. In a screening study conducted by Alabi *et al*. (2003, 2011), some of the cultivars assessed such as IT90k-277-2, KVx 404-8-1, Sewe, Moussa Local, and Sanzisabinli, showed some thrips resistance. Integrated pest management has been proposed to obtain a more realistic increase in cowpea output (Adati *et al*., 2007). It entails a cost-effective and ecologically friendly combination of two or more complementary pest control techniques.

2.8.3 Plant volatiles in synergy with pheromone baits

Insects release pheromones as a way of communicating with one another. These homones are harnessed for pest population monitoring and mass trapping by incorporating than into different types of traps (Witzgall *et al*., 2010). Sex pheromone could be better effective for trapping insects when combined with certain plant volatiles of host plants. Likewise, volatile compounds from host plants or non-host plants could inhibit the attractiveness of a pheromone to the target insect. The aphid, *Rhopalosiphum padi* L., was better attracted when the sex pheromone, nepetalactol, was combined with volatile compounds from the host plant *Prunus padus* L. in the field (Hardie *et al*., 1994). Baroffio *et al*. (2018) also reported that the attractiveness of the aggregation pheromone to male and female *Anthonomus rubi* Herbst was stronger when it was combined with 1, 4 dimethoxybenzene (DMB), a volatile from strawberry flower. In a different scenario, the attraction of *Spodoptera littoralis* Boisduval to a single synthetic pheromone component was reduced when it was combined with the host plant volatiles (Borrero-Echeverry *et al*., 2018). In addition, when volatiles from leaf and bark non-host plant was combined with sex pheromone, it inhibited the attractiveness of the male moth, *Thaumetopoea pityocampa* Denis and Schiffermüller (Jactel *et al*., 2011).

2.8.4 Plant volatiles as attractants of natural enemies of pests

Plants are protected by volatiles emitted from vegetative parts, particularly those released after herbivory, referred to as Herbivore Induced Plant Volatiles (HIPVs). They deter herbivores and/or attract herbivore enemies. Herbivores crop pests often employ volatiles as cues when selecting host plants, likewise organisms in the next trophic level, which are the natural enemies of crop pests. Predators and parasitoids use plant scents to locate plants that are hosts to their prey (Kessler and Heil, 2011).

These natural enemies also take advantage of the fact that many plants release mixtures of volatiles after herbivory that are different from those before herbivory in terms of amount and composition. The production of unique mixtures of herbivore-induced volatiles by over 50 different plant species is documented. These volatiles are known to attract a variety of herbivore enemies, including predators and parasitoids from five insect groups as well as predatory mites, nematodes, and birds (Turlings and Wäckers, 2004; Mumm and Dicke, 2010). Methyl salicylate (MeSA) has attracted increased attention among the HIPVs used as bait to boost natural enemies in the field (Rodriguez-Saona *et al*., 2012). In the vineyard crop, methyl salicylate attracted five predatory insect species (*Chrysopa nigricornis* Burmeister, *Hemerobius* sp., *Deraeocoris brevis* Uhler, *Stethorus punctum picipes* Casey, and *Orius tristicolor* White) (James and Price, 2004).

Common compounds among various HIPV blends of different plants include: the monoterpenes (E)-β-ocimene and linalool, the sesquiterpenes (E, E)-α-farnesene and (E)-β-caryophyllene, the C11 homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and the fatty acid derivatives known as green leaf volatiles (GLVs), including (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate (Kigathi *et al*., 2009; Schaub *et al*., 2010). Despite their role in recruiting natural enemies to the field, HIPVs must be used with caution as they can also be an attraction to new or existing pest, resulting in a devastating effect on crop (Simpson *et al*., 2011).

2.8.5 Push-pull strategy

The strategy is a companion cropping system used to manipulate the behaviour of insect pests and their natural enemies by blending cues from companion crops that make the protected crop undesirable or unsuitable to the pests (push), while drawing them toward an attractive source (pull), from which the pests are then removed (Cook *et al*., 2007). The technique is also described as stimulo-deterrent diversionary strategy (Pickett *et al*., 2014). Push-pull has been used to manage stemborer population on maize fields by intercropping insect-repellent forage legumes from the genus *Desmodium*, for example *Desmodium uncinatum* (silver leaf Desmodium) with maize and planting Brachiaria (Mulato II) or *Pennisetum purpureum* (Napier grass) around the border of the intercrop (Midega *et al*., 2018; Njeru *et al*., 2020). Desmodium releases volatile organic compounds such as ocimene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and cedrene (Khan *et al*., 2016), that repel adult stemborer, and serves as an attractant to *Cotesia sesamiae*, which is a natural enemy, Napier grass on the other hand releases VOCs such as nonanal, linalool, naphthalene, octanal, eugenol, and 4-allylanisole, were identified in maize and Napier grass, they were also found attractive to stemborer moths, but do not support their survival (Khan *et al*., 2016; Midega *et al*., 2018). This strategy has been reported to successfully control stemborers on maize fields (Khan *et al*., 2000; Midega *et al*., 2018; Njeru *et al*., 2020). Before deploying the strategy, however, extensive study is required to understand the behavioral response of both pests and natural enemies to the attractant (pull) and repellent companion plants (push) (Pickett *et al*., 2014; Khan *et al*., 2016; Eigenbrode *et al*., 2016).

The push-pull strategy was adapted to suppress populations of thrips, *Frankliniella occidentalis* Pergande, where the push components consisted of ultraviolet (UV)-reflective mulch and foliar applications of kaolin and the pull component was the companion plant, *Bidens alba* (L.). The companion plants were attractive to *Frankliniella occidentalis* and hosts for the thrips predator *Orius insidiosus* Say (Tyler-Julian *et al*., 2018). This technique was also combined with netting technology for the management of *Megalurothrips sjostedti*. The push stimuli were produced by the plants *Cymbopogon citratus* de Candolle and *Tagetes minuta* L., and the attraction stimuli came from visual response from blue and yellow sticky traps (Diabate *et al*., 2021). The push-pull strategy uses non-toxic push and pulls components, therefore a perfect component of an integrated pest management strategy. It also has the advantage of reducing reliance on insecticides, allowing natural enemies to naturally reduce insect pests. The companion, Desmodium has also been reported useful in suppressing the parasitic weed striga through allelopathy and contributes to soil health improvement through nitrogen fixation (Khan and Pickett, 2008), therefore improving soil health.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental locations

This study was carried out at the following locations: at the Insect Chemical Ecology Laboratory and Crop Garden, both located in the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria, 7.4433° N, 3.9003° E; Insect Rearing (Entomology) Unit, International Institute of Tropical Agriculture (IITA),Ibadan, Nigeria 7.4962° N, 3.9076° E; Department of Behavioural and Chemical Ecology, International Center for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, 36.8967°N, 1.2219°E; Department of Entomology Laboratory, Wageningen University, The Netherlands, Latitude: 51.9669 Longitude: 5.6552 and International Institute of Tropical Agriculture, Abomey-Calavi, Republic of Benin, 2.3221622° N, 6.4663884°E.

3.2 Sources of cowpea cultivars

The study to evaluate the headspace volatile organic compound of cowpea was initially conducted with eight cowpea materials which included two susceptible varieties: Ife Brown and Vita-7, five resistant cowpea types: Sanzibanili, Moussa Local, Sewe, TVu1509, KVx404-8-1 and one moderately resistant variety: IT90K-277-2 (Table 3.1). The number of cultivars studied was reduced to seven after KVx404-8-1 was dropped because the seeds were not readily available. Seeds of Ife Brown, TVu1509, Vita7 and IT90K-277-2 were obtained from the Genetic Resources Center, IITA Ibadan, while Moussa Local, Sewe and Sanzibanili were obtained from IITA Republic of Benin.

Table 3.1 The origin and status of the cowpea materials used for olfactory studies.

Cowpea types	**Origin**	**Status**
IT90K-277-2	Nigeria, IITA	Moderately Resistant
Moussa Local	Burkina Faso	Highly Resistant
Sanzibanili	Ghana	Highly Resistant
Sewe	Republic of Benin	Highly Resistant
TVu 1509	Nigeria, OAU	Resistant
Vita-7	Nigeria	Susceptible
Ife Brown	Nigeria, OAU	Susceptible
KVx 404-8-1	Burkina Faso	Moderately Resistant

IITA: International Institute for Tropical Agriculture

TVu: Tropical *Vigna unguiculata*

IT: IITA identification title

OAU: Obafemi Awolowo University

Source: (Alabi *et al*., 2004; Sidibe *et al*., 2018)

3.3 Rearing of *Megalurothrips sjostedti*

The initial population of *Megalurothrips sjostedti* was collected from cowpea plants on the demonstration plot at IITA Ibadan. Thrips were reared in 10 cm by 16 cm ventilated plastic jars covered with thrips proof mesh. Two pods of *Vigna unguiculata* subspecies sesquipedalis commonly referred to as Yardlong beans (Plate 3.1) averagely measuring 45 cm long were put into the rearing jars to serve as feed and substrate for the insects to lay eggs because they stayed fresh longer than cowpea pods. The bean was supplied weekly by a small holder farmer who grows them on his farm without the application of insecticides. Petals of infested cowpea flower were carefully severed to expose thrips and aspirator was used to pick 300 insects by siphoning (Plate 3.2) into each of the rearing jars (Plate3.3). At 48 hours, the insects had laid eggs on the bean pods. Pods with eggs were removed into an empty jar and replaced with fresh pods.

Fresh pods were re-introduced into the jars containing the initial insect population every 48 hours to ensure a continuous supply of new adults. Dry and decaying pods were removed from the other jars and replaced with fresh pods until the eggs developed through the larvae stages to become adults. The culture was maintained in the Entomology Laboratory, IITA, Ibadan at a temperature of 25 ±1ºC; relative humidity 70 ±5% and 12 hours light – 12 hours darkness photoperiod.

3.4 Olfactory response of *Megalurothrips sjostedti* to cowpea headspace volatiles

3.4.1 Experimental location

This experiment was carried out in then Insect Chemical Ecology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan.

3.4.2 Growing of test plant

The seeds of seven cowpea cultivars were sown in platic pots containing 5 kg soil at two seeds /pot. Soil type is sandyloam, collected from the crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan. The pots were arranged in rows, with ten pots per row. A cowpea cultivar was sown in each row, making a total of seven rows. The rows were sown at two weeks' interval to prevent cross pollination. Plants were grown in the screen house (Plate. 3.4) at a temperature of 29±6ºC

Plate 3.1: **Pods of *Vigna unguiculata* subspecies *sesquipedalis* (Yardlong beans) used for rearing *Megalurothrips sjostedti* in the laboratory**

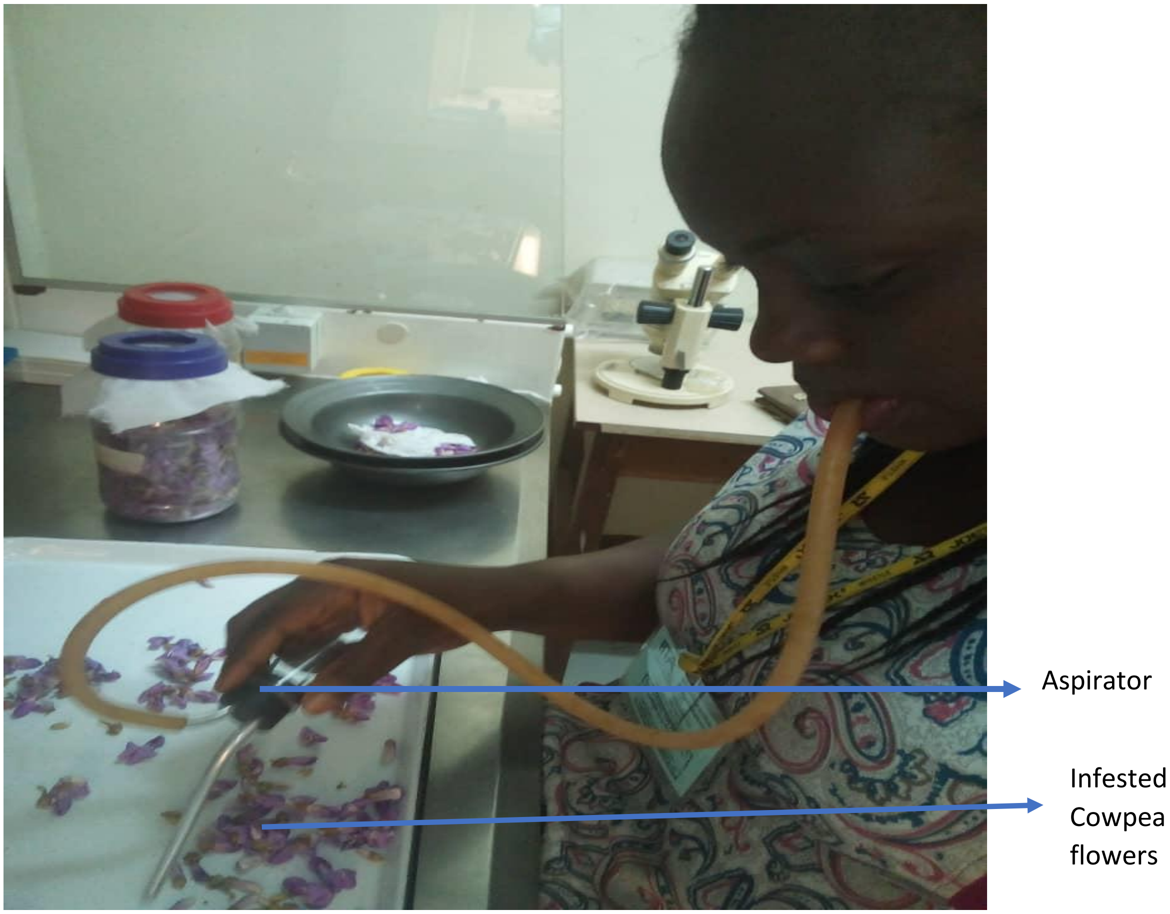


Plate 3.2: Student using an aspirator to pick out *Megalurothrips sjostedti* from excised, thrips infested cowpea flowers

Plate 3.3: Laboratory population of *Megalurothrips sjostetdti* in plastic jars with pods of *Vigna unguiculata* subspecies sesquipedalis

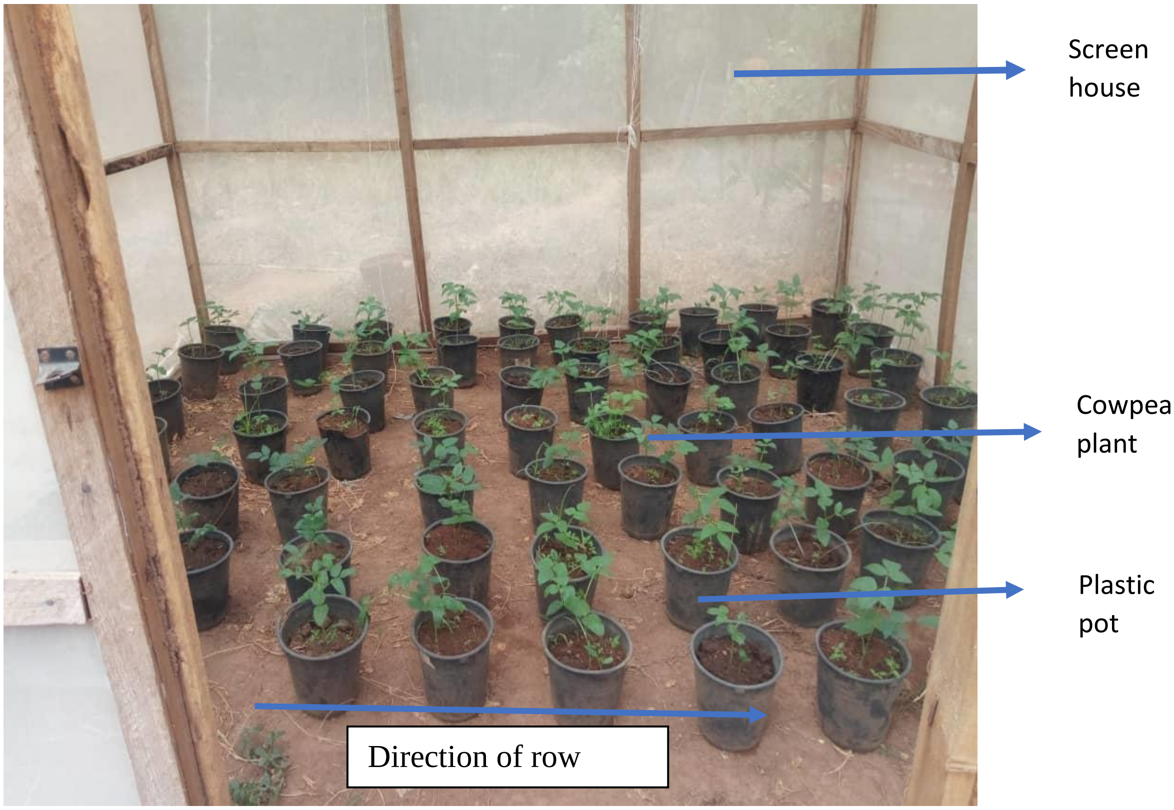


Plate 3.4: Seven rows of potted cowpea with a different cowpea type growing in each row in the screen house at the crop garden, Department of Crop Protection and Environmental Biology, University of Ibadan.

and relative humidity of 72 ±5%. The plants were allowed to grow without the application of any chemical. When the plants reached their reproductive stage with at least one open flower and one raceme, they were ready for the experiment.

3.4.3 Olfactometer choice assay

Olfactory response of adult female *Megalurothrips sjostedti*, to odours from seven cowpea types at their reproductive stage were evaluated using a Y-tube olfactometer (internal diameter: 0.5 cm, stem length: 4 cm, arm length: 5.5 cm) at 27 ± 1 °C and 40–50% relative humidity as described by Diabate *et al*. (2019). A dynamic headspace was created with continous supply of air through the clean air delivery system to cowpea heaspace and at same time, air flow out through the Y-tube (Figure 3.1). The Y-tube was positioned on a white board placed at a 25° angle to a plane surface (Plate 3.5) (Koschier *et al*., 2017), within a cardboard box (50 cm × 48 cm × 33 cm). The box was slightly cut opened at to permit a white florescent light at the top, for illuminating the bioassay arena to eliminate light bias. The entire arena was lined with white paper to prevent any visual cues interrupting the olfactory response of thrips to the odours from the cowpea plants. Adult female thrips were isolated and starved for one hour before commencing bioassay.

The shoot of potted cowpea plants that were grown in the screen house (plate 3.4) was covered with an oven bag to ward off odours emanating from the soil from interacting with those from the plants and held tightly with Teflon tape around the stem just above the soil. The olfactory bioassay set up with potted cowpea plants is shown on Plate 3.6. Two teflon tubes carried filtered compressed air from the Volatile Assay Systems (VAS) field pump into the bagged cowpea head space, which is the first odour source, and into an empty but tied oven bag, which is the second odour source, serving as the control. The two odour sources were connected to the right and left arm of the olfactometer, respectively. The flow of odour was allowed to run for 20 minutes before the insects were introduced to the tail of the Y tube. Individual adult female *Megalurothrips sjostedti* was released at the inlet of the Y- tube olfactometer. Each *Megalurothrips sjostedti* had only 3 minutes to choose its preferred odour by moving in the direction the odour emanates from. When each thrips reached the end of an arm of the olfactometer, it was entered as a

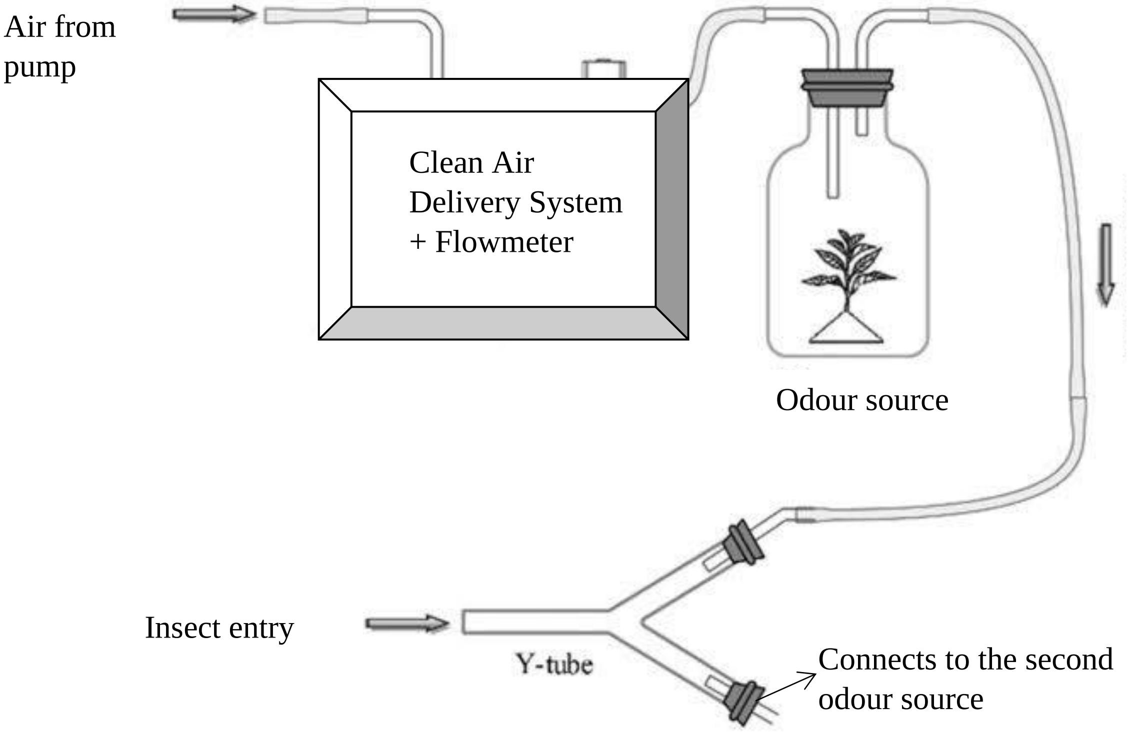


Figure 3.1: A schematic diagram of the Y-tube olfactometer bioassay setup, used to test the olfactory preference of *Megalurothrips sjostedti* for cowpea headspace volatile of clean air

Source: Saad *et al.*, 2015

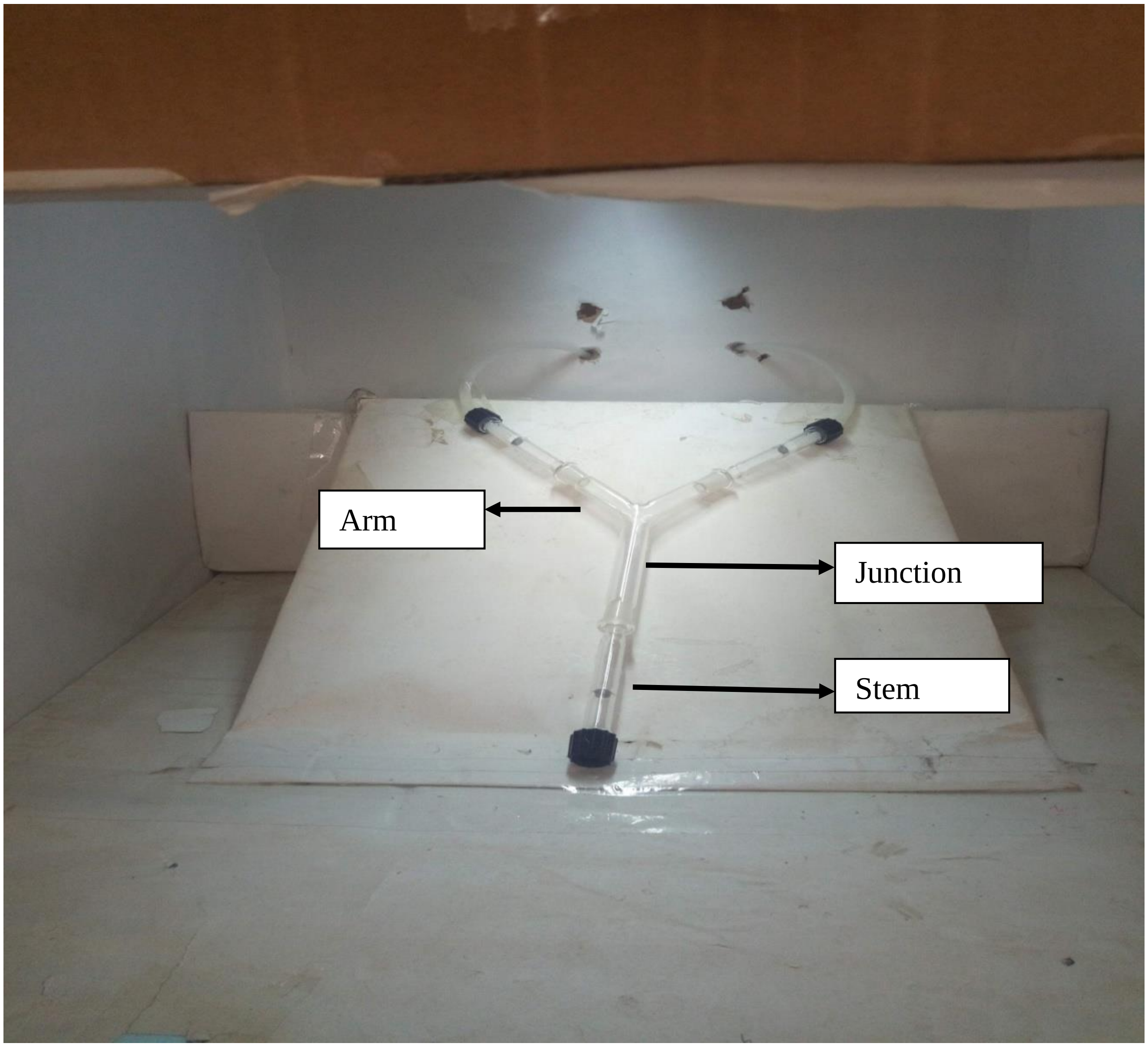


Plate 3.5: A Y- tube olfactometer positioned in a white box inclined at an angle of 25°

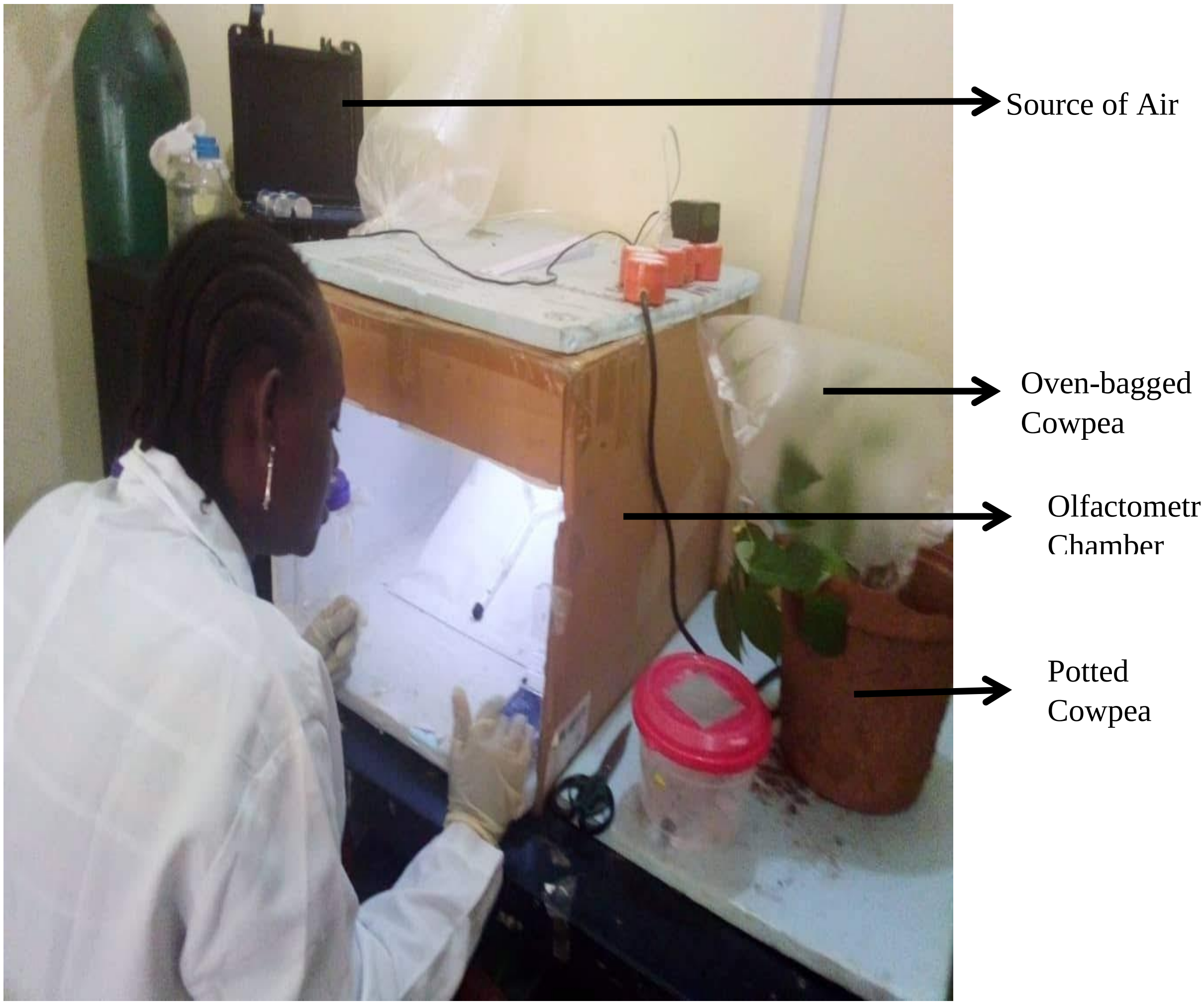


Plate 3.6: The set-up of olfactory bioassay with live cowpea plant in the Insect Chemical Ecology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan.

choice. If no choice was made after 3 minutes, such trips were considered non-responsive. Experimental errors and bias were minimised by changing potted plants after testing twenty insects and changing the positions of the odour sources after every five thrips tested. A total of sixty insects were tested per cultivar.

The Y-tube was washed with clean water and ethanol after testing for each cultivar and left to dry overnight. A different oven bag was used for each of the seven cultivars. Assay was conducted for (a) the reproductive stage of the cultivars versus clean air b) the reproductive stage of selected resistant and susceptible cultivars, tested against each other.

3.4.4 Data collection and analysis

Data were collected on the choice of the insects and the time taken by the insects to move through the stem of the Y-tube, the time taken at the junction before it makes a choice and the time it took the insect to move to the end of the chosen arm of the olfactometer. Thrips preference was presented on a bi-directional barchart, and choice data collected were analysed with Chi-square (χ^2) test to test relatedness and preferences of *Megalurothrips sjostedti* amongst the cowpea types tested, while time data was analysed with T-test.

3.5 Extraction and identification of headspace volatiles from cowpea cultivars

3.5.1 Experimental location

Volatile extraction and identification were first carried out at the Entomology Laboratory, Wageningen University, the Netherlands. In a repeat experiment, volatile extraction was carried out at the Insect Chemical Ecology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, while identification of volatiles was carried out at the Department of Behavioural and Chemical Ecology, International Centre for Insect Physiology and Ecology, Nairobi, Kenya

3.5.2. Growing of test plants in Wageningen and Ibadan

Eight cowpea materials (Ife Brown, TVu1509, Vita-7, IT90K-277-2, Moussa Local, Sewe, Sanzibanili and KVx404-8-1) were sown at Wageningen, The Netherlands, while seven cultivars were sown in Ibadan, Nigeria because the seed of Kvx404-8-1 was not sufficiently available. Seeds were sown in 5 kg soil in the screen house at two seeds per pot and cultivars were sown at two weeks' interval. Cowpea plants at Netherlands did flower as expected, therefore some organic soil amendment were applied to the soil to stimulate flowering. There was no application of any type of chemical to the cowpea in Ibadan and the crops were watered only when rainfall was insufficient. Cowpea plants were ready for volatile extraction when at least two flowers were opened

3.5.3 Constituent volatile profiling from cowpea

Cowpea headspace volatiles were collected using the dynamic headspace sampling technique described by Murungi *et al*. (2016). The setup encloses plant's headspace in a way that excludes external factors. Clean air is metered into the system at one point, and pulled out at another point through an adsorbent trap (Figure 3.2). Volatiles from the flowering cowpea cultivars were sampled by covering the shoot of cowpea plant with a transparent oven bag that was tied with Teflon tape around the stem just above the soil. Air provided by an Air compressor/pump was passed through the Clean Air Delivery System (CADS) by Sigma Scientific before it was metered into the oven bag covering cowpea headspace, at 300 mL/ min. Air was pulled out from the covered headspace through the adsorbent Hayesep-Q trap (Plate 3.7) at 200 mL/min for seven hours between 8:30 am and 3:30 pm. The Volatile Assay Systems (VAS) field pump was also used for collecting headspace volatiles from the cowpea cultivars. Volatiles were also collected from empty oven bags under the same conditions to serve as a check. Volatile was trapped from 6 different plants per cowpea type, and six excised flowers per cultivars. Headspace volatile collection set up is shown in Plates 3.8, 3.9 and 3.10 after seven hours of headspace volatile collection, the Haysep Q trap was unmounted; the two ends of the traps were sealed with Teflon tapes before they were wrapped in foil paper and stored in a -80ºC freezer. Traps were sent to ICIPE for analysis 3 days after the last sample was collected.

3.5.4: Chemical analysis of volatiles

Volatile compounds trapped in hayesep-Q traps were eluted with 150 µl dichloromethane. Thereafter, the eluted volatiles were analysed with an Agilent Technologies 7890A gas chromatography which had a HP-5 MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) coupled to a 5975C mass spectrometer. A 1-µl aliquot of each sample

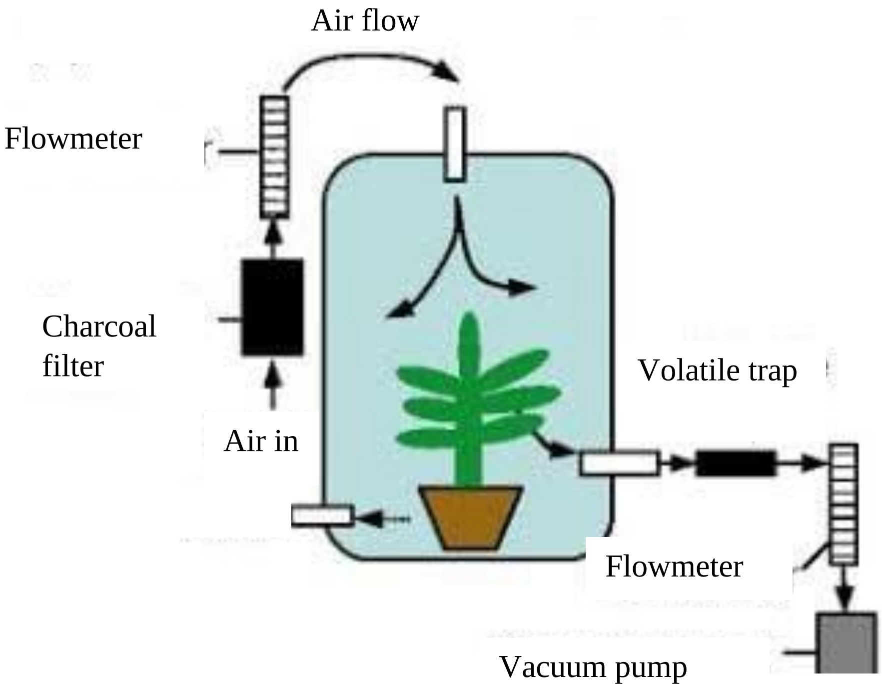


Figure 3.2: A schematic setup for headspace volatile collection from plants using an adsorbent trap

Source: **Tholl *et al.*, 2006**

Plate 3.7: Haysep-Q volatile adsorbent trap used for trapping cowpea headspace volatile

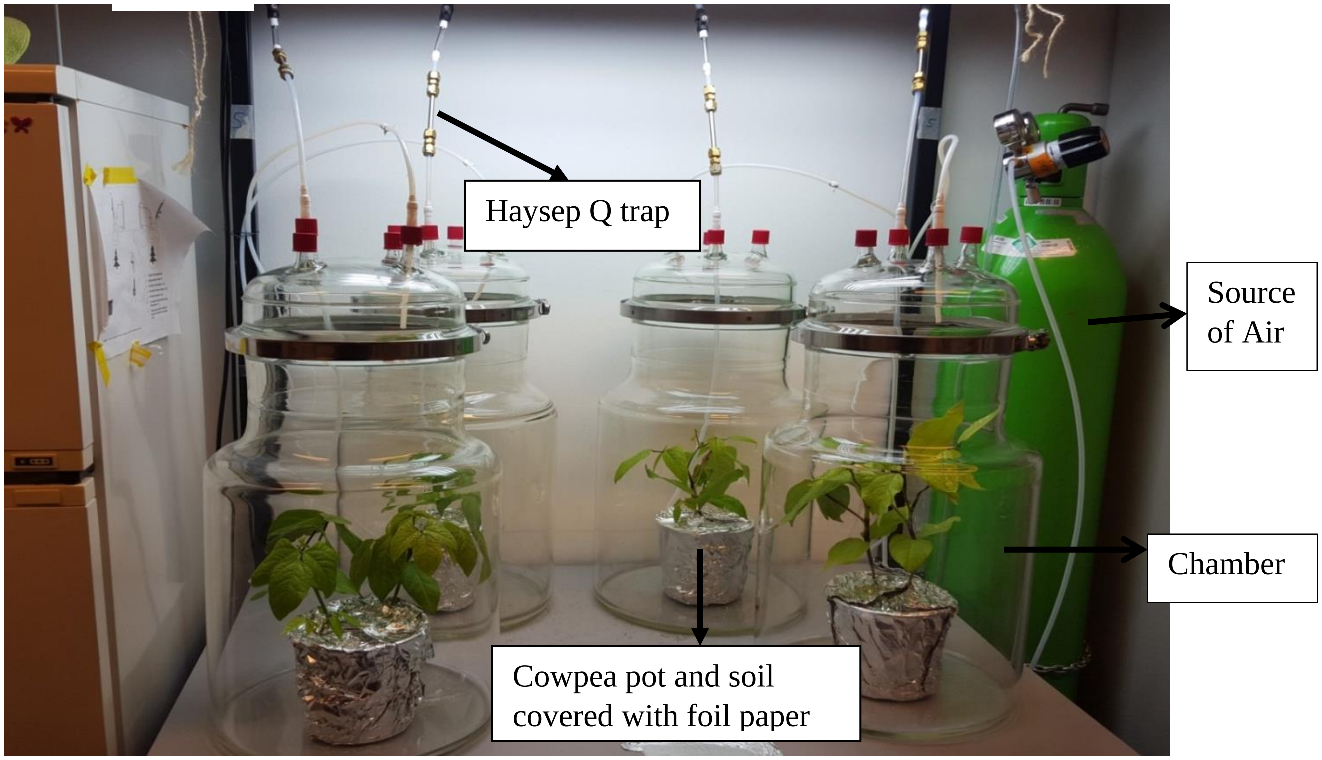


Plate 3.8: Dynamic headspace sampling apparatus used for cowpea headspace volatile extraction at Wageningen, the Netherlands

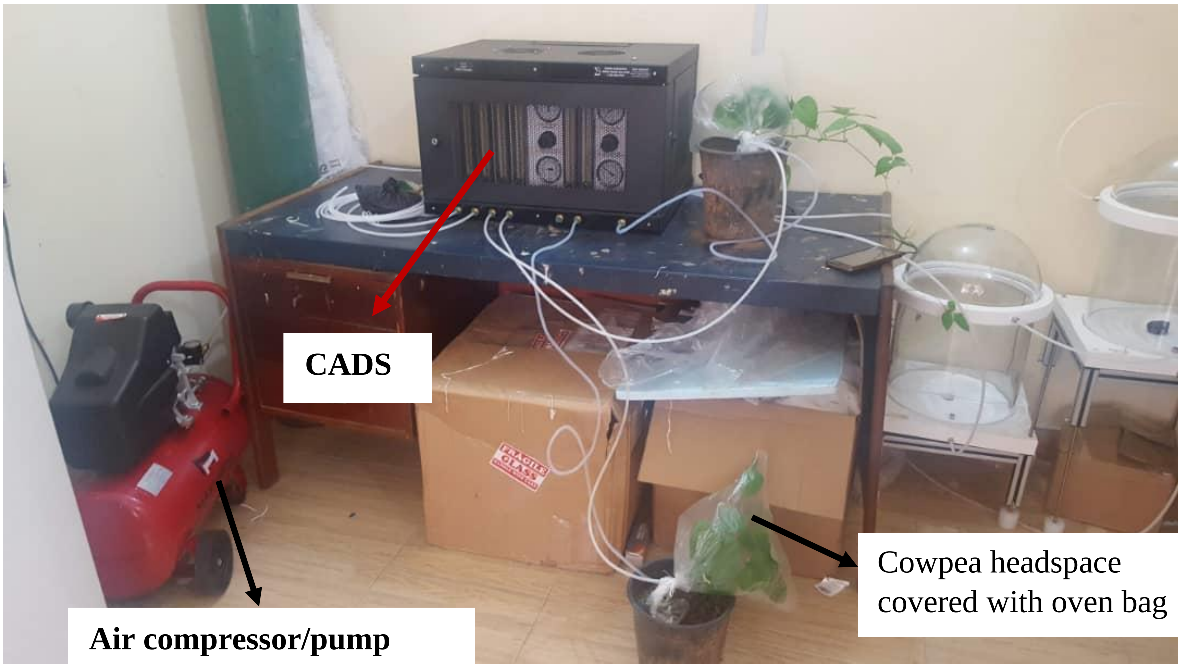


Plate 3.9: Trapping of cowpea headspace volatiles using clean air delivery system (CADS) alongside an air compressor at the Insect Chemical Ecology Laboratory Department of Crop Protection and Environmental Biology, University of Ibadan

Plate 3.10: The set-up for trapping of cowpea headspace volatiles using the volatile assay systems (VAS) field pump, at the Insect Chemical Ecology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan

was analysed in a splitless mode using helium as a carrier gas at a flow rate of 1.2 ml/min. after sample had been injected. The temperature of the oven was set at 35 °C for 5 minutes, after which it was programmed to increase to 280 °C at the rate of 10 °C/min; this temperature was maintained for 5.5 min. Spectra were recorded at 70 eV in the Electron Impact (EI) ionization mode. Compound identification was achieved by comparing the mass spectra data with library data: Adams2 terpenoid/natural product library (Adams 1995) and National Istitute of Standards and Technology (NIST) (2008) (MSD ChemStation F.01.00.1903, MS HP, USA). The retention times of some compounds and mass spectra were also compared to those of authentic standards.

3.5.5: Data analysis

The relative amount of volatile organic compounds among eight cultivars was determined using the non-parametric Kruskal-Wallis one-way analysis and Dunn's method of multiple comparisons. Partial least square discriminant analysis 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 carried out in R version 3.3.2 (R Core Team, 2016).

3.6: Olfactory response of *Megalurothrips sjostedti* to synthetic standards of identified cowpea volatiles.

3.6.1: Experimental location

The experiment was carried out in the Entomology Unit, International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria

3.6.2. Source of synthetic standards used for the experiment

The standard compounds, Alpha terpinene (purity ≥ 98%), γ-Terpinene (purity ≥ 98%), (R) - (+) - Limonene (purity ≥ 98%), Tetradecane (purity ≥ 98%), Sabienene (purity ≥ 98%), Methylsaliscylate (purity ≥ 98%), Hexadecane (purity ≥ 98%), Dodecane (purity ≥ 98%), Nonanal, 1- Tetradecene, Undecane, were purchased from Sigma Aldrich United Kingdom. All standards came in liquid state. Selection of compounds was based of availability and toxicity level of the compounds. Harzadous VOCs were not selected.

3.6.3. Behavioural assay

Exactly 10 µl of selected synthetic standard was applied to 90 mm Whatman filter paper at 100% concentration, using a 1000 µl micro syringe. The filter paper was tied in an oven bag as an odour source. The second odour source was clean air pumped into an empty oven bag. Olfactory assay was carried out as described in 3.4 above. The position of odour source was alternated after every five thrips and the filter paper changedhourly. A new bag was used for every treatment. A total of 60 female thrips were tested for each synthetic standard.

3.6.4. Data analysis

Data were collected on the choice of the insects and the time taken by the insects to move through the stem of the Y-tube, the time taken at the junction before it makes a choice and the time it takes the insect to move to the end of the chosen arm of the olfactometer. Thrips preference was presented on a bi-directional barchart, and choice data collected were analysed with Chi-square (χ^2) test to evaluate the relatedness of *Megalurothrips sjostedti to* the Volatile Organic Compounds, while time data were analysed with T-test.

3.7 Evaluation of volatile organic compounds as effective lures for *Megalurothrips sjostedti* on cowpea fields

3.7.1 Experimental location:

The experiment was carried out at the research field (EN 6) located in International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and the research field at IITA, Abomey-Calavi, Republic of Benin.

3.7.2 Sources of experimental material

The yellow sticky traps and ethanol were purchased from a science equipment supplier in Ibadan, twine was purchased from the University of Ibadan Bookshop and the bamboo pegs were sources from bushes around IITA, Ibadan and Abomey-Calavi. Lambdacyhalothrin was purchased from an agrochemical shop located at Ojoo, Ibadan, while Ziploc bags were provided by IITA Abomey-Calavi and synthetic standards of VOCs were purchased as listed in 3.6.2 above.

3.7.3 Land Preparation

The land allocated was ploughed twice, and then harrowed. It was left to rest for two days after which plots were measured and marked out into six plots per replication and three replications, for planting according to treatments.

3.7.4 Experimental design

The experiment comprised of six treatments, each represented on a plot of 4 m by 3 m each Plots were spaced at 3.71 m apart and replicates spaced at 7.75 m, including the borders apart. The experiment was laid out in a randomized complete block design with three replicates (Figure 3.3).

3.7.5 Planting of cowpea

Ife Brown cowpea was grown at Ibadan while Kpodiguegue was grown at Abomey-Calavi. Both cowpea types well adapted susceptible materials in the respective locations. Two seeds were sown at 75 cm by 25 cm inter and intra row spacing as modified from Jakusko (2013). At two weeks after planting, cowpea stands were thinned to one plant per stand.

3.7.6 Agronomic practices

Cowpea fields were sprayed with lambdacyhalothrin at two weeks and four weeks after sowing following recommended rate bythe manufacturer, to prevent aphids infestation. After all data and sample collections were done, there was another application of lambdacyhalothrin, to prevent yield losses caused by the pod sucking bugs complex. Hand weeding was carried out once during the vegetative growth and once during the reproductive growth stages of cowpea.

3.7.7 Treatment combinations

. The treatments were: (i) Yellow sticky traps only (ii) Yellow sticky trap baited with Methyl salicylate (iii) Yellow sticky trap baited with Hexadecane (iv)Yellow sticky trap baited with Tetradecane (v) Lambda-cyhalothrin only (vi) Control, plots with neither traps nor insecticides. Chemical structures of volatile organic compounds are shown in Figure 3.4 and the laid out plots in Plate 3.11.

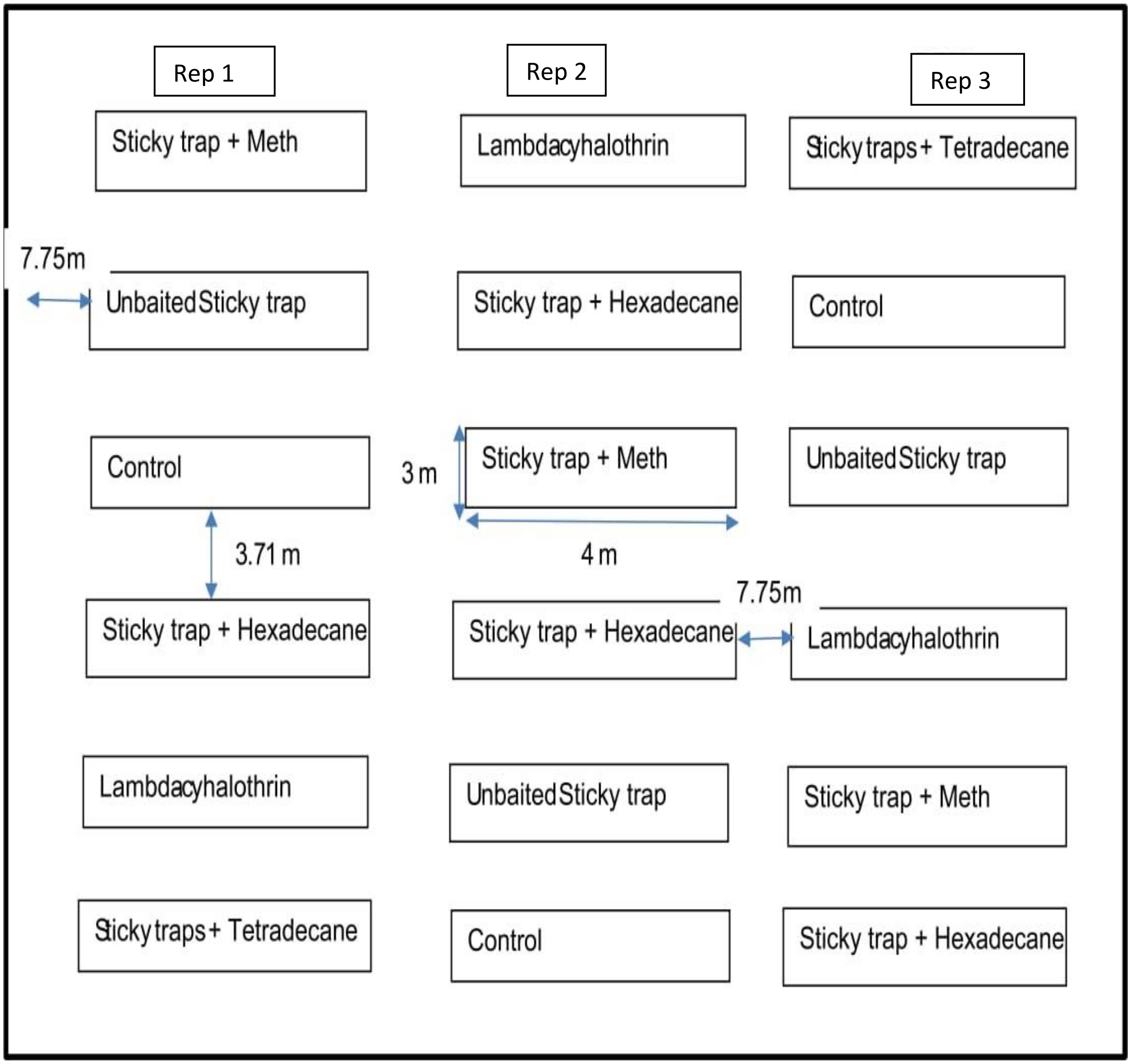
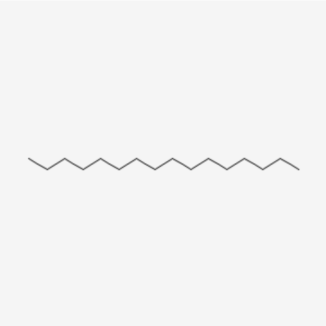
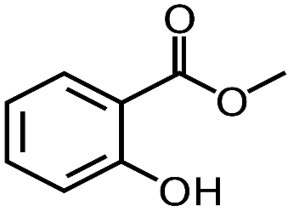


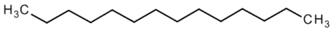
Figure 3.3: Field layout of cowpea plots showing six plots replicated three times



Hexadecane ($C_{16}H_{34}$)



Methyl salicylate ($C_8H_8O_3$)



Tetradecane ($C_{14}H_{30}$)

Figure 3.4 Chemical structure of volatile organic compounds used as bait on cowpea field

Plate 3.11: Experimental field plots layout with yellow sticky traps and bait installed

3.7.8 Trap installation and baiting

Three bamboo sticks of about 1m height above the ground were arranged in a triangular pattern across each plot by digging two holes at one side on the plot and one on the opposite side of the plot, and firmly installing the sticks in the holes. A twine was tied in a continuous stretch round the bamboo pegs. The twine served as an anchor for yellow sticky traps (20 cm by 12.5 cm) and Ziploc bags (10 cm by 6 cm) containing volatile organic compound (Plate 3.12)

1ml of each volatile compound was sealed up in a Ziploc bag, which served as a slow-release dispenser and suspended on the twine right in front of the sticky trap (Plate 3.12). The traps were arranged randomly on each side of the triangle. Traps and volatile compounds were replaced every week. Other cowpea insect pests were controlled by applying lambdacyhalothrin 5% EC at two weeks after planting and two times at podding stage to prevent pod sucking bugs infestation and damage.

3.8 Data Collection

3.8.1 Insect count in cowpea flowers and racemes

One week after trap installation, 43 days after sowing in Ibadan and 42 days after planting in Abomey-Calavi, 20 flowers were picked randomly from each plot. In a case where there were less than 20 flowers, racemes were picked to make up the number. The flowers were preserved in 70% ethanol (Plate 3.13) until all insects in the flowers were rinsed out completely and identified as thrips, aphids, or others, and counted. ‘Others’ comprised of all the other insects apart from thrips and aphids that were present in the flowers. Insect count was done weekly for three weeks, which coincided with 43 days after sowing, 50 days after sowing, 57 days after sowing in Ibadan, and 42 days after sowing, 49 days after sowing and 56 days after sowing in Abomey-Calavi. Insects were counted by pouring 70% ethanol containing the insects into a grided Petri dish (Plate 3.14) and counted under the microscope.

3.8.2 Insect count on yellow sticky traps

After the installation of yellow sticky traps, all the traps on the plots were removed and replaced with new ones weekly for three weeks. All insects trapped after one week were

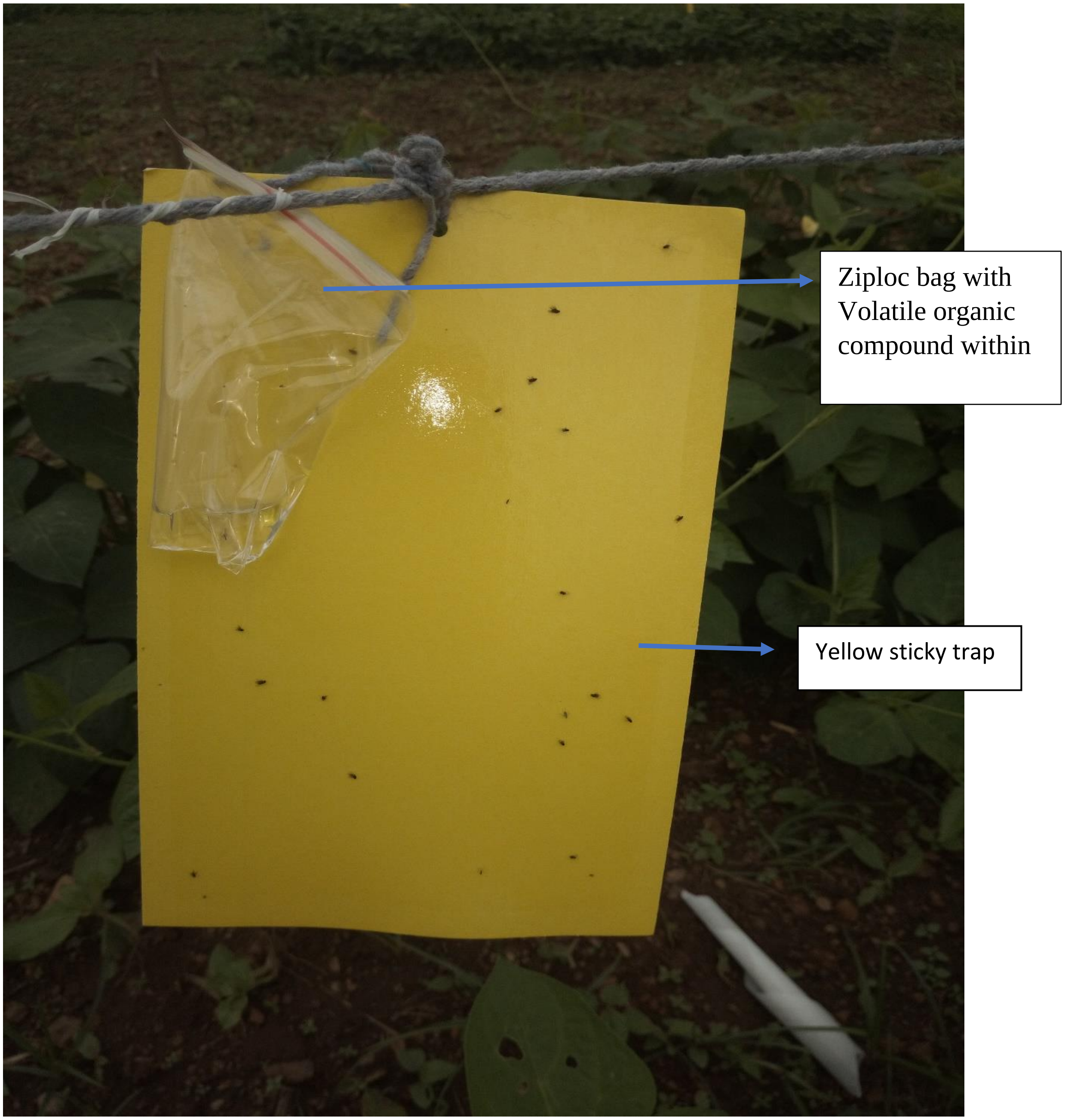


Plate 3.12: Yellow sticky trap with ziploc bag containing volatile organic compound

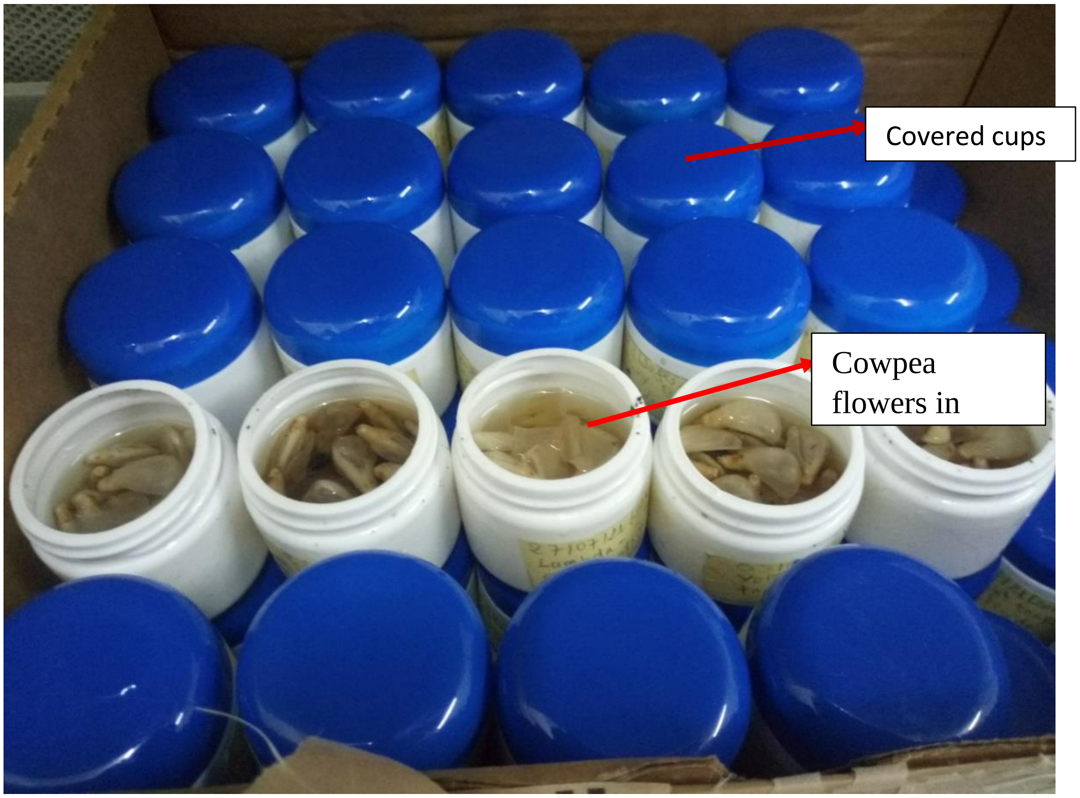


Plate 3.13: Plastic cups containing sampled cowpea flowers collected in 70% ethanol

Plate 3.14: Adult *Megalurothrips sjostedti* put in grided petri dish under a microscope for observation and counting.

Identified up to the family level and counted. Sticky trap was gridded with a pen before counting to minimize error of omission or multiple counting.

3.8.3 Yield assessment

At maturity when cowpea pods were dry and colour turned brown, pods from the whole plot were harvested by hand picking. The pods were further sun dried, shelled and the grain yield was estimated. Yield per plot was measured initially in grams per square meter (g/m^2) but was later converted to Kilogram per hectare (kg/ha) using the formular:

$$Yield\ in\ Kg/ha = Yield\ in\ g/m^2 \times 100$$

$$\text{Yield loss} = \frac{Yield\ of\ Lam - Yield\ of\ Trmt}{Yield\ of\ Lam} \times 100$$

3.9 Data analysis

Count data rarely conform to the assumption of normal distribution; they were therefore transformed using the logarithm transformation to create a linear relationship. Mean number of insects trapped on sticky traps were determined and count of insect infestation on cowpea flowers was analysed using analysis of variance with the volatile organic compounds as treatments while *Megalurothrips sjostedti* and other insects trapped were the dependent variables. Differences were determined using Least Significant Difference (LSD) method at 5% level of significance. Data on yield and yield lossess were also analysed using analysis of variance and LSD at 5% level of significance for means separation. Mean population of thrips were presented on bar charts, while their population trends over a period of three weeks were presented using line graphs.

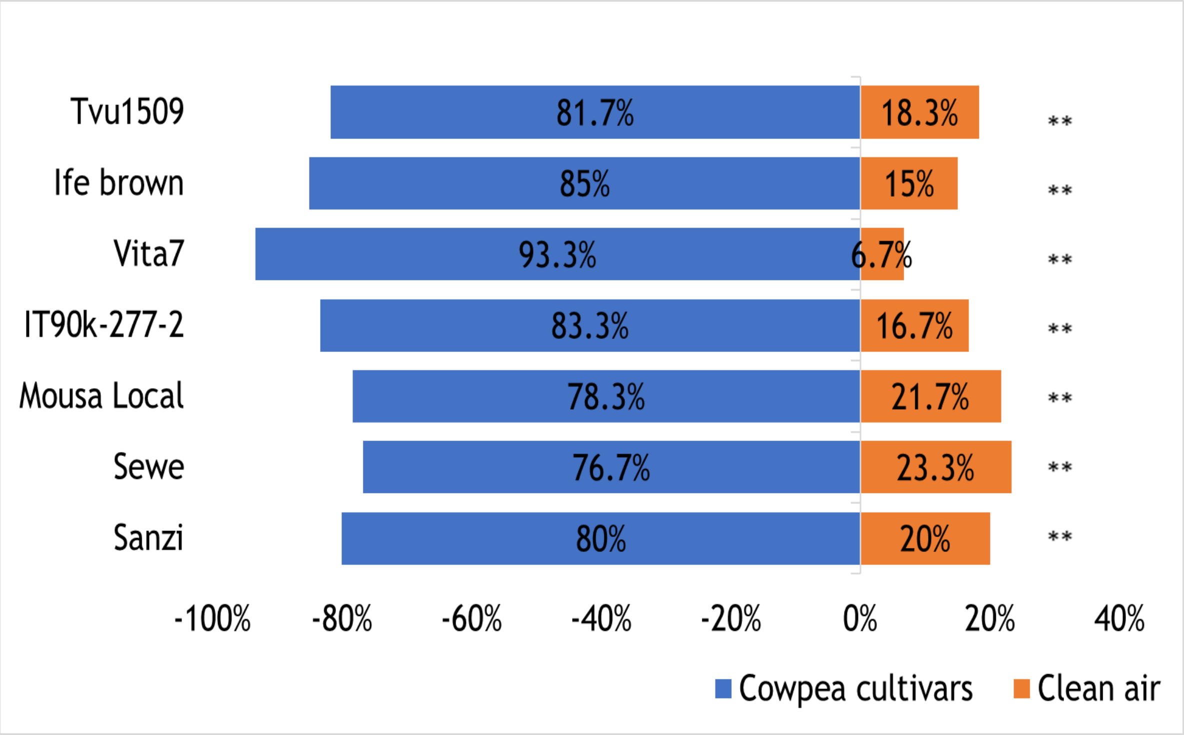
CHAPTER FOUR

RESULTS

4.1 Olfactory response of female *Megalurothrips sjostedti to* headspace volatiles of flowering cowpea cultivars

Adult female *Megalurothrips sjostedti* showed similar response to all the seven cultivars by choosing their headspace volatiles over clean air (Blank). The percentage number of thrips that chose the different arms of the olfactometer out of sixty insects that were tested per cultivar is presented in Figure 4.1. The highest percentage of thrips chose the Ife Brown and Vita-7 arm of the olfactometer, compared to clean air. Vita-7 had 93.3% and Ife Brown had 85.0% of thrips tested. The resistant cultivar Sewe attracted the lowest percentage of thrips among all seven cultivars tested. Each of the cowpea types attracted higher percentage of thrips than the clean air in the following decreasing order: Vita7 (93.3%) <Ife Brown (85.0%) < IT90K-277-2 (83.3%) < TVu1509 (81.7%) <Sanzibanili (80.0%) < Moussa Local (78.3%) <Sewe (76.7%).

Observed differences in the number of *Megalurothrips sjostedti* that chose either of the two arms of the olfactometer for each of the cowpea types were significant at $p < 0.01$ (Table 4.1). This is reflected in the statistics; Vita7: $\chi^2 = 45.07$, $p < 0.01$, Ife Brown: $\chi^2 = 29.4$, $p < 0.01$, IT90K-277-2: $\chi^2 = 29.4$, $p < 0.01$, TVu1509: $\chi^2 = 24.07$, $p < 0.01$, Sanzibanili: $\chi^2 = 24.07$, $p < 0.01$, Moussa Local: $\chi^2 = 19.27$, $p < 0.01$, Sewe: $\chi^2 = 17.07$, $p < 0.01$.



**** = Significant at $p < 0.01$**

Figure 4.1:Percentage of female *Megalurothrips sjostedti* that responded to headspace volatiles from cowpea and clean air from the y-tube olfactometer experiment

Table 4.1: Chi-square statistics, probability value and value of significance of observed differences between the choice of cowpea odour and clean air by *Megalurothrips sjostedti* in the olfactometer test

Choices	X^2	Prob. Value	Significance
Sanzi vs Blank	24.07	9.31E-07	$p < 0.01$
Sewe vs Blank	17.07	3.61E-05	$p < 0.01$
Moussa Local vs Blank	19.27	1.14E-05	$p < 0.01$
IT90K-277-2 vs Blank	29.40	5.89E-08	$p < 0.01$
Vita-7 vs Blank	45.07	1.9E-11	$p < 0.01$
Ife Brown vs Blank	29.40	5.89E-08	$P < 0.01$
TVu1509 vs Blank	24.07	9.31E-07	$p < 0.01$

4.2: Time taken for thrips to respond to headspace volatile from resistant and susceptible cowpea cultivars

At the stem, the mean time the insects spent before moving to Sanzibanili, a resistant cultivar was 26.24 seconds (SD = 20.13), higher than the time spent at the stem beforemoving to clean air (blank), which was 7.45 seconds (SD = 2.52). The observed difference was significant at $p < 0.05$. At the junction and arm of the Y-tube olfactometer, there was no significant difference in the time of response by *Megalurothrips sjostedi* to both Sanzibanili and clean air ($p>0.05$).

The mean time taken for thrips to respond to either Ife Brown (susceptible cultivar) or clean air was significantly different from each other ($p < 0.05$), only at the junction of the Y-tube olfactometer. *Megalurothrips sjostedi*s pent 9.51 seconds at the junction before moving in the direction of Ife Brown, while it spent 33.77 seconds before moving in the direction of clean air. The differences in the response time of thrips to the other cowpea cultivars (Sewe, Moussa Local, Vita-7, TVu1509, IT90K-277-2) relative to clean air (blank) was not significant ($p > 0.05$) at the stem, junction, and arm of the Y-tube olfactometer. There was also no specific trend observed in the time of response.

4.3: Comparison of olfactory response of *Megalurothrips sjostedti* females to cowpea headspace volatiles from Ife Brown and other resistant cultivars of flowering cowpea

Cowpea cultivar IT90K-277-2 had the highest thrips attraction of 63.7% of thrips (Figure 4.2.). Moussa Local had the lowest percentage of thrips (36.7%) attracted to it. TVu1509 had 50% of thrips relative to Ife Brown. There was no definite trend observed in the percentage number of thrips response to the cowpea cultivars.

Table 4.2: Time taken for *Megalurothrips sjostedti* to move through the stem, junction and arm of the Y-tube olfactometer in response to cowpea volatiles or clean air (Blank)

Test Area	Choices (cultivar/clean air)	Time (seconds)	Std.Dev.	t-value	p-value	Significance
Stem	Sanzibanili	26.24	20.14	2.769	0.009	$p < 0.05$
	Clean Air	7.45	2.52			
Junction	Sanzibanili	6.18	9.04	1.204	0.236	$p > 0.05$
	Clean Air	2.50	1.80			
Arm	Sanzibanili	10.31	10.64	1.790	0.082	$p > 0.05$
	Clean Air	3.81	3.38			
Stem	Moussa-Local	27.48	30.02	1.232	0.223	$p > 0.05$
	Clean Air	16.88	13.98			
Junction	Moussa-Local	4.46	4.87	0.185	0.884	$p > 0.05$
	Clean Air	4.15	7.23			
Arm	Moussa-Local	9.66	8.05	1.762	0.083	$p > 0.05$
	Clean Air	5.64	2.98			
Stem	Vita7	26.81	20.61	1.944	0.057	$p > 0.05$
	Clean Air	14.46	8.07			
Junction	Vita7	10.37	15.16	1.485	0.143	$p > 0.05$
	Clean Air	19.95	32.64			
Arm	Vita7	8.05	11.08	1.297	0.369	$p > 0.05$
	Clean Air	11.19	6.08			
Stem	TVu1509	14.02	15.67	1.171	0.246	$p > 0.05$
	Clean Air	20.75	23.28			
Junction	TVu1509	6.72	9.78	0.993	0.325	$p > 0.05$
	Clean Air	3.76	1.81			
Arm	TVu1509	7.87	11.42	0.447	0.657	$p > 0.05$
	Clean Air	6.27	6.47			

Stem	Sewe	31.81	28.66	1.441	0.155	$p > 0.05$
	Clean Air	47.24	51.46			
Junction	Sewe	10.24	15.68	1.124	0.266	$p > 0.05$
	Clean Air	15.64	15.94			
Arm	Sewe	5.53	6.81	0.571	0.571	$p > 0.05$
	Clean Air	4.46	2.87			
Stem	IT90K-277-2	27.28	20.30	1.136	0.261	$p > 0.05$
	Clean Air	19.07	16.97			
Junction	IT90K-277-2	7.63	10.64	0.303	0.763	$p > 0.05$
	Clean Air	6.52	4.95			
Arm	IT90K-277-2	5.78	5.07	1.414	0.163	$p > 0.05$
	Clean Air	3.36	1.25			
Stem	Ife Brown	33.48	30.36	1.821	0.074	$p > 0.05$
	Clear Air	14.52	15.73			
Junction	Ife Brown	9.51	16.71	2.995	0.004	$p < 0.05$
	Clear Air	33.77	43.52			
Arm	Ife Brown	9.08	13.68	0.418	0.678	$p > 0.05$
	Clear Air	11.16	14.31			

Std.Dev: Standard Deviation
t-value: Calculated T value
p- value: Probalility value

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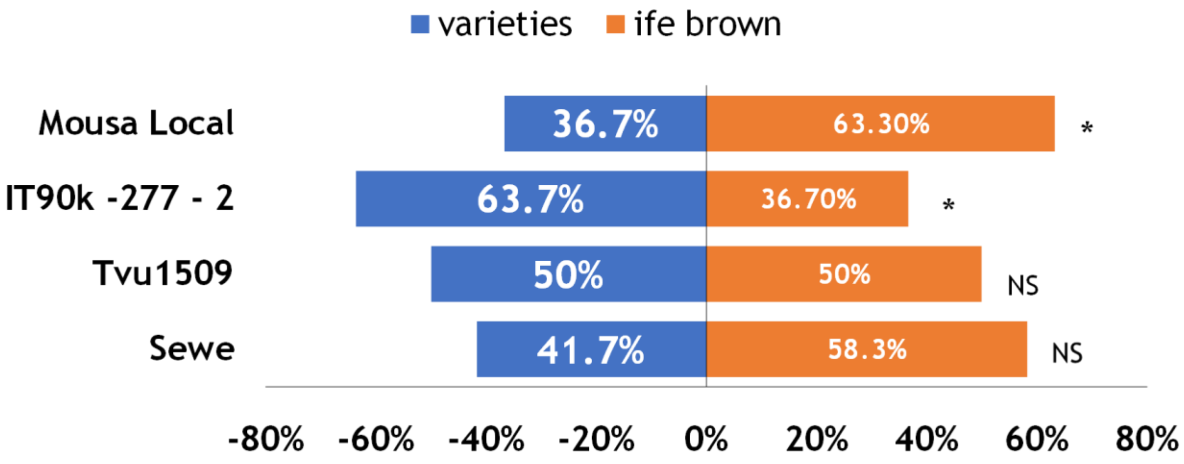


*** = Significant at $p < 0.05$**

Figure 4.2: Response of female *Megalurothrips sjostedti* to headspace volatiles from Ife Brown and four resistant cultivars of cowpea

The differences in the percentage number of thrips attracted to five cowpea cultivars as presented on Table 4.3, showed that the response of thrips to Moussa Local relative to Ife Brown was significant (Moussa Local: $\chi2 = 4.27$, $p< 0.05$). Also, the response of thrips to IT90K-277-2relative toIfe Brown was significant (IT90k-277-2: $\chi2 = 4.27$, $p<0.05$). A

4.4 Time taken for *Megalurothrips sjostedti* to respond to headspace volatile from four resistant cultivars relative to Ife Brown

The response time of *Megalurothrips sjostedti* through the stem, junction, and arm of the Y- tube olfactometer was compared between each of the four resistant cultivars (Sewe, Moussa Local, TVu1509, and Sanzibanili) relative to Ife Brown. The results presented in Table 4.4 showed that there was no significant difference ($p> 0.05$) in the response time of thrips at the stem, junction, and arm to all the cowpea cultivars. There was no definite trend observed in the time of response of thrips to all the cultivars.

4.5 Volatiles organic compounds identified from flowering cowpea cultivars grown at Wageningen

Sixty-eight volatile organic compounds were identified (Table 4.5), 1-Propanol (compound 1) had the least retention time of 3.61 minutes while 4-Acetyl-alpha-cedrene (compound 68) had the highest retention time of 33.32 minutes. Each volatile organic compound belonged to one of seventeen classes; the class Alcohol had 7 volatile organic compounds (1, 2, 6, 15, 25, 31 and 34), oxime, 6 (4, 7, 16, 18, 19 and 23), cyclic ketone, 1 (3), nitrile, 4 (5, 12, 13 and 14), ester, 10 (10, 26, 35, 36, 37, 38, 51, 63, 64 and 67), aldehyde, 6 (17, 21, 22, 24, 27 and 30), isothiocyanate, 2 (9 and 11) furan, 1 (8), hydrocarbon, 1 (20), pyrazine, 1 (28), monoterpene, 8 (29, 32, 33, 39, 40, 41, 43 and 50), sesquiterpene, 9 (44, 46, 54, 55, 58, 61, 62, 66 and 68) monoterpenoid, 4 (42, 45, 47 and 49), alkatetraenes, 1(48), indole, 1 (52) ketone, 4 (53, 56, 57 and 59), benzofurans, 1 (60), sesquiterpene alcohol, 1(65). The esters were the most occurring compound. The relative amount of each of the compounds with each cowpea type varied widely from as low as 1% to 16.49 (Table 4.6)

Table 4.3: Chi-square statistics, probability value and value of significance of observed differences between the odour choices of Ife Brown and resistant cowpea types by *Megalurothrips sjostedti* in the olfactometer test

Choices	Chi Test Stat	Prob. Value	Significance
Ife Brown vs Sewe	1.67	0.20	$p > 0.05$
Ife Brown vs TVu1509	0.00	1.00	$p> 0.05$
Ife Brown vs IT90K-277-2	4.27	0.04	$p < 0.05$
Ife Brown vs Moussa Local	4.27	0.04	$p < 0.05$

Table 4.4: Time taken for *Megalurothrips sjostedti* to move through the Y-tube olfactometer in response to four resistant cowpea cultivars relative to Ife Brown

Y- Tube	Choices (resistant cultivar/Ife Brown)	Time (seconds)	Std.Dev.	t-value	p-value	Significance
Stem	Ife Brown	14.20	13.79	1.356	0.180	p> 0.05
	Sewe	9.77	10.32			
Junction	Ife Brown	8.66	12.80	1.465	0.151	p> 0.05
	Sewe	4.81	3.67			
Arm	Ife Brown	3.70	3.20	1.918	0.060	p> 0.05
	Sewe	2.38	1.48			
Stem	TVu1509	11.98	12.41	0.154	0.878	p> 0.05
	Ife Brown	11.45	14.60			
Junction	TVu1509	10.22	16.06	1.383	0.173	p> 0.05
	Ife Brown	5.58	8.96			
Arm	TVu1509	5.47	9.017	0.929	0.357	p> 0.05
	Ife Brown	3.62	6.16			
Stem	IT90k-277-2	14.19	14.93	1.911	0.061	p> 0.05
	Ife Brown	7.96	3.96			
Junction	IT90k-277-2	12.43	26.63	1.006	0.318	p> 0.05
	Ife Brown	6.47	10.07			
Arm	IT90k-277-2	3.16	3.64	0.188	0.855	p> 0.05
	Ife Brown	2.97	3.89			

Stem	Moussa local	12.97	13.63	0.742	0.462	p> 0.05
	Ife Brown	21.69	50.05			
Junction	Moussa local	4.36	5.08	1.515	0.136	p> 0.05
	Ife Brown	10.34	16.73			
Arm	Moussa local	2.50	3.99	0.808	0.423	p> 0.05
	Ife Brown	3.81	6.45			
Stem	Sanzibanili	8.40	7.34	0.202	0.843	p> 0.05
	Ife Brown	9.04	5.77			
Junction	Sanzibanili	10.18	18.60	1.043	0.314	p> 0.05
	Ife Brown	25.18	34.52			
Arm	Sanzibanili	1.51	.58	1.450	0.168	p> 0.05
	Ife Brown	4.68	5.70			

Std.Dev: Standard Deviation
t-value: Calculated T value
p- value: Probalility value

Table 4.5: Volatile organic compounds identified from headspace volatile of eight flowering cowpea cultivars at Wageningen, The Netherlands

Compound number	Compound name	Retention time (min)	Class of Compound
1	1-Propanol	3.61	Alcohol
2	2-Butanol	4.24	Alcohol
3	2-Methylfuran	4.46	Cyclic ketone
4	2-Propanone, O-methyloxime	4.55	Oxime
5	2-Methylpropanenitrile	4.68	Nitrile
6	1-Penten-3-ol	5.92	Alcohol
7	2-Methylpropanal, O-methyloxime	5.94	Oxime
8	2-Ethylfuran	6.32	Furan
9	Isopropyl isothiocyanate	6.51	Isothiocyanate
10	Propyl acetate	6.63	Ester
11	Propyl isothiocyanate	6.65	Isothiocyanate
12	2-Methyl-3-butenenitrile	6.83	Nitrile
13	2-Methylbutanenitrile	6.93	Nitrile
14	3-Methylbutanenitrile	7.14	Nitrile
15	3-Methyl-1-butanol	7.22	Alcohol
16	2-Methylpropanal oxime	7.58	Oxime
17	(E)-2-Pentenal	7.79	Aldehyde
18	2-Methylbutanal, O-methyloxime-	8.52	Oxime
19	3-Methylbutanal, O-methyloxime	8.61	Oxime
20	2-Methyl-1-nitropropane	8.90	Hydrocarbon
21	(Z)-3-Hexenal	9.00	Aldehyde

22	(Z)-2-Hexenal	10.41	Aldehyde
23	(1Z)-2-Methylbutanal oxime	10.51	Oxime
24	(E)-2-Hexenal	10.65	Aldehyde
25	(Z)-3-Hexen-1-ol	10.69	Alcohol
26	(Z)-2-Penten-1-ol, acetate	12.27	Ester
27	(E,E)-2,4-Hexadienal	12.36	Aldehyde
28	2,6-Dimethylpyrazine	12.45	Pyrazine
29	alpha-Thujene	12.95	Monoterpene
30	(E)-4-Oxo-2-hexenal	13.76	Aldehyde
31	1-Octen-3-ol	14.35	Alcohol
32	Sabinen	14.39	Monoterpene
33	beta-Myrcene	14.69	Monoterpene
34	3-Octanol	14.86	Alcohol
35	(Z)-2-Hexen-1-ol, acetate	14.96	Ester
36	(Z)-3-Hexen-1-ol, acetate	15.08	Ester
37	Hexyl acetate	15.25	Ester
38	(E)-2-Hexen-1-ol, acetate	15.30	Ester
39	alpha-Phellandrene	15.35	Monoterpene
40	alpha-Terpinene	15.66	Monoterpene
41	beta-Phellandrene	16.12	Monoterpene
42	trans-beta-Ocimene	16.32	Monoterpenoid
43	gamma-Terpinene	16.79	Monoterpene

44	(Z)-DMNT	17.63	Sesquiterpene
45	Linalool	17.81	Monoterpenoid
46	(E)-DMNT	18.16	Sesquiterpene
47	Allo-ocimene	18.58	Monoterpenoids
48	(E,E)-Cosmene	18.71	Alkatetraenes
49	Neo-allo-ocimene	18.95	Monoterpenoid
50	4-Terpineol	20.23	Monoterpene
51	Methyl salicylate	20.52	Ester
52	Indole	23.04	Indole
53	(E)-alpha-Ionone	26.00	Ketone
54	(E)-beta-Caryophyllene	26.22	Sesquiterpene
55	alpha-Caryophyllene	27.03	9Sesquiterpene
56	alpha-Isomethyl ionone	27.17	Ketone
57	(E)-beta-Ionone	27.25	Ketone
58	gamma-Muurolene	27.84	Sesquiterpene
59	alpha-Methyl ionone	28.02	Ketone
60	Dihydroactinidiolide	28.66	Benzofurans
61	(E)-Nerolidol	28.84	Sesquiterpene
62	(E,E)-TMTT	29.06	Sesquiterpene
63	(Z)-3-Hexen-1-ol, benzoate	29.23	Ester
64	Ethyl dodecanoate	29.34	Ester
65	Cedrol	30.72	Sesquiterpene alcohol
66	Isocurcumenol	31.26	Sesquiterpene
67	Methyl tetradecanoate	31.93	Ester
68	4-Acetyl-alpha-cedrene	33.32	Sesquiterpene

Table 4.6a: Relative amount of headspace volatile organic compounds collected from eight cowpea types in their reproductive stages at Wageningen University, Netherlands, expressed as percentage peak area by weight of each type

	Relative Amount of VOC (%)																
Cowpea Varieties	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
IT	1.81	0.20	0.44	0.09	1.26	2.04	6.80	0.33	0.06	0.98	0.11	0.31	4.24	0.21	0.10	0.62	0.26
IB	1.84	0.26	0.73	0.28	1.59	3.47	5.53	0.50	0.14	0.68	0.26	0.55	7.26	0.44	0.14	1.00	0.40
VT	1.87	0.17	0.58	0.11	2.63	2.18	2.94	0.40	0.08	0.69	0.16	2.65	11.69	0.38	0.11	2.22	0.33
SW	3.90	0.17	0.77	1.73	0.52	4.74	1.98	0.80	0.65	1.57	1.23	0.01	0.86	0.08	0.65	0.09	0.67
SZ	4.45	0.28	1.29	0.34	0.44	6.05	2.21	1.01	0.18	1.46	0.33	0.07	1.53	0.22	0.78	0.24	0.86
ML	4.19	0.14	0.87	1.72	0.29	4.62	2.83	1.30	0.74	2.92	1.52	0.03	0.99	0.04	0.49	0.08	1.14
TV	0.28	0.31	0.69	0.45	1.19	7.81	9.35	0.92	0.26	0.22	0.50	0.33	4.86	0.31	0.32	0.59	0.66
KV	0.41	0.09	0.58	0.09	0.89	6.19	3.57	0.87	0.07	0.58	0.12	0.06	1.54	0.11	0.24	0.75	0.71

IT90K=IT90k-277-2; IB= Ife Brown; VT=Vita-7; SW= Sewe; SZ= Sanzibanili; ML= Moussa Local; TV= TVu1509; Kv=Kvx404-8-1

***Numbers on the header row refers to number identity of compounds in Table 4.5**

Table 4.6b: Relative amount of headspace volatile organic compound collected from eight cowpea type in their reproductive stages at Wageningen University, Netherlands, expressed as percentage peak area by weight of each type

	Percentage Amount of VOC (%)																
Cowpea Varieties	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**
IT90K	11.43	0.28	1.38	0.60	0.05	0.13	0.28	4.60	0.08	0.06	0.03	1.46	0.68	1.62	0.35	1.21	0.49
IB	8.76	0.28	1.12	0.64	0.05	0.18	0.28	8.36	0.15	0.09	0.07	2.31	1.10	1.12	0.58	2.79	0.37
VT	10.50	0.11	2.33	0.32	0.04	0.91	0.27	7.41	0.06	0.07	0.05	0.62	1.11	1.49	0.13	1.56	0.43
SW	1.26	0.04	0.15	1.17	0.10	0.01	0.53	14.18	0.07	0.15	0.77	0.15	2.13	2.09	0.05	5.75	0.72
SZ	2.85	0.04	0.18	0.93	0.10	0.04	0.80	10.42	0.12	0.18	0.15	0.05	2.71	2.44	0.03	5.52	0.46
ML	3.05	0.06	0.15	1.12	0.15	0.01	0.99	15.54	0.13	0.22	0.19	0.20	3.77	2.71	0.05	0.18	0.35
TV	11.75	0.46	1.07	2.37	0.24	0.10	1.35	9.82	0.24	0.18	0.07	0.05	2.57	1.02	0.02	1.40	0.62
KV	5.09	0.11	0.97	6.36	0.41	0.11	1.12	13.37	0.47	0.25	0.06	1.10	3.09	2.05	0.28	0.69	0.36

IT90K=IT90k-277-2; IB= Ife Brown; VT=Vita-7; SW= Sewe; SZ= Sanzibanili; ML= Moussa Local; TV= TVu1509; Kv=Kvx404-8-1

***Numbers on the header row refers to number identity of compounds in Table 4.5**

Table 4.6c: Relative amount of headspace volatile organic compound collected from eight cowpea types in their reproductive stages at Wageningen University, Netherlands, expressed as percentage peak area by weight of each type

	Percentage Amount of VOC (%)																
Cowpea Varieties	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**43**	**44**	**45**
IT90K	0.46	5.85	0.52	0.24	0.47	0.74	0.54	11.76	0.72	0.17	0.42	8.54	0.47	0.23	0.72	0.17	0.42
IB	0.78	10.01	0.82	0.06	0.97	1.31	1.05	2.02	1.41	0.11	2.33	6.02	0.59	0.07	1.41	0.11	2.33
VT	0.39	6.10	0.48	0.05	0.25	0.38	0.31	8.87	0.39	0.07	1.64	4.73	0.77	0.34	0.39	0.07	1.64
SW	0.91	11.47	1.54	0.07	0.08	0.08	0.11	2.92	0.08	0.01	0.70	1.21	1.20	0.11	0.08	0.01	0.70
SZ	0.82	9.28	0.91	0.12	0.04	0.03	0.15	2.28	0.03	0.02	0.53	1.72	0.81	0.09	0.03	0.02	0.53
ML	1.15	8.90	0.98	0.11	0.11	0.09	0.10	1.23	0.09	0.02	0.39	1.89	0.04	0.02	0.09	0.02	0.39
TV	1.45	9.62	1.73	0.74	0.03	0.04	0.05	0.86	0.05	0.08	0.53	4.91	0.33	0.04	0.05	0.08	0.53
KV	1.11	17.14	2.00	0.64	0.38	0.63	0.43	3.17	0.62	0.07	0.67	3.10	0.16	0.05	0.62	0.07	0.67

IT90K=IT90k-277-2; IB= Ife Brown; VT=Vita-7; SW= Sewe; SZ= Sanzibanili; ML= Moussa Local; TV= TVu1509; Kv=Kvx404-8-1

***Numbers on the header row refers to number identity of compounds in Table 4.5**

Table 4.6d: Relative amount of headspace volatile organic compound collected from eight cowpea type in their reproductive stages at Wageningen University, Netherlands, expressed as percentage peak area by weight of each type

	Percentage Amount of VOC (%)																
Cowpea Varieties	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**
IT90K	8.54	0.47	0.23	0.07	0.07	13.46	2.81	0.41	0.66	0.03	1.52	0.71	0.03	0.10	0.30	0.43	0.19
IB	6.02	0.59	0.07	0.05	0.18	6.13	3.25	0.71	0.30	0.04	2.64	1.17	0.06	0.15	0.31	0.13	0.18
VT	4.73	0.77	0.34	0.07	0.06	5.31	5.70	0.24	0.73	0.03	0.86	0.57	0.11	0.07	0.20	0.14	0.18
SW	1.21	1.20	0.11	0.09	0.00	7.41	12.97	0.58	0.21	0.01	2.33	1.14	0.01	0.15	0.33	0.16	0.11
SZ	1.72	0.81	0.09	0.05	0.00	2.79	16.49	0.88	1.84	0.55	3.44	1.94	0.05	0.25	0.56	0.31	0.12
ML	1.89	0.04	0.02	0.00	0.00	1.26	6.43	1.93	2.64	1.17	7.36	3.23	0.05	0.45	0.62	0.13	0.02
TV	4.91	0.33	0.04	0.02	0.00	5.38	4.21	0.48	0.91	0.04	1.64	0.80	0.03	0.10	0.28	0.23	0.45
KV	3.10	0.16	0.05	0.01	0.09	4.02	4.18	0.41	1.54	0.19	1.53	0.77	0.06	0.09	0.32	0.38	0.07

IT90K=IT90k-277-2; IB= Ife Brown; VT=Vita-7; SW= Sewe; SZ= Sanzibanili; ML= Moussa Local; TV= TVu1509; Kv=Kvx404-8-1

***Numbers on the header row refers to number identity of compounds in Table 4.5**

Table 4.6d: Relative amount of headspace volatile organic compound collected from eight cowpea varieties in their reproductive stages at Wageningen University, Netherlands, expressed as percentage peak area by weight of each type

	Percentage Amount of VOC (%)					
Cowpea Varieties	**63**	**64**	**65**	**66**	**67**	**68**
IT90K	1.86	0.71	0.12	0.22	0.57	0.05
IB	1.36	1.08	0.19	0.32	0.81	0.07
VT	3.25	0.47	0.09	0.19	0.35	0.04
SW	2.16	0.85	0.19	0.34	0.65	0.07
SZ	1.29	1.61	0.26	0.56	1.29	0.13
ML	1.48	2.25	0.66	0.86	1.40	0.16
TV	1.28	0.55	0.09	0.17	0.45	0.04
KV	1.82	0.69	0.08	0.17	0.61	0.06

IT90K=IT90k-277-2; IB= Ife Brown; VT=Vita-7; SW= Sewe; SZ= Sanzibanili; ML= Moussa Local; TV= TVu1509; Kv=Kvx404-8-1

***Numbers on the header row refers to the number identity of compounds in Table 4.5.**

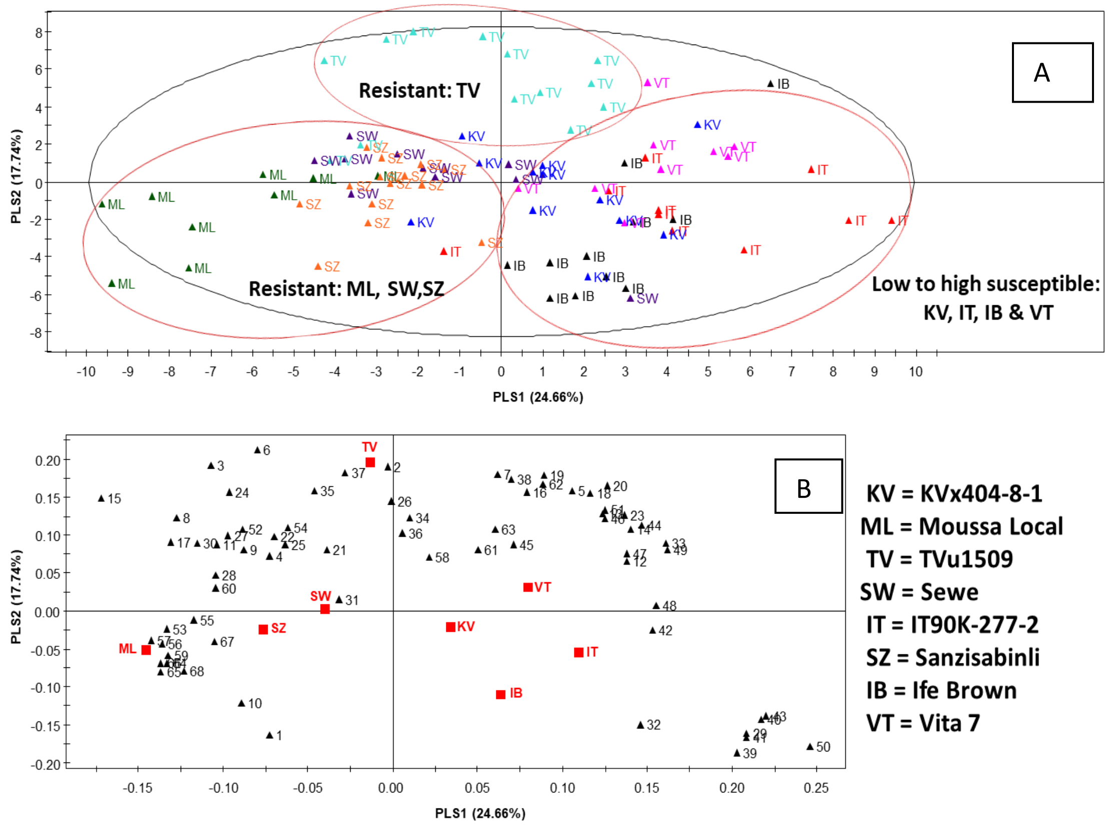
4.6 Projection to Latent Structures Discriminant Analysis (PLS-DA) of volatile organic compounds identified from flowering cowpea cultivars at Wageningen, The Netherlands

All the cowpea types formed three independent clusters i (Moussa Local, Sanzibanili, Sewe), ii (TVu1509), and iii (KVx-404-8-1, IT90K-277-2, Ife Brown, Vita-7) (Figure 4.3a). i and ii consist of resistant cultivars, while iii consists of cultivars with low to high susceptibility. The characteristics of the susceptible cultivars lay between the clusters of resistant cultivars, and they all remained within the big eclipse, indicating 95% similarity. PLS1 contributed 24.66% to the variability along the horizontal axis, while PLS2 contributed 17.74% to the variability along the vertical axis.

Figure 4.3b shows the loading plot of compounds, showing the association of the Volatile Organic Compounds with the different cultivars and between the VOCs themselves. The distance between the cowpea cultivars showed how closely related they are, while the numbers that are closest to the cowpea cultivars represent the specific VOCs that are closely associated with the cultivars. The numbers correspond to compound numbers presented on Table 4.5.

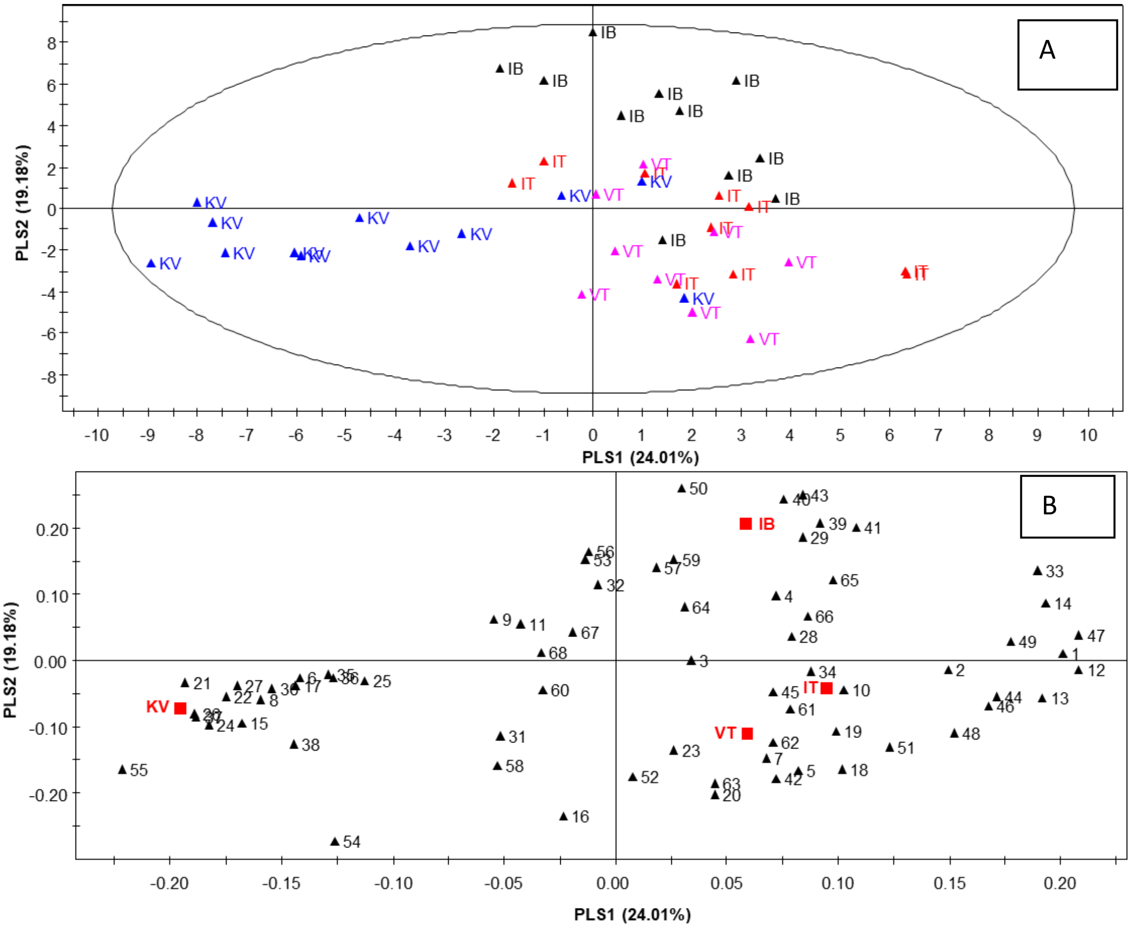
4.7 Projection to Latent Structures Discriminant Analysis (PLS-DA) of volatile organic compounds identified from flowering cowpea cultivars with different degrees of resistance and different degrees of susceptibility.

The separation pattern of resistant cowpea cultivars is shown in the score plot (Figure 4.4a). The cultivars Moussa Local, Sanzibanili, Sewe and TVu1509 were all within the 95% similarity eclipse. TVu1509, however, skewed more to the negative side of the graph. PLS1 contributed 24.36 % to variability along the horizontal axis, while PLS2 accounted for 22.34 % variability on the vertical axis. The loading plot (Figure 4.4b) showed that the relationship between cowpea cultivar TVu1509, and the other three resistant cultivars is wider apart from the other three resistant cultivars.



A = Score plot
B = Loading plot

Figure 4.3: Partial least square discriminant analysis of eight flowering cowpea types of varying level of resistance and susceptibility to *Megalurothrips sjostedti*, using the relative amount of volatile organic compound present in the cowpea types



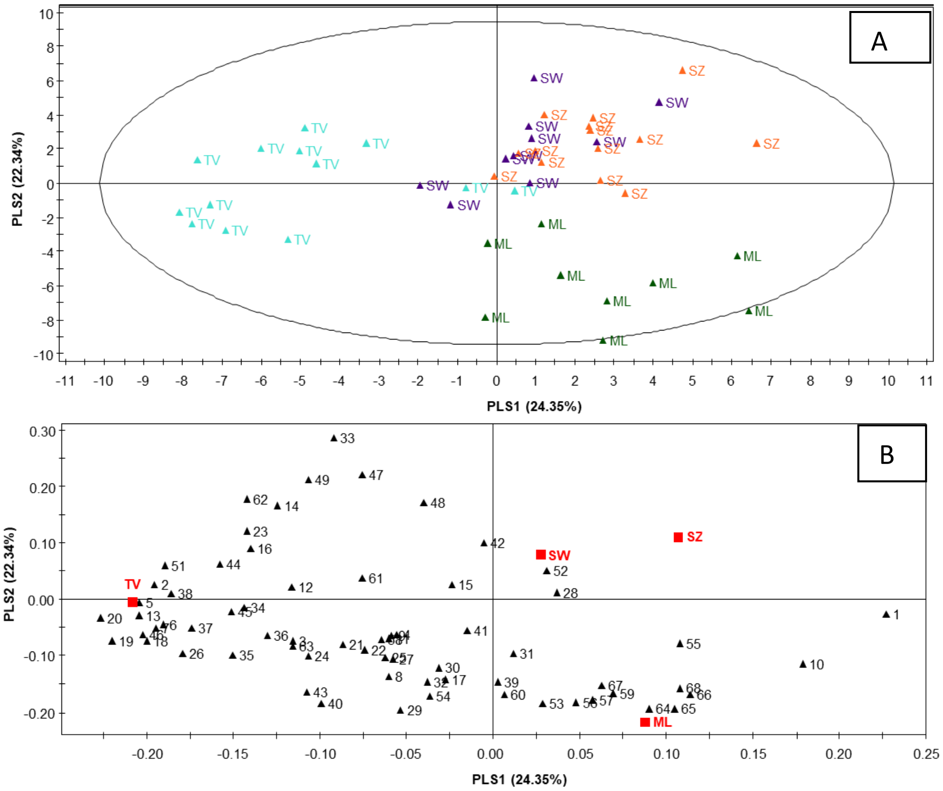
KV = KVx404-8-1, IT = IT90K-277-2 IB = Ife BrownVT = Vita-7

A = Score plot
B = Loading plot

Figure 4.4: Partial least square discriminant analysis of four full flowering cowpea types with varying levels of susceptibility to *Megalurothrips sjostedti* using the relative amount of volatile organic compound present in the cowpea types

The most important compounds that resolved TVu1509 as indicated by the compound numbers around the cultivars are 2- methylpropanenitrile, 2, Butanol, Methyl salicylate 2-methylbutanenitrile, 2-Methyl-1-nitropropane, (E)-2-Hexen-1-ol, acetate (compounds 5, 2, 51, 13, 20, and 38). Sewe is resolved by 2, 6 dimethylpyrazine and Indole (compounds 28 and 52). Moussa Local is resolved by Ethyl dodecanoate, Cedrol, Isocurcumenol and 4-Acetyl-alpha-cedrene (compounds 64, 65, 66, 68).

Cultivars KVx-404-8-1, IT90K-277-2, Ife Brown, and Vita-7 all fell with the (95%) similarity eclipse, with PLS1 responsible for 24.01% of variability along the horizontal axis and PLS2 responsible for 19.18% of variability along the vertical axis (Figure 4.5a). The loading plot in Figure 4.5b shows that the distance between cultivar KVx-404-8-1 and the other three cultivars is wider, indicating a higher level of variability between them. IT90K-277-2, Ife Brown and Vita-7 are more closely related as indicated by their distance apart. KVx-404-8-1 was resolved by (Z)-3-Hexenal (compound 21), (E)-2-Hexenal (24) and Sabinen (32) among others, Vita-7 was resolved by (E, E)- TMTT (62) and 2-Methylpropanal, O- methyloxime (7). IT90K-277-2 was resolved by Propyl acetate (10), 3 - Octanol (34), Linalool (45), (E) - Nerolidol (61) and Ife Brown was resolved by Alpha thujene (29) and Alpha terpinene (40).



ML = Moussa Local, TV = TVu1509, SW = Sewe, SZ = Sanzisabinli

A = Score plot
B = Loading plot

Figure 4.5: Partial least square discriminant Analysis of four full flowering cowpea types with varying levels of resistance to *Megalurothrips sjostedti* using the relative amount of volatile organic compound present in the cowpea types

4.8 Volatile organic compounds identified from excised cowpea flowers.

A total of forty- five volatile organic compounds were identified from the flowers of eight cowpea materials (Table 4.7) in the order of increasing retention time. The VOCs belong to fourteen different classes: ketones, alcohols, oxime, nitrile, alkadienes, aldehydes, monoterpenes, ester, monoterpenoids, sesquiterpenoids, alkatetraenes, indole and sesquiterpenoids. The VOCs, 3-pentanone, 3 -pentanol, 1,3-octadiene, (E)-2-hexen-1-ol, 3-octanone, beta-myrcene, 1,8-cineole, 1-octen-3-ol, acetate were unique to the flowers of the cowpea plant alone but were not present in the headspace volatile collected from full flowering plants. The compounds identified from excised flowers of the susceptible cowpea materials (Tables 4.8) were also forty – five, belonging to fourteen different classes of compounds.

4.9 Projection to Latent Structures Discriminant Analysis (PLS-DA) of volatile organic compounds identified from excised flowers of cowpea materials grown at Wageningen, The Netherlands

The relationship observed between the resistant, moderately resistant, and susceptible (2) cowpea materials based on the type and quantity of organic compound identified from excised cowpea flowers is shown in the score plot presented in Figure 4.6a. All the cowpea types fell within distinct clusters without overlapping, and the features of VOCs from the moderately resistant plants fell in between those of the resistant and susceptible cowpea materials. PLS1 contributed 22.56 % variability on the horizontal axis, while PLS2 contributed 15.48 % variability on the vertical axis. All three clusters fell within the big eclipse that indicates 95% similarity.

Table 4.7: Volatile organic compounds identified from excised flowers of eight cowpea types

Compound number	Compound name	Retention time (min)	Classof compound
1	2-Methylfuran	4.46	Cyclic ketone
2	1-Penten-3-ol	5.92	Alcohol
3	2-Methylpropanal, O-methyloxime-	5.94	Oxime
4	3-Pentanone	6.22	Ketone
5	3-Pentanol	6.34	Secondary Alcohol
6	2-Methyl-3-butenenitrile	6.83	Nitrile
7	2-Methylbutanenitrile	6.93	Nitrile
8	3-Methylbutanenitrile	7.15	Nitrile
9	2-Methylpropanal oxime	7.58	Oxime
10	2-Methylbutanal, O-methyloxime-	8.52	Oxime
11	3-Methylbutanal, O-methyloxime-	8.61	Oxime
12	1,3-Octadiene	9.91	Alkadienes
13	(1Z)-2-Methylbutanal oxime	10.51	Oxime
14	(E)-3-Hexen-1-ol	10.52	Fatty Alcohol
15	(E)-2-Hexenal	10.65	Aldehyde
16	(Z)-3-Hexen-1-ol	10.68	Alcohol
17	(E)-2-Hexen-1-ol	10.94	Fatty Alcohol
18	alpha-Thujene	12.95	Monoterpene
19	(E)-4-Oxo-2-hexenal	13.76	Aldehyde
20	1-Octen-3-ol	14.37	Alcohol
21	3-Octanone	14.55	Ketone
22	beta-Myrcene	14.69	Monotepenes
23	3-Octanol	14.86	Alcohol
24	(Z)-3-Hexen-1-ol, acetate	15.07	Ester
25	Hexyl acetate	15.23	Ester
26	beta-Phellandrene	16.12	Monoterpene

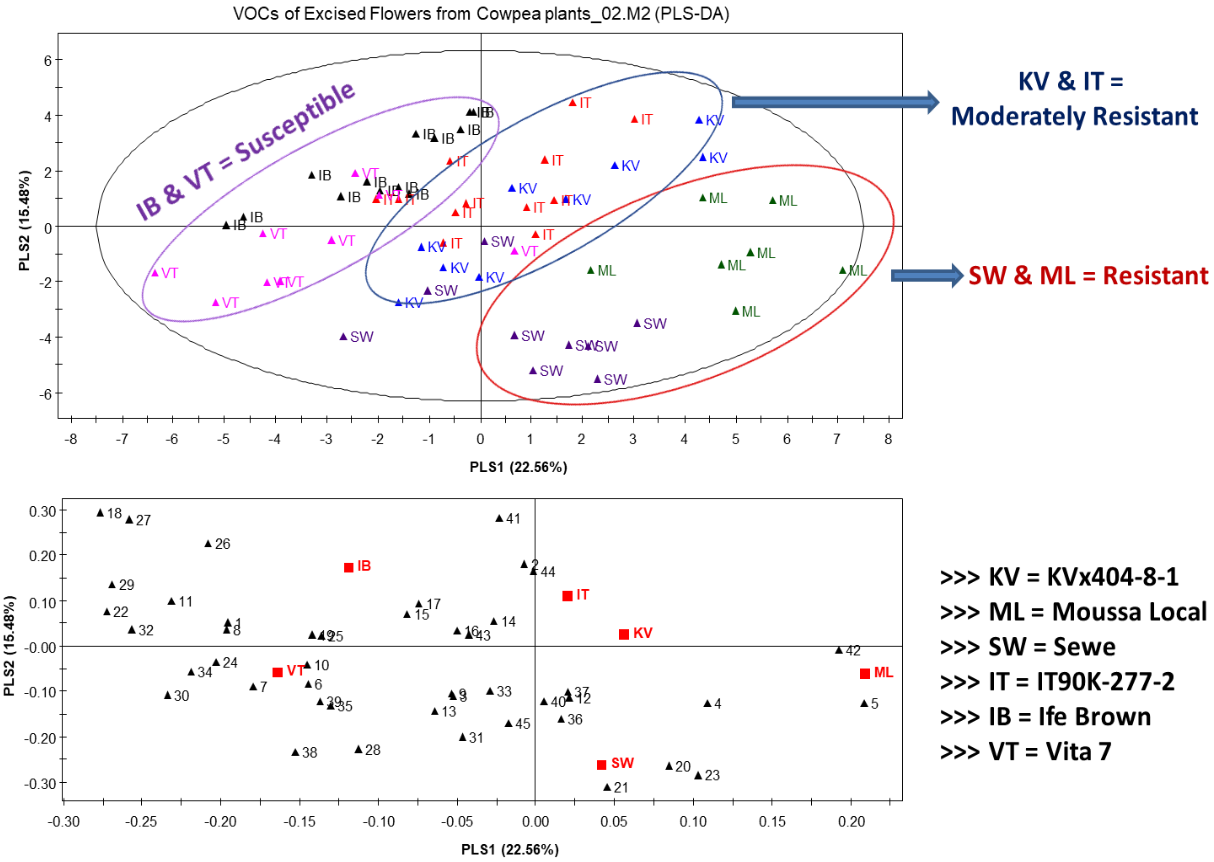
27	1,8-Cineole	16.17	Monoterpene
28	trans-beta-Ocimene	16.32	Monoterpenoid
29	(Z)-DMNT	17.63	Sesquiterpene
30	Linalool	17.81	Monoterpenoid
31	1-Octen-3-ol, acetate	17.88	Ester
32	(E)-DMNT	18.17	Sesquiterpene
33	3-Octanol, acetate	18.21	Ester
34	(E, Z)-Allo-ocimene	18.58	Monoterpenoids
35	(E, E)-Cosmene	18.71	Alkatetraenes
36	(Z)-3-Hexen-1-ol, butanoate	20.01	Ester
37	(Z)-3-Hexen-1-ol,3-methylbutanoate	21.19	Alcohol
38	Indole	23.04	Indole
39	Methyl anthranilate	24.22	Ester
40	(Z)-3-Hexenyl hexanoate	24.78	Ester
41	(E)-beta-Caryophyllene	26.25	Sesquiterpenes
42	alpha-Caryophyllene	27.03	Sesquiterpenes
43	(Z)-Nerolidol	27.85	Sesquiterpenoid
44	(E)-Nerolidol	28.84	Sesquiterpenoid
45	(Z)-3-Hexen-1-ol, benzoate	29.23	Ester

Table 4.8: Volatile organic compounds identified from excised flowers of susceptible cowpea types

Compound no	Compound name_RT (min)	Retention time (min)	Class of compound
1	2-Methylfuran	4.46	Cyclic ketone
2	1-Penten-3-ol	5.92	Alcohol
3	2-Methylpropanal, O-methyloxime-	5.96	Oxime
4	3-Pentanone	6.22	Ketone
5	3-Pentanol	6.34	Secondary Alcohol
6	2-Methyl-3-butenenitrile	6.83	Nitrile
7	2-Methylbutanenitrile	6.93	Nitrile
8	3-Methylbutanenitrile	7.15	Nitrile
9	2-Methylpropanal oxime	7.58	Oxime
10	2-Methylbutanal, O-methyloxime-	8.52	Oxime
11	3-Methylbutanal, O-methyloxime-	8.61	Oxime
12	1,3-Octadiene	9.91	Alkadienes
13	(1Z)-2-Methylbutanal oxime	10.51	Oxime
14	(E)-3-Hexen-1-ol	10.52	Fatty Alcohol
15	(E)-2-Hexenal	10.65	Aldehyde
16	(Z)-3-Hexen-1-ol	10.68	Alcohol
17	(E)-2-Hexen-1-ol	10.94	Fatty Alcohol
18	alpha-Thujene	12.95	Monoterpene
19	(E)-4-Oxo-2-hexenal	13.76	Aldehyde
20	1-Octen-3-ol	14.37	Alcohol
21	3-Octanone	14.55	Ketone
22	beta-Myrcene	14.69	Monotepenes
23	3-Octanol	14.86	Alcohol
24	(Z)-3-Hexen-1-ol, acetate	15.07	Ester
25	Hexyl acetate	15.23	Ester

26	beta-Phellandrene	16.12	Monoterpene
27	1,8-Cineole	16.17	Monoterpene
28	trans-beta-Ocimene	16.32	Monoterpenoid
29	(Z)-DMNT	17.63	Sesquiterpene
30	Linalool	17.81	Monoterpenoid
31	1-Octen-3-ol, acetate	17.88	Ester
32	(E)-DMNT	18.17	Sesquiterpene
33	3-Octanol, acetate	18.21	Ester
34	(E,Z)-Allo-ocimene	18.58	Monoterpenoids
35	(E,E)-Cosmene	18.71	Alkatetraenes
36	(Z)-3-Hexen-1-ol, butanoate	20.01	Ester
37	(Z)-3-Hexen-1-ol,3-methylbutanoate	21.19	Alcohol
38	Indole	23.04	Indole
39	Methyl anthranilate	24.22	Ester
40	(Z)-3-Hexenyl hexanoate	24.78	Ester
41	(E)-beta-Caryophyllene	26.25	Sesquiterpenes
42	alpha-Caryophyllene	27.03	Sesquiterpenes
43	(Z)-Nerolidol	27.85	Sesquiterpenoid
44	(E)-Nerolidol	28.84	Sesquiterpenoid
45	(Z)-3-Hexen-1-ol, benzoate	29.23	Ester

The loading plot presented in Figure 4.6b shows the separation pattern of the cowpea types, based on the type and quantity VOCs identified from them. It also shows the association of the various VOCs with the different cowpea type. The distance between IT90K-277-2 and KVx 404-8-1 is closest, which implies that those two cowpea types share more similarities than the other four (Moussa Local, Ife Brown, Sewe, Vita-7), that are farther apart. Also, the number closest to each of the cowpea types represents the compound number of VOCs as previously presented in Table 4.7., and it indicates the most important VOCs associated with each of the types. The most important VOCs that resolve Vita-7 are 2-Methylbutanal, O-methyloxime- (10), 2-Methyl-3-butenenitrile (6) and 2-Methylbutanenitrile (7). Moussa Local has two VOCs 3-Pentanol (5) and Z)-Nerolidol (42), Sewe is resolved by 1-Octen-3-ol (20), 3-Octanone (21), and 3-Octanol (23) and IT90K-277-2 is resolved by 1-Penten-3-ol (2) and (E)-Nerolidol (44). KVx404-8-1 did not have VOCs surrounding it.



KV & IT = Moderately Resistant

SW & ML = Resistant

>>> KV = KVx404-8-1
>>> ML = Moussa Local
>>> SW = Sewe
>>> IT = IT90K-277-2
>>> IB = Ife Brown
>>> VT = Vita 7

Figure 4.6: Partial least square discriminant analysis of volatile organic compounds from excised flowers of cowpea types with varying resistance and susceptibility status

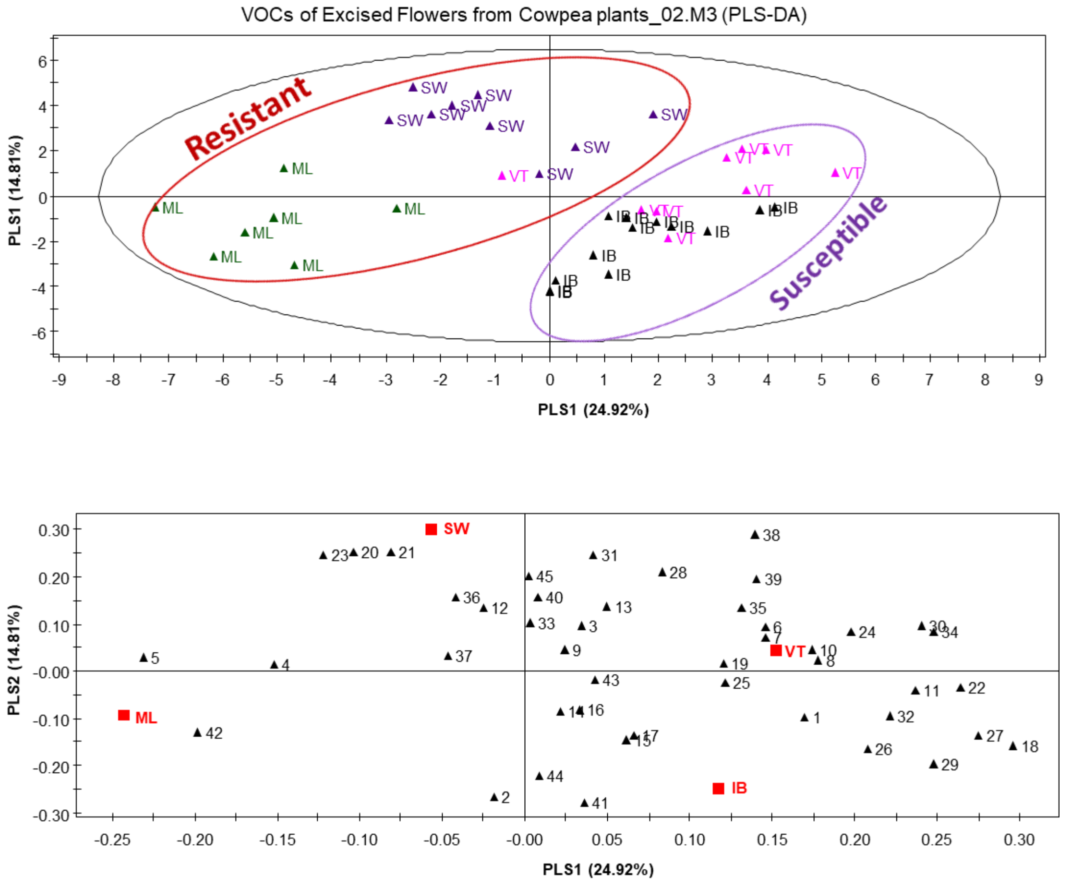


Figure 4.7: Partial least square discriminant analysis of volatile organic compounds from excised flowers of resistant and susceptible cowpea types

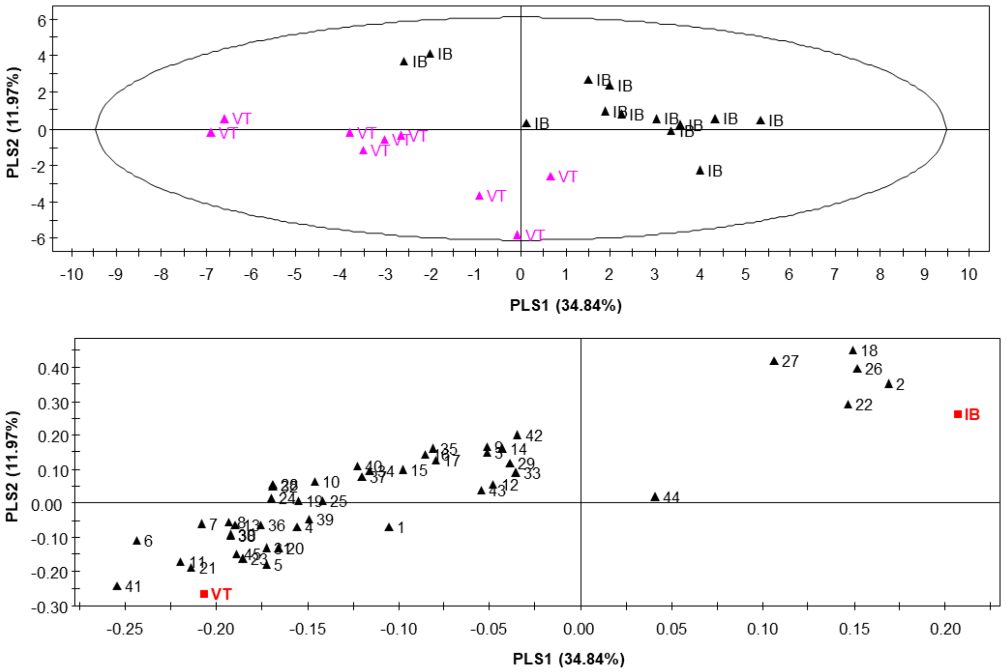


Figure 4.8: Partial least square discriminant analysis of volatile organic compounds from excised flowers of susceptible cowpea types

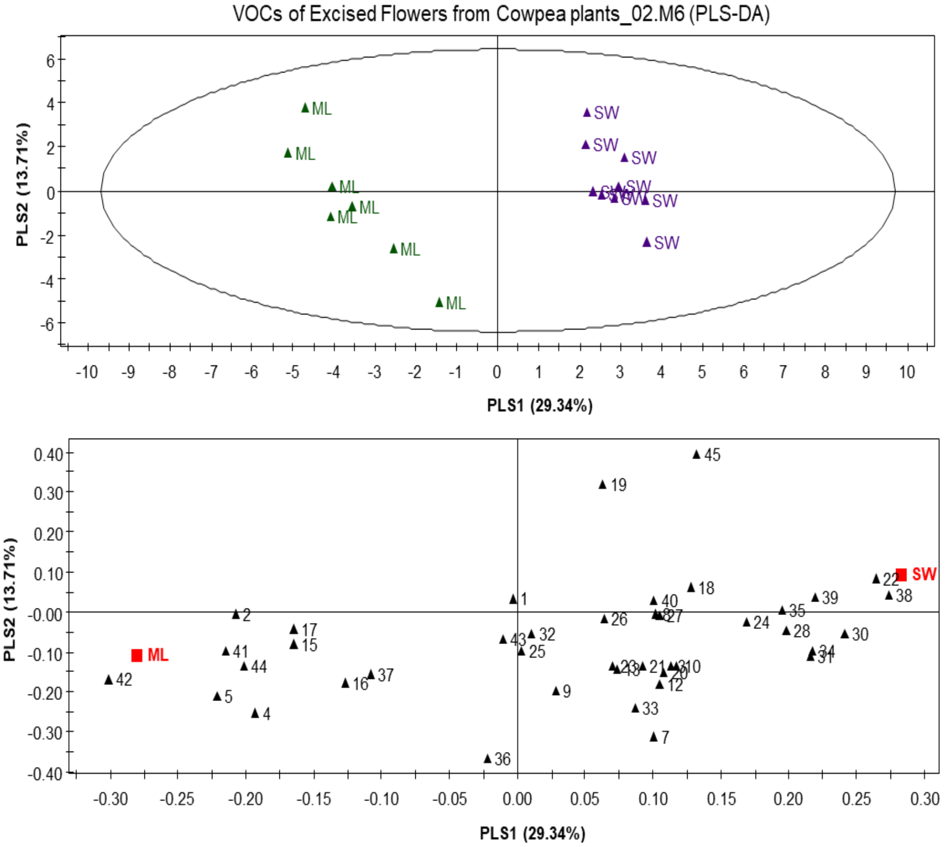


Figure 4.9: Partial least square discriminant analysis of volatile organic compounds from excised flowers of resistant cowpea types

4.10: **Volatiles organic compounds identified from flowering cowpea materials grown at Ibadan**

A total of twenty-nine volatile organic compounds were identified from seven cowpea materials of varying resistance and susceptibility status (Table 4.9). The compounds belonged to thirteen different classes of compounds; Alkene 1(2,4-Dimethyl-1-heptene), Benzenoid 3 (o-xylene, styrene, p-xylene), alkane 8 (decane, undecane, nonane, dodecane, tridecane, tetradecane, hexadecane, octadecane), monoterpene 4 (alpha-pinene, camphene, beta-pinene, limonene), alcohol 2 (1-octen-3-ol, phenol), indane 1 (indane), ketones 3 (acetophenone, o-ethyl acetophenone, p-ethyl acetophenone), aldehydes 1 (nonanal), ester 2(2-ethylhexyl ester, Methyl salicylate), Naphthalenes 1 (Naphthalene), monoterpenoid1 (4,8-Dimethyl-1,3 (E)7-nonatriene), phenols1 (butylated hydroxytoluene) , unsaturated hydrocarbon 1 (1 – tetradecene), phenols, esters, among others were identified from seven cowpea types with their varying retention time Figure 4.10 shows the abundance of the of the different volatile organic compounds found in all the seven cowpea types. It also shows that the VOCs were present in all the seven cowpea cultivars. The percentage of tetradecane was significant across all the cowpea types, same as hexadecane, although to a lesser extent. Methyl salicylate, camphene, styrene was among the VOCs occurring in minute quantities. Principal component analysis of identified VOCs showed clusters representing each variety at 14.4% and 44.4% vertical and horizontal variation. The clusters interacted closely as indicated by a web of several overlaps (Fig 4.11).

Table 4.9: Volatile organic compounds identified from seven full flowering cowpea types grown at Ibadan

No.	Compound	Retention time	Classof Compound
1	2,4-Dimethyl-1-heptene	7.3809	Alkene
2	o-Xylene	8.1005	Benzenoid
3	Styrene	8.6328	Benzenoid
4	p-Xylene	8.6738	Benzenoid
5	Nonane	8.8727	Alkane
6	alpha-Pinene	9.6098	Monoterpene
7	Camphene	9.932	Monoterpene
8	beta-Pinene	10.5282	Monoterpene
9	1-Octen-3-ol	10.629	Alcohol
10	Phenol	10.8032	Alcohol
11	Decane	11.0079	Alkane
12	Limonene	11.5812	Monoterpene
13	Indane	11.6748	Indane
14	Acetophenone	12.2598	Ketones
15	Undecane	12.8097	Alkane
16	Nonanal	12.9033	Aldehyde
17	4,8-Dimethyl-1,3 (E)7-nonatriene	13.096	Monoterpenoid
18	2-ethylhexyl ester	13.645	Ester
19	Naphthalene	14.2196	Naphthalenes
20	Methyl salicylate	14.386	Ester
21	Dodecane	14.4068	Alkane
22	o-Ethyl acetophenone	15.4072	Ketones

23	p-Ethyl acetophenone	15.6763	Ketones
24	Tridecane	15.8517	Alkane
25	1-Tetradecene	17.1036	Unsaturated aliphatic hydrocarbons
26	Tetradecane (C14)	17.2206	Alkane
27	Butylated hydroxytoluene	18.7183	Phenols
28	Hexadecane (C16)	19.6718	Alkane
29	Octadecane (C18)	21.8831	Alkane

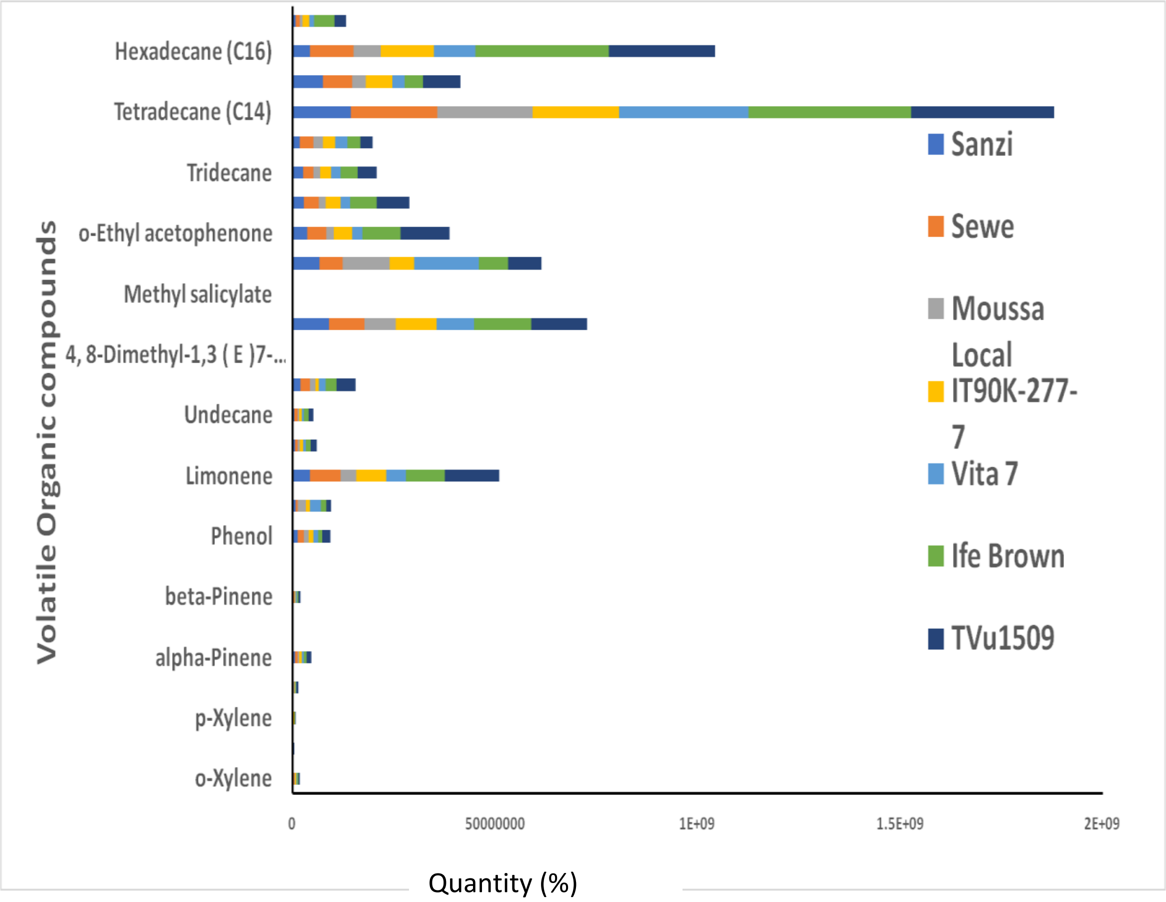


Figure 4.10: Presence and percentage abundance of volatile organic compounds in different cowpea materials grown at Ibadan

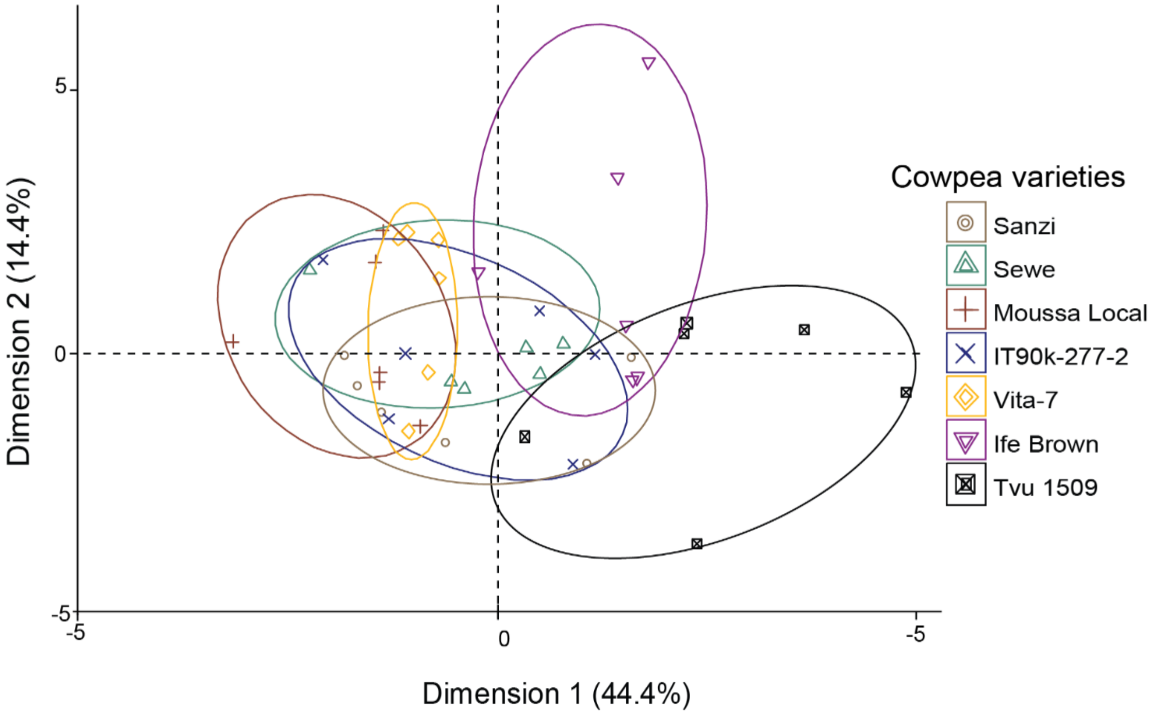


Figure 4.11: Principal component analysis of volatile organic compounds from seven cowpea types of varying resistant status

4.11: Olfactory response of *Megalurothrips sjostedti* females to selected volatile organic compounds identified from cowpea headspace volatile

The olfactory preference of *Megalurothrips sjostedti* for eight volatile organic compounds tested against clean air was observed and presented in Figure 4.12. The preference of thrips was reflected in the number of thrips that chose the arm of the Y-tube olfactometer carrying volatile organic compounds relative to clean air. More thrips chose the volatile organic compounds except in Nonanal, where morethrips chose clean air (91 %) over the VOC (9 %). Table 4.10 shows the result of chi-square analysis that tested the choices of thrips. *Megalurothips sjostedti* showed significant preference for Tetradecane at $p < 0.05$ and highly significant preference for Methyl salicylate, 1-Tetradecene and Gamma Terpinene at $P < 0.01$. The response of thrips to Nonanal was also significant at $p < 0.05$. There was no significant difference in the number of thrips that chose the other VOCs over cleanzb air. More thrips moved away from Sabinene and Dodecane towards clean air, but their choices were not significant ($p > 0.05$)

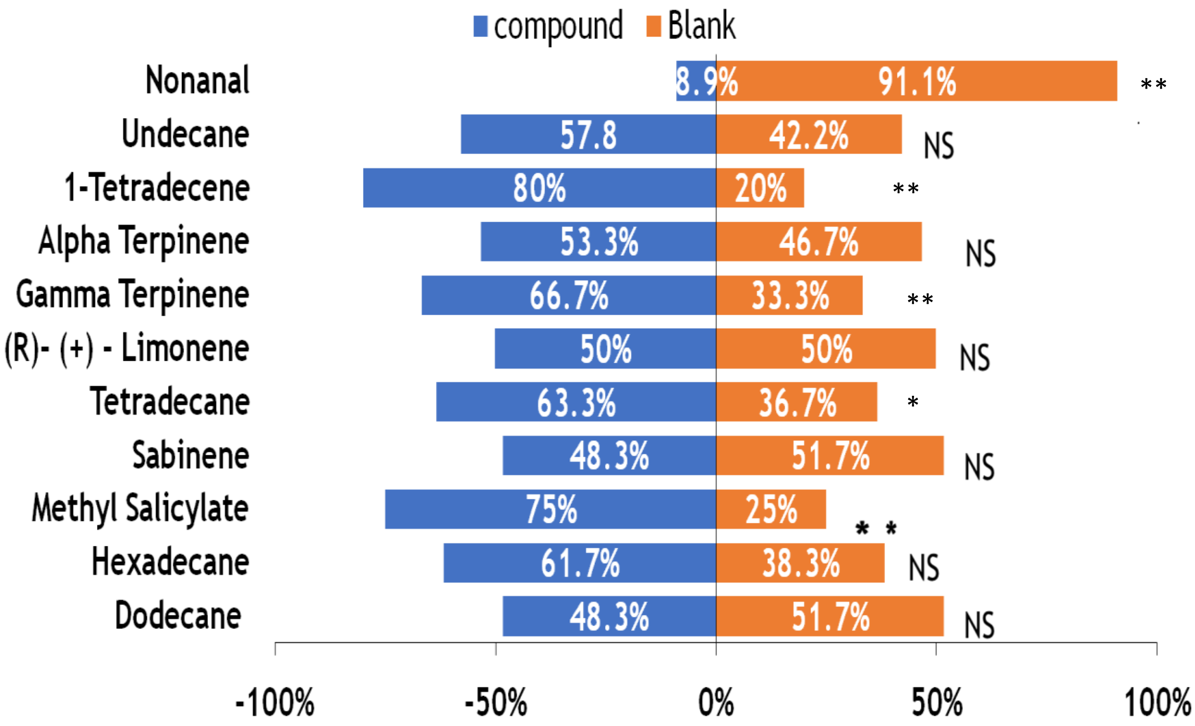


Figure 4.12: Percentage response of female *Megalurothrips sjostedti* to volatile organic compounds identified from cowpea headspace volatiles

Table 4.10: Chi-square statistics, probability value and value of significance of observed differences between the odour choices of volatile organic compounds and clean air by *Megalurothrips sjostedti* in the olfactometer test

Choices	Chi Test Statistic	Prob. Value	Significance
Dodecane vs Blank	0.067	0.80	$p > 0.05$
Hexadecane vs Blank	3.27	0.07	$p > 0.05$
Methyl Salicylate vs Blank	15	0.00	$P < 0.01$
Sabinene vs Blank	0.07	0.80	$p > 0.05$
Tetradecane vs Blank	4.27	0.04	$P < 0.05$
(R)-(+)- Limonene vs Blank	0	1	$p > 0.05$
Gamma Terpinene vs Blank	6.67	0.01	$P \leq 0.01$
Alpha Terpinene vs Blank	0.27	0.61	$p > 0.05$
Nonanal vs clean air	30.42	3.48 x 10 -8	$P < 0.01$
Undecane vs clean air	1.09	0.29	$p > 0.05$
1-tetradecene vs clean air	16.2	5.7x 10 -5	$P < 0.01$

4.12: Number of *Megalurothrips sjostedti* caught on baited and unbaited yellow sticky traps at Abomey-Calavi, Benin Republic and Ibadan, Nigeria

The effectiveness of volatile organic compounds as suitable lures for the attraction of thrips to sticky traps on cowpea field was partly determined by the number of thrips caught on baited and unbaited yellow sticky traps. Yellow sticky traps baited with methyl saliscylate lure caught the highest number of thrips (5.3) which was not significantly different (p>0.05) from thrips caught on other treatments. Traps baited with Tetradecane caught 4.6 thrips. Unbaited yellow sticky traps caught more thrips (2.7) than traps baited with Hexadecane (1.6) (Figure 4.13). There was no significant difference in the number of thrips caught across all the treatments.

Observations on the number of thrips caught on traps at Abomey-Calavi were different from what was observedat Ibadan. The number of thrips caught in Ibadan, presented in Figure 4.14, shows that thrips population was highest on Unbaited traps (224.6), closely followed by Hexadecane traps (224.1). The lowest number of thrips was caught on Methyl salicylate traps (189.2) and next to it was tetradecane traps (219.7). The observed differences were significantly different (p <0.05) and the number of thrips caughtat Ibadan was generally higher than the numbers in Abomey-Calavi for all three volatile organic compounds.

4.13: Orders of insects caught on sticky traps in Abomey-Calavi and Ibadan

Apart from *Megalurothrips sjostedti*, some other insects were caught on sticky traps on cowpea fields. The most prominent of them was *Aphis craccivora*, which belong to the order Homoptera. Eight insect Orders were identified in sticky traps after three weeks of collection at Abomey-Calavi. (Table 4.11): Lepidoptera, Diptera, Coleoptera, Hymenoptera, Orthoptera, Hemiptera, Thysanoptera and Odonata. The Order Diptera had the highest number of insects on the traps across all the treatments, ranging from 11.7 (tetradecane trap, 42 days after sowing) to 42.3 (Unbaited trap, 56 days after sowing). The order Odonata had the lowest number of insects on the sticky traps and was only present on Hexadecane trap at 49 days after sowing (2.67) and unbaited trap, also at 49 days after sowing (0.33).

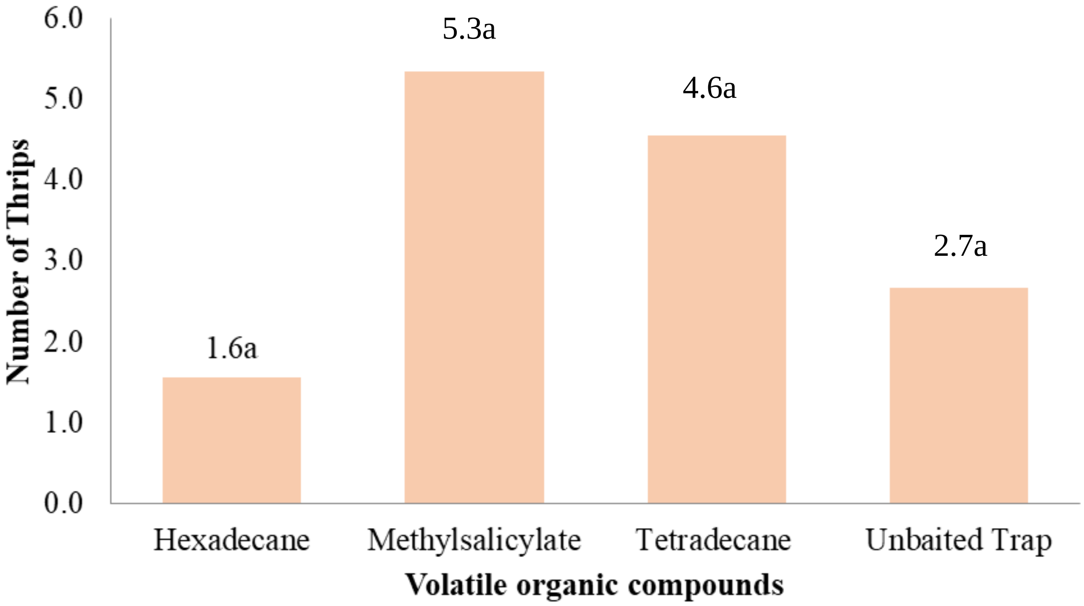


Figure 4.13: Mean number of thrips on baited and unbaited traps at Abomey-Calavi, Benin Republic

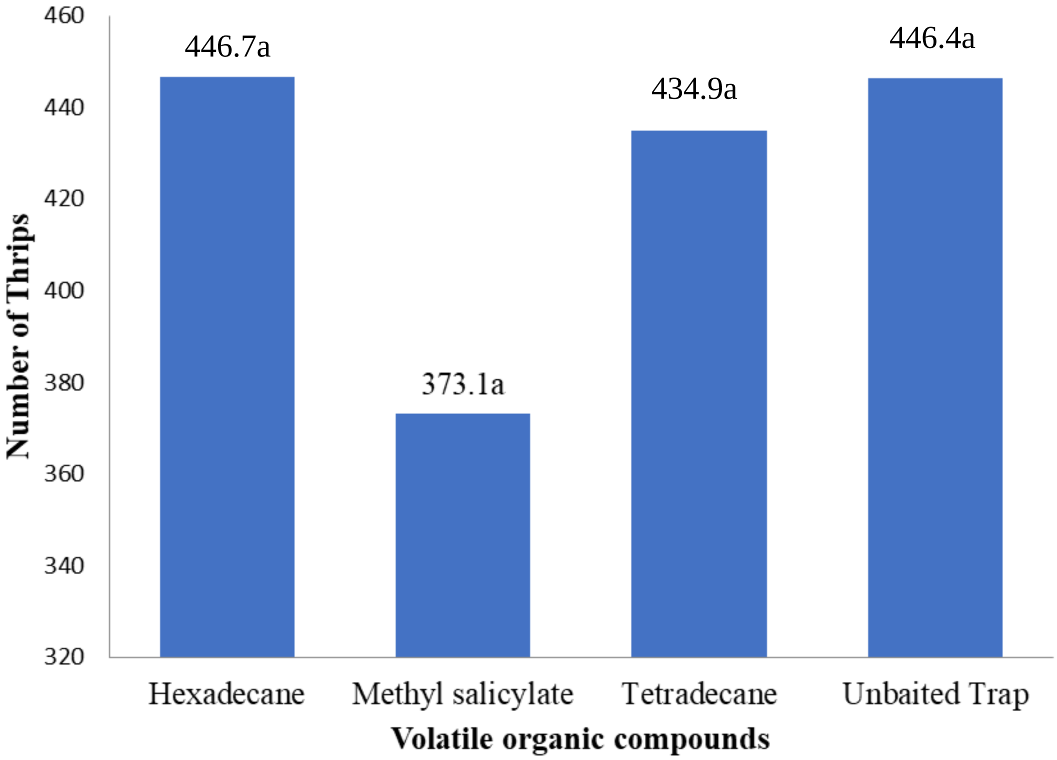


Figure 4.14: Mean number of thrips on baited and unbaited traps at Ibadan

Table 4.11: Insect orders identified from yellow sticky traps on cowpea fields at Abomey-Calavi, Benin Republic

VOCs	DAS	Homoptera (Aphids)	Lepidoptera	Diptera	Coleoptera	Hymenoptera	Orthoptera	Hemiptera	Odonata
Methyl salicylate	42	5.7	1.7	27.3	2.3	1.0	0.0	1.0	0.0
	49	11.7	1.0	32.3	9.00	16.0	0.0	0.3	0.0
	56	12.7	1.0	32.3	6.7	4.3	0.0	1.0	0.0
Tetradecane	42	0.7	1.0	11.7	0.7	1.7	0.0	2.0	0.0
	49	7.6	0.3	22.3	2.7	11.7	0.7	0.7	0.0
	56	6.6	2.3	27.3	3.3	14.0	0.7	2.3	0.0
Hexadecane	42	12.7	1.0	27.0	1.7	0.7	0.7	0.7	0.0
	49	3.7	1.0	24.3	3.0	1.3	1.3	1.0	2.7
	56	16	1.3	29.7	8.7	5.3	0.3	0.7	0.0
Unbaited Trap	42	12.3	0.0	27.7	1.0	1.0	0.3	0.3	0.0
	49	38.6	1.0	40.0	3.0	3.0	0.3	1.3	0.3
	56	2.5	1.3	42.3	7.0	3.0	0.3	1.7	0.0

Coleopterans and Hymenopterans were present on all the baited and unbaited traps, while the Order orthoptera was present on all the traps except the Methyl salicylate baited traps. *Aphis craccivora* was the only insect identified from the order homoptera. The number of aphids caught on traps ranged from 0.7 (Tetradecane traps at 42 days after sowing), to 38.6 (unbaited traps at 49 days after sowing). All the treatments had Aphids caught on their traps.

Seven insect orders were identified in Ibadan after weeks (43, 50 and 57) of collection (Table 4.12): Lepidoptera, Diptera, Coleoptera, Hymenoptera, Orthoptera, Hemiptera and Thysanoptera. *Aphis craccivora* is the only insect identified in the order homoptera, and they were present on all the baited and unbaited traps, ranging from0.3 (Methyl salicylate trap at 57 days after sowing) to 194 (Tetradecane trap at 50 days after sowing). The population of aphids on traps peaked in the second week of flowering (50 days after sowing) across all baited traps. The order Diptera had the highest number of insects caught on sticky traps across all the treatments. It ranged from 28.0 (Tetradecane at 43 days after sowing) to 176.3 (unbaited traps at 50 days after sowing). The population of dipterans peaked in the second week of flowering (50 days after sowing) for all the treatments. The population of hymenopterans was also high, next to Dipterans, it ranged from 6.0(Hexadecane at 57 days after sowing) to 84.7 (Methyl salicylate at 50 days after sowing. The Order Hemiptera had the lowest population of insects on sticky traps, ranging from 0 (on most of the traps) to 2.7 (Hexadecane at 50 days after sowing). The Order Odonata which was present at Abomey-Calavi, was completely absent in Ibadan.

4.14: Thrips Infestation in cowpea flowers on cowpea materials at Abomey-Calavi

The number of thrips in the flowers of cowpea ranged from 152 in Methyl salicylate baited plots to 212 in Tetradecane baited plots weekly at Abomey-Calavi (Figure 4.15). Number of thrips

Table 4. 12: Mean number of insect species and insect orders identified from yellow sticky traps in Ibadan, Nigeria

VOCs	DAS	Homoptera (Aphids)	Lepidoptera.	Diptera	Coleoptera	Hymenoptera	Orthoptera	Hemiptera
Methyl salicylate	43	1.3	0.7	40.0	1.3	18.7	0.0	1.0
	50	16.3	1.0	144.0	2.7	84.7	0.7	1.0
	57	0.3	1.0	14.7	0.3	5.3	0.0	1.0
Tetradecane	43	3.6	0.0	47.3	1.7	14.3	0.3	0.0
	50	194	0.3	76.0	1.0	35.3	0.7	0.7
	57	0.0	0.0	16.3	1.3	10.0	1.7	1.7
Hexadecane	43	2.6	0.3	28.0	1.3	12.3	0.0	0.0
	50	24.3	1.3	160.0	3.7	63.0	0.5	2.7
	57	0.6	1.3	14.3	3.3	6.0	0.3	0.0
Unbaited Trap	43	10.3	0.3	21.3	1.3	13.7	0.3	0.0
	50	7.0	2.0	176.3	4.0	45.0	0.0	0.0
	57	2.3	0.7	22.7	1.7	9.7	0.7	0.7

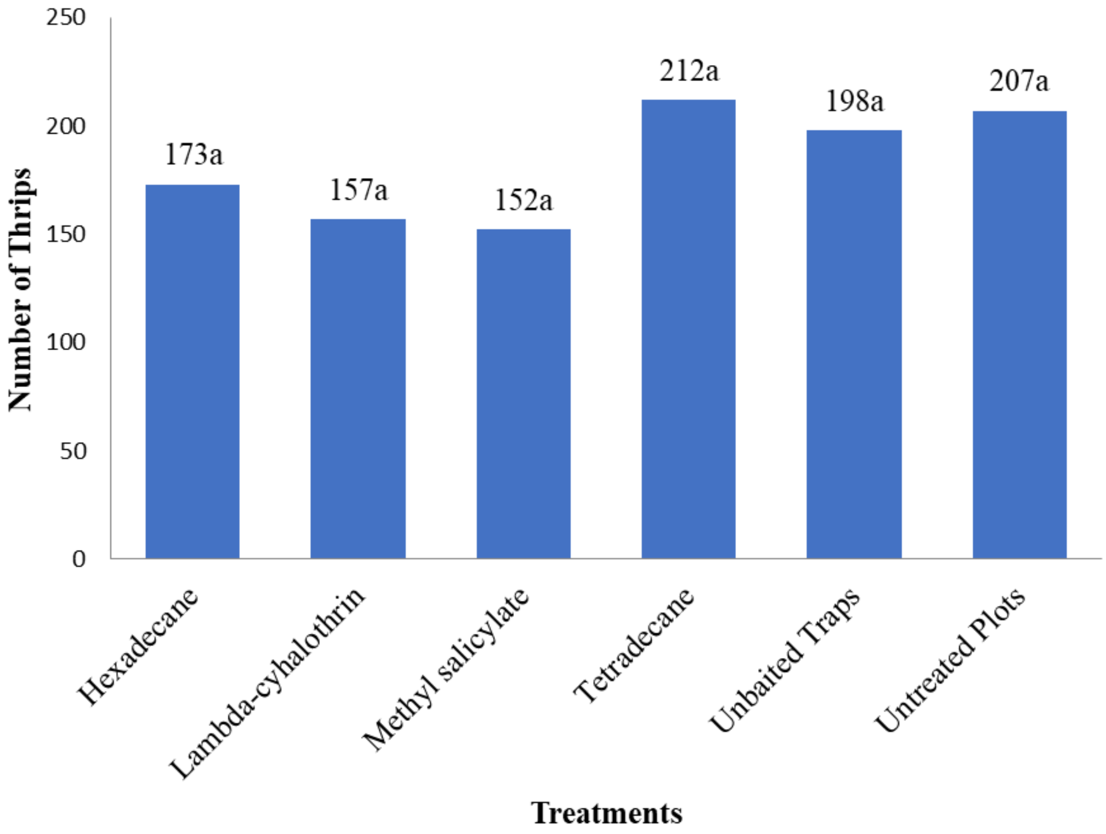


Figure 4.15: Mean population of *Megalurothrips sjostedti* in flowers of cowpea plants at Abomey- Calavi

in the lambda-cyhalothrin treated plots was lower than those in Hexadecane (173±) and Tetradecane treated plots. However, there was no significant difference ($p>0.05$) in the number of thrips present in the flowers of cowpea across all the treatments. The weekly trend of the population of thrips in cowpea flower is presented in Figure 4.16. The populationof thrips peaked in the second week of flowering (49 days after sowing) and began to drop by the third week of flowering (56 days after sowing). This was true for all the treatments at Abomey-Calavi.

4.15: Thrips infestation in cowpea flowers at Ibadan field plots

The number of thrips in cowpea flowers increased in the order Hexadecane (494) < Methyl salicylate (590) < Tetradecane (620) among the volatile organic compound treatments, closely followed by untreated plots (618) (Figure 4.17). Lambda-cyhalothrin plots had the lowest number of thrips in the flowers of cowpea plant (199). Analysis of variance showed that there was significance ($p < 0.05$) in the number of thrips observed in the flowers. Table 4.13 shows the pairwise comparison of number of thrips in cowpea flowers across treatments. A total of fifteen pairs of comparisons were made, but only the comparison between Tetradecane and Lambda-cyhalothrin showed marginal significant difference with a probability value of 0.0540.

The population trend of *Megalurothrips sjostedti* in cowpea flowers over a three-week period (43, 50 and 57 days after sowing) at Ibadan is presented in Figure 4.18. The population trend was likethe observation at Abomey-Calavi. The population rose to a peak in the second week (50 days after sowing) and began to drop by the third week, the only exception being unbaited traps treatment in Ibadan where thrips population dropped in the second week and increased in the third week.

4.16: Infestation of other insects in cowpea flowers

Apart from thrips, cowpea flowers were also infested with aphids, and a few other types of insects that were grouped together as ‘Others’ (Table 4.14).

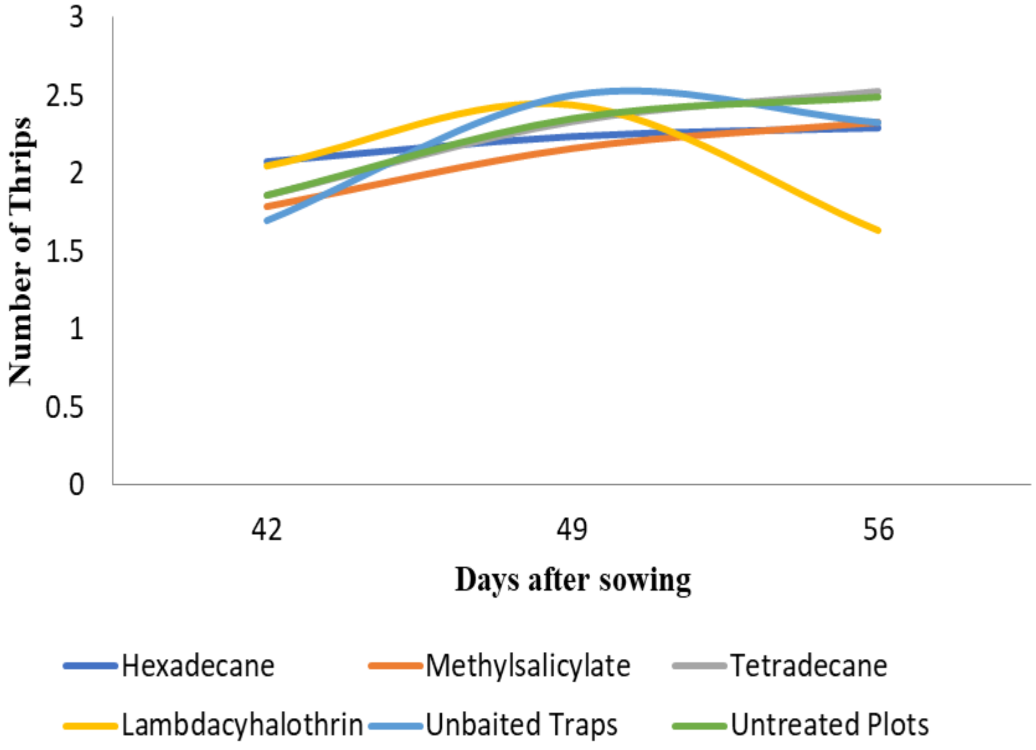


Figure 4.16: Population trend of *Megalurothips sjostedti* in cowpea flowers over a three-week period at Abomey- Calavi

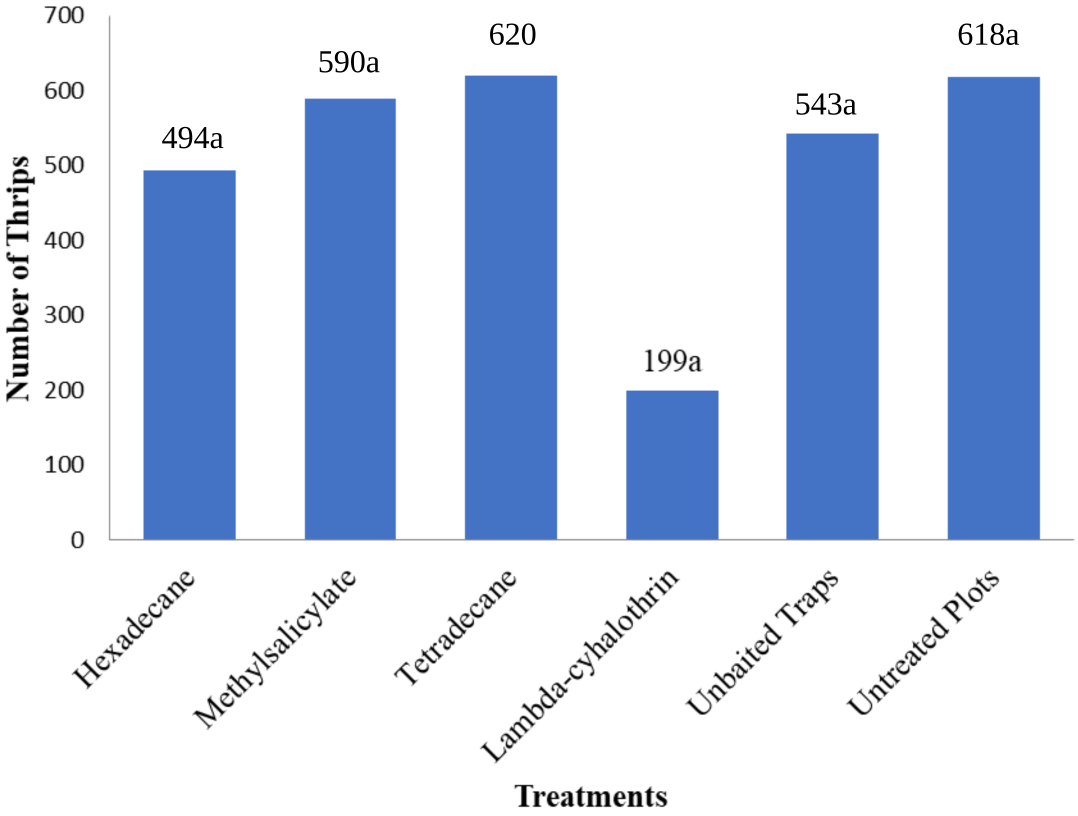


Figure 4. 17: Population of *Megalurothrips sjostedti* in flowers of cowpea plants in Ibadan

Table 4.13: Pairwise comparison of number of thrips in the flowers of cowpea plants in Ibadan

Contrast	Estimate	p.value	Significance
Hexadecane – Lambda-cyhalothrin	294.56	0.2438	Not Significant
Hexadecane – Methyl salicylate	-96.00	0.9648	Not Significant
Hexadecane – Tetradecane	-126.22	0.8986	Not Significant
Hexadecane - Unbaited Traps	-49.44	0.9982	Not Significant
Hexadecane - Untreated plots	-124.00	0.9049	Not Significant
Lambda-cyhalothrin - Methyl salicylate	-390.56	0.0784	Not Significant
Lambda-cyhalothrin – Tetradecane	-420.78	0.0540	$p \leq 0.05$
Lambda-cyhalothrin - Unbaited Traps	-344.00	0.0555	Not Significant
Lambda-cyhalothrin - Untreated plots	-418.56	0.0555	Not Significant
Methyl salicylate – Tetradecane	-30.22	0.9998	Not Significant
Methyl salicylate - Unbaited Traps	46.56	0.9986	Not Significant
Methyl salicylate - Untreated plots	-28.00	0.9999	Not Significant
Tetradecane - Unbaited Traps	76.78	0.9864	Not Significant
Tetradecane - Untreated plots	2.22	1.0000	Not Significant
Unbaited Traps - Untreated plots	-74.56	0.9881	Not Significant

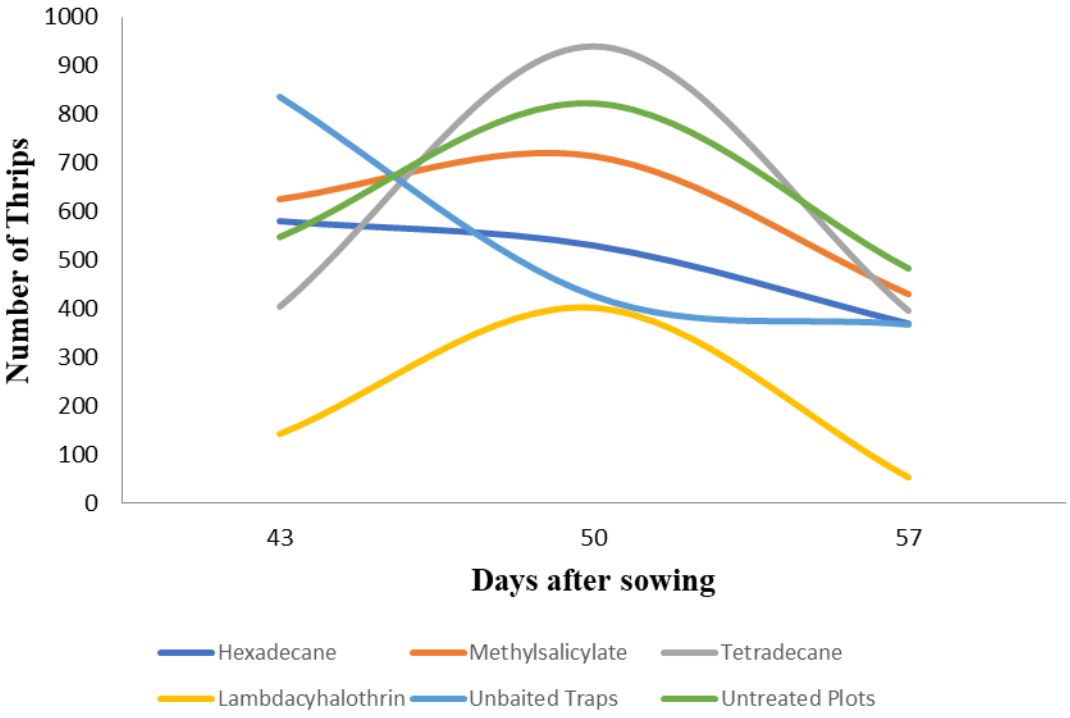


Figure 4.18: Population trend of *Megalurothips sjostedt* in cowpea flowers over a three week period in Ibadan

Insect orders that make up the category ‘others’ are: hemiptera, hymenoptera, diptera, odonata, coleoptera, lepidoptera and orthoptera. Aphids were present in all treatments in Ibadan, ranging from 3.0 on Lambda-cyhalothrin plots to 14.0 on Methyl salicylate plots. The number of other insect species also ranged from 10.6 on Lambda-cyhalothrin plots to 22.0 on Hexadecane plots. At Abomey-Calavi, the number of aphids in cowpea flowers was very high, ranging from 117.3 on Untreated plot to 932.3 on Lambda-cyhalothrin plots. The number of others was not as high as the aphids. it ranged from 11.0 on untreated plots to 16.7 on Tetradecane plots (Table 4.14).

4.17: Cowpea grain yield and percentage yield lossin Ibadan

The highest observed grain yield was from plots with unbaited traps (21,972.7 Kg/ha), followed by Lambda-cyhalothrin treated plots (19, 071.6 Kg/ha), while the lowest yield was from untreated plots (15,163 Kg/ha) (Table 4.15). Despite the variation in yield across the treatments, there were no significant differences among them. However, there was a significant difference in the percentage yield loss across the treatments. There was no significant difference between the percentage yield loss in Tetradecane (14.5 %), Hexadecane (3.8%) and Methyl salicylate (8.8 %), but they were significantly lower than yield loss in untreated plot (26. 4 %). There was no yield loss recorded in plots with unbaitedtraps but yield increase. When compared with the standard check (Lambda-cyhalothrin), there was an 18.1 % yield increase recorded from unbaitedplot.

4.18: Cowpea grain yield and percentage yield loss in Abomey-Calavi

The grain yield of cowpea in Abomey-Calavi was generally lower than the yield in Ibadan (Table 4.16). However, there was no significant difference in cowpea grain yield across all the treatments. Yield ranged from 552.2 Kg/ha in Methyl salicylate plots to 723.6 in Lambda-cyhalothrin plots. Grain yield from untreated plots (578.6 Kg/ha) was higher than all the other treatments.

Table 4.14: Number of aphids and other insect species found within flowers of cowpea at Calavi and Ibadan

	Ibadan		**Abomey-Calavi**	
Treatment	**Aphids**	**Others**	**Aphids**	**Others**
Hexadecane	10.0	22.0	353.7	11.3
Methyl salicylate	14.0	18.3	498.3	11.3
Tetradecane	6.3	14.6	596.0	16.7
Lambda-cyhalothrin	3.0	10.6	932.3	15.3
Unbaited Traps	4.0	10.7	459.3	14.7
Untreated Plots	3.0	19.0	117.3	11.0

Table 4.15: Yield and yield losses on cowpea plots with baited and unbaited traps at Ibadan

Treatments	Yield (Kg/ha)	Yield loss (%)
Hexadecane	18, 515.9a	3.8 ± 4.436 ab
Methyl salicylate	17, 666.4a	8.8 ± 36.438 ab
Tetradecane	18, 887.5a	14.5 ± 8.976 ab
Unbaited traps	21, 972.7a	-18.1 ± 13.731 b
Untreated plots	15, 163.6a	26.4 ± 4.861 a
Lambda-cyhalothrin	19, 071.6a	0.0 ab

Means in each column followed by the same letter(s) are not significantly different at $p<0.05$

Table 4.16: Yield and yield losses on cowpea plots with baited and unbaited traps at Abomey-Calavi

Treatments	**Yield (Kg/ha)**	**Yield loss (%)**
Hexadecane	608.3a	15.9 ± 14.203 ab
Methyl salicylate	554.2a	23.4 ± 20.217 ab
Tetradecane	558.6a	22.8 ± 12.165 ab
Unbaited traps	432.8a	40.2 ± 8.286 a
Untreated plots	578.6a	20.1 ± 21.278 ab
Lambda-cyhalothrin	723.6a	0.0b

Means in each column followed by the same letter(s) are not significantly different at $p<0.05$

except hexadecane (608.3 Kg/ha) and lambda-cyhalothrin. Analysis of variance showed that there was significant difference in Percentage yield loss among treatments. Percentage yield loss was highest in plots with unbaited traps, followed by Methyl salicylate treated plots. Hexadecane plots had the lowest yield loss of 15. 9%.

CHAPTER FIVE

DISCUSSION

Insect's behaviour is generally a response to several factors operating in their environment. Some of these behaviours include activities such as locomotion, mating, communicating, grooming, reproduction, feeding, and host selection among others. Insect chemical ecology as partly determined by volatile organic compounds, given off by plants plays a major role in insect host finding. Several researches highlight the role of antennal olfactory receptors for plant volatiles, which enables orientation and movement towards the host plant from a distance (Visser, 1986; Conchou *et al*., 2019).

5.1 Olfactory bioassay with living cowpea plants

This study hypothesizes that headspace volatiles from resistant and susceptible cowpea materials are different, and the difference is a factor in their attractiveness to cowpea flower thrips *Megalurothrips sjostedti*. The cowpea materials in this study, both resistant and susceptible, were strongly attractive to *Megalurothrips sjostedti*. The scenario here suggests that in the absence of alternative host plants which are known to enhance the pest status of *Megalurothrips sjostedti* (Tamo *et al*., 1993), all the cowpea types were similarly predisposed to thrips infestation. Therefore, a sole cowpea crop without any form of control measure will always be an attraction for thrips. The emphasis on sole cowpea crop was also reported by Ekesi *et al*. (1998) who stated that the olfactory attractiveness of cowpea to *Megalurothrips sjostedti* decreased when it was intercropped with maize. However, whether the population of thrips on the field corresponds to the level of damage to crop would depend on the resistant status as well as the mechanism of resistance in operation in the cowpea type. Similarly, Feng *et al*. (2017) reported that after an insect has been attracted to a plant, the physical and chemical properties of a plant are important in determining its suitability for oviposition. The suitability of the host crop for the success of the insect is.

determined by the reactions of chemoreceptors located on the tarsi, mouthparts, or ovipositors to the plant odours. In a comparison between the susceptible Ife Brown and five resistant types, the finding shows that the attractiveness of cowpea headspace volatiles to thrips differs with cowpea materials. Similarly, Diabate *et al*. (2019) reported that apart from phenological stage of cowpea and sex of thrips, behavior of thrips towards cowpea volatile also differ with the variety of cowpea. Since organic compounds identified were found in different amount in the materials, the variation in amount could be responsible for the choices made by thrips. The observed difference in attractiveness of the cowpea types did not reflect any pattern in favour of resistants or susceptibles. This suggests that the resistant status of cowpea cultivar does not translate to the olfactory attractiveness or repulsion of the crop to *M sjostedti*. A study on thrips, *Frankliniella occidentallis* showed that it responded positively to all volatiles of its host plant (Ekesi *et al*., 1998). However, it has been reported that some compounds are important in the identification of host plant volatiles by insects therefore, when they are not present, the behavioural response of the insect is altered (Bruce and Pickett, 2011). Studies have shown that female *M. sjostedti* are more attracted to floral volatiles than those from other parts of cowpea plant (Ngakou *et al*., 2008; Niassy *et al*., 2016). Since this study was carried out with female *Megalurothrips sjostedti*, it is possible that high quality and quantity of floral volatile was produced by the preferred cowpea type. Volatile compounds from cowpea leaves such as (E)-2-Hexenal makes cowpea flowers less appealing to female *Megalurothrips sjostedti* (Diabate *et al*., 2019). This interference could be a result of a blocked olfactory receptorscaused by the compound, thus interrupting the signal flow to the glomeruli of the insect. This compound, however, was present in both the resistant and susceptible materials.

5.2 Headspace volatile organic compounds identified from cowpea plants in Wageningen, The Netherlands and Ibadan, Nigeria

Observed differences in the number of volatile organic compound identified in the same cowpea materials planted in two completely different locations: Ibadan and Wageningen is an indication that environmental factors play a major role in the quality and quantity of volatile organic compound given off by plants. Zang *et al*. (2018) reported that due to biotic and abiotic stressors, plants typically exhibit temporal and geographical variability in the composition of their volatiles. Because of occurrences such as this, a successful herbivore is required to exibit behavioral plasticity that allows it recognise these differences and distinguish between host and non-host. It also enables it to recognize the phenology and physiology of hosts (Magalhães *et al*., 2016).

Head space volatiles of different cowpea types reported by different researchers at different locations have shown a level of variation (Lwande *et al*., 1989; Ager, 2009; Feng *et al*., 2017. Some of the compounds found in this study such as" (Z)-3-Hexen-1-ol, acetate, (E)-beta-caryophyllene, Linalool have been reported as floral volatiles of cowpea and other plant species (Farré-Armengol *et al*., 2017). Some of the compounds from excised flowers were not present in the whole plant volatile profile such as 1, 8-cineole which is also referred to as eucalyptol, has been reported as an aromatic component of many plants; (E)-3-Hexen-1-ol, also known as green alcohol, was present in only flowers of susceptible cowpea types but absent in whole plant and resistant ones. It has also been reported as an allelochemical that is released by plant in response to mechanical damage (Cofer *et al*., 2018). This compound could be a focus for future research on lures for managing cowpea flower thrips population.

The class of organic compound Benzenoid was a major difference as it was identified in the cowpea planted in Ibadan and absent in cowpea planted in Wageningen. Report from an experiment carried out in a controlled environment showed that benzenoids are mostly produced when plants alter their metabolism under stress conditions. Although it wasn't stated what their function is, it may be related to chemical communication and protection against stress (Misztal *et al*., 2015). This compound might therefore be an indication of stress condition present in the cowpea materials grown in Ibadan. This stress condition could also be responsible for the fewer compounds identified in cowpea grown in Ibadan. The addition of several soil amendments into the soil in which cowpea was grown in Wageningen, which was necessary to enhance the growth of cowpea in a place that was not its natural habitat, could also be responsible for the large number of volatile organic compounds collected and identified at Wageningen.

5.3 Olfactory bioassay with volatile organic compounds

Although the headspace volatile profile from seven cowpea materials in this study were similar, only eight of the identified compounds were further investigatedin a Y-tube olfactometer, based on abundance of the compound in the headspace profile and its availability for further studies.

Methyl salicylate, tetradecane and gamma terpinene were most attractive to *Megalurothrips sjostedti*in the Y-tube olfactometer. Timing of the insect movement through the Y- Tube could be a pointer to the clarity of the odour identified by the thrips. *Megalurothrips sjostedti* showed a definite preference for all cowpea types and spent minimum time at the junction. Clean air is odourless, so it might be safe to say that at the stem, the only motivating odour is that from the treatment, it is at the junction that the insect detects that the odour is from one side and gets to decide to move towards it or move away from it. A strongly appealing odour would imply that the thrips move quickly towards it. The longer time at the junction showed some level of confusion as to which odour source to choose. This explains why treatments that a significant higher number of positive responses had had lower junction time as seen in the time of response to tetradecane, methyl salicylate and Tetradecane, which was lesser than the time taken to respond to dodecane which shared a close response rate with clean air.

Methyl salicylate have been reported as attractants for some beneficial insects in the familie schrysopidae, coccinellidae (Lee, 2010) and the dance fly *Rhamphomyia gibba* (Shamshev and Selitskaya, 2016). Gamma-terpinene has been used as an attractant in the control of *Leptocybe invasa*, an insect pest of Eucalyptus trees, by blending with limonene in the ratio 8:2, and tetradecane was reported as a likely component of sex pheromone of female clearwing moth (Minaeimoghadam *et al*., 2017). There have been no reports where any of these compounds were found attractive to *Megalurothrips sjostedti*on the field. However, it has been reported that among the various volatile organic compounds present in a host, it is expedient to find the one (s) with physiological importance for host plant location among a complex blend of volatiles (Collatz and Dorn, 2013). Since VOCs were the same in both susceptible and resistant materials, it implies that blends at different proportions will produce other types of compounds and elicit different responses from the insect (Magalhães *et al*., 2016).

Some lures have been reported for other thrips species but not *Megalurothrips sjostedti*. Some of them includeethyl nicotinate for *Thrips obscuratus* (Newzealand flower thrips), Ethyl isonicotinate for *Thrips tabaci (*Onion *thrips),* Methyl salicylate was named lure for*Frankliniellaoccidentallis*based on findings from a laboratory bioassay. (Koschier *et al*., 2000). Similarly, the compound was found attractive to *Amblyomma sculptum* (Ticks) only in a laboratory bioassay, but not on the field (Ferreira *et al*., 2020)

5.4 Evaluation of volatile organic compounds as lures on cowpea fields in two agro - ecologies

These compounds methyl salicylate, tetradecane and gamma terpinene were selected for furtherinvestigation as possible attractants for*Megalurothrips sjostedti*onthe field, for the development of IPM strategies for the management of thrips. However, gamma terpinene, was not available and was replaced with hexadecane which followed closely after Tetradecane in attraction in the Y-tube bioassays.

Several studies have been carried out varying the concentration of different organic compounds for the purpose of identifying the most effective and economical concentrations (Boer and Dicke, 2004; James, 2005; Diabate *et al*., 2019; Frederickx *et al*., 2012.

In this study however, volatile organic compounds were used undiluted at two locations but the response of *Megalurothrips sjostedti* to the treatments varied widely from one location to the other. The population of thrips both on the traps and in cowpea flowers at Abomey-Calavi was significantly lower that the occurrence at Ibadan. Difference in genetic structure of thrips or environmental factors could be responsible for the observed differences in the response of *M. jostedti* in the different location. A similar occurrence was reported for Peach Fruit Moth, *Carposina sasakii* (Wang *et al*., 2017).The bioecology in the Abomey-Calavi fields are clearly different from what was observed in Ibadan. This could be due to the prevailingagro- ecology or weather condition as at the time of planting or the history of agricultural practices in the area. For instance, there has been several studies on the use of bio-control agents *Cerenisus femoratus* for the management of *Megalurothrips sjostedti* in Cotonou, in which bio-control agents *Cerenisus femoratus* were experimentally released on the nursery plots of *T. candida* and were later found on the leguminous trees *Millettiathonningii* (Schum. & Thonn.) Bak. and *Pterocarpus santalinoides* L'Hér. ex DC. (Fabaceae) as far as 65 km north of the original release sites (Abomey-Calavi) in southern Benin, after one year. After two years, *C. femoratus* were foundtohave established steadily in wider areas of Benin.(Tamo *et al*., 2003, 2013). The successful establishment of the population of *C. femoratus* in southern Benin must have impacted on thrips population, therefore, reducing the pressure of infestation on cowpea plants sown in that agroecology.

The abundant presence of aphids in cowpea flowers could be an indication of its gradual emergence as pest of flowering cowpea, or simply a response to the volatile organic compounds presents on cowpea field. Occurrences where experimental uses of volatile organic compounds elicit responses from non-target pest, is one of the draw-backs of its draw-backs in insect pest behavioural modification. For instance, the release of green leaf volatiles in the field had a synergistic effect on isoprenoid emission from maize plants and increased herbivore damage, without significantly attracting beneficial insects (Mérey *et al*., 2011). Also, in wheat plants, Z-3-hexenyl acetate improved their resistance to pathogenic fungus *F. graminearum*, but increases the production of deoxynivalenol, a mycotoxin that is harmful to human health (Ameye *et al*., 2015).

The effect of the individual volatile organic compounds did not impact on the yield of cowpea at the two locations studied. This could be so because the VOCs were tested singly; if it was a blend of different compounds, there could be difference in the number of thrips on baited trap. This suggests that the attraction of the volatile compounds needs to be enhanced for better effectiveness on the fields. Attractive volatile organic compounds are sometimes combined with existing pheromone lure to enhance the lure for a better catch (Hilker and Mcneil, 2008).This occurrence was observed in field trapping experiments with volatile organic compound 1-undecene, which is a male sex pheromone from bean seed beetle, *Bruchusrufimanus*. The pheromone was EAG active with female antennae, and attractive to females in an olfactometer. However, in the field it only enhanced trap catches when it was released together with the floral volatiles (Bruce *et al*., 2011). Also, field evaluations showed that mixtures of (E)-2-hexenyl acetate female sex pheromone, L-isoleucine methyl ester:(R) -(-)-linalool (6:1), resulted in significantly higher catches of *Holotrichia parallela*than the sex pheromone alone.

This study has demonstrated that a suitable attractant must be attractive beyond the space of a Y- tube. In the open field, the strength of appeal of a suitable attractant for *Megalurothrips sjostedti* must overshadow that of the scents produced by the host plant. Due to the highly biodegradable nature of volatile organic compounds, it may not travel far before it is exhausted in the atmosphere, therefore requiring a more frequent supply of the compounds than what was carried out in this study (once every week), to ensure that it persists long enough to elicit desired level of attraction of thrips. itself and other background odours. This is to prevent a masking effect that makes the attractant stay unnoticed by thrips.

The use of semio chemicals for the management of thrips has largely been for population monitoring rather than direct field population management (James and Price, 2004; Sugimoto *et al*., 2014; Brilli *et al*., 2019). There in need to intensify studies and modify available methods to fully utilize the potentials of semiochemicals for behaviour modification of *Megalurothrips sjostedti*, that will eventually translate to population control of thrips on cowpea fields.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

Megalurothrips sjostedti is an important insect pest of cowpea crops that is mostly managed with conventional insecticides which are hazardous to the environment. Volatile Organic Compounds developed into attract and kill mechanisms are safer alternatives for insect pest management on agricultural crops, but have been scarcely documented in cowpea insect pest management. This study identified headspace volatiles of resistant and susceptible cowpea materials and investigated them for their attractant and repellant properties for management of *Megalurothrips sjostedti.*

Olfactory response of *M. sjostedti* to headspace volatiles from five resistant cultivars: Moussa Local, Sewe, TVu1509, Sanzibanili, IT90K-277-2 and two susceptible cultivars: Ife brown and Vita7 at the flowering stage, was investigated in the laboratory with a Y-tube olfactometer and their choices determined. The VOC of resistant and susceptible cowpea cultivars were collected at Wageningen, The Netherlands, and Ibadan, Nigeria, using dynamic headspace volatile collection method and profiled with a Gas Chromatography-Mass Spectrometry. Olfactory response of thrips to eleven VOC: α-terpinene, γ-terpinene, (R)-(+)–limonene, tetradecane, sabienene, methyl saliscylate, hexadecane, dodecane, 1-tetradecene, nonanal and undecane were also evaluated, thrips preferences were determined. Three VOC baits: methyl salicylate, hexadecane and tetradecane, unbaited traps, lambda-cyhalothrin and untreated plot (control) were evaluated on cowpea plots of 30x40 m^2 dimension, in a Derived Savanna (Ibadan) and Guinea Savanna (Abomey-Calavi) Agro-Ecologies, established with Ife brown and Kpodiguegue cowpea cultivars, respectively. Number of thrips and orders of insects in cowpea flowers and Sticky traps were recorded, respectively; Grain Yield (kg/ha) and Yield Losses were determined.

Attraction of *Megalurothrips sjostedti* to headspace volatile of cowpea cultivars relative to clean air was significantly higher in all the cultivars. The VOC identified in Wageningen and Ibadan were 68 and 29, respectively, belonging to 22 different classes of compounds. Attraction of *M. sjostedti* to VOC relative to clean air was significantly higher in the order: hexadecane, tetradecane, γ-terpinene, methyl salicylate, 1-tetradecene, while nonanal repelled *M. sjostedti*. In Abomey-Calavi, *M. sjostedti* was highest in tetradecane plot and lowest in methyl salicylate. Also in Ibadan, tetradecane plot had the highest *M. sjostedti*, while lambda-cyhalothrin had the lowest. Eight insect orders: Thysanoptera, Hemiptera, Hymenoptera, Diptera, Odonata, Coleoptera, Lepidoptera and Orthoptera, were identified on sticky traps. Grain yield ranged from 21,927.7 (Unbaited trap) to 15,163.6 (untreated) in Ibadan and 723.6 (lambda-cyhalothrin) to 432.8 (Unbaited trap) in Abomey-Calavi. Hexadecane elicited the lowest yield loss in Abomey-Calavi and Ibadan

This study showed that cowpea materials, irrespective of their resistant status give off headspace volatiles (odour) that are attractive to cowpea flower thrips *Megalurothrips sjostedti.* Therefore, when the following cowpea types; Vita-7, Ife Brown, which are susceptible, and IT90k-277-2, Moussa Local, Sanzisabinli, Sewe, TVu 1509, KVx404-8-1 (resistant materials) are cultivated on the field as sole crops, they are highly attractive to *Megalurothrips sjostedti*. This implies that cowpea odour studied may not be used as a biomarker for differentiating resistant materials from susceptible ones. Furthermore, headspace volatiles from different cowpea types, grown in the same environment, show qualitative similarities by emitting the same type of volatile organic compounds, and quantitative differences by emitting those organic compounds at varying amounts.

Undiluted isolated organic compounds: methyl salicylate, tetradecane, gamma-terpinene, and 1-tetradecene are strong attractants to *Megalurothrips sjostedti* in a Y-Tube bioassay, while nonanal is a strong repellant. On the field, the effect of individual volatile organic compounds did not impact on the yield of cowpea at the two agro- locations studied. There exist geographical differences in the behaviour of thrips towards volatile organic compounds in the two locations as evidenced in the number of thrips trapped and in the flowers of cowpea.

The use of semiochemicals as attract and kill will minimise the use of synthetic insecticides in cowpea production and consequently preserve the environment.

6.2 Recommendations

1. Entomologists in the industry can harness the attractive properties of hexadecane and methyl saliscylate, to develop a technologyy for the management of *Megalurothrips sjostedti.*
2. Various blends of isolated organic compounds should be tested to identify a variety of attractants and repellants suitable for population monitoring and management of thrips *Megalurothrips sjostedti* in different agro-ecologies.
3. Cultivation of resistant cowpea materials alone may not sufficiently reduce the population of *Megalurothrips sjostedti* in a cowpea farm. Farmers need to employ other thrips management statrgies for maximum output.

6.3 Contributions to Knowledge

This work has contributed to knowledge in the following ways:

1. *Megalurothrips sjostedti* is highly attracted to headspace volatiles of cowpea plants irrespective of their resistance or susceptibility status
2. Volatile organic compounds: methyl salicylate, 1-tetradecene, tetradecane, and gamma terpenene are attractants of *Megalurothrips sjostedti*.
3. Nonanal, a volatile organic compound of cowpea is a repellant *of Megalurothrips sjostedti.*
4. Volatile organic compound, Hexadecane at 1 mL in yellow sticky trap, minimises yield loss, caused by *Megalurothrips sjostedti*in cowpea plants in Ibadan and Abomey Calavi, Republic of Benin

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