Exploiting early herbivory-induced defense traits in *Zea* species for the management of *Chilo partellus* in East Africa

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Thesis submitted for the degree *Philosophiae Doctor* in Environmental Sciences at the Potchefstroom Campus of the North-West University

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Exploiting early herbivory-induced defense traits in *Zea* species for the management of *Chilo partellus* in East Africa

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DECLARATION BY THE CANDIDATE

I, **DANIEL MUNYAO MUTYAMBAI**, declare that this thesis which I submit to North-West University, Potchefstroom Campus, in compliance with the requirements set for the *Philosophiae Doctor* (Environmental Sciences) degree is my own original work and has not been submitted to any other university for a similar or any other degree award.

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DECLARATION AND APPROVAL BY SUPERVISORS

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

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DEDICATION

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ACRONYMS AND ABBREVIATIONS

CIMMYT- International Maize and Wheat Improvement Center

DMNT- (*E*)-4,8-dimethyl-1,3,7-nonatriene

EAG - Electroantennography

FAO - Food and Agricultural Organization

FEWS - Famine Early Warning Systems

FID – Flame Ionization Detector

GC - Gas Chromatography

GLV – Green Leaf Volatile

HIPV - Herbivore-Induced Plant Volatiles

HP – Hewlett Packard

ICIPE- International Centre of Insect Physiology and Ecology

IRMA – Insect Resistant Maize for Africa

KARI - Kenya Agricultural Research Institute

Ltd – Limited

ML - Mililitre

MS - Mass Spectroscopy

NIST – National Institute of Standards and Technology

OPV - Open Pollinated Variety

SCPRID - Sustainable Crop Production Research for International Development

SE – Standard Error

SEM – Standard Error of the Mean

TMTT – (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene

VOC - Volatile Organic Compound

ABSTRACT

Maize, a genetically diverse crop, is the third largest cereal crop in the world and the most important staple cereal in sub-Saharan Africa, supplying 50% of the calorie intake in this region. The stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is a key constraint to cereal production in most resource-poor smallholder farming systems in sub-Saharan Africa causing crop losses accruing up to 88%. Previous studies have shown that feeding by herbivorous insects induces maize to emit volatiles attractive to natural enemies. However, these antagonists are recruited when damage has already been inflicted on the plant. Recent investigations revealed that egg deposition can induce maize landraces of Mesoamerican origin to emit volatiles attractive to *C. partellus* parasitoids, a trait previously reported to be absent in maize hybrids. However, genotypic variation in this indirect defence trait within maize varieties adapted to local agroclimatic conditions and the effect of processes such as domestication and breeding on this trait are not known. Moreover, it is not known whether maize varieties possessing this indirect defence trait can directly deter further herbivore colonization and constitutively suppress the herbivore's larval development or whether they can induce the same defence trait in neighbouring unattacked plants. This study sought to fill these knowledge gaps with the aim of exploiting these plant defence traits in the development of ecologically sound crop protection strategies. Experiments were conducted in which headspace volatile samples were collected from plants of wild, landrace and hybrid maize with and without *C. partellus* eggs. Chemical analyses were done using gas chromatography (GC), coupled GC-mass spectrometry (GC-MS) and coupled GC-Electroantenography (GC-EAG). Behavioural bioassays were done using egg (*Trichogramma bournieri* Pintureau (Hymenoptera: Trichogrammatidae)) and larval (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) parasitoids in a 4-arm olfactometer using volatiles collected from the plants. Moreover, *C. partellus* larval preference, growth and development as well as subsequent oviposition behaviour of gravid *C. partellus* moths on these plants were determined. Behavioural assays showed that both *T. bournieri* and *C. sesamiae* preferred volatiles from four of the five wild teosinte species, five landraces and one of two maize hybrids exposed to egg deposition. Similarly, volatiles collected from unoviposited maize landrace plants exposed to oviposited landrace maize plants emitting oviposition-induced volatiles, were attractive to both egg and larval parasitoids. Moreover, maize varieties emitting these oviposition-induced volatiles deterred further herbivore colonization and suppressed larval development. Volatile analysis by GC and GC-MS revealed marked increases in volatile emission as well as qualitative changes in the odour blends in four wild types, five landraces and one hybrid, following stemborer oviposition. Coupled GC-EAG analysis of attractive samples revealed that C. sesamiae was responsive to (E)-2-hexenal, (Z)-3-hexen-1-ol, nonane, 6-methyl-5-heptene-2-one, α-pinene, myrcene, limonene, (E)-4,8-dimethyl-1,3,7-nonatriene, decanal, 3,4-dimethylacetophenone and (E)-β-farnesene. Results from this study provide insights into tritrophic interactions thus paving the way for designing novel and ecologically sound pest management strategies through breeding crops with this novel oviposition-induced defence trait.

Key Words: *Chilo partellus*, indirect defence, maize, oviposition, parasitoids.

UITTREKSEL

Mielies, ‘n geneties-diverse gewas, is die 3^{e} grootste landbougewas ter wêreld en die belangrikste gewas in sub-Sahara Afrika waar dit 80% van die kalorie-inname verskaf. Die stamboorder, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is ‘n belangrike plaag van mielies in kleinboerstelsels waar dit oesverlies van tot 80% veroorsaak. Navorsing het getoon dat voeding deur herbivore mielieplante induseer om vlugtige stowwe vry te stel wat natuurlike vyande aanlok, maar eers wanneer skade reeds aangerig is. Onlangse navorsing het bevind dat eierlegging op mielieplante van Meso-Amerikaanse landrasse lei tot vrystelling van vlugtige stowwe wat aanloklik is vir parasitoïde van *C. partellus,* ‘n voorheen onbekende eienskap van mielies. Dit is egter onbekend of daar variasie is in hierdie indirekte verdedigingseienskap tussen mielievariëteite wat aangepas is by plaaslike omstandighede en of die prosesse van domestikasie en plantteling hierdie eienskap beïnvloed het. Dit is ook onbekend of variëteite wat hierdie verdedigingseienskap besit in staat is om verdere herbivoor-kolonisasie af te weer, of dit larvale ontwikkeling van die herbivoor beïnvloed, en of hierdie plante dieselfde eienskap kan induseer in naburige plante wat nie besmet is nie. Hierdie studie poog om kennisgapings aan te spreek ten einde plantverdedigingsmeganismes te benut in ontwikkeling van ekologies-verantwoordbare gewasbeskermingstrategieë. Versameling van vlugtige stowwe is gedoen vanaf wilde mielietipes, landrasse en bastermielies waarop *C. partellus* motte eiers gelê het en dit is vergelyk met vlugtige stowwe vanaf onbesmette plante. Chemiese analises is gedoen d.m.v. gas-kromatografie (GK), gekoppelde-GK massa-spektrofotometrie (GK-MS) en gekoppelde GK-elektroantennografie (GK-EAG). Gedragseksperimente is gedoen met eier- (*Trichogramma bournieri* Pintureau (Hymenoptera: Trichogrammatidae)) en larfparasitoïde (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) in 4-arm-olfaktometers met vlugtige stowwe wat vanaf plante versamel is. Larfvoorkeur, groei en ontwikkeling asook daaropvolgende eierleggingsgedrag van *C. partellus* motte op hierdie plante is ook bepaal. Gedragstudies toon dat beide *T. bournieri* en *C. sesamiae* die vlugtige stowwe van vier van die vyf wilde teosinte spesies, vyf landrasse en een van twee bastermielies verkies waarop *C. partellus* eiers gelê het. Daar is ook bevind dat eier- en larfparasitoïde die eierlegging-geïnduseerde vlugtige stowwe verkies van onbesmette mielie-landrasplante wat blootgestel was aan plante waarop voorheen eiers gelê is. Mieliekultivars wat eierlegging-geïnduseerde vlugtige stowwe vrystel het ook verdere eierlegging afgeweer en larvale ontwikkeling onderdruk. Analises van vlugtige stowwe d.m.v. GK en GK-MS het aangetoon dat *C. partellus* eierlegging lei tot aansienlike toename in vrystelling van vlugtige stowwe asook kwalitatiewe veranderinge in die reukprofiele van vier van die wilde-tipe mielies, vyf landrasse en een mieliebaster. Gekoppelde GK-EAG analises van monsters wat *C. sesamiae* individue aanlok, het getoon dat die aktiewe verbindings waarop hierdie spesie reageer die volgende is: (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, nonaan, 6-metiel-5-hepteen-2-oon, α-pineen, mirseen, limoneen, (*E*)-4,8-dimetiel-1,3,7-nonatrien, dekanaal, 3,4-dimetielasetophenoon en (*E*)-β-farneseen. Hierdie resultate verskaf insigte rakende tritrofiese interaksies en fasiliteer verdere ontwikkeling van ekologies-verantwoordbare plaagbestuurstrategieë deur die teling van gewasse met hierdie eierlegging-geinduseerde plantverdedigingseienskap.

Slutelwoorde: *Chilo partellus,* indirekte verdediging, mielies, eierlegging, parasitoïde.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Cereal crops, particularly maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are vitally important sources of food for humans and livestock in sub-Saharan Africa (Harris & Nwanze, 1992; Polaszek & Khan, 1998). These crops contribute significantly both to local and national economies (Smith & Wiedenmann, 1997). Maize is the third largest cereal crop in the world and the most important crop in sub-Saharan Africa supplying 50% of the calory intake in this region (Oluwafemi *et al*., 2013). Cultivated maize is the domesticated variant of teosinte (Wang *et al*., 1999) which originated from Mesoamerica and by the 16th century was already cultivated in parts of sub-Saharan Africa (Polaszek & Khan, 1998). In the sub-Saharan region, maize is mainly grown by millions of resource-constrained farmers under smallholder systems (Odendo *et al*., 2001). For a long time, many countries in sub-Saharan Africa have remained net importers of maize. This is attributed to a rapidly growing population and stagnating yields over the years (FAO, 1999). It is forecasted that by year 2020, the global demand for maize will have grown by 45% of which 72% will be in developing countries and only 18% in the developed nations (James, 2003). In order to deal with this surging demand, new production methods need to be developed while reinforcing the existing ones to better manage the myriads of problems facing maize production in sub-Saharan Africa (FAO, 2002). Sub-Saharan countries such as Kenya, South Africa, Tanzania and Nigeria are principal producers of maize but it is only South Africa that regularly exports maize (Polaszek & Khan, 1998). Maize yields in Africa are generally low, averaging less than half of Asian and Latin American yields (Polaszek & Khan, 1998). The average yield for industrialized countries is 6.2t/ha compared to only 2.5 t/ha for developing countries and less than 2.0t/ha for sub-Saharan Africa (Inside Track, 2013).

There has been a significant advancement in technology towards maize production in Africa. However, despite this advancement, the productivity is hampered by several abiotic and biotic constraints, which may cause losses up to 80% (Pingali & Pandey, 2001). Abiotic constraints in eastern Africa are mostly due to seasonal unreliability of rain-fed agriculture, poverty and limited access to remedial inputs. In the densely populated areas of eastern Africa that have a high yield potential, maize is grown on the same plot year after year due to population pressure and land constraints. This has led to steady decline in soil fertility and a net reduction in yields (FEWS, 2008). In Kenya, only about 2% of arable land is farmed under irrigation systems while the rest of farming is rainfall dependent. This over-reliance on rainfall for production poses a major hindrance to sustainable maize production because the rainfall is often low and unreliable (FAO, 2004).

Biotic stresses, which include diseases, weeds and pests are ever present and require effective management processes to support productivity and environmental protection. Among the various insect pests attacking maize in Africa, lepidopteran stemborers are the most destructive causing severe damage to the crop (Ingram, 1958; Youdeowei, 1989; Kfir *et al*., 2002). Yield losses ranging between 10% and 75% have been recorded on maize and sorghum depending on cultivar, phenological stage of plant at infestation, infestation level, agro-ecological zone and prevailing environmental conditions (Kfir *et al*., 2002). In Kenya, losses due to stemborer damage fluctuate between 10-12% in high-potential areas and 15-21% in low-potential areas (De Groote, 2002). Thus, these insect pests present a major constraint to maize production in areas where they are abundant (Youdeowei, 1989). In addition to cultivated Poaceae such as maize, sorghum and millet, stemborers have also been recorded in a wide range of wild grasses belonging to the Poaceae, Cyperacae and Typhaceae (Nye, 1960; Khan *et al*., 1997; Le Ru *et al*., 2006; Moolman *et al*., 2014).

Stemborers in Africa are generally considered to be geographically widespread. Approximately 21 economically important species of stemborers occur in Africa, belonging to either Crambidae, Noctuidae or Pyralidae families (Seshu Reddy, 1983; Harris, 1990; Maes, 1998). In East Africa, there exists a complex of 12 species of stemborers attacking cereal crops with the crambids *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand), the noctuids *Busceola fusca* (Fuller) and *Sesamia calamistis* Hampson and the pyralid *Eldana saccharina* (Walker) being among the economically important and widely distributed species (Nye, 1960; Youdeowei, 1989). Of this complex, the exotic *C. partellus* is the most damaging lepidopterous pest of maize in eastern and southern Africa causing yield losses of up to 88% (Kfir *et al*., 2002).

1.2 Problem statement

Cereal crops present enormous opportunities, both in terms of income and food for improving the livelihood of many smallholder farmers in sub-Saharan Africa. The production of cereal crops is severely constrained by lepidopteran stemborers with reported yield loss due to these pests ranging between 20-80%. Thus crop infestations by these pests can lead to food insecurity in Sub-Saharan Africa where cereals are the main food crops. Efforts to control these pests through chemical pesticides are hampered by development of resistance and elimination of natural enemies which can lead to secondary pest outbreaks. The high costs of chemical control, especially for farmers in the subsistence farming environment, and the cryptic and nocturnal habits of the adult moths and the protection provided to larvae by the stem of the host crop further limits control of these pests. At the same time, the effectiveness of the cultural control methods employed alone cannot empirically keep these pests below economic injury levels. Therefore, the management of these lepidopteran pests requires sustainable methods which involve an integrated approach. Plants have evolved innate defence mechanisms against herbivores. Whereas breeding for higher yield and grain quality may have compromised the innate plant defence systems of conventional cereal hybrids there are some maize varieties that possess innate defence mechanisms against attacking herbivores. There is therefore the need to select maize varieties possessing these innate defence traits to exploit these novel traits in integrated cereal stemborer control strategies.

1.3 Justification of the study

As polyphagous pests attacking a wide range of both cultivated and wild plants belonging to Poaceae, Cyperaceae and Typhaceae, stemborers can cause devastating yield losses if not controlled. Being widely distributed in Africa both the indigenous and invasive species of stemborers can cause almost total crop loss if not controlled. These insect pests are difficult to control by chemical means. Integrated pest management approaches like stimulo-deterrent diversionary tactics through habitat diversification provide a good approach especially to smallholder resource-poor farmers of sub-Saharan Africa. Plants have evolved a wide range of defensive tactics to protect themselves against attack by herbivores. These tactics may involve emission of repellent compounds as well as recruitment and sustenance of natural enemies to a damaged plant through its induced volatiles. Many wild relatives and landraces of grass species from which crop plants and fodder crops have been selected continue to survive today. These may possess defence traits that are absent in mainstream crop cultivars, and which might have been lost in the due course of breeding as other traits such as yield and grain quality were considered (Migui & Lamb, 2003; Köllner *et al*., 2008). Recently, maize landraces of Mesoamerican origin have been shown to produce volatile compounds that attract egg and larval parasitoids in response to egg deposition by a stemborer herbivore (Tamiru *et al*., 2011). African open pollinated maize varieties that are locally adapted to local agroecosystems and are grown approximately by 80% of smallholder farmers (Odendo *et al*., 2001) may present a good opportunity for stemborer control if they possess innate defence mechanisms, inducible by moth oviposition like the Mesoamerican landraces, since they are already adapted to the adverse climatic conditions of the region. There is therefore the need to investigate this trait in locally adapted maize varieties both open pollinated and hybrids with a view of exploiting these inherent plant defences for pest management. Additionally, for a better understanding of the ecological relevance and evolutionary history of herbivore-induced plant signalling, it is necessary to study these signals in the wild systems. It is therefore prudent to explore tritrophic interactions of the wild ancestor of maize, teosinte, and stemborer oviposition signals as these can lead to making better use of the indirect defence traits when selecting new crop varieties. Plants are known to 'eavesdrop' on the volatile signals from attacked plants through airborne signalling (Chamberlain, 2014). As such it is prudent to investigate whether oviposition-induced volatile compounds emitted by maize plants that were ovipositied on can induce the same indirect defence into neighbouring intact maize plants which can help increase the signal strength and foraging efficiency of the parasitoids. Poaceous plants are known to produce secondary defence metabolites that play an important role in defence against bacteria, fungi and insects (Erb *et al*., 2009). However, no studies have been done on the constitutive larval suppression and deterrence of further herbivore colonization on maize varieties emitting egg-induced volatiles. Therefore, there is need to fully explore the early-herbivory inducible defence traits and select crops that are able to adjust their innate defences by adjusting metabolism of their compounds in response to initial stage of herbivore attack. This will provide important key to the development of new crop protection strategies based on switching on of inherent plant defences through either companion cropping or incorporation of the these traits into crops lacking these traits. As such, this study aimed at investigating the inherent defence traits in maize and their tritrophic interactions with a view of utilizing these innate defence traits for the development of an integrated approach for cereal stemborer control.

1.4 Objectives

1.4.1 General objective

The main objective of this study was to exploit innate plant defences in development of an integrated pest management approach for cereal stemborers

1.4.2 Specific objectives

This study had four specific objectives, namely:

1. to investigate ovipostion-induced indirect defence traits in the wild ancestor of maize, teosinte,
2. to determine prevalence of oviposition-induced indirect defence traits amongst African maize landraces and hybrids,
3. to determine induction of indirect defence traits by oviposition-induced maize volatiles to a neighbouring maize plant,
4. to determine *Chilo partellus* moth and larval behaviour, growth and development on different maize landraces possessing egg-inducible defence traits.

1.5 Hypotheses

1. Oviposition-induced semiochemical emission in maize is an ancestral trait present even in pre-domestication wild maize,
2. The oviposition-induced volatile emission indirect defence trait is prevalent amongst open pollinated maize varieties grown by smallholder farmers in East Africa,
3. Although breeding may have caused loss of egg-induced semiochemical emission defence traits in hybrid maize, some improved maize lines possess this indirect defence trait,
4. Oviposition-induced maize volatiles can induce emission of volatiles attractive to parasitoids in neighbouring intact conspecific maize plants,
5. Both *Chilo partellus* moth and larvae show different behavioural and physiological responses to maize varieties possessing egg-induced indirect defence traits in comparison to varieties lacking this trait.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Economic importance of *Chilo partellus*

In smallholder farmers' fields in Sub-Saharan Africa, maize yield losses due to this pest range between 20-88% (Kfir *et al.*, 2002; Khan *et al*., 2008a). The larval stage is the destructive stage of the pest. Crop losses are caused by feeding and stem tunnelling by larvae which results in destruction of growing point, stem breakage, disruption of nutrient translocation, stunting, lodging and direct damage to ears (Polaszek, 1998; Kfir *et al*., 2002) (Plate 2.1). Stemborer infestation may also enhance incidence and severity of stalk rots (Bosque-P'erez & Mareck, 1991). In addition to maize and sorghum, this pest is also known to attack other important crops such as pearl millet, finger millet, rice, wheat, sugar cane, foxtail and various grass species including Sudan grass and Napier grass (Khan *et al*., 2000; Matama-Kauma *et al*., 2008).

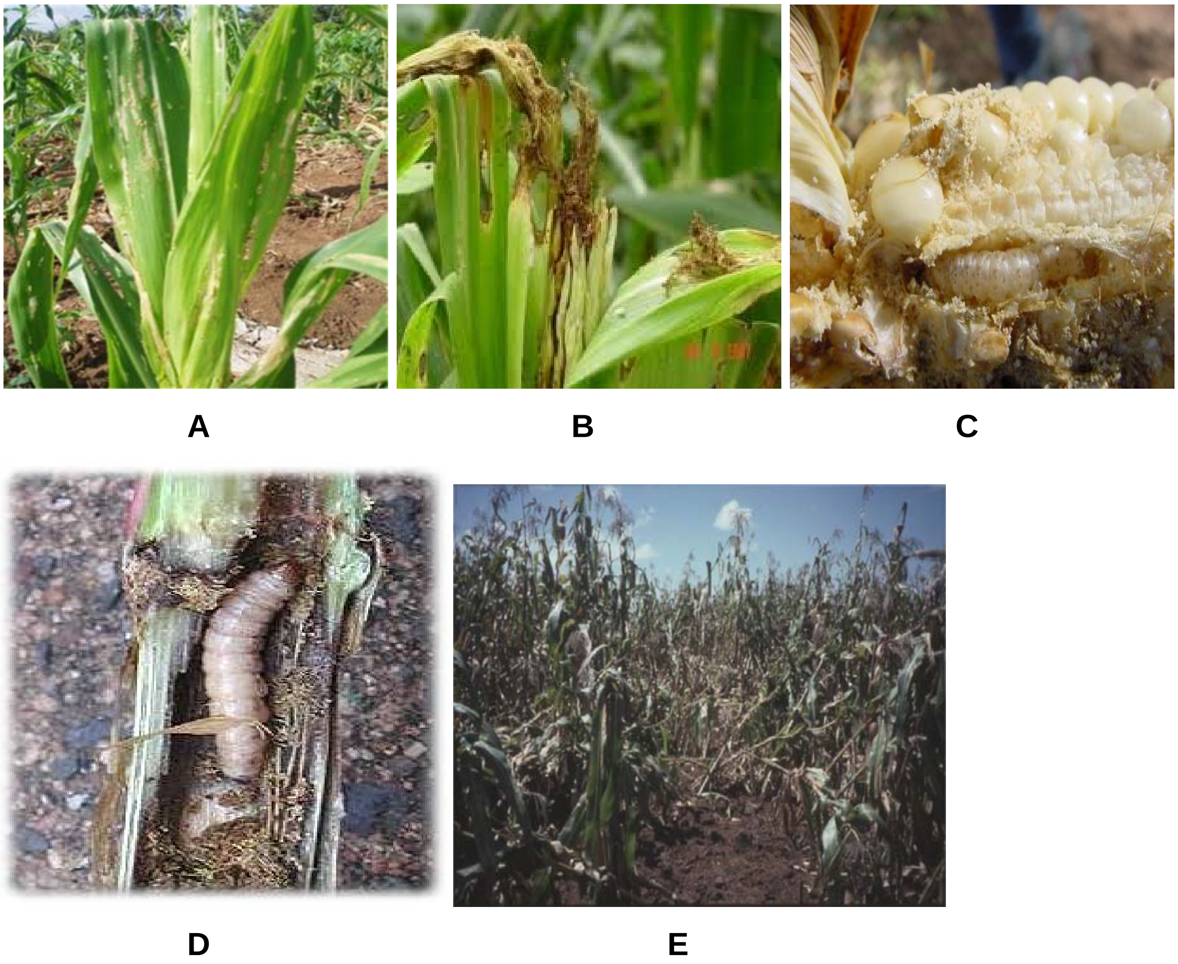

Plate 2.1: Damage caused by *Chilo partellus* on different maize parts: (A) leaves (B) tassel (C) ear (D) stem and (E) lodging damage (source: http://www.infonet-biovision.org)

2.2 Distribution of *Chilo partellus* in Africa

Chilo partellus is an exotic species of Asian origin. It was first reported in Africa in 1928 in Malawi (Tams, 1932), then 1953 in Tanzania (Duerdon, 1953) and has since spread to most countries in eastern and southern Africa (Sithole, 1990; Kfir, 1998; Kfir *et al*., 2002) (Fig. 2.1). The predicted eventual distribution included several countries in south-western and western Africa where the pest is not yet known to occur (Overholt *et al*., 2000). This invasive stemborer has proved to be a highly competitive colonizer in many areas it has invaded in eastern and southern Africa, often becoming the most injurious stemborer (Kfir, 1997; Seshu Reddy, 1983), displacing native species (Kfir, 1997; Overholt, 1998). In Coastal Kenya, there is evidence that *C. partellus* has partially displaced the indigeneous stemborer, *C. orichalcociliellus* (Overholt, 1998; Ofomata *et al.*, 1999a; Ofamata *et al*., 1999b; Ofomata *et al*., 2000). However, *C. orichalcociliellus* continues to be found at a relatively high frequency (10-30% of the stemborer complex), which suggests displacement of *C. orichalcociliellus* will not proceed to extirpation (Zhou *et al*., 2001). Investigations have found that *C. orichalcociliellus* completed development in two native grasses, in which *C. partellus* could not develop (Ofamata *et al*., 2000), which may be one factor that allows continued co-existence. In Eastern part of Kenya, *C. partellus* was present in the early 1980s but was less abundant than *B. fusca* (Seshu Reddy, 1983). However, in the same area in the period 1996-1998, *B. fusca* was rare and *C. partellus* was dominant (Songa, 1999). The pest has also been known to co-exist in many areas with *B. fusca*, in the moist mid-altitude and moist transitional agroecological zones (Polaszek, 1998; Abate *et al*., 2000).

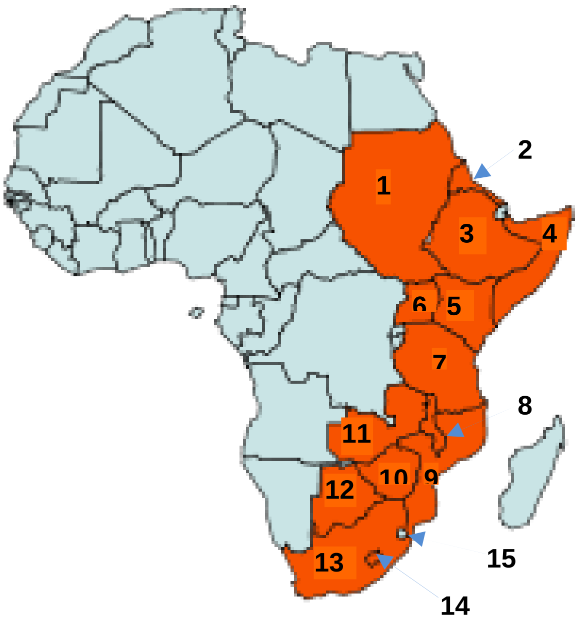

Figure 2.1. Geographical distribution of *Chilo partellus* in Africa (orange area). Countries marked are: (1) Sudan, (2) Eritrea, (3) Ethiopia, (4) Somalia, (5) Kenya, (6) Uganda, (7) Tanzania, (8) Malawi, (9) Mozambique, (10) Zimbabwe, (11) Zambia, (12) Botswana, (13) South Africa, (14) Lesotho and (15) Swaziland (adapted from http://www.infonet-biovision.org).

2.3 Biology of *Chilo partellus*

Like other stemborers, *C. partellus* is a holometabolous insect (Fig. 2.2). Adults emerge from pupae late afternoon and early evening and are active at night. Mating usually takes place soon after the female emerges. A gravid female lays up to 350 eggs in batches of 10-80 eggs on the upper and underside of leaves mainly near the midribs. Female moth prefers the young stage of 3-4 weeks old maize and oviposits for a period of 3-4 subsequent nights. The eggs hatch into larvae in 4-10 days depending on prevailing environmental conditions, after which they move upward on the plant to begin feeding in the leaf whorl. After a few days, mid to late instar larvae leave the leaf whorl and tunnel into the stem where they feed and grow for 2-3 weeks. The larvae may also move outwardly and bore into the stem just above an internode and maize ears. When the larvae are fully grown, pupation occurs inside the stem for 6-14 days. An adult emerges from the pupa, mates and lays eggs on plants again to continue their life cycle and damage to the plant. The whole life cycle takes 25-60 days depending on temperature and other prevailing environmental conditions (Kfir *et al*., 2002; http://www.infonet-biovision.org).

During a growing season, five or more successive generations of *C. partellus* may develop depending on the climatic conditions and availability of host plants (Polaszek, 1998). In warm low-altitude regions with ample hosts, *C. partellus* will reproduce and develop throughout the year. However, fully grown larvae may enter a resting period towards the end of cropping season in an area with long dry periods and will pupate with the onset of rains (Aghali, 1985; Harris, 1990; Kfir *et al*., 2002).

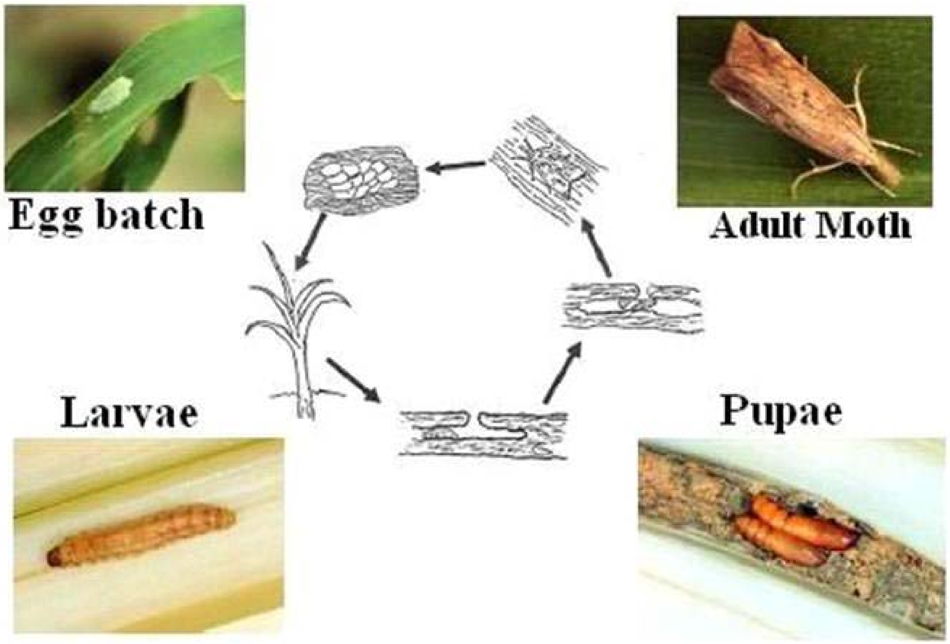

Figure 2.2. Life cycle of *Chilo partellus* (adapted from push-pull.net, ICIPE)

2.4 Management strategies for stemborers

To suppress lepidopteran stemborer damage, various control strategies have been developed. These range from chemical, cultural, and biological control to host-plant resistance which can either be used singly or in an integrated strategy (Bosque-Perez, 1995; Kfir *et al*., 2002).

2.4.1 Chemical control

Chemical control forms the basis of pest control in commercial farming systems. Based on the knowledge regarding the ecology and larval behaviour of stemborers such as *Chilo* species, insecticide application can be used with great success in controlling these pests (Polaszek, 1998). The identification of the most susceptible stage in the stemborer life cycle is important to ensure timely and effective chemical control. The application of insecticides according to economic threshold levels rather than on a fixed schedule basis may reduce the number of insecticide applications or at least ensure that the number of applications is economically viable (Dent, 1991). This therefore requires the monitoring of the stemborer populations. Pheromone-baited traps are useful devices for monitoring moth population levels of stemborers. Moth catches can provide useful information for timing of insecticide applications (Van Rensburg *et al*., 1985; Revington, 1987; Van Rensburg, 1997).

Although the use of insecticides can be of benefit to farmers in the short term, the use thereof has not been without the problems. Recommended chemical control strategies are often not practical and economical to smallholder farmers (Van Rensburg *et al*., 1988). Additionally, the adverse effects on non-target species including the stemborer natural enemies, insecticide resistance, hazards of pesticide residues in the environment and direct health risks to sprayers make the use of chemicals not viable in the long run (Minja, 1990; Bruce *et al*., 2010).

Biopesticides and natural products can also be used to control stemborers. Traditionally, smallholder farmers in Africa have been using botanical extracts to protect their crops from pest damage (Polazsek, 1998). Trials carried out with various botanical extracts such as *Azadirachta indica* A. Juss (neem), *Tephrosia vogelii* (Hemsley) A. Gray, *Neurautanenia mitis* (A. Rich) Verdc, *Cassia didymobotrya* Fresen, *Phytolacca dodecandra* L., *Schinus molle* L., *Lantana camara* L., *Tephrosia vogelii* Hook and *Tagetes minuta* L. have shown potential in controlling stemborers (Mallya, 1986; Marandu *et al*., 1987; Polaszek, 1998; Ogendo *et al*., 2013).

2.4.2 Cultural control

This is the most relevant and economic stemborer control strategy available for resource-poor farmers in Africa. Various methods of cultural control of stemborers in Africa have been investigated (Lawani, 1982; Minja, 1990; Seshu Reddy, 1985; 1990; Van den Berg *et al*., 1998). It is considered the first line of defence against pests and includes techniques such as destruction of crop residues, intercropping, crop rotation, manipulation of planting dates and tillage methods (Van den Berg *et al*., 1998; Kfir *et al*., 2002). In the past decade, scientists have exploited the rich botanical biodiversity in Africa and developed cropping systems that have been able to control stemborers and parasitic weeds. A case example is the pro-poor ‘push-pull’ strategy developed at ICIPE which has been adopted by thousands of smallholder farmers in East Africa (Khan *et al*., 1997b; 2008b; 2014). In this system, certain companion crops are grown in between and around the main crop. These companion crops release semiochemicals that repel stemborers from the main crop using the intercrop which is the ‘push’ component and attract stemborers away from the main crop using a trap crop which is the ‘pull’ component (Cook *et al*., 2007).

Several factors limit the reliance of some of the cultural practices in controlling stemborers. Employed alone, most of these practices are unable to keep stemborers below economic injury levels. Destruction of crop residues by burning leads to loss of organic matter, beneficial soil microorganisms and exposes farms to severe soil erosion from wind and rains (Van den Berg *et al*., 1998). Besides, crop residues have multiple uses in mixed smallholder systems and their destruction is not feasible. Intercropping, crop rotation and early planting have been practiced by farmers across Africa but studies show that their impact in stemborer control is limited (Skovgård & Päts, 1996). In subsistence farming systems in Africa where farmers intercrop cereals with other crops and lack of water is a major constraint, manipulation of sowing dates and management of plant densities is not always practical as farmers often plant after the first rains (Van den Berg *et al*., 1998). Cultural control entails labour intensive practices and implementation of these practices is always a challenge to farmers (Van den Berg *et al*., 1998). For cultural control to be effective, co-operation of farmers within a particular area is required because moths emerging from untreated fields can infest adjacent crops. Cultural control is severely constrained by lack of management capabilities among farmers especially in areas where farmers lack the support of adequate extension services (Harris, 1989).

2.4.3 Biological control

Biological control involves use of living organisms antagonistic to stemborers to suppress their population. Several indigenous predators such as ants (*Tetramorium guineense*, *Pheidole megecephala*, *Cardiocondyla badonei*, *C. emeryi, Camponotus* sp. and *Dorylus* sp.), earwigs (*Diaperasticus erythrocephalus* and *Forticula* spp.), *Chrysopa* sp., ladybird beetles (*Cheilomenes* sp.) and several spiders have been shown to predate on stemborer eggs and neonate larvae (Girling 1978; Leslie 1988; Dwumfour, 1990; Greathead 1990). However, later stages which feed in a protected environment inside the stem are less vulnerable to predation. It has been reported that indigenous predators alone are not able to keep stemborer populations below economic injury levels (Skovgård & Päts, 1996; Bonhof, 2000).

A complex of native parasitoid species attack stemborers in Africa, including species that attack eggs, larvae and pupae. However, native parasitoids such as *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) in most cases do not seem to be able to maintain stemborer populations at economically acceptable levels (Williams, 1983; Oloo, 1989; Kfir, 1992; Kfir & Bell, 1993; Overholt *et al*., 1994). Indigeneous parasitoids may have a greater impact on stemborer populations residing in wild-grass communities than on the populations that periodically invade annual crops (Conlong, 1994). Over the years, efforts have been made to introduce the exotic parasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for control of *C. partellus* in Africa (Overholt, 1993). Since its first release in Kenya in 1993, the parasitoid has established in Kenya, Tanzania, Uganda, Zambia and Ethiopia and has caused a 32-55% reduction in stemborer densities (Kfir *et al*., 2002; Getu *et al*., 2003). The biological control of stemborers is only partially successful if employed alone (Kfir *et al*., 2002) hence the need for other methods that can control stemborers with more success or those that can integrate with biological control. A compelling motivation for adoption of biological control is the potential for permanent return to ecological conditions similar to those seen prior to the arrival of the invasive pest, no harm to the environment and a reduced ongoing expenditure on pesticides, labour and specialised equipment (Hoddle, 2004).

2.4.4 Host plant resistance

This method exploits intrinsic plant resistance to pests. It is generally farmer friendly, economically feasible and ideal pest control option, posing no environmental hazard and being generally compatible with other control methods (Singh *et al*., 1983; Nwanze, 1997). Several mechanisms are utilized by resistant maize cultivars against the attack by stemborers. These include non-preference for oviposition, reduced feeding and tunnelling, tolerance to leaf and stem damage, antibiosis and antixenosis (Polaszek, 1998). A holistic breeding strategy which aimed at developing varieties resistant with acceptable agronomic characteristics, yield and resistance to major diseases, yielded moderate resistance to stemborers in West Africa (Bosque-Pérez *et al*., 1997; Schulthess & Ajala, 1999). Use of recombinant biotechnological techniques which allows introgression of genes of unrelated organisms into plants has resulted in the development of genetically modified insect resistant maize varieties. The most well known of these are *Bt* maize which is resistant to stemborers. This maize was produced by introduction of genes from the soil bacterium *Bacillus thuringiensis* into the host plant genome. The Bt gene encodes for production of toxic proteins that, when ingested, kill stemborer larvae. The transformed maize plant produces the same toxic proteins thus conferring resistance against lepidopteran pests (Estruch *et al.*, 1997). However, the transgenic approach is controversial due to the possibility of ecological and toxicological side effects (van Emden, 1999). Conventional breeding approaches for host-plant resistance are still ongoing and efforts are underway to develop insect resistant maize varieties for sub-Saharan Africa (KARI & CIMMYT, 2007). Incorporation of innate plant defences into these breeding programs for insect resistance can provide a sustainable approach without the ecological interference.

2.5 Innate plant defence against herbivory

Although lacking an immune system comparable to animals, plants have developed a remarkable array of structural and chemical defences designed to detect and stop attacking organisms before they are able to cause extensive damage. These defences can be classified generally as constitutive or induced (Traw & Dawson, 2002). Constitutive defences are always present in the plant, while induced defences are produced (locally or systematically) and mobilized to the site where a plant is injured. In terms of composition and concentration of constitutive defences, there exists a wide variation ranging from mechanical to chemical defences such as toxins and digestion inhibitors. Constitutive defences require large amounts of resources to produce and are difficult to mobilize (Traw & Dawson, 2002). Many of external mechanical and large quantitative defences fall under this category.

Induced defences include plant secondary metabolic products as well as morphological and physiological changes that occur upon herbivory and which are detrimental to the herbivores (Karban *et al*., 1997). As opposed to constitutive defences, inducible defences have an advantage in that they are produced only when needed and are therefore potentially less costly, especially when herbivory is variable (Karban *et al*., 1997). Many plants produce secondary defence metabolites in response to herbivory that influence the behaviour, growth or survival of the herbivores. These chemicals can act as repellents or toxins to herbivores or reduce plant digestibility (Duffey & Stout, 1996; De Moraes *et al*., 2001; Kessler & Baldwin, 2001).

Indirectly, plants also protect themselves by emitting semiochemicals that attract the natural enemies of the herbivores (Dicke & van Loon, 2000; Heil, 2008). Indirect plant defences such as semiochemical emissions have been exploited in the development of various pest management strategies that are sustainable, environmentally benign and low-cost alternatives to the use of pesticides.

2.6 Semiochemicals

Semiochemicals (Greek *semeon*, a sign or signal), are natural organic compounds that transmit chemical messages (Nordlund and Lewis, 1976). They are also known as behaviour-modifying chemicals and convey a signal from one organism to another so as to modify the behaviour of the recipient (Law & Regnier, 1971; Dicke & Sabelis, 1988).

They are emitted by one individual and cause a behavioural response in another without having direct effect on physiology of the receiving organism other than interacting with sensory systems (Howse *et al*., 1998). Semiochemicals can be volatile or non-volatile. Volatiles semiochemicals are perceived through olfaction while non-volatile ones are perceived through contact chemoreception.

Based on effect, semiochemicals are broadly classified into two major categories, namely pheromones and allelochemicals. In terms of structure, they can be classified into 24 categories according to functional groups. Pheromones mediate intraspecies interactions and include aggregation pheromones (organic compounds that attract and increase the concentration of insects at the pheromone source); alarm pheromones (they stimulate the insect's escape or defence behaviour) and sex pheromones (chemical signals that help in mate location). Others include trail pheromones which are used mainly by social insects to mark the way to a food source and marking pheromones, organic compounds used by insects to mark territorial boundaries or by ovipositing females to deter conspecifics from ovipositing at the same site (Nordlund, 1981).

Allelochemicals, on the other hand mediate interspecies communication. They are classified into allomones, kairomones or synomones. Allomones benefit the producer but not the receiver while kairomones are beneficial to the receiver. Synomones are beneficial to both the producer and the receiver. Plants make use of allelochemicals to modify their interactions with other organisms including beneficial insects and harmful herbivores (Nordlund, 1981).

2.7 Utilization of semiochemicals in pest management

Plants use semiochemicals to modify their interactions with other organisms including beneficial and harmful insects. This knowledge has been exploited in the development of alternative pest management strategies preferably due to its non-toxic mode of action, cost-effectiveness, environmental friendliness and the possibility of integrating it with other control options (Khan *et al*., 2010). In the past decades, scientists have made tremendous efforts to develop pest control methods that make use of semiochemicals. Several pest management strategies including the “push-pull” or stimulo-deterrent diversionary strategy have been developed in which semiochemicals are used (Kfir *et al*., 2002; Cook *et al*., 2007; Degenhardt *et al*., 2009).

2.7.1 Utilization of pheromones

Being species-specific, pheromones have been used as a pest management tool in three ways, namely monitoring, mass trapping and mating disruption (Ridgeway *et al*., 1990). Pheromone-baited traps have been used for surveying and monitoring the presence or absence of pests. Monitoring of pest population is the cornerstone in integrated pest management since it informs the decision of applying control measures when the pest population reaches an economic injury level. Monitoring also helps farmers to detect migration of migratory pests and predict their eventual outbreaks. Since pheromone traps are species specific, they provide an accurate monitoring tool by catching only the target insect pests. Pheromone traps are available for many insect pests including lepidoterans, dipterans and coleopterans. These traps are baited either with pheromone gland extracts or synthetic pheromone compounds (Carde, 1976). Lepidopteran pheromone traps were among the first traps to be developed since lepidopteran sex pheromones were among the first to be identified and synthesised. A good example is the use of sticky traps baited with synthetic of the natural pheromone, a 9:1 admixture of *cis-11* and *cis-9*-tetradecenyl acetate, in monitoring summer fruit tortrix moth (*Adoxophyes orana* Fischer (Lepidoptera: Tortricidae)), a serious pest of apples in Netherlands (Minks & Voerman, 1973). This resulted in a marked change of insecticide application which was previously determined by calendar date (Minks, 1975). Fewer insecticide applications with more control effectiveness were realised (Minks, 1975).

In addition to pest monitoring, pheromone-baited traps can be used in direct pest control through attraction-annihilation mass trapping systems. Suppression of pest populations has been achieved in areas where mass trapping has been deployed with the correct pheromone and desirable number of traps. These include area wide mass trapping of codling moth in pear and apple orchards where the pheromone-baited traps were mass deployed leading to reduced populations of the moths (Weissling & Knight, 1994).

Indirectly, pheromone-baited traps can be used to reduce pest populations through the disruption of mating using the sex pheromones (Wright, 1964). Sex pheromones for several stemborers including *C. partellus, B. fusca, S. calamistis, S. cretica, S. naonagroides* and *C. ignefusalis* have been identified, synthesised and are commercially available (Youm & Beevor, 1995; Van den Berg & Nur, 1998). Mating disruption can provide effective stemborer reduction of mating thus reducing population density (Campion & Nesbitt, 1983).

2.7.2 Plant semiochemicals and their utilization in pest management

In nature, especially flowering plants continuously produce an array of chemical compounds. The emission of these chemical substances is not necessarily related to abiotic or biotic environmental stress, since intact plants, which are under no stress also emit chemicals (Cole, 1980; Agelopoulos & Keller, 1994; Loughrin *et al*., 1996; McAuslane & Alborn, 1998). These chemicals, produced from flowers, leaves, stem or roots can be volatile or non-volatile organic compounds (Knudsen *et al*., 1993).

Volatile organic compounds (VOCs) are synthesised and emitted by plants from vegetative plant parts, flowers and roots (Knudsen *et al*., 1993; Steeghs *et al*., 2004; Rasmann, *et al*., 2005). These VOCs, being typically lipo-philic compounds with high vapour pressures, often evaporate into the atmosphere across the plant membrane from their intracellular sites of synthesis when there are no barriers to diffusion (Goodwin *et al*., 2003; Pichersky *et al*., 2006). There exists variability in the types and amounts of volatiles emitted between and amongst plant genera. Some volatiles are commonly produced by all species while others are specific to only one or a few related taxa (Pichersky & Gershenzon, 2002). These volatile chemicals play an important ecological role mediating a range of interactions including multitrophic interactions such as plant-plant, plant-microbe, plant-herbivore and inter-trophic interactions between the plant and organisms acting beneficially for the plant like pollinators and herbivores' natural enemies (Vet & Dicke, 1992; Bernays & Chapman, 1994; Langenheim, 1994; Dudareva *et al*., 2004). Thus, there is an intricate interaction 'arms race' between plants, pollinators, herbivores and herbivores' natural enemies as they have been coevolving for almost 400 million years, resulting in inter-trophic 'conversations' (Metcalf & Metcalf, 1992; Cook *et al*., 2007). Organisms including herbivores in these inter-trophic interactions are able to detect specific semiochemicals or specific ratios of these semiochemicals which induce behavioural modification of the perceiving herbivores, for example attraction or repellency (Pickett *et al*., 2006; Bruce & Pickett, 2011). Researchers have utilized these behaviour modifying semiochemicals released by intact plants to develop habitat diversification crop protection strategies against injurious herbivorous pests. Such novel crop protection approaches include the stimulo-deterrent diversionary or 'push-pull' strategy for cereal stemborer control in East Africa (Khan *et al*., 2000).

2.7.3 Stimulo-deterrent diversionary (Push-Pull) strategy

This is a cropping system in which specifically selected crops are grown in between and around the main crop. This technology exploits semiochemicals produced by the companion crops. The companion crops are chosen based on emission of specific semiochemicals that have an effect in the plant-herbivore interaction. The companion plants release semiochemicals that repel insects pests away from the main crop (push component) and attract insect pests away from the main crop (pull component) (Miller & Cowles, 1990; Cook *et al*., 2007). A very successful push-pull strategy for control of cereal stemborers in smallholder systems in eastern Africa has been developed by ICIPE, Rothamsted research and partners and has been adopted by farmers (Khan *et al*., 2010; 2014). This technology involves intercropping a cereal crop, maize or sorghum with desmodium (silverleaf, *Desmodium uncinatum* DC and greenleaf, *D.intortum* (Mill) Urb) and molasses grass, *Melinis minutiflora* P. Beauv as intercrops. Napier grass, *Pennisetum purpureum* Schumach and Sudan .grass, *Sorghum sudanense* Stapf. are planted around the intercrop (Fig. 2.3) (Cook *et al*., 2007; Hassanali *et al*., 2008). Intact intercrops (desmodium and molasses grass) emit semiochemicals such as (*E*)-4,8-dimethyl-1,3,7 nonatriene, (*E*)-ocimene, β-caryophyllene, humulene and α-terpinolene that are repugnant to stemborer moths but attractive to parasitic wasps (Khan *et al*., 1997a; 2000; Pickett *et al*., 2006). Thus, not only do the intercrops repel moths from the main crop but they also increase parasitism of the pests. Volatile organic compounds such as hexanal, (*E*)-2-hexanal, (*Z*)-3-hexen-1-ol and (*Z*)-3-hene-1-yl acetate released by trap crops (Napier and Sudan grass) and maize attract stemborer moths for oviposition (Khan *et al*., 2000). However, trap plants emit significantly higher amounts of the attractive compounds than the main crop, (Birkett *et al*., 2006), which increase 100-fold during the scotophase (time when moths do actively seek host plants for oviposition) in trap crops, with the increase in the main crop being 10 times less that in trap crops (Chamberlain *et al*., 2006). This makes the trap crops much more attractive to the ovipositing moths thus attracting them away from the main crop.

In addition to stemborer control, the intercrop desmodium mediates a striga (*Striga hermonthica* (Del.) Benth.) control effect. Striga is a parasitic weed of cereals in sub-Saharan Africa. The mechanism of striga suppression by desmodium involves allopathic roots exudates released by the roots of *D. uncinatum* (Khan *et al*., 2002). The root exudates contain biologically active isoflavonones that stimulate germination of *S. hermonthica* seeds while others, C-glycosylflavones inhibit radical growth (Tsanuo *et al*., 2003; Khan *et al*., 2008b; Hooper *et al*., 2010). This causes suicidal germination of the *S. hermonthica* seeds resulting in depletion of the seed bank in the soil even in the presence of host plants (Khan *et al*., 2008b). The semiochemistry of push-pull companion plants demonstrates the value and potential of exploiting intact plants with inherent abilities for constitutive emission of biogenic compounds in the development of effective crop protection strategies. Thus, selection or breeding of main crops with the inherent ability to emit such attractive stimuli could pave way for development or optimisation of novel, sustainable and ecologically sound pest management strategies.

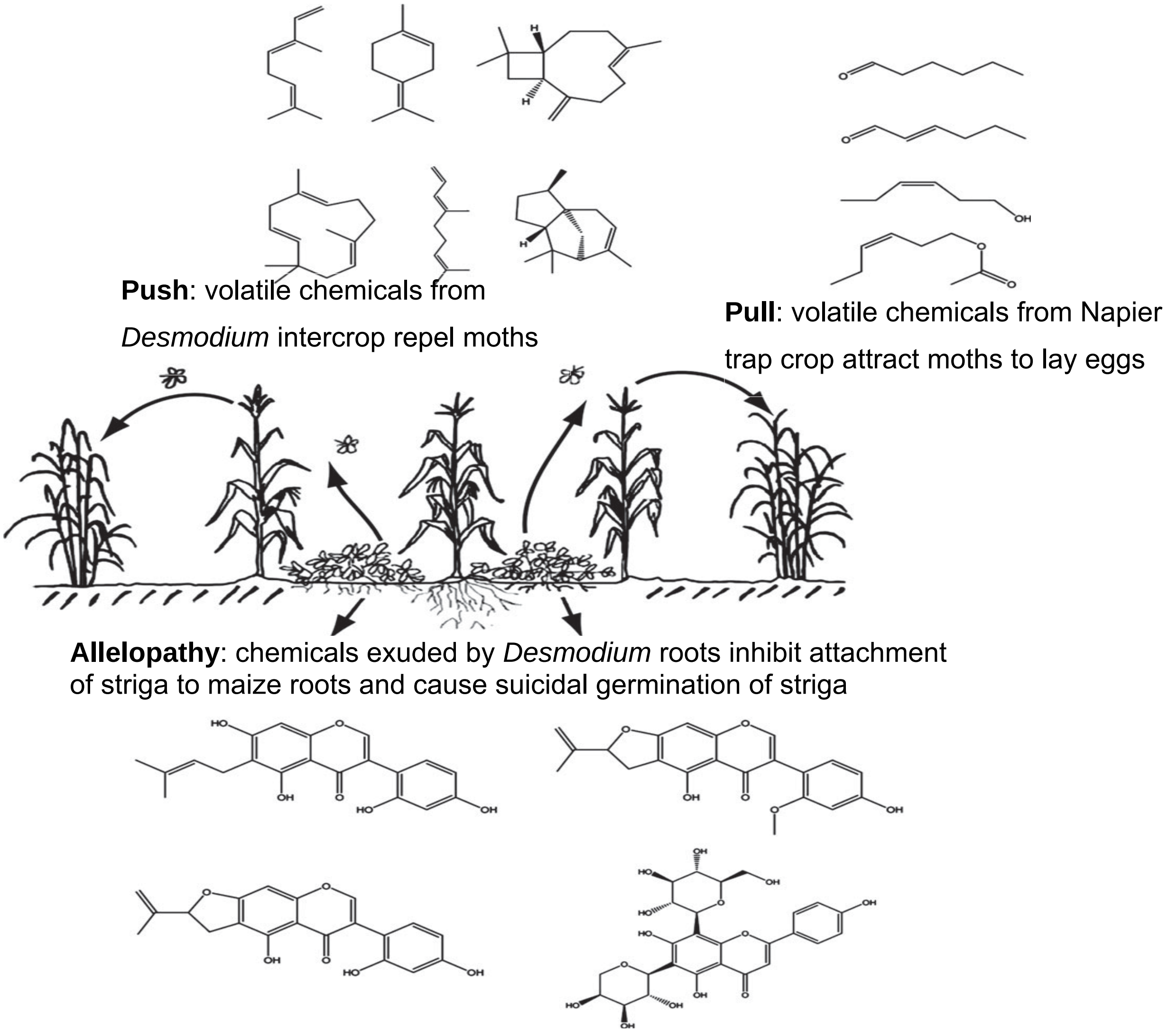

Figure 2.3: The functioning of the push-pull system: stemborer moths are repelled by intercrop volatiles while attracted by trap crop volatiles. Root exudates from *Desmodium* intercop cause suicidal germination of *Striga* and inhibit attachment to maize roots. 1, (*E*)-β ocimene; 2, α-terpinolene; 3, β-caryophyllene; 4, humulene; 5, (*E*)-4,8-dimethyl-1,3,7-nonatriene; 6, α-cedrene; 7, hexanal; 8, (*E*)-2-hexanal; 9, (*Z*)-3-hexen-1-ol; 10, (*Z*)-3-hexen-1-yl acetate; 11, 5,7,2',4'-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone (uncinone A); 12, 4'',5''-dihydro-5,2',4'-trihydroxy-5''-isopropenylfurano-(2'',3'';7,6)-isoflavanone (uncinone B); 13, 4'',5''-dihydro-2'-methoxy-5,4'-dihydroxy-5''-isopropenylfurano-(2'',3'';7,6)-isoflavanone (uncinone C) and 14, di-*C*-glucosylflavone 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin (Adapted from Khan *et al*., 2010).

2.7.4 Herbivore induced plant volatiles (HIPVs)

Plants have evolved sophisticated defence mechanisms against attacking phytophagous herbivores (Takabayashi & Dicke, 1996; De Moraes *et al*., 2001; Kessler & Baldwin, 2001). They respond to herbivore damage through morphological, biochemical and molecular alterations (Sharma *et al*., 2009; War *et al*., 2011). These alterations lead to production of a wide range of volatile semiochemicals which play an important role in the plant's indirect defence against the attacking herbivores (Pichersky *et al*., 2006). Typically, insect attack leads to an increase in volatile emission by plants that is used as 'cry for help' to attract natural enemies in tritrophic interactions (Whitfield, 2001; Turlings & Ton, 2006) and to repel further colonization by the herbivore (De Moraes *et al*., 2001). HIPVs are released both locally and systematically from leaves, flowers and fruits into the atmosphere or from roots into the soil when plants are under herbivore attack (Kessler & Baldwin, 2001; Karban, 2011). Previous studies from 900 plant families have shown that about 2,000 volatile compounds are released in response to herbivore attack (Rodriguez-Saona *et al*., 2009; Dicke *et al*., 2009). These studies have demonstrated that odour profiles display an enormous range of diversity among plant species (Turlings *et al*., 1993; Loughrin *et al*., 1995). However, different plant taxa exhibit a considerable overlap in producing a number of HIPVs (Dicke, 1994; Pare & Tumlinson, 1999). Most of the HIPVs generally belong to terpenoids, phenylpropanoids/benzenoids and fatty-acid and amino-acid derivatives (Dudareva *et al*., 2006). Based on the category, these compounds are synthesized through different metabolic pathways upon herbivory. Various metabolic pathways can be stimulated by herbivore attack at the same time, resulting in several compounds from different pathways playing a role in indirect defence. For example, a maize plant emits a range of HIPVs in response to herbivory including the green-leaf volatile, (*Z*)-3-hexen-1-ol (fatty-acid derivative); aromatic indole (benzenoid) and terpenoids such as (*E*)-4,8-dimethyl-1,3,7-nonatriene, *S*(-)-linalool, (*E*)-β-bergamotene and (*E*)-β-caryophyllene (Fig. 2.4).

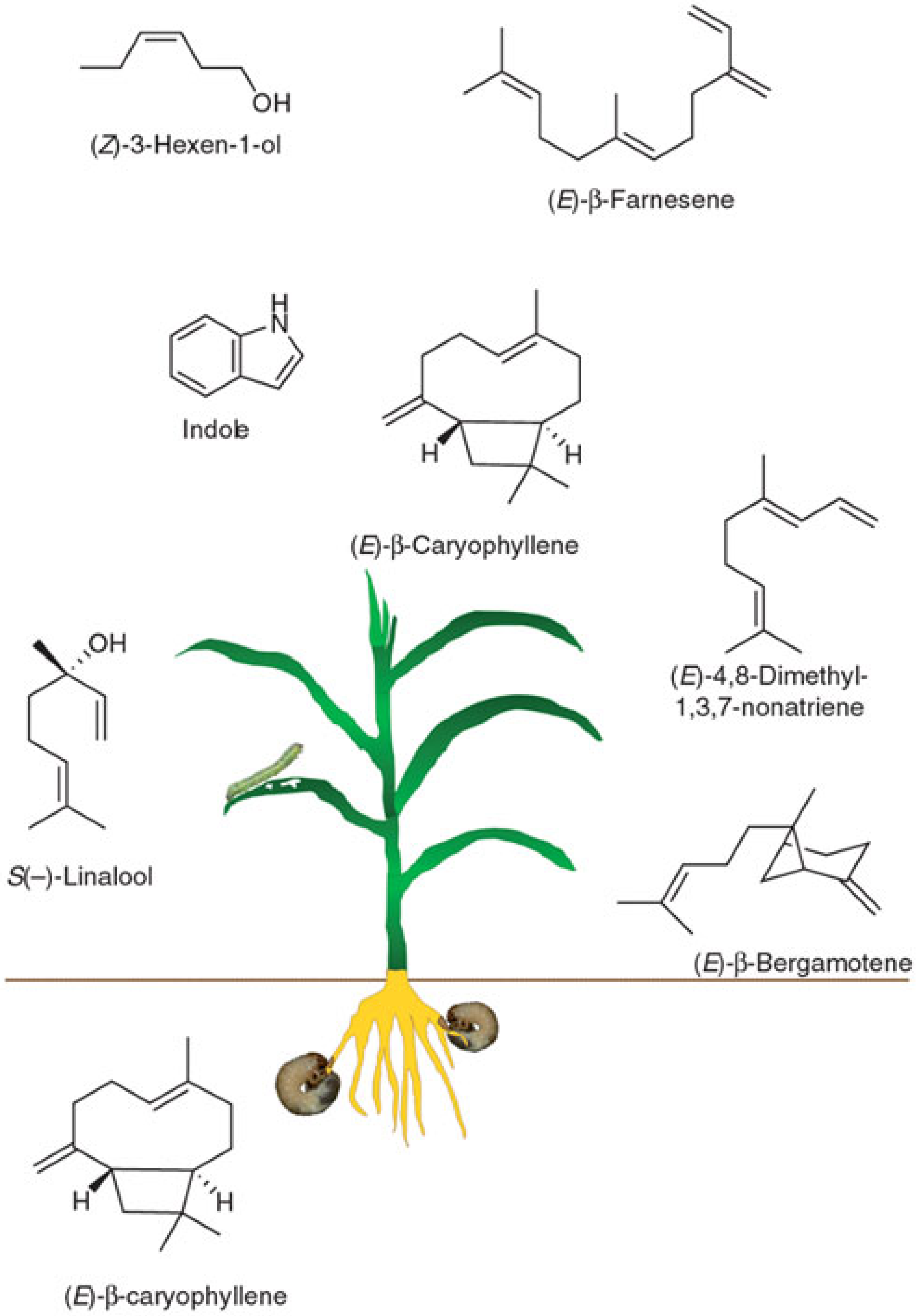

Figure 2.4: Emission of HIPVs aboveground and belowground by a maize plant induced by leaf feeding larvae, *Spodoptera exigua,* and larvae of corn rootworm, *Diabrotica virgifera* (Adapted from Dicke *et al*., 2009)

Herbivore-induced plant volatiles that are induced by larval feeding are generally not effective in reducing pest damage in farmers' fields because the 'cry for help' comes after damage has already been inflicted on the crops. The activity of the antagonistic insects does therefore not prevent yield losses (Khan *et al*., 2010). Recent studies have shown that some plants are able to respond to the initial stage of herbivore attack (oviposition) through the alteration of volatile emission. For example, the African forage grass, *Brachiaria brizantha* (Hochst. Ex A. Rich.) Stapf., responds to oviposition by *C. partellus* through suppression of the main green leaf volatile (*Z*)-3-hexenyl acetate (Z3HA), used in host location by the pest, thereby making the plant 'invisible' to ovipositing females and thus preventing further colonization by conspecifics (Bruce *et al*., 2010). However, despite suppression of Z3HA, the ratio of the other compounds relative to Z3HA, increase in plants exposed to *C. partellus* egg deposition, making the volatile blend more attractive to the parasitic wasp *C. sesamiae* than that of plants without eggs. A similar trait has been found in maize landraces of Mesoamerican origin and two of African landraces (Tamiru *et al*., 2011; 2012), where egg deposition by *C. partellus* resulted in emission of volatiles attractive to both egg and larval parasitoids. However, none of the elite commercial maize hybrids screened so far was found to have this trait. Varietal and genotypic differences in herbivore-induced volatile emissions have been reported for several plant species. Considerable intraspecific variation in quality and quantity of odours released by different maize varieties have also been reported (Degen *et al*., 2004). There is therefore the need to screen more maize varieties adapted to local agroclimatic conditions as well as high yielding commercial varieties to select the ones which can switch on their defence systems early during the initial stages of pest attack. Moreover, it is prudent to investigate this trait in the wild maize system from which our crops were domesticated in order to understand changes of this important trait when subjected to forces such as domestication or breeding. This will provide full utilization of this vital trait in development of novel crop protection strategies.

Plant volatile emission by damaged plants can also affect the defence responses of neighbouring plants by adjusting their metabolism to increase their resistance to the attacking herbivores (Karban *et al*., 2003). These plants with increased defence-related volatiles are thus avoided by herbivores (Glinwood *et al*., 2003) because the volatile compounds from neighbouring attacked plants stimulate resistance gene expression resulting in the emission of higher levels of defence compounds (Arimura *et al*., 2000). Exposure to HIPVs also primes the plant's defences to future attack (Engelberth *et al*., 2004; Oluwafemi *et al*., 2013). Attractiveness of an uninfested plant to antagonistic insects increases after exposure to volatiles released from infested plants or intact plants emitting these volatile cues (Birkett *et al*., 2000). For example, *Melinis minutiflora* P. Beauv. (Poales: Poaceae) which constitutively releases volatile cues associated with damaged maize [primarily *(Z)*-4,8-dimethyl-1,3,7-nonatriene] (Khan *et al*., 1997a), has been shown to induce a defence response in the neighbouring non-damaged maize plants leading to production of similar 'cry for help' cues that attract larval parasitoids (Khan *et al*., unpublished data). It therefore of paramount importance to investigate whether maize plants that have been oviposited on can emit defence volatiles that can induce defence responses in neighbouring intact maize plants. This will pave way towards understanding the effects of induced defence volatiles in maize and may provide an important key to development of new crop protection strategies through companion cropping.

Integrated approaches in the past decade involving ecological, chemical and molecular techniques have yielded important progress in our understanding of induced plant defences. Large-scale transcriptomic changes have been documented in plants in response to herbivore attack, revealing the upregulation of defence-related genes and downregulation of photosynthesis-related genes (Voelckel & Baldwin, 2004; Broekgaarden *et al*., 2007). Metabolic engineering of herbivory-induced plant semiochemicals has been investigated and the possibility demonstrated to induce defensive functions in model plants under laboratory conditions (Schnee *et al*., 2006; Cheng *et al*., 2007). For example, under laboratory conditions *Arabidopsis thaliana* (L.) Heynh. (Brassicales: Brassicaceae) has been transformed to emit (E)-β-farnesene through cloning with the (E)-β-farnesene gene from *Mentha piperita* L. (Lamiales: Lamiaceae) (Beale *et al*., 2006). The transformed *A. thaliana* plants emitted (E)-β-farnesene at levels sufficient to elicit a potent repellent response on the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and an arrestment response on its parasitoid *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae) (Beale *et al*., 2006). Therefore, knowledge of early herbivory-induced defences in maize can lead to development of maize hybrids with desirable defence traits either through conventional breeding or biotechnological approaches. Henceforth, this study aimed at investigating the innate defence traits in maize and their tritrophic interactions.

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CHAPTER THREE

3.0 OVIPOSITION-INDUCED SEMIOCHEMICAL EMISSIONS IN TEOSINTE, A WILD ANCESTOR OF MAIZE

3.1 Abstract

Maize, an important agricultural crop, is the domesticated descendent of its wild ancestor, teosinte. Recently, it has been shown that certain maize landraces possess a valuable indirect defence trait not present in commercial hybrids. Plants of these landraces release herbivore-induced plant volatiles (HIPVs) that attract both egg (*Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)) and larval (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) parasitoids in response to stemborer egg deposition. This study tested whether this trait also exists in pre-domesticated wild *Zea* species germplasm. Headspace samples were collected from plants exposed to egg deposition by *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) moths and unexposed control plants. Four-arm olfactometer bioassays with parasitic wasps *T. bournieri* and *C. sesamiae* indicated that both egg and larval parasitoids preferred HIPVs volatiles from plants with eggs in four of the five teosinte varieties sampled. Headspace samples from oviposited plants released higher amounts of EAG-active compounds such as (*E*)-4,8-dimethyl-1,3,7-nonatriene. In oviposition choice bioassays, plants without eggs were significantly preferred for subsequent oviposition by moths compared to plants with prior oviposition. These results suggest that this valuable oviposition-induced indirect defence trait is not limited to landraces but occurs widely in wild *Zea* species and appears to be an ancestral trait. Hence these species possess a valuable trait that could be introgressed into domesticated maize lines to provide indirect defence mechanisms against stemborers.

Key Words: Induced defense, oviposition, plant volatiles, tritrophic interactions.

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3.2 Introduction

In natural ecosystems, plants are exposed to an array of both abiotic and biotic stress factors. They are under selection pressure to maximize their fitness in an environment where biotic interactions can have positive or negative outcomes (Dicke *et al*., 2009). In particular, plants respond to attack by herbivorous insects through activation of induced chemical defences which can be either direct or indirect. Direct defence mechanisms comprise chemical cues such as toxins, digestion inhibitors or deterrents that adversely affect the behaviour and/or physiology of the herbivore (De Moraes *et al*., 2001, Kessler & Baldwin, 2001; Roda & Baldwin, 2003). Indirect defence mechanisms include herbivore-induced volatiles (HIPVs) that attract natural enemies of the herbivores (Turlings *et al*., 1990; Dicke & van Loon, 2000; Heil, 2008). Activation of induced defences means that individual plants can change their chemical phenotype in response to biotic stress which can profoundly affect tritrophic interactions on an ecological and evolutionary time scale (Agrawal, 2001). Integrated approaches to research into ecological, chemical and molecular techniques yielded important progress in our understanding of induced plant defences over the past decade. Large-scale transcriptomic changes have been documented in plants in response to herbivore attack, revealing the up-regulation of defence-related genes and down-regulation of photosynthesis-related genes (Voelckel & Baldwin, 2004; Broekgaarden *et al*., 2007).

Emission of HIPVs in response to herbivore feeding damage has been studied intensively during the past two decades. Recently, a relatively small but growing number of investigations have considered the reactions of plants towards egg deposition. These studies revealed that oviposition can induce production of plant volatiles that attract parasitoids (Colazza *et al*., 2004; Hilker & Meiners, 2006; Bruce *et al*., 2010, Tamiru *et al.*, 2011; 2012). Detection of egg deposition and subsequent changes in chemical phenotype prepare plants for the impending attack by the emerging phytophagous larvae (Hilker *et al*., 2002; Bruce *et al*., 2010).

Tritrophic signalling via HIPVs has been demonstrated for several systems, mostly cultivated plants such as Lima bean (Fabaceae), cabbage (Brassicaceae), cucumber (Cucurbitaceae), apple (Rosaceae), cotton (Malvaceae) and maize (Poaceae) (Dicke *et al.*, 1990; Turlings *et al*., 1990; 1993; Takabayashi *et al*., 1991; Agelopoulos & Keller, 1994; Mattiacci *et al*., 1994; McCall *et al*., 1994; Pallini *et al*., 1997; Röse *et al*., 1997; Krips, 2000; Gouinguenė *et al*., 2001; Tamiru *et al*., 2011; 2012). Natural enemies of the herbivores attacking these plants make use of HIPVs for long-range prey or host location (Dicke & Sabelis, 1988; Turlings *et al*., 1990; Powel, *et al*., 1998). Different plant species release entirely different HIPV blends and even within one plant species, there are clear differences among genotypes (Takabayashi *et al*., 1991; Turlings *et al*., 1998; Gouinguenė *et al*., 2001). Information regarding HIPV variability comes mainly from studies of cultivated plants (Takabayashi *et al*., 1991; Turlings *et al*., 1998; Krips, 2000; Gouinguenė *et al*., 2001). The only studies done on wild systems are those of a wild cotton variety which was found to release much higher quantities of induced volatiles than cultivated varieties (Loughrin *et al*., 1995) and studies of larval regurgitant applied to mechanically damaged maize leaves (Gouinguenė *et al*., 2001). However, no tritrophic interactions have so far been investigated in wild maize systems following egg deposition by a herbivore. For a better understanding of the ecological relevance and evolutionary history of oviposition-induced plant signalling, it is necessary to study these signals in wild systems.

The spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most damaging lepidopteran pests of maize in eastern and southern Africa and Asia, causing yield losses of up to 88% (Kfir *et al*., 2002). Effective chemical control of this pest is difficult, mostly due to the protection provided to larvae by plants when feeding inside plant whorls and stems (Slabbert & Van den Berg, 2009). Furthermore, insecticides are not economical for smallholder farmers. Thus, the ecology of tritrophic interactions presents an opportunity for development of crop protection approaches that make use of induced innate plant defences. Such defences, together with high levels of antibiosis resistance against *C. partellus* larvae identified in maize breeding lines (Van

Rensburg & Van den Berg, 1995), can contribute to development of environmentally benign and cost effective pest management strategies.

For better understanding and utilization of indirect plant defence traits in pest management strategies we need to consider their evolutionary and ecological history when subjected to forces such as domestication or plant breeding. The objectives of this study were therefore to determine: (1) volatile profile changes in response to *C. partellus* egg deposition on wild maize, (2) behavioural responses of parasitoids to HIPVs collected from wild maize exposed to stemborer oviposition, and (3) the effect of oviposition-induced volatiles on subsequent moth oviposition on these plants.

3.3 Materials and methods

3.3.1 Plants

Seeds of teosinte varieties were obtained from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico. The following teosinte varieties were used: *Zea diploperennis* Iltis, Doebley & Guzman, *Z. huehuetenangensis* (Iltis & Doebley) Doebley*, Z. mays* spp. *mexicana* (Schrader) Iltis, *Z. m.* spp. *parviglumis* Iltis & Doebley, and *Z. perennis* (Hitcht.) Reeves & Manglesdorf*.* Seeds were grown individually in pots filled with fertilised soil in an insect-proof screen house at the *icipe*-Thomas Odhiambo campus, Mbita point (0^o 25'S, 34^o 12'E; 1200m above sea level), in western Kenya. All the seedlings were grown under natural conditions (25^oC, 65%RH; 12L: 12D) and used in the experiments when 3-4 weeks old.

3.3.2 Insects

Chilo partellus moths were obtained from the insect mass rearing unit at the *icipe*-Thomas Odhiambo campus. The larvae originated from field-collected stemborers, principally from sorghum (*Sorghum bicolor* L. Moench) fields in the Mbita region. Larvae were reared on a semi-synthetic diet containing sorghum leaf powder (Ochieng *et al*., 1985). Field collected egg parasitoids, *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae) and larval parasitoids, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) were reared on stemborer eggs and larvae respectively, using methodologies described by Overholt *et al*. (1994). The insects were maintained at 24 ± 3^{O}C, 70 ± 5% RH, 12L: 12D. The mass-reared culture was infused with a field-collected population every three months to avoid genetic decay and maintain the original behavioural characteristics of the species.

3.3.3 Volatile organic compound (VOC) collection

Headspace sampling (Agelopoulos *et al*., 1999) was used to collect volatile compounds from whole maize plants, with and without stemborer eggs (Plate 3.1). Prior to volatile collection, seedlings were placed in oviposition cages (80 X 40 X 40 cm) into which five gravid naïve female stemborer moths were introduced and kept overnight for oviposition. A wad of cotton wool (10 cm in diameter) moistened with water was placed into the cage for the moths to feed on the water from the wet cotton wool. Control plants were kept inside similar cages but without *C. partellus* moths. Volatiles were collected from these plants for a period of 48 hours, starting at the last two hours of photophase of the following day. Leaves with or without eggs were enclosed in polyethylene terephthalate (PET) bags (3.2 L,~12.5 mm thick) heated to 150^{O}C before use and fitted with a swagelock inlet and outlet ports. Charcoal-filtered air was pumped (600 mL min^{-1}) through the inlet port. Volatiles were collected on Porapak Q (0.05g, 60/80 mesh; Supelco) filters inserted into the outlet through which air was drawn at 400 mL min^{-1}. Elution of the entrained volatiles was done using 0.5 mL dichloromethane. The eluted samples were stored in tightly capped microvials in a -20^{o}C freezer prior to bioassays and further analysis. Entrainments from both oviposited and control plants were replicated four times and each plant was used only once.

3.3.4 Four-arm olfactometer bioassay

Responses of parasitoids to plant derived volatiles were tested in a Perspex four-arm olfactometer (Pettersson, 1970). A choice-test was carried out to compare insect responses to headspace samples from oviposited and control plants. The two opposite arms held the test stimuli (10 µL aliquots of headspace sample). The remaining two arms were solvent controls. The experiment was replicated 12 times. Headspace samples (10 µL aliquots) were applied, using a micropipette (Drummond ‘microcap’, Drummond Scientific Co., Broomall, PA, USA), to a piece of filter paper (4 x 25 mm) placed in the inlet port at the end of each olfactometer arm. Gravid female parasitoids without any prior exposure to plants or hosts were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Air was drawn through the four arms towards the centre at 260 mL min^{-1}. Time spent by parasitoids in each olfactometer arm was recorded with ‘Olfa’ software (F. Nazzi, Udine, Italy) for 12 minutes.

3.3.5 Gas Chromatography (GC) analysis

Entrained VOCs were analyzed using a Hewlett-Packard 7890 GC machine (Agilent Technologies) equipped with a cool-on column injector, a non-polar HP-1 capillary column (50 m, 0.32mm internal diameter, 0.52 µm film thickness) and a flame ionization detector (FID). Four µL of headspace sample was injected into the injector port of the GC instrument. Oven temperature was maintained at 30^{o}C for 2 minutes and then programmed at 5^{o}C min^{-1} to 250^{o}C. The carrier gas was hydrogen. Data was analyzed using HP Chemstation software.

3.3.6 Coupled GC-Electroantennography (GC-EAG) analysis

GC-EAG was carried out using the antennae of female *C. sesamiae* with the headspace samples of the different teosinte varieties that elicited positive responses during olfactometer bioassays. The GC-EAG system, in which the effluent from GC column is simultaneously directed to the antennal preparation and GC detector, has been previously described by Wadhams (1990). EAG recordings were made using Ag-AgCl glass electrodes filled with saline solution, as described by Maddrell (1969), but without glucose. A female parasitoid was chilled for one minute and the head excised and the tips of both antennae removed to ensure good conduct. The indifferent electrode was placed within the head capsule. Signals were then passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and analysed using a customised Syntech software package. Separation of volatiles was done on a 6890N GC (Agilent Technologies) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm internal diameter, 0.52μm film thickness). The oven temperature was maintained at 30 ^{o}C for 2 minutes and then programmed at 15 ^{o}C min^{-1} to 250 ^{o}C. The carrier gas was helium. Outputs from EAG amplifier and the FID were analysed using Syntech software.

3.3.7 Coupled GC-Mass Spectrometry (GC-MS) analysis

Aliquots of attractive headspace samples were analysed using a Hewlett-Packard 5890 GC machine (Agilent Technologies) on a capillary Gas Chromatography HP-1 column (50 m, 0.32 mm internal diameter, 0.52 μm film thickness) directly coupled to a mass spectrometer (VG Autospec; Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionisation was performed by electron impact (70 eV at 250^{o}C). The oven temperature was maintained at 30^{o}C for 5 minutes, and then programmed at 5^{o}C min^{-1} to 250^{o}C. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005). Tentative identifications of the compounds were confirmed through co-injections with authentic standards.

3.3.8 Oviposition bioassays

Two-choice tests were conducted using modifications of the methodology of Khan *et al*. (2007) in oviposition cages (80 X 40 X 40 cm) covered by fine cloth mesh netting with a cloth access flap. Prior to the two-choice test, a 3 to 4-week old potted maize plant, was caged overnight with five gravid naïve *C. partellus* moth to allow oviposition. A wad of cotton wool (10 cm in diameter) moistened with water was introduced into the cage for the moths to feed on. After 24 hours, the positions of egg batches on leaves were marked. The following day another maize plant of the same variety and age but without prior exposure to moths was placed into each of the oviposition cages, adjacent to the previously exposed plant. Thus, each cage had two potted maize plants positioned at opposite sides, one exposed to moths the day before and the other without any prior exposure to moths. Five new gravid naïve *C. partellus* moths were then introduced into the cage and allowed to oviposit for 48 hours under natural conditions of approximately L12:D12. Plants were then removed and the number of newly laid eggs and egg batches on each plant counted under a light binocular microscope. 'Preference' was taken in this context to be differential oviposition on a plant when the insect is given a choice between two plants of the same variety but with different treatments. Data collected were expressed as mean proportion (percentage) of total number of eggs oviposited during the second oviposition period on plants in the two-choice test. This experiment was replicated 10 times.

3.3.9 Statistical analysis

Statistical analyses were done using R software (R, 2013). Time spent in each arm of the four-arm olfactometer bioassay was compared by analysis of variance (ANOVA) after conversion of the data into proportions followed by a logratio transformation. Means were separated using the Tukey test with α set at 0.05. The two-sample (unpaired) student's t-test was used to determine if there were any differences between the numbers of eggs and egg batches laid on plants that were previously either exposed or non-exposed to oviposition.

3.4 Results

3.4.1 Behavioural responses of parasitoids to headspace samples of VOCs

Female *T. bournieri* individuals spent significantly more time in olfactometer arms containing volatiles from plants exposed to oviposition in comparison to those with volatiles from plants without eggs and solvent controls for four of the five teosinte varieties (*Z. huehuetenangensis* $F_{2,33}$ = 6.505; *P*=0.004; *Z. m.* spp. *mexicana* $F_{2,33}$ = 4.41; *P*=0.020; *Z. m.* spp. *parviglumis* $F_{2,33}$ = 7.357; *P*=0.002 and *Z. perennis* $F_{2,33}$ = 6.492; *P*=0.004) (Fig. 3.1A). Similar results were observed for the larval parasitoid, *Cotesia sesamiae* (*Z. huehuetenangensis* $F_{2,33}$ = 8.428; *P*=0.001; *Z. m.* spp. *mexicana* $F_{2,33}$ = 10.15; *P*<0.001; *Z. m.* spp. *parviglumis* $F_{2,33}$ = 10.15;*P*<0.001; *Z. perennis* $F_{2,33}$ = 5.488; *P*=0.009) (Fig. 3.1B). In the case of *Z. diploperennis*, there were no significant differences in time spent in arms with volatiles from plants exposed to egg deposition, plants without eggs and solvent controls for both egg and larval parasitoids ($F_{2,33}$ = 0.434; *P*=0.651; $F_{2,33}$ = 0.391; *P*=0.679 respectively) (Fig. 3.1A&B).

3.4.2 Comparison of volatiles emitted from plants with and without eggs

GC analysis revealed quantitative and qualitative changes in the volatile blend profile emitted by plants exposed to egg deposition (Table 3.1). All teosinte varieties except *Z. diploperennis* emitted more EAG active compounds when exposed to *C. partellus* oviposition compared to unexposed plants (Figs. 3.2-3.6).

3.4.3 Identification of attractive volatile organic compounds

GC-EAG recordings with the attractive samples from teosinte varieties and subsequent GC-MS identification showed that *C. sesamiae* antennae were responsive to 1-butanol,3-methyl-, hexenal, 2,3-butanenediol, octane, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, nonane, 6-methyl-5-heptene-2-one, α-pinene, myrcene, limonene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, decanal, 3,4-dimethylacetophenone and (*E*)-β-farnesene (Fig. 3.7).

3.4.4 Oviposition preference

In two-choice tests, a significantly higher number of eggs was laid on unexposed control plants in comparison to plants with prior egg deposition, except in variety *Z. diploperennis* (*Z. diploperennis t* = 0.122; *P*= 0.905; *Z. huehuetenangensis t* = -2.141; *P*=0.048; *Z. m.* spp. *mexicana t* = -2.914; *P*=0.009; *Z. m.* spp. *parviglumis t* = -2.804; *P*= 0.014 and *Z. perennis t* = -3.314; *P*=0.008) (Table 3.2). Similarly, a higher number of egg batches and number of eggs per batch were laid in *Z. huehuetenangensis, Z. mays* spp. *mexicana*, *Z. m.* spp. *parviglumis,* and *Z. perennis*, although the difference was only significant in *Z. m.* spp. *parviglumis* (*t* = 2.193*; P*= 0.04) (Table 3.2).

3.5 Discussion

These findings provide evidence that indirect defence through insect egg-induced HIPV emission is an ancestral trait in maize, present in several species of teosinte. Previous research (Tamiru *et al*., 2011) showed that the egg-induced HIPV emission trait was present in certain landraces but not in the commercial maize hybrids tested to date. Together with the findings of the current research, it appears that this valuable indirect defence trait may have been inadvertently lost during the development of commercial maize hybrids. The latter were developed primarily with the aim of higher yield and improved grain quality. In hybrid breeding programmes pesticide applications are often used to protect breeding lines, which may have resulted in elite maize breeding material losing some of its natural defence traits. However, smallholder farmers in Africa often do not have access to insecticides which means that when crops are grown under unprotected conditions, the loss of natural plant resistance traits such as the indirect defence trait described here, can lead to yield instability due to crop losses caused by insect pests.

Similar to earlier reports on maize landraces (Tamiru *et al*., 2011; 2012), analysis of volatiles collected from teosinte seedlings in this study revealed both quantitative and qualitative changes in the volatile blend emitted by plants following oviposition by *C. partellus*. In behavioral bioassays, both egg and larval parasitoids preferred volatiles from plants exposed to egg deposition compared to those from unexposed plants. Conversely, the herbivore itself avoided egg exposed plants with gravid female *C. partellus* moths laying fewer eggs on plants that were emmiting these semiochemicals.

Previous studies have shown that egg deposition by herbivorous insects on plants can induce indirect defence responses by means of volatile emissions that attract parasitoids (Hilker & Meiners, 2006; Tamiru *et al*., 2011). Parasitoids and predators of herbivorous insects make use of these induced HIPVs to locate plants colonised by their hosts, thus enhancing their foraging efficacy (Dicke *et al*., 1990; De Moraes *et al*., 1998; Colozza *et al*., 2004; Bruce *et al*., 2010). The attraction of both egg and larval parsitoids is considered a preventive defence strategy since larval parasitoids are recruited in advance, before the phytophagous larvae emerge from eggs and start causing plant damage (Bruce *et al*., 2010). Interspecific as well as intraspecific variation in HIPV emission following larval damage of different maize breeding lines as well as teosinte varieties have also been reported (Gouinguene *et al*., 2001; Degen *et al.*, 2004). However, egg induced effects have not previously been investigated in wild maize species.

Most of the oviposition-induced HIPVs identified in the teosinte varieties were similar to those previously identified in maize landraces (Tamiru *et al*., 2011; 2012). Qualitative and quantitative variations were however observed in the volatile composition between landraces and teosinte varieties. Even within the teosinte varieties, there was variation in the quality and quantity of the volatiles emitted following egg deposition. *Zea diploperennis*, for example, showed little change in the volatile emission. DMNT, a key semiochemical known to attract *C. sesamiae* larval parasitoids (Khan *et al*., 1997), was released in larger amounts in three of the teosinte varieties exposed to egg deposition: *Z. m.* spp. *mexicana*, *Z. m.* spp. *parviglumis* and *Z. perennis.* Noticeably, *Z. m.* spp. *parviglumis*, which is considered the closest relative to cultivated maize (Doebley & Wang, 1997; Kellogg, 1997), produced more HIPVs after egg deposition. Comparing the quality of odour blends from teosinte and those previously reported for maize landraces, the volatile profiles appear largely conserved in maize germplasm since key compounds like DMNT were reported for maize landraces (Tamiru *et al*., 2011; 2012) and teosinte varieties in this study.

Gravid *C. partellus* moths preferred to oviposit on teosinte plants with no prior exposure to egg deposition in four of the five varieties tested. Volatile analysis showed significant changes in volatile profiles of these four varieties with increased emissions of volatiles attractive to parasitoids. The presence of the semiochemicals that were attractive to the herbivore's natural enemies could have influenced the oviposition behaviour of the moths. Furthermore, there was no preferential oviposition behaviour observed for *Z. diploperennis*, the teosinte variety which showed limited volatile profile changes after exposure to oviposition. Previous studies showed that HIPVs may result in increased pressure from natural enemies and the risk of competition for resources on plants emitting HIPVs, resulting in female moths avoiding these plants (Dicke & Baldwin, 2010; Heil & Karban, 2010). Zakir *et al*. (2013) observed that HIPVs alone were sufficient to affect oviposition behaviour in female *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) on cotton, supporting the current observation of decreased oviposition on teosinte varieties with elevated HIPVs profiles.

In summary, results of this study showed that oviposition-induced plant signalling is an ancestral trait in maize that exists even in pre-domestication germplasm. It is therefore not limited to the *Zea mays* landraces in which it was originally discovered (Tamiru *et al*., 2011). The observation of egg-induced HIPV emission in wild *Zea* species and landraces, but not in the commercial hybrids investigated to date, suggests that breeding for yield and palatability could have resulted in the loss of secondary defence metabolites in improved maize breeding lines (Benrey *et al*., 1998). In addition to recruitment of parasitoids, teosinte varieties emitting oviposition-induced volatiles deter further herbivore colonization directly through oviposition deterrence.

3.6 References

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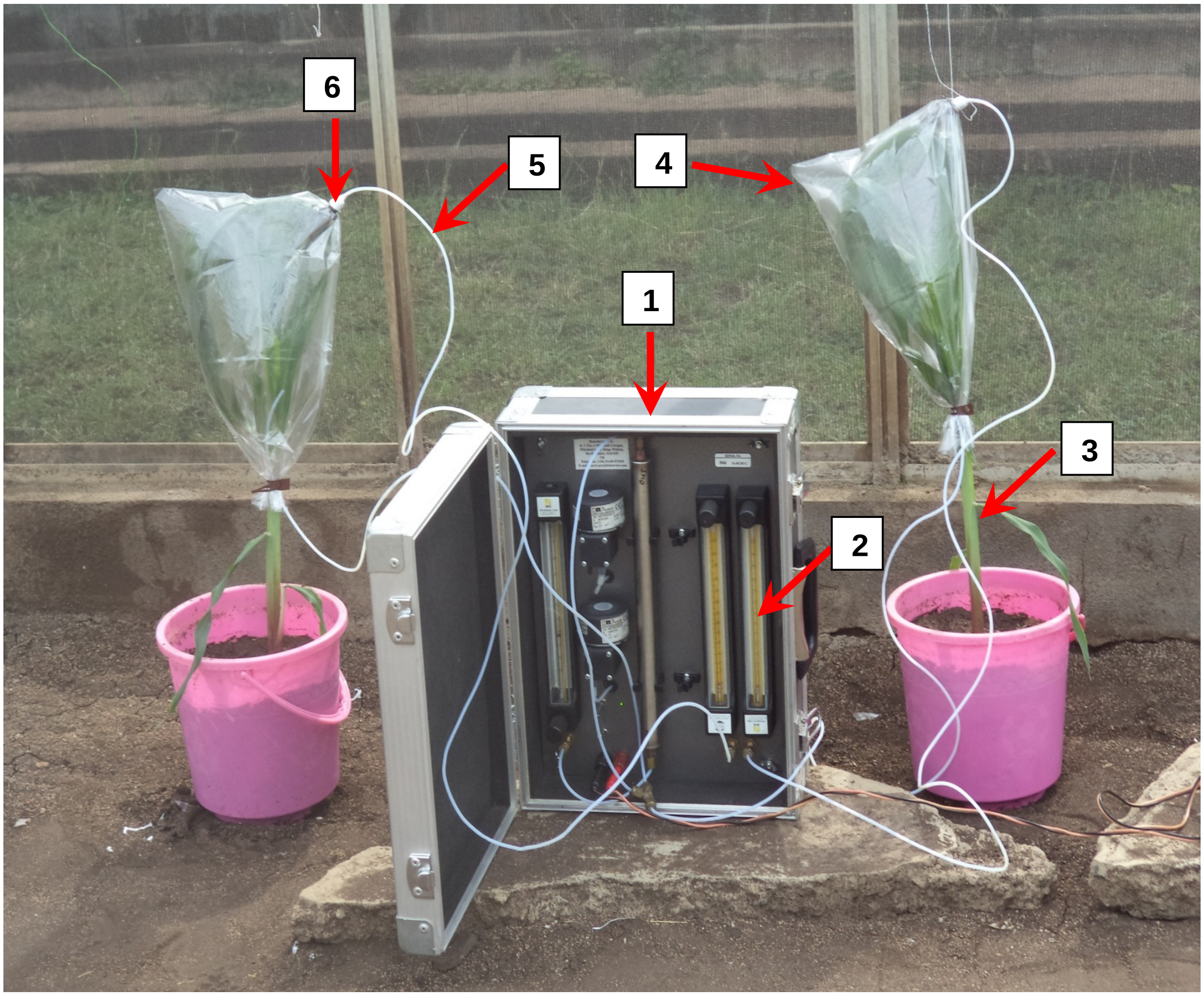


Plate 3.1 Headspace sampling set-up for volatile collection from teosinte seedlings exposed and unexposed to egg deposition. The labels represent (1) Portable air entrainment kit, (2) Flow-metre controlling air flow rate, (3) Teosinte seedling from which volatiles are collected, (4) Polyethyleneterephthalate bags enclosing teosinte leaves, (5) Ethylene terephthalate tubes transporting air to/from the pump, (6) Porapak Q tubes trapping volatiles.

Table 3.1: Volatile emission (ng / plant / h) (Mean ± S.E.) from teosinte species with and without *Chilo partellus* eggs (N = 4)

	Zea mays parviglumis		*Zea mays mexicana*		*Zea huehuetenangensis*		*Zea perennis*		*Zea diploperennis*	
Compound	With eggs	No eggs	With eggs	No eggs	With eggs	No eggs	With eggs	No eggs	With eggs	No eggs
(*E*)-2-hexenal	0.012(± 0.012)	n.d.	n.d.	n.d.	n.d.	n.d.	0.005(± 0.005)	n.d.	n.d.	n.d.
(*Z*)-3-hexen-1-ol	0.014(± 0.014)	n.d.	n.d.	n.d.	n.d.	n.d.	0.004(± 0.004)	n.d.	n.d.	n.d.
nonane	0.004(± 0.004)	n.d.	n.d.	n.d.	n.d.	n.d.	0.001(± 0.001)	n.d.	n.d.	n.d.
α-pinene	0.075(± 0.074)	0.007(± 0.007)	n.d.	n.d.	n.d.	n.d.	0.020(± 0.020)	0.001(± 0.001)	n.d.	0.002(± 0.002)
6-methyl-5-hepten-2-one	0.073(± 0.071)	0.005(± 0.005)	n.d.	n.d.	n.d.	n.d.	0.013(± 0.012)	0.002(± 0.002)	0.003(± 0.003)	n.d.
(*Z*)-3-hexenyl acetate	0.039(± 0.033)	0.021(± 0.010)	0.042(± 0.019)	0.013(± 0.005)	0.031(± 0.004)	0.013(± 0.006)	0.026(± 0.017)	0.009(± 0.009)	0.036(± 0.008)	0.008(± 0.008)
limonene	0.020(± 0.020)	0.009(± 0.009)	0.003(± 0.003)	n.d.	0.014(± 0.014)	0.007(± 0.004)	0.016(± 0.016)	0.003(± 0.003)	n.d.	0.021(± 0.021)
(*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT)	0.169(± 0.128)	0.065(± 0.029)	0.043(± 0.043)	n.d.	0.029(± 0.011)	0.010(± 0.007)	0.012(± 0.009)	0.014(± 0.009)	n.d.	0.036(± 0.021)
decanal	0.008(± 0.008)	0.006(± 0.006)	0.016(± 0.016)	n.d.	n.d	n.d.	0.053(± 0.047)	0.009(± 0.009)	n.d.	0.045(± 0.045)
3,4-dimethylacetophenone	0.017(± 0.017)	0.019(± 0.009)	0.064(± 0.045)	n.d.	0.026(± 0.005)	0.008(± 0.006)	0.013(± 0.011)	n.d.	n.d.	n.d.
(*E*)-β-farnesene	0.012(± 0.008)	0.002(± 0.002)	0.003(± 0.003)	n.d.	0.055(± 0.041)	0.024(± 0.014)	0.026(± 0.026)	0.004(± 0.004)	0.008(± 0.008)	n.d.

n.d. = not detected

Table 3.2: Percentages of *Chilo partellus* eggs oviposited per plant (± SEM), number of egg batches per plant (± SEM), and number of eggs per egg batch (± SEM) for five teosinte varieties exposed and unexposed to prior egg deposition

Oviposited (T) vs. unoviposited (C) teosinte variety	Mean percentage of eggs oviposited/test plant	Mean number of egg batches/test plant	Mean number of eggs/batch
Zea diploperennis (T)	50.79 (± 9.17) a	2.83 (± 0.65) a	25.92 (± 4.42) a
Z. diploperennis (C)	49.21 (± 9.17) a	3.00 (± 0.52) a	25.10 (± 8.93) a
Z. huehuetenangensis (T)	45.99 (± 2.65) a	9.89 (± 0.98) a	34.62 (± 3.08) a
Z. huehuetenangensis (C)	54.01 (± 2.65) b	10.33 (± 0.73) a	36.62 (± 5.58) a
Z. mays spp. *mexicana* (T)	37.43 (± 6.10) a	3.90 (± 0.98) a	30.72 (± 4.76) a
Z. mays spp. *mexicana* (C)	62.57 (± 6.10) b	6.40 (± 0.86) a	38.92 (± 8.90) a
Z. m. spp. *parviglumis* (T)	36.46 (± 6.83) a	4.50 (± 1.07) a	29.60 (± 2.96) a
Z. m. spp. *parviglumis* (C)	63.54 (± 6.83) b	7.63 (± 1.31) a	37.02 (± 6.32) b
Z. perennis (T)	34.10 (± 6.79) a	10.17 (± 2.40) a	20.30 (± 2.76) a
Z. perennis (C)	65.90 (± 6.70) b	13.00 (± 2.73) a	32.77 (± 5.90) a

Values in a column between oviposited and unoviposited teosinte variety followed by the same letter are not significantly different at P = 0.05 (two-sample t-test).

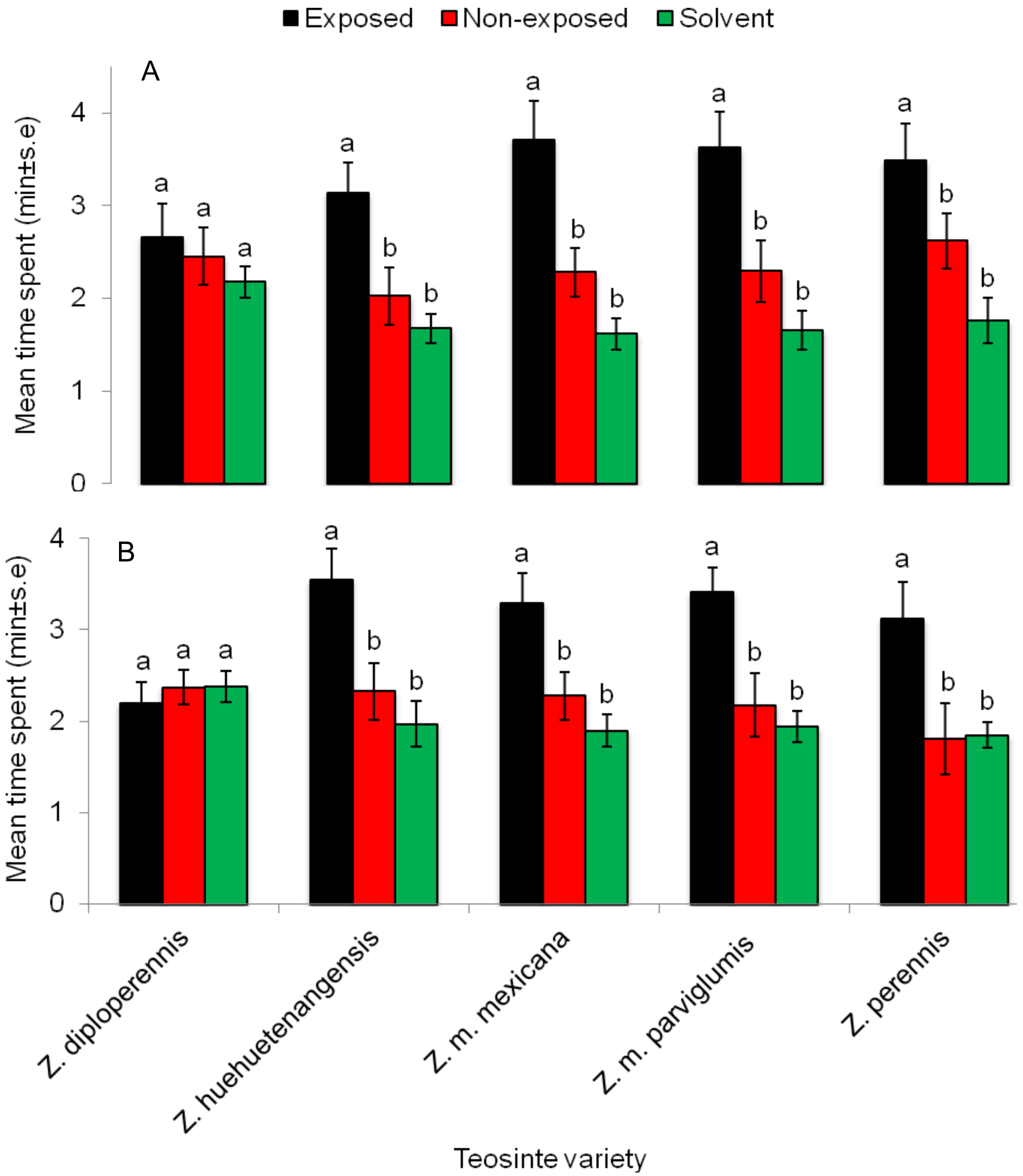


Figure 3.1: Behavioural response of female parasitoids to volatiles collected from teosinte exposed and not exposed to *Chilo partellus* eggs and solvent control in a four-arm olfactometer bioassay. (A) response of *Trichogramma bournieri*; (B) response of *Cotesia sesamiae*. Parasitoid responses were compared by ANOVA after conversion of the data into proportions and log-ratio transformation. Different letters on bars indicate a significant difference using Tukey studentized range test ($P < 0.05$).

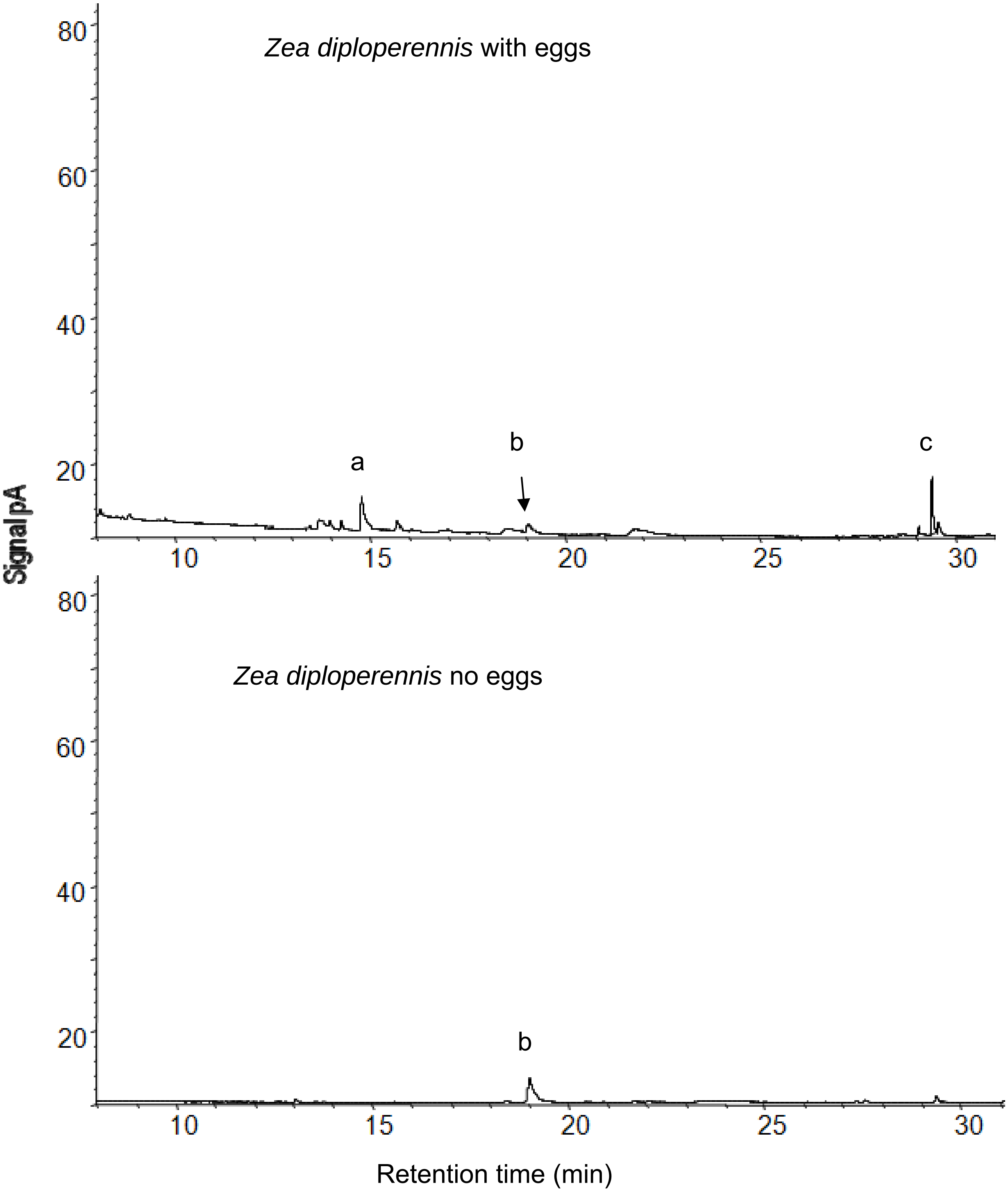


Figure 3.2: GC profiles of headspace volatiles from teosinte variety *Zea diploperennis* with and without *Chilo partellus* eggs. The identities of the EAG active compounds are as follows: (a) myrcene, (b) (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT), (c) (*E*)-β-farnesene.

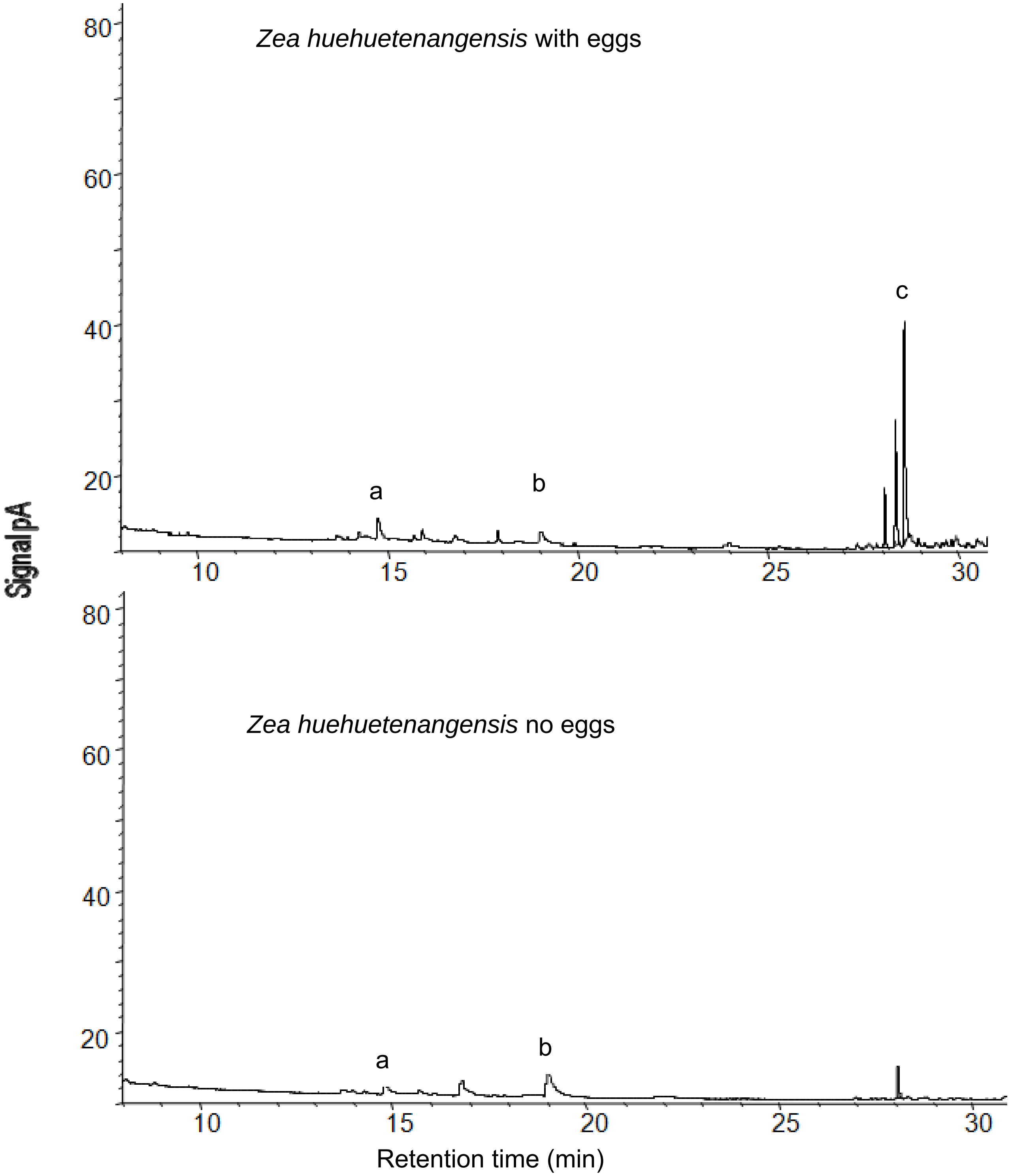


Figure 3.3: GC profiles of headspace volatiles from teosinte variety *Zea huehuetenangensis* with and without *Chilo partellus* eggs. The identities of the EAG active compounds are as follows: (a) myrcene, (b) (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT), (c) (*E*)-β-farnesene.

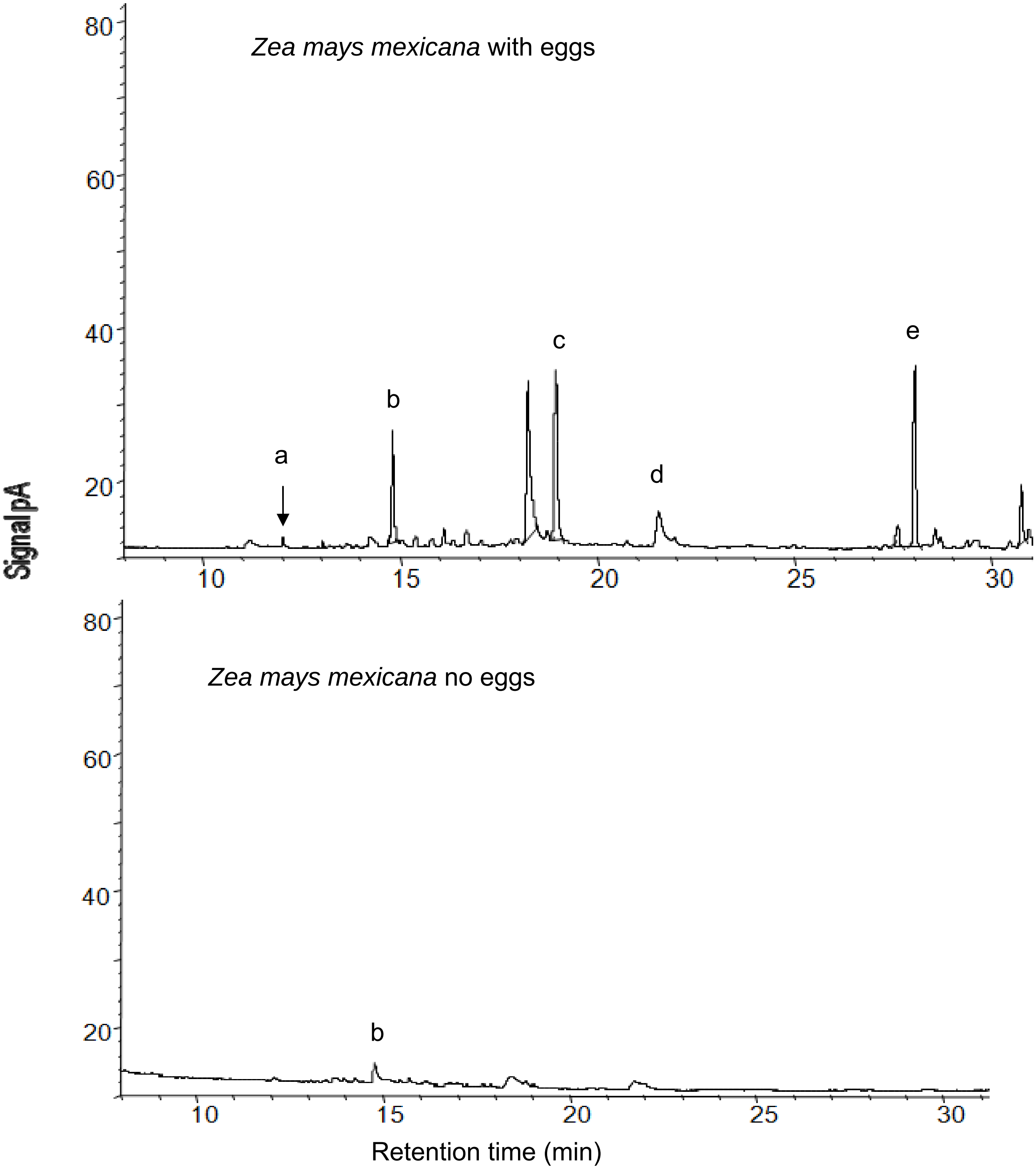
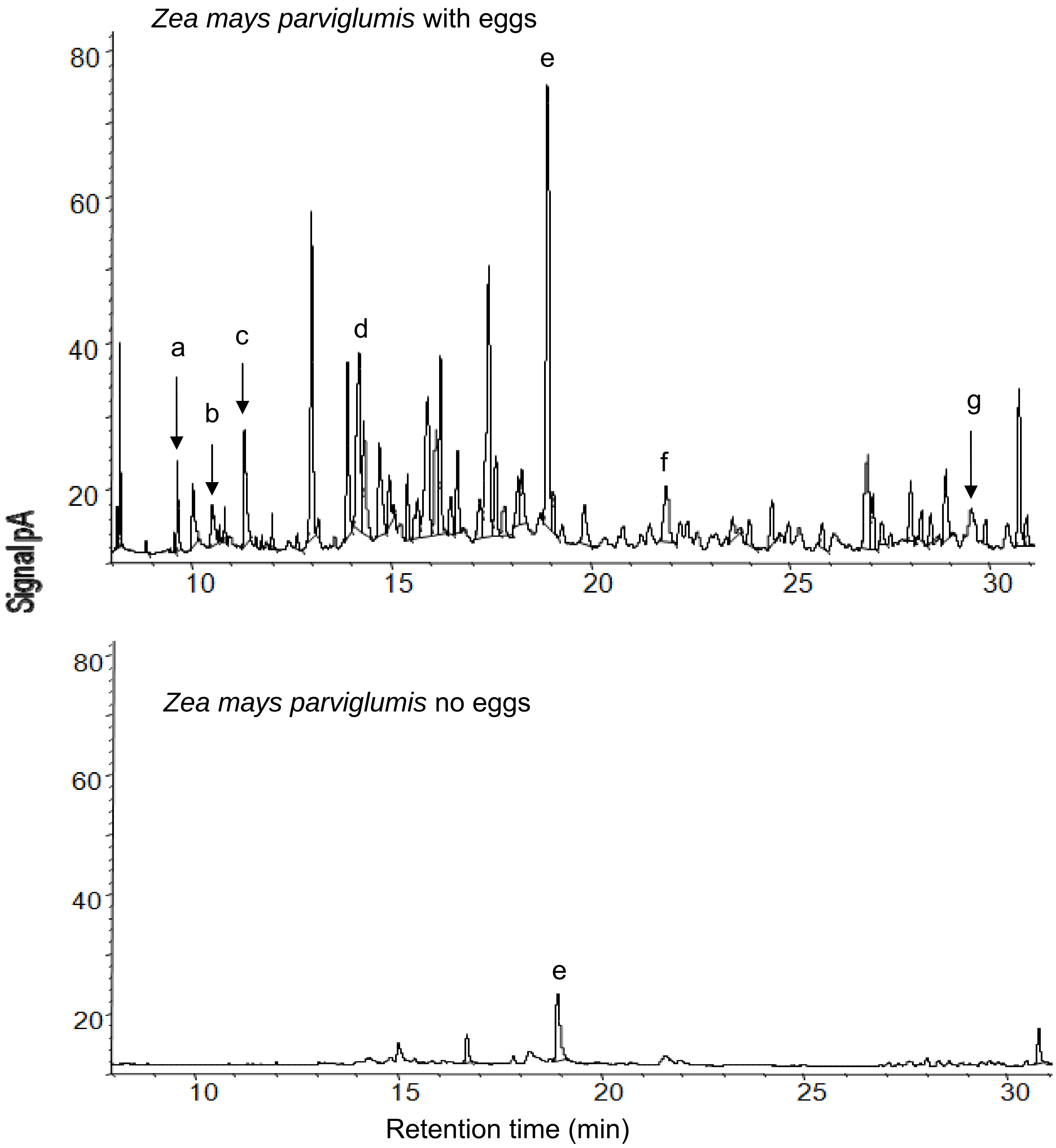


Figure 3.4: GC profiles of headspace volatiles from teosinte variety *Zea mays mexicana* with and without *Chilo partellus* eggs. The identities of the EAG active compounds are as follows: (a) α-pinene, (b) myrcene, (c) (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT), (d) decanal, (e) 3,4-dimethylacetophenone.



Zea mays parviglumis with eggs

Zea mays parviglumis no eggs

Figure 3.5: GC profiles of headspace volatiles from teosinte variety *Zea mays parviglumis* with and without *Chilo partellus* eggs. The identities of the EAG active compounds are as follows: (a) (*E*)-2-hexenal, (b) (*Z*)-3-hexen-1-ol, (c) nonane, (d) 6-Methyl-5-hepten-2-one, (e) (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT), (f) decanal, (g) (*E*)-β-farnesene.

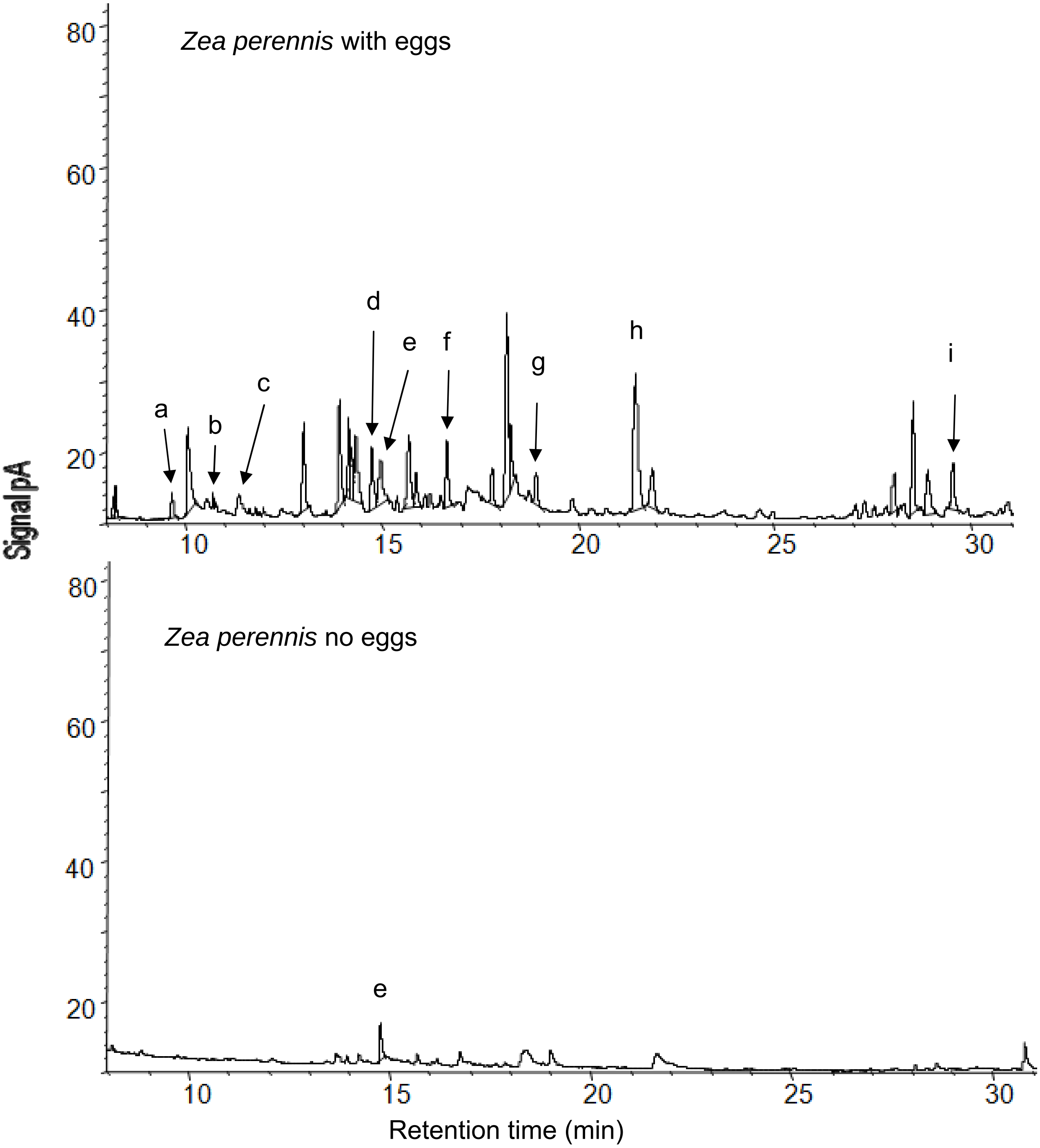


Figure 3.6: GC profiles of headspace volatiles from teosinte variety *Zea perennis* with and without *Chilo partellus* eggs. The identities of the EAG active compounds are as follows: (a) (*E*)-2-hexenal, (b) (*Z*)-3-hexen-1-ol, (c) nonane, (d) 6-Methyl-5-hepten-2-one, (e) myrcene, (f) limonene, (g) (*E*)-4,8-dimethyl-1,3,7 nonatriene (DMNT), (h) decanal, (i) (*E*)-β-farnesene.

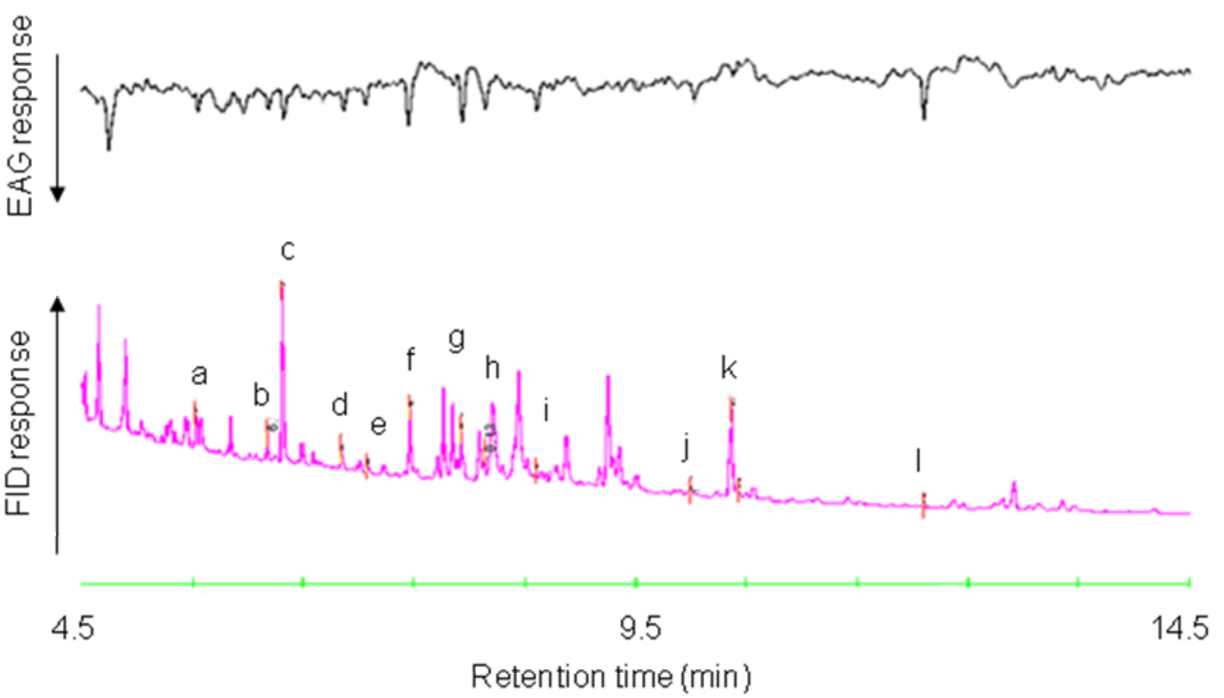


Figure 3.7: A representative GC-EAG response of female *Cotesia sesamiae* to volatiles collected from *Zea perennis* with eggs. FID peaks marked are those which elicited antennal response in coupled runs: a = hexanal, b = 2,3-butanediol, c = (*E*)-2-hexenal, d = (*Z*)-3-hexen-1-ol, e = (*E,E*)-2,4-hexadienal, e = nonane, f = (*Z*)-2-heptenal, g = 6-methyl-5-heptene-2-one, h = (*E,E*)-2,4-heptadienal, i = limonene, j = (*E*)-4,8-dimethyl-1,3,7-nonatriene, k = decanal, l = (*E*)-β-farnesene.

CHAPTER FOUR

4.0 PREVALENCE OF OVIPOSITION-INDUCED VOLATILE EMISSION TRAITS AMONG AFRICAN MAIZE LANDRACE VARIETIES AND HYBRIDS

4.1 Abstract

Maize is a genetically diverse crop. Recent studies on maize-herbivore-parasitoid tritrophic interactions revealed that herbivore egg deposition triggers volatile semiochemical emissions attractive to lepidopteran parasitoids in two African maize landraces. However, this trait was reported to be absent in the elite commercial hybrid maize screened then. This study tested how widespread this important trait is among five African maize landraces and two hybrids. Headspace volatile samples were collected from plants exposed to egg deposition by *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) moths as well as unexposed control plants. Behavioural bioassays were carried out in a four-arm olfactometer using both egg (*Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)) and larval (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) parasitoids. Coupled Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Electroantennography (GC-EAG) were used for volatile analysis. GC-MS analysis of volatiles revealed enhanced production of EAG-active compounds such as (*E*)-4,8-dimethyl-1,3,7-nonatriene in all landraces and one hybrid, SC Duma 43, upon *C. partellus* oviposition on these plants. Olfactometer bioassays with *T. bournieri* and *C. sesamiae* indicated that both egg and larval parasitoids preferred volatiles from plants with eggs compared to plants without eggs. These results suggest that this valuable oviposition-induced indirect defence trait is not limited to the two landraces it was initially discovered in but occurs widely in most maize landraces. It therefore seems that although breeding for yield and palatability may have caused loss of this trait, some elite commercial hybrids like SC Duma 43 posses it. These varieties could provide a genetic resource for introgressing the trait into high yielding maize lines to increase indirect defence against stemborers.

Key Words: Egg deposition, maize, parasitoids, plant volatiles, tritrophic interactions.

4.2 Introduction

Although plants are sessile organisms unable to flee from attack, they are not passive victims of the organisms that attack them. They have evolved several defence mechanisms including direct and indirect defence strategies in response to attacking organisms (Sabelis *et al*., 1999; Karban & Baldwin, 1999; Dicke & van Loon, 2000; Howe & Jander, 2008). Direct defences involve production of toxins, digestion inhibitors and herbivore-induced plant volatiles (HIPVs) repellent to phytophagous insects (De Moraes *et al*., 2001; Kessler & Baldwin, 2001). Indirectly, plants use HIPVs to attract natural enemies, both parasitoids and predators that are antagonistic to the herbivores (Turlings *et al*., 1990; De Moraes *et al*., 1998; Dicke & van Loon, 2000; Heil, 2008). Induced defences may be either amplified/or suppressed by mechanical damage caused by chewing larvae of phytophagous insects or elicitors present in the insect oral regurgitant, saliva and/or eggs (Turlings *et al*., 1993; Musser *et al.*, 2002; Alborn *et al*., 2007; Tian *et al*., 2012; Louis *et al*., 2013).

Egg deposition on plants is the first stage of attack by lepidopteran insects. Several studies have shown that plants are able to detect egg deposition and respond by activating direct and indirect defences early enough before larvae can hatch and cause damage by feeding (Hilker & Meiners, 2006). For instance, the African forage grass, *Brachiaria brizantha*, responds to ovipositing stemborers through suppression of green leaf volatile emissions used during host location by pests, thereby making the plant “invisible” to ovipositing females thus preventing further egg laying (Bruce *et al*., 2010). Parasitoids, however, can recognise the volatile blend and are attracted to it. Intact Molasses grass, *Melinis minutiflora*, constitutively releases volatile cues that repel stemborer moths but attract the pest’s natural enemies without being damaged. This unique property has been used in push-pull crop protection strategies for resource-poor farmers in Africa who do not use pesticides (Cook *et al*., 2007; Khan *et al*., 2010). If these properties were present in the main crop, it would confer much advantage to the ‘push-pull’ system, especially now that intercrops are often chosen primarily for their striga suppression qualities.

HIPV-mediated indirect defences are known to play a role in maize following larval feeding (Tumlison *et al*., 1993; Turlings *et al*., 1998; Ngi-Song *et al*., 2000; Köllner *et al*., 2004). Recently, Mesoamerican and two African maize landraces have been shown to recruit both egg and larval parasitoids in response to egg deposition by stemborer moths (Tamiru *et al*., 2011; 2012). However, this trait was absent in the commercial mainstream maize hybrids tested at that time, an indication that these natural occurring defences may have been lost whilst selective breeding favoured other traits such as yield (Sotelo, 1997; Migui & Lamb, 2003; Köllner *et al*., 2008). Maize is a genetically diverse crop with many farmers’ own varieties and commercially bred hybrids being available to farmers. Knowledge of induced plant defence in maize germplasm locally adapted to agroclimatic conditions is key since this could lead to selection and/or development of varieties with more durable resistance, through enhanced constitutive or induced plant defence (Stout & Davies, 2009). This study therefore tested how prevalent this indirect oviposition-induced trait is among African maize landraces and hybrids grown in Kenya with a view of utilizing this trait in developing environmentally benign alternative crop protection strategies.

4.3 Materials and methods

4.3.1 Plants

Five landrace maize varieties were used. These are known by local names as ‘Endere’, ‘Jowi’, ‘Kongere’, ‘Nyamula’ and ‘Sefensi’ and were obtained from smallholder farmers in western Kenya. The two maize hybrids used in this study were obtained from commercial seed suppliers. These were HB 515 (Western Seed Company Ltd., Kitale, Kenya) and SC Duma 43 (Agri SeedCo Ltd, Nairobi, Kenya). Seeds were planted individually in pots filled with fertilised soil in an insect-proof screen house under natural conditions (25°C, 65%RH; 12L: 12D) at *icipe*'s Thomas Odhiambo Campus, Mbita Point (0° 25'S, 34° 12'E, 1200 m above sea level). The seedlings were used in experiments when they were 3-4 weeks old, approximately 45 cm tall.

4.3.2 Insects

The *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) moths used in this study were obtained from the insect mass rearing unit at the *icipe*-Thomas Odhiambo campus. The larvae originated from field-collected stemborers, principally from sorghum fields in the Mbita region in western Kenya. Larvae were reared on a semi-synthetic diet containing sorghum leaf powder (Ochieng *et al*., 1985). Field collected egg parasitoids, *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae) and larval parasitoids, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) were reared on stemborer eggs and larvae respectively, using methodologies described by Overholt *et al*. (1994). These insects were maintained at 24 ± 3°C, 70 ± 5% RH, 12L: 12D. The mass-reared culture was infused with a field-collected population every three months to avoid genetic decay and maintain the original behavioural characteristics of the species. Naive mated female moths and parasitoids obtained from second to third generation of the original field collected culture were used in the experiments.

4.3.3 Volatile organic compound (VOC) collection

Volatile compounds from whole maize plants, with and without stemborer eggs, were collected using headspace sampling (Agelopoulos *et al*., 1999) (Plate 4.1). Prior to volatile collection, seedlings for oviposition were placed in oviposition cages (80 X 40 X 40 cm) into which five gravid naïve female moths were introduced and kept overnight to oviposit. A wad of cotton wool (10 cm in diameter) moistened with water was placed into the cage for the moths to feed on. Control plants were kept inside similar cages but without *C. partellus* moths. Volatiles were collected from these plants for a period of 48 hours, starting at the last two hours of photophase of the following day. Leaves with or without eggs were enclosed in polyethyleneterephthalate (PET) bags (3.2 L,~ 12.5 mm thick) heated to 150^{O}C before use and fitted with a swagelock inlet and outlet ports. Charcoal-filtered air was pumped (600 ml min^{-1}) through the inlet port. Volatiles were collected on Porapak Q (0.05g, 60/80 mesh; Supelco) filters inserted into the outlet through which air was drawn at 400 ml min^{-1}. Elution of the entrained volatiles was done using 0.5 ml dichloromethane. The eluted samples were stored in tightly capped microvials in a -20^{o}C freezer prior to bioassays and further analysis. Entrainments of both oviposited and control plants were replicated four times and each plant was used only once.

4.3.4 Four-arm olfactometer bioassay

Responses of parasitoids to plant derived volatiles were evaluated in a Perspex four-arm olfactometer (Pettersson, 1970). Headspace samples (10 µL aliquots) were applied, using a micropipette (Drummond ‘microcap’, Drummond Scientific Co., Broomall, PA, USA), to a piece of filter paper (4 x 25 mm) subsequently placed in an inlet port at the end of each olfactometer arm. Gravid female parasitoids without any prior exposure to plants or hosts were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Air was drawn through the four arms towards the centre at 260 ml min^{-1}. Time spent in each olfactometer arm was recorded with ‘Olfa’ software (F. Nazzi, Udine, Italy) for 12 minutes. A choice-test was carried out to compare insect responses to headspace samples from oviposited and control plants. The two opposite arms held the test stimuli (10 µL aliquots of headspace sample). The remaining two arms were solvent controls. The experiment was replicated 12 times.

4.3.5 Gas Chromatography (GC) analysis

Entrained VOCs were analyzed using a Hewlett-Packard 7890 GC machine (Agilent Technologies) equipped with a cool-on column injector, a non-polar HP-1 capillary column (50 m, 0.32mm internal diameter, 0.52 µm film thickness) and a flame ionization detector (FID). Four µL of headspace sample was injected into the injector port of the GC instrument. Oven temperature was maintained at 30^{o}C for 2 minutes and then programmed at 5^{o}C min-1 to 250^{o}C. The carrier gas was hydrogen. Data were analyzed using HP Chemstation software.

4.3.6 Electrophysiological analysis

Coupled Gas Chromatography-electroantennography (GC-EAG) was carried out using the antennae of female *C. sesamiae* with the headspace samples of the different maize varieties that elicited positive responses during olfactometer bioassays. The GC-EAG system, in which the effluent from GC column is simultaneously directed to the antennal preparation and GC detector, was previously described by Wadhams (1990). EAG recordings were made using Ag-AgCl glass electrodes filled with saline solution, compositions as in Maddrell (1969), but without glucose. A female parasitoid was chilled for one minute and the head excised and the tips of both antennae were removed to ensure good conduct. The indifferent electrode was placed within the head capsule. Signals were then passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and analysed using a customised Syntech software package. Separation of volatiles was done on a 6890N GC (Agilent Technologies) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm internal diameter, 0.52µm film thickness). The oven temperature was maintained at 30 ^{o}C for 2 minutes and then programmed at 15 ^{o}C min^{-1} to 250 ^{o}C. The carrier gas was helium. Outputs from EAG amplifier and the FID were analysed using Syntech software package.

4.3.7 Coupled GC-Mass Spectrometry (GC-MS) analysis

Aliquots of attractive headspace samples were analysed using a Hewlett-Packard 5890 GC machine (Agilent Technologies) on a capillary Gas Chromatography HP-1 column (50 m, 0.32 mm internal diameter, 0.52 μm film thickness) directly coupled to a mass spectrometer (VG Autospec; Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionisation was performed by electron impact (70 eV at 250^{o}C). The oven temperature was maintained at 30^{o}C for 5 minutes, and then programmed at 5^{o}C min^{-1} to 250^{o}C. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005). Tentative identifications of the compounds were confirmed through co-injections with authentic standards.

4.3.8 Statistical analysis

Four-arm olfactometer bioassay data, i.e. time spent in each arm by parasitoid, were compared by analysis of variance (ANOVA) after conversion of the data into proportions and a logratio transformation. Means were separated using Tukey test with α set at 0.05. Statistical analyses were done using R software (R, 2013).

4.4 Results

4.4.1 Behavioural responses of parasitoids to headspace samples of volatiles from maize with and without eggs

Female egg parasitoids, *T. bournieri,* were significantly attracted to volatiles from all maize landraces exposed to egg deposition (Endere: $F_{2,33}$= 8.24, *P*<0.001; Jowi: $F_{2,33}$= 3.73, *P*=0.03; Kongere: $F_{2,33}$= 9.06, *P*<0.001; Nyamula: $F_{2,33}$=11.56, *P*<0.001; Sefensi: $F_{2,33}$= *P* 10.12, <0.001) compared to volatiles from unexposed plants and solvent controls (Fig. 4.1). Similarly *C. sesamiae*, spent significantly more time in the olfactometer arms with volatiles from maize landraces exposed to egg deposition by *C.*

partellus compared to arms with volatiles from unoviposited plants and the solvent control (Endere: $F_{2,33}$ = 9.79, *P* <0.001; Jowi: $F_{2,33}$ = 12.77, *P*<0.001; Kongere: $F_{2,33}$=6.67, *P* = 0.004; Nyamula: $F_{2,33}$=11.17, *P* <0.001; Sefensi: $F_{2,33}$=13.34, *P* <0.001) (Fig. 4.1B). Similar observations were made in the bioassay involving hybrid SC Duma 43 ($F_{2,33}$=4.23, *P*=0.024 for *T. bournieri* and $F_{2,33}$=9.47, *P*<0.001 for *C. sesamiae* (Fig. 4.2). In contrast, volatiles collected from the oviposited commercial hybrid, HB 515, were not attractive to either *T. bournieri* or *C. sesamiae* ($F_{2,33}$=0.12, *P*=0.88 and $F_{2,33}$=0.32, *P*=0.73 respectively) (Fig. 4.2).

4.4.2 Changes in volatile profiles in plants with and without eggs

Gas chromatography analysis of headspace samples revealed quantitative and qualitative changes in the volatile profiles emitted by all maize landraces and hybrid SC Duma 43 exposed to egg deposition in comparison to the unoviposited maize plants of the same varieties. There were marked increases in the levels of electrophysiologically active compounds produced after *C. partellus* egg deposition on maize landraces and hybrid SC Duma 43 (Figs. 4.3; 4.5 - 4.9). However, there was no change in the volatile profile of HB 515 hybrid maize (Fig. 4.4).

4.4.3 Identification of attractive volatile organic compounds

GC-EAG recordings with the attractive samples from different maize varieties revealed that *C. sesamiae* antennae were responsive to certain compounds in the headspace sample aliquots (Figs. 4.10 and 4.11). Subsequent GC-MS identification showed that *C. sesamiae* antennae were responsive to 3-methyl-1-butanol, hexenal, 2,3-butanenediol, octane, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, nonane, 6-methyl-5-heptene-2-one, α-pinene, myrcene, limonene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, decanal, 3,4-dimethylacetophenone and (*E*)-β-farnesene.

4.5 Discussion

This study demonstrated that the oviposition-induced volatile emission trait is prevalent in all the locally adapted African open pollinated maize varieties (OPVs) that were screened. All the OPVs in this study showed enhanced volatile profiles when *C. partellus* eggs were laid on plants. Comparison of volatile profiles revealed a close correspondence between egg-induced changes in the volatile profile and attraction observed in the olfactometer bioassays. Previous studies reported similar volatile profile changes and parasitoid attraction in three maize OPVs of Latin American origin (Tamiru *et al*., 2011). However, this effect occurred only in the landraces but not the hybrid maize screened then. In this study, one commercial maize hybrid, SC Duma 43, was identified to also possess this defence trait.

Studies on plant-insect interactions have shown that oviposition by herbivorous insects can induce indirect plant defence through emission of volatiles that attract parasitoids (Hilker & Meiners, 2006). However, volatile suppression following egg deposition may also occur. For example, oviposition by *Spodoptera frugiperda* (J.E.Smith) (Lepidoptera: Noctuidae) on maize plant (*Zea mays* L. var. Delprim) showed that oviposited plants emitted linalool in lower amounts in comparison with unoviposited maize plants over time (Penaflor *et al*., 2011). Bruce *et al.* (2010) also found that oviposition by *C. partellus* on African forage grass*, (Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf.*)* resulted in marked reduction in emission of main volatile, *Z*-3-hexenyl acetate (*Z*3HA) but the ratio of certain other minor component volatiles to *Z*3HA was increased. HIPVs provide natural enemies of the herbivores with early-alert cues for plants colonised by their host and thus enhance their foraging behaviour (Hilker & Meiners, 2006; Bruce *et al*., 2010). Recruitment of natural enemies through HIPVs emission following oviposition is considered a preventive defence strategy against herbivores as plants are able to defend themselves prior to damage caused by emerging larvae (Hilker & Meiners, 2002). Emission of volatile cues that attract larval parasitoids following egg deposition means that these parasitoids are recruited in anticipation of larval emergence.

Parasitized lepidopteran eggs do not develop into larvae and parasitised larvae feed less than non-parasitized ones and die upon emergence of the adult wasp, this greatly reduces damage to the plant (Hoballah *et al*., 2002; 2004).

Since *C. partellus* has a short life cycle, with eggs hatching to larvae in four to five days after laying under tropical conditions (Harris, 1990), the presence of *C. partellus* eggs indicates the likely presence of larvae in maize fields. This best explains the observed attraction of larval parasitoids to the volatile cues emitted following egg deposition. Being an annual plant with a short life cycle, maize varieties possessing this oviposition-induced defence trait will benefit from recruiting parasitoids even more than perennial plants with similar oviposition-induced traits (Hilker & Meiners, 2006). Parasitoids are also under selection pressure to respond to cues such as HIPVs emitted following herbivore egg deposition and even distinguish between cues caused by mechanical damage and those induced by the presence of their hosts as this enhances their foraging efficiency eventually improving their ecological fitness.

While this oviposition-induced defence trait was found to be prevalent in all the five maize landraces in the current study, the occurrence of the trait in only one commercial hybrid maize varieties screened so far is a clear indication that these defensive responses induced by the early herbivory may have been lost during the process of maize breeding for grain quality and yield. Although only two hybrids and five landraces were tested in this study, ongoing work on the Sustainable Crop Production Research for International Development (SCPRID) programme at *icipe* has screened over 25 landraces and 60 hybrids and only one inbred line (CKIR12001) and 60% of landraces were observed to posess this trait (*personal communication*, Amanuel Tamiru). Possible loss of defensive traits during crop breeding has been postulated. Sotelo (1997) reported possible loss of direct defences and below-ground indirect defence losses have been reported by Rasmann *et al*. (2005) and Köllner *et al*. (2008). The occurrence of this above-ground indirect defence trait elicited by insect eggs in commercial hybrid maize bred for high yield and drought tolerance (http://www.seedco.co/) indicates that this hybrid can be a source for the genetic material for introgression of this trait into other commercial hybrids. This may allow for exploitation of this trait in pest management in high yielding maize hybrids.

Qualitative and quantitative variation in oviposition-induced semiochemical emission was observed amongst the maize varieties possessing this inducible defence trait. Even within the landraces, there was variation in the quantity of emitted compounds as well the quality of the odour blends induced by egg deposition. Different groups of compounds were induced by egg deposition. These included Green Leaf Volatiles (GLVs) like (*Z*)-3-hexen-1-ol; monoterpenes like myrcene and limonene; homoterpenes like (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); sesquiterpenes like (*Z*)-β-farnesene and α- humulene; phenyl propanoids such as methyl salicylate and aldehydes such as decanal. Methyl salicylate was only shown to have elevated emission following egg deposition in the hybrid SC Duma 43. DMNT, a key compound known to attract *C. sesamiae* (Khan *et al*., 1997), was elevated by egg deposition in all the maize varieties that exhibited this trait. This strongly suggests that there is potential to exploit maize plants possessing this trait in biological control of the stemborer pests.

Although there was variability in the composition of the volatile compounds induced by egg deposition, most of the egg-induced volatiles in these maize varieties were similar to those induced by *C. partellus* eggs on wild maize (chapter 3) and those reported in mesoamerican maize landraces (Tamiru *et al*., 2011). This indicates that this oviposition-induced defence mechanism is conserved in maize germplasm and is only affected by interventions such as controlled breeding. Differences in induced volatile emissions have been reported in different plant species and different herbivores and their natural enemies are known to discrimate between these odour cues (Dicke & Vet, 1999). Interspecific and genotypic variation in herbivore induced odour emission has been shown among maize cultivars and inbred lines (Gouinguene *et al*., 2001; Degen *et* *al*., 2004). Different herbivores can also cause different plant responses through different feeding habits or elicitors present in the insect egg or regurgitant (Hopke *et al*., 1994; Turlings *et al*., 1998).

Results of this study revealed that the oviposition-induced indirect defence trait is prevalent in maize landraces which comprise locally adapted crop germplasm bred through open pollination and maintained by farmers. Although breeding may have caused loss of this important trait in hybrid maize, some hybrids still possess this trait. Variability in the emission of egg-induced semiochemicals also suggests that maize genotypes will vary in their attractivensss to natural enemies of herbivores. Therefore selecting and breeding maize cutlivars that release compounds attractive to antagonistic insects can be exploited for developing benign crop protection strategies based on this type of host plant resistance.

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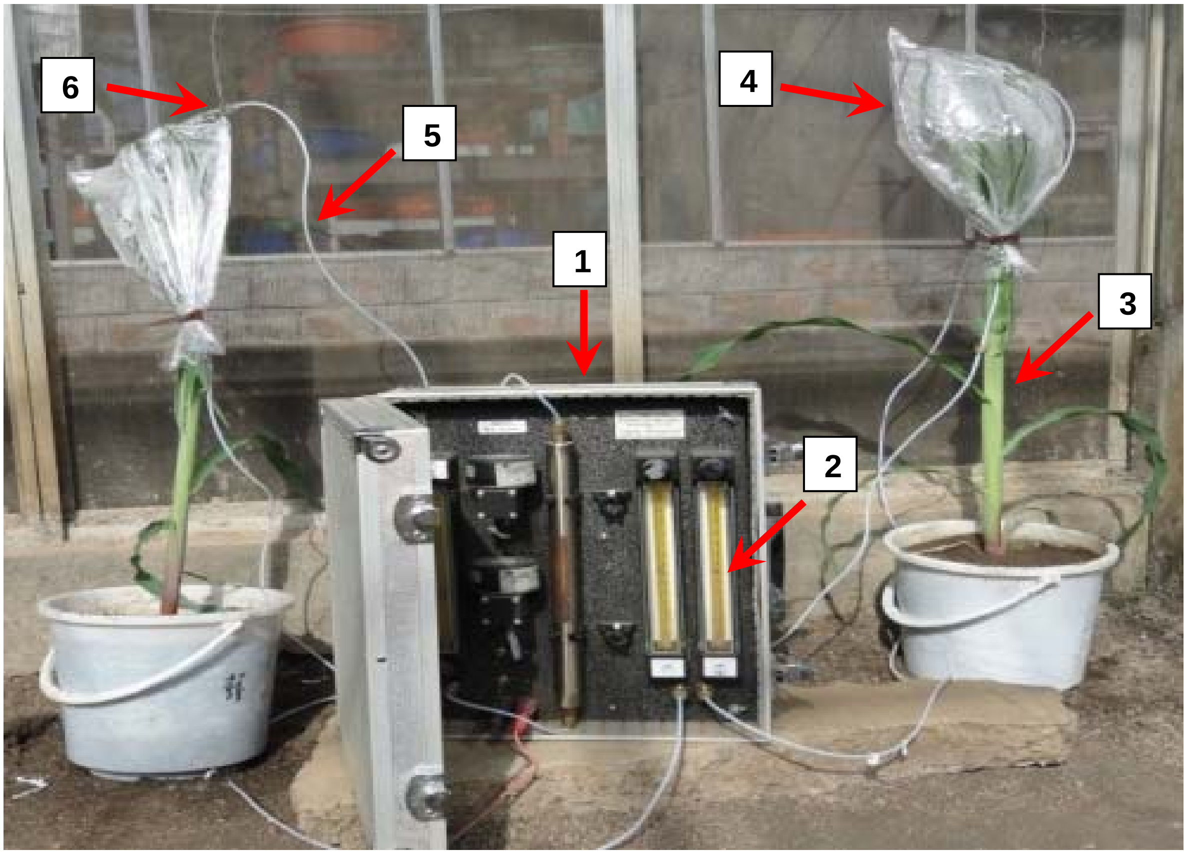

Plate 4.1 Headspace sampling set-up for volatile collection from maize seedlings exposed to egg deposition and unexposed control plants. The labels represent (1) Portable air entrainment kit, (2) Flow-metre controlling air flow rate, (3) Maize seedling from which volatiles are collected, (4) Polyethyleneterephthalate bags enclosing maize leaves, (5) Ethylene terephthalate tubes transporting air to/from the pump, (6) Porapak Q tubes trapping volatiles.

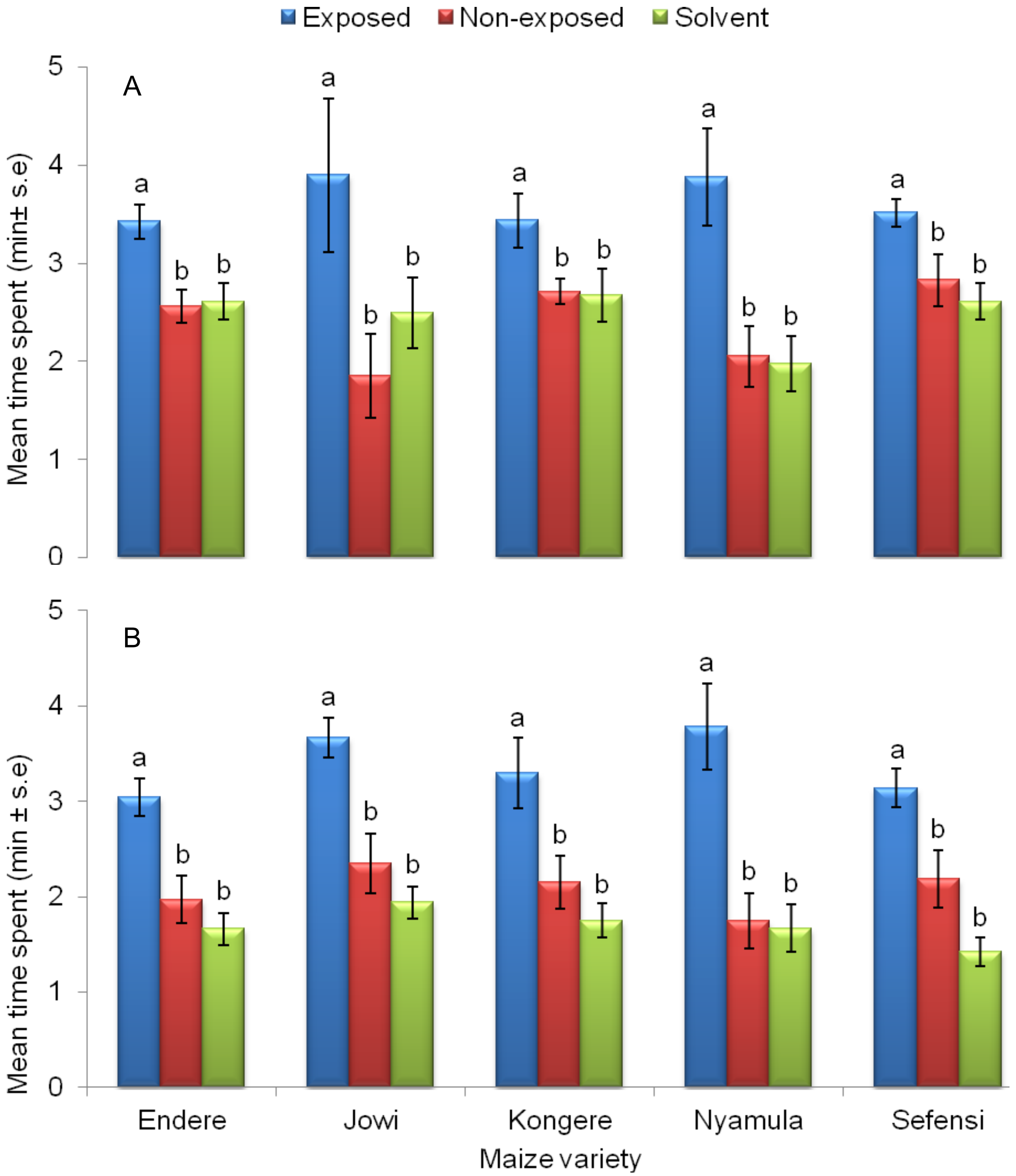

Figure 4.1: Behavioural response of female parasitoids to volatiles collected from landrace maize plants exposed and not exposed to *Chilo partellus* eggs and solvent control in a four-arm olfactometer bioassay. Response of (A) *Trichogramma bournieri*; (B) *Cotesia sesamiae*. Bars marked with different letters are significantly different (Tukey studentized range test: P<0.05).

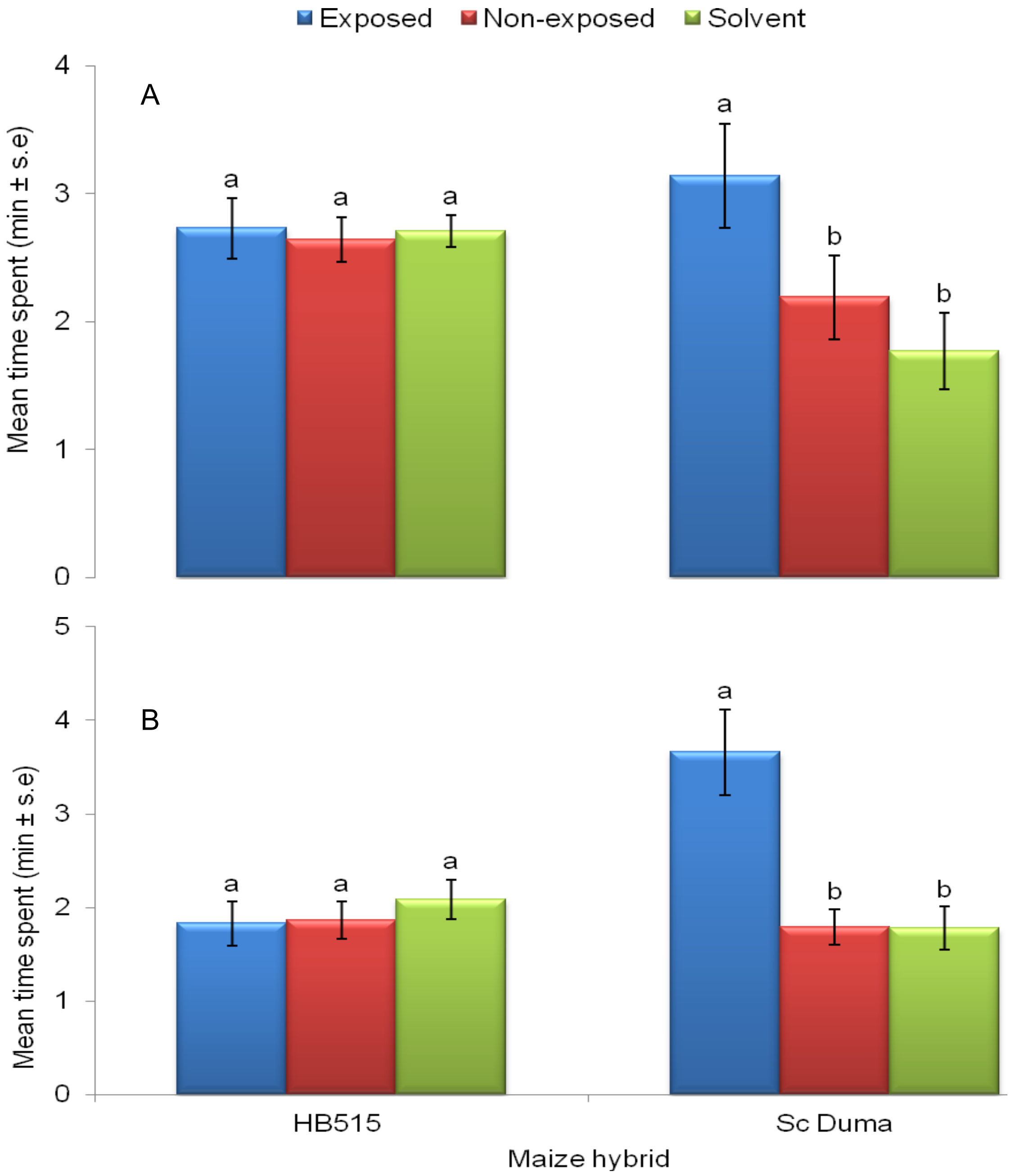

Figure 4.2: Behavioural response of female parasitoids to volatiles collected from hybrid maize plants exposed and not exposed to Chilo partellus eggs and solvent control in a four-arm olfactometer bioassay. Response of (A) *Trichogramma bournieri*; (B) *Cotesia sesamiae*. Bars marked with different letters are significantly different (Tukey studentized range test: $P<0.05$).

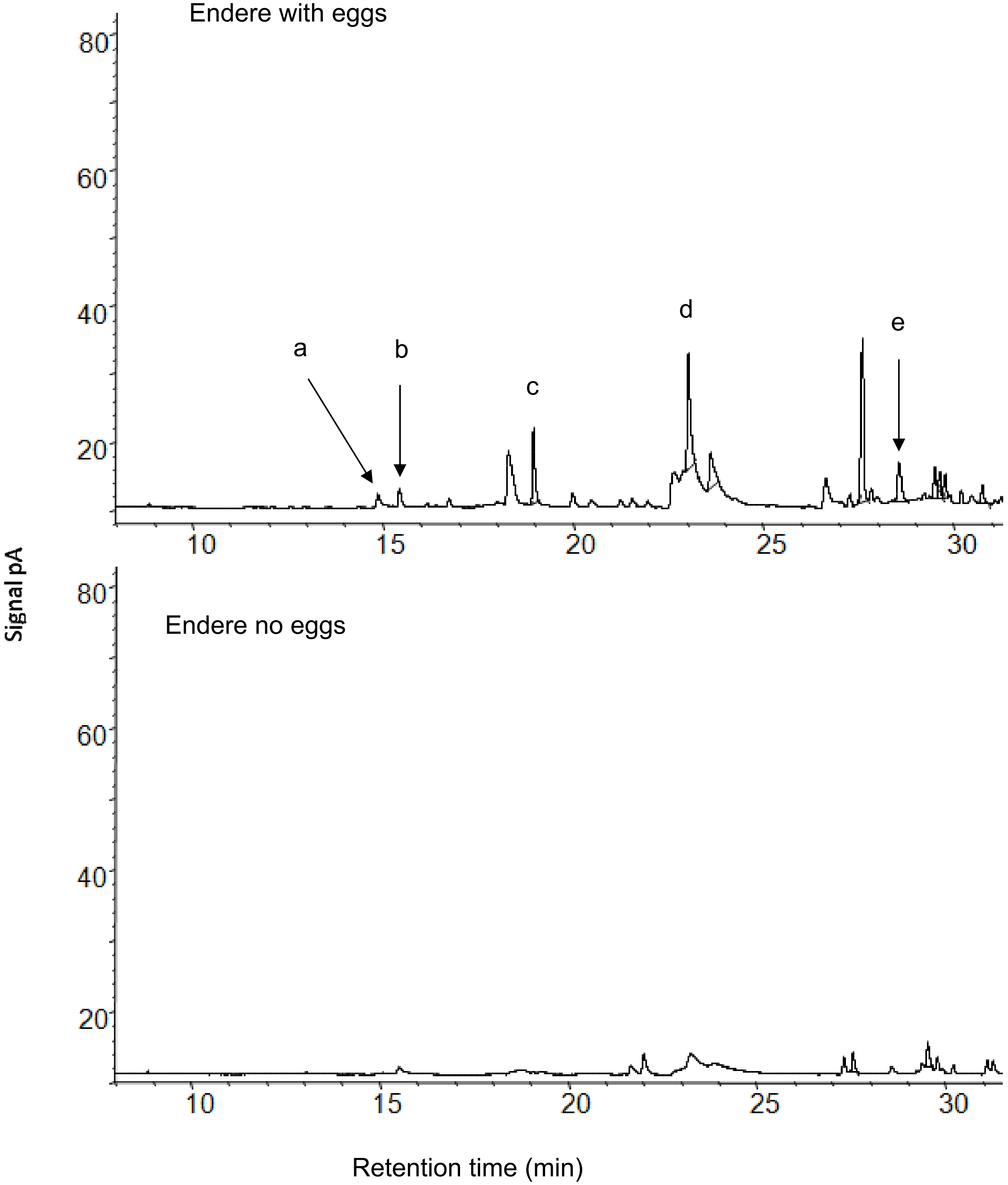

Figure 4.3: GC profiles of headspace volatiles from the landrace maize variety, Endere, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) myrcene; (b) limonene; (c) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (d) decanal; (e) (*E*)-β-farnesene.

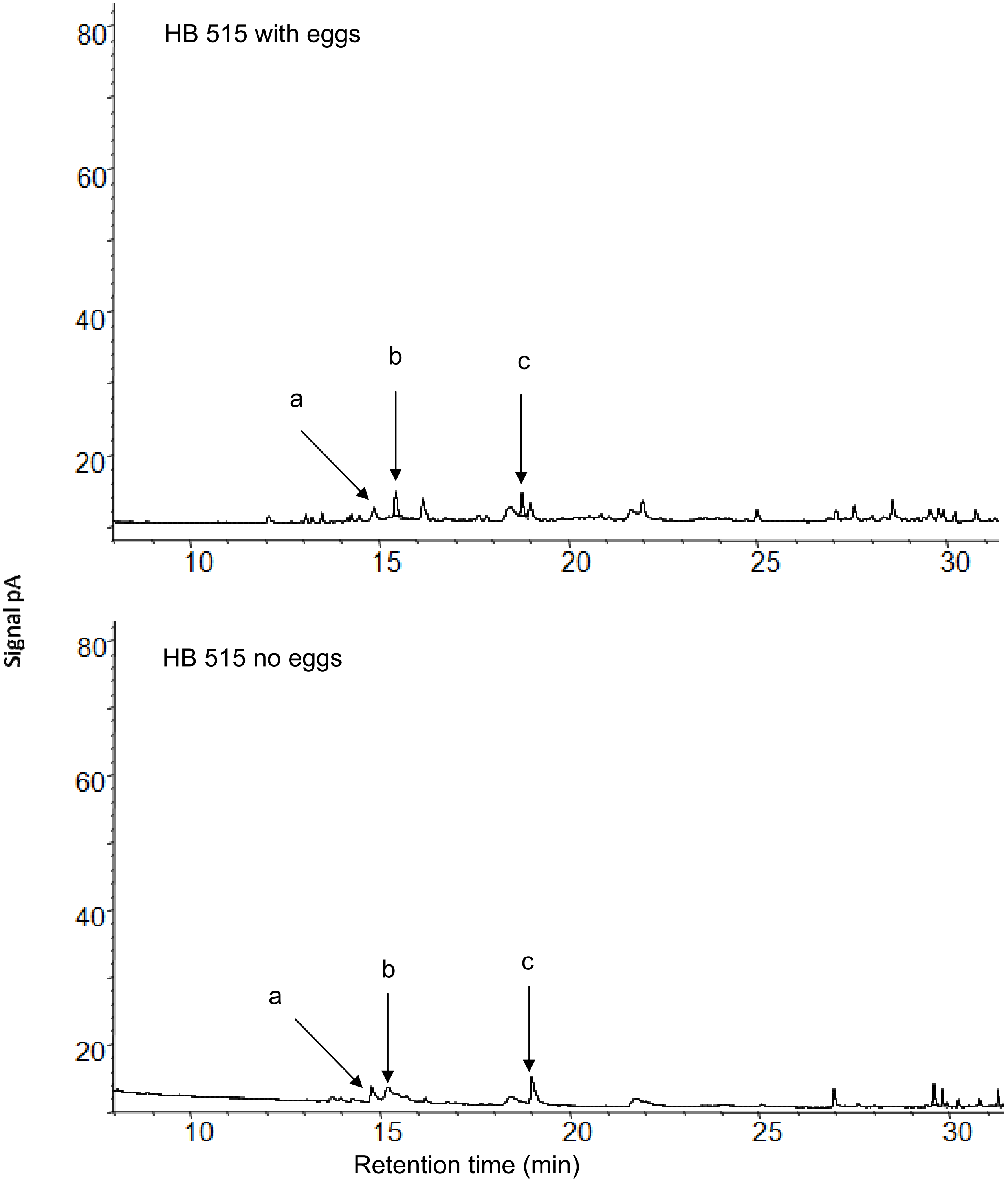

Figure 4.4: GC profiles of headspace volatiles from hybrid maize, HB 515, with and without *Chilo partellus* eggs. The identities of some of the compounds identified in this hybrid are as follows: (a) myrcene; (b) limonene; (c) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT). No marked changes were observed in oviposited and unoviposited plants.

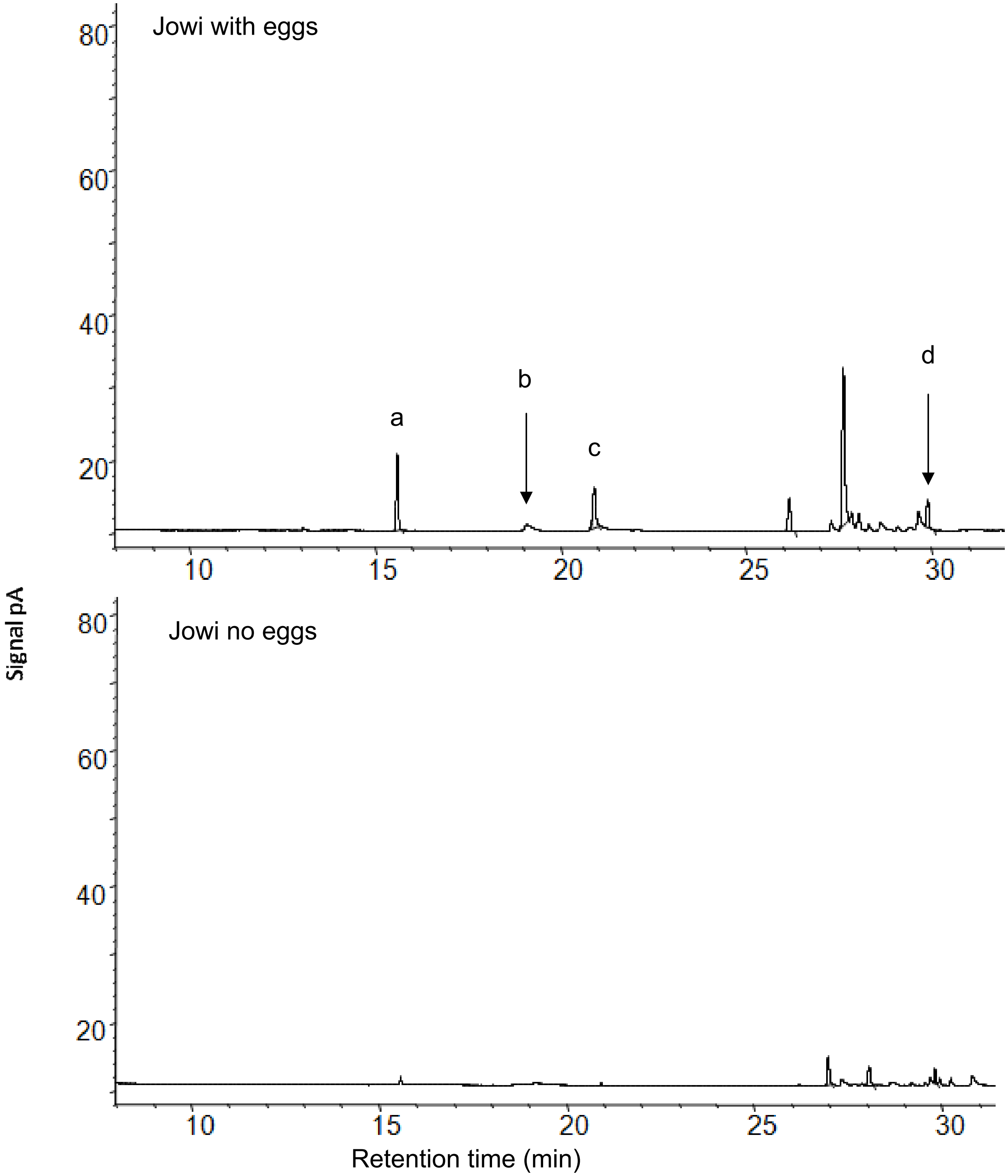

Figure 4.5: GC profiles of headspace volatiles from landrace maize, Jowi, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) limonene; (b) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (c) decanal; (d) (*E*)-β-farnesene.

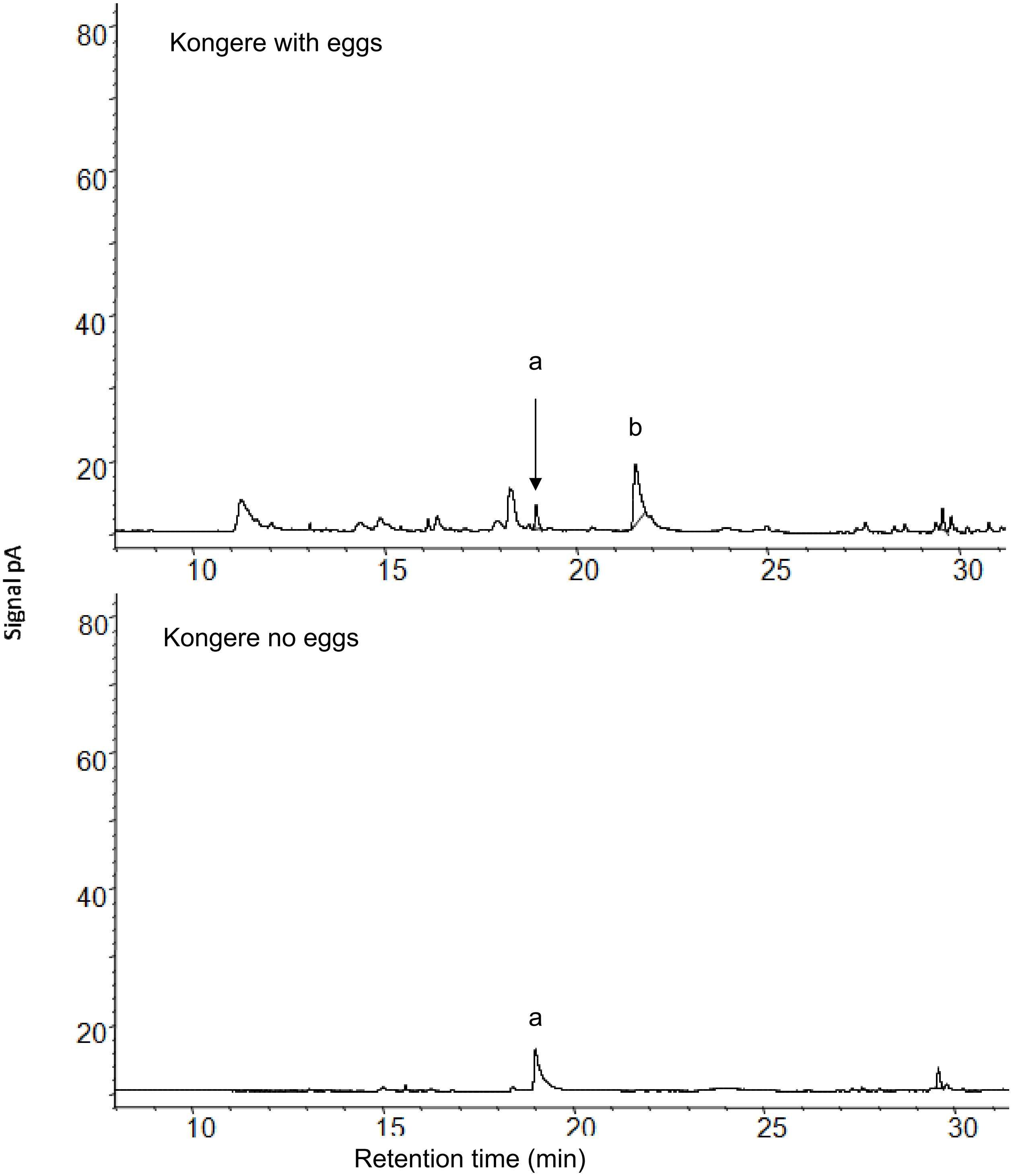

Figure 4.6: GC profiles of headspace volatiles from landrace maize, Kongere, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (b) decanal.

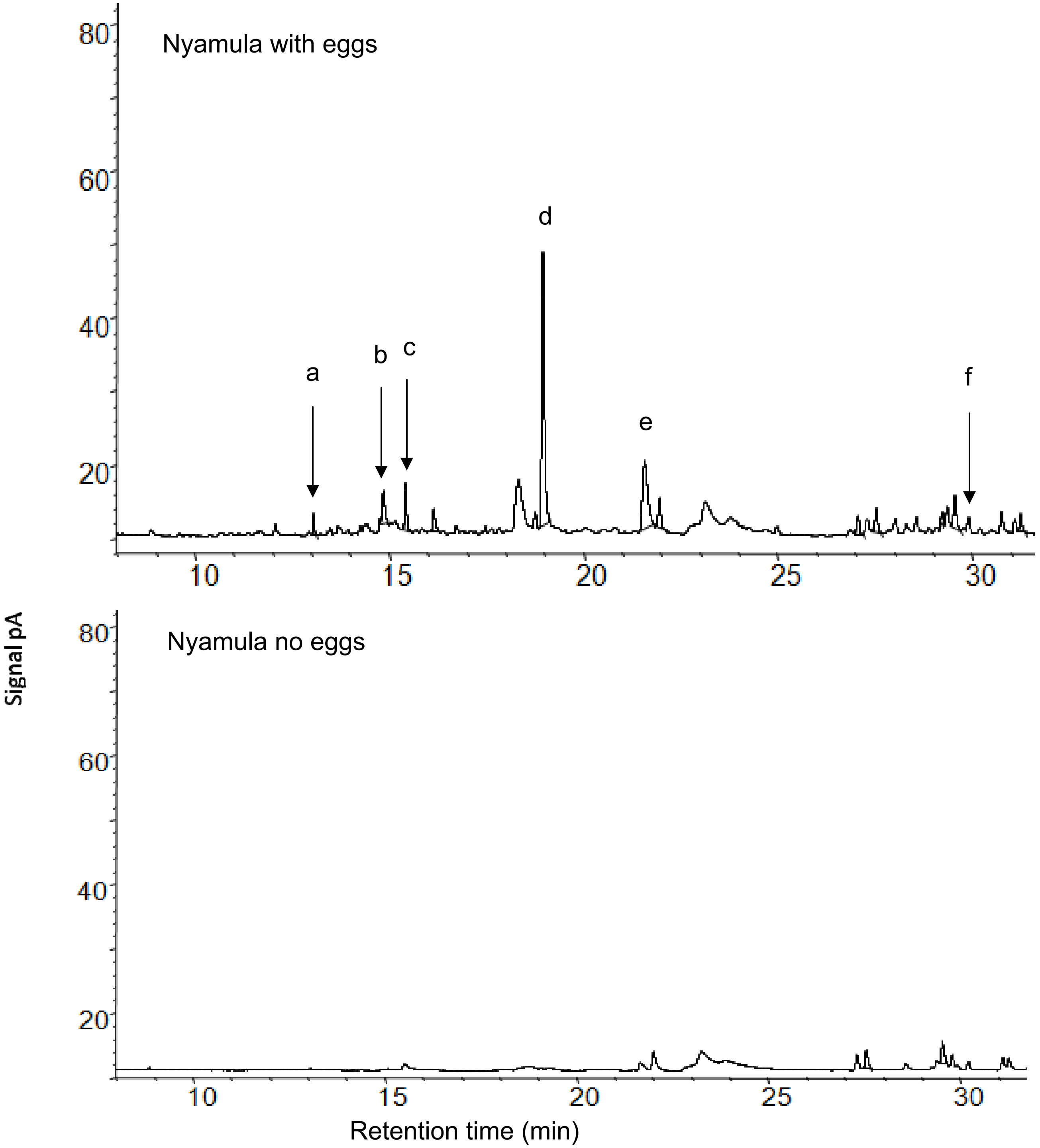

Figure 4.7: GC profiles of headspace volatiles from landrace maize, Nyamula, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) (*Z*)-3-hexen-1-ol; (b) myrcene; (c) limonene; (d) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (e) decanal (f) (*E*)-β-farnesene.

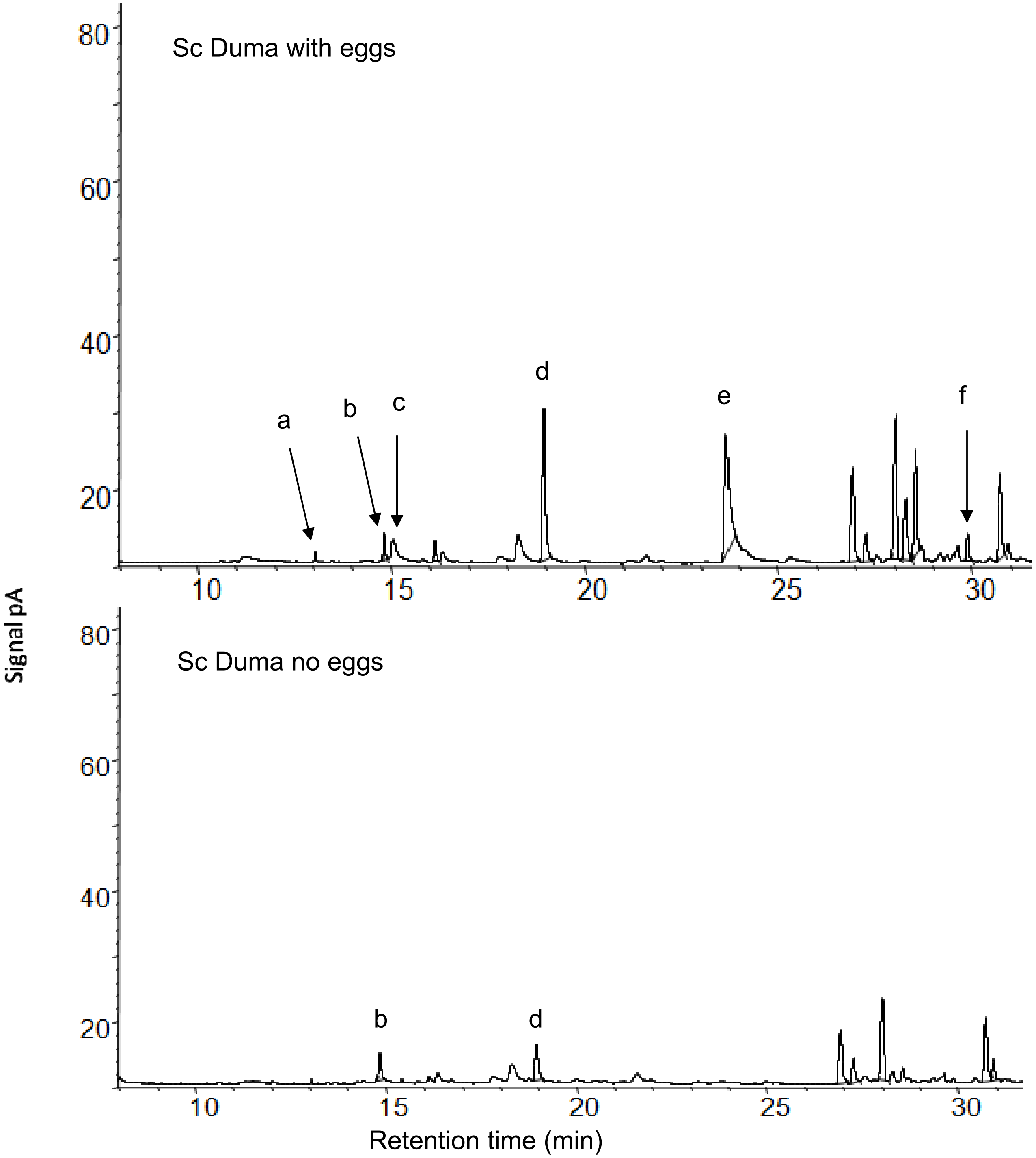

Figure 4.8: GC profiles of headspace volatiles from hybrid maize, SC Duma 43, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) (*Z*)-3-hexen-1-ol; (b) myrcene; (c) limonene; (d) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (e) methyl salicylate; (f) (*E*)-β-farnesene.

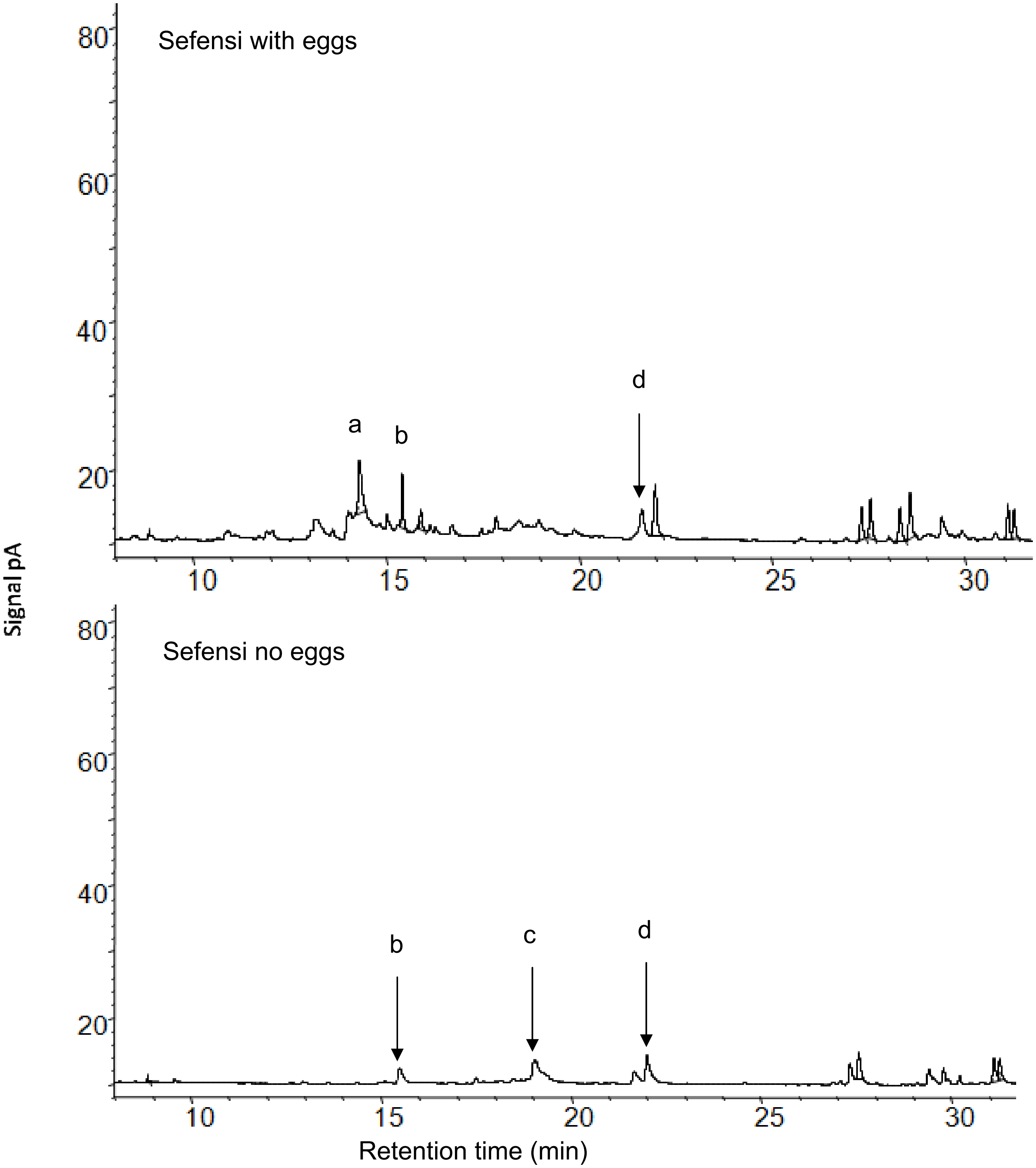

Figure 4.9: GC profiles of headspace volatiles from landrace maize, Sefensi, with and without *Chilo partellus* eggs. The identities of some of the compounds whose emission was elevated by oviposition are as follows: (a) myrcene; (b) limonene; (c) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (d) decanal.

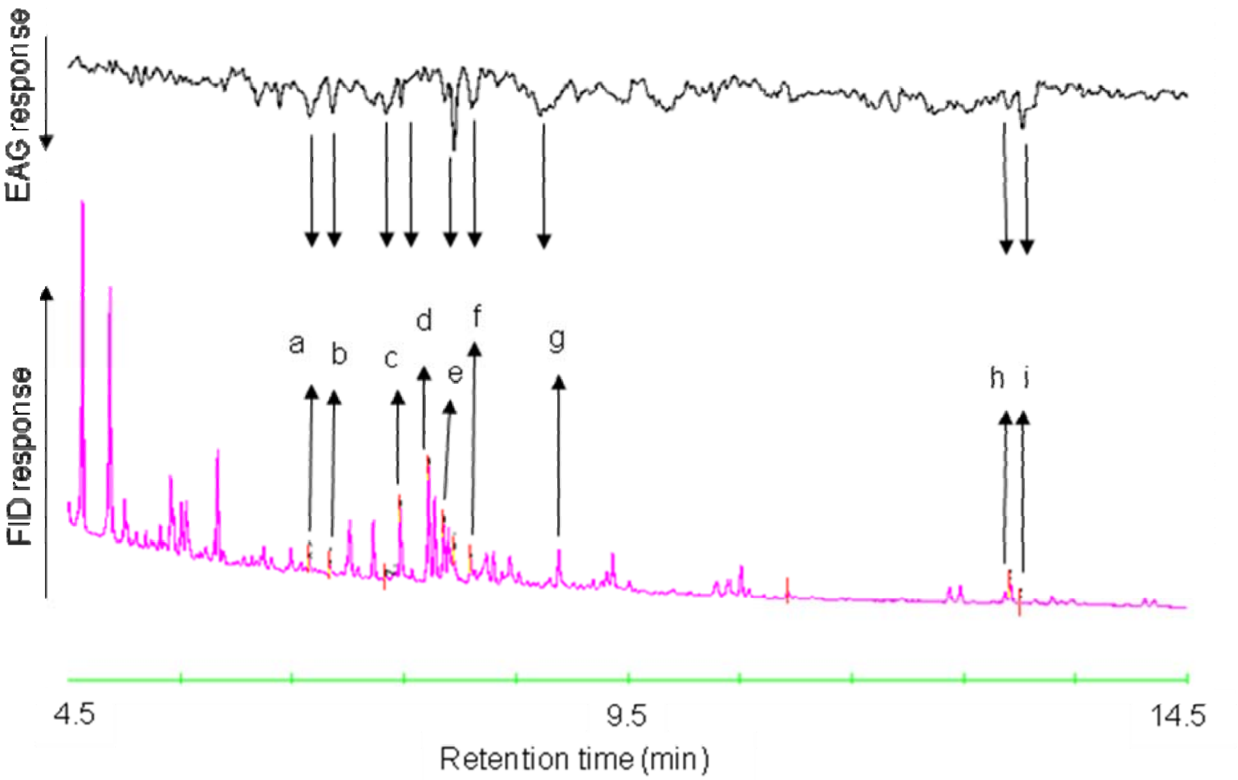

Figure 4.10: A representative GC-EAG response of female *Cotesia sesamiae* to volatiles collected from landrace Nyamula with eggs. FID peaks marked are those which elicited antennal response in coupled runs: a = 3-methyl-1-butanol acetate, b= (*E,E*)-2,4-hexadienal, c = (*Z*)-2-heptenal, d = (*Z*)-2-hepten-1-ol, e = 2-octenal, f = (*E,E*)-2,4 heptadienal, g = (*E*)-4,8-dimethyl-1,3,7-nonatriene, h = (*Z*)-β-farnesene, i = α-humulene.

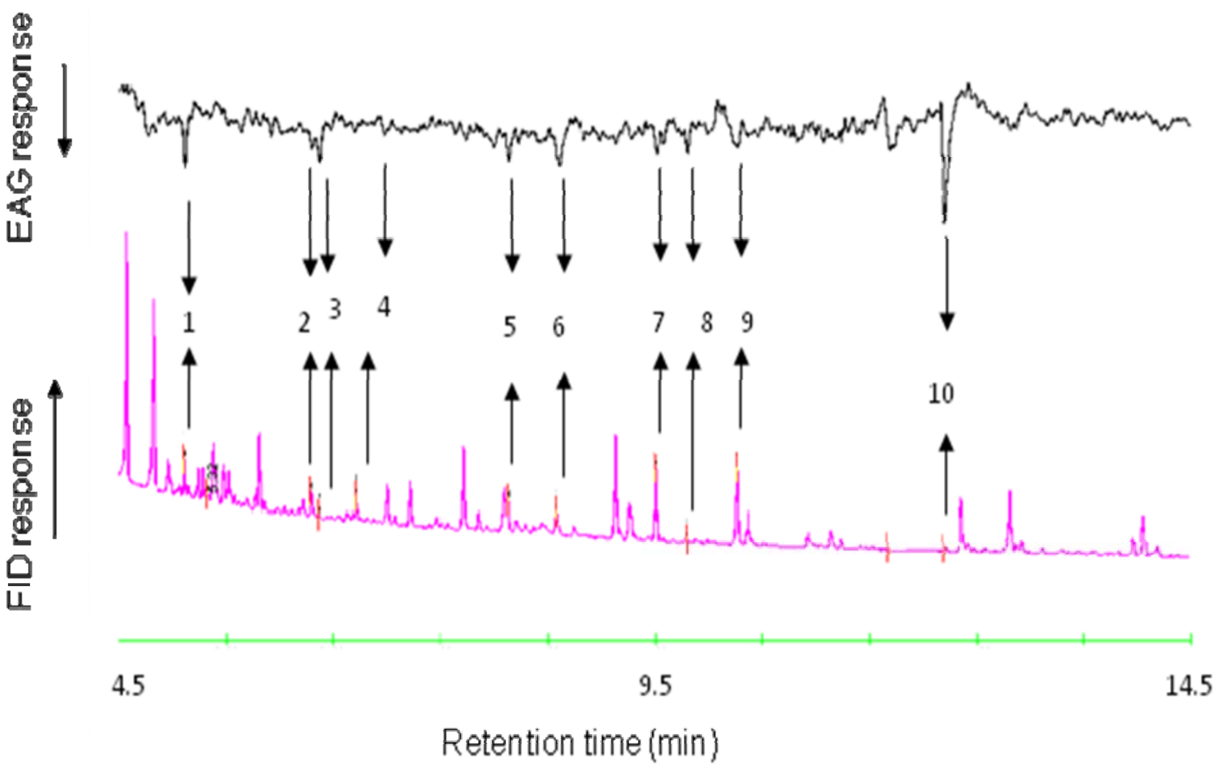

Figure 4.11: A representative GC-EAG response of female *Cotesia sesamiae* to volatiles collected from oviposited hybrid maize, SC Duma 43. The numbered FID peaks are those which elicited antennal response in coupled runs: 1= Propanal; 2= (*Z*)-3-hexen-1-ol; 3= Ethylbenzene; 4= 2-heptanone; 5=myrcene; 6= limonene; 7= (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); 8= 1-ethenyl-4-methoxy-benzene; 9= methyl salicylate; 10= (*Z*)-β-farnesene.

CHAPTER FIVE

5.0 INDUCTION OF AN INDIRECT DEFENCE TRAIT IN NEIGBOURING PLANTS THROUGH EGG-INDUCED MAIZE VOLATILES

5.1 Abstract

The attack of plants by herbivorous arthropods can result in considerable changes in the plant's chemical phenotype with emission of herbivore-induced plant volatiles (HIPVs). These HIPVs have been shown to act as repellents to the attacking insects as well as attractants for the insects antagonistic to these herbivores. Plants can also respond to HIPV signals from other plants that warn them of impending attack. Recent investigations have shown that certain maize varieties are able to emit volatiles following stemborer egg deposition. These volatiles attract the herbivore's parasitoids and directly deter further oviposition. However, it is not known whether these oviposition-induced maize volatiles can induce chemical phenotypic changes in neighbouring unattacked maize plants. Therefore, this study sought to investigate the effect of oviposition-induced maize volatiles on intact neighbouring maize plants. Headspace volatile samples were collected from maize plants exposed to *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) egg deposition, unoviposited neighbouring maize plants as well as control plants kept away from the volatile emitting ones. Behavioural bioassays were carried out in a four-arm olfactometer using egg (*Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae)) and larval (*Cotesia sesamiae* Cameron (Hymenoptera: Braconidae)) parasitoids. Coupled Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Electroantennography (GC-EAG) were used for volatile analysis. GC-MS analysis revealed enhanced profile changes in the oviposited and induced maize plants. Higher amounts of EAG-active biogenic volatiles such as (*E*)-4,8-dimethyl-1,3,7-nonatriene were emitted from these plants compared to control plants. Subsequent behavioural assays with female parasitic wasps *T. bournieri* and *C. sesamiae* indicated that these parasitoids preferred volatiles from oviposited and induced maize plants compared to those from the control plants. These results suggest that a maize plant emitting oviposition-induced volatiles attractive to the herbivore's natural enemies can induce this indirect defence trait in conspecific neighbouring undamaged maize plants. Maize plants growing in a field may thus benefit from this indirect defence through airborne signalling which enhances the fitness of the volatile-emitting plant by increasing predation pressure on herbivores.

Key Words: indirect defence, maize, oviposition-induced volatiles, parasitoids, plant-plant communication

5.2 Introduction

In nature, plants live in complex communities comprising herbivores, pollinators, microbes, carnivores and neighbouring conspecific and other plants (Kessler & Baldwin, 2002; Pieterse & Dicke, 2007; Schaller, 2008). These plants are thus under selection pressure to maximize fitness within a complex setting of biotic interactions with positive and negative outcomes (Dicke *et al*., 2009). As such, plants have evolved a diverse array of defence strategies against the attacking organisms including herbivores and parasitic plants (Khan *et al*., 2010). In particular, plants respond to herbivore attack through production of a number of chemical signals known as herbivore-induce plant volatiles (HIPVs), which have direct or indirect effect on the attacking herbivore. Directly, these chemical cues negatively affect the physiology or behaviour of the herbivore either as toxins, digestibility reducers or deterrents (Dicke *et al*., 2009). Indirectly, plants use these HIPVs to attract the natural enemies of the herbivores as well as increase the foraging success of these natural enemies, thereby facilitating imporved control of herbivores (Karban *et al*., 2000; Kessler & Baldwin, 2001).

HIPVs play a role in multitrophic community interactions (Turlings *et al*., 1995; Kessler & Baldwin, 2001; van Poecke & Dicke, 2004). Not only do they communicate between the infested plant and natural enemies of the attacking herbivores but also warn undamaged neighbouring plants of the same or another species, of the impending attack (Baldwin & Schultz, 1983; Arimura *et al*., 2000; Karban & Maron, 2002). They also systematically communicate between different parts of the same plant (intraplant signalling) (Heil, 2008; Arimura *et al*., 2009; Karban, 2011; Chamberlain, 2014). The HIPVs are emitted not only from the infested plant parts but also systematically from uninfested parts of the plant which increases the detectability of the signal cues (Dicke *et al*., 2009; Rodriguez-Saona *et al*., 2009; War *et al*., 2011).

Undamaged plants that can activate and tailor their defences according to information derived from their attacked neighbouring plants may gain a selective advantage over plants that are unable to make use of the signal cues (Kost & Heil, 2006). Evidence of plants being capable of ‘eavesdropping’ on airborne signals has been documented (Fowler & Lawton, 1985; Bruin *et al*., 1992; Shonle & Bergelson, 1995; Chamberlain *et al*., 2000; 2001; Karban *et al*., 2000; Pickett & Poppy, 2001; Tscharntke *et al*., 2001; Kost & Heil, 2006). HIPVs can immediately induce defence in neighbouring plants at artificially high levels (Heil & Ton, 2008) while at the same time, physiologically relevant levels of induced volatile organic compounds (VOCs) can prime plants to prepare themselves for future pest and pathogen attack (Heil & Ton, 2008). Perceived plant volatiles can also have physiological effects on the receiving plant as evidenced by changes in the transcription of defence-related genes (Arimura *et al*., 2000; Gomi *et al*., 2003; Paschold *et al*., 2006). Exposure of plants to herbivore-induced volatile organic compounds can result in changes in the abundance of phyto-hormones (Arimura *et al*., 2002; Engelberth *et al*., 2004) and increase production of defence-related metabolites such as terpenoids (Engelberth *et al*., 2004; Ruth & Kleir, 2005), proteinase inhibitors (Tscharntke *et al*., 2001) and phenolic compounds (Tscharntke *et al*., 2001).

HIPVs are produced by plants long after damage has been inflicted to the plant by feeding larvae. However, recent studies indicate that plants are able to respond to the initial stage of herbivore attack (egg deposition) by emission of volatile organic compounds that are attractive to certain natural enemies (Hilker & Meiners, 2002; Hilker & Meiners, 2006; Tamiru *et al*., 2011; 2012). Investigations involving maize-herbivore-natural enemy tritrophic interactions have shown that egg deposition by stemborers can induce volatile emission in certain maize varieties. These volatiles attract natural enemies and deter further herbivore colonization (Chapters 3 and 4; Tamiru *et al*., 2011; 2012). This is seen as a preventative defence mechanism since parasitoids are recruited in advance, before the phytophagous larvae hatch and cause damage to plants. However, it is not known whether these oviposition-induced volatiles can induce the same indirect defence trait in neighbouring unattacked conspecific maize plants.

This study sought to fill this gap. As such the objectives of this study were to determine whether 1) oviposition-induced volatiles also induce volatile emission in neighbouring unattacked maize plants; 2) induced volatiles from unattacked maize plants attract stemborer parasitoids.

5.3 Materials and methods

5.3.1 Plants

'Nyamula', a local maize landrace variety was used in this experiment. This landrace variety has been shown to emit oviposition-induced volatiles upon stemborer egg deposition. Seeds were obtained from local smallholder farmers in the Mbita region, western Kenya. The seeds were planted individually in pots filled with fertilised soil in an insect-proof screen house under natural conditions (25^{o}C, 65%RH; 12L: 12D) at *icipe*'s Thomas Odhiambo Campus, Mbita Point (0^{o} 25'S, 34^{o} 12'E, 1200 m above sea level). The seedlings were used in experiments when they were 3-4 weeks old, approximately 45 cm tall.

5.3.2 Insects

The *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) moths used in this study were obtained from the insect mass rearing unit at the *icipe*-Thomas Odhiambo campus. The larvae originated from field-collected stemborers, principally from sorghum *Sorghum bicolor* L. Moench fields in the Mbita region in western Kenya. Larvae were reared on a semi-synthetic diet containing sorghum leaf powder (Ochieng *et al*., 1985). Field collected egg parasitoids, *Trichogramma bournieri* Pintureau & Babault (Hymenoptera: Trichogrammatidae) and larval parasitoids, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) were reared on stemborer eggs and larvae respectively, using methodologies described by Overholt *et al*. (1994). The insects were maintained at 24 ± 3^{O}C, 70 ± 5% RH, 12L: 12D. The mass-reared culture was infused with a field-collected population every three months to avoid genetic decay and maintain the original behavioural characteristics of the species. Naive mated female moths and parasitoids obtained from second to third generation of the original field collected culture were used in the experiments.

5.3.3 Volatile collection

Volatile compounds were collected using headspace sampling (Agelopoulos *et al*., 1999) (Plate 5.1) from whole maize plants subjected to the following treatments: with stemborer eggs (*inducing* plants), without stemborer eggs but exposed to oviposited plants (*induced* plants) and, without stemborer eggs and not exposed to oviposited plants (control plants). Prior to volatile collection, seedlings for oviposition were placed in oviposition cages (80 X 40 X 40 cm) into which five gravid naïve female moths were introduced and kept overnight for oviposition. A wad of cotton wool (10 cm in diameter) moistened with water was placed into the cage for the moths to feed on. The following day, 20 oviposited plants were moved into an insect-proof screen house and arranged in two rows, 60 cm apart and 30cm between the plants to act as the inducing plants. Ten unoviposited and undamaged maize plants of the same variety and age were then introduced between the two rows and placed 30cm apart. The set up (Plate 5.2) was left for three days. Control plants were kept inside similar cages but without *C. partellus* moths in an insect-proof screen house. Volatiles were collected from these plants for a period of 48 hours, starting at the last two hours of photophase. Maize leaves were enclosed in polyethyleneterephthalate (PET) bags (3.2 L,~ 12.5 mm thick) heated to 150^{O}C before use and fitted with a swagelock inlet and outlet ports. Charcoal-filtered air was pumped (600 ml min^{-1}) through the inlet port. Volatiles were collected on Porapak Q (0.05g, 60/80 mesh; Supelco) filters inserted into the outlet through which air was drawn at 400 ml min^{-1}. Elution of the entrained volatiles was done using 0.5 ml dichloromethane. The eluted samples were stored in tightly capped microvials in a -20^{o}C freezer prior to bioassays and further analysis. Entrainments from both oviposited (inducing), induced and control plants were replicated four times and each plant was used only once.

5.3.4 Behavioural bioassay

Parasitoids responses to plant derived volatiles were evaluated in a Perspex four-arm olfactometer (Pettersson, 1970). Headspace samples (10 µL aliquots) were applied, using a micropipette (Drummond 'microcap', Drummond Scientific Co., Broomall, PA, USA), to a piece of filter paper (4 x 25 mm) subsequently placed in an inlet port at the end of each olfactometer arm. Gravid female parasitoids without any prior exposure to plants or hosts were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Air was drawn through the four arms towards the centre at 260 ml min^{-1}. Time spent in each olfactometer arm was recorded with 'Olfa' software (F. Nazzi, Udine, Italy) for 12 minutes. A choice-test was carried out to compare insect responses to headspace samples from induced and control plants, as well as from oviposited and control plants. The two opposite arms held the test stimuli (10 µL aliquots of headspace sample). The remaining two arms were solvent controls. The experiment was replicated 12 times.

5.3.5 Chemical analysis

Entrained VOCs were analyzed using a Hewlett-Packard 7890 GC machine (Agilent Technologies) equipped with a cool-on column injector, a non-polar HP-1 capillary column (50 m, 0.32mm internal diameter, 0.52 µm film thickness) and a flame ionization detector (FID). Four µL of headspace sample was injected into the injector port of the GC instrument. Oven temperature was maintained at 30^{o}C for 2 minutes and then programmed at 5^{o}C min^{-1} to 250^{o}C. The carrier gas was hydrogen. Data was analyzed using HP Chemstation software. Aliquots of attractive headspace samples were analysed using a Hewlett-Packard 5890 GC machine (Agilent Technologies) on a capillary Gas Chromatography HP-1 column (50 m, 0.32 mm internal diameter, 0.52 µm film thickness) directly coupled to a mass spectrometer (VG Autospec; Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionisation was performed by electron impact (70 eV at 250^{o}C). The oven temperature was maintained at 30^{o}C for 5 minutes, and then programmed at 5^{o}C min^{-1} to 250^{o}C. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005). Tentative identifications of the compounds were confirmed through co-injections with authentic standards. Quantification of DMNT, a key compound known to attract larval parsitoids *C. sesamiae* (Khan *et al*., 1997) in both oviposited (inducing), induced and control maize plants was done by comparing the peak area of these treatments to the peak area of 100 nanograms of synthetic DMNT. This was done by first injecting 1 μl of synthetic DMNT which contained 100 ng of DMNT prepared in redistilled hexane. The peak area was recorded and a response factor calculated using equation 1. Then an equal amount of natural headspace sample containing unknown concentration of DMNT was analysed and the amount of compound was obtained using equation 2.

Equation 1:

$$\text{Response factor} = \frac{\text{peak area}}{\text{Sample amount}}$$

Equation 2:

$$\text{Amount of analyte} = \frac{\text{peak area}}{\text{Response factor}}$$

5.3.6 Electrophysiological analysis

Coupled Gas Chromatography-electroantennography (GC-EAG) was carried out using the antennae of female *C. sesamiae* with the headspace samples of the oviposited and induced maize plants that elicited positive responses during olfactometer bioassays. The GC-EAG system, in which the effluent from GC column is simultaneously directed to the antennal preparation and GC detector, was previously described by Wadhams (1990). EAG recordings were made using Ag-AgCl glass electrodes filled with saline solution, compositions as in Maddrell (1969), but without glucose. A female parasitoid was chilled for one minute and the head excised and the tips of both antennae were removed to ensure good conduct. The indifferent electrode was placed within the head capsule. Signals were then passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and analysed using a customised Syntech software package. Separation of volatiles was done on a 6890N GC (Agilent Technologies) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm internal diameter, 0.52μm film thickness). The oven temperature was maintained at 30 ^{o}C for 2 minutes and then programmed at 15 ^{o}C min^{-1} to 250 ^{o}C. The carrier gas was helium. Outputs from EAG amplifier and the FID were analysed using Syntech software package.

5.3.7 Statistical analysis

Four-arm olfactometer bioassay data, i.e. time spent in each arm by parasitoids, were compared by analysis of variance (ANOVA) after conversion of the data into proportions and a logratio transformation. Means were separated using Tukey test with α set at 0.05. Statistical analyses were done using R software (R, 2013).

5.4 Results

5.4.1 Behavioural responses of parasitoids to headspace samples of volatiles from oviposited, induced and control maize plants

Both egg (*T. bournieri*) and larval (*C. sesamiae*) parasitoids were significantly attracted to volatiles from oviposited maize plants compared to those from unoviposited plants and solvent controls ($F_{2,33}$= 10.37, P<0.001; $F_{2,33}$=12.76, P<0.001 respectively) (Fig. 5.1). Similarly, both egg and larval parasitoids spent significantly more time in the olfactometer arms with volatiles from undamaged maize plants exposed to oviposition-induced volatile-emitting maize plants compared to arms holding volatiles from unexposed plants and solvent controls ($F_{2,33}$=18.39, P<0.001; $F_{2,33}$=10.54, P<0.001) (Fig. 5.2).

5.4.2 Chemical analysis

Gas chromatography analysis of the headspace samples revealed quantitative and qualitative changes in the volatile profiles emitted by the landrace maize plants exposed to egg deposition in comparison to the unoviposited control maize plants of the same variety. Similar observations were made for the headspace samples from the unoviposited maize plants which had been exposed to plants emitting egg-induced volatiles (Fig. 5.3). There were marked increases in the levels of compounds which have been shown to be electrophysiologically active and play a key role in parasitoid attraction such as (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) in the oviposited and the neighbouring maize plants exposed to those emitting egg-induced volatiles (Fig. 5.3). Quantification of DMNT from the maize treatments showed that the oviposited maize plants produced on average 3.28 ± 1.53 ng/μl compared to 0.84 ± 0.56 ng/μl of the intact neighbouring maize plant exposed to egg-induced maize volatiles and 0.04 ± 0.03 ng/μl from the control plant. Comparison of the quantities of the DMNT emitted by both treatments revealed that the undamaged maize plants exposed to egg-induced maize volatiles emitted by neigbouring plants produced 21 times more DMNT compared to the control plant. At the same time, the amount of DMNT emitted by the undamaged maize plants exposed to egg-induced maize volatiles was 3.9 times less compared to the amount emitted by the oviposited maize plant which was used for inducing the indirect defence to the neighbouring undamaged plant (Table 5.1).

5.4.3 Identification of electrophysiologically active volatile compounds

Coupled GC-EAG recordings with the attractive samples in the bioassays, from intact plants exposed to egg-induced volatiles and oviposited maize plants showed that *C. sesamiae* antennae were responsive to certain compounds in the headspace sample aliquots (Fig. 5.4). Subsequent coupled GC-MS identification showed that the EAG-active compounds included 2-heptanol, decane, (*R*)-limonene, methyl benzoate, (*R*)-linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate and decanal.

5.5 Discussion

Results from this study showed that *C. partellus* egg deposition on plants of the landrace, Nyamula, induced emission of VOCs that are attractive to stemborer parasitoids. Interestingly, the current findings also show that the egg-induced maize volatiles can induce an indirect defence response in a conspecific neighbouring maize landrace through airborne signalling. This demonstrates that egg-induced volatile organic compounds were recognized by undamaged, neighbouring maize plants. This response to signalling of an impending herbivore attack, prompts plants to activate their defence mechanisms, eventually emitting volatile semiochemicals that attract natural enemies of the herbivores. Previous studies have shown that herbivore egg deposition on certain maize varieties induces volatile profile changes and emissions that are attractive to both egg and larval parasitoids. This trait has been found to be prevalent in wild and landrace maize varieties and rare in maize hybrids (Chapters 3 & 4; Tamiru *et al*., 2011; 2012). This study reports for the first time that egg-induced maize volatiles induce an indirect defence trait in undamaged neighbouring plants of maize landraces.

Comparing the egg-induced volatile organic compounds (VOCs) from the oviposited maize plants and those from neighbouring intact maize plants exposed to the egg-induced VOCs revealed quantitative rather than qualitative differences. Larger quantities of VOCs were emitted from the oviposited maize plant compared to the induced plant. The mechanism of emission of physiologically-active volatile compounds from exposed plants can have two possible explanations. Firstly, the egg-induced maize volatiles might have been adsorbed to the surface of the exposed maize plants and later volatilized once more. Secondly, it is possible that volatiles from oviposited plants triggered production of electrophysiologically active semiochemicals by exposed plants. Given that VOCs have been shown to induce expression of defence genes in uninfested plants (Bate & Rothstein, 1998; Arimura *et al*., 2001; Kost & Heil, 2006), the latter explanation seems viable. The VOCs released from oviposited and VOCs-exposed intact maize plants generally matched those of previous studies on egg-induced volatiles in maize varieties (Chapters 3 & 4; Tamiru *et al*., 2011; 2012). These included compounds like Green Leaf Volatiles (GLVs); monoterpenes like myrcene and limonene; homoterpenes like (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); sesquiterpenes like (*Z*)-β-farnesene and (*E*)-β-caryophyllene; phenyl propanoids like methyl salicylate and aldehydes such as decanal. DMNT, a key compound known to attract *C. sesamiae* (Khan *et al*., 1997), was elevated by egg deposition as well as exposure of intact maize to egg-indued VOCs. The emission of these compounds explains the observed behavioural responses of the parasitoids in the olfactometer bioassays.

The role of HIPVs in influencing defence pathways and responses in neighbouring undamaged plants has been previously described (Chamberlain *et al*., 2000; Pickett & Poppy, 2001; Chamberlain, 2014). Volatile organic compounds (VOCs) have been shown to induce defence responses in plants. For example, the Green-leaf volatile (GLV), (*Z*)-3-hexenyl acetate induces defence genes in unifested leaves of Lima bean (Arimura *et al*., 2000; Kost & Heil, 2006) and *Arabidopsis* (Bate & Rothstein, 1998). Other compounds which have been identified to elicit VOC-induced plant responses include (*Z*)-jasmone (Birkett *et al*., 2000; Bruce *et al*., 2003), (*E,Z*)-β-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (Arimura *et al*., 2000) and methyl salicylate (Shulaev *et al*., 1997). GLVs have also been shown to prime maize plants against subsequent herbivore attack (Engelberth *et al*., 2004). Whilst HIPVs can immediately induce defence signalling in neighbouring plants at artificially high levels, physiologically relevant levels of HIPVs appear instead to prime plants to prepare for future herbivore and pathogen attack (Heil & Ton, 2008). Since most of these volatile compounds had elevated levels and emissions following egg deposition, it is suggested that they are the ones responsible for the induction of indirect defence mechanisms in neighbouring undamaged maize plants.

Plants that are able to ‘eavesdrop’ volatile cues from neighbouring attacked plants and use these cues to activate and tailor their defences according to information derived from their attacked neighbours in anticipation of herbivore attack may gain a selective advantage over plants that are unable to make use of these signal cues (Kost & Heil, 2006). Additionally, egg-induced volatiles provide natural enemies of herbivores with early-alert cues indicating the presence of hosts (Hilker & Meiners, 2006; Bruce *et al*., 2010). Thus, the emission of volatile organic compounds attractive to natural enemies of herbivores from both the oviposited and neighbouring undamaged maize plants increases the signal strength of these attractive cues thereby increasing the recruitment and foraging efficiency of the antagonists. This eventually increases the preventive defence strategy of the plant community against herbivores since large numbers of natural enemies are recruited to parasitize eggs as well as emerging larvae before causing damage to the plants (Hilker & Meiners, 2002). Furthermore, parasitized lepidopteran eggs do not develop into larvae and parasitised larvae feed less than non-parasitized ones and die upon emergence of the adult wasp, which greatly reduces damage to the plant (Hoballah *et al*., 2002; 2004).

The induction of defensive responses in maize plants through airborne signals from oviposited plants even before any damage is inflicted to the crop plant could contribute to the natural protection of crop plants against stemborers. For instance, intercropping maize varieties that are able to respond defensively to early-herbivory with varieties that can perceive the emitted chemical signals can enhance natural enemy recruitment as opposed to crop monocultures that cannot respond to egg deposition or volatile signals induced by oviposition. This could be of practical importance as demonstrated by Pettersson *et al*. (1999) and Ninkovic *et al*. (2002) who planted mixtures of barley cultivars emitting volatiles that negatively affected host plant acceptance by aphids. Since there is variability in emission of egg-induced semiochemicals in maize germplasm, and the levels of emission may be too low for practical crop protection purposes, it may be possible to boost the strength of the signal by companion planting with maize varieties that emit larger amounts of effective volatile compounds as described by Pickett *et al*. (2014).

5.6 References

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Table 5.1: Total amount of (DMNT) produced by oviposited (inducing), induced and control maize plants

Treatment	Mean ± S.E concentration of DMNT (ng/μl)
Inducing plant	3.28 ± 1.53
Induced plant	0.84 ± 0.56
Control plant	0.04 ± 0.03

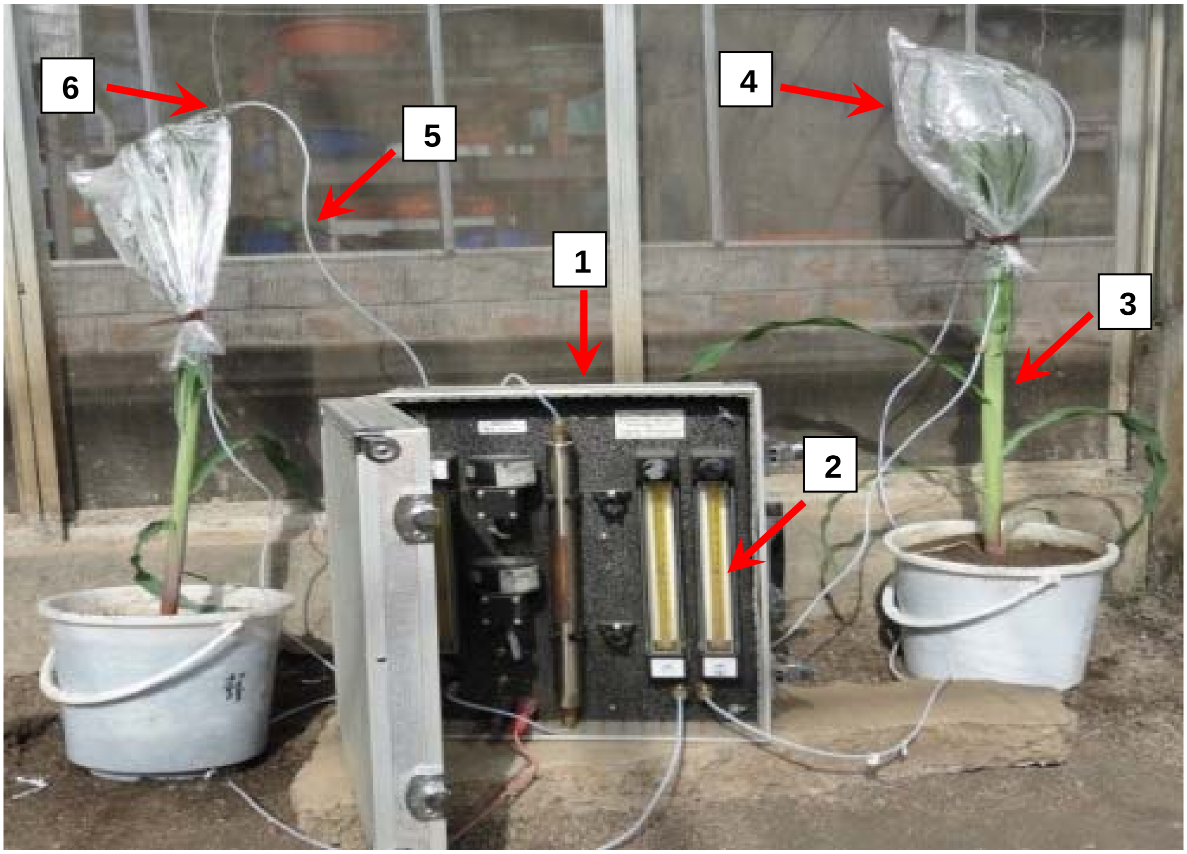

Plate 5.1 Headspace sampling set-up for volatile collection from maize seedlings exposed to egg deposition, exposed to egg-induced maize volatiles and unexposed control plants. The labels represent (1) Portable air entrainment kit, (2) Flow-metre controlling air flow rate, (3) Maize seedling from which volatiles are collected, (4) Polyethyleneterephthalate bags enclosing maize leaves, (5) Ethylene terephthalate tubes transporting air to/from the pump, (6) Porapak Q tubes trapping volatiles.

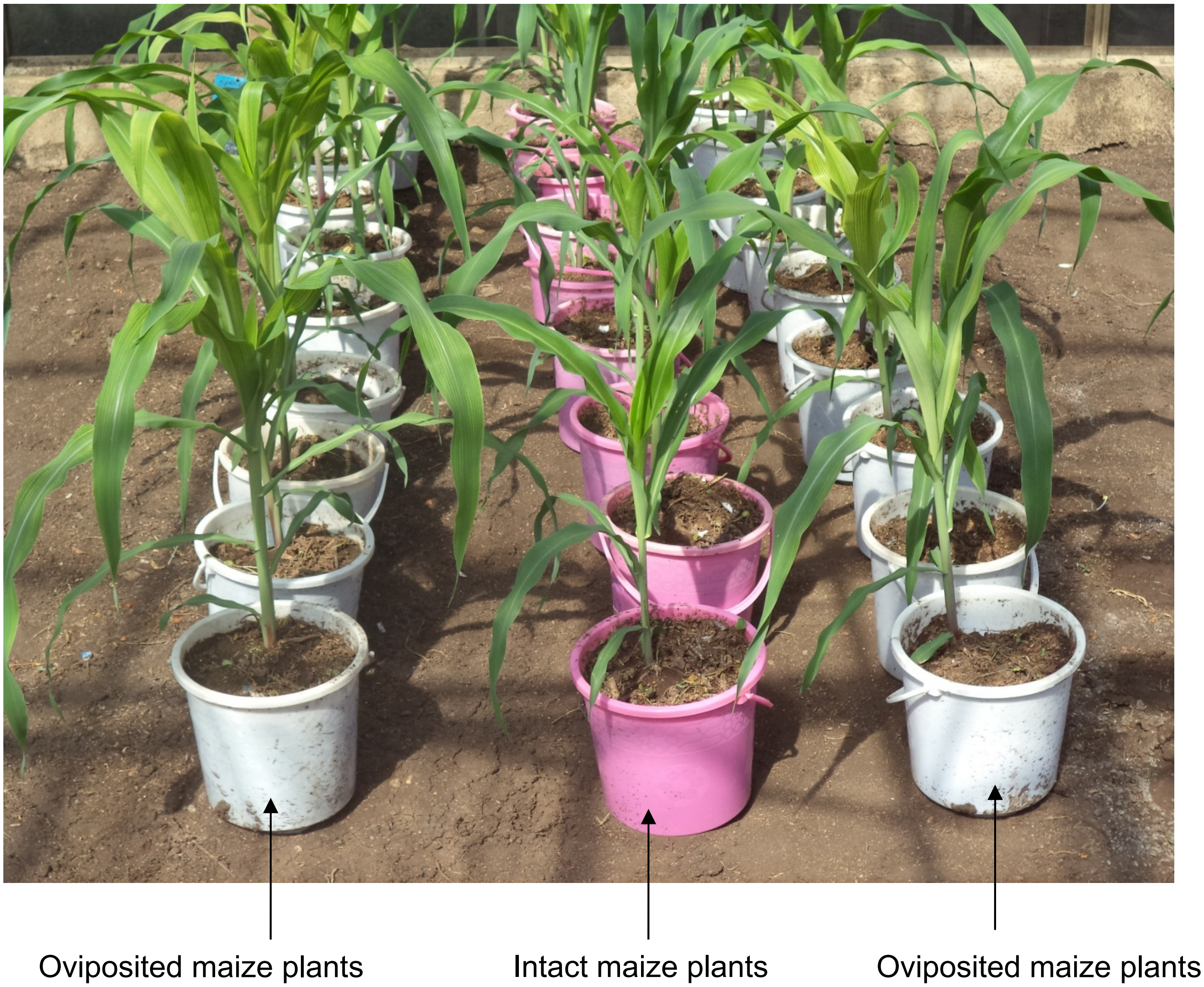

Plate 5.2: Experimental set up exposing undamaged maize plants to egg-induced maize volatiles from neighbouring maize plants.

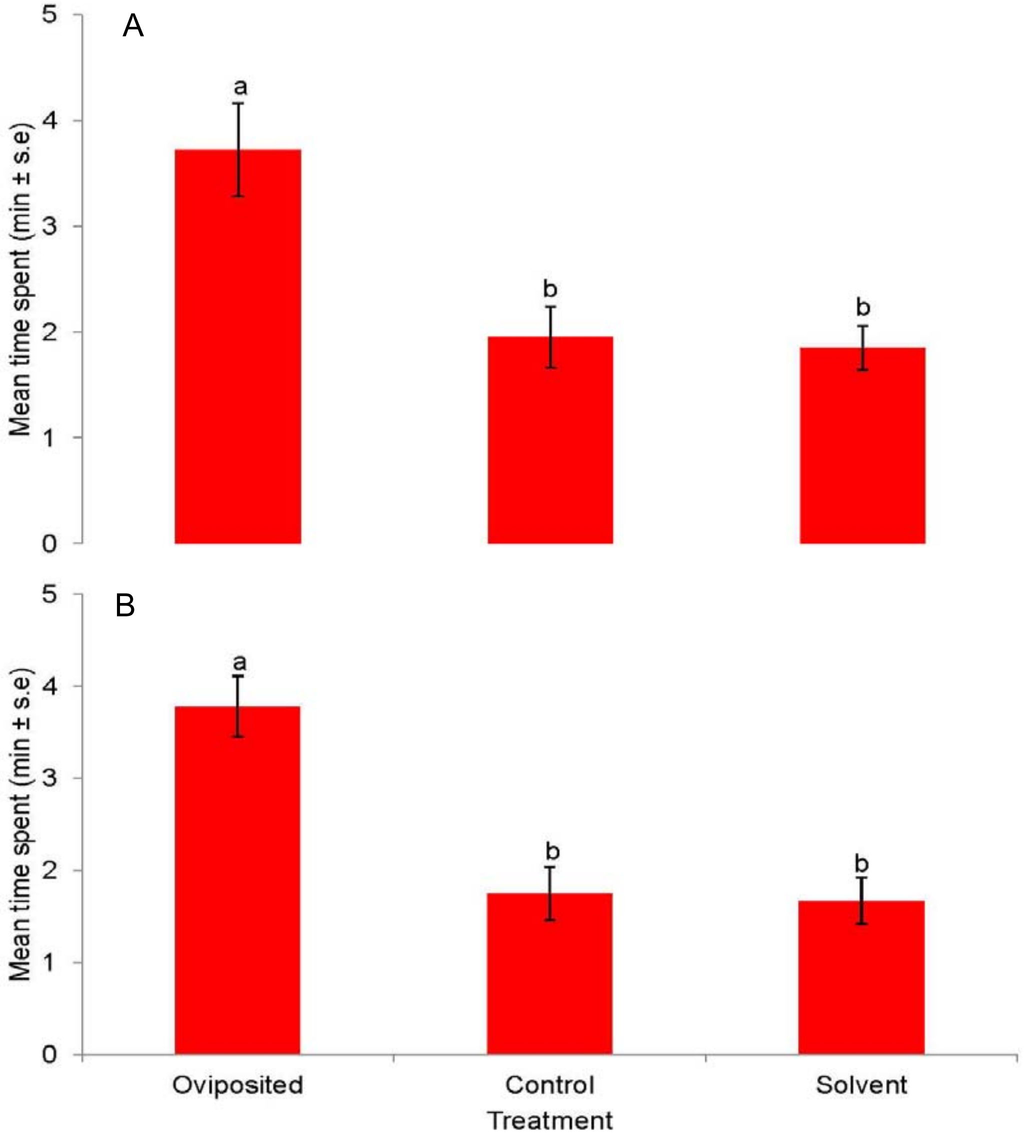

Figure 5.1: Behavioural response of female parasitoids to volatiles collected from maize seedlings exposed and not exposed to *Chilo partellus* eggs and solvent control in a four-arm olfactometer bioassay. Response of (A) *Trichogramma bournieri*; (B) *Cotesia sesamiae*. Parasitoid responses were compared by ANOVA after conversion of the data into proportions and log-ratio transformation. Different letters on bars indicate a significant difference using Tukey studentized range test ($P < 0.05$).

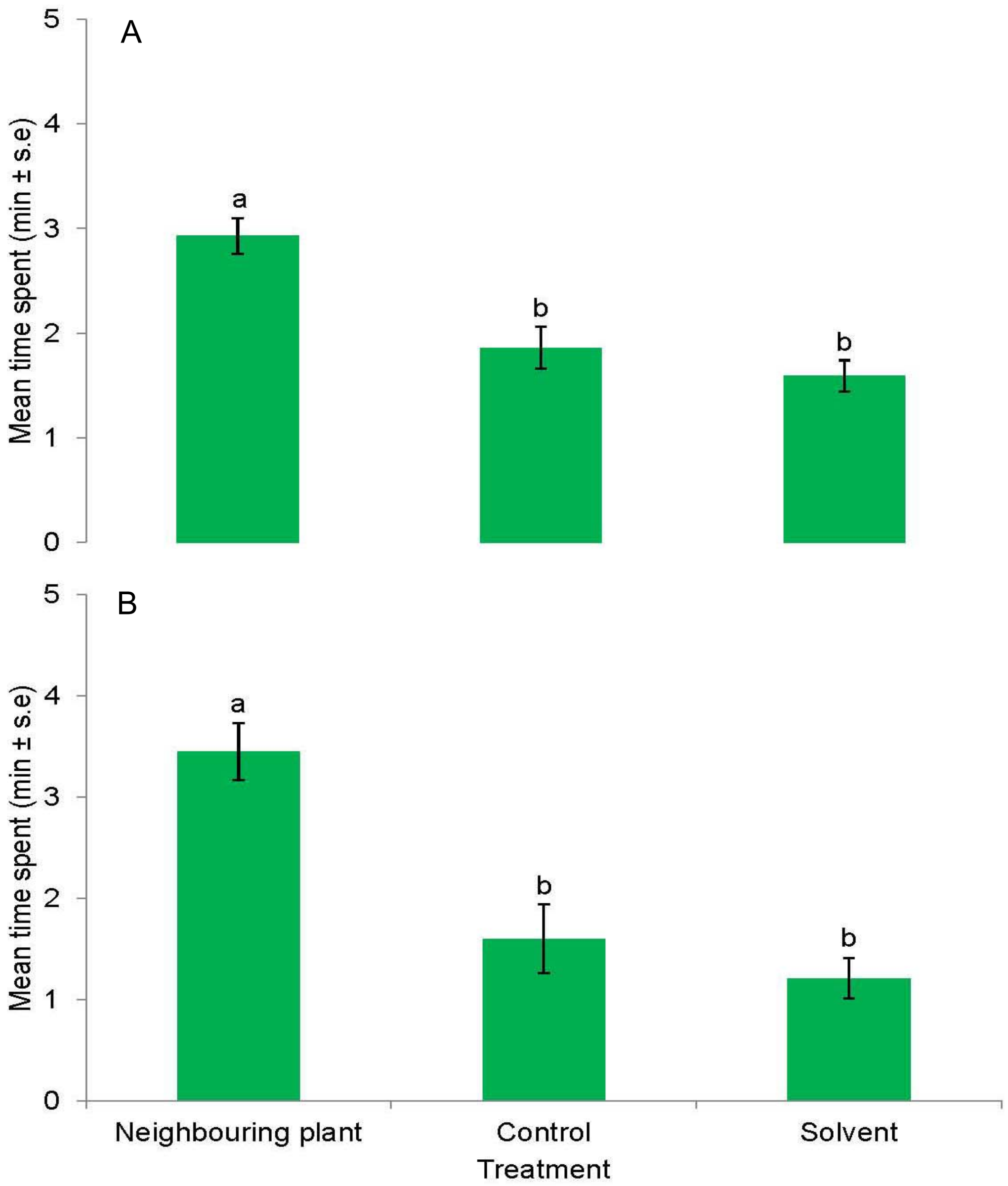

Figure 5.2: Behavioural response of parasitoids to volatiles collected from unoviposited neighbouring maize plant exposed to maize plant emitting egg-induced volatiles, unoviposited control plant and solvent control in a four-arm olfactometer bioassay. Response of (A) *Trichogramma bournieri*; (B) *Cotesia sesamiae*. Parasitoid responses were compared by ANOVA after conversion of the data into proportions and log-ratio transformation. Different letters on bars indicate a significant difference using Tukey studentized range test ($P < 0.05$).

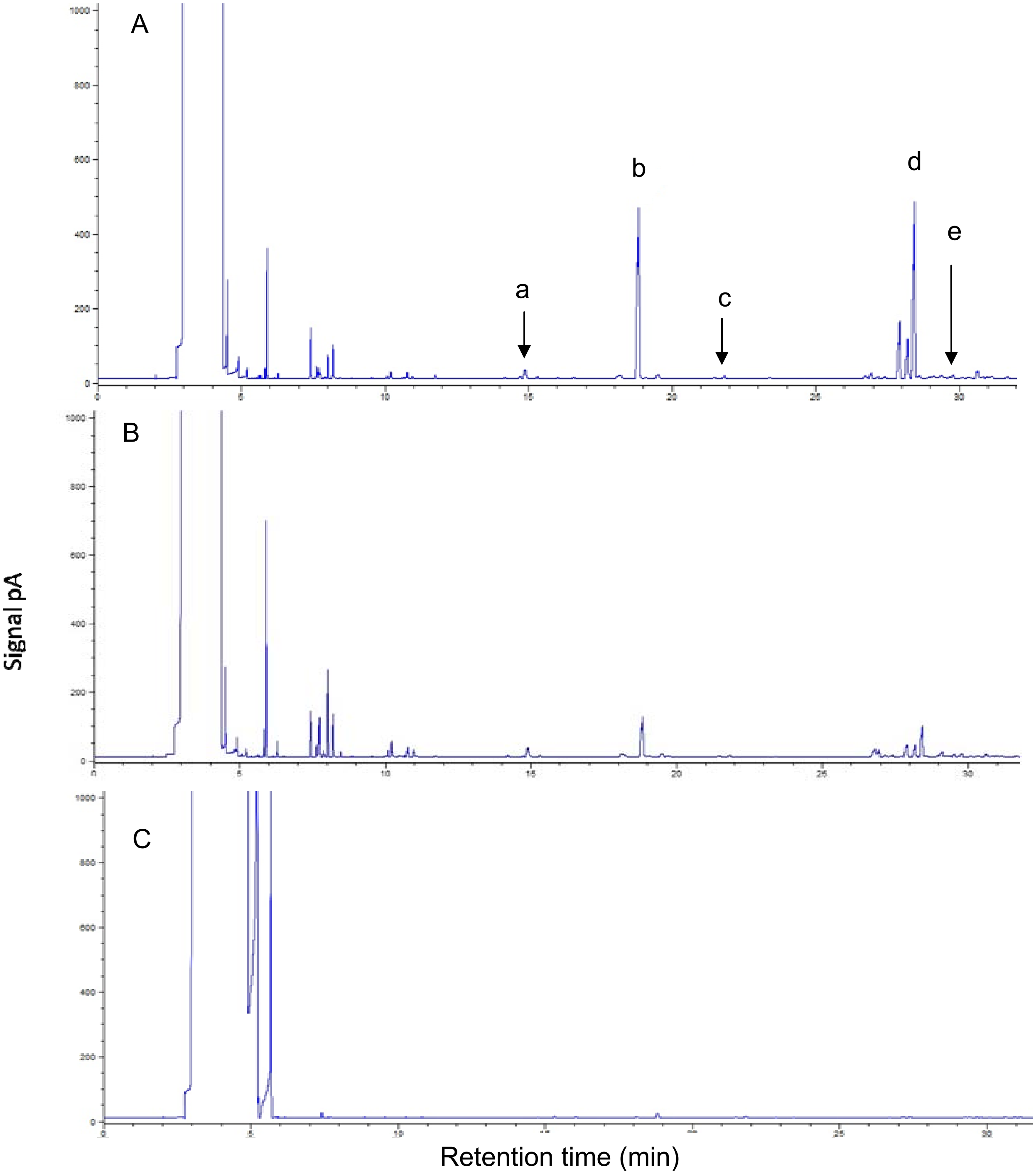

Figure 5.3: GC profiles of headspace volatiles from maize landrace, Nyamula: (A) oviposited, (B) unoviposited but exposed to egg-induced maize volatiles from neighbouring plant (C) unexposed control plant. The identities of some of the EAG-activecompounds whose emission was highly elevated by oviposition and induction are as follows: (a) limonene; (b) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); (c) methyl salicylate; (d) decanal; (e) (*E*)-β-farnesene.

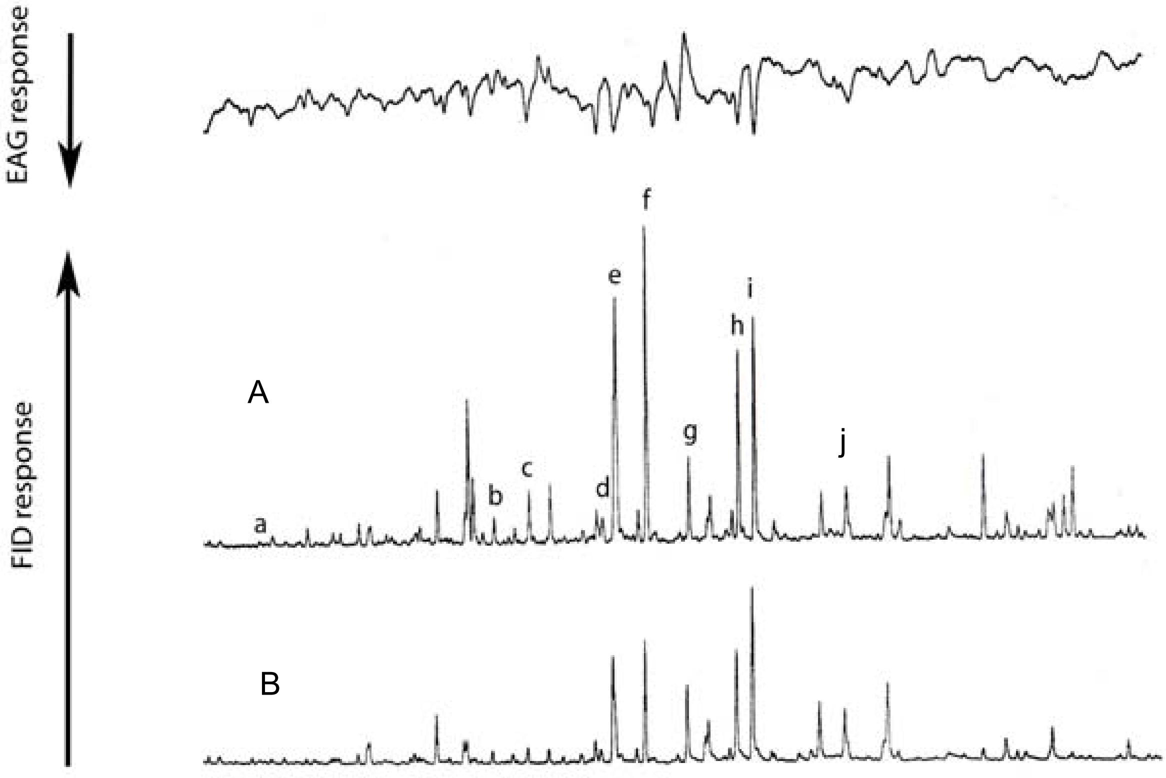

Figure 5.4: GC-EAG response of female *Cotesia sesamiae* to volatiles collected from landrace Nyamula (A) without eggs but exposed to egg-induced maize volatiles and (B) without eggs and not exposed to egg-induced maize volatiles. FID peaks marked are those which elicited antennal response in coupled runs: a = 2-heptanol, b= decane, c = (*R*)-limonene, d = methyl benzoate, e = (*R*)-linalool, f = (*E*)-4,8-dimethyl-1,3,7-nonatriene, g = *cis*-*p*-mentha-2,8-dien-3-ol, h = methyl salicylate, i = decanal, j = 2,3-dimethylacetophenone.

CHAPTER SIX

6.0 BEHAVIOUR AND BIOLOGY OF *CHILO PARTELLUS* (SWINHOE) (LEPIDOPTERA: CRAMBIDAE) ON MAIZE LANDRACES EXHIBITING OVIPOSITION-INDUCED VOLATILE EMISSION TRAITS

6.1 Abstract

Plants defend themselves against herbivores through actiation of both constitutive and induced defences. Previous studies reported that egg deposition on maize landraces induces the release of volatiles that attract parasitoids, but little is known on the effect of these volatiles on subsequent herbivore oviposition. Additionally, larval preference and development on these maize landraces is unknown. This study evaluated six landraces and one hybrid maize variety for their resistance to *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). Larval orientation, settling, arrest and dispersal, feeding, development, survival, and subsequent oviposition of moths were determined for individuals reared on each of these varieties under laboratory and screen house conditions. For oviposition preference studies, all treatments were initially exposed to egg deposition whereas the control treatments were not. Larval preference was generally higher for hybrid maize, compared to the landraces. Similarly, first instar feeding on maize leaves was more intense in hybrid maize than in four of the six landraces. The amount of food consumed and assimilated by third instars over a 24-h period was, however, not different among the maize varieties. Larval survival was significantly lower in maize landraces (32%) compared to hybrid maize (54%). However, there was no difference in the larval development period between any of the treatments. Two-choice oviposition assays showed that moths preferred non-exposed maize landraces for subsequent oviposition, whereas in the hybrid, there was no difference in oviposition preference between exposed and non-exposed plants. Although the mechanism of larval suppression was beyond the scope of this study, it was inferred that the landraces showed some resistance to *C. partellus* feeding and that initial egg deposition on these landraces deter further colonization by the herbivore.

Key Words: *Chilo partellus*, constitutive and induced defences, larval feeding, survival and development, maize, oviposition, Lepidoptera, Crambidae

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6.2 Introduction

In natural and agro-ecosystems, plants are exposed to an array of biotic stresses, including attack by herbivores. To counter this threat, plants have developed complex defence mechanisms such as constitutive and induced defences. Many plants activate induced defences in response to herbivore attack (Chan, 2008), which can be direct or indirect. Directly, plants produce toxins, digestion inhibitors, and herbivore-induced plant volatiles (HIPVs) repellent to phytophagous insects (De Moraes *et al*., 2001; Kessler & Baldwin, 2001). Indirectly, plants use HIPVs to attract both parasitoids and predators antagonistic to the herbivores (Turlings *et al*., 1990; De Moraes *et al*., 1998; Dicke & van Loon, 2000; Heil, 2008). Induced plant defences may be either amplified or suppressed by mechanical damage caused by chewing larvae or elicitors present in the oral regurgitant, saliva, and/or eggs of herbivores (Turlings *et al*., 1993; Musser *et al.*, 2002; Alborn *et al*., 2007; Tian *et al*., 2012; Louis *et al*., 2013).

Constitutively, plants synthesize a wide range of secondary metabolites, including alkaloids and terpenoids, which act as defence compounds against herbivores and pathogens (Wittstock & Gershenzon, 2002). Due to the resources involved in the biosynthesis processes, toxicity of the metabolites to the plant, and the ecological consequences of their accumulation, biosynthesis of defensive metabolites by plants has been thought to be a costly activity (Purrington, 2000). Plants can reduce these costs by synthesising defence compounds only after initial attack by herbivores. In cases in which initial attack results in severe damage or is too rapid for induced-defences to be deployed effectively, damage-triggered defence systems may not be effective to protect plants. Plants that are likely to suffer frequent or serious damage may therefore maximise fitness by investing mainly in constitutive defences, whereas those that are attacked rarely may rely mostly on induced defences (McKey, 1979). Notably, plants that possess both constitutive and induced defences may have optimal protection against attacking herbivores, and herbivores tend to avoid these plants (Mckey, 1979).

In Lepidoptera, host-plant recognition and selection is primarily a function of ovipositing females (Konstantopoulou *et al*., 2002), and correct host plant choice is vital for the fitness and survival of progeny (Schoonhoven *et al*., 2005). The choice and acceptance of a plant for oviposition is based on the balance between attractants and stimulants (positive stimuli) and repellents and deterrents (negative stimuli) (Renwick & Chew, 1994). Volatile cues from host plants provide crucial information for many herbivores during orientation towards a plant (Bruce & Pickett, 2011). In addition to chemical cues, physical plant characteristics also play a role in assessment of host plant suitability (Waladde *et al*., 1990). The HIPVs produced as a result of herbivore attack have been shown to reduce further herbivore colonization by affecting the host selection behaviour in herbivores or attracting natural enemies of herbivores (Karban, 2010; Zakir *et al*., 2013). Whereas the choice of oviposition site is the preserve of female herbivores, larval dispersal of most Lepidoptera is usually restricted to the neonates (Zalucki *et al*., 2002; Moore & Hanks, 2004) and is similarly influenced by physical and chemical characteristics of plants. The suitability of a plant for larval growth and development depends on the plant’s nutritional quality, chemical properties, occupation by other insects, or attractiveness to natural enemies (Bernays, 2001). These properties therefore affect the preference for, and resistance to, herbivores.

The spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), is a generalist herbivore that feeds on several species of cultivated and wild plants belonging to the Poaceae (Ong’amo *et al*., 2006; Moolman *et al*., 2014). Although it is the most damaging Lepidopteran pest of maize in eastern and southern Africa (Kfir *et al*., 2002), causing significant losses in grain yields, its control remains a challenge among smallholder farmers. Some maize landraces have been shown to respond to early herbivory (egg deposition) by *C. partellus* by producing HIPVs both locally (at site of oviposition) and systemically (distal to oviposited sites), which subsequently attract parasitoids (Tamiru *et al*., 2011; 2012). There is therefore an opportunity to exploit this trait in an integrated management approach for the pest. However, there is no information available regarding the moths’ subsequent oviposition behaviour and constitutive plant defences on larval preference and development in these landrace maize varieties. The objectives of this study were therefore to establish (1) larval preference for various maize landraces, (2) larval development in various maize landraces, and (3) effect of prior *C. partellus* egg deposition on subsequent oviposition preference of the moths.

6.3 Materials and methods

6.3.1 Study site

Experiments were carried out at Thomas Odhiambo Campus, Mbita Point (0^{o}25'S, 34^{o}12'E, 1200 metres above sea level), a field station of International Centre of Insect Physiology and Ecology (icipe) located in western Kenya. This station is situated in the eastern shores of Lake Victoria, where cereal stemborers are a serious constraint to maize cultivation. The station is approximately 24.5 hectares. The vegetation type around the station is mainly savannah grassland with mixed combretum and acacia trees to the north and papyrus along the shores of the lake.

6.3.2 Plants

A total of six landrace maize varieties were used; five, known by local names as 'Endere', 'Jowi', 'Kongere', 'Nyamula', and 'Sefensi' were obtained from smallholder farmers in western Kenya, and a South American landrace, Cuba2101, which was obtained from the International Maize and Wheat Improvement Centre (CIMMYT), Nairobi, Kenya. A hybrid maize variety, HB 515, was obtained from a commercial seed supplier (Western Seed Company, Kitale, Kenya). Seeds were planted individually in pots filled with fertilised soil in an insect-proof screen house under natural conditions (25 ^{o}C, 65% r.h., L12:D12) and plants were used in experiments when they were 3-4 weeks old, approximately 45 cm tall (Plate 6.1).

6.3.3 Insects

Chilo partellus moths and larvae used in this study were obtained from the insect mass rearing unit of the icipe-Thomas Odhiambo campus. The founder colony originated from larvae collected from sorghum fields, around the locality of the study site. These larvae were subsequently reared on a semi-synthetic diet containing sorghum leaf powder (Ochieng *et al*., 1985) under laboratory conditions at 24 ± 3 ^{o}C, 70 ± 5% r.h., L12:D12. The mass-reared culture was infused with a field-collected insect population every 3 months to avoid genetic decay and maintain the original behavioural characteristics of the species. The insects used in the experiments were of the second generation of the founder colony with infestations being carried out early in the morning.

Larval behaviour and feeding

6.3.4 Experiment I: Larval orientation and settling

Larval orientation and settlement on different maize varieties was assessed in two-choice tests using a modification of the methodologies described by Khan *et al*. (2007). Experiments were conducted inside 15-cm-diameter Petri dishes lined with moist filter paper discs. Four 3 × 3-cm leaf cuts of each landrace and hybrid maize variety were laid alternately and radially, two each for a landrace and hybrid maize variety at a time, with their adaxial surfaces facing upwards. The leaf cuttings were excised early in the morning. At the centre of each Petri dish, 10 first instar *C. partellus* larvae were introduced. The Petri dishes were then placed in a dark room. Larvae were allowed to orientate and settle on their preferred leaf cuts, between the landrace and hybrid. The number of larvae on/underneath each leaf cut was counted after 1 and 24 h to determine orientation and settling preference, respectively. This experiment was replicated 10 times.

6.3.5 Experiment II: Arrest and dispersal of first instars

In a no-choice test, a 6-cm-long leaf cut of each maize variety together with a moist cotton wad at either end was placed at the centre of a 9-cm-diameter Petri dish, with its adaxial surface facing upwards. Ten first instar *C. partellus* larvae were released in the centre, on top of each leaf cut. The Petri dishes were then placed in a dark room. The number of larvae remaining on/underneath each leaf cut was counted after one and 24 h. The experiment was replicated 10 times.

6.3.6 Experiment III: Larval leaf and stem feeding and food assimilation

To evaluate the extent of feeding of *C. partellus* larvae on various maize varieties, experiments were conducted with leaves and stem cuttings of test plants. A piece of the second-youngest leaf (2.5 × 2.5 cm) from a 3-week-old maize plant was placed in a 6-cm-diameter Petri dish lined with wet filter paper to prevent wilting. Five unfed neonate larvae were then placed on each leaf cut. Each Petri dish was covered and sealed with parafilm to prevent larvae from escaping. The Petri dishes were kept in a dark room with 10 replicates for each variety. Twenty-four hours after larval release, the leaf area (mm^2) consumed by larvae was measured using graph paper (Mohammed *et al*., 2004). Preference was indicated for the variety with the largest leaf area consumed.

To assess stem feeding and the amount of food assimilated by larvae, 4-cm-long stem segments of each of the 3-week-old potted plants were cut. Each segment was weighed (S1) and then placed in a vial (4.1 × 1 cm). A newly molted third-instar *C. partellus* larva, starved for 3 h in the presence of high humidity, was weighed (W1) on a microbalance (Mettler PM460; Mettler Instrument, Greifensee, Zurich, Switzerland) and introduced into the vial. The vial was then kept in a dark room after being covered with a cotton wool plug. After 24 h, the remaining unconsumed parts of the stem segments were weighed again (S2) after removing the larvae and excreta. To determine weight loss due to evaporation, 10 4-cm stem segments of each treatment were weighed (CE1), kept in the vials alongside the experimental ones, and weighed again after 24 h (CE2). The difference between the initial weight (S1) and the final weight (S2) of the stem after the adjustment of the weight loss from evaporation represented the amount of fresh weight consumed by larvae (Khan & Saxena, 1985). Each treatment was replicated 10 times.

To determine the amount of food assimilated, each larva was weighed again (W2). To determine weight loss due to metabolism, 10 larvae were weighed (C1), kept alongside the experimental vials in vials without stem pieces and weighed again after 24 h (C2). The amount of food metabolized by each larva was determined using the equation from Khan & Saxena (1985): Assimilation of food = W1 x (C1-C2)/C1 + (W2-W1), where W1 = initial weight of larva, W2 = final weight of larva, C1 = initial weight of control larva, and C2 = final weight of control larva.

6.3.7 Experiment IV: Larval mortality and development

To evaluate mortality and development of *C. partellus* larvae on different maize varieties, a study on time sequence of development and mortality of larvae was carried out under semi-controlled conditions in a room with no climate control and poor lighting as described by Khan *et al*. (2007). Temperatures were cooler inside by day due to shading (mean 26.5 ^{o}C), but similar to outside temperatures by night (mean 23.5 ^{o}C). Relative humidity was maintained at 65%. The lack of sunlight meant that the laboratory was darker than under natural light. Five sections from one 3-week-old potted maize plant grown in a screen house were placed in a screw-top glass jar, 20 cm in height and 8 cm in diameter. The sections were made up of leaves and stems approximately 15 cm long and 0.5–1.5 cm in diameter. This provided a variety of material for larval feeding. Twenty-five newly hatched larvae were introduced into each jar using a fine camel-hair brush. The lids of the jars were tightly closed and paper towelling was used to make a tighter seal, so that the larvae could not escape from jars (Khan *et al*., 2007). Fifty larvae were used for each maize variety. The larvae were allowed to feed for 5 days, the least number of days taken between instars (Päts, 1992). After 5 days, the plants were removed and carefully dissected to recover the larvae, which were then assessed with regard to their developmental stage. The surviving larvae were placed into similar jars containing five freshly cut sections of the same maize variety previously fed on. Larvae were allowed to feed for another 5 days, after which the plants were dissected and the larvae reassessed. Additional stem sections were introduced to replace some of the whorl sections as the larvae reached the third instar and started to bore into stems (Leuschner, 1990). As the larvae neared pupation, white absorbent paper towelling was placed at the bottom of the jars as pupation medium (Khan *et al*., 2006). In order to calculate survival indices of larvae on the different maize varieties, time to pupation of each larva was recorded. Larval survival indices were calculated by dividing the number of larvae surviving to pupation by the number of days taken to pupation.

6.3.8 Experiment V: Larval survival and plant damage (screen-house trial)

This experiment was conducted over a 25-day period under uninterrupted, semi-natural conditions using modifications of methods used by Smith *et al*. (1994), as described by Khan *et al*. (2006). Three-week-old potted maize plants were placed in 80 × 40 × 40-cm cages, one plant per cage. Water in the pots was regularly replenished and benches checked for spiders and other invading insects. Cages were placed approximately 80 cm apart, to prevent dispersal of the larvae between cages and arranged in a randomized block design within a 15 × 5 × 4-m screen house. Thirty-five cages were tested in each screen house at a time. Five newly hatched larvae were introduced into the whorl region of each of the plants early in the morning using a fine camel-hair brush. At 25 days after infestation, leaf feeding and larval mortality data were collected. Leaf feeding, an important indicator of the level of infestation in maize and sorghum (Leuschner, 1990) was assessed qualitatively for each plant by estimating leaf area eaten using a graph paper. A visual scoring system with one representing little or no leaf feeding and five representing heavy leaf feeding was then applied to assess the extent of plant damage. Larval survival was determined on each plant by dissecting the plants and counting live larvae. The number of survivors was expressed as a percentage of the total number introduced per plant. This experiment was replicated ten times.

Oviposition preference

6.3.9 Experiment VI: Oviposition preference (two-choice test)

This experiment was conducted to determine moth preference for either exposed or non-exposed maize plants. Modifications of the methodology of Khan *et al*. (2007) were used in this experiment. Two-choice tests were conducted in oviposition cages measuring 80 × 40 × 40 cm, covered by fine cloth netting. Prior to these tests, a 3-4-week-old potted maize plant of each variety, the age at which maize is most susceptible to *C. partellus* damage (Kumar & Asino, 1993), was caged overnight with five gravid naïve *C. partellus* moth for oviposition. A wad of cotton wool (10 cm in diameter) moistened with water was introduced into the cage for the moths to feed on. After 24 h, the number of egg batches oviposited on each plant were marked. The following day, two potted maize plants of each variety and of the same age, one exposed to moths the day before and the other one without any prior exposure to moths, were placed at opposite sides of the same cage. Five gravid naïve moths were introduced into the cage and a wad of wet cotton wool was provided for moths to feed on. The moths were allowed to oviposit for 48 h under natural conditions of approximately L12:D12. Afterwards, the number of fresh eggs oviposited on each plant were counted under a light microscope at 6.5×. ‘Preference’ in this context was taken to be differential oviposition on a plant when the moth is given a choice between two plants of the same variety but with different treatments (exposed vs. non-exposed plants). The number of eggs per plant was expressed as mean percentage of the total number of eggs laid during the period that alternate plants were available. This experiment was replicated 10 times.

6.3.10 Statistical analysis

Two-sample (unpaired) Student’s t-tests were used to analyze differences between exposed and non-exposed maize plants of the different varieties with regards to the number of eggs laid on each plant and larval orientation on maize leaves. One-way analysis of variance (ANOVA) was used for analyses of larval arrest, dispersal, survival, and leaf feeding scores. Prior to analysis, data on proportion of larvae surviving (percentage of survival) and larval mortality were subjected to square root and arcsine transformation, respectively, and conformed to the assumptions of ANOVA as indicated by tests of normality. Data on larval arrest and dispersal was subjected to log transformation (log x+1) prior to analysis. Thereafter, Tukey’s studentized test was used to separate the means at $P<0.05$. Means of non-transformed data are represented in tables. All data was analysed using R3.0 software (2013).

6.4 Results

6.4.1 Experiment I: Larval orientation and settling

When larvae were provided a choice of leaf cuts from landraces and the commercial hybrid HB515, most larvae oriented to those of hybrid HB515 maize compared to landraces in most of the tests. The difference was however only significant in the two-choice test with Kongere and Sefensi, in which a significantly higher number of larvae orientated to leaf cuts of the commercial hybrid HB515 than to those of Kongere and Sefensi (Fig. 6.1A). Similarly, most larvae settled on leaf cuts of hybrid HB515 maize compared to landraces after 24 h of release although the difference was only significant with the local landrace Nyamula (Fig. 6.1B).

6.4.2 Experiment II: Arrest and dispersal of first instars

The number of *C. partellus* larvae arrested on leaf cuts of the various maize varieties did not differ significantly after the first hour of release ($F_{6,69}$ = 1.84, P = 0.106; Fig. 6.2A). However, significantly more larvae were found on leaf cuts of the commercial hybrid HB515 maize than on leaf cuts of the landraces Cuba, Endere, and Sefensi after 24 h ($F_{6,69}$ = 19.80, P<0.001; Fig. 6.2B).

6.4.3 Experiment III: Larval leaf and stem feeding and food assimilation

First-instar *C. partellus* consumed a significantly larger area of leaf cuts from hybrid HB515 maize than they did from leaf cuts of landraces Cuba, Endere, Jowi, and Sefensi ($F_{6,69}$ = 3.93, P = 0.002). However, the consumed leaf area did not differ significantly between the commercial hybrid HB515 maize variety and the landraces Nyamula and Kongere, although larger leaf areas were consumed in the hybrid HB515 maize variety (Table 6.1). Similarly, the amount of material from stem pieces consumed by the larvae did not differ significantly between the commercial hybrid HB515 variety and the landraces, except in the test with the landrace Sefensi ($F_{6,69}$ = 2.62, P = 0.025). Relatively more material, in terms of weight, was consumed from the commercial hybrid HB515 variety than from the landraces (Table 6.1). Similarly, the amount of food assimilated by larvae fed on stem pieces of different landraces did not significantly differ from the amount assimilated by larvae fed on the commercial hybrid HB515 variety except in the case of the landrace Sefensi ($F_{6,69}$ = 2.80, P = 0.018).

6.4.4 Experiment IV: Larval mortality and development under laboratory conditions

Monitoring *C. partellus* larval survival and development over a 60-day period showed that the greatest mortality occurred within the first 15 days after egg hatch in all maize varieties (Fig. 6.3). This was followed by a period of considerably reduced mortality, reaching 68 and 46% at 30 days in landraces and hybrid HB515 maize varieties, respectively (Fig. 6.3). Significantly more larvae survived to the pupal stage in the commercial hybrid HB515 variety compared to the landraces (t = 3.42, P = 0.019; Table 6.2). The recovered larvae were at different developmental stages, with the standard hybrid HB515 showing fast progression of subsequent stages as compared to the landraces (Fig. 6.4). The time taken to pupation was approximately 30.8 and 31.1 days on average in the landraces and the commercial hybrid HB515 variety, respectively, and did not differ significantly (Table 6.2). The mean percentage survival to adult stage was significantly different between landraces and the commercial hybrid HB515 variety: it was 50% in the commercial hybrid HB515 variety compared to 29% in the landraces. Amongst the landraces, Jowi had the lowest survival (16%; Fig. 6.3). Significantly more female moths than males emerged from the commercial hybrid HB515 variety. In landraces more males were recorded although this difference was not significant, except in the landrace Sefensi (Table 6.2). Growth index was significantly higher (t = 3.80, P = 0.009) with the commercial hybrid HB515 variety (1.736) than on all individual landraces, and the overall mean for landraces (1.036; Table 6.2). The lowest growth index was observed in the landrace Jowi. Consequently, lower pupal weight, though not statistically significant, was observed in the landraces Sefensi and Jowi than in all other maize varieties (Table 6.2).

6.4.5 Experiment V: Larval survival and plant damage

Higher larval survival was recorded on the commercial hybrid HB515 variety than on the landraces although the differences were not statistically significant (Table 6.3). Plant damage through leaf feeding was significantly higher on the commercial hybrid HB515 variety than on the landraces Jowi, Kongere, Sefensi, and Nyamula ($F_{6,69}$ = 4.85, P<0.001). The lowest damage among the landraces was recorded in Sefensi, with a mean damage score of 2.5 (Fig. 6.5).

6.4.6 Experiment VI: Oviposition preference (two-choice tests)

The proportions of eggs laid on plants of non-exposed landraces (with no prior oviposition) were significantly higher than those laid on plants that were exposed previously (overnight) (with prior oviposition), except in the landrace Endere ($P<0.05$). The number of eggs laid, did however, not differ significantly between exposed and non-exposed plants of the commercial hybrid HB515 variety ($P>0.05$; Fig. 6.6).

6.5 Discussion

Both larvae and moths of *C. partellus* showed different behavioural and physiological responses to the different maize varieties used in this study. Generally, most first instars preferred leaf cuts of the standard commercial hybrid variety to those of the maize landraces for orientation and settling. Similar trends were observed with regards to larval arrestment. Once a preferred host plant has been identified initially by an ovipositing moth, plant suitability for larval feeding and development is the next step in the host colonization process, and is the preserve of the larvae. Larval migration off plants is usually ascribed to low-quality, non-preferred, or unsuitable host species (Ramachandran, 1987). Previous studies ascribed poor acceptance and subsequent dispersal by *C. partellus* neonate larvae on resistant maize cultivars in comparison to more susceptible cultivars to factors such as plant chemicals, physical characters, and/or poor nutrient quality.

Host-plant quality determines the biological performance of herbivores, including larval survival and/or development (Awmack & Leather, 2002). In this study, stem feeding and damage to plants were significantly higher on the commercial hybrid variety than on the landraces. Several factors influence larval feeding, including plant chemicals, physical and/or anatomical characters such as hairiness, or presence of high concentration of waxes on the leaf surface (Norris & Kogan, 1979, Setamou *et al*., 1993). Reduced feeding on leaves and whole plant damage of maize landraces by *C. partellus* larvae suggests that these landraces may have antibiosis properties, or that leaf feeding may have induced secondary defence metabolites making plants unpalatable (Roda & Baldwin, 2003). This may represent an opportunity for exploitation in the management of *C. partellus*.

Differences in food assimilation and growth of insects have been attributed to varying nutrient proportions in the host plant (Hoo & Frankel, 1966). Some herbivores such as *Anticarsia gemmatalis* (Hübner) are known to do compensatory feeding when exposed to host plants of low nutritional value (Slansky & Wheeler, 1989). In this study, no such compensatory feeding was observed. Instead, first instars of *C. partellus* fed more on leaf cuts of the commercial hybrid variety than on those of most landraces. Similarly, there was more intense feeding by the larvae on leaves of the hybrid variety (whole plant) than those of most landraces. However, third instars did not significantly differ with regards to the amount of food consumed and assimilated between the hybrid and most landraces. Similar observations were made by Khan *et al*. (2007) when *C. partellus* larvae were fed on a susceptible maize cultivar and a less suitable Napier grass variety. However, the limited time period (24 h) that the larvae were allowed to feed may not have been sufficient enough to draw a conclusion on food assimilation in larvae fed on different maize varieties.

The amount of feeding by *C. partellus* larvae on some landraces, such as Jowi, was comparable to that on the commercial hybrid variety. However, larval growth index scores indicated that larvae feeding on Jowi had the lowest growth index (0.635), implying that the landrace may possess some antibiotic effect against *C. partellus* larvae. An overall lower larval growth index (1.03 on average) was observed in maize landraces as opposed to the 1.73 for the commercial hybrid variety. Sekhon & Sajjan (1987) reported growth index scores on a number of maize varieties resistant to *C. partellus* ranging from 0.3 to 0.7. Previous studies reported growth indices for *C. partellus* in susceptible maize as 1.3 (Khan *et al*., 2006) and 3.8 (Mohammed *et al*., 2004), values which are higher than the mean growth index for the landraces but within the range of those of the hybrid variety used in this study. The higher growth index on the commercial hybrid variety can be attributed to its suitability for the development of *C. partellus,* whereas the lower index in the landraces can be attributed to some factors that inhibit larval development in these varieties.

Lower larval survival was recorded in maize landraces, with high larval mortality being observed within the first 15 days after inoculation. This is an indication that either the landraces were of poor nutritional value or had an antibiotic effect on the larvae. Larval survival on the landrace Jowi was the lowest in spite of larval preference for, and a higher amount of stem weight consumed and assimilated by the larvae feeding on this landrace. This could indicate that Jowi had low nutritional quality but also an antibiotic effect once consumed. Lower larval survival was also reported in sorghum by van den Berg & van der Westhuizen (1997) where there were high levels of antibiosis and larval antixenosis but high oviposition preference by *C. partellus*. Kumar & Asino (1993), reported mortality of 80%, 14 days after egg hatching for maize cv. MP704, a cultivar known for its antibiotic qualities against *C. partellus*. Nutritional indices have been used to determine the mechanisms of growth-reducing factors in plants (Reese & Beck, 1976; Reese & Schmidt, 1986). For example, Kumar (1993) used neonate *C. partellus* larvae to measure nutritional indices on maize cultivars where the results showed a low growth of larvae on resistant ‘Mp704’ cultivar as a result of low efficiency with which digested food was converted to body matter. Similarly, Arabjafari & Jalali (2007) found varying nutritional constituents between *C. partellus* susceptible cultivar HY-4642 and resistant variety CM-137, with sugars and nitrogen being higher in susceptible cultivar. Although determination of the causal mechanism of larval mortality in maize landraces was beyond the scope of this study, previous studies on maize resistance mechanisms to lepidopteran larvae have shown that secondary metabolites such as the hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) play a role (Tseng 1994). Analysis of the level of secondary metabolites in the tested maize landraces can provide an insight into the resistance mechanism employed by these varieties, and should be pursued.

Van den Berg (2006) and Khan *et al*. (2006) reported high mortality of *C. partellus* larvae on Napier grass. Production of a sticky substance around the sheath region, which restricts larval movement, has been attributed to stemborer resistance in certain Napier grass varieties (Khan & Pickett, 2004). In landraces, a similar white sticky substance was observed oozing from the site of tunnelling into the stem, notably in two landrace varieties, Jowi and Sefensi. In nature, exudates are produced by a number of plant species when attacked or damaged to seal the wound (Hill, 1983). However, the consistency of the exudates has important implications for stemborer mortality because a viscous substance would act to trap or restrict larval movement (Khan & Pickett, 2004) and cause it to die (Hill, 1983) or expose it to natural enemies.

Gravid *C. partellus* moths preferred to oviposit on landrace plants with no prior oviposition in comparison to those previously exposed to egg deposition. This was not the case in regard to the commercial hybrid variety where there was no preference between non-exposed and exposed plants for oviposition. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of volatiles from these maize landraces revealed qualitative and quantitative changes in the volatile profiles. Several compounds including (*E*)-2-heptenal, myrcene, (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT), decanal, (*E*)-β-farnesene, and α-humulene were emitted in higher quantities following *C. partellus* egg deposition in these maize landraces (Chapter 4; Tamiru *et al*., 2011; 2012). However, there were no changes in the volatile profiles in the HB 515 hybrid maize variety following *C. partellus* oviposition (chapter 4). These volatile compounds such as DMNT are known to attract parasitoids (Khan *et al*., 1997; Tamiru *et al*., 2011; 2012) and this suggests that emission of these compounds could have influenced the oviposition decisions of the moth. Furthermore, production of constitutive compounds such as linalool, which are produced by undamaged maize plants (D’Alessandro et al.,

2006) and which are attractive to moths (Malo *et al*., 2004), are suppressed upon egg deposition. Moths are known to respond to HIPVs from damaged plants in different ways, with some being attracted (Anderson & Alborn, 1999; Rojas, 1999) whereas others are repelled (Kessler & Baldwin, 2001; von Mérey *et al*., 2013). For ovipositing females, HIPVs can indicate lowered food quality, increased pressure from natural enemies, and risk of competition on the plant emitting HIPVs (Rasmann *et al.*, 2005; Dicke & Baldwin, 2010; Heil & Karban, 2010). The presence of conspecifics is used in animal systems as an indicator of resource quality and the level of competition, but also to reduce the costs of sampling by ovipositing moths (Dall *et al*., 2005; Pasqualone & Davis, 2011). This fact best explains the observation that more eggs, though not statistically significant, were deposited on hybrid maize exposed to *C. partellus* moths as opposed to non-exposed maize plants. Previous studies have demonstrated that induction of plant volatiles modifies plants' interactions with herbivores, carnivores, and/or with competing plants (Dicke & van Loon, 2000; Dicke *et al.*, 2009), hence maize landraces tested in this study could have employed this property to deter further colonization by the herbivore. Therefore, by combining constitutive chemical/physical characteristics and oviposition-induced plant chemistry changes, maize landraces can profoundly influence oviposition, predation rates, and larval development, which in turn influence both "bottom-up" as well as "top-down" control over its herbivore populations.

In summary, the current study demonstrates different behavioural and physiological responses of *C. partellus* to different maize varieties. Maize landraces showed limited preference and survival of the stemborer larvae. Additionally, these landraces further deterred subsequent colonization by the herbivore through oviposition deterrence. These traits were, however, absent in the standard commercial hybrid variety. Elucidations of the specific mechanism or metabolites responsible for the behavioural and physiological responses open up new avenues for further investigation. Introgression of these traits into commercial hybrids may pave the way for development of novel and ecologically benign approaches for stemborer management.

6.6 References

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Table 6.1: Feeding and food assimilation by *Chilo partellus* larvae on different maize varieties after 24 h

Maize Variety	Mean (± SE) leaf area (mm2) consumed by five first instars	Mean (± SE) weight of stem (g) consumed per third instar	Mean (± SE) weight (g) of food assimilated per third instar
Cuba	0.58 ± 0.01)a	0.15 ± 0.04ab	0.20 ± 0.003ab
Endere	1.06 ± 0.03a	0.19 ± 0.04ab	0.026 ± 0.004ab
HB 515	2.24(± 0.03b	0.39 ± 0.08b	0.032 ± 0.003b
Jowi	1.0 ± 0.03a	0.35 ± 0.07ab	0.034 ± 0.004ab
Kongere	1.52 ± 0.04ab	0.29 ± 0.09ab	0.028 ± 0.005ab
Nyamula	1.4 ± 0.02ab	0.22 ± 0.07ab	0.023 ± 0.004ab
Sefensi	1.11 ± 0.03a	0.11 ± .04a	0.016 ± 0.003a
F6,69	3.93	2.62	2.80
P	0.002	0.025	0.018

Means within a column followed by same letters are not significantly different (Tukey's studentized range test: P>0.05).

Table 6.2: Development and survival of *Chilo partellus* larvae on different maize varieties under laboratory conditions

Maize variety	Survival to pupation (P)	Mean (± SE) days to pupation (D)	Growth index (P/D)	Mean (± SE) weight (g) of pupae	% adult emergence	
					Female	Male
Cuba	0.32	33.437 ± 1.691	0.957	0.081 ± 0.008	60.0	40.0
Endere	0.38	32.105 ± 1.395	1.183	0.123 ± 0.029	52.9	47.1
HB 515	0.54	31.111 ± 1.541	1.736	0.086 ± 0.005	64.0	36.0
Jowi	0.18	28.333 ± 2.764	0.635	0.067 ± 0.007	50.0	50.0
Kongere	0.32	31.250 ± 1.070	1.024	0.085 ± 0.002	56.2	43.8
Nyamula	0.40	29.00 ± 1.686	1.379	0.083 ± 0.001	35.3	64.7
Sefensi	0.32	30.937 ± 1.781	1.034	0.060 ± 0.006	28.6	71.4
Landraces (Mean)	0.32	30.845 ± 0.78	1.036	0.083 ± 0.006	46.6	53.4
Hybrid (Mean)	0.54	31.111 ± 1.541	1.736	0.086 ± 0.005	64.0	36.0

Table 6.3: Mean (± SE) recovery, mortality and developmental stage of *Chilo partellus* larvae in different maize varieties under semi-natural conditions in the screen house

Maize variety	Mean (± SE) number of larvae recovered per plant	Mean (± SE) percentage of larval mortality per plant	Developmental stage of the recovered larvae: mean (± SE) percentage of total larvae recovered		
			Third instar	Fourth instar	Pupa
Cuba	2.7 ± 0.33a	46 ± 6.70a	32.50 ± 14.93a	67.50 ± 14.93a	0.00a
Endere	2.7 ± 0.26a	46 ± 5.21a	15.00 ± 10.67a	80.00 ± 11.06a	5.00 ± 5.00a
HB 515	3.0 ± 0.30a	40 ± 5.96a	6.67 ± 6.67a	60.00 ± 14.74a	33.33 ± 13.15b
Jowi	2.3 ± 0.37a	54 ± 7.33a	5.50 ± 5.00a	85.00 ± 10.67a	0.00a
Kongere	2.0 ± 0.45a	60 ± 8.94a	17.50 ± 10.57a	72.50 ± 13.15a	0.00a
Nyamula	2.5 ± 0.43a	48 ± 8.54a	10.83 ± 7.86a	71.67 ± 13.16a	7.5 ± 5.34a
Sefensi	2.2 ± 0.42a	56 ± 8.32a	5.00 ± 5.00a	76.67 ± 13.19a	0.00a
$F_{6,69}$	0.86	0.88	1.11	0.40	4.60
P	0.53	0.52	0.37	0.88	<0.001

Means within a column, followed by same letters are not significantly different (Tukey’s studentized range test: P>0.05).

Plate 6.1: Maize seedlings growing in an insect-proof screen house.

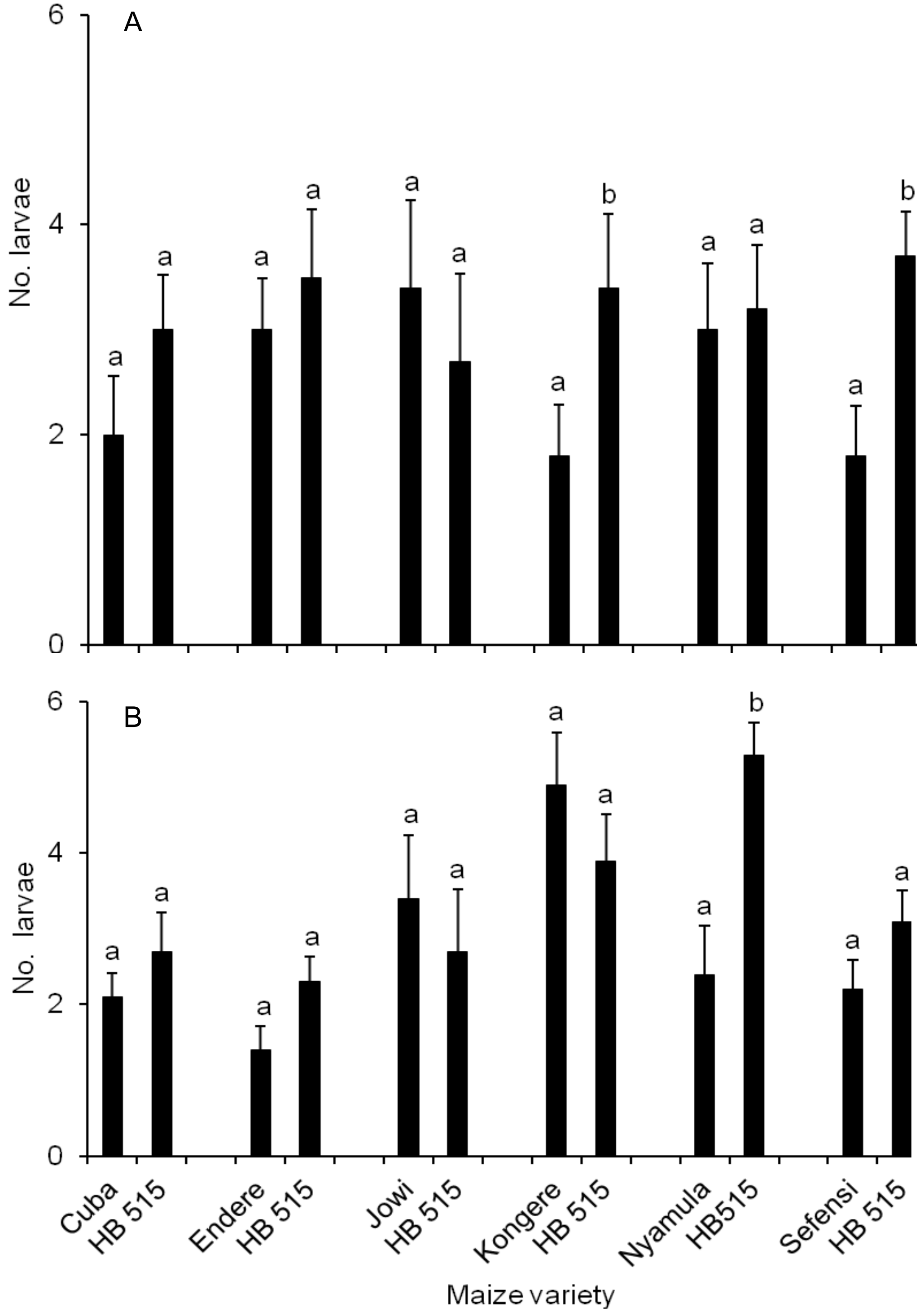

Figure 6.1: Mean (± Standard Error) number of *Chilo partellus* larvae (A) oriented and (B) settled on leaf cuts of different maize varieties. Bars marked with different letters are significantly different (Tukey studentized range test: P<0.05).

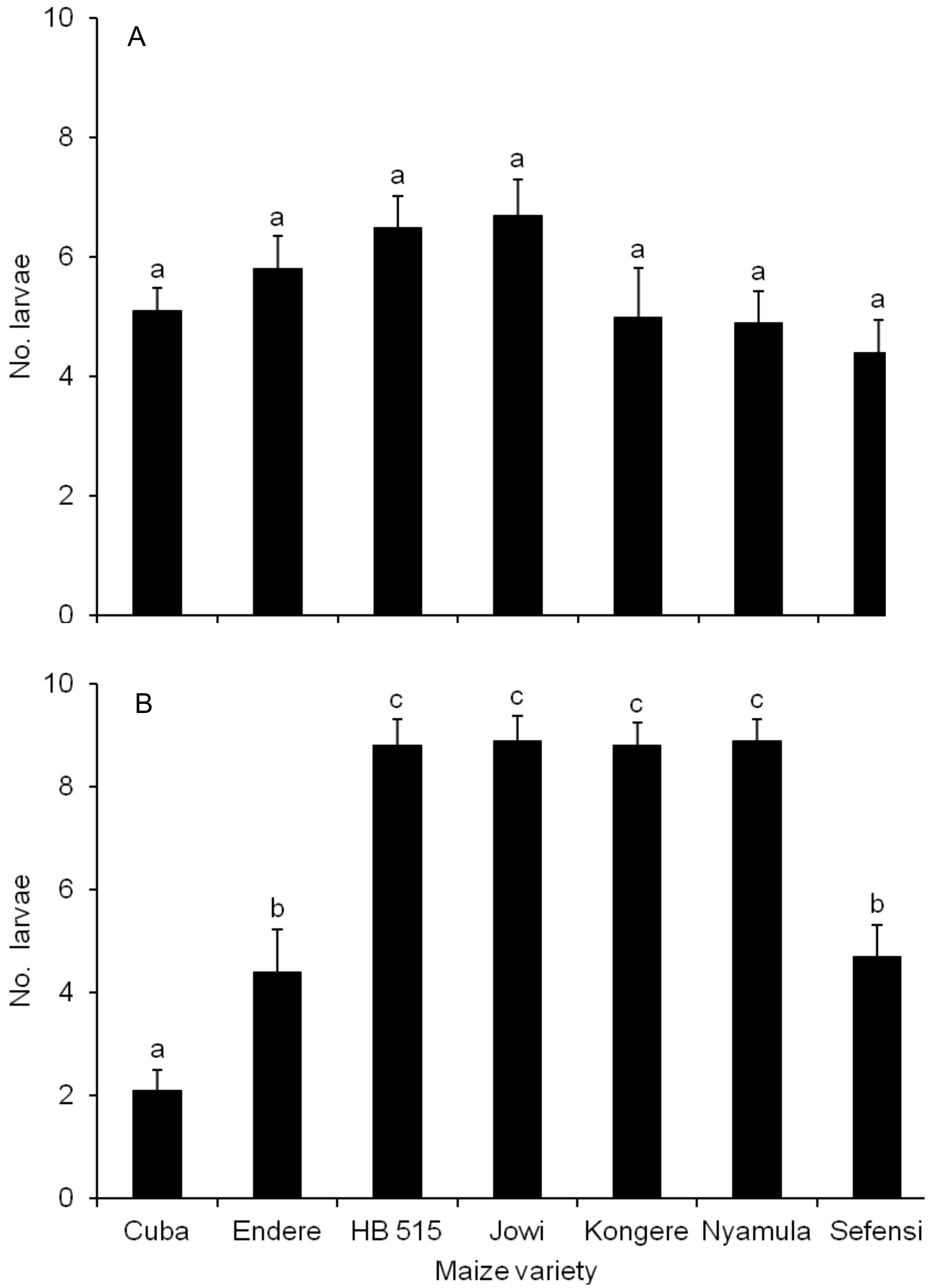

Figure 6.2: Mean (± Standard Error) number of *Chilo partellus* larvae occurring on leaf cuts of different maize varieties (A) 1 h and (B) 24 h after release. Bars marked with different letters are significantly different (Tukey studentized range test: $P<0.05$).

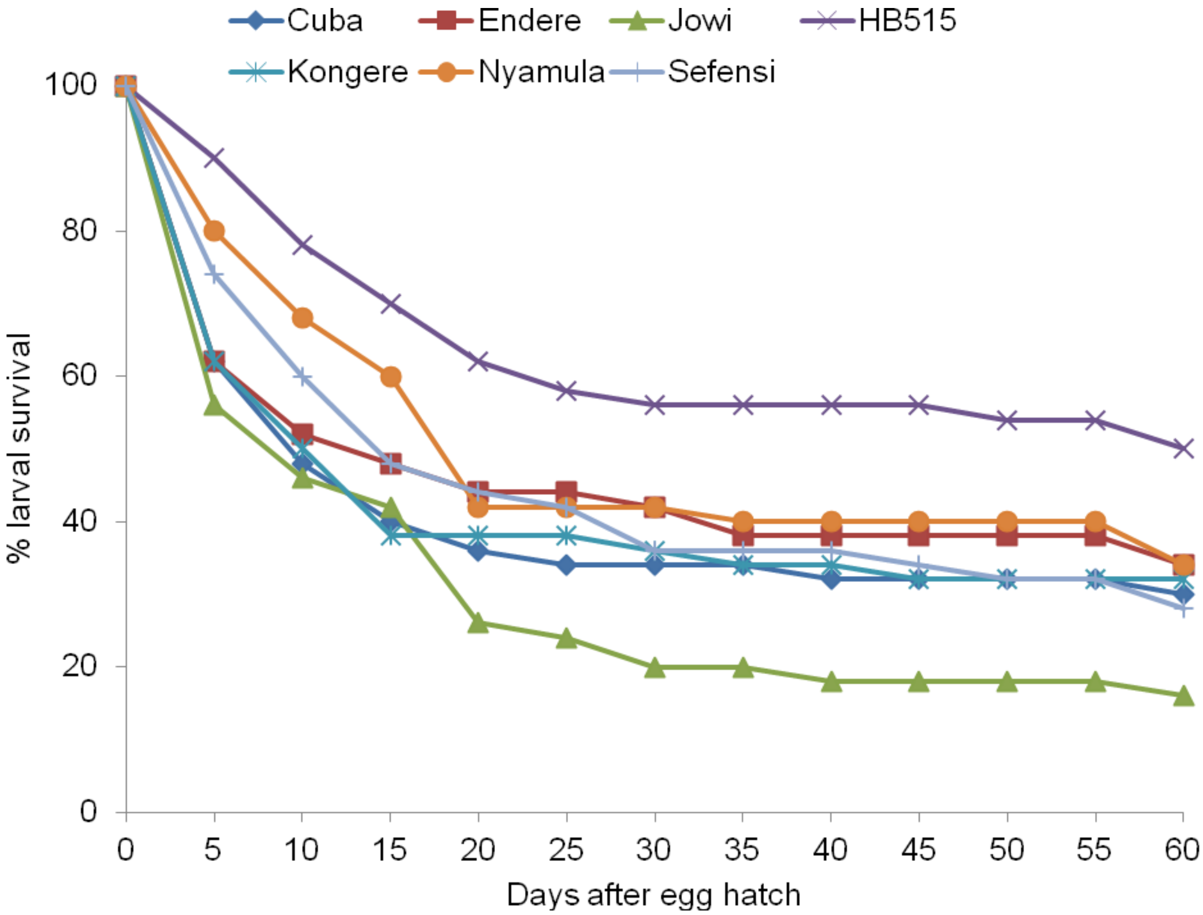

Figure 6.3: Percentage survival of *Chilo partellus* larvae on different maize varieties at different times following egg hatch.

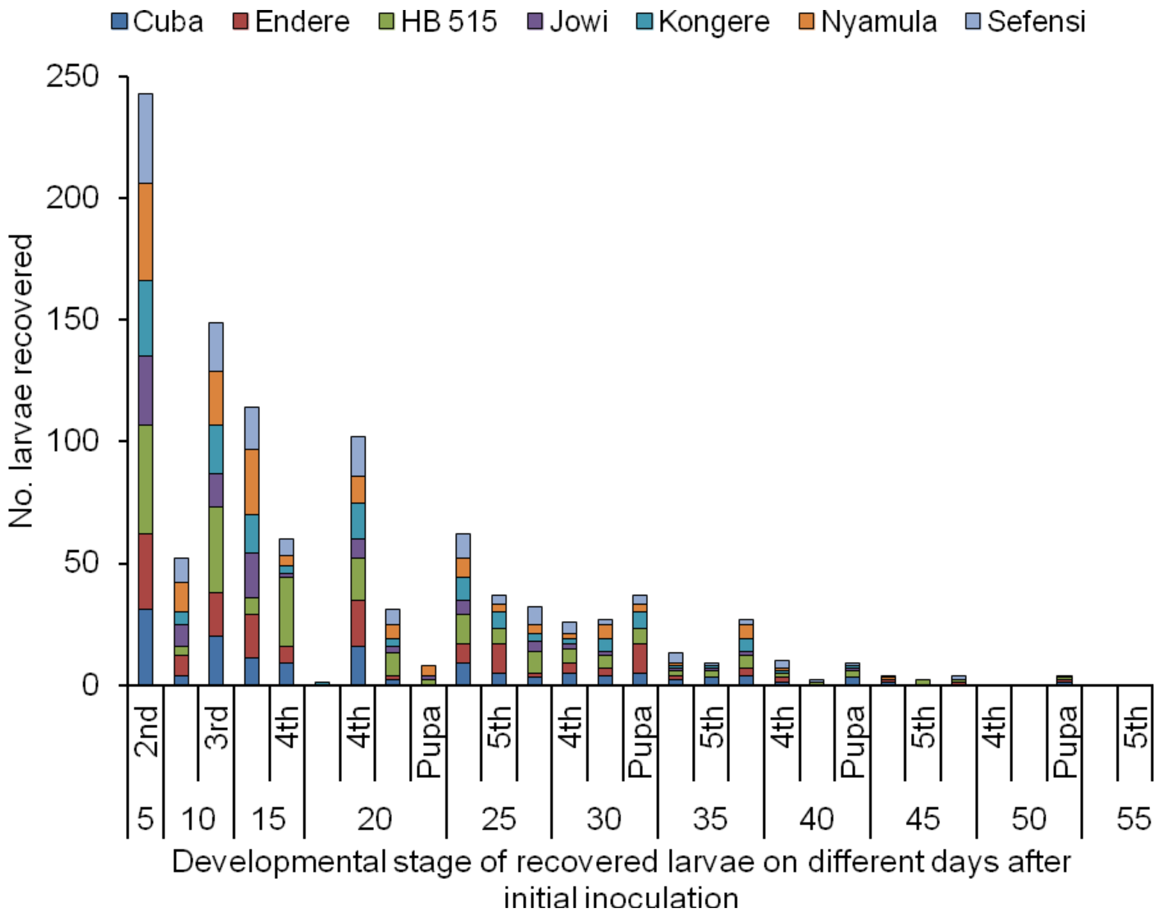

Figure 6.4: Number of larvae recovered at different developmental stages on different maize varieties following the initial inoculation with neonate larvae.

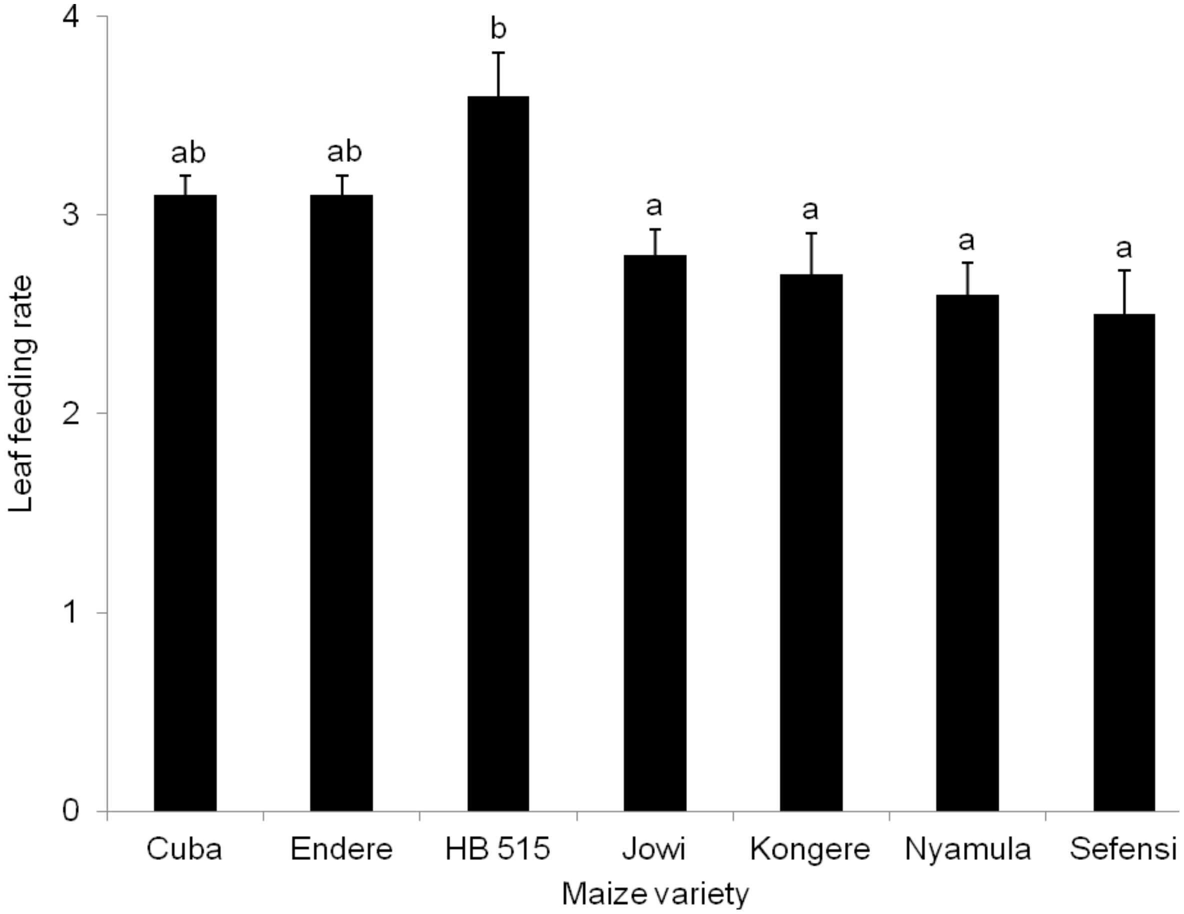

Figure 6.5: Average (± Standard Error) larval leaf feeding score in different maize varieties under semi-natural conditions in screen house after 25 days of larval release. Bars marked with different letters are significantly different (Tukey studentized range test: $P<0.05$); 1 = little or no feeding, 5 = heavy leaf feeding.

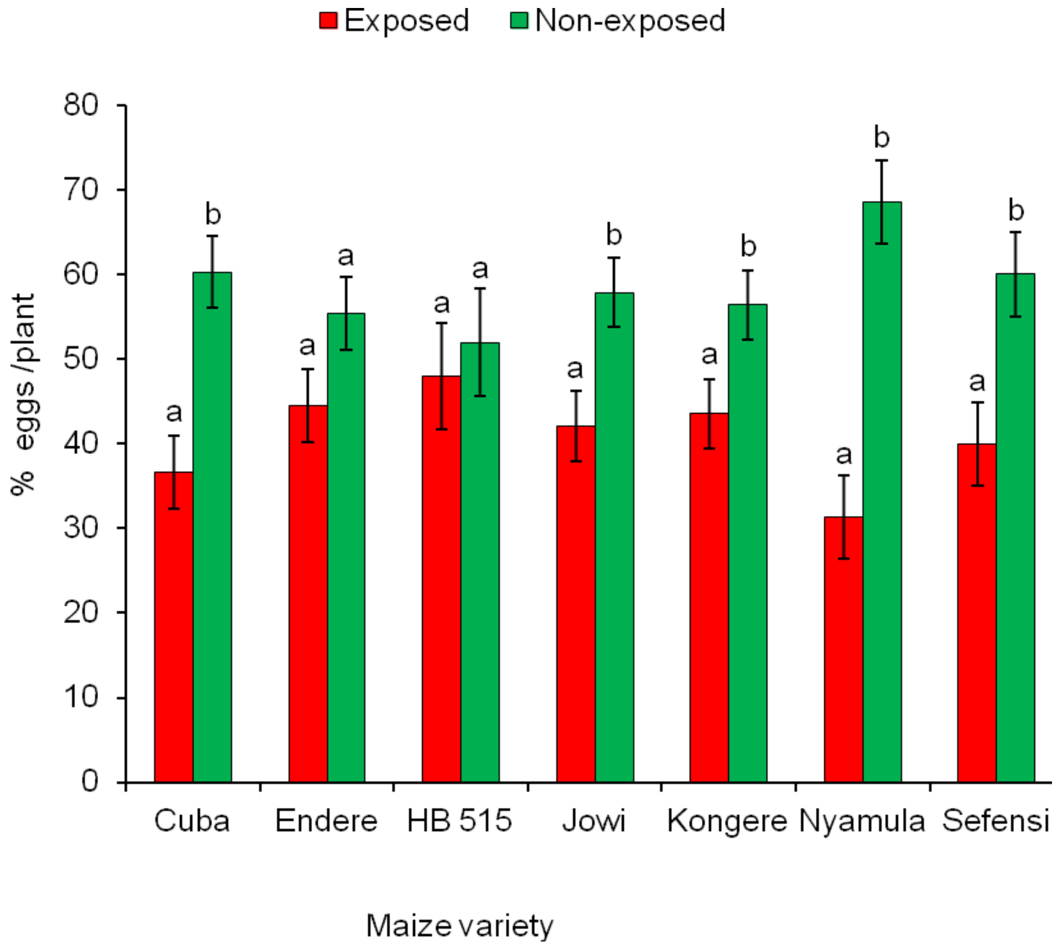

Figure 6.6: Percentage *Chilo partellus* eggs per plant (± SEM) laid in two-choice tests with exposed and unexposed maize plants of different varieties. Different letters above the bars indicate significant difference (Student's t-test: $P<0.05$).

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

The control of lepidopteran stemborers in many maize farming systems has relied mostly on the use of chemical pesticides (Khan *et al*., 2003), and crop losses to the pests have occurred when they have not been used, for example, in subsistence smallholder farming systems. However, the indiscriminate and frequent use of these pesticides can result in pest resistance evolution, pollution of the environment as well as elimination of the natural enemies leading to secondary pests outbreaks. Additionally, chemical control of stemborers is uneconomical and impractical for many resource-poor, smallholder farmers (Khan *et al*., 2003; 2010). Moreover, stemborers are difficult to control, largely due to the cryptic and nocturnal habits of the adult moths and the protection provided by the stem of the host plant for the immature stages (Ampofo, 1986; Seshu Reddy & Sum, 1992; Kfir *et al*., 2002). Alternative control strategies for the stemborers using companion cropping and biological agents have been exploited and shown potential for the control of cereal stemborers in small-scale farmers of sub-Saharan Africa (Khan *et al*., 2010; 2014). This study sought to exploit the innate plant defences in maize for the development of ecologically sound and sustainable stemborer crop protection strategies.

Plants have evolved intricate defence strategies, both directly and indirectly against the attacking herbivores (Sabelis *et al*., 1999; Dicke & van Loon, 2000; Howe & Jander, 2008; Mithoefer & Boland, 2012). Directly, they produce toxins, digestion inhibitors and herbivore-induced plant volatiles (HIPVs) that are repellent to phytophagous insects (Duffey & Stout, 1996; De Moraes *et al*., 2001, Kessler & Baldwin, 2001). Indirectly, they use HIPVs to attract natural enemies which are antagonistic to herbivores (Turlings *et al*., 1990; Loughrin *et al*., 1995; De Moraes *et al*., 1998; Dicke & van Loon, 2000; Roda & Baldwin, 2003; Heil, 2008; Tamiru *et al*., 2011; 2012). Some wild plants possess defence traits that are absent in mainstream crops and have sophisticated responses to herbivory that involve multitrophic interactions with natural enemies (Bruce *et al*., 2010). For example, the African molasses grass (*Melinis minutiflora* P. Beauv. (Poaceae)) defends itself against pest attack by constitutively releasing volatile semiochemicals that have dual effects: repelling the pests and attracting the pests' natural enemies (Khan *et al*., 1997). These properties have been used in developing a 'push-pull' cropping system for management of stemborers in sub-Sahran Africa (Cook *et al*., 2007; Khan *et al*., 2010; 2014). If these properties were present in commonly cultivated maize cultivars, it would confer much advantage to the above mentioned stemborer control strategy.

Previous studies with maize demonstrated that larval feeding by herbivorous insects induces emission of volatiles that attract the herbivores' natural enemies (Turlings *et al*., 1998; Ngi-Song *et al*., 2000; Kollner *et al.*, 2008). However, these antagonists are recruited long after damage has already been inflicted onto the plants. Many wild ancestors of grass species from which our crop plants were domesticated continue to survive to date. These wild ancestors have evolved adaptations that allow them to survive in their natural habitats where they are exposed to attacking organisms. They may thus posses defence traits absent in the domesticated crop cultivars as improved crop varieties were selected primarily for yield and quality by humans over thousands of years. Maize is the domesticated variant of teosinte (Wang *et al*., 1999).

Recent investigations involving maize-herbivore-parasitoid tritophic interactions have shown three maize landraces of Mesoamerican origin emit volatile semiochemicals that attract egg (*Trichogramma bournieri*) and larval (*Cotesia sesamiae*) parasitoids following egg deposition by *Chilo partellus* (Tamiru *et al*., 2011) as well as two African landraces (Tamiru *et al*., 2012). The current study demonstrated that oviposition by *C. partellus* on five maize landraces adapted to local Kenyan agroclimatic conditions causes changes in volatile emission profiles. These emitted volatile semiochemicals were attractive to both egg and larval parasitoids of the herbivore. These maize landraces are the ones popularly grown by smallholder farmers in western Kenya (Odendo *et al*., 2001). This study demonstrates that this egg-induced volatile emission trait is not limited to the Mesoamerican maize landraces but also occurs widely in the African maize landraces. Furthermore, the study revealed that one commercial hybrid maize, SC Duma 43, also possesses the trait. This means that it is not limited to landrace germplasm as previously thought and widens the scope for utilising it in breeding programmes. It was also shown that the majority of wild teosinte maize species emit HIPVs following *C. partellus* oviposition which suggests that the ability to respond in this way is an ancestral trait that has become rarer in hybrid maize germplasm.

Any defences elicited by the presence of eggs, even before any damage is inflicted to the plant, would indicate a finely-tuned and coevolved defence response sensitive even to the earliest stage of herbivore attack (Hilker & Meiners, 2006; Bruce *et al*., 2010). Plants that are able to produce volatile organic compounds (VOCs) in response to egg deposition have the advantage of defending themselves early on, before the hatching larvae can cause any damage to the plant. These volatile compounds provide the parasitoids with reliable cues regarding plants colonized by their hosts thus increasing their foraging efficiency (Colazza *et al*., 2004; Hilker & Meiners, 2006; Bruce *et al*, 2010). This in turn improves the parasitoids' ecological fitness by enhancing its host searching efficiency in a complex environment. The latter is critical to their successful establishment and effective functioning as biological control agents (Nordlund *et al*., 1998; Waage, 1990). The recruitment of antagonists by a plant following the initial stage of herbivore attack (oviposition), increases plant fitness as most parasitized eggs do not hatch into larvae. Moreover, parasitized lepidopteran larvae feed less than non-parasitized ones and die upon emergence of the adult wasp which results in a reduction in damage to plants (Hoballah *et al*., 2002; 2004).

The observed attraction of larval parasitoids following egg deposition indicates that these parasitoids are recruited in anticipation of larval emergence and parasitism can start early enough before the larvae can cause damage to the plant. Since *C. partellus* has a short life cycle, with eggs hatching to larvae in four days after oviposition under tropical conditions (Harris, 1990), it implies that the presence of *C. partellus* eggs indicates presence of larvae in maize fields where this pest is of economic importance. As such, being an annual plant with a short life cycle, maize varieties possessing this oviposition-induced defence trait will benefit from recruiting parasitoids even more than perennial plants whose defence is induced by egg deposition (Hilker & Meiners, 2006).

In this study, the egg-induced indirect defence trait was identified to be prevalent in the wild ancestor of maize, teosinte, occurring widely in four of the five teosinte varieties screened. This is the first study to report egg-induced indirect defence trait in wild ancestor of maize. These teosinte varieties showed volatile profile changes upon egg deposition by *C. partellus* both quantitatively and qualitatively in terms of the volatile blend. There was also significant attraction of both egg and larval parasitoids of the herbivore by the emitted volatiles. These observations demonstrated that oviposition-induced plant signalling is an ancestral trait in maize that exists even in pre-domestication germplasm. Previous studies have shown that, processes of natural evolution, domestication and breeding for increased yield may have an associated effect in reducing plant defences (Rosenthal & Dirzo, 1997). During these processes, changes in plant features occur through adaptation, mutation, hybridization, selection, drift and other genetic processes (van Raamsdonk, 1993). As a result, changes in the whole plant physiology occur and physiological trade-offs between defence and yield typically assume both a metabolic cost of defence and limiting resources for plant development (Mooney & Gulmon, 1982; Gulmon & Mooney, 1986). Studies on maize herbivory comparing maize defences in teosinte, cultivated landraces and hybrid maize have shown a negative correlation between defence and yield over life history, domestication and agronomic selection. Wild teosinte has been shown to be more defended but low yielding, with modern hybrid maize being less defended but more yielding (Rosenthal & Dirzo, 1997; Davila-Flores *et al*., 2013). Thus, as a genetic resource, teosinte maize types are potentially valuable for the improvement of maize production as unravelling the defence mechanisms of teosinte offers interesting possibilities to identify novel strategies for protection of improved maize cultivars (de Lange *et al*., 2014).

The occurrence of egg-induced indirect defence trait in hybrid maize seems to be rare. In this study only one hybrid maize, SC Duma 43 was identified to possess this important trait. Screening over 60 inbred lines from CIMMYT, identified only one inbred line CKIR12001 to possess this trait (personal communication, Amanuel Tamiru). It was not possible to test the parental lines for SC Duma 43 due to commercial sensitivities but the three parental lines for the CKIR12001 namely CML 312, CML 442 and CKSBL10027 were all tested and found to have the trait (personal communication, Amanuel Tamiru). Previous studies had reported loss of this trait in all the hybrid maize screened then (Tamiru *et al*., 2011). Crop breeding has been shown to alter emissions of volatile semiochemicals that mediate attraction of natural enemies, an indirect defence strategy in many plants (Gols *et al*., 2011; Rodriquez-Saona *et al*., 2011; Tamiru *et al*., 2011). The evident trade-off between yield and defence could lead to the disruption of species interactions causing reduced biological control mediated by natural enemies of herbivorous pests (Macfadyen & Bohan, 2010). It has been shown for example, certain modern maize lines lack the ability to produce an important volatile that attracts insect-killing nematodes, with possible consequences for the overall resistance (Rasmann *et al*., 2005; Kollner *et al*., 2008). The current study revealed a kind of reverse progression with regard to egg-inducible indirect defence traits in maize where there is a steady decline in the prevalence of the trait going from teosinte to landraces to higher yielding hybrids. This can perhaps be attributed to the conditions under which crops are artificially selected during domestication and breeding processes. Modern breeding and agronomic selection begins with a domesticated plant, and through recurrent selection practices under pesticides treated, fertilized conditions, a hybrid that produces higher yields under near optimum conditions is developed

(Buddenhagen, 1981; Mayo, 1987; Kennedy & Barbour, 1992). Eventually, even if defence traits are maintained during domestication, through selection pressure, these traits become effectively invisible under pest-free conditions (Rosenthal & Dirzo, 1997). Indeed, the one commercial hybrid in which the trait was found appears to have been bred to withstand stresses such as drought to make it more suitable for African smallholder market (http://www.seedco.co/). Most importantly, identification of maize hybrids possessing this oviposition-induced indirect defence trait, may lead to identification of sources of genetic material for introgression of this trait into commercial hybrids.

Maize varieties that were responsive to *C. partellus* egg deposition emitted a complex mixture of volatile compounds. From the volatile mixtures, electrophyiologically active compounds were detected by antenna of *C. sesamiae.* The EAG-active compounds were identified and characterised through GC and GC-MS analysis. Some of the identified electrophysiologically relevant compounds which occurred predominantly across the maize varieties included (*Z*)-3-hexen-1-ol, a Green Leaf Volatile (GLV); monoterpenes like myrcene and limonene; homoterpenes like (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT); sesquiterpenes like (*Z*)-β-farnesene; phenyl propanoids like methyl salicylate and aldehydes such as decanal. DMNT, a key compound known to attract *C. sesamiae* (Khan *et al*., 1997), was predominantly elevated by egg deposition in both wild, landrace and the hybrid maize variety which possessed the indirect defence trait. Although there were variations in the amounts emitted across the different maize varieties, the volatile profiles appear highly conserved across the maize germplasm.

Maize landraces emitting egg-induced volatile compounds were shown to induce an indirect defence trait into neighbouring undamaged conspecific maize landrace. Naturally, undamaged healthy plants emit volatile semiochemicals such as green-leaf volatiles and terpenoids in trace amounts as part of their secondary metabolism (Dudareva *et al*., 2006; Pichersky *et al*., 2006; Chamberlain, 2014). These volatile compounds are produced in significantly higher amounts following mechanical damage or biotic stress such as feeding by herbivorous insects and mites (Hare, 2011), plant diseases caused by fungi (Kishimoto *et al*., 2008), bacteria (Yi *et al*., 2009) or even viral infections (Eigenbrode *et al*., 2002). Many of these compounds emitted by plants following biotic stress have been found to have defensive roles against the herbivores either directly or indirectly. Directly, they deter insects of same or different species from colonizing the plants emitting the volatiles while indirectly, they recruit parasitoids and predators of herbivores (Turlings *et al*., 1990; De Moraes *et al*., 2001; van Poecke & Dicke, 2004).

Previous studies demonstrated that volatile signals that act in defence of plants are induced systemically with the undamaged leaves remote from the site of herbivore attack also emitting defensive compounds (Turlings & Tumlinson, 1992; Röse *et al*., 1998; Mattiaci *et al*., 2001; Tamiru *et al*., 2011). Gómez & Stuefer (2006) pointed out that these signals are transmitted through the plant vascular system. There is also growing evidence of undamaged neighbouring plants either of the same or different species, responding to airborne signals produced by attacked plants, with over twenty plants so far reported to have this inter-plant signalling ability (Review by Chamberlain, 2014). Findings of the current study that egg-induced defensive volatile cues from oviposited maize plants can induce neighbouring maize plants to emit similar defensive compounds is important in a pest management context since it increases the recruitment and foraging efficiencies of natural enemies.

Both moths and larvae of *C. partellus* showed different behavioural responses to maize varieties emitting egg-induced volatiles and those which were not responsive to egg deposition. The moths showed non-preference for maize varieties emitting oviposition-induced defensive semiochemicals as opposed to hybrid maize which did not show volatile profile changes up on egg deposition. Similarly, *C. partellus* larvae showed preference for hybrid maize which was not emitting oviposition-induced semiochemicals as opposed to the maize varieties possessing this trait. Previous studies showed that female moths avoid plants emitting HIPVs as these chemical cues can indicate increased pressure from natural enemies and risk of competition on the plant emitting HIPVs (Rasmann *et al*., 2005; Dicke & Baldwin, 2010). Neonate *C. partellus* larvae were observed to move away and feed less on leaf tissue of maize varieties exhibiting early-herbivory defence traits as opposed to hybrid maize that did not exhibit this trait. Up on hatching, lepidopteran neonates have been observed to move away from host plant if oviposition occurred on unpreferred host plant (Zalucki *et al*., 2002). The observed behaviour of both the moth and larvae indicate that maize landraces exhibiting early-herbivory alert traits are less preferred hosts of *C. partellus* compared to hybrid maize that do not possess this trait. As such less plant damage is likely to be inflicted in these plants as compared to the preferred hybrid maize.

Different physiological responses were observed in *C. partellus* larvae exposed to different maize varieties used in this study. The maize varieties possessing the egg-induced defence trait had lower food assimilation and higher larval mortality both under laboratory and semi-field conditions compared to the hybrid maize which lacked the egg-inducible defence trait. Likewise, plant damage through leaf feeding was higher in hybrid maize lacking the egg-inducible defence trait compared to the maize varieties possessing this trait. These observations indicate that growth and development of *C. partellus* larvae is adversely affected in these maize varieties. As such, it has been reported by Awmack & Leather (2002) that host-plant quality determines the biological performance of the herbivores. Maize varieties possessing egg-inducible defence traits may also be possessing antibiosis and antixenosis properties against the *C. partellus.* These properties can be exploited for the development of novel crop protection strategies against cereal stemborers.

7.2 Conclusions

Of the five teosinte varieties screened for the presence of the egg-induced indirect defence trait, four possessed this trait. This suggests that this trait exists even in the pre-domesticated *Zea* species and that it could be an ancestral trait present in maize germplasm for defence against herbivores. In addition to recruitment of parasitoids, teosinte varieties emitting oviposition-induced volatiles deterred further herbivore colonization directly through oviposition deterrence.

The egg-induced volatile emission trait was prevalent in all the African maize landraces screened in this study. Parasitoids were attracted by these volatiles while female moths showed non-preference for subsequent oviposition on the maize landraces emitting these volatile cues. The volatile profiles in the landraces were generally similar to those obtained in the teosinte types, which is an indication that this trait is generally conserved especially in *Zea* species.

This study discovered the oviposition-induced volatile emission trait in one maize hybrid, SC Duma 43. This is after screening over 60 maize hybrids (both in this study and SCPRID ongoing work at *icipe*). The discovery of the trait in only one hybrid means that crop breeding for yield and quality has resulted in decline or loss of this trait in the improved maize lines.

Maize landraces emitting egg-induced defensive volatiles were shown to induce the same indirect defence trait in neighbouring undamaged conspecific maize plants through airborne signalling. This is ecologically important as signal cues increase in strength thus improving the recruitment and foraging efficiency of the natural enemies.

Both *C. partellus* moth and larvae showed non-preference behaviour for maize varieties exhibiting egg-induced volatile emissions trait compared to the hybrid maize lacking this trait.

Maize varieties exhibiting oviposition-induced volatile emissions constitutively suppressed *C. partellus* larval growth and development. There was limited larval survival of stemborer larvae as well as leaf feeding damage in these maize varieties as opposed to hybrid maize which lack this trait.

In general, this study has demonstrated that oviposition-induced plant signalling in maize is an ancestral trait which is prevalent in wild teosintes, landraces and rare in improved maize hybrids.

7.3 Recommendations

The study identified research gaps which need further investigation and recommends the following areas for future study:

1) Study the genetics underpinning the production of egg-induced VOCs in wild, landrace and hybrid maize.
2) Investigate signal pathways of oviposition-induced volatile compounds.
3) Investigate the parasitism rate in maize varieties exhibiting the egg-induced volatile emission trait under field conditions.
4) Elucidate specific mechanisms or metabolites responsible for behavioural and physiological responses of *C. partellus* on maize varieties exhibiting the egg-induced volatile emission trait.
5) Investigate the effect of stemborer oviposition on secondary defence metabolites such as hydroxamic acids in maize varieties possessing the egg-induced volatile emission trait.

6) Introgress the egg-inducible defence trait into high yielding improved maize lines for stemborer resistance.

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