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SCHOOL OF GRADUATE STUDIES

ELECTROPHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF SORGHUM CHAFER, *PACHNODA INTERRUPTA* (COLEOPTERA: SCARABAEIDAE) TO HOST PLANT VOLATILE COMPOUNDS

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A Dissertation Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Insect Science

MARCH, 2008

Dedicated to my Father, sister and brother who passed away during this study

ABSTRACT

The sorghum chafer, *Pachnoda interrupta,* (Coleoptera: Scarabaeidae) is a polyphagous herbivore and an important pest of sorghum (*Sorghum bioclor*). In this thesis work, plant volatile compounds from different hosts were identified with the purpose of using it as bait in traps for monitoring and/or control of *P. interrupta* adults.

Behavioral and antennal responses of the sorghum chafer, *Pachnoda interrupta* (Olivier) were tested to eugenol, methyl salicylate, methyl anthranilate, isoamyl acetate and butyl butyrate. In the field, all odor-baited traps except isoamyl acetate applied on cotton dispensers, were significantly more attractive than unbaited traps. All compounds, except isoamyl acetate, elicited dose dependent electrophysiological responses from both male and female antennae. Dispenser type (cotton wick or rubber septum), trap location (inside or outside sorghum fields) and season (mating/July or feeding/September) affected the performance of different compounds as lures. In July, methyl salicylate applied on cotton was the most attractive lure, whereas the most attractive treatments in September were eugenol and isoamyl acetate on rubber septum.

Headspace volatiles were collected from sorghum, *Sorghum bicolor* (Poaceae) and abutilon, *Abutilon figarianum* (Malvaceae). Combined gas chromatography-electroantennographic detection (GC-EAD) analysis was carried out on the antennae of both sexes of *P. interrupta*. Compounds active in GC-EAD were identified by coupled gas chromatography-mass spectrometry (GC-MS) as (*Z*)-3-hexen-1-ol, tridecane, 1-octen-3-ol, and 1-octanol from sorghum. From abutilon, (*Z*)-3-hexen-1-ol, tetradecane, methyl salicylate and methyl anthranilate were identified. Similarly, EAD-active compounds were identified from ripe banana as: ethyl butanoate, butyl

isobutanoate, isobutyl isobutanoate, isopentyl isobutanoate, isoamyl butanoate, cis-3-hexyl-2-methyl butanoate and 2-heptyl butanoate.

In the field, there was no significant difference in number of beetles caught in traps baited with individual sorghum compounds as compared to a blend of all four compounds in ratios mimicking the ones found in the sorghum extract. However, when methyl salicylate and/or eugenol were added to the blend, trap catches increased significantly. Similar results were obtained when eugenol was added to the synthetic abutilon blend in this study.

Behavioral responses tested using the Y-tube olfactometer showed no significant different in response between the banana extract and the blend of synthetic compounds. This indicates that the compounds identified are behaviorally relevant and can be used as potential candidate for further field test.

Scanning electron microscopy (SEM) revealed four morphological types of sensilla in the sorghum chafter, *Pachnoda interrupta*: grooved peg, smooth peg, grooved placodea, and smooth placodea. Olfactory receptor neurons (ORNs) were screened for response in single sensillum recordings with a set of 80 food-related compounds. From the 50 responding ORNs, 23 classes were identified. The classes were tuned to single compounds or sets of structurally and functionally similar compounds. The different ORN classes responded to green leaf volatiles (GLVs), flower and fruit odours and fermentation products. This indicates a sensory strategy for food search where multiple classes of ORNs each have a narrow detection window. Such a strategy could rely on common compounds present in hosts such as fruit or flowers, which advertise to symbionts for pollination and seed-dispersal with stereotyped odour signals.

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ABBREVIATIONS

AAU = Addis Ababa University

CAS # = Chemical Abstract Service number

CNS = Central nervous system

CSA = Central Statistics Authority

EAG = Electroantennography

EARO = Ethiopian Agricultural Research Organization

FAO = Food and Agriculture Organization-

FID = Flame Ionization Detector

GC-EAD = Gas Chromatogaphic-Electroanennographic Detection

GC-MS = Gas Chromatogaphy- Mass-spectrometry

GLV = Green leaf volatiles

IPM = Integrated Pest Management

ISP = International Science Program

MASL = Meters above see level

MOA = Ministry of Agriculture

OBP = Odor binding protein

ORN = Olfactory Receptor Neurone

PC = Personal Computer

PPRC = Plant Protection Research Center

SIDA-SAREC= Swedish International Development Coopration Agency-The Department for Research Coopration

SPME = Solid-Phase Micro-extraction

SSR = Single Sensillum Recording

CHAPTER I

GENERAL INTRODUCTION

1.1. Background

Sorghum chafer, *Pachnoda interrupta* (Olivier) belongs to the order Coleoptera, of the family Scarabaeidae and subfamily Cetoniinae (Clark and Crowe, 1978; Grunshaw, 1992). The distribution of *Pachnoda* is restricted to the African continent, although a few species have been recorded in Arabia (Grunshaw, 1992). *P. interrupta* was known in Ethiopia as early as the 1940s from specimens collected from different parts of the country (Zoological Natural History Museum, Addis Ababa University). In the late 1970s, a countrywide survey was carried out and nine species of *Pachnoda* were identified, one representing two subspecies (Clark and Crowe, 1978). In 1993, *P. interrupta* became a serious pest in the northeastern part of the country (MOA and EARO, 1999). Since then, the pest has been noted to increase in population density, geographical distribution and host range (MOA and EARO, 1999).

P.interrupta is a polyphagous insect, feeding on fruits, flowers and seeds from more than 35 plant species, some of which are important crops (Hiwot lemma 2000). *P.interrupta*s are particularly attracted to sorghum, *Sorghum bicolor* (L.) Moench, and *Abutilon figarianum* (Webb) - a shrub that grows naturally in dry low-land areas along the rift valley (Edwards *et al.,* 1995). Sorghum is the most important cereal crop in eastern Africa, in in particular in Ethiopia, (Brihane Gebrekidan, 1986) center of origin and diversity of sorghum (Thomas and Waage, 1966). In Ethiopia, sorghum is grown mainly by smallholder farmers who consume most of the produce themselves (Thomas and Waage, 1966). It ranks third both in area coverage and total cereal

production (CSA, 2006). However, the yield remains low (1365 kg/ha). Mean percent yield loss due to *P.interrupta* infestation has been recorded as high as 70% (Yitbarek Wolde-Hawariat and Hiwot Lemma 2000). In addition to sorghum, the beetle aggregates and feeds on large numbers on *Abutilon figarianum* (Webb). The total area infested by *P.interrupta* in Ethiopia had increased from only 1,375 ha in 1996 to 112,739 ha in 2000 (Hiwot Lemma 2000; Yeraswork Yilma 2000).

Since the first outbreak, farmers have been trying to control the pest using one or a combination of different methods such as bending stalks of sorghum and hand-pick adult beetles, smoking, burning compost heaps to kill the larvae and use of chemical insecticides. However, in most cases, these methods have not been fully successful in reducing beetle populations in the field because of continuous re-infestation, the height of the local sorghum varieties (above 2.5 m) and inappropriate spraying equipment.

Currently, the lure and kill technique developed by farmers is considered one of the best options available for reduction of the beetle population. The approach involves baiting of locally available traps, e.g. plastic containers or cans, with mashed fruits as attractant and a synthetic insecticide (Sevin®, a.i. carbaryl) as a killing agent.

Ripe fruit, banana especially, is an effective attractant for *P. interrupta* (MOA and EARO, 1999) but the use of fruit as bait in traps is inconvenient and not economical due to the need for high frequency of bait-replacement and trap maintenance. In some places, fruit is scarce and this may affect the control program.

The need for alternative control of beetle populations has stimulated investigations aimed at the characterization of plant kairomones (MOA, 2003). Damage to agricultural and horticultural crops has been a driving force to study the

chemical ecology of phytophagous scarab beetles during the past decades (Leal, 1998). Kairomones can provide a means of monitoring and controlling insects that is non-toxic to animals and plants. Olfaction has been shown to be important sense for food search in insects (Hartlieb and Rembold, 1996; Metcalf and Metcalf, 1992; Visser, 1986) and several studies have shown phytophagous scarabs to be attracted to plant-related compounds (Donaldson *et al.,* 1990; Imai *et al.,* 1997; Ruther and Mayer, 2005). Attempts to identify scarab attractants can be divided into two main categories: the ecologically oriented, where researchers have tested constituents of plants observed to be attractive, and the trial-and-error method, testing more or less randomly selected compounds (Leal, 1998). The identification and synthesis of a female-released sex pheromone, japonilure for Japanese beetle, opened the way for further investigations on intraspecific chemical communication in scarab beetles (Tumlinson, 1977).

The identification of attractive plant compounds has been facilitated by the adaptation of coupled gas chromatographic with electroantennographic detection (GC-EAD) (Arn *et al.,* 1975) to scarab antenna morphology (Leal *et al.,* 1996, Leal *et al.,* 1992b) and the development of bioassay coupled to gas chromatography also suitable for scarabs (Leal and Mochizuki, 1993, Leal *et al.,* 1992a). Utilizing GC-EAD technique, the major constituents of plant volatiles attractive to *P. interrupta* were identified (Chapters, 4 and 5).

Despite the abundance and economic importance of *P.interrupta*, until recently not much has been known about sorghum chafer olfaction. Gathering knowledge of the morphology and the physiology of the olfactory system is an essential first step in the process of finding efficient attractants, which may be used to improve *P. interrupta*

control. To achieve this goal, much can be gained by improving chemical formulations that either mimic natural substances or form new attractive odor compositions from *P. interrupta* hosts or from floral and fruit compounds which are known to be attractive to other scarab beetles. In this thesis, results of morphology, electrophysiological and behavioral responses of *P. interrupta* to host plant compounds are presented.

1. 2. Objectives of the study

1. 2. 2 General objective

- To investigate attractive host plant volatile compounds in order to formulate and use their synthetic forms for *P.interrupta* management.

1. 2. 3. Specific objectives

- To collect and identify volatile compounds from host plants of *P. interrupta*
- To test the electrophysiological and behavioral responses of attractve synthetic compounds in both laboratory and field experiments.
- To test a suitable dispenser and trap type for trapping experiments
- To study how the peripheral olfactory system in *P. interrupta* has been adapted to finding a wide array of hosts.

1.3. DESCRIPTION OF THE STUDY SITE

The field experiments were conducted during the 2004-2007 cropping seasons in Kewet Woreda, Rasa kebele, North Showa Zone of the Amhara Region, Ethiopia. The study site is located 255 km northeast of Addis Ababa, at altitudes of 1300 to 2600 m.a.s.l. and 11^{o} 55' N and 37^{o} 20' E (Fig.1.1). The area has a bimodal rainfall pattern – one between March and May (short rain) and the other from July to October (main rainy season). The average annual rainfall of 500-700 mm that is distributed unevenly and the annual temperature ranges from 8°C to 37°C. Major crops grown in the area are sorghum, tef, maize, and cowpea. Kewot woreda is one of the areas where *P.interrupta* infestation is most prevalent.

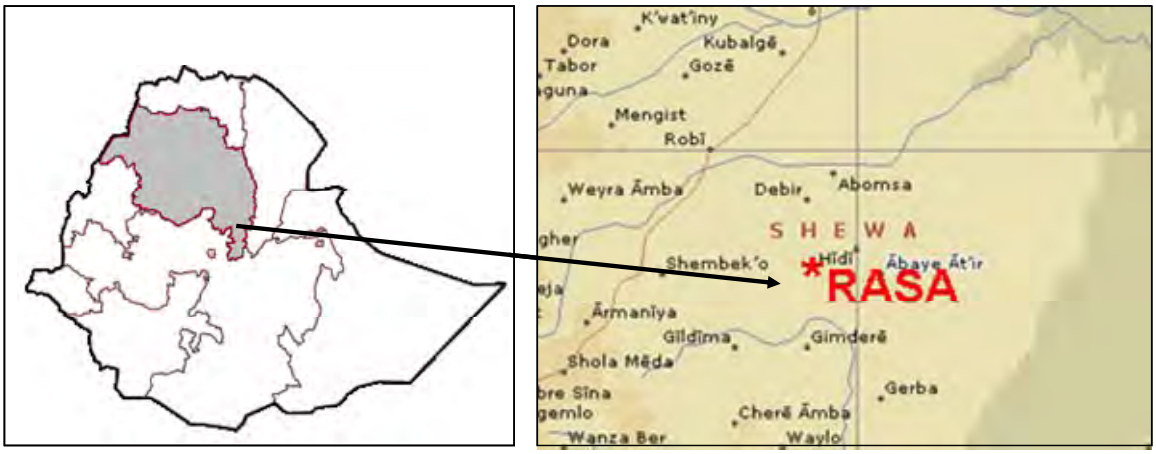


Fig. 1.1. Map of the study area.

1.4. Thesis plan

Little is known about the host plant selection in *P.interrupta* and there is no documentation of the role of host chemicals for this species. This work was initiated to provide further insight into the chemical ecology of *P. interrupta*, by studying the electrophysiological and behavioral basis for host plant selection. Chapter I is a general introduction to the subject, while chapter II presents an overview of relevant literature with the purpose of consolidating information known about the species, as well as generating further information on the topics covered in this study. Chapter III presents behavioral and electrophysiological responses of *P. interrupta* to selected floral and fruit volatiles. Factors affecting the efficiency of trap catches: effect of season, comparison of traps and dispenser types used as bases for field trapping experiments are also presented. Chapter IV deals with identification of volatile compounds from sorghum and abutilon headspace volatiles. As a step in the process of identifying behaviorally-active volatile compounds electrophysiological analysis were made on *P. interrupta* antennae. Coupled gas chromatographic-electroantennographic detection (GC-EAD) was used to determine which components of the host odors that were detected detected by the insect antennae. EAD-active compounds in extracts were identified using GC-MS. Investigation of behavioral activity of the insect to electrophysiologicallly-active compounds and blends of compounds were made in field experiments. Chapter V describes the identification of the volatile compounds found in banana that *P*. *interrupta* antennae can detect as well as the behavioral responses to headspace sample and synthetic blend in a Y-tube olfactometer. Chapter VI describes the antennal morphology and how the

peripheral olfactory system in *P. interrupta* has been adapted to the challenge of finding a wide array of hosts. Using single sensillum recordings, olfactory receptor neurons (ORNs) have been screened for response with 80 synthetic compounds that represent different functional groups. In Chapter VII the conclusion and recommendations of the study are presented.

CHAPTER II

LITERATURE REVIEW

2.1. Taxonomy of *Pachnoda interrupta*

Sorghum chafer, *Pachnoda interrupta* (Olivier) belongs to the order Coleoptera, family Scarabaeidae and subfamily Cetoniinae (Clark and Crowe, 1978; Grunshaw, 1992). The genus *Pachnoda* contains some 130 species (Krikken, 1994) but the taxonomy is poorly understood (Grunshaw, 1992). Nine species, one represented by two sub-species have been reported in Ethiopia (Clark and Crowe, 1978). Sorghum chafer is the smallest species in the genus *Pachnoda,* reported in Ethiopia.

2.2. Biology and Ecology of *P. Interrupta*

2.2.1. Eggs

The life cycle of the *P. interrupta* is depicted in Fig. 2.2. The eggs are laid singly and are stratified in the oviposition medium. Newly laid eggs are white, ovoid-spherical, and about 1.40 x 1.23 mm in size. At eclosion, they become brown, swollen and attain a size of 2.0 x 1.77 mm (Grunshaw, 1992). Reports vary concerning oviposition rate by single female and the duration of egg laying. According to Grunshaw (1992) a single female lays up to 24 eggs in one night and hatching takes seven to nine days. Contrary to this, Seneshaw Aysheshum and Mulugeta Negeri (2002) reported the ovipostion rate being 1.8 eggs per day and that the priod of oviposition would last for about two months. Gebeyehu Feleke (2002) reported 0.58

eggs per female per day during a period of 25 days. The average hatching period for eggs was reported to be 11.3 days (Seneshaw Aysheshum and Mulugeta Negeri, 2002) and 9.6 days (Gebeyehu Feleke, 2002). This inconsistency of results might be attributed to variations in the food provided for sexually mature insects, soil type, moisture level and methodologies adopted in egg collection. The diet of the beetles and constant disturbances of egg laying adults have been suggested to determine egg development and rate of ovipositon (Giliomee and Donaldson, 1992).

2.2.2. Larvae

A detailed description of the larvae including chaetotaxy of the third instar is given by Grunshaw (1992). The head width of the first instar is 1.17-1.24 mm, the second instar is 2.06 mm and the third instar is 3.05-4.05 mm. The third-instar larva is well developed. The head is smooth, light brownish-yellow and has well developed mouthparts for chewing. Ocelli are present and unpigmented. Prothoracic spiracles are 0.042 x 0.034-0.044 x 0.037 mm, with the opening of the respiratory plates directed posteriorly. Claws are cylindrical with 6-8 terminal setae. Anal slit is transverse and covered with hairs. The distinctiveness of the third-instar larva is used as the identification feature of the scarabs. The larval stage lasts between 41 and 71 days with a mean of 55.7 days (Seneshaw Aysheshum and Mulugeta Negeri, 2002; Gebeyehu, 2002; Grunshaw, 1992).

2.2.3. Pupae

The pupae are enclosed in earthen pupation cells, which are 10-15 mm long. Fully developed pupae are 12 x 8 mm. The duration of the pupal stage is 18.9 days

accorging to Seneshaw Aysheshum and Mulugeta Negeri, (2002) and 24.5 days according Gebeyehu Feleke (2002). Differences have been observed due to variations in temperature regimes used, 28 ± 2 °C (Seneshaw Aysheshum and Mulugeta Negeri, 2002) and 25 °C (Gebeyehu, 2002), respectively.

2.2.4. Adults

The adult is 12 to 16 mm long. Males can be distinguished from females by the presence of a shallow groove on the underside of the abdomen, whereas the abdomen of the female is convex (Fig 2.1b; Clark and Crowe, 1978). The elytra and pronotum have yellow-brown or red-brown spots and stripes (Grunshaw, 1992; Matthews and Jago, 1993). The mouthparts are small, poorly developed and do not project in the front of the head. The antennae have scape, pedicel and five flagellomeres with the apex extended on one side into the lamellae. The body of the adult beetle is flattish when viewed from above (Borror *et al.,* 1976). Color of the adults is very variable, from nearly complete black to complete pale yellow individuals, but usually with a distinct pattern as shown in Fig. 2.1a.

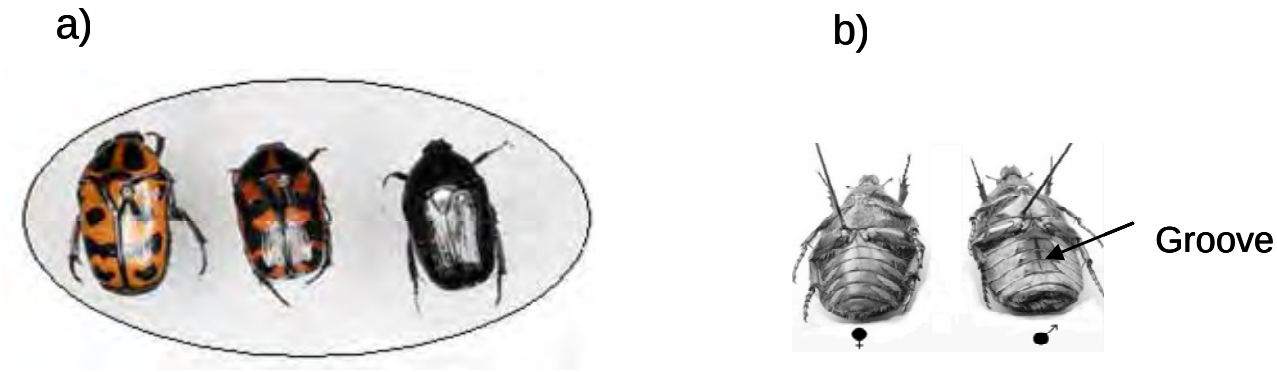


Fig. 2.1. Adult beetles, 3 color varieties of *P. interrupta* (a) male and (b) female morphs.

According to Clark and Crowe (1978) once sexual maturity is attained, mating is soon followed by egg laying and death, however, laboratory results of Seneshaw Aysheshum and Mulugeta Negeri (2002) demonstrated that sexually matured adult beetles could live for up to six months after egg laying.

Feeding and mating on host plants (June/July)

October/November feeding on sorghum; December- July (aestivates in the soil)

Eggs (6-16 days)

***P. interrupta* life cycle**

Pupal cell -20 days

Larvae (56 days)

Fig. 2.2. The life cycle of *P. interrupta*.

2.2.5. Seasonal occurrence

Adult *P. interrupta* carries out its various activities at specific times of the year which often coincide with the arrival of the rains subsequent flowering of plants and crops (Clark and Crowe, 1978). The life cycle is associated with varying habitats, including, favorable breeding sites, host plants on which the adult feeds and mate; and shaded vegetation for eastivation. The adults are diurnal, requiring warm and sunny conditions for their dispersal and are most active during the warmest part of the day (Clark and Crowe, 1978; Jago, 1995). *P. interrupta* is a non-migratory pest (Jago, 1993b). The movement of early season adult beetles may be restricted by the amount and distribution of rainfall as well as the type and pattern of local vegetation. Fairly high, widely distributed rainfall, which is usually accompanied by an abundance of alternative hosts, may favor *P. interrupta* to localized movements. Later, beetles are capable of gradual displacement over short to long distances. This dispersal is aimed at searching for suitable hosts (Jago, 1993b).

2.3. Geographic distribution

Many species of brightly colored Cetoniinae are distributed over the African continent (Giliomee and Donaldson, 1992). Although a few species from the genus *Pachnoda* have been recorded from the Arabian peninsula to Madagascar, the genus is mainly restricted to and widely distributed over the African continent (Clark and Crowe, 1978; Grunshaw, 1992); Schmutterer, 1969). *P. interrupta* was not on the list of crop pests of East Africa reported by De Pury (1968) but Schmutterer (1969) reported its occurrence in the region a year later suggesting that it may have been

introduced from West or Central Africa in 1969. *P. interrupta* has been recorded in Sudan and Cameroon (Risbec, 1950) and in Gambia, Brundi and Burkina Faso (Descamps, 1954), Senegal (Appert, 1957) and Somalia (Schmutterer, 1969). It is widely widespread in Mali (Risbec, 1950; Lock *et al*., 1988; Doumbia and Bronzi, 1985, 1989; Grunshaw, 1992; Jago, 1993a, b; Jago, 1995; Ratnadas and Ajayi, 1995) and Nigeria (Sastawa and Lale, 2000).

In Eritrea, it is the major pest of sorghum and millet (Andemeskel Woldehaimanot, 1987) and in Ethiopia, the beetle is widely distributed in the eastern lowland areas that boarder the Afar region and the Awash River (Clark and Crowe, 1978). Tsedeke Abate (1988) also reported *P. interrupta* as a serious pest of sorghum at altitudes below 2000 m.a.s.l. in several parts of Ethiopia where the dominant crop is sorghum.

2.4. Host range

Adults of many species of Cetoniinae feed on pollen, nectar, ripening or overripe fruits. Most species are diurnal in habit (Ritcher, 1958). *P. interrupta* is a polyphagous insect, feeding on a wide range of wild and cultivated flowering plants (Caswell, 1962; Hill, 1989). The main host plants among cultivated crops are probably *Sorghum bicolor* (common sorghum) and *Pennisetum glaucum* (pearl millet). Pearl millet is recorded as the main host of *P. interrupta* in Mali, Cameroon, and Nigeria (Grunshaw, 1992; Troure and Yehouienou, 1995; Jago, 1995; Ratnadas and Ajayi, 1995). Grunshaw (1992) listed nine host plants of *P. interrupta* in Mali. Leaky and Wills (1977) reported that it also attacks rice, maize and immature grains. In Somalia, roses, cucumber and okra are attacked (Schmutterer, 1969).

In Ethiopia, *P. interrupta* feeds on about 35 wild and cultivated plants, among the major ones are field crops like sorghum, maize, safflower, niger seed and sesame, and fruit crops like guava and banana (MOA and EARO, 1999). The beetles are highly aggregated and feed on sorghum seeds, *Acacia* and abutilon (Fig. 2.3a, b and c) respectively. Apart from plants it is also attracted to sugar cane wastes, and local beer residue, called "*atela"* (MOA and EARO, 1999).

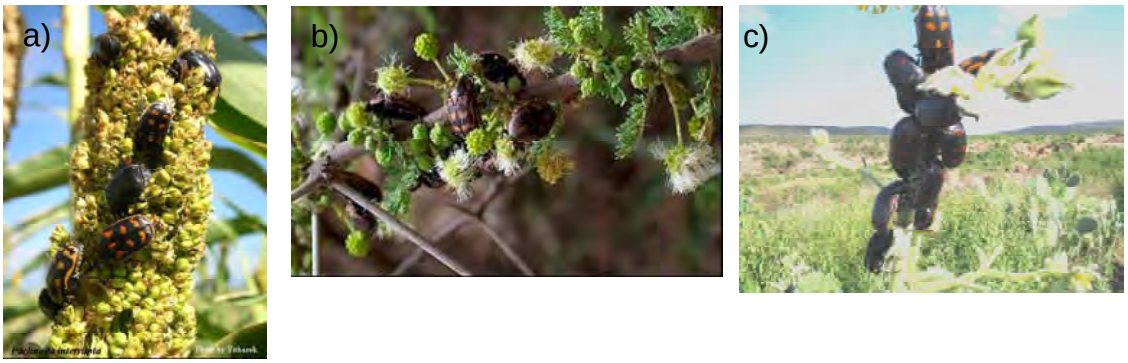


Fig. 2.3. *P. interrupta* adults aggregating and feeding on (a) sorghum panicle (b) Acacia flowers and (c) Abutilon

2.5. Economic importance

P. interrupta is the most damaging chafer beetle among in many West African countries and in the Sudan (Schmutterer, 1969). It can cause up to 50% yield loss in cases of heavy infestation (Troure and Yehouienou, 1995). According to loss assessment studies conducted in Mali in caged millets, the pest caused from 7.7 to 44.6% grain loss depending on the number of beetles, from 1 to 10 per millet head, respectively and the relationship between infestation level and grain yield reduction was linear (Grunshaw, 1992).

In Ethiopia, *P. interrupta* is the most destructive pest of sorghum and during huge outbreak seasons entire fields of sorghum were destroyed at the milk stage (Clark and Crowe, 1978; Tsedeke Abate, 1988). In addition to sorghum, *P. interrupta* has also been reported as major pest of maize (Adhanom Negassi and Abraham Tadesse, 1985; Hill, 1989). Since 1993, it has widened its host range from only sorghum and maize up to 35 plant families, and increased the size of land infested, which was only 1375 ha in 1996 to 112739 ha in 2000 (Hiwot Lemma, 2000; Yeraswork Yilma, 2000).

The adults are the damaging stage that feed on flowers and grains at the milky stage (Grunshaw, 1992; Jego, 1993a; Troure and Yehouienou, 1995). By aggregating on sorghum heads (Fig. 2.4a) they devour the pollen and stigma and suck out all the contents of the grain at milky and dough stages. They are also responsible for grain abortion and panicle sterility causing seeds to dry out before maturity (Fig. 2.4b). In Ethiopia, sorghum grain yield loss caused by *P. interrupta* has been reported as high as 70% (Yitbarek Wolde-Hawariat and Hiwot Lemma, 2000).

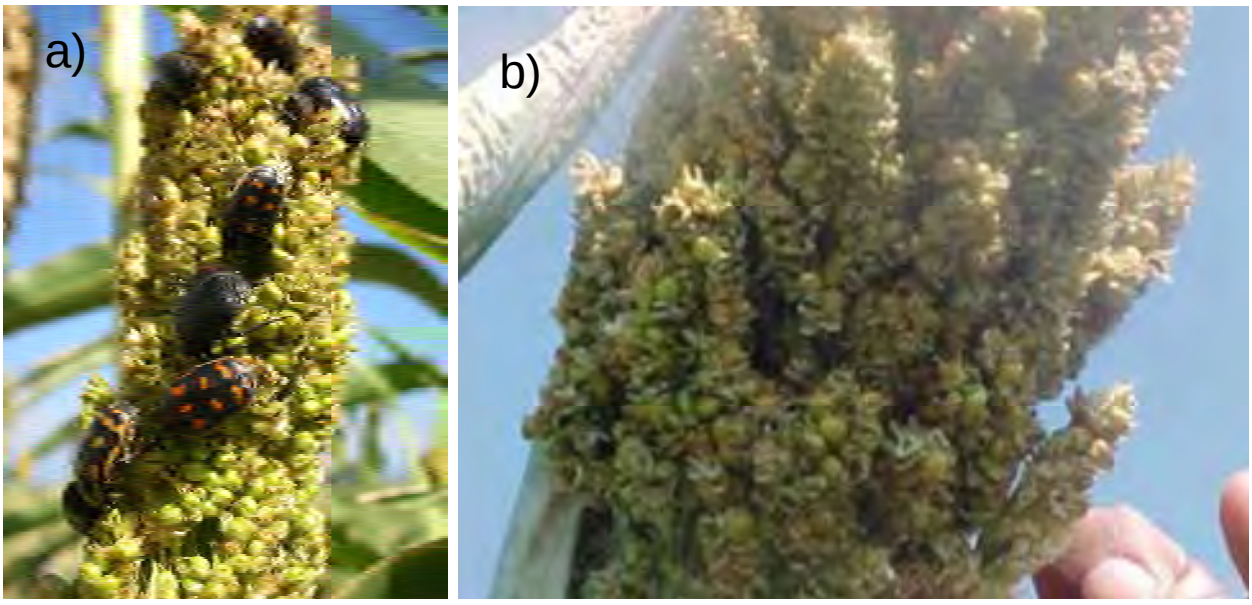


Fig. 2.4. a) *P.interrupta* adults aggregate and feed on sorghum panicle b) damage caused after feeding

Damages due to *P. interrupta* and other panicle pests can cause both qualitative and quantitative losses that generally cannot be compensated by further plant growth and recovery (Ratnadas and Ajayi, 1995; Rana and Singh, 1995).

2.6. *P. interrupta* management

Details of pest management practices used in a particular area depend on the pest incidence and socio-economic conditions. Farmers have tried to control the pest by using one or a combination of various methods (MOA and EARO, 1999), described in detail below.

2.6.1. Use of insecticides

The main control method that has been used for the control of *P. interrupta* was the application of insecticides mainly Sevin® 85% WP and Malathion 50% EC (MOA and EARO, 1999). Insecticides like endosulfan, trichlophone and pyrethrins have been recommended (Clark and Crowe, 1978) and here are reports that synthetic pyrethroids are effective (Grunshaw, 1992; Jago, 1993a, b). In spite of the attempt made to use chemical control for *P. interrupta*, the result has not been as good as expected because of several technical problems associated with the insecticide efficacy, application equipment, time of application and the height of the crop (MOA and EARO, 1999). During spray applications of insecticides directed at the tall crops (2-4 m) people have been exposed to spray drift. In some places, colonies of bees in nearby hives have been reported to be adversely affected (FAO-TCP, 2002).

In general, chemical pest control in millet and sorghum is best used as a component of Integrated Pest Management (IPM) (Matthews and Jago, 1993). Under laboratory conditions, Bekele Jembere *et al.,* 2005 reported that water extract of the Birbira, *Milletia Ferruginea,* seed caused 45-60% mortality of adult *P.interrupta* within 24-48 hrs. This mortality rate was significantly higher than the one caused by the standard insecticide, Sevin®, applied at the recommended rate. Jago (1991, 1993b) suggested that IPM for insect pests of millet should include the adoption of cultural techniques, the selection of appropriate varieties and minimal use of pesticides. The selection of appropriate millet varieties, suitable sowing date and a selective insecticide significantly reduced crop losses attributable to *P. interrupta* in Nigeria (Sastawa and Lale, 2000).

2.6.2 Cultural control methods

Farmers have been trying to control *P.interrupta* using combinations of indigenous control methods. The most widely used practices are hand collection or picking, bending of sorghum stalk to the ground, fogging with smoke, shifting the cropping system and use of baited traps. However, combinations of all these interventions could not reduce the beetle populations below levels causing economic damage (Hiwot Lemma, 2000; Yeraswork Yilma, 2000).

Hand collection has been practiced early in the morning and late in the afternoon when the beetle are inactive (Fig. 2.5). The need for manpower for repeated collection in the same field and the height of sorghum which creates difficulties during collection, are some of the limitations of this method (MOA and EARO, 1999). Bending of sorghum stalks to the ground has disadvantages since it leads to shrinkage of seeds and poor quality because of physiological disorder, exposure of the sorghum head to rodent and termite damage and molding of grains because of favorable micro-environment created for fungi. Hence, this practice is not technically advisable (MOA and EARO, 1999).

2.5. Hand collected beetles by farmers measured in sacks

2.6.3. Lure and kill technique

Currently, a lure and kill technique developed by farmers is considered one of the best options available for reduction of the beetle population. The approach involves baiting of locally available traps, e.g. plastic containers or cans, with mashed fruits or local brew residue as attractant and a synthetic insecticide (Sevin®, a.i. carbaryl) as a killing agent. However, in some places, fruit is scarce and this may affect the control program (MOA and EARO, 1999; FAO-TCP-Ethiopia project (TCP/ETH/0166) interim report, 01/2002).

2.6.4 Biological control

Two parasitoids, *Eutrixopsis* spp (Tachinidae) and *Adapsila latipennis* (Walker) Pyrgotidae) have been reported parasitizing adults of *P. interrupta* (Kasahun Yitaferu *et al.,* 2006). According to Seneshaw Aysheshum (2001) over 80 isolates of fungal pathogens (mainly *Beauveria* and *Metarhizium* spp.) have been collected from adult *P. interrupta* and various beetles and most of these isolates have been identified and characterized. Preliminary efficacy study of ten native fungal isolates revealed that *Metarhizium* isolates PPRC-19 and PPRC-14 showed good potential in killing the larvae and adult beetles (Seneshaw Aysheshum, 2001). Using the attract and infect method under field conditions, the local isolate *Metarhizium* sp. (AAU Insect-D) was found to be the most effective killing 75% of the infected adult beetles within 15 days (Emiru Seyoum *et al.,* unpulished data). According to Klein and Lacey (1999) using attract and infect with pathogens resulted in up to 97% mortality of adult Japanese beetle, *Popillia japonica*. This indicates that there is a potential to use the native entomopathogenic fungal isolates in the management of *P.interrupta*.

2.7. Types of semiochemicals

Law and Regnier (1971) proposed the term semiochemicals derived from the Greek term *semen*, meaning, a mark or signal for chemicals that mediats interactions between organisms. Semiochemicals are divided into pheromones and allelochemicals (Fig. 2.6). Pheromones are intraspecific chemicals, i.e signals working between individuals of the same species. Several types of pheromones have been identified and these include sex, aggregation, alarm and trail pheromones (Jutsum and Gordon, 1989; Wyatt, 2003).

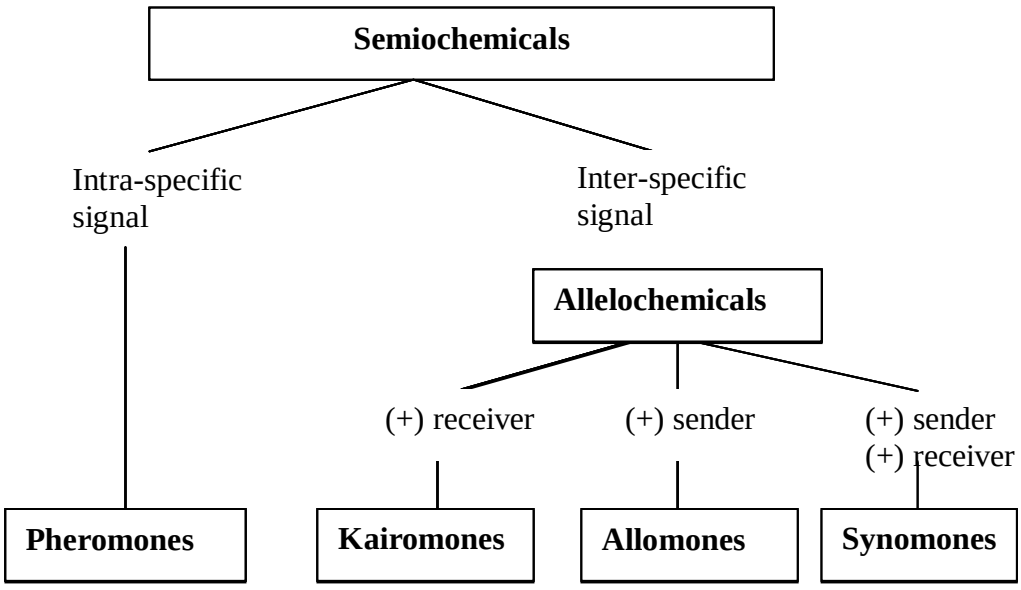


Fig. 2.6. Classification of chemical compounds that mediate interaction between organisms semiochemicals, according to the effect they have on the involved organisims. (+) = benefit

Allelochemicals are interspecific communication chemicals divided into three classes: allomones, kairomones and synomones (Nordlund and Lewis, 1976, Gullan

and Cranston, 1994). Allomones are signals that benefit the emitter while being negative or of no significance to the receiver. An example of allomones are the odors released by the plant include dead horse arum, *Helicodiceros muscivorus*, mimicking a carcass inorder to attract and trap flies for unrewarded pollination (Stensmyr *et al.,* 2002). Kairomones are signals that benefit the receiver. These odors could be used as an attractant either for pests using plant chemicals, or for natural enemies using herbivore emitted chemicals (Nordlund, 1981). There are also synomones that benefit both emitter and receiver such as chemicals mediating pollination. This terminology is somewhat limited since the same chemical compound may have several functions, e.g. a pheromone that also acts as a kairomone for another species. Hence, Dicke and Sabelis (1988) proposed the use of the term “infochemical” which may be particularly appropriate in situations where tritrophic interactions are being considered.

Allelochemicals have been further grouped grouped by Ruther *et al.,* (2002) by introducing terms such as foraging kairomone (used in the context of food location), enemy-avoidance kairomone (used to reduce the negative impact of natural enemies), sexual kairomone (used for sexual purposes), and aggregation kairomone (attracting/arresting both sexes of an organism) based on the function of the benefiting organism (Cork, 2004). The difference between semiochemical and infochemical terminology is that, whereas in semiochemical the origin of the chemical determines its designation as a kairomone, allomone, or synomone, in infochemical terminology the adaptive value of the use of the information that the chemical carries is the central issue.

In addition to the classification described above, semiochemicals are also classified according to the behavioral responses they elicit in insects (Table 2.1).

Table 2.1. Definitions of chemicals in terms of the responses they elicit in insects (Dethier *et al.,* 1960, In Schoonhoven *et al.,* 1998)

Attractant	A chemical that causes insects to make oriented movements towards its source
Repellent	A chemical that causes insects to make oriented movements away from its source
Arrestant	A chemical that may slow the linear progression of an insect by reducing actual speed of locomotion, by increasing turning rate or prolonging stay in a place
Feeding or ovipostional stimulant	A chemical that elicits feeding or oviposition in insects ('feeding stimulant' is synonymous with 'phagostimulant')
Deterrent	A chemical that inhibits feeding or oviposition when present a in a possible feeding or ovipostion site

2.7.1. Uses of semichemicals

The uses of semiochemicals as pest management tools show considerable promise in reducing damage. One example is the reduced mortality of trees, by bark beetles, where semiochemicals have been used successfully to monitor population trends or as a mass trapping and/or disruption tactic (Borden, 1993). The exploitation of semiochemicals as management tools requires a thorough understanding of the mechanisms involved in the production and release of compounds as well as the response by the target species. In addition, it is important to have an understanding of

the effects of semiochemicals applied on the target species and associated organisms (Leal, 1999).

Methyl anthranilate, for example, has been demonstrated to be 2.4 times more attractive for males of *Anomala rufocuprea* Motschulsky than the female-produced pheromone methyl (Z)-5-tetradecenoate, whereas female catches with the kairomone were 65 times higher (Imai *et al.,* 1997). Attraction to plant volatiles has been demonstrated in numerous scarabs such as *P. japonica* (Ahmad, 1982, Loughrin *et al.,* 1996, 1998) and *Maladera matrida,* Argaman (Harari *et al.,* 1994). A survey of floral compounds demonstrated that 2-phenylethanol is a potent attractant to the long-legged chafer, *Hoplia communis* Waterhouse (Imai *et al.,* 1998). The search for plant kairomones for control and survey of scarab populations was carried out a few decades before the first insect pheromone was identified. Later on, several studies have demonstrated synergistic effects of combined pheromone/kairomone lures, one example being the successful control programs of *P. japonica* (Leal, 1998). Host plant volatiles (kairomones) have also been shown to enhance the effectiveness of pheromone traps in attracting weevils (Dickens *et al*., 1984; Phillips *et al*., 1984; Giblin-Davis *et al*., 1994; Cerda *et al*., 1999).

2.7.2. Plant volatiles as guiding stimuli

The processes of finding and choosing a host for feeding or ovipostion can be divided into different, but overlapping stages: 1) orientation, 2) landing and 3) assessment (Renwick, 1989; Schoonhoven *et al.,* 1998) or as described for parasitoids: 1) habitat or host community location, 2) microhabitat location and acceptance, 3) host location and acceptance (Vinson, 1998). Olfactory and visual

stimuli are generally most important in the first steps of host location; orientation and landing while mechanosensory and gustatory stimuli are more important during the final assessment of the plant (Schoonhoven *et al.,* 1998). The volatile blend released from a plant can be species specific either by releasing unique compounds or by having characteristic ratios of compounds. The volatile blend can also contain more information about the physiological state of the plant and about presence of herbivores (Schoonhoven *et al.,* 1998).

The search behavior of different insects depends on species, environment and cues available. In some insects a random search can be observed. But also during random search responses to stimuli can occur in change in speed or change in turning frequencies (Schoonhoven *at al.,* 1998; Bernays and Chapman, 1994).

Insect orientation to an odor source exists only when they are very close to odor concentration gradient. The technique used by many insects is to approach the odor source from downwind. The air contains pockets of odor-carrying air. Flying insects navigating in such odor plumes detect and correct for the wind direction visually. This is called potomotor (means that it is driven vissualy) anemotaxis (means only flying against the wind) and involves an oriented movement by maintaining a set angle to the wind direction (Bell *et al.,* 1995; Schoonhoven *et al.,* 1998). Insect flight to the odor plume is in zigzag movments upwind directions. These zigzag turns are not induced by reaching the edges of the plume; they are the results of a program in the central nervous system (CNS). When the plume is lost the insect stops for a while and moves from side to side without progress towards the source. This technique for oriented approach towards an odor source is well documented in attraction to

pheromones but has also been observed in attraction to plant volatiles (Bernays and Chapman, 1994; Bell *et al.,* 1995; Schoonhoven *et al.,* 1998).

2.8. Morphology of olfactory sensilla and odor detection

An insect in the environment encounters thousands of odor compounds. If the insect uses this information as a means to locate a suitable food source or a potential mate, it naturally must be equipped with sensory organs capable of detecting and discriminating between different odor compounds. The olfactory receptor cells in insects are usually found on the antennae (Schneider *et al.,* 1964). Scarab beetles are characterized by lamellate antennae, formed by leaf-like extensions of the terminal segments (Chapter VI; Fig. 6.1). The receiving part of the receptor cell is located within the olfactory organ or sensilla, of which the outer structure can be seen on the surface of the antenna as hairs, plates or pores (Kaissling, 1987). Regardless of their differences in outer morphology they are all built around the same basic concept (Keil, 1999; Fig. 2.7).

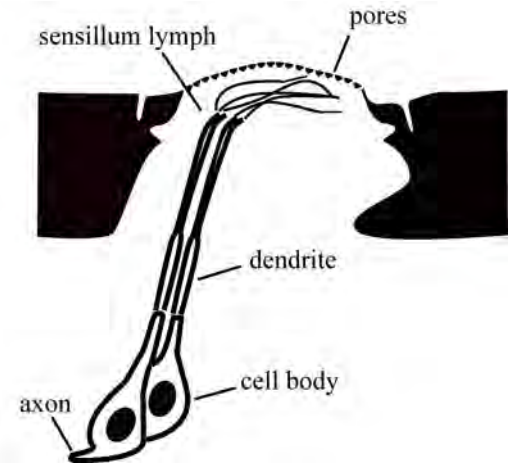


Fig. 2.7. Schematic drawing of a sensillum placodeum of a type common in scarab beetles.

Odor molecules enter through the pores in the sensillum cuticle and are transported in the lymph to receptors situated in the dendritic membrane. A receptor potential is elicited in the dendrite and propagated to the cell body, triggering action potentials in the axon leading to the central nervous system. Redrawn from (Keil, 1999).

The sensilla are covered with a porous cuticle (Fig. 2.7). The odor molecules are thought to reach the sensory neurons by being absorbed on the cuticle and diffuse through numerous pores in the sensillum walls after which they bind to and odor binding protein (OBP) in the receptor lymph (Vogt and Riddiford, 1981; Kaissling, 1987). OBPs are responsible for the recognition of specific odor molecules (Clyne *et al.,* 1999; Vosshall *et al.,* 1999). Odor molecule-receptor interactions give rise to a change in receptor potential and if the potential is above a threshold, action potentials are triggered in the neuron (Todd and Baker, 1999). After receptor activation, the odor- OBP complex is deactivated and dissolved, followed by degradation of the odor molecule complex by enzymes in the lymph (Stengl *et al.,*

1999). When the relevant compounds are present at the receptor site, a receptor potential is elicited. The receptor potential is a slow graded depolarization of the cell membrane which is propagated along the dendrite until it reaches a spike initiation site, where the signal is converted to a number of action potentials (spikes). The action potentials of different odor receptor neurons have different amplitudes and different waveforms, which often allow discrimination between recorded neurons within one sensillum (Todd and Baker, 1999). These action potentials are transmitted in the olfactory axons of the antennal nerves ending in the antennal lobe, where the olfactory signal is processed before being transferred to higher integrative centers of the brain (Todd and Baker, 1999).

There are two different mechanisms through which the receptor neurons could transmit information to the brain concerning encountered odors (Boeckh, 1984; Masson and Mustaparta, 1990). One way would be to have very specific receptor proteins each binding to one or a few compounds. Information about each particular compound in an odor would then be transmitted to the brain by the so called labeled lines, i. e. in each type of neuron signals the presence of one compound. These very specific neuron types are termed “specialists”. The other alternative is to have neurons with less specific receptor proteins, with partly overlapping response spectra, which bind to numerous different chemicals. The information about odor quality would then be transmitted in a so called across-fiber pattern as the combined response from many “generalist” receptors (Hansson and Christenson, 1999).

A typical example of a specialist neuron would be those responsible for detecting the long-distance sex pheromones found in many insect species. Generalist neurons would typically be involved when the numbers of relevant compounds are high, such as in the complex volatile blends originating from plants. However these two types represent the two extremes of a continuous scale. Plant odor receptors with high selectivity and sensitivity have been found in several insect species (Anderson *et al.,* 1995; Wibe *et al.,* 1997; Hansson *et al.,* 1999), indicating that information about food quality is transmitted via a combination of labeled lines and across fiber pattern mechanisms.

2.9. Methods and techniques in chemical ecology

2.9.1 Collection of volatiles for chemical analysis

The methods most commonly used for isolation of plant volatiles are solvent extraction or distillation and headspace techniques. By solvent extraction or distillation, only volatiles present at one time in the plant material are collected, together with a range of non-volatile compounds. In addition, distillation methods can induce damage-related volatile production (Buttery and Ling, 1984). This results in the collection of a blend of compounds, which is not representative of what the plant actually releases (Tollsten and Bergstrom, 1988). In comparison, headspace techniques are non-destructive. However, if the plant is transferred to an artificial environment for headspace collection, under a modified light, temperature, and humidity regime, modifications of the volatile emission are to be expected. For the study of insect-plant chemical communication, it is necessary to collect volatile

compounds that mediate the interactions. The collection method must prevent enrichment of contaminants during sampling to facilitate analysis (Millar and Sims, 1998).

Solid-Phase Micro-extraction (SPME) is a relatively new, solvent free headspace technology. Volatiles are absorbed from the headspace on a fiber coated with liquid (polymer), a solid (sorbent) or a combination of both. The SPME fiber is then directly inserted into the GC for thermal desorbtion and analysis (Flamini *et al.,* 2003). This technology offers several advantages, including simplicity of handling, reduced carryover of impurities and shorter sampling time. However, once injected, no volatiles are left for further investigations due to the limited storage capacity of the fiber. Moreover, compounds can not be stored over a long time.

The most frequently used method to concentrate volatiles in ecological studies is headspace collection (Millar and Sims, 1998). With the use of headspace techniques, volatile substances can be enriched in time as they are emitted by the plants, and sampling can be performed outdoors (Knudsen *et al.,* 1993). The odor source, for example a plant or an insect, is placed within an aeration chamber. Often different glass chambers are used, but these can be difficult to handle when larger plants are enclosed (Millar and Sims, 1998). As an alternative, in the present study collections of volatiles were done from intact plants, using a cooking bag (Toppist Scandinavia AB, 35 x 43 cm) wrapped around the plant stem (Fig. 2.8).

To avoid contamination during odor collection both in the field and in laboratory, charcoal filtered air was pumped into the bags and the samples were trapped on an adsorbent (Agelopoulos *et al*., 1999). Different adsorption materials are available, and the choice depends on compounds of interest and method used

(Agelopolous and Pickett, 1998; Raguso and Pellmyr, 1998). In the present study a polymer mesh (Super Q®, Alltech) was used as adsorbent and volatiles were eluted with hexane (Chapter IV and V).

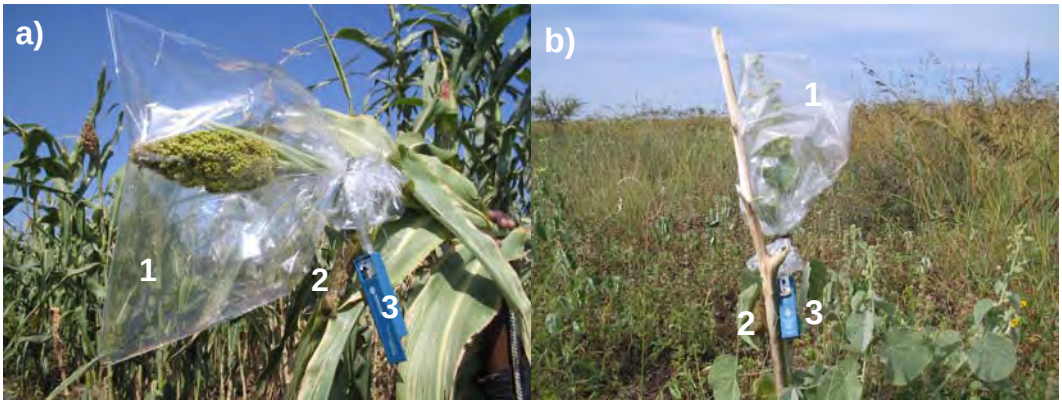


Fig. 2.8. Headspace collection of volatile odor from a) sorghum and b) abutilon.

The plant is enclosed with a polyester (cooking bag) (1) air is pushed into the bag through a charcoal filter (2) passed over the plant and pulled out using a pump through a filter (Super Q) that traps emitted volatiles (3).

2.9.2 Electrophysiological methods

Different electrophysiological methods are reviewed by Millar and Haynes (1998). The Electroantennogram (EAG) technique was originally developed by Schneider (Schneider, 1957a, 1957b). An antenna that is freshly cut is placed between two electrodes connected to an amplifier. A change in potential can then be recorded when the antenna is stimulated with relevant odors. An extended use of the EAG technique is when the antennal detector is used in parallel with a conventional flame ionization detector (FID) of a gas chromatograph (GC).

In a Coupled Gas Chromatography-Electroantennographic Detection (GC-EAD) setup (Fig. 2.9), the effluent of the GC column is split between the flame ionization detector (FID) of the GC and the antennal detector, the EAD (Arn *et al.,* 1975). EAD active compounds present in natural mixture volatiles are identified by gas chromatography/mass spectrometry (GC/MS). These combined techniques can be powerful tools to identify biologically-active compounds in a natural mixture of volatiles (Moorhouse *et al.,* 1969; Struble and Arn, 1984; Cork *et al.,* 1990; Wadhams, 1990), a technique that has also been adapted for single sensillum recording (Wadhams, 1984; Blight *et al.,* 1995; Wibe *et al.,* 1997; Stensmyr *et al.,* 2001; 2003). The EAD approach has numerous applications, including characterization of responses to pheromones (Wadhams, 1990; Burger *et al.,* 1991; Leal *et al.,* 1992b; Leal *et al.,* 1995) and host odors (Thiery *et al.,* 1990; Henning and Teuber, 1992; Wadhams *et al*., 1994; Cosse *et al*., 1995; reviewed by Bjostad, 1998).

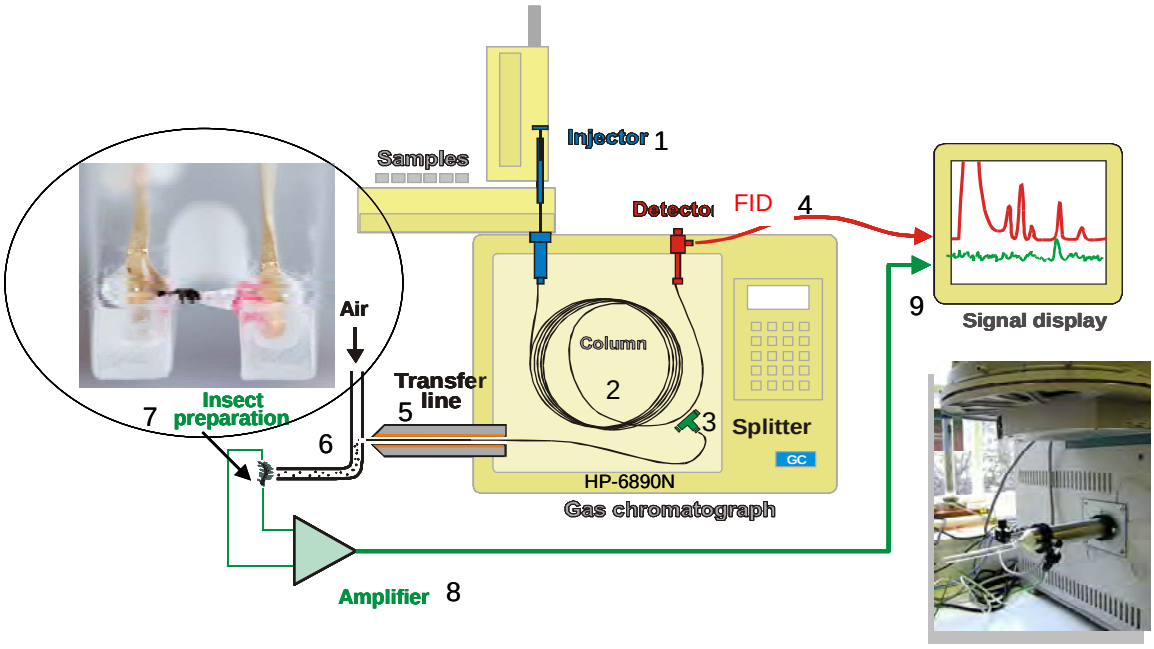


Fig. 2.9. Combined gas chromatography and electroantennographic detection (GC-EAD) technique.

Headspace extracts for analysis are injected using a microsyringe (1) into the column of a gas chromatogram (2). As the oven temperature increases the components of the extract are separated and move down through the column and reach a spliter (3) from where half of the effluent goes to a flame ionaization detector (FID) (4). The other half simultaneously passes through the transfer line (5) to glass tube (6) where a continuous humidified/purified airflow blows over the antennal preparation placed in the two wells in the holder with gold wire electrodes (7) and connected to an amplifier (8). Signals recorded from the FID and the antennae are recorded in parallel, and the peaks from the GC can be monitored by responses from the antenna (9).

With the single Sensillum recording (SSR) technique, responses of individual olfactory receptor neurons can be recorded. This technique is often more difficult to perform than the EAG technique but provides more detailed information. An electrolytically sharpened tungsten electrode is inserted at the base of sensilla as recording electrodes (Fig. 2.10) was used for the present study.

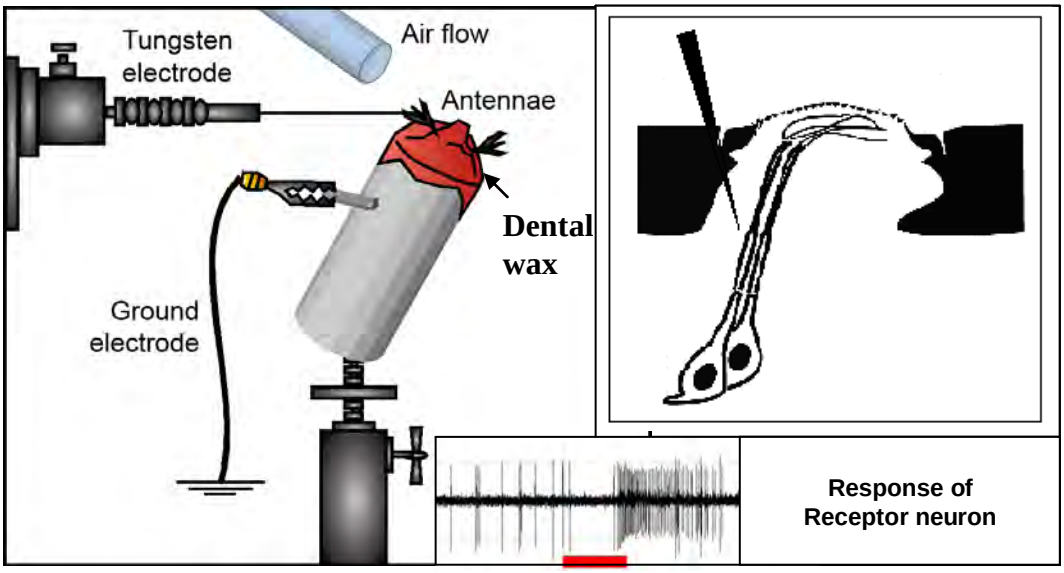


Fig. 2.10. Electrophysiological recordings from single sensilla on the *P.interrupta* antenna.

The beetle is restrained in a plastic tube with the head covered with dental wax only exposing the antenna. An electrode in the abdomen serves as a reference electrode, while recordings are made through a tungsten electrode inserted in sensillum (upper right). Odor stimulus is given in an air stream continuously flushing the antenna through a glass tube. Responses from the receptor neurons can be recorded as action potentials (lower right). Adapted from (Larsson, 2001).

The signals from SSR are amplified and then monitored visually on an oscilloscope or PC. The volatile stimulus is delivered in a purified and humidified air stream continuously passed over the mounted antenna. The compound to be tested is normally placed on a small piece of filter paper inside a Pasteur pipette; a puff of air through the pipettes brings the odor molecules into the air-stream passing over the antenna.

2.9.3. Bioassay on response to odors

Insect responses to odors can be studied in various laboratory bioassays and in the field by direct observations or catches in odor-baited traps. It can be advantageous to study insect behavior in the laboratory as experimental conditions can be kept constant and variation in responses thereby minimized (Hare, 1998). On the other hand, laboratory experiments can be misleading due to the absence of factors important for natural behavior. When behavioral studies are conducted to explain fundamental questions about insect natural behavior, laboratory studies always need to be related to field observations (van Alphen and Jervis, 1996; Hare, 1998). A Y-tube olfactometer was used in the present study (Chapter V). A Y- tube allows the insect to choose between the two air streams carrying odors of different treatments or purified air (Fig. 2.11). An early description of a Y-tube olfactometer was published by McIndoo (1926). In that experiment, the first evidence for insect attraction to plant odors was demonstrated in tests with Colorado beetle and potato plant odors.

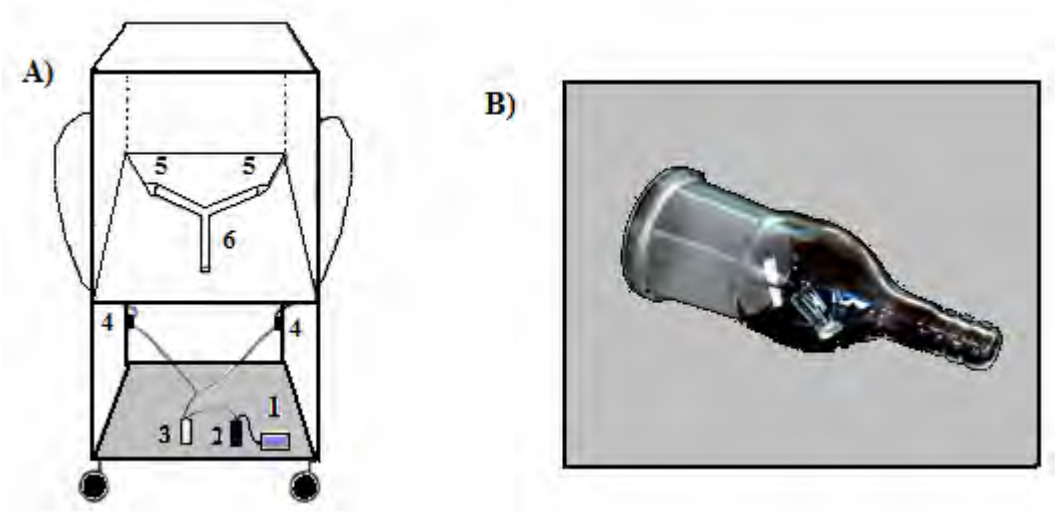


Fig. 2.11. A) Y-tube olfactometer.

1) pump, 2) charcoal filter, 3) air humidifier, 4) flow meters, 5) custom made glass tubes, 6) Y-tube. B) Custom made glass tube holding filter paper with stimulus. Modified after Jönsson, 2005 and Andersson, 2007.

A glass Y-tube was constructed similar to that reported by Jönsson (2005) and modified by Andersson (2007). Both in Jönsson (2005) and in the present study a light source behind the fork was used to stimulate insect movement. A disadvantage with the Y-tube is that choice is no longer possible when the insect has passed the decision line in one of the arm (van Alphen and Jervis, 1996) and the effects of non-odor decisions can be large if the insects are attracted to the light source. In the present experiments, parts of the tubing before the decision line were shaded to decrease the photo-tactic response in favour of odor-guided decisions. For final verification of synthetic compounds as host plant components, field trapping

experiments are crucial. In the field bioassays, factors such as: trap design, trap placement, and type of dispenser used for emission of compounds will affect the outcome of the experiment (Cardé and Elkinton, 1984; Wall, 1990; Anderbrant *et al.,* 1992). The effect of these factors on trapping efficiency was investigated (Chapter III).

CHAPTER III

BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSE OF SORGHUM CHAFER, *PACHNODA INTERRUPTA* (COLEOPTERA: SCARABAEIDAE) TO SYNTHETIC PLANT COMPOUNDS

3.1. Introduction

Among a wide range of insect pests on sorghum, the sorghum chafer, *P. interrupta* (Olivier) (Coleoptera: Scarabaeidae: Cetoniinae), is a key pest in the northern, eastern and Rift valley areas of Ethiopia (Hiwot Lemma, 2000). Mean percent yield loss due to *P. interrupta* infestation can be as high as 70% on sorghum (Yitbarek Wolde-Hawariat and Hiwot Lemma, 2000). It is the adult beetles that cause the damage to the crop. In September to October, the newly emerged adults feed on sorghum flowers and grains at the milky/soft dough stage, causing sterilization of florets and kernel damage (Clark and Crowe, 1978; Grunshaw, 1992; Jago, 1995). The intense feeding period is followed by a period of aestivation, outlasting the dry season. Aestivating beetles can be found in the soil and among plant litter under trees and bushes. After the short rain period in May, the beetles become active again and start mating. During this period, they feed on various flowering plants, notably acacias. The end of the mating period approximately co-occurs with the beginning of the long rains. The females then oviposit in the soil and the hatching grubs feed on plant litter until they pupate in August (Seneshaw Aysheshum and Mulugeta Negeri, 2002).

Management strategies for *P. interrupta* have been restricted to insecticidal treatments and traditional control methods (MOA and EARO, 1999). Due to continuous re-infestation, insecticides have to be applied several times throughout the growing season (MOA, 2003), which is both costly and hazardous to human health and the environment. The traditional control methods, such as hand-picking of beetles from the plants or smoking of the crop, are both labor intensive and time consuming and yet inefficient. Currently, the lure and kill technique developed by farmers is considered one of the best options available for reduction of the beetle population. The approach involves baiting of locally available traps, e.g. plastic containers or cans, with mashed fruits as attractant and a synthetic insecticide (Sevin®, a.i. carbaryl) as a killing agent. In some places, fruit is scarce and this may affect the control program.

It has been known for a long time that insect attraction to host or food plants is influenced by chemical cues (Kevan and Baker, 1983; Tasin *et al.,* 2005). For scarabs, field screening of synthetic floral scent compounds have revealed potent attractants (Ladd *et al.,* 1981; Donaldson *et al.*, 1990) that for some species are used for pest monitoring and control (Potter and Held, 2002; Ruther and Mayer, 2005). Electrophysiological and behavioral studies on *P. marginata* Drury – a species closely related to and with similar food preferences as *P. interrupta* – also revealed several attractive, synthetic floral and fruit odor compounds (Larsson *et al.,* 2003). The objective of the present study was to test the antennal response and the attractivity of some of these compounds on *P. interrupta* in the field, with the purpose of finding candidate attractants for mass trapping. The impact of trap design and dispenser type on the efficacy of the traps was compared. In addition, an investigation on the

attraction dynamics of these compounds in different seasons and locations was made.

3.2. Materials and methods

3.2.1. Electrophysiology

Field collected adult insects were kept in a climate chamber at a temperature of 26 °C, RH=70%, and 16/8 L/D photoperiod. Insects were taken out of the climate chamber and kept in a plastic jar 10 to 30 min before being tested. The antennal preparation was similar to the one described by Leal *et al.,* (1992b). One antenna was excised with a fine forceps and placed in an antennal holder (Hillbur, 2001) and the signal was amplified (JoAC, Lund, Sweden) and analyzed with ElectroAntennoGraphy software (Syntech®, Hilversum, The Netherlands). Clean, humidified air was passed continuously over the antenna at 1.5 l/min. The antennal response was tested to methyl salicylate, methyl anthranilate, butyl butyrate, isoamyl acetate and eugenol. Doses of 0.1, 1, 10, 100 ng and 1, 10 and 100µg of the compounds in 10 µl redistilled hexane (Lab-scan, Analytical Sciences, Ireland) were applied to filter paper strips (0.5 cm x 3.0 cm, Munktell No. 1, Sweden) placed in Pasteur pipettes. Fresh stimulus pipettes were prepared before each set of experiments. Stimuli were delivered as 0.2 s puffs at a flow rate of 10 ml/s by a stimulus controller, SC-01 (Syntech) into the continuous air stream 22 cm from the antenna. Stimulations were made at intervals of 1 min beginning with the lowest dose of the compounds and ending with the highest dose. Presentation order of the test compounds was randomized within doses. A blank stimulus (solvent on filter paper) was presented before and after each dose

level of the test compounds. To compensate for variation in response amplitude between antennae and for antennal fatigue, a reference stimulus (100 ng methyl salicylate) was presented to the antenna after every second test stimulus. Each test response was then divided by the average response to the two adjacent reference stimuli.

3.2.2. Estimation of evaporation rate of volatile compounds under laboratory conditions

Rubber and cotton dispensers (n=4) were loaded with 100 mg of neat compounds that were tested in the field. The dispensers were kept in a fume hood at 22 ± 2 °C for 11 days. The dispensers were weighed daily using a scale (LA-114 electronic balance, UK) and the daily weight loss was determined with a resolution of ± 0.1 mg. The weight loss was attributed to the emission of compound from the dispensers.

3.2.3. Field experiments

Field experiments were carried out at Rasa (09^{o} 54' N, 40^{o} 03' E) located 255 km north east of Addis Ababa, Ethiopia, at 1441 masl. Experiments were conducted during two periods: July 11 - 16 and 29 September to 7 October 2004. The September to October period is the cropping season when sorghum is in the milky/soft dough stage.

3.2.4. Evaluation of traps and dispensers

The trapping efficiency of a commercially available Japanese beetle trap (Trécé, Palo Alto, California, USA) was compared to that of a similar, locally produced trap (Fig. 3.1). The Japanese beetle trap collection canister (height: 20 cm, diameter: 16.5 cm) was dark green and the funnel (height above canister: 12 cm, top diameter: 18 cm) and the vanes (height: 20 cm) were chrome yellow. The locally produced trap had a light green collection canister (height: 30 cm, diameter: 21 cm), an orange funnel (height above canister: 9 cm, top diameter: 12 cm) and yellow vanes (height: 9 cm). The Japanese beetle trap canister was more, as well as more evenly, perforated than the local trap.

Fig. 3.1. The traps tested in the field experiments a) Japanese beetle trap and b) locally made trap.

Approximately 30 g of mashed banana placed inside the canister was used as lure. Empty traps were used as control. All traps were suspended on wooden poles

approximately 2.5 m above the ground. For the other experiments (treatments described below), only Japanese beetle traps were used. Cotton dental rolls (IVF, Shaffhauser No.3, Switzerland) cut in thirds and red rubber septa (VWR International, Sweden) were used as dispensers for the synthetic compounds. Dispensers were placed in a cardboard holder (8 x 3.4 cm) that was placed in a slot on the vane. In July only cotton dispensers were used, but in September both cotton and rubber septa were tested.

3.2.5. Treatments and experimental design

Five synthetic compounds - methyl salicylate, methyl anthranilate, butyl butyrate, isoamyl acetate and eugenol - of known purity (Table 3.1) were tested. Approximately 30 g mashed banana was used as positive control in all experiments and as a lure for comparison of trap design (see above). Empty, unbaited traps were used as negative control.

A dose of 100 µl (approximately 100 mg) pure compound was applied to the dispenser. Applications were renewed daily throughout the experimental period. All treatments were replicated ten times. Replicates were separated by at least 50 m, depending on the topography. Traps within each replicate were placed 10 m apart. The position of the treatments was randomized within replicates and kept unchanged during the experiment. In July, all ten replicates were placed in open grassland with only scattered acacia trees as food sources. In September, when the sorghum crop is in the field, five replicates were placed in sorghum fields and five replicates were placed 300 meters outside the fields in sites comparable to the July trapping sites.

Traps were emptied daily and the beetles were counted and sorted by sex. The average daily temperature during the experimental period was 30 °C in July and 24 °C in September and the relative humidity 39 and 44 %, respectively.

Table 3.1. Source and purity of synthetic compounds tested and their documented behavioral activity in other insects.

Compound	Source/Purity %	Species attracted	References
Methyl salicylate	Aldrich, 98%	*P. marginata*	Larsson *et al*., 2003
		Argyresthia conjugella	Bengtsson *et al*. 2006
Isoamyl acetate	Aldrich, 95%	*P. marginata*	Larsson *et al*., 2003
		Rhynchophorus palmarum	Jaffé *et al*., 2000
Butyl butyrate	Aldrich, 98%	*P. marginata*	Larsson *et al*., 2003
Methyl anthranilate	Aldrich, 99%	*P. marginata*	Larsson *et al*., 2003
		Anomala rufocuprea	Imai *et al*., 1997
		Thrips species	Murai *et al*., 2000
Eugenol	Aldrich, 98%	*P. marginata*	Larsson *et al*., 2003
		Maladera matirida	Ben-Yakir *et al*., 1995
		Anomala transvaalesis	Donaldson *et al*., 1986

3.2.6. Data analysis

The EAG data were subjected to analysis of variance (ANOVA) and differences of means were separated using Tukey's studentized range test, at α = 0.05. T-test was used to compare EAG responses between male and female. Before analysis, field trapping data were transformed using square-root transformation (Zar, 1984). Analysis of variance (ANOVA) was performed and Tukey's studentized range test was used for mean comparison. Season and location effects were compared using Chi square test. Untransformed means and standard errors (SE) are presented in the graphs. All data were analyzed using SPSS version 13.0 for Windows (SPSS, 2001).

3.3. Results

3.3.1. Electrophysiological responses

All tested volatile compounds elicited dose-dependent responses in the antennae of both sexes, except isoamyl acetate. Methyl salicylate elicited stronger responses in male than in female antennae (Fig 3.2).

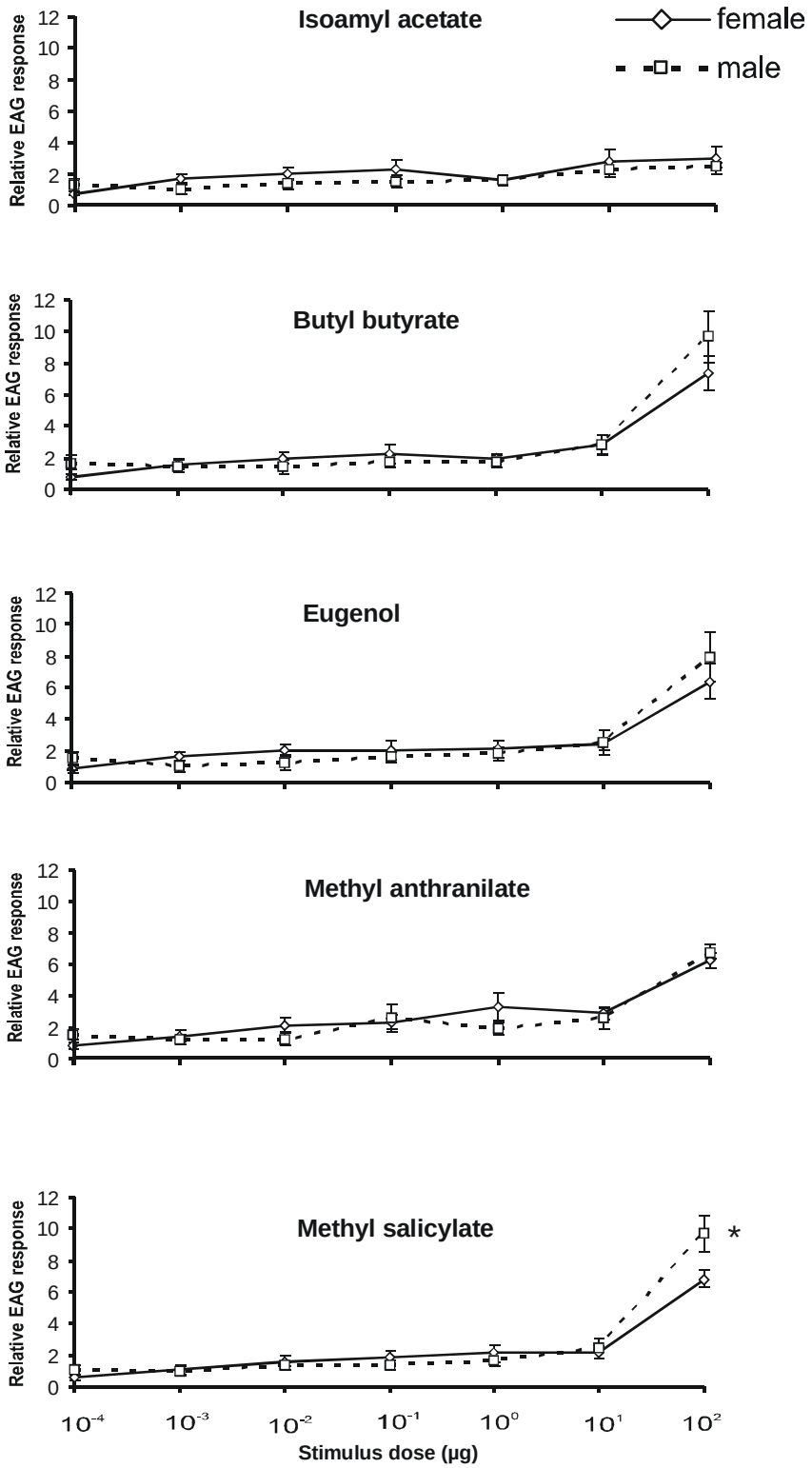


Fig. 3.2. Electroantennogram (EAG) responses from male and female *P. interrupta* antennae to different doses of methyl salicylate, methyl anthranilate, butyl butyrate,isoamyl acetate and eugenol.

Responses were standardized according to the antennal responses to methyl salicylate (100 ng). An asterisk indicates significant difference in response between male and female antennae (N=6, ANOVA followed by Tukey test $P < 0.05$). $F = 5.72$, $df = 4,25$, $P< 0.005$ (male) and $F = 6.00$, $df= 4,25$, $P< 0.005$ (female); t-test = 2.28, N= 6, $P< 0.005$).

3.3.2. Evaporation rate of tested compounds

Overall, compounds were released at a slower rate from rubber than from cotton dispensers (Fig. 3.3). The difference in release rate was most pronounced for eugenol and methyl anthranilate. Eleven days after the initial application of 100 mg, 60 mg of eugenol had been released from the cotton dispensers but only 3 mg from rubber. Similar values were obtained for methyl anthranilate. When applied on cotton, the more volatile compounds isoamyl acetate and butyl butyrate, had evaporated completely after 11 days, whereas on rubber, 85 and 65 mg of isoamyl acetate and butyl butyrate, respectively, had been released. Methyl salicylate showed an intermediate release pattern on both dispenser types.

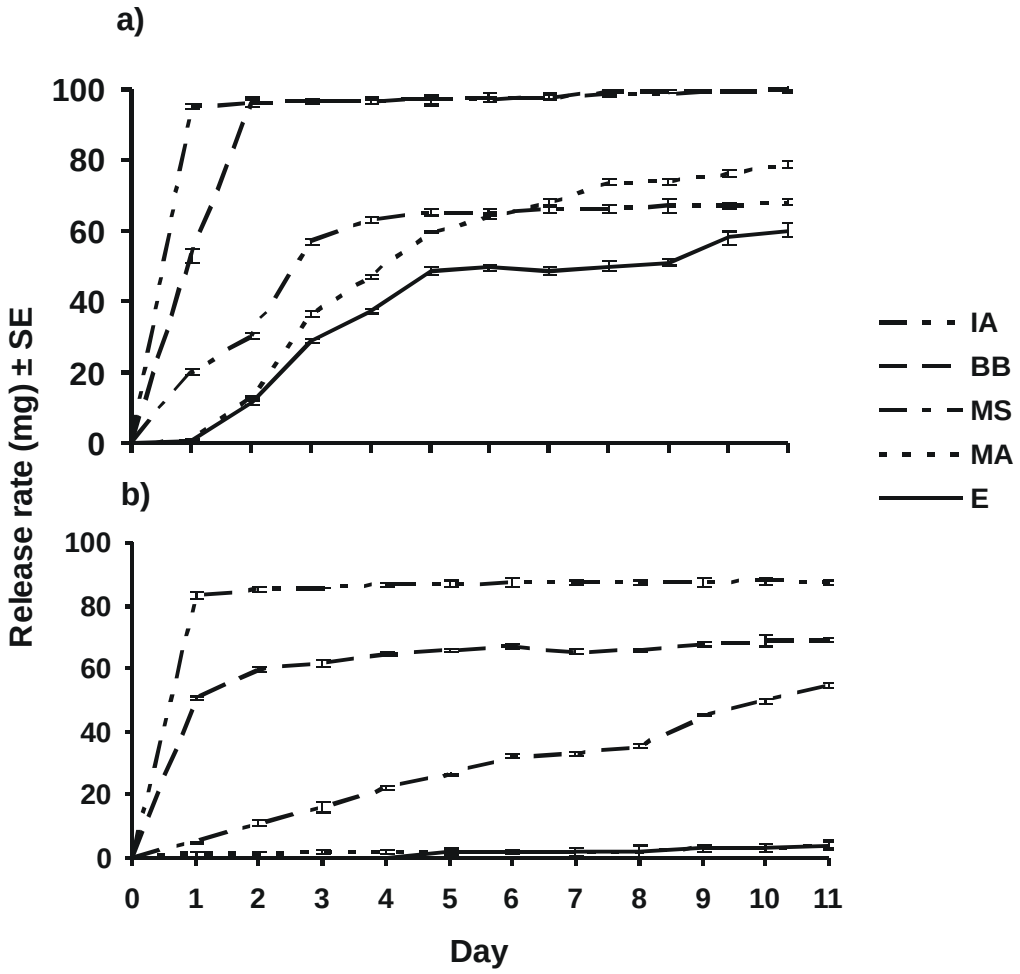


Fig. 3.3. Emission of eugenol (E), methyl salicylate (MS), methyl anthranilate (MA), isoamyl acetate (IA) and butyl butyrate (BB) from a) cotton dispensers and b) rubber septa under laboratory conditions.

3.3.3. Trap comparison

The Japanese beetle traps caught significantly more *P. interrupta* than the locally made traps (ANOVA, F= 214.79, df = 3, 36, P< 0.001). The local banana-baited trap, however, caught significantly more beetles than the unbaited traps (Fig. 3.4).

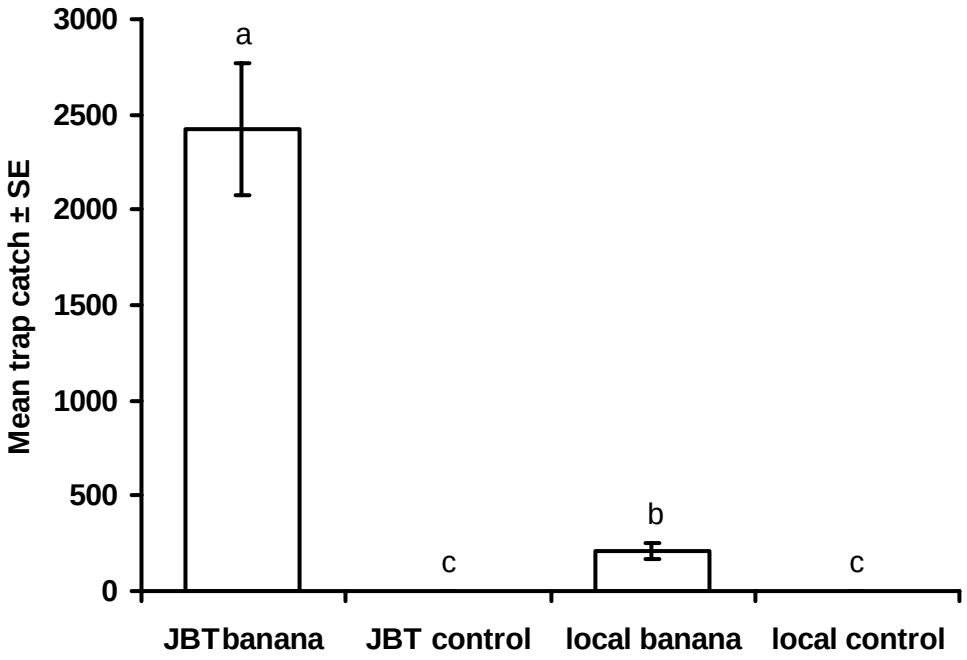


Fig. 3.4. Captures of *P. interrupta* in Japanese beetle traps (JBT) and local traps (Local) baited with banana. Empty traps were used as control. Values followed by different letters are significantly different (N=10, ANOVA followed by Tukey test, $P < 0.05$).

3.3.4. Effects of season and location

Significantly more beetles were caught in July than in September in traps placed outside the crop field, a comparable site ($\chi^2 = 10151.1$, df = 1, $P < 0.001$) (Fig. 3.5). The traps placed outside the crop caught more beetles than traps placed inside the crop ($\chi^2 = 3960.7$, df = 1, $P < 0.001$).

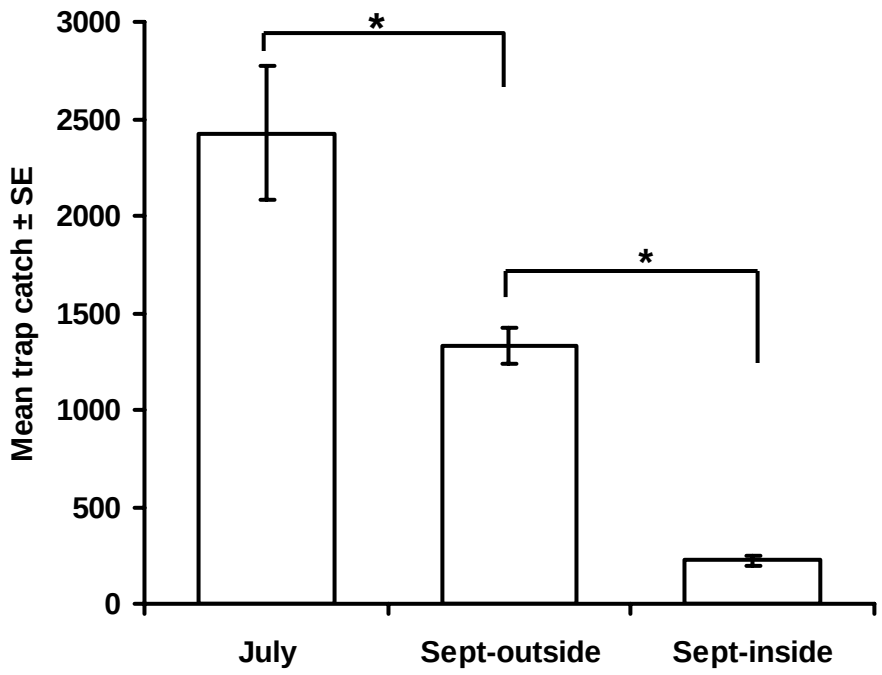


Fig. 3.5. Captures of *P. interrupta* in banana baited traps in July (N=10) and in September, inside (N=5) and outside (N=5) the sorghum field (χ^2 = 3960.7, *P* < 0.001, df = 1).

3.3.5. Effect of dispenser types on trapping efficiency of tested compounds

In July, only cotton dispensers were used and all traps baited with synthetic compounds, except the traps baited with isoamyl acetate, attracted significantly more beetles than the control (ANOVA, *F* = 34.26, df = 5, 54, *P* < 0.001) (Fig. 3.6). Methyl salicylate attracted the highest number of beetles, followed by eugenol. Methyl-anthranilate and butyl butyrate baited traps caught significantly fewer beetles, but more than the unbaited traps. The average female: male sex ratio was 1: 1.4 for all treatments.

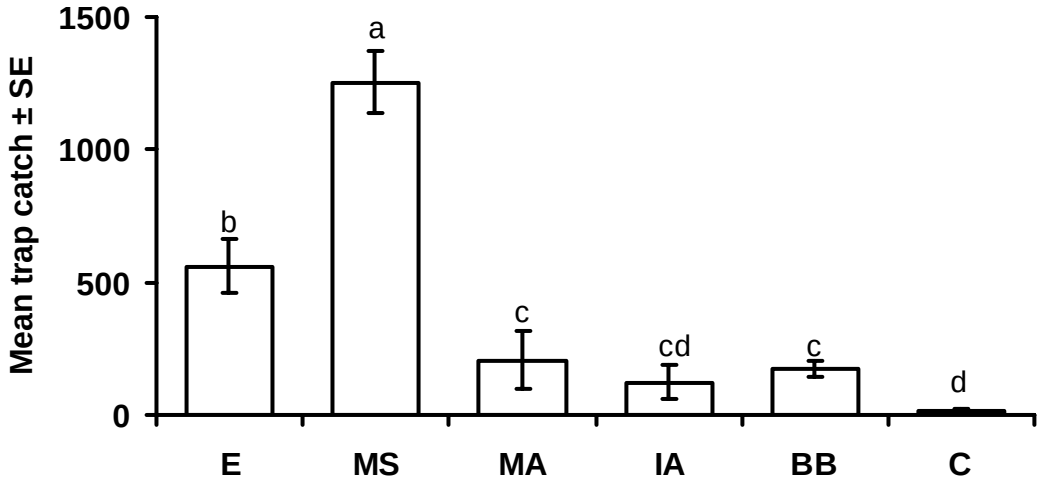


Fig. 3.6. Captures of *P. interrupta* in traps baited with eugenol (E), methyl salicylate (MS), methyl anthranilate (MA), isoamyl acetate (IA), butyl butyrate (BB) and in unbaited control traps (C).

All compounds were applied on cotton dispensers. Values followed by different letters are significantly different (N=10, ANOVA followed by Tukey test, $P < 0.05$). The experiment was performed from 11 to 16 July 2004.

In September, all compounds were tested on both cotton dispensers and rubber septa (Fig. 3.7). Eugenol and isoamyl acetate caught significantly more beetles when applied on rubber ($\chi^2 = 42.12$, df = 1, $P < 0.001$ and $\chi^2 = 116$, df = 1, $P < 0.001$, respectively) and were the two treatments that attracted the highest number of beetles (Fig. 3.7). Methyl anthranilate, however, caught more beetles when applied on cotton ($\chi^2 = 79.39$, df = 1, $P < 0.001$). For the other compounds, dispenser type had no significant effect on trap catches ($P > 0.05$). The sex ratio was the same for all treatments and comparable to the one in July.

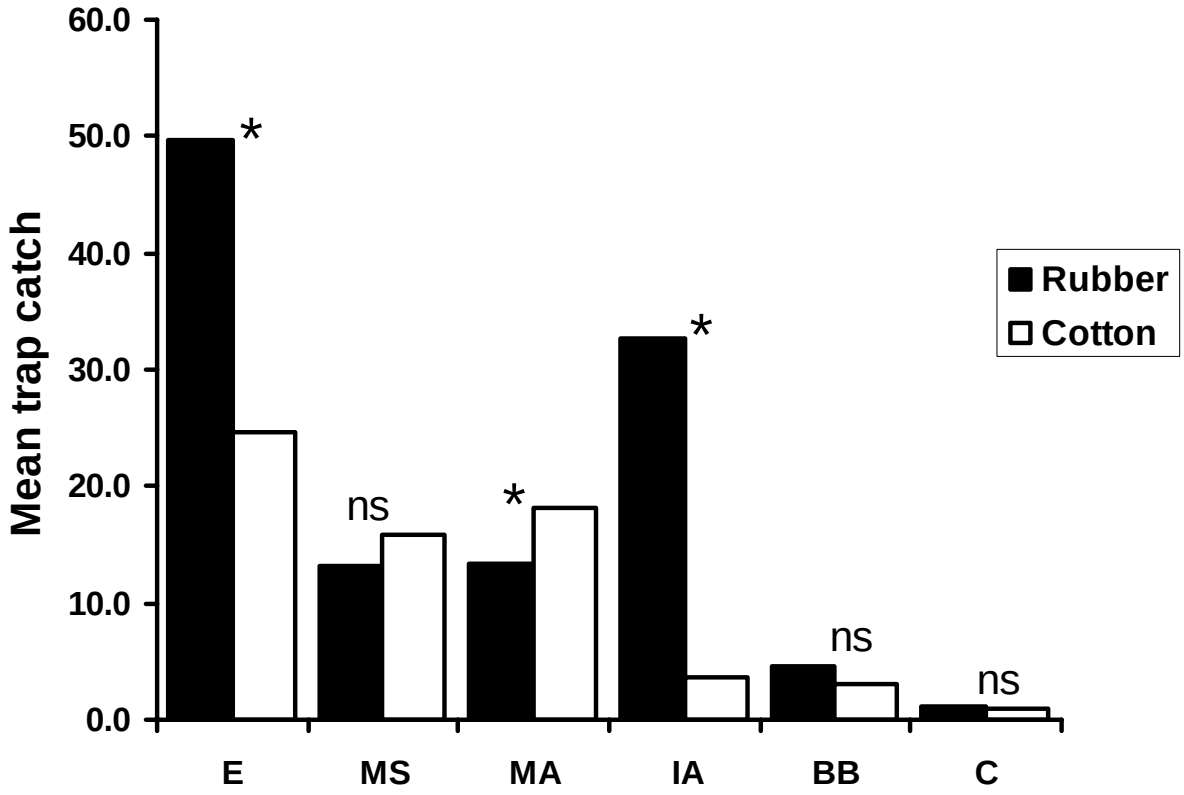


Fig. 3.7. Comparison of captures of *P. interrupta* in traps baited with compounds applied on cotton and rubber dispensers.

The compounds tested were eugenol (E), χ^2 = 42.12, df = 1, P < 0.001; methyl salicylate (MS), χ^2 = 1.17, df = 1, P > 0.05; methyl anthranilate (MA), χ^2 = 79.39, df = 1, P < 0.001; isoamyl acetate (IA), χ^2 = 116, df = 1, P < 0.001; butyl butyrate (BB), χ^2 = 1.68, df = 1, P > 0.05; unbaited traps (C) χ^2 = 0.091, df = 1, P > 0.05, served as control (Chi square test, N=5). The experiment was performed from 29 September to 7 October 2004.

3.4. Discussion

Our study demonstrates that *P. interrupta* is attracted to traps baited with individual synthetic fruit and floral compounds in the field and that the attraction varies with season, trap location, trap design, compound and dispenser type, i.e release rate. All compounds tested were perceived at the antennal level, i.e. electrophysiologically active, and were also attractive to the beetles in the field. The sex ratio of the trapped beetles was similar for all treatments and the significantly higher sensitivity of the male antenna to methyl salicylate (Fig. 3.2) was thus not reflected in the field trap catches.

Depending on season and dispenser type, methyl salicylate, eugenol and isoamyl acetate attracted more *P. interrupta* than the other compounds tested (butyl butyrate and methyl anthranilate), indicating that they may act as host compounds for the beetles. Methyl salicylate and eugenol have been identified as flower volatiles in different *Acacia* species (Lamarque *et al.,* 1998) – one of the main food sources for the beetles – and isoamyl acetate is a dominant compound in ripe banana (Macku and Jennings, 1987). Attraction to individual floral and fruit compounds has been demonstrated in other scarab beetles both in the field (Donaldson *et al.,* 1986; Klein and Edwards, 1989; Reed *et al.,* 1991; Crocker *et al.,* 1999; Ruther and Tolasch, 2004) and in the laboratory (Larsson *et al*., 2003) and eugenol, specifically, has been shown to be at least as attractive alone as in mixtures to several other Cetoniinae species (Donaldson *et al*., 1986), and to *Maladera matrida* (Argaman) (Coleoptera: Scarabaeidae) (Ben-Yakir *et al*., 1995). However, trapping of *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) was more effective with the mixture of

chemicals (McGovern and Ladd, 1984) and the effect of blends on *P. interrupta* attraction will be tested.

The seasonal differences in catches in the banana-baited traps could be attributed to differences in population density and to competition from natural food sources. In July, when most beetles were caught, food sources consisting of scattered acacias were scarce compared to September, when sorghum fields in the susceptible stage were abundant in the area. The competition with natural hosts is also illustrated by the significantly lower trap catches inside compared to outside the sorghum fields in September. Inside the field, traps are likely to be subjected to both visual (Held and Potter, 2004) and odor competition from the sorghum crop (Domek and Johnson, 1988; Harari *et al.*, 1994; Loughrin *et al*., 1995).

In addition to the seasonal variation in catches in the banana-baited traps, there is also a variation in the response to the different synthetic compounds as is shown by the catches with cotton dispensers in July and in September. Methyl salicylate was significantly more attractive than all other compounds in July, but in September eugenol and methyl anthranilate were at least as attractive. This shift may be due to changes in the odor background against which the lures compete, but it is also conceivable that experience or physiological change, induced e.g. by aestivation, may alter the beetle's preferences. In addition, the average temperature was 6 °C higher in July than in September and these seasonal differences in temperature may affect the release rates of the compounds and thus the performance of the lures.

In addition to season and trap location, trap design had a large impact on trap catches, with Japanese beetle traps being far more effective than the locally made trap. One reason for the poor performance of the local trap might be the

comparatively low number of vents in the collection containers. Low ventilation could affect catches negatively both by reduced dispersal of banana odour and – due to limited ventilation of the container – by an accumulation of odors from dying beetles that could repel approaching beetles. Odor from dying beetles in traps has been reported to be repellent to *P. japonica* (Klein *et al.*, 1973b; Alm *et al.*, 1996), but in our study, few dying beetles were observed in the traps and it seems more likely that reduced release of attractive compounds rather than accumulation of repelling odor is a key factor. Differences in catches between the two trap types should then be less pronounced when synthetic lures are used, since they are placed on the vanes on top of the traps. However, in addition to more vents, the Japanese beetle trap has larger vanes and different coloring than the local trap and these factors may also affect trap performance (Blight and Smart, 1999; Alm and Dawson, 2003).

In combination with the laboratory data, the field trapping results also demonstrate the impact of dispenser material on the release rate of different compounds and, consequently, trapping efficiency. Isoamyl acetate being the most prominent example, not catching more than the blank traps when applied on cotton but being among the most attractive on rubber (Fig. 3.7). Given the high release rate of isoamyl acetate from cotton in the laboratory (Fig. 3.3), it is likely that under field conditions, all the isoamyl acetate applied to cotton dispensers is released shortly after application and that for the rest of trapping period, these traps are perceived as unbaited traps by the beetles. The comparatively lower release rate from rubber (Fig. 3.3) thus seems to favour isoamyl acetate in that it is released for a longer time during the trapping period. Eugenol, on the other hand, had an extremely low release rate from rubber – 3 mg over 11 days – but it was still the most attractive treatment in the

field, indicating that high concentrations of eugenol might be deterring. The effect of dispenser material and dispenser type, i.e. release rate, on the performance of different lures has been shown for several other species (e.g Anderbrant *et al*., 1992; Hillbur *et al*., 2000).

To conclude, this study shows that synthetic plant compounds may be a basis for the development of mass trapping as a method for control of *P. interrupta*. The results also indicate that trapping would be most efficient in July, during the mating season, and if it is the cropping season traps should be placed outside the crop. However, in order to provide the farmers with an efficient, reliable and economical system, the design of the local trap must be improved and a high-longevity dispenser needs to be developed. More compounds, especially, blends of compounds should be tested as lures. Furthermore, it has been demonstrated for several species that a comparatively low attraction to floral compounds is highly synergized by pheromones (Ladd *et al*., 1981; Ladd, 1986; Jaffè *et al.,* 2000) and future projects should focus on deciphering the pheromone signal of *P. interrupta*.

CHAPTER IV

IDENTIFICATION AND FIELD EVALUATION OF HOST PLANT VOLATILES ATTRACTIVE TO THE SORGHUM CHAFER, *PACHNODA INTERRUPTA*

4.1. Introduction

The sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae: Cetoniinae), is a polyphagous insect known to feed on fruits, flowers, and seeds from several plant species, some of which are important crops (Hiwot Lemma, 2000). Sorghum chafers are particularly attracted to and form large aggregations on sorghum, *Sorghum bicolor* (L.) Moench – the most important staple cereal in semi-arid areas of eastern Africa, especially in Ethiopia (Brihane Gebrekidan, 1986). The feeding of the beetles on the panicles causes sterilization of florets and kernel damage (Clark and Crowe, 1978; Grunshaw, 1992; Jago, 1995). Mean yield loss can be as high as 70% (Yitbarek Wolde-Hawariat and Hiwot Lemma, 2000) and an environmentally sustainable management method is much needed (MOA, 2003).

It is well documented that insect attraction to host or food plants is influenced by chemical cues (Kevan and Baker, 1983; Stensmyr *et al.,* 2001; Tasin *et al.,* 2005) and several studies have demonstrated the potential of synthetic host plant compounds for management of scarab pests (Donaldson *et al.,* 1990; Imai *et al.,* 1997; Ruther and Mayer, 2005). Individual synthetic plant compounds used as lures in traps have been shown to be attractive to *P. interrupta* in the field (Yitbarek Wolde-Hawariat *et al.,* 2007). However, the compounds tested were compounds that have

been identified as attractants for other scarabs, e.g. a closely related species, *P. marginata* (Larsson *et al*., 2003), and it is possible that analyses of stimuli of ecological relevance to *P. interrupta* may reveal more powerful attractants.

The objective of this study was to identify volatile compounds in sorghum responsible for the attraction of *P. interrupta*. In addition to sorghum, the beetle also aggregate and feed in large numbers on *Abutilon figarianum* Webb – a shrub that grows naturally in dry low-land areas in the tropics (Edwards *et al.,* 1995). To this effect abutilon volatiles were also tested for activity. as well as eugenol and methylsalicylate, found to be highly attractive to *P. interrupta* in a previous study (Yitbarek Wolde-Hawariat *et al.,* 2007).

4.2. Materials and Methods

4.2.1. Insects

Adult insects were collected from the field in Ethiopia (see study site below) and transported to Alnarp, Sweden, where they were kept in a climate chamber at 26 °C, RH 70%, and 16/8 L/D photoperiod until used for electrophysiological recordings. Insects were taken out of the climate chamber and kept in a plastic jar 10 to 30 minutes before they were tested.

4.2.2. Headspace plant volatile collection

Flowers and leaves of Abutilon and sorghum panicles at the soft dough/milky stage – the stage most attractive to the beetles – were enclosed in polyethylene bags (Toppits Scandinavia AB, 35 x 43 cm) that were sealed with steel wire around the stem of the plant. Next to the stem an activated charcoal filter was placed in order to

filter the incoming air. Volatiles were collected using a glass tube (3.5 mm i.d x 50 mm) containing an adsorbant 25 mg SuperQ®, mesh 80/100 (Alltech, Deerfield, IL, USA) with glass wool and Teflon stoppers at both ends (Birgersson and Bergström, 1989). The filter was placed in the polyethylene bag and connected by PVC tubing to a small battery operated pump (PAS-500 Personal Air Sampler, Supelco, Bellefonte, Pennsylvania, USA). Collections were made in the field for two hours. Immediately after collection, the filters were rinsed with 200 µl of redistilled hexane into 1.5 ml glass vials (Scandinaviska GenTec AB, Sweden). Vials with extracts were kept in an icebox for transportation to the laboratory and thereafter kept at–18 °C until analysis.

4.2.3. Coupled Gas Chromatographic–Electroantennographic Detection (GC-EAD)

The response of *P.interrupta* antennae to volatiles was studied by GC-EAD (Arn *et al.,* 1975) using a Hewlett-Packard HP-6890 gas chromatograph (GC) equipped with a HP-INNOWAX (30 m x 0.2 mm x 0.25 µm) capillary column (J&W Scientific, Agilent Technologies, USA). Nitrogen was used as carrier gas. The column temperature was programmed from 40 °C (5 min hold) to 230 °C at 5 °C /min. Compounds eluting from the column were delivered to the antenna through a glass tube (12 cm x 8 mm inner diameter) by a charcoal-filtered and humidified air stream at 1.5 l/min. The antennae were mounted according to Leal *et al.* (1992b) and Yitbarek Wolde-Hawariat *et al*. (2007). The antenna was excised with a fine forceps and placed in an antennal holder (Hillbur, 2001; JoAC, Lund, Sweden) and the signal was amplified (JoAC) and analyzed with EAD software (Syntech, Hilversum, The Netherlands).

4.2.4. Chemical identification

Samples of plant volatile collections were analyzed with coupled gas chromatography-mass spectrometry (GC-MS) Hewlett Packard 6890 and 5973-MS. Extracts were injected with a HP 7683 auto injector in splitless mode 30 seconds. The GC was fitted with a fused silica capillary column (30 m x 0.25 mm x 0.25 µm, Hewlett-Packard, Palo Alto, CA, USA) coated with INNOWAX. Helium was used as a carrier gas at a linear velocity of 30 cm/s. The temperature program was the same as that described for the GC-EAD analyses. Identifications of compounds were confirmed by comparison of mass spectra with those of authentic standards and with those found in a NIST mass-spectral database (1998).

4.2.5. Synthetic compounds

The synthetic standards were purchased from Sigma/Aldrich, purity and CAS number indicated: (*Z*)-3-hexen-1-ol (>98% purity, CAS 928-96-1), tridecane (> 99%, CAS 692-50-5); 1-octen-3-ol (>98%, CAS 3391-86-4); 1-octan-1-ol (>99%, CAS 111-87-5); tetradecane (>99%, CAS 629-59-4); methyl anthranilate (>99%, CAS 134-20-3); methyl salicylate (>98, CAS 119-36-8) and eugenol (98%, CAS 97-53-0).

4.2.6. Field experiment

Field experiments were carried out at Rasa (09° 55' N, 40° 05' E) located 255 km north east of Addis Ababa, Ethiopia. The altitude in the area ranges between 1359 and 1458 masl. Experiments were done during two periods: July 11-16 and October 7-13, 2006. The latter tests were done during the cropping season, when the

sorghum had seeds in the milky stage. The average daily temperature during the experimental period was 34 °C in July and 27 °C in September and the relative humidity 32 and 42%, respectively.

In July, trapping experiments were carried out in a non-cropping area characterized by scattered *Acacia* trees. In October, however, traps were placed along the borders of five sorghum fields located approximately 500 m from the July test sites. In all experiments, traps baited with mashed banana were used as a positive control to monitor the population density of beetles in the area. Unbaited traps were used as a negative control. The experimental design was a randomized complete block design with 10 replicates. The position of the treatments within replicates was kept unchanged during the experiment. The distance between traps was l0 m and the distance between blocks was at least 50 m. Commercially available Japanese beetle traps (Trécé, Alto, CA, USA) were used for trapping experiments. Traps were suspended 2.5 m above ground from wooden poles. Traps were emptied daily, lures were reloaded and trapped beetles were sorted by sex.

All individual sorghum compounds were applied at a dose of 100 mg as follows: 100 mg (*Z*)-3-hexen-1-ol (blend code 1), 100 mg tridecane (blend code 2), 100 mg 1-octen-3-ol (blend code 3) and 100 mg 1-octanol (blend code 4) (Fig. 4.2a and b). In addition to the sorghum compounds, 100 mg eugenol (blend code 5) and 100 mg methyl salicylate (blend code 6) were also tested. Two of the sorghum related blends were tested both in July and in October: a blend of the four sorghum compounds with the same total dose as for the individual compounds and in a ratio mimicking what was found in the sorghum headspace, i.e. 10 mg (*Z*)-3-hexen-1-ol + 30 mg tridecane + 30 mg 1-octen-3-ol + 30 mg 1-octanol (blend code 8), and the

same blend – the sorghum blend – with the addition of 30 mg methylsalicylate (blend code 9) (Fig. 4.2a and b). In addition to these, three more blends were tested in October: the sorghum blend with the addition of 30 mg eugenol (blend code 10), the sorghum blend with the addition of 30 mg eugenol and 30 mg methylsalicylate (blend code 11), and a blend of 100 mg eugenol and 100 mg methyl salicylate (code 7) (Fig. 4.2b).

The individual abutilon compounds were also tested at a dose of 100 mg: 100 mg (*Z*)-3-hexen-1-ol (blend code 1 – the same as in the sorghum experiment), 100 mg tetradecane (blend code 2), 100 mg methyl anthranilate (blend code 3), and 100 mg methyl salicylate (blend code 4) (Fig. 4.3a and b). Methyl anthranilate was only tested individually in October. An abutilon blend consisting of 20 mg (*Z*)-3-hexen-1-ol, 20 mg tetradecane, 5 mg methyl anthranilate and 55 mg methyl salicylate (blend code 6), i.e. a total dose of 100 mg and ratios mimicking the headspace collections, was also tested. In addition, a blend without methyl salicylate was tested (blend code 7) (Fig. 4.3a and b). Furthermore, in October a third blend consisting of the abutilon blend with the addition of eugenol (blend code 8) was added to the experiment (Fig. 4.3b). Compounds were applied to dental cotton rolls (No. 3, Q-dent®, Germany).

4.2.7. Data analysis

Data for total catch per trap of *P. interrupta* was transformed with a (x+1) ^0.14 power transformation to homogenize the variance. It was thereafter subjected to an analysis of variance (ANOVA), and when there was a significant difference, means were separated using a Tukey multiple comparisons test ($P<0.05$). Data are

presented in the graph as untransformed means ± SE. Data analysis was carried out using SPSS version 13.0 for Windows (SPSS, 2001).

4.3. Results

4.3.1. GC-EAD analyses and identification of host plant volatiles

Several compounds in both the abutilon (Fig. 1, upper trace) and the sorghum (Fig. 4.1, lower trace) headspace collections elicited consistent responses in *P.interrupta* antennae. The compounds collected from abutilon headspace extracts were identified as (*Z*)-3-hexen-1-ol (1), tetradecane (2), methyl salicylate (3), and methyl anthranilate (4) (Fig. 4.1, upper trace), and in sorghum tridecane (5), (*Z*)-3-hexen-1-ol (6), 1-octen-3-ol (7), and 1-octanol (8) (Fig. 4.1, lower trace). (*Z*)-3-hexen-1-ol was present in both sorghum and abutilon extracts. Representative mass spectra of (*Z*)-3-hexen-1-ol is presented in Appendix 1. In abutilon headspace extracts, methyl salicylate was found to be a major constituent, making up approximately 14.4 % of the extract (Fig. 4.1, upper trace). In sorghum extract, however, it was only present at low concentrations. Despite a low concentration in abutilon extract, methyl anthranilate elicited a strong antennal response.

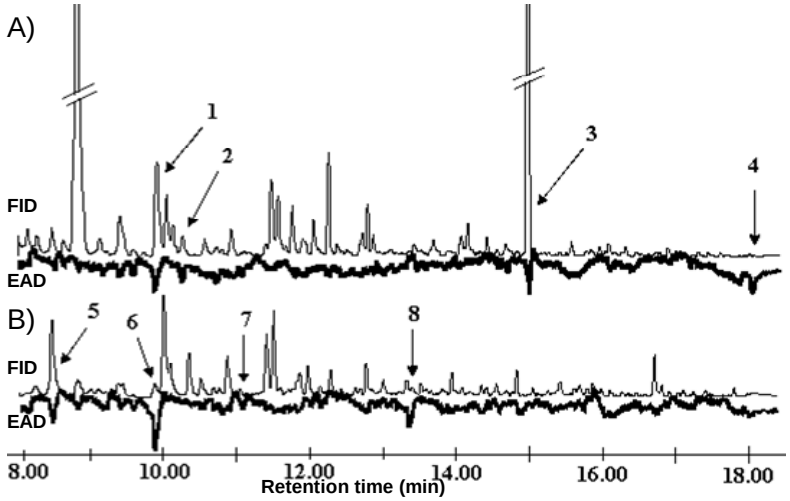


Fig. 4.1. Simultaneous responses of FID and EAD using antenna of *P. interrupta* adult to headspace volatile compounds collected from A) *Abutillon* (male antennae) and B) sorghum (male antennae).
The upper traces in each figure represent the signal from the flame ionization detector (FID) and the lower traces represent the signal from electroantennographic detector (EAD).

4.3.2. Field experiments

In July, there were no significant differences in trap catches between the sorghum blend and the individual sorghum compounds, except for tridecane that caught significantly fewer beetles (Fig. 4.2a). Eugenol baited traps, however, caught significantly more beetles, as did the sorghum blend with the addition of methyl salicylate. In October, the three multi-component blends – the sorghum blend with eugenol and/or methyl salicylate – attracted by far the highest numbers of beetles, followed by the two-component eugenol-methylsalicylate blend and methylsalicylate alone (Fig. 4.2b). In this season, the four-component sorghum blend caught significantly more beetles than two of the individual compounds, (*Z*)-3-hexen-1-ol and 1-octen-3-ol (Fig. 4.2b).

In both July and October, the four-component abutilon blend caught significantly more beetles than the single compounds (*Z*)-3-hexen-1-ol and tetradecane (Fig. 4.3a). However, there were no significant differences in trap catches between traps baited with the abutilon blend, the blend without methyl salicylate, methyl salicylate presented as a single compound or eugenol as a single compound (Fig. 4.3a). When methyl anthranilate was added as a single compound in October, it was as attractive as the previously tested blends and eugenol and methyl salicylate (Fig. 4.3b). The other treatment that was added, the four-component abutilon blend combined with eugenol, caught significantly more beetles than any other treatment (Fig 4.3b). During both seasons, all treatments – sorghum as well as abutilon related – caught significantly more beetles than the unbaited control traps.

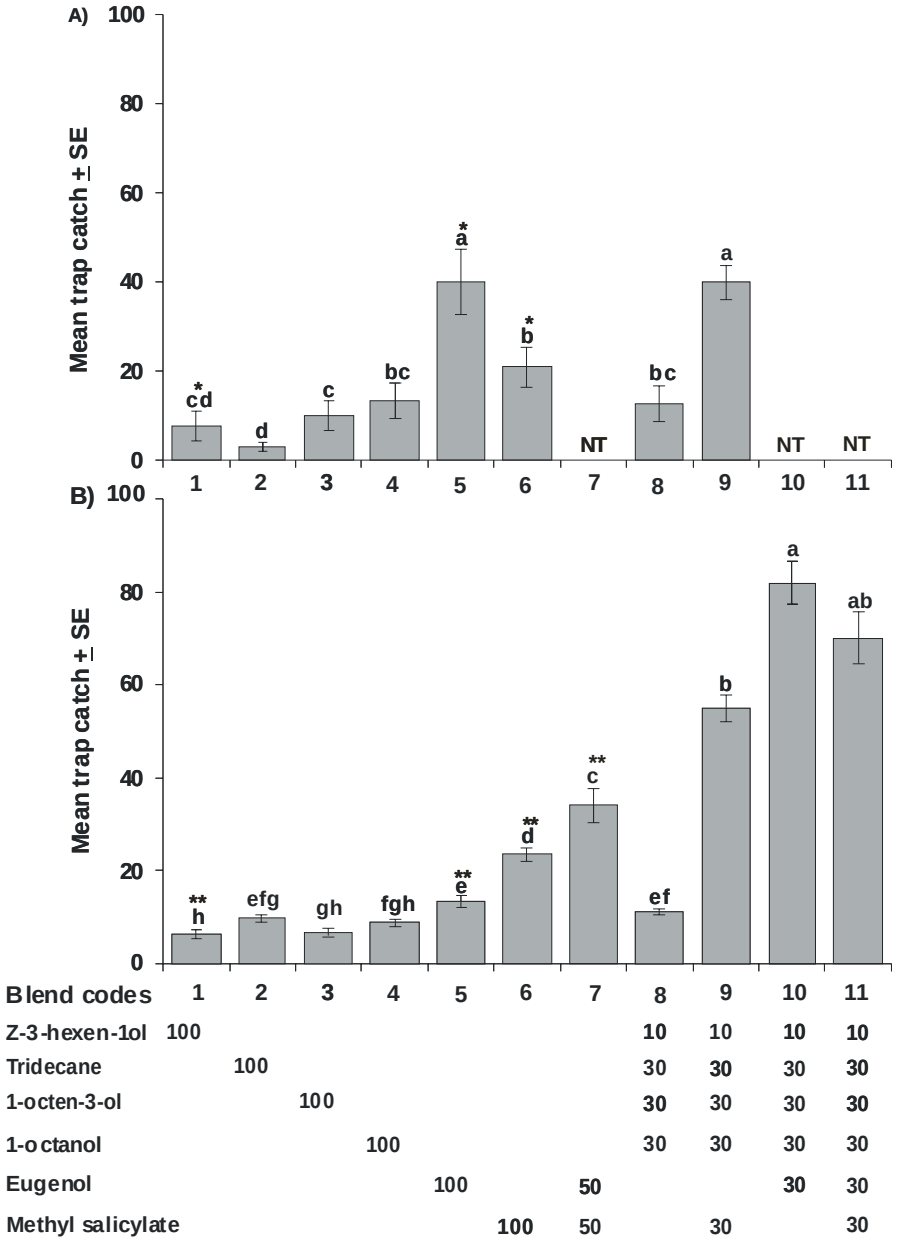


Fig. 4.2. Mean ± SE number of *P. interrupta* captured in the traps baited with synthetic plant odors in A) July, 2006 and B) October, 2006.

The different treatments tested were 1) Z-3-hexen-1-ol, 2) tridecane 3) 1-octen-3-ol 4) 1-octenol 5) eugenol 6) methyl salicylate 7) eugenol + methyl salicylate 8) sorghum blend (four components 1- 4), 9) sorghum blend + methyl salicylate 10) sorghum blend + eugenol and 11) sorghum blend + eugenol + methyl salicylate. Unbaited traps caught no insects and are thus not included in the comparison. ANOVA; July, N=10. ($F= 162.97$, $df= 10, 99$, $p< 0.001$).October, N= 10. ($F= 162.97$, $df= 10, 99$, $p< 0.001$). Bars with the same letters above them are not significantly different at $\alpha = 0.05$. NT- indicates the compound not tested.

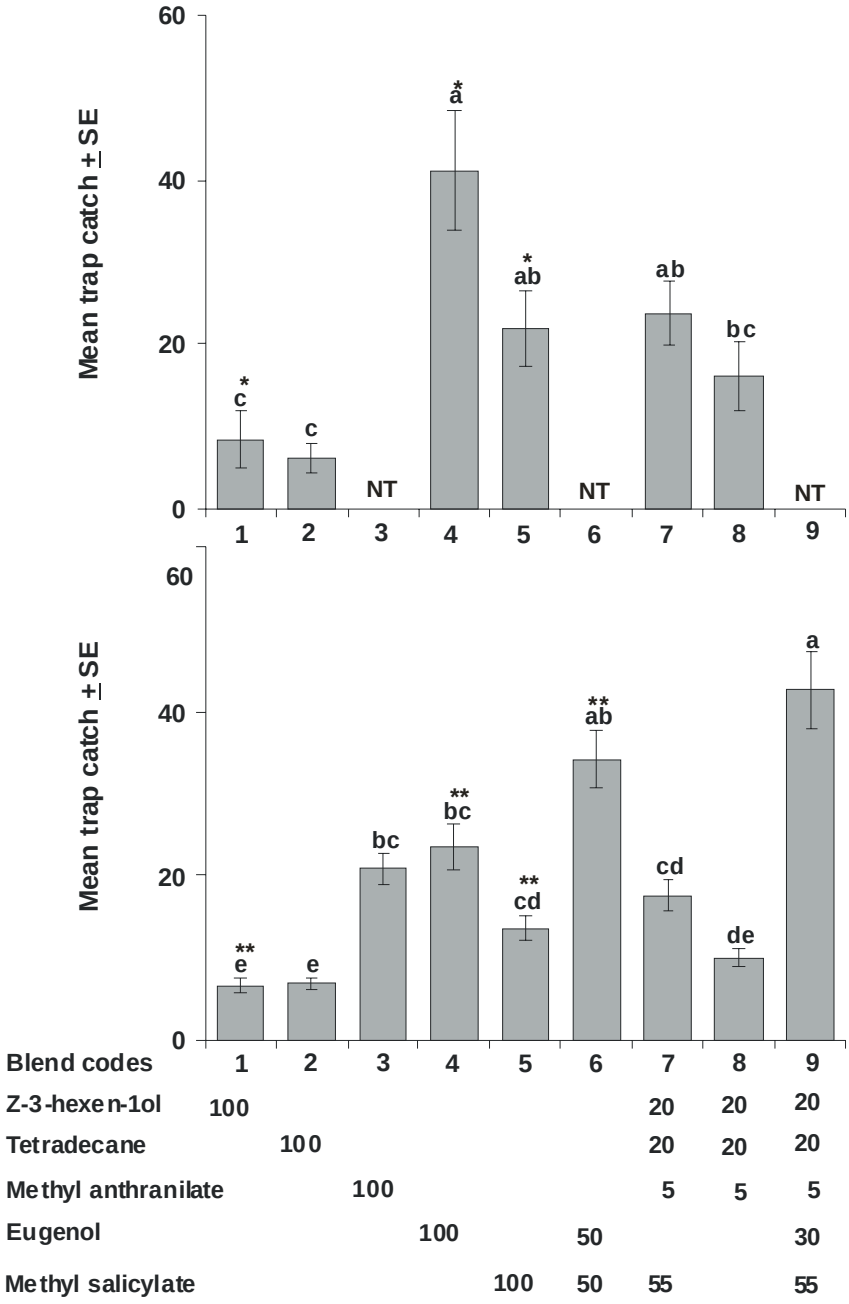


Fig. 4.3. Mean ± SE number of *P. interrupta* captured in the traps baited with synthetic abutilon compounds and blends in: A) July, 2006 and B) October, 2006.
The different treatments were 1) Z-3-hexen-1-ol, 2) tetradecane 3) methyl anthranilate 4) methyl salicylate 5) eugenol 6) *Abutillon* blend (four components 1-4) 7) abutilon blend without methyl saicylate (three components 1-3) 8) abutilon blend + eugenol. Unbaited traps caught no insects and are thus were not included in the comparison. ANOVA; July 2006, N= 10. (F= 10.74, df= 5, 54, p< 0.001). October 2006, N= 10. (F= 24.9, df= 7, 72, p< 0.001). Bars with the same letter above them are not significantly different at α = 0.05. NT- indicates the compound not tested.

4.4. Discussion

This study shows that antennae of *P. interrupta* respond selectively to some of the volatiles emitted by the host plants sorghum and abutilon. Antennal response to key compounds in host plant odor has been demonstrated in several other insects (Cossé and Baker, 1999; Jönsson and Anderson, 1999; Zhang *et al.,* 1999; Huber *et al.,* 2000). The EAD-active compounds from sorghum and abutilon belong to several functional groups – alcohols, esters and alkanes – and are common foliar and floral odors (Gibbs, 1974; Knudsen *et al.,* 1993; Bernays and Chapman, 1994; Schoonhoven *et al.,* 1998).

Furthermore, in the field, trap catch showed synergistic effects for some blends, which has been shown in several studies for other scarab beetles (Ladd and McGovern, 1980; Ladd *et al.,* 1981; Loughrin *et al.,* 1998; Ruther, 2004). One reason for the comparatively low trap catch of the four-component sorghum and abutilon blends could be the effect of varying release rates of the different compounds from the dispensers (Yitbarek Wolde-Hawariat e*t al.,* 2007). Although dispensers were reloaded every 24 hours in the field in this study, the variation in the release rate of the different compounds may affect the emitted ratio of the blends as compared to the applied ratio. In a review, Bruce *et al*. (2005) stated that insects recognize suitable hosts by using key volatiles present in specific ratios. Similarly, host finding in the grapevine moth, *Lobesia botrana*, has been shown to be encoded by a ratio-specific blend of three ubiquitous plant volatiles (Tasin *et al*., 2006). Furthermore, in the Colorado potato beetle, *Leptinotarsa decemlineata*, directed host orientation ceased if host odors were presented in inappropriate ratios (Visser, 1978).

In addition to ratio, the comparatively low trap catches with the natural blend mimics might indicate the absence of essential components in the blend, as indicated by the significant increase in trap catches when methyl salicylate and/or eugenol were added. Both compounds have been identified as flower volatiles in several different acacia species (Lamarque *et al.,* 1998) and, as acacia is another important host plant for the beetles, this may explain their strong behavioral impact. Attraction to flower volatiles, e.g. eugenol, is common in many scarab species (Ladd *et al.,* 1976; Ladd and McGovern, 1980; Donaldson *et al.,* 1986; Williams *et al.*, 1990; Leal *et al.,* 1994; Ben-Yakir *et al.,* 1995; Cherry *et al.,* 1996; Imai *et al.,* 1997; Crocker *et al.,* 1999; Larsson *et al.,* 2003), particularly from the subfamilies Rutelinae and Cetoniinae that visit flowers for both feeding and mating (Ritcher, 1958; Leal, 1998).

Methyl salicylate is commonly emitted by plants but may have different behavioral impact, depending on the context in which it is emitted. It is commonly found in leaf odors (Andersen *et al.,* 1988; Buttery *et al.,* 1982, 1986; Loughrin *et al.,* 1996, 1997a, 1997b; Shulaev *et al.,* 1997), but also in flower volatiles where it is active in pollinator attraction in several plant species (Armbruster *et al.,* 1989; Knudsen and Tollsten, 1993; Knudsen *et al.,* 1993; Pham-Dèlegue *et al.,* 1997, Raguso and Light, 1998).

Furthermore it has been reported as an attractant for the bigeyed bug, *G. pallens*, (James, 2005) and several hoverfly species (James, 2003) as well as western corn rootworm females, *Diabrotica virgifera virgifera* (Hammack 1997). Methyl salicylate is also common as a defensive compound in higher plants in

response to herbivory (Turlings *et al.,* 1998; Kessler and Baldwin, 2001), and has been shown to have a repellent effect on e.g. aphids (Hardie *et al.,* 1994).

Apart from eugenol and methyl salicylate, methyl anthranilate – a minor component of the abutilon headspace collections – was highly attractive when applied singly as trap lure (Fig. 4.3b). Methyl anthranilate is a floral compound (Knudsen *et al.,* 1993) and has also been identified in headspace collections from vegetative maize that has been subject to insect herbivory (Bernasconi *et al*., 1998; Turlings *et al.,* 1998). It is known to be a powerful attractant to insects, notably polyphagous scarab beetles (Imai *et al.,* 1997; Maekawa *et al.,* 1999; Larsson *et al*., 2003).

This study shows that synthetic sorghum or abutilon volatile blends in combination with eugenol and/or methyl salicylate constitute powerful attractants for *P. interrupta*. The blends could be used for monitoring of the pest or in direct control measures, e.g. mass trapping. In addition, the compounds might be used as attractants in programs to infect *P. interrupta* with entomopathogens using modified traps with subsequent auto-dissemination of infected beetles into the population (Klein and Lacey, 1999).

CHAPTER V

IDENTIFICATION OF VOLATILE COMPOUNDS FROM RIPE BANANA AND ATTRACTION OF SORGHUM CHAFER *P. INTERRUPTA* USING Y-TUBE OLFACTOMETER

5.1. Introduction

The sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae: Cetoniinae) is among the major insect pests causing a serious damage on cereals, fruits and forage crops in Ethiopia (Clark and Crow, 1978). The genus *Pachnoda* comprises over 130 species (Grunshaw, 1992), and nine of those have been recorded in Ethiopia (Clark and Crowe, 1978). Farmers have adopted different control strategies such as e.g. smoking to push the beetles out of the field or hand collection. These techniques are labor intensive and time consuming and yet inefficient. The use of insecticides as a control measure for *P. interrupta* has proven inefficient, mainly due to re-infestations of the beetle from the surroundings (MOA and EARO, 1999).

An alternative control method developed by the farmers is the use different fruits, especially banana, to trap adult sorghum chafer in order to reduce population density. The use of different fruit attractants is dependent on the availability of fruits at the time of the outbreak. In some places fruits are scarce and this may affect the control program. Therefore, another tool for management of *P. interrupta* was required and trapping with semiochemicals has been shown to be a potential solution (Yitbarek Wolde Hawariat *et al*., 2007).

Volatiles from banana have been widely studied, especially with respect to banana aroma (Macku and Jennings, 1987; Jordan *et al.,* 2001; Boudhrioua *et al.,*

2003). In the present study, we report on the identification of volatiles that elicit responses from antennae of *P. interrupta* and the attractiveness to the beetles of a blend of these compounds.

5.2. Materials and methods

5.2.1. Insects

Adult insects were collected from the field at Rasa (09^{o} 55' N, 40^{o} 05' E) located 255 km north east of Addis Ababa, Ethiopia. The altitude ranges between 1359 and 1458 m above sea level. Insects were transported to Alnarp, Sweden, and kept in a climatic chamber at a temperature of 26 °C, RH=70%, and 16/8 L/D photoperiod. Insects were taken out of the climate chamber and kept in a plastic jar (30 cm x 12 cm x 22 cm) 10 to 30 minutes before they were tested for GC-EAD analysis. Whereas the behavioral experiments were done with the insects collected from the field (same place as above) and maintained in the laboratory at room temperature in Addis Ababa, Ethiopia.

5.2.2. Headspace volatile collection

Cavendish bananas (Chiquita Panama) were purchased from a local supermarket in Sweden. The bananas used were all yellow color. Mashed banana was enclosed in polyethylene bags (Toppits Scandinavia AB, 35 x 43 cm) and air was pumped into the system through an activated charcoal filter. The filter was placed in the bag and connected by Teflon tubing to a pump (12V, KNF-Neuberger) with an air flow of 1.0 l/min. Volatiles were collected using a glass tube (3.5 mm i.d x 35 mm)

containing SuperQ® absorbent (35 mg, mesh 80/100, Alltech, Deerfield, IL, USA) with glass wool and Teflon stoppers in both ends (Birgersson and Bergström, 1989).

Two odor collections were made from banana: the first one was made from mashed ripe banana for 1 hr. After the first odor collection the mashed banana sample was placed in bag for 36 hrs at room temperature. A second collection was then made from the bag for 1 hr. In both cases, the filters were rinsed with 200 µl of redistilled hexane into 1.5 ml glass vials (Genetec AB, Sweden) immediately after collection. Vials with extracts were kept at -18°C until analysis.

5.2.3. Coupled gas chromatographic electroantennographic detection (GC-EAD).

The response of sorghum chafer antennae to banana volatiles was studied by GC-EAD (Arn *et al.,* 1975). The setup for mounting the antenna for EAG/GC–EAD recordings was the same as reported by Leal *et al.,* (1992b) and Yitbarek Wolde-Hawariat *et al.,* (2007). One antenna was excised with a fine forceps and placed in the holder and the signal was amplified and analyzed with EAD software (Syntech, Hilversum, The Netherlands).

The banana extract was analyzed on a Hewlett-Packard HP-6890 gas chromatograph (GC) equipped with a HP-INNOWAX (30 m x 0.2 mm x 0.25 µm) capillary column (J&W Scientific, Agilent Technologies, USA). Nitrogen was used as carrier gasat a flow rate of 30cm/s, and oven temperature was held at 50°C for 5 min then programmed at 5°C /min to 230°C and held for 10 min. The column effluent was split (50:50) between the FID and the antenna. Both signals were recorded simultaneously on a PC using a GC-EAD software program (Syntech). Compounds

eluting from the column were delivered to the antenna through a glass tube (12cm x 8mm inner diameter) by a charcoal-filtered and humidified air stream at a flow rate of 1.5 l/min.

5.2.4. Chemical identification

Volatile collections were analyzed with gas chromatography-coupled mass spectrometry (GC-MS) on a Hewlett Packard 6890 and 5973-MS. Two µl of headspace collection was injected with a HP 7683 auto injector in splitless mode in 30 seconds. The GC was fitted with a fused silica capillary column coated with INNOWAX (30m x 0.25mm x 0.25µm, Hewlett-Packard, Palo Alto, CA, USA). Helium was used as a carrier gas. The temperature program was the same as that described for the GC-EAD analyses. Identifications of compounds were confirmed by mass spectra matches to the NIST mass-spectral database (1998) as well as by retention time matches to synthetic standards.

5.2.5. Y-tube bioassay

The behavioral response of mashed banana headspace collections and synthetic blends was tested in a Y-tube olfactometer, similar to the one developed by Jonsson *et al.,* (2005). The olfactometer setup consisted of a Y-shaped glass tube with an entry arm (14 cm) and two side arms (16 cm) with the inner diameter of 3.1 cm. Activated charcoal-filtered and humidified air was pumped through the olfactometer arms using a pump (KNF-Neuberger, Freiburg, Germany). The airflow was adjusted by a flow meter (Muurame, Finland) to 1 l/min and passed through Teflon tubes and entered each arm via a custom made 9.5 cm long glass tube. The

air inlet side of the tube had an outer diameter of 1.0 cm and was connected to the Teflon tube. The other side had a female ground and was connected to the male ground on each of the Y-tube arms. In the center of the glass tube, there was a glass rod (*ca* 1 cm long; 0.5 cm diameter) containing a small hole. During experiments, the stimulus was applied on a small piece of filter paper (2.0 x 0.5 cm) attached to a ca 1.4 cm long steel wire. The wire was placed in the hole of the glass rod to position the stimulus in the centre of the tube.

Two stimuli were tested: banana headspace collection and a blend of synthetic compounds mimicking the ratios of compounds found in banana headspace (Table 5.1). The amount of each of the electrophysiologically active compounds was quantified by injecting known quantities of the corresponding synthetic compounds. Each compound was injected three times and the average peak area was used for calculating the response factor. 10 µl of banana extract and 10 µl synthetic blend was applied to a piece of filter paper (2.0 x 0.5 cm). One of the tubes contained the experimental stimulus and the other hexane (control) on filter paper. Prior to testing, the hexane was allowed to evaporate for 1 min. Beetles used in the bioassay were kept without food for 48h before the experiment. A single individual was introduced into the Y- tube and observed until it had walked at least 10 cm up one of the arms or until 10 min had elapsed. Individuals that did not choose a side arm within 10 min were recorded as 'no choice'. After testing 10 females or males, the Y-tube was cleaned with ethanol (95%) and dried at 250°C for 10 min. Subsequently, odour sources were switched between the left and right side arms to minimize any directional effect. Each experiment was repeated on four different experimental days

with new sets of odor sources and insects. The experiments were conducted in the laboratory at 25 ± 2°C and 45 ± 5% R.H.

5.2.6. Data analysis

The behavioral data for each odor in each test were pooled as a response category and compared to a hypothesized 50:50 ratio to determine significant preferences by chi square test. The non-responsive individuals were omitted from further analysis, and the choice of one arm was compared to the choice of the other.

5.3 Results

5.3.1. GC-EAD and chemical analysis

GC-EAD analysis showed that seven compounds from freshly mashed banana and one from 36 hrs old mashed banana, repeatedly elicited responses in *P. interrupta* antennae. No significant difference in antennal sensitivity was observed between male and female antennae. The EAD active compounds in volatiles from freshly mashed banana were identified as isobutyl acetate (peak 1), ethyl butanoate (peak 2), butyl isobutanoate (peak 3), isobutyl isobutanoate (peak 4), isopentyl isobutanoate (peak 5), isoamyl butanoate (peak 6), cis-3-hexyl-2-methyl butanoate (peak 7) (Fig. 5.1A and Table 5.1). The one compound that was antennally active in volatiles from 36 hrs old mashed banana was 2-heptyl butanoate (peak 8) (Fig. 5.1B and Table 5.1). The odor compositions of the volatiles released from mashed banana changed over time. Early eluting, presumably highly volatile compounds were

abundant in freshly mashed banana, but decreased after 36 hrs, when, on the other hand, more late eluting (after 7 min) compounds increased in abundance.

5.3.2. Behavioral response in the Y-tube olfactometer

In the Y-tube, *P. interrupta* were significantly more attracted to both banana extract and synthetic blend compared to the control (hexane). However, there was no significant difference between the number of beetles being attracted to banana headspace extract and to the synthetic blend ($P>0.05$) (Fig. 5.3a, and b). Some beetles walked into the treatment arm in less than two minutes whereas others required as much as ten minutes reaching to either side.

Table 5.1. Volatile compounds identified from mashed banana.

#	Compound	CAS#	Purity,%	µg/µl in extract	µg/µl in synthetic blend
1	Isobutyl acetate	110-19-0	99.8	0.26	0.23
2	Ethyl butanoate	105-54-4	99	0.04	0.02
3	Butyl isobutanoate	97-87-0	97	0.06	0.02
4	Isobutyl iso butanoate	97-85-8	99	0.1	0.11
5	Isopentyl isobutanoate	2050-01-3	98	1.01	1.01
6	Isoamyl butanoate	106-27-4	98	0.8	0.79
7	Cis-3-hexyl-2-methyl butanoate	10094-41-4	97	0.06	0.02
8	2-heptyl butanoate*			0.04	0.02

(#) = Peak number in figure
(t_R) = Retention time (min)
(CAS) = Chemical Abstract Service #; * = Synthesized

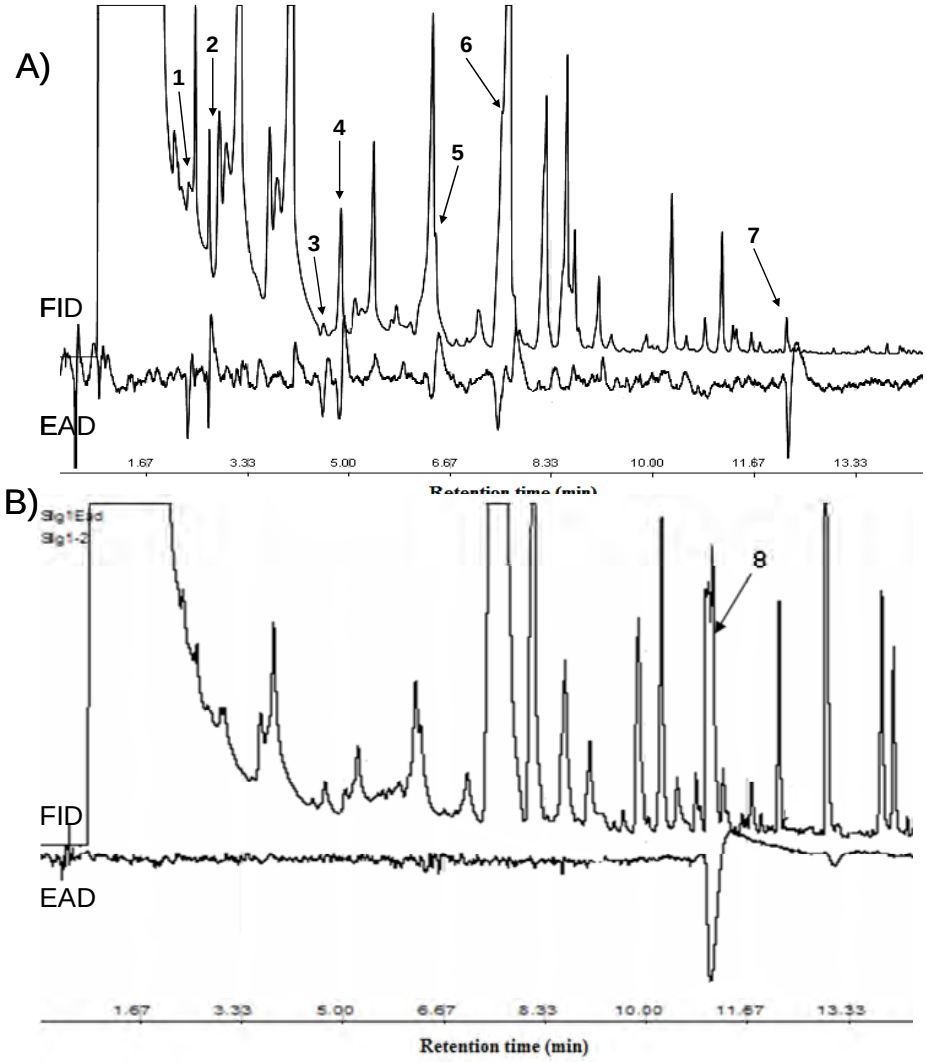


Fig. 5.1. GC-EAD analysis of volatiles collected from mashed banana using antenna of male *P. interrupta*. A) Volatiles from freshly mashed banana and B) volatiles from 36 hrs old mashed banana. The lower trace indicates the flame ionization detector (FID) response and the upper trace indicates EAD response. Number on each peak refers to compounds listed in Table 5.1.

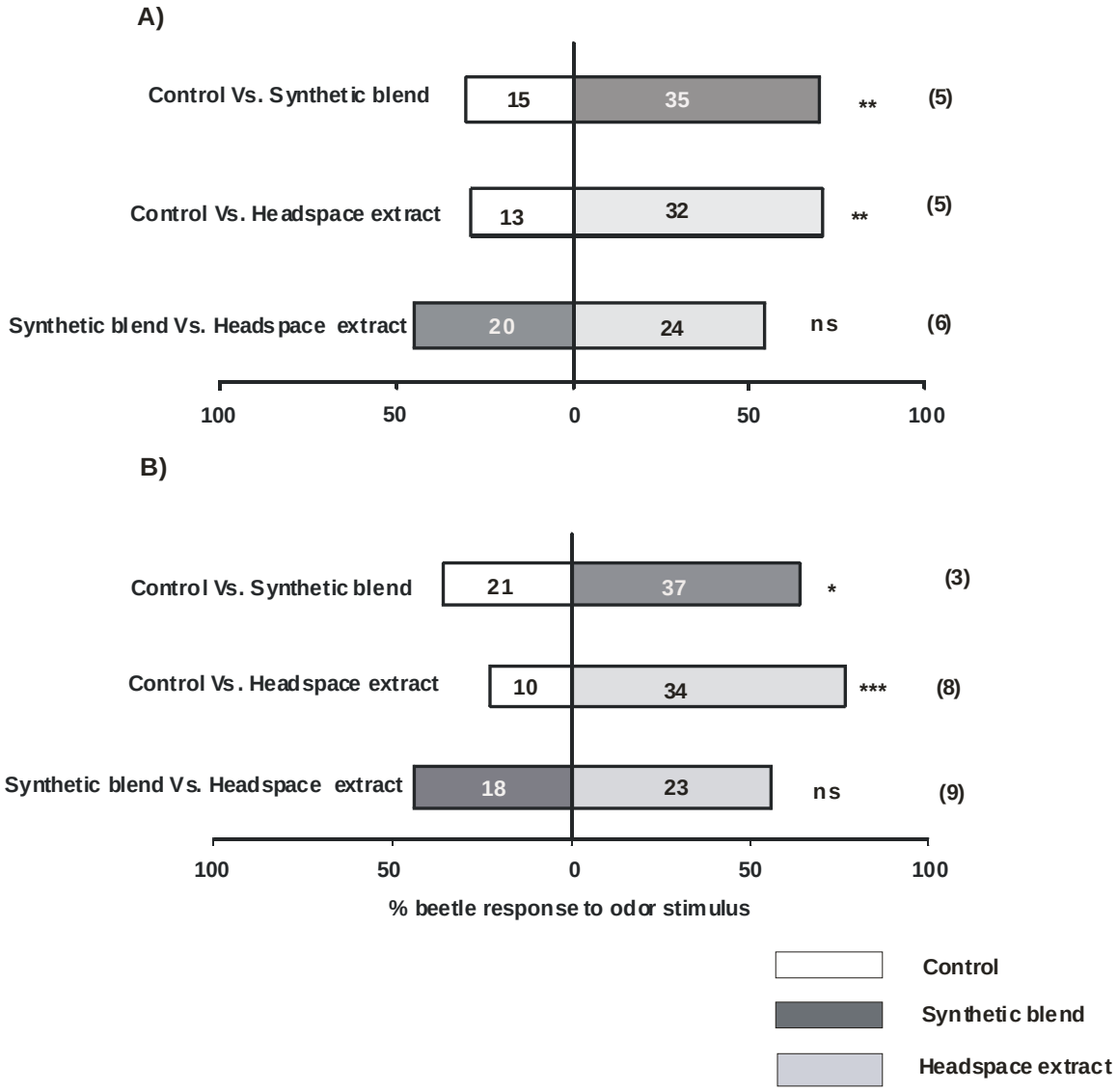


Fig. 5.2. Response of male (A) and female (B) adults of *P. interrupta* in a Y-tube olfactometer to banana extract and a synthetic banana blend. The treatments were compared with a control (hexane). In each bar the actual number of beetles that made a particular choice is given while the x-axis indicates the percentages of choosing beetles that number represents. Figures in the parenthesis indicate the number of beetles in each test that did not choose within 10 min after introduction. Asterisks indicates a significantl between the treatments using Chi square tests (* χ^2, $P < 0.05$; ** χ^2, $P < 0.01$; *** χ^2, $P < 0.001$; ns = not significant). N= 50 for all treatments, except the extract vs. control in females.

5.4. Discussion

Plant odors are complex blends of many different chemical compounds, e.g. the odor emitted from the banana is made up of more than 230 different compounds; a complex mixture of several classes of components, mainly esters, alcohols, aldehydes, ketones, acids and other minor compounds are reported (Pelayo *et al.,* 2003, Macku and Jennings, 1987; Shiota, 1993). The results of this study indicate that *P. interrupta* antenna only detect eight ester compounds from banana. Polyphagous species such as *P. interrupta* rely on being able to identify various key compounds, characteristic for certain common aspects of suitable host plants, in this case ripe fruit. Other investigations of scarab attractants and of potential food sources like flowers and fruits showed that a limited number of compounds could be enough for identification of these preferred food sources of high quality (Stensmyr *et al.,* 2001).

Most of the compounds identified in the current study have also been reported in other studies on banana fruit volatiles (Shiota, 1993, Jordan *et al.,* 2001). Some compounds have also been reported as odour constituents in other ripe fruits, e.g. 2-heptyl butanoate in passion fruit (Strohalm *et al.,* 2007) and feijoa (pineapple guava) (Shawa *et al.,* 1983), isobutyl acetate in golden delicious apple (De Pooter *et al.,* 1983) and ethyl butanoate in mango and papaya (Pino *et al*., 2003, 2005).

The odor composition of fruits changes over time and the stage of fruit ripeness. Certain compounds that are predominant at one stage might be lacking at a later or earlier stage (Flath *et al.,* 1990; Umano *et al.,* 1992). In this study, 2-heptyl butanoate was found in small amounts in freshly mashed banana, but was abundant

when the banana was aged for 36 hrs and it also elicited a strong antennal response (Fig. 5.1 B).

The results of this study show that both male and female *P. interrupta* are attracted to banana headspace extract as well as to the synthetic banana blend in a Y-tube olfactometer (Fig. 5.2a and b). Since there was no significant difference in attractivty between the extract and the synthetic blend it seems likely that the behaviorally relevant key compounds in banana volatiles have been identified. The compounds are potential candidates for further improvement of trapping for control of *P. interrupta*, but further field testing of the individual compounds and blends will be necessary.

CHAPTER VI

OLFACTORY RECEPTOR NEURONS DETECTING FRUIT AND FLOWER COMPOUNDS IN THE SORGHUM CHAFER, *PACHNODA INTERRUPTA* (COLEOPTERA: SCARABAEIDAE)

6.1. Introduction

Adult beetles of the superfamily Scarabaeoidea (commonly known as scarabs) have a wide range of life styles: some are coprophagous (i.e., they feed on dung), while others are monophagous or polyphagous herbivores feeding on fruit, flowers, sap flow and other food sources of high sugar content (Scholtz and Chown, 1995). The adult sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae) is an example of such a highly polyphagous herbivore, and has been recorded to feed on a number of hosts, such as the fruit of mango and banana, and the flowers of orange, papaya, guava and acacia (Donaldson *et al.,* 1990; Grunshaw, 1992). Other hosts on which the beetle feeds include food crops such as pearl millet, *Pennisetum glaucum* (Sastawa and Lale, 2000) and sorghum, *Sorghum bicolor* (Schmutterer, 1969; Grunshaw, 1992; Jago, 1995), the herbaceous weed abutilon, *Abutilon figarianum*, as well as the fruit-bearing plants gumero (*Capparis tomentosa*), inkoy (*Ximenia americana*) and agam (*Carissa edulis*) (Donaldson *et al.,* 1990; Grunshaw, 1992).

Olfaction has been shown to be an important sense for food search in insects (Visser, 1986; Metcalf and Metcalf, 1992; Hartlieb and Anderson, 1999), and several studies have shown attraction of phytophagous scarabs to plant-related compounds

(Klein *et al.*, 1973a; Donaldson *et al.,* 1990; Imai *et al.,* 1997). Findings of field experiments conducted on *P. interrupta* show that adult beetles are attracted to traps baited with odors identified from sorghum and abutilon using gas chromatograph-coupled electroantennographic detection, GC-EAD (Chapter IV).

The olfactory system of a polyphagous herbivore such as the *P.interrupta* needs to be adapted to detect a diverse array of different hosts. How this is exactly reflected in the population of olfactory receptor neurons (ORNs) on the antenna has been a matter of debate. Earlier single sensillum recording (SSR) studies seemed to indicate that generalist ORNs detected a wide range of different host volatiles (Schneider, 1964; Ma and visser, 1978; Selzer, 1981; Den Otter *et al.,* 1980; Dickens *et al.,* 1984; Kafka, 1987), while recent studies have found multiple classes of highly specific ORNs each responding to single host odors, or small groups of structurally similar compound (Dickens, 1990; Todd and Baker, 1993; Anderson *et al.,* 1995; Blight *et al.,* 1995; Wibe *et al.,* 1997; Hansson *et al.,* 1999; Jönsson and Anderson 1999; Larsson *et al.,* 1999; Larsson *et al.,* 2001; Stensmyr *et al.,* 2001; Barata *et al.,* 2002; Lopes *et al.,* 2002; Bichao *et al.,* 2003; Stranden *et al.,* 2003).

The fruit chafer, *Pachnoda marginata*, a close relative of the *P.interrupta*, is also a highly polyphagous herbivore, feeding on the fruit and flowers of guava and mango, as well as flowers of roses, and milk-ripe grain of dura (Schmutterer, 1969). The two species have overlapping geographic ranges, with both being widely distributed in sub-Saharan Africa (Rigout, 1989). Coupled Gas chromatographic single sensillum recordings (GC-SSR) on host plants and SSR with synthetic plant

volatiles performed with *P. marginata* identified several classes of ORNs with narrow response spectra to odors from suitable hosts, such as fruits, and flowers, and fermentation products (Stensmyr *et al.,* 2001).

Using SSR, studies have been conducted to study how the peripheral olfactory system in *P. interrupta* has been adapted to the task of finding a wide array of hosts. The population of ORNs was screened with a set of 80 food-related compounds to determine whether neurons were narrowly or broadly tuned. Narrowly tuned neurons would respond to single compounds or small sets of functionally or structurally similar compounds, while broadly tuned ORNs would respond to multiple functionally and / or structurally different compounds.

6.2. Materials and methods

6.2.1. Insects

Male and female *P. interrupta a*s were collected from the field in Ethiopia. After transport to Alnarp, Sweden, they were kept in clear plastic boxes (30 cm x 12 cm x 22 cm) with a mixture of planting soil, peat and composted cow dung. These boxes were kept in a climate controlled room, set to 25ºC and 70% relative humidity, and a 16/8 L/D cycle. They were fed apples and bananas ad libitum. Beetles were sexed based on the presence of an abdominal groove in males (Rigout, 1989).

6.2.2. Scanning electron microscopy

For scanning electron microscopy (SEM), antennae were excised from the head by fine scissors and immersed in 70% ethanol for three days, dehydrated in absolute ethanol and air-dried. Antennal club lamellae were cut at the joint and both lateral and medial lamellae were mounted individually on stubs and coated with gold-palladium (3:2) using a JEOL T330 ion sputter. Specimens were studied with a LEO 435VP scanning electron microscope operated at 10 kV (Stensmyr *et al.,* 2001).

6.2.3. Single sensillum recordings

The beetles maintained as described above were taken from rearing chambers and kept in a plastic container 15-45 min prior to experiments. Insects were restrained with Parafilm (PM-992, Pechiney plastic packaging, USA) and fixed on microscope slides (ca. 76 x 26 mm, Menzel-Gläser, Germany) using dental wax (Surgident periphery wax, Heraeus Kulzer Inc, USA), with the lamellae held open on a wax surface using 2-3 mm long pieces of thin tungsten wire (diameter 0.12 mm, Harvard Apparatus Ltd, United Kingdom). A silver grounding electrode was inserted in the abdomen, and sensilla were contacted with a tungsten electrode (diameter 0.12 mm, Harvard Apparatus Ltd) electrolytically sharpened using a saturated KNO_2 solution (Hubel, 1957).

Synthetic compounds were diluted to 1μg/μl in acetone or hexane, depending on polarity. Highly volatile compounds were diluted to 1μg/μl in paraffin oil. Stimuli were prepared by applying 10μl of 1μg/μl solution to a 1.5 x 1 cm piece of Whatman

filter paper (No. 3, Whatman, USA), which was placed in a Pasteur pipette (150mm soda lime glass, VWR International, Sweden). For stimuli diluted in hexane or acetone, solvent was allowed to evaporate before stimuli were used in experiments. Between trials, stimulus pipettes were kept at -18ºC, to avoid evaporation or contamination. For comparison, stimulus pipettes containing only solvent as well as empty pipettes were prepared. New stimulus pipettes were prepared once per week.

A constant flow of 0.5 m/s of charcoal-filtered and humidified air was delivered through a glass tube with its outlet approximately 15 mm from the antenna. Stimuli were presented to the insect by inserting the stimulus pipette through a hole in the glass tube, and blowing an air puff of 2.5 ml during 0.5 seconds through the pipette into the air stream, using a stimulus controller (Syntech SFC-1/b, Hilversum, the Netherlands).

The signal from the ORNs was registered and amplified 10 times with a probe (INR-02, Syntech), amplified by 20 times with a Syntech UN-06 AC/DC amplifier, and transferred to a computer through an IDAC-4-USB (Syntech), where it was visualized and analysed with the software Autospike v. 2.2 (Syntech). In cases where two neurons were located in the same sensillum, they were separated based on differences in the amplitude of their action potentials as described below and depicted in Fig. 6.3.

6.2.4. Compounds used for screening

ORN responses to 80 synthetic compounds (Table 6.1; Sigma-Aldrich) were tested. The compounds include common flower and fruit volatiles, and volatiles related to fermentation processes (Knudsen *et al.,* 2006). The majority of these compounds have previously been found to elicit behavioral or electrophysiological activity in related scarab beetles (Stensmyr *et al.,* 2001; Larsson *et al.,* 2003), and some have previously been identified as active in GC-EAD or behavior (or both) for the *P.interrupta* (Chapter III-V).

6.3. Results

The club-shaped antenna of *P. interrupta* exhibits typical scarab morphology with three distal segments flattened into lamellae (Fig. 6.1). Olfactory sensilla were found on the inner sides of the lamellae (those sides that, once the club is closed, are not exposed). Four morphological types of sensilla were identified using SEM: grooved sensilla placodea, smooth sensilla placodea, grooved peg sensilla, and smooth peg sensilla (Fig. 6.2B-E). Approximately 55% of the sensilla are smooth placodea, while around 40% are grooved placodea, and the remaining 5% of the sensilla are of the two peg morphologies.

All lamellar surfaces have a similar distribution of the morphological types of sensilla: a large part of the lamellar surface almost exclusively contains smooth placodea. Near the edge of the lamella that is upwards when the antenna is extended, is a morphologically heterogeneous area (circled in Fig. 6.2A). In this area,

a large proportion of the sensilla are of the grooved placodea type. This area contains almost all of the smooth and grooved peg sensilla that can be found on the lamella. The border between the smooth and heterogeneous area is not sharp, but has a gradual transition in the proportion of grooved to smooth placodea. The position of the border between the areas – the dashed line (Fig. 6.2A) – is based on SEM and light microscope observations at higher magnifications.

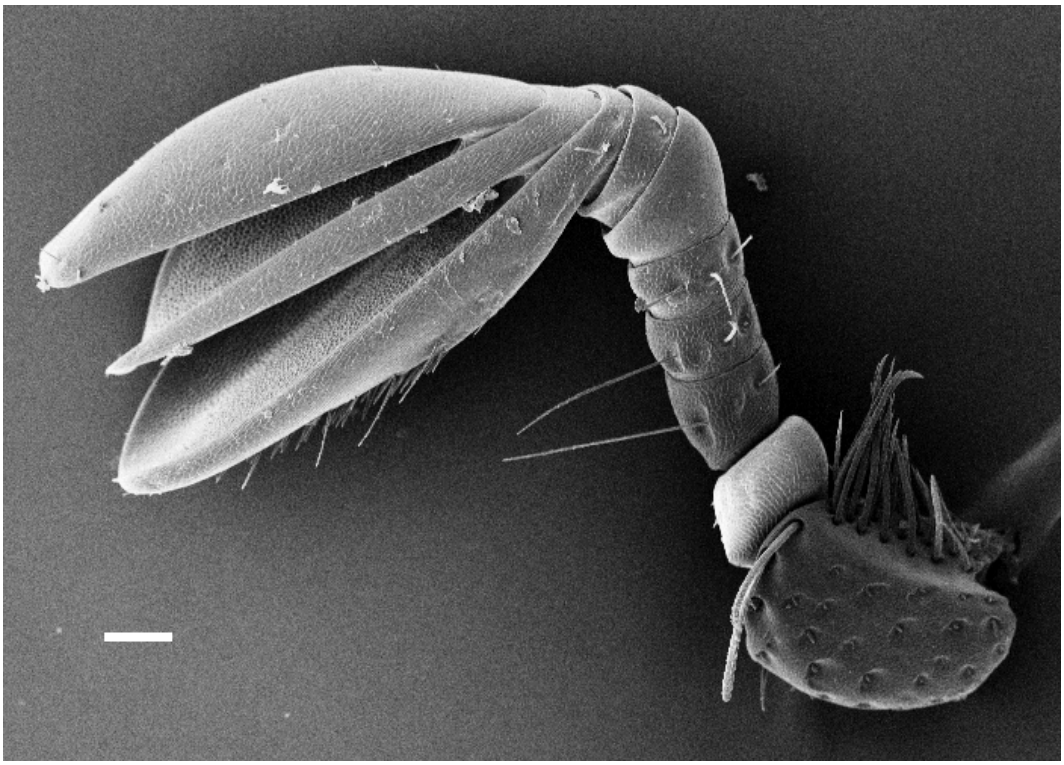


Fig. 6.1. Scanning electron micrograph of *P.interrupta* antenna with lamellae parted to show inner sides with sensilla. Scale bar is 100μm.

Table 6.1. Synthetic compounds used for screening. Compounds active in single sensillum recordings are marked with an asterisk: *.

Compound	CAS no.	Purity, %	Compound	CAS no.	Purity, %
Acetic acid	64-19-7	99	Hexyl hexanoate	6378-65-0	97
* Acetoin (racemic)	513-86-0	97	* Isoamyl acetate	123-92-2	98
Acetone	67-64-1	99.9	* Isoamyl alcohol	123-51-3	98
* Anethole	4180-23-8	99	* Isoamyl butyrate	106-27-4	98
* Benzaldehyde	100-52-7	99.5	Isobutyl acetate	110-19-0	99,8
* Benzyl alcohol	100-51-6	99	* Isobutyl isobutyrate	97-85-8	99
* 2,3-Butane diol (racemic)	513-85-9	99	Isopentyl isobutyrate	2050-01-3	98
* Butyl butyrate	109-21-7	98	Isopropyl acetate	108-21-4	99,8
* Butyl isobutyrate	97-87-0	97	* Isovaleric acid	503-74-2	98
Butyric acid	107-92-6	99	* (±)-Linalool	78-70-6	97
* Carvacrol	499-75-2	98	* Linalool oxides	5989-33-3	97
* beta-Caryophyllene	87-44-5	98.5	* 6-Methyl-5-hepten-2-one	78-70-6	99
Cinnamic aldehyde	104-55-2	98	* Methyl anthranilate	134-20-3	99
* beta-Citronellol	106-22-9	95	* Methyl benzoate	93-58-3	99
(±)-delta-Decalactone	705-86-2	98	Methyl butyrate	623-42-7	99
(±)-gamma-Decalactone	706-14-9	97	* Methyl cinnamate	103-26-4	99
Ethanol	64-17-5	99	* Methyl hexanoate	106-70-7	99
Ethyl acetate	141-78-6	99.5	Methyl jasmonate	1211-29-6	95
* Ethyl butyrate	105-54-4	99	* Methyl octanoate	111-11-5	99
* Ethyl hexanoate	123-66-0	99	Methyl propionate	554-12-1	99
Ethyl propionate	105-37-3	99	* Methyl salicylate	119-36-8	99
* Ethyl-3-hydroxy-butyrate	5405-41-4	97	* Nerolidol	7212-44-4	98
* Eugenol	97-53-0	98	* Nonanal	124-19-6	95
* Geraniol	106-24-1	98	gamma-Nonalactone	104-61-0	97
Geranyl acetate	105-87-3	98	1-Nonanol	143-08-8	99,5
gamma-Hexalactone	695-06-7	98	gamma-Octalactone	104-50-7	97
Hexanal	66-25-1	98	1-Octanol	111-87-5	99,5
* Hexanoic acid	142-62-1	99.5	3-Octanol	589-98-0	99
* 1-Hexanol	111-27-3	98	* 1-Octen-3-ol	3391-86-4	98
* *(E)*-2-hexenol	928-95-0	96	Pentanoic acid	109-52-4	99,8
* *(E)*-2-hexenal	6728-26-3	98	Phenylacetaldehyde	122-78-1	90
* *(E)*-2-hexenyl acetate	2497-18-9	98	Phenylacetonitrile	140-29-4	99
* *(E)*-3-hexenol	928-97-2	98	2-Phenyl ethanol	60-12-8	98
* *(Z)*-3-hexenol	928-97-2	98	2-Phenylethyl propionate	122-70-3	98
* *(Z)*-3-hexenyl acetate	3681-71-8	98	Propionic acid	79-09-4	99,5
* *(Z)*-3-hexenyl butyrate	16491-36-4	98	* Propyl butyrate	105-66-8	99
* *(Z)*-3-hexenyl isobutyrate	41519-23-7	98	Tetradecane	629-59-4	99,5
* *(Z)*-3-hexenyl tiglate	67883-79-8	97	Thymol	89-83-8	99,5
* Hexyl acetate	142-92-7	98	Tridecane	629-50-5	99,5
Hexyl butyrate	2639-63-6	98	gamma-Undecalactone	104-67-6	99

Single sensillum recording data for males and females were pooled. Most recordings were done from sensilla on the lamella closest to the base of the antenna, but a few recordings were done on other lamellae furthest from the base of the antenna. Stable contacts were only possible with sensilla placodea, from which all of our results stem. Among the 50 ORNs for which we could find at least one ligand eliciting a response, 23 classes were identified (Table 6.2.). The ORN classes responded to single compounds or a small group of functionally and structurally similar compounds. From the contacted grooved sensilla placodea, 68% contained at least one responding neuron (41 out of 60), in contrast to only 11% of the contacted smooth sensilla placodea (2 out of 18).

Correspondingly, responding ORNs (Fig. 6.4.) are mainly located in the heterogeneous area (Fig. 6.2A). ORNs responding to nonanal were commonly found in the same sensillum as those responding to isovaleric acid (Fig. 6.4). ORNs responding to linalool oxides were often co-compartmentalized with those responding to methyl salicylate (Fig. 6.4.).

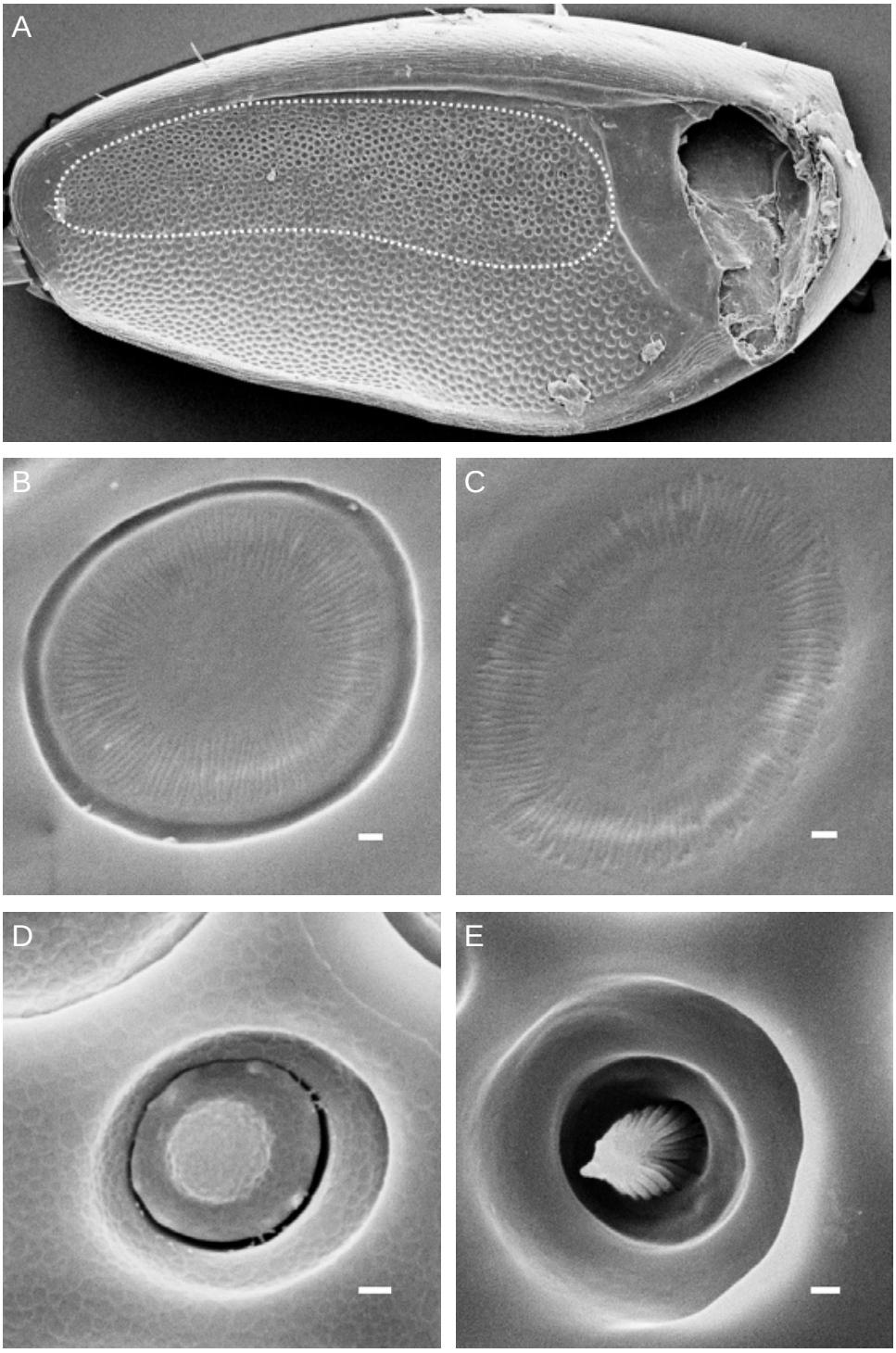
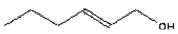
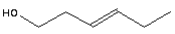
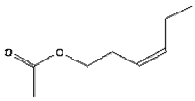
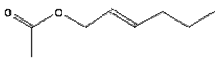
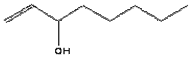
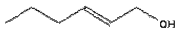


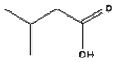
Fig. 6.2. A: Scanning electron micrograph of *P. interrupta* lamella. B-E: Morphological types of sensilla; B: grooved placodea, C: smooth placodea, D: smooth peg, E: grooved peg. Scale bars for B-E are 500nm. The circled area in A predominantly contains sensilla of the grooved placodea morphological type. In remaining areas, the majority of sensilla are of the smooth placodea type. Smooth and grooved peg-type sensilla are mainly found in the circled area.

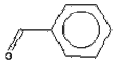
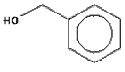
Table 6.2. ORN classes, including molecular formulas for key ligands.
Net response in Hz is shown in brackets. All responses above 40 Hz are included, except in the case of ORN class 14 (butyl butyrate), where some ligands beyond the eleven included elicited responses above 40 Hz.

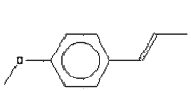
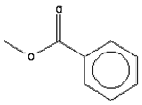
ORN class 1: (e)-2-hexenol	n = 2
 [112] (e)-2-hexenol	 [95] (z)-3-hexenol
 [91] 1-hexanol	 [88] (e)-3-hexenol
 [72] (z)-3-hexenyl acetate	 [64] (e)-2-hexenal
 [64] (e)-2-hexenyl acetate	 [44] 1-octen-3-ol

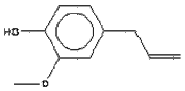
ORN class 2: (z)-3-hexenol	n = 3
 [125] (z)-3-hexenol	 [110] (e)-3-hexenol
 [68] (e)-2-hexenol	

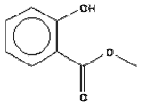
ORN class 3: Nonanal	n = 4
 [112] Nonanal	

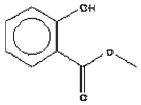
ORN class 4: Isovaleric acid	n = 5
 [81] Isovaleric acid	

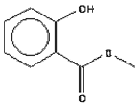
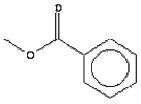
ORN class 5: Benzaldehyde	n = 2
 [90] Benzaldehyde	 [46] Benzyl alcohol

ORN class 6: Anethole	n = 2
 [107] Anethole	 [48] Methyl benzoate

ORN class 7: Eugenol	n = 3
 [123] Eugenol	

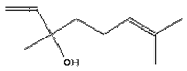
ORN class 8: Methyl salicylate	n = 2
 [158] Methyl salicylate	

ORN class 9: Secondary methyl salicylate	n = 2
 [65] Methyl salicylate	

ORN class 10: Methyl salicylate / methyl benzoate	n = 3
 [125] Methyl salicylate	 [106] Methyl benzoate

ORN class 11: Linalool oxides n = 4

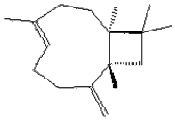
Several different structural formulas



[93] Linalool oxides (mix of several)

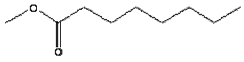
[80] Linalool

ORN class 12: Secondary β-caryophyllene n = 4



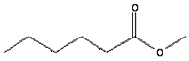
[68] β-caryophyllene

ORN class 13: Methyl octanoate n = 4



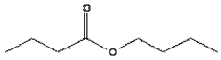
[101] Methyl octanoate

[65] Hexyl acetate



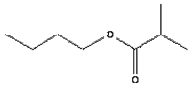
[56] Methyl hexanoate

ORN class 14: Butyl butyrate n = 1

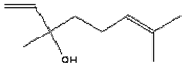


[175] Butyl butyrate

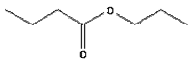
[151] Methyl hexanoate



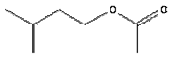
[131] Butyl isobutyrate



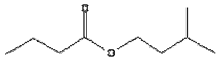
[129] 6-methyl-5-hepten-2-one



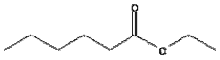
[121] Propyl butyrate



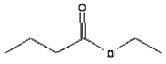
[120] Isoamyl acetate



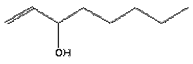
[113] Isoamyl butyrate



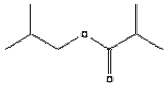
[99] Ethyl hexanoate



[99] Ethyl butyrate

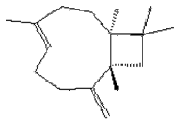


[91] 1-octen-3-ol



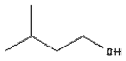
[73] Isobutyl isobutyrate

ORN class 15: β-caryophyllene n = 1

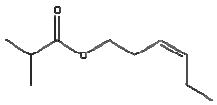


[137] β-caryophyllene

ORN class 16: Isoamyl alcohol n = 1

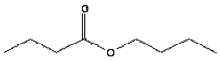


[132] Isoamyl alcohol

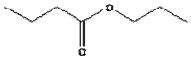


[98] (z)-3-hexenyl isobutyrate

[88] Methyl octanoate



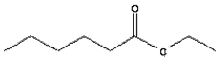
[72] Butyl butyrate



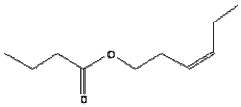
[70] Propyl butyrate

Several different structural formulas

[68] Linalool oxides (Mix of several)



[66] Ethyl hexanoate



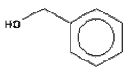
[66] (z)-3-hexenyl butyrate

ORN class 17: 2,3-butane diol n = 1

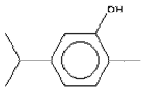
[125] 2,3-butane diol

[94] Acetoin

ORN class 18: Benzyl alcohol n = 1



[101] Benzyl alcohol

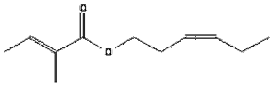


[59] Carvacrol

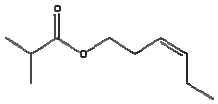
ORN class 19: Hexanoic acid n = 1

[87]
Hexanoic acid

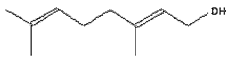
ORN class 20: (z)-3-hexenyl tiglate n = 1



[81]
(z)-3-hexenyl tiglate

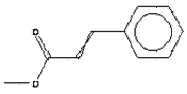


[69]
(z)-3-hexenyl isobutyrate

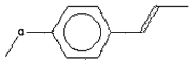


[53]
Geraniol

ORN class 21: Methyl cinnamate n = 1

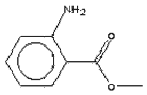


[59]
Methyl cinnamate

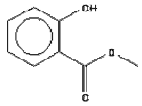


[49]
Anethole

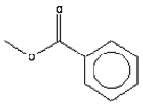
RN class 22: Methyl anthranilate n = 1



[50]
Methyl anthranilate

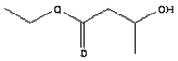


[38]
Methyl salicylate

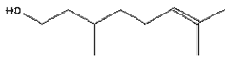


[38]
Methyl benzoate

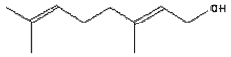
ORN class 23: Ethyl-3-hydroxy butyrate n = 1



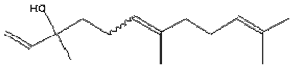
[49]
Ethyl-3-hydroxy butyrate



[43]
β-citronellol



[39]
Geraniol



[39]
Nerolidol

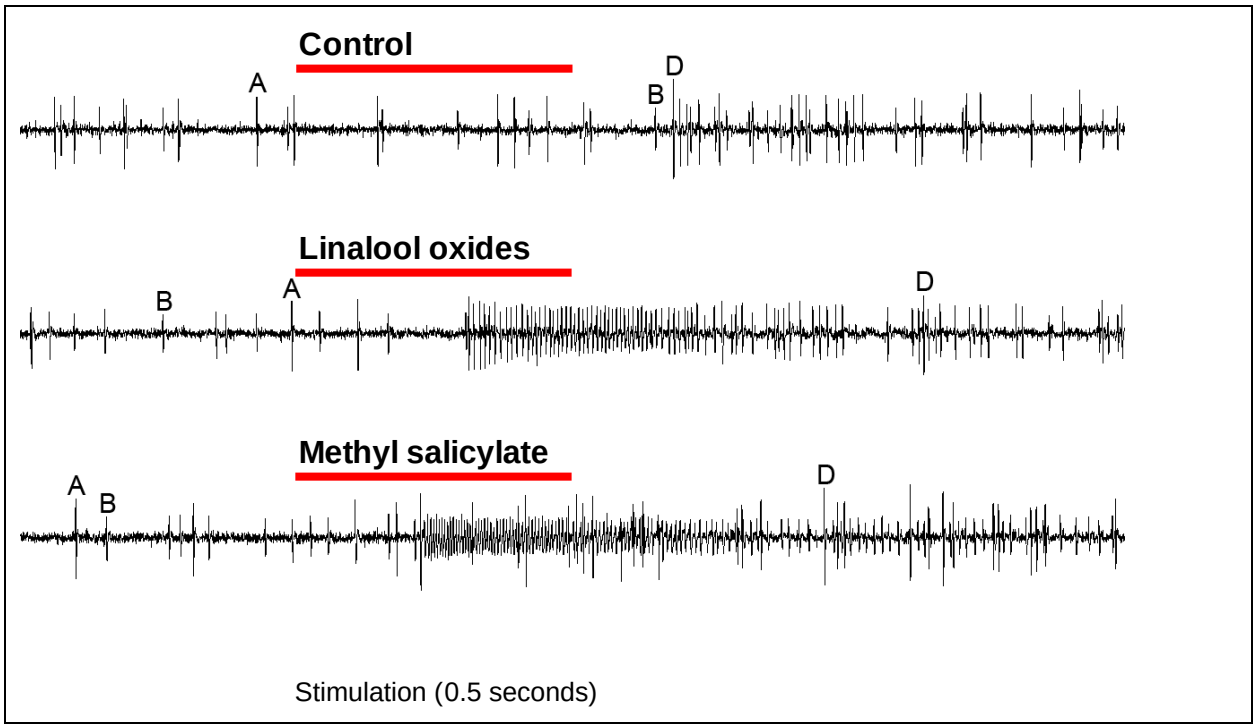


Fig. 6.3. The two ORNs present in this sensillum are distinguished by the amplitudes of their action potentials. The ORN responding to linalool oxide (denoted “A” above) has a higher amplitude than the ORN responding to methyl salicylate (B). There are also double spikes, where both neurons fire simultaneously (D), that have a greater amplitude than either neuron by itself.

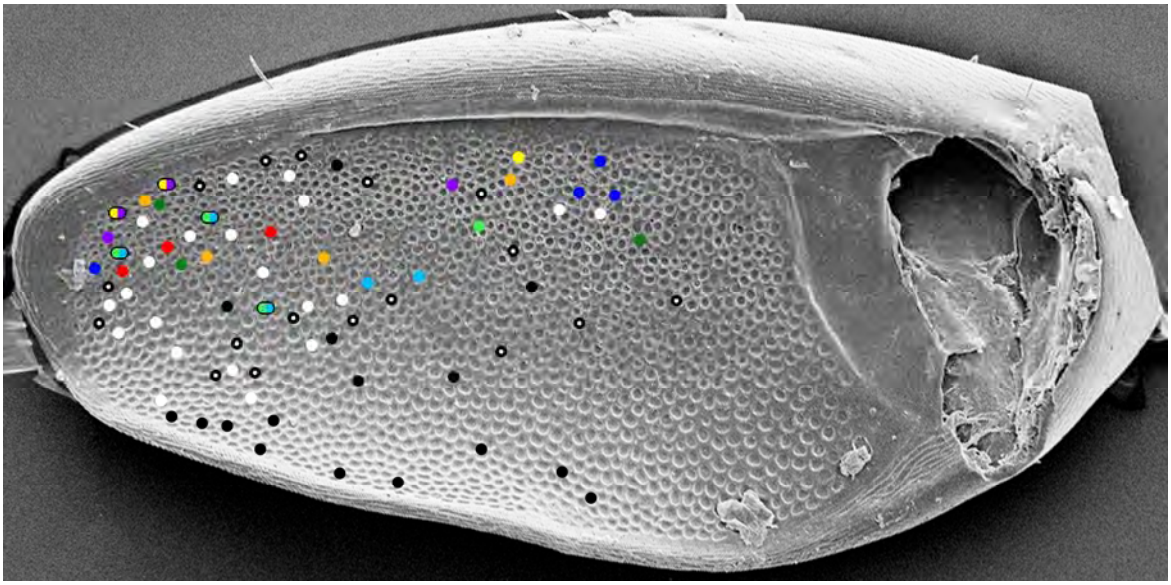


Fig. 6.4. Distribution of responding olfactory receptor neurons.

Two colors within border indicate that the ORNs are present in the same sensillum.

Legend:

- No response, grooved placodea
- No response, smooth placodea
- Isovaleric acid
- beta-Caryophyllene
- Linalool oxides
- Eugenol
- Methyl octanoate
- Methyl salicylate/Methyl benzoate

6.4. Discussion

Initial SSR studies on insects found ORNs with a wide response range (Schneider *et al.,* 1964; Ma and Visser, 1978; Den Otter *et al.,* 1980; Selzer, 1981; Dickens *et al.,* 1984; Kafka, 1987). These results can most likely be attributed to a lack of key stimuli, as well as the use of stimuli at concentrations above those encountered in nature. A majority of SSR studies during the last two decades have found ORNs that are narrowly tuned: they respond to single compounds or small sets of structurally and functionally similar compounds (Harris and Todd, 1980; Dickens, 1990; Anderson *et al.,* 1995; Blight *et al.,* 1995; Wibe *et al.,* 1997; Hansson *et al.,* 1999; Jönsson and Anderson, 1999; Larsson *et al.,* 1999; Larsson *et al.,* 2001; Stensmyr *et al.,* 2001; Barata *et al.,* 2002; Lopes *et al.,* 2002; Bichao *et al.,* 2003; Stranden *et al.,* 2003). Narrowly tuned ORNs have been found in mono- or oligophagous insects as well as polyphagous ones. This indicates that narrowly tuned ORNs are used in host search, no matter if the host range is wide or narrow. Herbivores with a wide host range could either look for a distinctive compound for each host, or use compounds that are shared by multiple hosts. Such shared compounds could for instance be the stereotyped odour signals used by plants to attract symbionts for pollination and seed-dispersal, that a herbivore could effectively be overhearing.

Since *P. interrupta* feeds on fruits and flowers, such pollination- and seed-dispersal signals would form a reliable cue to find suitable hosts. Narrowly tuned ORNs responding to these signals could thus be used in host search. The closely

related scarab *P. marginata* seems to have adopted a host search strategy similar to that of *P. interrupta* (Stensmyr *et al.,* 2001).

Sorghum chafer ORNs responding to nonanal were frequently found inhabiting the same sensillum as those responding to isovaleric acid, and ORNs responding to linalool oxides were often found together with neurons responding to methyl salicylate. The co-compartmentalization of ORNs may be important for detecting the synchronous arrival of multiple odour components (reviewed in Ayres *et al.,* 2001). It has been implied as the mechanism for separating pheromones from their behavioural antagonists, where adult corn earworm, *Helicoverpa zea*, males are able to distinguish between odour plumes with a temporal and spatial resolution of less than 0.001 s and 1 mm (Fadamiro *et al.,* 1999).

Co-compartmentalization could enable insects to detect odour packages in the air that originate from a host, where the host odour lacks unique compounds, but does have a unique combination of compounds, or ratio between compounds (Ayres *et al.,* 2001). For the sorghum chafer, methyl salicylate has been found to be attractive in the field (Yitbarek Wolde-Hawariat *et al.,* 2007, Chapter V). Linalool oxides, nonanal and isovaleric acid, that all elicited strong responses from their respective ORNs, have so far not been identified as electrophysiologically or behaviourally active ligands from headspace extractions of *P*. *interrupta* hosts. However, as these compounds have previously been found to be components of flower odours (Knudsen *et al.,* 2006), and several hosts of the sorghum chafer remain to be investigated, they could potentially be involved in food searching behavior.

The comparatively low percentage of smooth sensilla placodea that contain responding ORNs indicates that key ligands for the ORNs in this morphological type of sensillum arenot identified. These ligands could be host-search related ligands that were not included in our set of compounds. They could also be related to other parts of the life cycle of the sorghum chafer, such as mate finding, search for oviposition site, or search for aestivation site. The use of chemical cues in finding an oviposition site is well documented in insects, e.g. in the onion fly, *Delia antiqua*, the cabbage root fly, *Delia radicum*, the diamondback moth, *Plutella xylostella*, the spruce budworm, *Choristoneura fumiferana*, and the codling moth, *Laspeyresia pomonella* (Renwick, 1989). Behavioural experiments indicate that chemical compounds are important oviposition cues for a related scarab species, the Japanese beetle, *Popillia japonica* (Szendrei and Isaacs, 2005).

In the ruteline scarab beetles *Anomala cuprea* and *Phyllopertha diversa*, sensilla in the heterogeneous area of the antenna responded to host odours, while sensilla in the smooth area responded to pheromone compounds (Hansson *et al.,* 1999; Larsson *et al.,* 2001). No responding neurons were found in the smooth area in *P. marginata*, but sensilla were not tested with pheromone compounds since they have not been identified (Stensmyr *et al.,* 2001).

While some compounds that elicit strong, repeated responses from ORNs have previously been shown to be powerful attractants in the field, e.g. eugenol and methyl salicylate (Chapter V). Some of the most active ligands in SSR have previously not been tested in field trapping experiments with the sorghum chafer.

Examples include anethole, benzaldehyde and methyl octanoate, and these compounds could be tested to see if they elicit a behavioural response for future mass trapping or monitoring.

7.0. CONCLUSIONS

Based on this research work the following conclusions can be made:

This study is the first to study the electrophysiological and behavioral responses of *P. interrupta* to host plant volatile compounds.

The performance of the different compounds as lures were affected by Dispenser type (cotton wick or rubber septum), trap location (inside or outside sorghum fields) and season (mating/July or feeding/September). The results indicated that trapping would be most efficient in July, during the mating season, and if it is the cropping season traps should be placed outside the crop.

The synthetic sorghum or abutilon volatile blends in combination with eugenol and/or methyl salicylate constitute powerful attractants for *P. interrupta*. The blends could be used for monitoring of the pest or in direct control measures, e.g. mass trapping.

Eight volatile compounds were identified from ripe banana. Behavioral responses tested using the Y-tube olfactometer showed no significant different in response between the banana headspace extract and the blend of synthetic compounds. This indicates that the compounds identified are behaviorally relevant. Thus, the compounds can be used as potential candidates for further improvement of the trapping of *P. interrupta.*

The scanning electron microscopy of *P. interrupta* revealed four types of sensilla: grooved peg, smooth peg, grooved placodea, and smooth placodea. The majority of responding ORNs were found in grooved sensilla placodea, and only a few in smooth placodea.

Olfactory receptor neurons (ORNs) were screened for response in single sensillum recordings with a set of 80 food-related compounds representing different odor sources: green leaf volatiles (GLVs), flower and fruit odors (aromatics, terpenes and esters) and fermentation products (acids). From the 50 responding ORNs, 23 classes were identified. The classes were tuned to single compounds or sets of structurally and functionally similar compounds.

The sensory strategy for food search where multiple classes of ORNs each have a narrow detection window. Such a strategy could rely on common compounds present in hosts such as fruits or flowers, which advertise to symbionts for pollination with stereotyped odour signals.

8.0 RECOMMENDATIONS

Based on the results from this research work, the following can be pointed out as foci of future studies

In order to provide the farmers with an efficient, reliable and economical system, the design of the local trap must be improved and a high-longevity dispenser needs to be developed.

In several species that a comparatively low attraction to floral compounds is highly synergized by pheromones, therefore, future projects should focus on deciphering the pheromone signal of *P. interrupta*.

The blends identified in current study can be used as attractants in programs to infect *P. interrupta* with entomopathogens. However, this needs further research on use of the local isolates and modified traps with subsequent auto-dissemination of infected beetles into the population.

This study was a further step to map the antennal sensillar ORNs. The next step should include the non-investigated sensilla types to plant compounds and search for new types of ORNs.

Using GC-EAD and/or GC-SSR techniques have proved to work well on the *P. interrupta*, Odor from suitable sources relating to oviposition, sexual attraction, aggregation, and aestivation, further research is needed in this area.

9.0. REFERENCES

Adhanom Negassi and Abraham Tadesse (1985). A Review of Research on Insect Pests of Maize and Sorghum in Ethiopia. In: *Proceedings of the First Ethiopian Crop Protection Symposium. IAR*, pp. 7-19. (Tsedeke Abate ed.) Addis Ababa, Ethiopia.

Agelopoulos, N.G. and Pickett, J.A. (1998). Headspace analysis in chemical ecology: Effects of different sampling methods on ratios of volatile compounds present in headspace samples. *J. Chem. Ecol.* **24**:1161-1172.

Agelopoulos, N.G., Hooper, A.M., Maniar, S.P., Pickett, J.A. and Wadhams, L.J. (1999). A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *J. Chem. Ecol.* **25**:1411-1425.

Ahmad, S. (1982). Host location by the Japanese beetle (*Popillia japonica*): evidence for a key role for olfaction in a highly polyphagous insect. *J. Exp. Zool.* **220**:117-20

Alm, S.R. and Dawson, C.G. (2003). Evaluation of two prototype traps and existing trap designs for captures of Japanese beetles (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* **96**:453-455.

Alm, S.R., Yeh, T., Dawson, C.G. and Klein, M.G. (1996). Evaluation of trapped beetle repellency, traps height, and string pheromone dispensers on Japanese beetle captures (Coleoptera: Scarabaeidae). *Environ. Entomol*. **25**:1274-1278.

Andemeskel Woldehaimanot. (1987). Handbook of insect pests of major crops in Eritrea Administrative Region, and their control. Asmara University. pp. 132.

Anderbrant, O., Bengtsson, M., Löfqvist, J. and Baeckström, P. (1992). Field response of the pine sawfly, *Neodiprion sertifer,* to controlled release of diprionyl acetate, diprionyl propionate and trans-perillenal. *J. Chem. Ecol*. **18**:1707-1725.

Andersen, R.A., Hamilton-Kemp, T.R., Loughrin, J.H., Hughes, C.G. Hildebrand, D.F. and Sutton, T.G. (1988). Green leaf headspace volatiles from *Nicotiana tabacum* lines of different trichome morphology. *J. Ag*ric. *Food Chem*. **36**:295-299.

Anderson, P., Hansson, B.S. and Lofqvist, J. (1995). Plant-odor-specific receptor neurons on the antennae of female and male *Spodoptera littoralis*. *Physiol. Entomol.* **20**:189-198.

Andersson, M. (2007). Behavioral electrophysiological response of male Hessian flies, *Mayetiola destructor*, to candidate sex pheromone components. Master project in biology. Lund University.

Appert, J. (1957). Les parasites animaux des plantes cultivers au Senegal et au Soudan francais. Paris, France: Gouvernement General de l'Afrique Occidentale Francaise.

Armbruster, R.A., Hamilton-Kemp, T.R., Loughrin, J.H., Hughes, C.G., Hildebrand, D. F. and Sultton, T.G. (1989). Pollination of *Dalechampia magnoliifolia* (Euphorbiaceae) by male euglossine bees (Apidae: Euglossinini). *Am. J. Bot*. **76**:1279-1285.

Arn, H., Stadler, E. and Rauscher, S. (1975). The electroantennographic detector - a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* **30**:722-725.

Ayres, B.D., Ayres, M.P., Abrahamson, M.D. and Teale, S.A. (2001). Resource partitioning and overlap in three sympatric species of *Ips* bark beetle (Coleoptera: Scolytidae). *Oecologia*. **128**:443-453.

Barata, E.N., Mustaparta, H., Pickett, J.A., Wadhams, L.J. and Araujo, J. (2002). Encoding of host and non-host plant odors by receptor neurones in the eucalyptus woodborer, *Phoracantha semipunctata* (Coleoptera: Cerambycidae). *J. Comp. Physiol.* **188**:121-133.

Bartelt, R.J and Zilkowski, B.W. (1999). Non-equilibrium quantification of volatiles in air streams by solid-phase microextraction. *Anal. Chem.* **71**:92-101.

Bekele Jembere, Daniel Getahun, Merid Negash, Emiru Seyoum, (2005). Toxicity of Birbira, *Milletia ferruginea,* seed crude extracts to some insect pests as compared to other botanicals and synthetic insecticide. In: *The 11th Symposium of the Natural Products Research Network for Eastern and Central Africa (NAPRECA), Antananarivo, Madagascar.*

Bell, W.J., Kipp, L.R. and Collins, R.D. (1995). In: *The Role of Chemo-Orientation in Search Behavior*, *Chemical of Ecology Insects,* pp. 105-153, (Carde, R.T. and Bell, W.J., eds). Chapman and Hall, New York.

Bengtsson, M., Jaastad, G., Knudsen, G., Kobro, S., Bäckman, A-C., Pettersson, E. and Witzgall, P. (2006). Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella*. *Entomol. Exp. Appl.* **118**:77-85.

Ben-Yakir, D., Bazar, A. and Chen, M. (1995). Attraction of *Maladera matrida* (Coleoptera: Scarabaeidae) to eugenol and other lures. *J. Econ. Entomol.* **88**:415-420.

Bernasconi, M.L., Turlings, T.C.J., Ambrosetti, L., Bassetti, P. and Dorn, S. (1998). Herbivore induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomol. Exp. Appl*. **87**:133-142.

Bernays, E.A. and Chapman, R.F. (1994). Host-plant Selection by Phytophagous Insects. Chapman and Hall, London.

Bichao, H., Borg-Karlson, A.K., Araujo, J. and Mustaparta, H. (2003). Identification of plant odors activating receptor neurones in the weevil *Pissodes notatus* F. (Coleoptera, Curculionidae). *J. Comp. Physiol.* **189**:203-212.

Birgersson, G. and Bergström, G. (1989). Volatiles released from individual spruce bark beetle entrance holes: Quantitative variations during the first week of attack. *J. Chem. Ecol.* **15**:2465-2483.

Bjostad, L.B. (1998). Electrophysiological methods. In: *Methods in Chemical Ecology, vol 1, Chemical methods*, pp. 339-369, (Millar J.G. and Kenneth, F. H., eds), Kluwer Academic Publishers, Norwell, Massachusetts, USA.

Blight, M.M. and Smart, L.E. (1999). Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aeneus* in field traps. *J. Chem. Ecol.* **25**:1501-1516.

Blight, M.M., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1995). Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae). *J. Chem. Ecol.* **21**:1649-1664.

Boeckh, J. (1984). Neurophysiological aspects of insect olfaction. In insect communication, pp. 83-104, (Lewis, T., ed). Acadamic Press, New York.

Borden, J.H. (1993). Strategies and tactics for the use of semiochemicals against forest insect pests in North America. In: *Pest Management: Biologically Based Technologies,* pp. 265-279, (Lumsden, R.D. and Vaughn, J.L., eds). ACS Conf. Proc., Washington, D.C.

Borror, D.J., Delong, D.M., Tripplehorn, C.A. (1976). An Introduction to the Study of Insects. Edition 4. New York, USA: Holt, Rinehart and Winston.

Boudhrioua, N., Giampaoli, P. and Bonazzi, C. (2003). Changes in aromatic components of banana during ripening and air-drying. *Lebensm. Wiss. Technol*. **36**:633-642.

Brihane Gebrekidan (1986). Sorghum improvement and production in eastern Africa. In: *Proceedings of an International Drought Symposium of food grain production in the semi-arid regions of sub-Saharian Africa.* pp. 141-154. (Menyonga, J. M., Bezuneh, T., and Youdeowei, A. eds). Nairobi, Kenya, May 19-23, OAU/STRC-SAFGRAD, Nairobi.

Bruce, T.J.A., Wadhams, L.J. and Woodcock, C.M. (2005). Insect host location: a volatile situation. *Trends Plant Sci*. **10**:269–274.

Burger, B.V., Nell, A.E. and Petersen, W.G.B. (1991). Analysis of pheromones: enrichment on thick film capillary traps and GC detection with a living detector (EAD). *J. High Resolut. Chromatogr.* **14**:718-725.

Buttery, R.G. and Ling, L.C. (1984). Corn leaf volatiles: identification using tenax trapping for possible insect attractants. *J. Agr. Food Chem*. **32**:1104-1106.

Buttery, R.G., Flath, R.A., Mon, T.R., Ling, L.C. (1986). Identification of germacrene D in walnut and fig leaf volatiles. *J. Agric. Food Chem.* **34**:820-822.

Buttery, R.G., Kamm, J.A., Ling, L.C. (1982). Volatile components of alfalfa flowers and pods. *J. Agric. Food Chem.* **30**:739-742.

Cardé, R.T. and Elkinton, J.S. (1984). Field trapping with attractants:.Method and interpretation, In: *Techniques in pheromone research*, pp. 111-129, (Hummel, H.E, Miller, T.A., eds). SpringerVerlag, Berlin, Heidelberg, New York.

Caswell, G.H. (1962). Agricultural Entomology in the Tropics. Edward Arnold (publishers) LTD. London. pp. 83-84.

Cerda, H., Fernandez, G., Lopez, A. and Varga, J. (1999). Olfactory attraction of the sugar cane weevil (Coleoptera: Curculionidae) to host plant odors, and its aggregation pheromone. *Fla. Entomol.* **82**:103-112.

Cherry, R.H., Klein, M.G. and Leal, W.S. (1996). Attraction of adult *Anomala marginata* (Coleoptera: Scarabaeidae) to anethole. *J. Agric. Entomol.* **13**:359–364.

Clark, R.O.S. and Crowe, T.J. (1978). The Genus *Pachnoda* in Ethiopia. Identification, Pest Status and Control of the Species. Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. 19.

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Junhyong, K. and Carlson, J.R. (1999). A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron.* **22**:327-338.

Cork, A. (2004). Pheromone Manual, Natural Resources Institute, Chatham ME4 4TB, UK.

Cork, A., Beevor, P.S. Gough, A.J.E. and Hall, D.R. (1990). Gas chromatography linked to electroantennography: a versatile technique for identifying insect

semiochemicals. In: *Chromatography and Isolation of Insect Hormones and Pheromones*, pp. 271-274. (McCaffery A.R. and Wilson, I.D. eds) Plenum Press, New York.

Cosse, A.A. and Baker, T.C. (1999). Electrophysiologically and behaviorally active volatiles of Buffalo gourd root powder for corn rootworm beetles. *J. Chem. Ecol.* **25**:51-66.

Cosse, A.A., Todd, J.L. Millar, J.G. Martinez, L.A. and Baker, T.C. (1995). Electroantennographic and coupled gas-chromatographic electroantennographic responses of the Mediterranean fruit-fly, *Ceratitis capitata*, to male-produced volatiles and mango odor. *J. Chem. Ecol*. **21**:1823-1836.

Crocker, R.L., Klein, M.G., Wie, X. and Nailon, W.T. (1999). Attraction of *Phyllophaga congrua, Phyllophaga crassissima, Phyllophaga crinita,* and *Cyclocephala lurida* (Coleoptera: Scarabaeidae) adults to selected volatile compounds. *South West Entomol*. **24**:315-320.

CSA (Central statistical Authority). (2006). Agricultural sample survey 2005/06: report on area and production for major crops (private peasant holdings, Meher season). Statistical Bulletin No 171. CSA. Addis Ababa, Ethiopia. pp. 106.

De Pooter, H.L., Montens, J.P., Willaert, G.A., Dirinck, P.J. and Schamp, N.M., (1983). Treatment of golden delicious apples with aldehydes and carboxylic acids: effect on the headspace composition. *J. Agric. Food Chem*. **31**:813-818.

De Pury, J.M.S. (1968). Crop pests of East Africa. Oxford, UK. Oxford University press. pp. 240.

Den Otter, C.J., Behan, M. and Maes, F.W. (1980). Single cell responses in female *Pieris brassicae* (Lepidoptera: Pieridae) to plant volatiles and conspecific egg odors. *J. Insect Physiol.* **26**:465-472.

Descamps, M. (1954). Insect pests of crops and insect predators recently observed in North Cameroon. *Agron. Tropic.* **9**:174-181.

Dethier, V.G., Barton, B.L. and Smith, C.N. (1960). The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol.* **53**:134-136.

Dicke, M. and Sabelis, M.W. (1988). Infochemicals terminology: Based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.* **2**:131-139.

Dickens, J.C. (1990). Specialized receptor neurons for pheromone and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). *Chemical Senses* **15**:311-331.

Dickens, J.C., Payne, T.L., Ryker, L.J. and Rudinsky, J.A. (1984). Single cell responses of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae) to pheromones and host odors. *J. Chem. Ecol.* **10**:583-600.

Domek, J.M. and Johnson, D.T. (1988). Demonstration of semiochemically induced aggregation in the green June beetle, *Cotinis nitida* (L) (Coleoptera: Scarabaeidae). *Environ. Entomol.* **17**:147-149.

Donaldson, J. M. I., Mcgovern, T. P. and Ladd, T. L. (1986). Trapping techniques and attractants for Cetoniinae and Rutelinae (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* **79**:374-377.

Donaldson, J.M.I., McGovern, T.P. and Ladd, T.L. (1990). Floral attractant for Cetoniinae and Rutelinae (Coleoptera: Scarabaeidae). *J. Econ. Entomol*. **83**:1298-1305.

Doumbia Y.O, Bronzi SM, (1985). Note sur les problemes des insects des panicles du sorgho au Mali. In: *Proceedings of the West African Regional Sorghum Network Workshop,* pp. 173-182, *21-24 October,* 1985,. Bamako, Mali.

Doumbia, Y.O. and Bronzi, S.M. (1989). Inventaire et distribution des insects du mil et du sorgho au Mali. *Agron. Tropic.* **44**:185-195.

Edwards, S., Tadesse, M. and Hedberg, H. (1995). Flora of Ethiopia and Eritrea. Vol. 2, Part- 2, Canellaceae to Euphorbiaceae. Addis Ababa, Ethiopia; Upsalla, Sweden.

Fadamiro, H.Y., Cosse, A.A. and Baker, T.C. (1999). Fine-scale resolution of closely spaced pheromone and antagonist filaments by flying male *Helicoverpa zea*. *J. Comp. Physiol.* **185**:131-141.

FAO-TCP (2002)-Ethiopia project (TCP/ETH/0166) interim report.

Flamini, G., Cioni, P.L. and Morelli, I. (2003). Volatiles from leaves, fruits, and virgin oil from from *Olea europaea* cv., *Olivastra Seggianese* from Italy. *J. Agri. Food Chem.* **53**:1382-1386.

Flath, R.A.,Light, D.M. Jang, E.B., Mon, T.R. and John, J.O. (1990). Headspace examination of volatile emissions from ripening papaya (*Carica papaya* L., Solo variety). *J. Agri. Food Chem.* **38**:1060-1063.

Gebeyehu Feleke, (2002). Study on the effect of physical factors and soil types on the development and food preference of sorghum chafer, *Pachnoda interrupta* (Oliver) Coleoptera: Scarabaeidae. MSc. Thesis, Addis Ababa University.

Gibbs, R. D. (1974). Chemotaxonomy of flowering plants. McGill-Queen`s University Press, Cambridge.

Giblin-Davis, R.M., Weissling, T.J., Oehlschlager, A.C. and Gonzalez, L. (1994). Field responses of (Coleoptera: Curculionidae) to its aggregation pheromone and fermenting plant volatiles. *Floida. Entomol*. **77**:196-111.

Giliomee, J.H. and Donaldson, J.M. (1992). Biology and Rearing Techniques for Twenty-Two Species of Cetoniinae (Coleoptera: Scarabaeidae). Technical Communication Department of Agricultural Development, Republic of South Africa. No. 237. pp. 122.

Grunshaw, J.P. (1992). Field studies on the biology and economic importance of *Pachnoda interrupta* (Coleoptera: Scarabaeidae) in Mali West Africa. *Bull*. *Ent*. *Res.* **82**:19-27.

Gullan, P.J. and Cranston, P.S. (1994). Insects: an outline of entomology. Chapman and Hall, London.

Hammack, L. (1997). Attractiveness of synthetic corn volatiles to feral northern and western corn rootworm beetles (Coleoptera: Chrysomelidae). *Environ. Entomol.* **26**:311-317.

Hansson, B.S. and Christensen, T.A. (1999). Functional characterstics of the antennal lobe, In: *Insect olfaction*, pp. 125-161, (Hansson, B.S., ed), Springer, Verlag, Berlin.

Hansson, B.S., Larsson, M.C. and Leal, W.S. (1999). Green leaf volatile-detecting olfactory receptor neurons display very high sensitivity and specificity in a scarab beetle. *Physiol. Entomol.* **24**:121-126.

Harari, A. R., Ben-Yakir, D. and Rosen, D. (1994). Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* **20**:361-371.

Hardie, J., Isaacs, R., Pickett, J.A., Wadhams, L. J. and Woodcok, C.M. (1994). Methyl salicylate and (-)-(lR, 5S)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae* Scop (Homoptera: Aphididae). *J. Chem. Ecol.* **20**:2847-2853.

Hare, J.D. (1998). Bioassay methods with terrestrial invertebrates. In: *Methods in Chemical Ecology, vol 2, Bioassay Methods,* pp.212-269 (Millar, J.G. and Haynes, K.F. eds.), Kluwar Academic Publishers, Bosten, Dordrecht, London.

Harris, V.E., and Todd, J.W. (1980). Male-mediated aggregation of male female and 5th-instar southern green stink bugs and concomitant attraction of a tachinid parasite, *Ttichopoda pennipes. Entomol. Exp. Appl.* **27**:117-126.

Hartlieb, E. and Anderson, P. (1999). Olfactory-released behaviors. *In: Insect olfaction,* pp. 315-349, (Hansson, B.S. ed). Springer Verlag, Berlin, Heidelberg.

Hartlieb, E. and Rembold, H. (1996). Behavioral response of female *Helicoverpa armigera* HB. (Lepidoptera: Noctuidae) moths to synthetic pigeon pea (*Cajanus cajan* L.) kairomone. *J. Chem. Ecol*. **22**:821-837.

Held, D.W. and Potter, D.A. (2004). Floral characteristics affect susceptibility of hybrid tea *Rosa* x *hybrida* to Japanese beetle (Coleoptera: Scarabaeidae). *J. Econ. Entomol*. **97**:353-360.

Henning, J.A. and Teuber, L.R. (1992). Combined gas-chromatography-electroantennogram characterization of alfalfa floral volatiles recognized by honey-bees (Hymenoptera: Apidae). *J. Econ. Entomol.* **85**:226-232.

Hill, D.S. (1989). Catalogue of Crop Pests of Ethiopia. Bulletin No.1. Alemaya University of Agriculture, Alemaya, Ethiopia, 104 pp.

Hillbur, Y. (2001). Tracking the tiny: Identification of sex pheromone of the pea midge as prerequisite for pheromone based monitoring. *PhD. Dissertation, Swedish University of Agricultural Sciences*.

Hillbur, Y., El-Sayed, A., Bengtsson, M., Löfqvist, J., Biddle, A., Plass, E. and Francke, W. (2000). Laboratory and field study of male midges, *Contarinia pisi*, to synthetic sex pheromone components. *J. Chem. Ecol*. **26**:1941-1952.

Hiwot Lemma (2000) Historical background on the pest status and control of sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae) in Ethiopia. In: *Proceedings of the workshop on the development of monitoring and control strategy against sorghum chafer, Pachnoda interrupta (Coleoptera: Scarabaeidae) in Ethiopia,* pp. 9-15. 28 February – 2 March, 2000, MOA, Addis Ababa.

Hubel, D.H. (1957). Tungsten microelectrode for recording from single units. *Science* **125**:549-550.

Huber, D.P.W., Gries, R., Borden, J.H. and Pierce, H.D.J. (2000). A survey of antennal response by five species of coniferophagous bark beetle (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. *Chemoecology* **10**:103-113.

Imai, T., Maekawa, M., Tsuchiya, S. and Fujimori, T. (1998). Field attraction of *Hoplia communis* to 2-phenylethanol, a major volatile component from host flowers, Rosa spp. *J. Chem. Ecol.* **24**:1491-1497.

Imai, T., Tsuchiya, S., Maekawa, M., Fujimori, T. and Leal, W.S. (1997). Methyl anthranilate, a novel attractant for the soybean beetle, *Anomala rufocuprea,* Motschulsky (Coleoptera: Scarabaeidae). *Appl. Entomol. Zool.* **32**:45-48.

Jaffé, K., Sánchez, P., Cerda, H., Hernández, J.V., Jaffé, R., Urdaneta, N., Guerra G., Martínez, R. and Miras, B. (2000). Chemical ecology of the palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): Attraction to host plants and to a male-produced aggregation pheromone. *J. Chem. Ecol*. **19**:1703-1720.

Jago, N.D. (1993a). Millet pests of the Sahel: biology, monitoring and control. Millet pests of the Sahel: Biology, monitoring and control. pp. 66.

Jago, N.D. (1993b). Methods of evaluation of harvest losses in millet. Bulletin–Natural Resources Institute, No. 62. pp. 65.

Jago, N.D. (1995). Population monitoring and crop loss assessment in integrated pest management of panicle pests of sorghum and pearl millet. In: *Proceedings of an international consultative workshop on panicle insect pests of sorghum and*

pearl millet, 4-7 October, 1993, pp. 103-113. (Nwanze, K.F, Youm, O. eds). ICRISAT Sahelian Centre, Niamey, Niger. Andhra Pradesh, India: ICRISAT.

Jago, N.D., (1991). IPM in the Sahelian zone, peasant-level farm environment of north-west Mali. In: *Proceedings of a Seminar on Crop Protection for Resource Poor Farmers*, 4-8 November, 1991. pp. 25-32 (Gibson RW, Sweetmore A, eds). East Sussex, UK: Technical Centre for Agricultural and Rural Cooperation and Natural Resources Institute.

James, D.G. (2003). Synthetic herbivore-induced plant volatiles as field attractants for beneficial Insects. *Environ. Entomol.* **32**:977-982.

James, D.G. (2005). Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *J. Chem. Ecol.* **31**:481-495.

Jennings, W. (1987). Analytical gas chromatography, Academic Press, London.

Jönsson, M. (2005). Responses to oilseed rape and cotton volatiles in insect herbivor and parasitoids. PhD dissertation. Alnarp, Sweden.

Jönsson, M. and Andersson, P. (1999). Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. *Physiol. Entomol.* **24**:377-385.

Jordan, M. J. Tandon, K.; Shaw, P. E. Goodner, K. L. J. (2001). Aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). *Agric. Food Chem*. **49**:4813-4817.

Jordán, M.J., Tandon, K., Shaw, P.E. and Goodner, K.L. (2003). Aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass

spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). *J. Agri.c Food Chem.* **51**:1421-1426.

Jutsum, A.R. and Gordon, R.F.S., (1989). Insect pheromones in plant protection. John Wiley and Sons Ltd, Chichester.

Kafka, W.A. (1987). Similarity of reaction spectra and odor discrimination: single receptor cell recordings in *Antheraea polyphemus* (Saturniidae). *J. Comp. Physiol.* **161**:867-880.

Kaissling, K-E. (1987). Peripherial mechanisims of pheromone reception in moths. *Chem. Senses*. **21**:257-268.

Kassahun Yitaferu, Hiwot Lemma, Tessema Megenassa and Harari, A. (2006). The field biology of sorghum chafer: its temporal occurrence and over-seasoning habits. *Pest Mgt. J. Eth.* **10**:1-13.

Keil, T.A. (1999). Morphology and development of the peripheral olfactory organs. In: *Insect Olfaction*, pp. 5-47, (Hansson, B.S. ed), Springer, Berlin.

Kessler, A. and Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science*. **291**:2141–2144.

Kevan, P.G. and Baker, H.G. (1983). Insect as flower visitors and pollinators. *Annu. Rev. Entomol.* **28**:407-453.

Klein, M.G. and Edwards, D.C. (1989). Captures of *Popillia lewisi* (Coleoptera: Scarabaeidae) and other scarabs on Okinawa with Japanese beetle lures. *J. Econ. Entomol*. **82**:101-103.

Klein, M.G. and Lacey, L.A. (1999). An attractant trap for autodissemination of entomopathogenic fungi into populations of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae). *Biocon. Sci. Technol.* **9**:151-58.

Klein, M.G., Ladd, T.L.J. and Lawrence, K.O. (1973a). Simultaneous exposure of phenethyl propionate, Eugenol 7: 3 and virgin female Japanese beetles as a lure. *J. Econ. Entomol.* **66**:373-374.

Klein, M.G., Lawrence, K.D. and Ladd, T.L. (1973b). A modified trap of Japanese beetle. *J. Econ. Entomol*. **66**:275-276.

Knudsen, J. T. and Tollsten, L. (1993). Trends in floral scents chemistry in pollination syndromes: floral scents composition in moth pollinated taxa. *Biol*. *J*. *Linn*. *Soc*. **113**:263-284.

Knudsen, J. T., Eriksson, R., Gershenzon, J. and Stahl, B. (2006). Diversity and distribution of floral scent. *Botan. Rev.* **72**:1-120.

Knudsen, J.T., Tollsten, L. and Bergström, G. (1993). Floral scents - A checklist of volatile compounds isolated by headspace techniques. *Phytochemistry* **33**:253-280.

Krikken, J. (1994). A new key to the Supragenic taxa in the beetle family Cetoniinae, with annotated list of the genera, *Z. Verh. Leiden.* **210**:1-75.

Ladd, T. L., Klein, M. G., and Tumlinson, J.H. (1981). Phenylethyl propionate + eugenol + geraniol (3:7:3) and Japonilure: a highly effective joint lure for Japanese beetles. *J. Econ. Entomol.* **74**:665-667.

Ladd, T.L, Mcgovern, T.P., Beroza .M, Buriff, C. R. and Klein, M.G. (1976). Japanese beetles: attractancy of phenylethyl propionate_ eugenol (3:7) and synthetic eugenol. *J. Econ. Entomol*. **69**:468-470.

Ladd, T.L. and McGovern, T.P. (1980). Japanese beetle: a superior attractant, phenethyl propionate +eugenol + geraniol 3:7:3. *J. Econ. Entomol.* **73**:689-691.

Ladd, T.L., and Klein, M.G. (1986). Japanese beetle (Coleoptera: Scarabididae) response to color traps with phenethyl propionate + eugenol + graniol (3: 7: 3) and japonilure. *J. Econ. Entomol.* **79**:84-86.

Ladd, T.L. (1986). Enhancement of Lures for Japanese Beetles (Coleoptera: Scarabaeidae) by Eugenol and Japonilure. *J. Econ. Entomol*. **79**:405-409.

Lamarque, A.L., Maestri, D.M., Zygadlo, J.A. and Grosso, N.R. (1998). Volatile constituents from flowers of *Acacia caven* (Mol.) Mol. var. *caven*, *Acacia aroma* Gill. ex Hook., *Erythrina crista-galli* L. and *Calliandra tweedii* Benth. *Flav. Fragr. J.* **13**:266-268.

Larsson, M. C. (2001). Neural interfaces to the odor world of scarab beetles. *PhD. Dissertation, Lund University, Sweden*.

Larsson, M. C., Leal, W. S., and Hansson, B. S. (1999). Olfactory receptor neurons specific to chiral sex pheromone components in male and female *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *J. Comp. Physiol.* **184**:353-359.

Larsson, M.C., Leal, W.S., and Hansson, B.S. (2001). Olfactory receptor neurons detecting plant odors and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *J. Insect Physiol.* **47**:1065-1076.

Larsson, M.C., Stensmyr, M.C., Bice, S.B. and Hansson, B.S. (2003). Attractiveness of electrophysiologically active fruit and flower volatiles to *Pachnoda marginata* (Coleoptera: Scarabaeidae). *J. Chem. Ecol*. **29**:1253-1268.

Leaky, C.L.A. and Wills, J.B. (1977). Food Crops of the Lowland Tropics. Oxford, UK: Oxford University Press.

Leal, W.S. (1998). Chemical ecology of phytophagous scarab beetles. *Ann. Rev. Entomol.* **43**:39-61.

Leal, W.S. (1999). Scarab beetles. In. *Pheromone of non-Lepidopteran insects associated with agricultural plant*. pp. 51-68. (Hardie, J. and Minksed, A.K. ed). CABI Publishing, UK.

Leal, W.S. and Mochizuki, F. (1993). Sex pheromone reception in scarab beetle *Anomala cuprea*: enantiomeric discrimination by sensilla placodea. *Naturwissenschaften* **80**:278–281.

Leal, W.S., Hasegawa, M. and Sawada, M. (1992a). Identification of *Anomala schonfeldti* sex pheromone by high-resolution GC behavior bioassay. *Naturwissenschaften* **79**:518–519.

Leal, W.S., Mochizuki, F., Wakamura, S. and Yasuda, T. (1992b). Electroantennographic detection of *Anomala cuprea* Hope (Coleoptera: Scarabaeidae) sex pheromone. *Appl. Entomol. Zool.* **27**:289–291.

Leal, W.S., Kuwahara, S., Ono, M. and Kubota, S. (1996). (*R, Z*)-7, 15-Hexadecadien-4-olide, sex pheromone of the yellowish elongate chafer, *Heptophylla picea*. *Bioorg. Med. Chem.* **4**:315–321.

Leal, W.S., Ono, M., Hasegawa, M. and Sawada. M. (1994). Kairomone from dandelion, *Traxacum officinale*, attractant for scarab beetle *Anomala octiescostata*. *J. Chem. Ecol.* **20**:1697–1704.

Leal, W.S., Shi, X., Liang, D., Schal, C. And Meinwald, J. (1995). Application of chiral gas chromatography with electroantennographic detection to the determination of the stereochemistry of a cockroach sex pheromone. *Proc. Nat. Acad. Sci.USA*. **92**:1033-1037.

Lock, C., Mahmoud, M. and Sidibe, A. (1988). Agricultural Economics Report. Mali millet pest control project. Chatham, UK: Natural Resources Institute, R1521 (R).

Lopes, O., Barata, E.N., Mustaparta, H. and Araujo, J. (2002) Fine structure of antennal sensilla basiconica and their detection of plant volatiles in the eucalyptus woodborer, Phoracantha semipunctata Fabricius (Coleoptera: Cerambycidae). *Arthr. Struc. Develop.* **31**:1-13.

Loughrin, J.H., Potter, D.A. and Hamilton-Kemp, T.R. (1998). Attraction of Japanese beetles (Coleoptera: Scarabaeidae) to host plant volatiles in field trapping experiments. *Environ. Entomol*. **27**:395–400.

Loughrin, J.H., Potter, D.A. and Hamilton-Kemp, T.R. (1995). Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *J. Chem. Ecol*. **21**:1457-1467.

Loughrin, J.H., Potter, D.A., Hamilton-Kemp, T.R. and Byers, M.E. (1996). Roll of feeding-induced plant volatiles in aggregative behavior of Japanese beetle (Coleoptera: scarabaeidae). *Environ. Entomol*. **25**:1188-1191.

Loughrin, J.H., Potter, D.A., Hamilton-Kemp, T.R. and Byers, M.E. (1997a). Diurnal emission of volatile compounds by Japanese beetle damaged grape leaves. *Phytochemistry* **45**:919-923.

Loughrin, J.H., Potter, D.A., Hamilton-Kemp, T.R. and Byers, M.E. (1997b). Response of Japanese beetle (Coleoptera: Scarabaeidae) to leaf volatiles of susceptible and resistant maple species. *Environ. Entomol.* **26**:334-342.

Ma, W.L. and Visser, J.H. (1978). Single unit analysis of odor quality coding by the olfactory antennal receptor system of the Colorado beetle. *Entomol. Exp. Appl.* **24**:320-333.

Macku, C. and Jennings, W.G. (1987). Production of volatiles by ripening bananas. *J. Agri. Food Chem*. **35**:845-848.

Maekawa, M., Imai, T., Tsuchiya, S., Fujimori, T. and Leal, W.S. (1999). Behavioral and electrophysiological responses of the soybean beetle, *Anomala rufocuprea* Motschulsky (Coleoptera: Scarabaeidae) to methyl anthranilate and its related compounds. *Appl. Entomol. Zool.* **34**:99-103.

Masson, C. and Mustaparta, H. (1990). Chemical information processing in the olfactory system of insects. *Physiol. Rev.* **70**:199-245.

Matthews, M. and Jago N.D. (1993). Millet pests of the Sahel: an identification guide. Millet pests of the Sahel: an identification guide. pp. 80.

McGovern, T.P. and Ladd, T.L. (1984). Japanese beetle (Coleoptera: Scarabaeidae) attractant: Tests-with eugenol substitute and phenethyl propionate. *J. Econ. Entomol*. **77**: 370-373.

McIndoo, N.E. (1926). An insect olfactometer. *J. Econ. Entomol*. **19**:545-571.

Metcalf, R.L. and Metcalf, E.R. (1992). Plant kairomones in insect ecology and control. Routledge, Chapman and Hall Inc, New York.168 pp.

Millar, J.G. and Sims, J.J. (1998). Preparation, cleanup, and preliminary fraction of extracts. In: *Methods in Chemical Ecology, vol 1, Chemical methods*, pp. 1-37, (Millar J.G. and Kenneth, F. H., eds), Kluwer Academic Publishers, Norwell, Massachusetts, USA.

MOA and EARO**.** (1999). The significance, distribution and control of sorghum chafer, *Pachnoda interrupta* (Olivier) Coleoptera: Scarabaeidae in Amhara and Afar regions. 10-13 March, 1999, MOA and EARO, Addis Ababa, Ethiopia. 71 pp.

MOA. (2003). Proceedings of a Workshop on Recent Developments in Sorghum Chafer, *P. interrupta* (Olivier) Research. June 25-26, 2003, Crop Protection and Regulatory Department (CPPTRD), Addis Ababa, Ethiopia. 65 pp.

Moorhouse, J.E., Yeadon, R., Beevor, P.S. and Nesbitt, B.F. (1969). Method for use in studies of insect chemical communication. *Nature*. **223**:1174-1175.

Murai, T., Imai, T. and Maekawa, M. (2000). Methyl anthranilate as an attractant for two thrips species and the thrips species parasitods *Ceranisus menes*. *J. Chem. Ecol*. **26**:2557-2565.

Nordlund, D.A. (1981) Semiochemicals: a review of the terminology. In: *Semiochemicals: their role in pest control*, pp 13-23, (Nordlund, D.A., Jones, R.L. and Lewis, W.J., eds). Wiley and Sons, New York.

Nordlund, D.A. and Lewis, W.J., (1976). Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *J. Chem. Ecol*. **2**:211–220.

Pelayo, C., Vilas-Boas, E.V.B., Benichou, M. and Kader, A.A. (2003). Variability in responses of partially ripe bananas to 1-methylcyclopropene *Post harv. Biol. Techno.* **28**:75-86.

Pham-Delègue, M. H., Blight, M. M., Kerguelen, V., Métayer, M., Le Marion-Poll, F., Sandoz, J.C. and Wadhams, L. J. (1997). Discrimination of oilseed rape volatiles by the honeybee: combined chemical and biological approaches. *Entomol. Exp. et Appl.* **83**:87-92.

Phillips, T.W., West, J.R., Foltz, J.L., Silverstein, R.M. and Lanier, G.N. (1984). Aggregation pheromone of the deodar weevil, *Pissodes nemorensis* (Coleoptera: Curculionidae): Isolation and activity of grandisol and grandisal. *J. Chem. Ecol.* **10**:1417-1423.

Pino, J., Almora, K. and Marbot, R. (2003). Volatile components of papaya *(Carica papaya L.,* maradol variety) fruit. *Flavour Fragr. J***. 18**: 492-496.

Pino, J.A., Mesa, J., Muñoz, Y., Martí, M.P. and Marbot, R. (2005).*Volatile components from mango (Mangifera indica L.) cultivars*, *J. Agric. Food* Chem**. 53**:2213-2223.

Potter, D.A. and Held, D.W. (2002). Biology and management of the Japanese beetle. *Ann. Rev. Entomol*. **47:**175-205.

Raguso, R.A. and Light, D.M. (1998). Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and “green-leaf volatiles”. *Entomol. Exp. Appl.* **86**:287-293.

Raguso, R.A. and Pellmyr, O. (1998). Dynamic headspace analysis of floral volatiles: A comparison of methods. *Oiokos.* **81**:238-254.

Rana, B.S. and singh, B.U. (1995). Insect Pests of Sorghum and Millet Panicles in Asia. In: *Proceedings of an international Consultative Workshop on Panicle Insect Pests of Sorghum and Pearl Millet*, 4-7 October. 1993, pp. 57-67. (Nwanze, K.F., and Youm, O. eds) ICRISAT, Sahalian Center, Niamey, Niger.

Ratnadas, A. and Ajayi, O. (1995). Panicle Insect pests of sorghum in west Africa. Pages 29- 38-113 In: *Panicle Insect Pests of Sorghum and Pearl Millet: Proceedings of an International Consultative Workshop*, 4-7 Oct. 1993, ICRISAT Sahalian Center, Niamey, Niger (Nwanze, K.F., and Youm, O. eds). Patanchera 502 324, Andhara Pradesh, India: International Crops Research for Semi-Arid Tropics.

Reed D. K., Lee M. H., Kim S. H. and Klein M. G. (1991). Attraction of scarab beetle populations (Coleoptera: Scarabaeidae) to Japanese beetle lures in the Republic of Korea. *Agric. Ecosys. Environ*. **36**:163-174.

Renwick, J.A.A. (1989). Chemical ecology of oviposition in phytophagous insects. *Experientia*. **45**:223-228.

Rigout, J. (1989). Beetles of the World, Vol 9. *Sciences Nat, Venette.*

Risbec, J. (1950). The entomological fauna of crops in Senegal and French Sudan. Work of the Entomology Laboratory of the Sudanese Sector of Agronomic Research. Paris, France: Gouvernment Général de l'Afrique Occidentale Française, pp. 9-500.

Ritcher, P.O. (1958). Biology of Scarabaeidae. *Ann. Rev. Entomol.* **3**:311–334.

Ruther, J. (2004). Male-biassed response of garden chafer, *Phyllopertha horticola* L. to leaf alcohol and attraction to both sexes to floral plant volatiles. *Chemoecology*. **14**:187-192.

Ruther, J. and Mayer, C.J. (2005) Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. *Entomol. Exp. Appl*. **115**:51-59.

Ruther, J. and Tolasch, T. (2004). Attraction of garden chafer, *Phyllopertha horticola*, to floral Japanese beetle lure. *J. App. Entomol.* **128**:158-160.

Ruther, J., Meiners, T. and Steidle, J.L.M. (2002). Rich in phenomena lacking in terms. A classification of kairomones, *Chemoecology.* **12**:161-167.

Sastawa, B.M. and Lale, N.E.S. (2000). Efficacy of host plant resistance, sowing date modification and intercropping as methods for the control of *Pachnoda interrupta* (Olivier) in pearl millet in the Nigerian Sudan savanna. *J. Arid Environ*. **46**:249-262.

Schmutterer, H. (1969). Pests of Crops in North East and Central Africa: with Particular Reference to the Sudan. Gustav Fisca Verlag, Stutgart. Portland USA. pp 124-125.

Schneider, D. (1957a). Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. *Experientia* **13**:89-91.

Schneider, D. (1957b). Elektrophysiologische Untesuchungen von Chemo- und Mechanotezeptorin der Antenne des Seidenspinners *Bombyx mori* L. *Z. Vergl. Physiol*. **40**:8-41.

Schneider, D., Lacher, V., and Kaissling, K.E. (1964). Die reaktionsweise und das reaktionsspektrum von riechzellen bei *Antheraea pernyi* (Lepidoptera, Saturniidae). *Z. Vergl. Physiol.* **48**:632-662.

Scholtz, C.H. and Chown, S.L. (1995). The evolution of habitat use and diet in the Scarabaeoidea: a phylogenetic approach. In: *Biology, Phylogeny and Classification of Coleoptera. Papers Celebrating the 80th Birthday of Roy A. Crowson*. pp. 355-374, (Pakaluk, J. and Slipiñski, A. eds). *Museum i Instytut Zoologii PAN*, Warsaw.

Schoonhoven, L. M., Jermy, T. and Van Loon, J.J.A. (1998). Insect-Plant Biology. From physiology to evolution. Chapmann & Hall, London.

Selzer, R. (1981). The processing of a complex food odor by antennal olfactory receptors of *Periplaneta americana*. *J. Comp. Physiol.* **144**:509-519.

Seneshaw Aysheshum and Mulugeta Negeri (2002). Study on the Biology of sorghum chafer, *P. Interrupta* (Coleopter: Scarabaeidae) under laboratory condition. *Pest Manag. J. Ethio.* **6**:31-36.

Seneshaw Aysheshum. (2001). Activity report on insect pest msnsgement with fungi: A mass production technique for farmers. Cooperative development research project C-16-125. Ambo PPRC, Ethiopia.

Shawa, J., Ellingham, J. and Birch, E.J. (1983). Volatile constituents of feijoa-headspace analysis of intact fruit. *J. Sci. Food Agri*. **34**:743-747.

Shiota, H. (1993). New ester components in the volatiles of banana fruit (Musa sapientum L). *J. Agric Food Chem*. **41**:2056-2062.

Shulaev, V., Silvermann, P., and Raskin, I. (1997). Airborne signaling by methyl salicylate in plant pathogen resistance, *Nature* **385**:718–720.

SPSS for Windows, Version 13.0. (2001) Chicago: SPSS Inc.

Stengl, M., Ziegelberger, G., Boekhoff, I. and Krieger, J. (1999). Perireceptor events and transduction mechanisms in insect olfaction. In: *Insect Olfaction*, pp. 49-66, (Hansson B.S., ed.). Springer-Verlag Berlin.

Stensmyr, M.C, Giordano, E., Balloi, A., Angioy A-M. and Hansson, B.S. (2003). Novel natural ligands for *Drosophila* olfactory neurones. *J. Exp. Biol.* **206**:715-724.

Stensmyr, M.C., Larsson, M.C., Bice, S.B. and Hansson, B.S. (2001). Detection of fruit and flower-emitted volatiles by olfactory receptor neurons in the polyphagous fruit chafer *Pachnoda marginata* (Coleoptera: Cetoniinae). *J. Com. Physiol.* **187**:509-519.

Stranden, M., Rostelien, T., Liblikas, I., Almaas, T.J., Borg-Karlson, A.K., and Mustraparta, H. (2003). Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. *Chemoecology* **13**:143-154.

Strohalm, H., Dregus, A., Wahl, A. And Engel, K. (2007). Enantioselective analysis of secondary alcohols and esters in purple and yellow passion fruits. *J. Agric. Food Chem*. **55**:10339-10344.

Struble, D.L. and Arn, H. (1984). Combined gas chromatography and electroantenogram recording of insect olfactory responses. In: *Techniques in Pheromone Research*, pp. 161-178. (Hummel, H.E. and Miller, T.A., eds). Springer-Verlag, New York.

Szendrei, Z. and Isaacs, R. (2005). Do plant cues influence the oviposition behavior of Japanese beetles? *Entomol. Exp. Appl.* **117**:165-174.

Tasin, M., Anfora, G., Ioriatti, C., Carlin, S., Decristofaro, A., Schmidt, S., Bengtsson, M., Versini, G. and Witzgall, P. (2005). Antennal and behavioral responses of grapevine moth, *Lobesia botrana,* females to volatiles from grapevine. *J. Chem. Ecol*. **31**:77-87.

Tasin, M., Bäckman, A.-C., Bengtsson, M., Loriatti, C. and Witzgall, P. (2006). Essential host plant cues in the grapevine moth. *Naturwiss.* **93**:141-144.

Thiery, D., Bluet, J.M. Phamdelegue, M.H. Etievant, P. and Masson, C. (1990). Sunflower aroma detection by the honeybee—study by coupling gas-chromatography and electroantennography. *J. Chem. Ecol*. **16**:701-711.

Thomas, M. and Waage, J. (1966). Integration of biological control and host plant resistance breeding; A Scientific Literature. CAB international and CTA.

Todd, J. L., and Baker, T.C. (1993).Response of single antennal neurons of female cabbage loopers to behaviorally active attractants. *Naturwissenschaften* **80**:183-186.

Todd, J.L. and Baker, T.C. (1999). Function of peripheral olfactory organs. In: *Insect olfaction*, pp. 67-96. (Hansson, B.S., ed). Springer-Verlag, Berlin.

Tollsten, L. and Bergström, G. (1988). Headspace volatile of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry*. **27**:4013-4018.

Troure, K, Yehouenou, A. (1995). Les insects de I'epi de mil en Afrique de I'Ouest. In: *Proceeding of an International Consultative Workshop on Panicle Insect Pests of Sorghum and Pearl Millet, 4-7 October, 1993*, (Nwanze KF, Youm O, eds). ICRISAT Sahelian Centre, Niamey, Niger. Andhra Pradesh, India: ICRISAT.

Tsedeke Abate (1988). Insect and mite pests of horticultural and miscellaneous plants in Ethiopia. IAR Hand Book No. 1, Institute of Agricultural Research. Addis Ababa. 115 pp.

Tumlinson, J.H., Klein, M.G, Doolittle, R.E, Ladd, T.L. and Proveaux, A.T. (1977). Identification of the female Japanese beetle sex pheromone: inhibition of male response by an enantiomer. *Science* **197**:789–792.

Turlings, T.C.J., Bernasconi, M., Bertossa, R., Bigler, F., Caloz, G. and Dorn, S. (1998). The induction of volatile emissions in maize by three herbivore species with different feeding habits: Possible consequences for their natural enemies. *Biol. Control* **11**:122–129.

Umano K., Hagi, Y., Nakahara, K., Shoji, A. and Shibamato, T. (1992). Volatile constituents of green and ripened pineapple (*Ananas comosus* (L) Merr.). *J. Agri. Food Chem.* **40**:599-603.

Van Alphen, J.J.M and Jervis, M.A. (1996). Foraging behavior. In: *Insect natural enemies , Practical Approaches to Their study and Evaluation*, pp.1-62. (Jervis, M. and Kidd, N. eds.), Chapman and Hall, London.

Vinson, S.B. (1998). The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species, *Biolo. Cont.* **11**:79-96.

Visser, J. H. and Avé, D. A. (1978). General green leaf volatiles in the olfactory orientation of the Colorado beetle *Leptinotarsa decemlineata. Entomol. Exp. Appl.* **24**:738-749.

Visser, J.H. (1986). Host odor perception in phytophagous insects. *Ann. Rev. Entomol*. **31**:121-144.

Vogt, G.R. and Riddiford, L.M. (1981). Pheromone binding and inactivation by moth antennae. *Nature* 293:161-163.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rhetsky, A. and Axel, R. (1999). A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**:725-736.

Wadhams, L.J. (1984). The coupled gas chromatography-single cell recording technique. In: *Techniques in Pheromone Research*, pp. 179-189, (Hummel, H.E. and Miller, T.A., eds). SpringerVerlag, New York..

Wadhams, L.J. (1990). The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones. In: *Chromatography and Isolation of Insect Hormones and Pheromones*, pp. 289-298, (McCaffery, A.R. and Wilson, I.D., eds). Plenum Press, New York.

Wadhams, L.J., Blight, M.M., Kerguelen, V., Le Metayer, M., Marion-Poll, F., Masson, C., Pham-Delegue, M.H. and Woodcock, C.M. (1994). Discrimination of oilseed rape volatiles by honey bee: novel combined gas chromatographic-elecctrophysiological behavioral assay. *J. Chem. Ecol.* **20**:3221-3231.

Wall, C. (1990). Principles in monitoring. In: *Behavior-modifying chemicals for insect management*, pp. 9-23. (Ridgway, R.L., Silverstem, R.M., Inscoe, M.N., eds). Marcel Dekker Inc. New York.

Wibe, A., Borg-Karlson, A.K, Norin, T., and Mustraparta, H. (1997). Identification of plant volatiles activating single receptor neurons in the pine weevil (*Hylobius abietis*). *J. Comp. Physiol. A* **180**:585-595.

Williams, R.N., Mcgovern, T.P., Kelin, M.G., and Fickle, D.S. (1990). Rose chafer (Coleoptera: Scarabaeidae): Improved attractants for adults. *J. Econ. Entomol.* **83**:111-116.

Wyatt, T.D. (2003). Pheromones and animal behavior: Communication by smell and taste. Cambridge University Press, Cambridge.

Yeraswork Yilma. (2000). The importance, distribution and current status of Sorghum chafer, *Pachnoda interrupta* (Olivier) in Amhara Region In*: Proceedings of The Workshop on the Development on Monitoring and Control Strategy Against sorghum shafer, Pachnoda interrupta* (Olivier), (Coleoptera: Scarabaeidae) in Ethiopia. Addis Ababa. pp 24-35.

Yitbarek Wolde-Hawariat and Hiwot Lemma. (2000). Preliminary yield loss assessment on sorghum due to sorghum chafer, *Pachnoda interrupta* (Olivier) in Amhara Region. In: *Proceedings of the Workshop on the Development, Moniotiring and control strategy against sorghum chafer, Pachnoda interrupta* (Olivier), (Coleoptera: Scarabaeidae) in Ethiopia. Addis Ababa. pp 39-43.

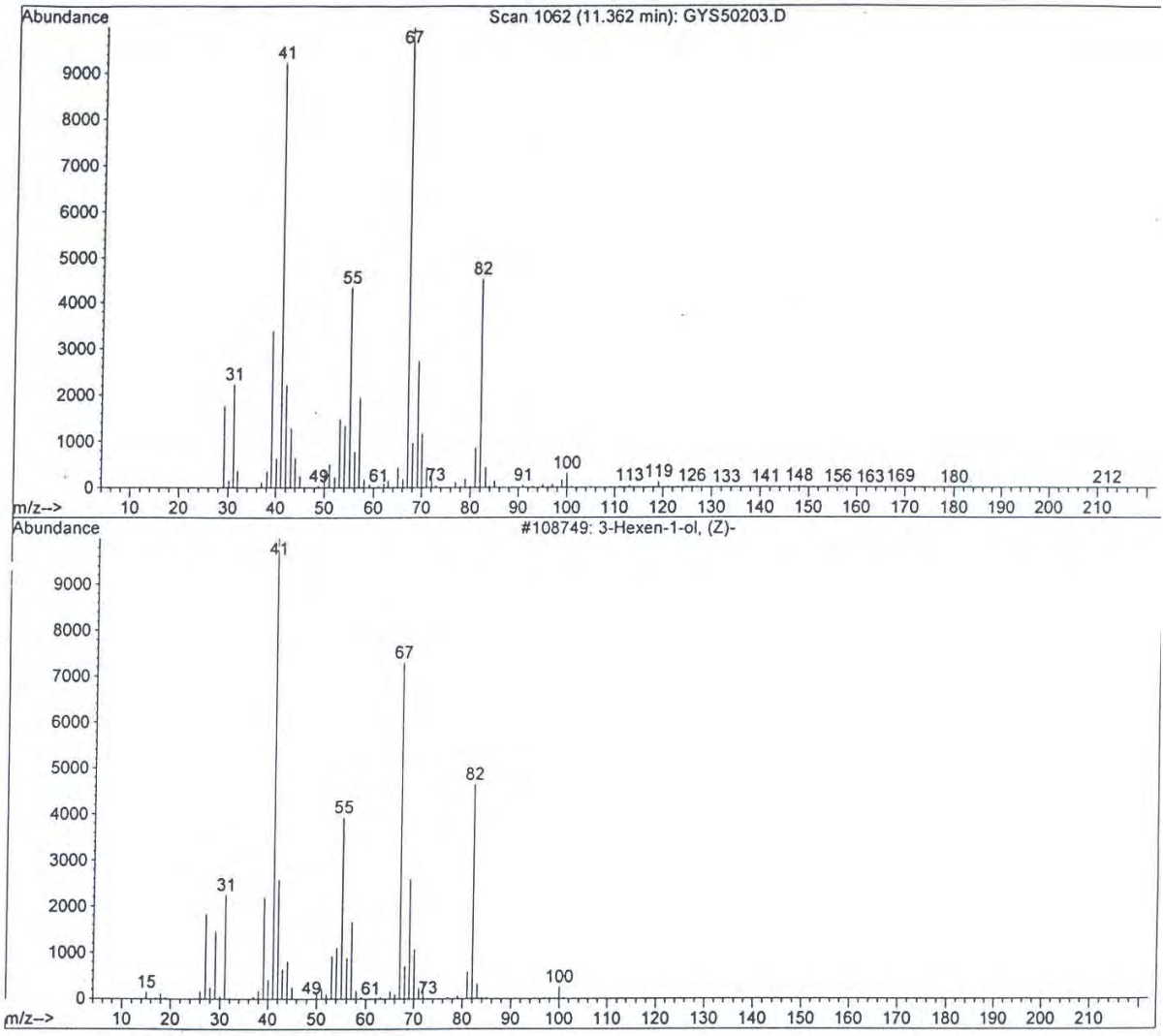
Yitbarek Wolde-Hawariat, Emiru Seyoum, Bekele Jembere, Merid Negash, Hansson, B. S. and Hillbur, Y. (2007). Behavioral and electrophysiological responses of sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae: Cetoniinae), to plant comounds. *Int. J. Trop. Insect Sci*. **27**:53-61.

Zar J. H. (1984) Biostatistical analysis. Prentice Hall Inc. Englewood Cliffs. 718 pp.

Zhang, A., Linn, C.E., Jr., Wright, S., Prokopy, R., Reissig, W. And Roelofs, W.L. (1999). Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *J. Chem. Ecol*. **25**:1221-1232.

10.0 APPENDIX

Appendix 1. Representative mass spectra of (Z)-3-hexen-1-ol a compound found in both sorghum and abutilon headspace extracts



DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other university, and that all sources of material used for the thesis have been duly acknowledged.

Name: Yitbarek Weldehawariat

Signature: _____

Date: 9 May, 2008

Place: Addis Ababa