Development of lures and local traps and their application for field management of *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae) in Sorghum in Ethiopia

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A Thesis Submitted to the Department of Zoological Sciences

Presented in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology (Insect Science)

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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.

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Submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Biology (Insect Science Stream)

By Getnet Atenafu

Department of Zoological Sciences (Insect Science Stream)

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) is one of the major nutritious cereals in Africa widely cultivated in eastern regions including Ethiopia. Sorghum chafer *Pachnoda interrupta* (Coleoptera: Scarabaeidae) is a polyphagous insect whose adults are serious pests of sorghum (*Sorghum bioclor*) in the fields. The damage by the sorghum chafer during the milky stage of the seed contributed to low production of sorghum in many regions of Ethiopia. The adult beetle uses visual and olfactory cues (specific blends of compounds) in their search for food sources, mate selection, and oviposition sites. The present study focused on evaluation of the attractiveness of lures and catch performance of locally designed cheap traps in *P. interrupta* field management strategies in naturally infested regions of northeastern Ethiopia. Optimal and constant release rates of previously identified volatile compounds phenylacetaldehyde, 2,3-butanediol, methyl salicylate, eugenol, isoamyl acetate were determined from dispensers in the laboratory. Field experiments testing the efficacy of different single- and multi-component baits and different types of traps were conducted in close collaboration with farmers. Various blends/single compounds and the natural attractant banana fruit were tested in both the mating season in July and the feeding season for newly emerged adults in October. Different locally affordable trap designs were evaluated and compared with the previously proven efficacy of a commercial Japanese beetle trap. In addition, novel attractant blends were also tested in the field. These blends were synthetic replicas of odors from overripe banana fruit and fermentation volatiles identified by solid-phase micro extraction combined with gas chromatography-mass spectrometry analysis in the laboratory. After a two-choice behavioral bioassay, the promising component blends tested in the laboratory were evaluated in the mating and feeding seasons in 2013. The results of the beetle catch revealed that traps baited with the multiple-dispenser blend of the five compounds, and the mixture of the five compounds formulated in one dispenser, were the best lures. It was also demonstrated that the longevity of the lure in the field coincided with the flying period of *P. interrupta* during the mating and feeding seasons. Among the field tested trap designs, four locally affordable cheap traps were found to be as efficient as the commercially produced Japanese beetle trap in trapping *P. interrupta* . Among the blends from volatile chemicals released from overripe banana and fermentation volatiles, a blend of eleven compounds was proven effective in behavioral responses both in the laboratory choice bioassay and the *P. interrupta* trapping in the field. This indicates that the compounds identified are behaviorally relevant and can be used as potential candidates for further field tests. In conclusion, the work has produced both efficient and cheap local traps baited with best attractant compounds blend.

Dedication

Dedicated to my supervisor Dr. Emiru Seyoum who passed away unexpectedly during the final year of this study

Acknowledgments

I would like to thank my supervisors and co-supervisors Dr. Emiru Seyoum, Dr. Habte Tekie, Dr. Mattias Larsson, Dr. Ylva Hillbur, and Dr. Yitbarek Wolde-Hawariat. I would like to gratefully express my sincere thanks to the late Dr. Emiru Seyoum for accepting me as a PhD student, his financial support from Bio-Innovate Project, advice, and encouragement in all aspects of my research, and especially for convincing me to study the chemical ecology of sorghum chafer. I appreciate his facilitation of my travel to Alnarp, Sweden for chemical ecology laboratory work and courses. I would like to extend my thanks to Dr. Habte Tekie for his facilitation with the department after the death of Dr. Emiru Seyoum. His commitments to arrange the program for examination, valuable comments, editing and formatting the final version of the thesis are unforgettable.

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Acronyms

AAU = Addis Ababa University

CAS # = Chemical Abstract Service number

CSA = Central Statistics Authority

DAs = Development Agents

EARO = Ethiopian Agricultural Research Organization

GC-MS = Gas Chromatogaphy- Mass-spectrometry

JBT= Japanese beetle trap

LAT= locally affordable trap

MOA = Ministry of Agriculture

SPME = Solid-Phase Micro-extraction

Chapter 1

General Introduction

1.1. Background

Sorghum (*Sorghum bicolor* (L.) Moench (Poaceae) is one of the major cereal crops in Africa, which makes the continent the world's largest sorghum producer, and it is primarily a crop of resource-poor smallholder farmers. The crop is adapted to a wide range of precipitation and temperature levels and is produced at various altitudes from sea level to above 2000 m. Sorghum is fifth in production in the world next to wheat, rice, maize and barley and the annual production of sorghum is about 60 million tons, which can be cultivated in about 43 million hectares in the regions (Paterson, 2008).

Sorghum is produced in most part of Ethiopia, particularly in areas with low annual rainfall in arid and semi-arid agro-ecologies, and is believed to have been the crop first domesticated in the country (Thomas and Waage, 1966). It is ranked third in total national cereal-based nutrition after tef (*Eragrostis tef*) and maize (*Zea mays*). This indicates that sorghum contributes a huge supply of important staple food and household income in the country; mainly by smallholder farmers who consume most of the produce themselves. More than 1.7 million hectares of land is covered with sorghum and produced over 3.6 million tons annually (CSA, 2013).

Like any other cereal crop, sorghum is also affected by different pests, mainly insects, during both pre- and post-harvesting periods. Insects such as stalk borers, army worm, sorghum shoot fly, and sorghum chafer are the common pests of sorghum in Ethiopia (Hiwot Lemma, 2000). Among the insect pests, Sorghum chafer, *Pachnoda interrupta* (Olivier) was recorded as the most destructive insect pest of sorghum in the North eastern part of the country over three decades (MOA and EARO, 1999; Hiwot Lemma, 2000). Some farmers in the study sites, however, described that Sorghum chafer was present in their crop fields already five decades ago. Moreover, there are specimens in the Zoological Natural History Museum, Addis Ababa University, collected from different parts of the country that indicate the presence of *P. interrupta* as early as the 1940s. The life history and control measures of this beetle have been described by several authors (Tsedeke Abate, 1988; Grunshaw 1992; MOA and EARO, 1999; Hiwot Lemma, 2000; Yitbarek Wolde-Hawariat *et al.*, 2007; Bengtsson *et al.*, 2009).

Pachnoda interrupta was recorded as an important pest of sorghum and other plants in the North and Eastern parts of the country along the rift valley areas during a country wide survey conducted in the 1970s (Clark and Crowe, 1978). After this survey, the pest was not reported as significant for more than twenty years (Hiwot Lemma, 2000). It became a serious pest of sorghum in 1993 and was noted to increase in population density, geographic distribution and host range (MOA and EARO, 1999). The total area infested by *P. interrupta* in Ethiopia reached 113,739 ha in 2000 (Hiwot Lemma, 2000; Yeraswork Yilma, 2000). Up to 70 % yield loss due to adult *P. interrupta* was recorded on sorghum production in the Amhara Regional State of Ethiopia (Yitbarek Wolde-

Hawariat and Hiwot Lemma, 2000). The beetles feed and damage in the milky stage of sorghum (Figure 1). During major outbreaks, 100% damage has been recorded due to *P. interrupta* in the field (Tsedeke Abate, 1988), even on insecticide treated sorghum fields (Yeraswork Yilma, 2000).

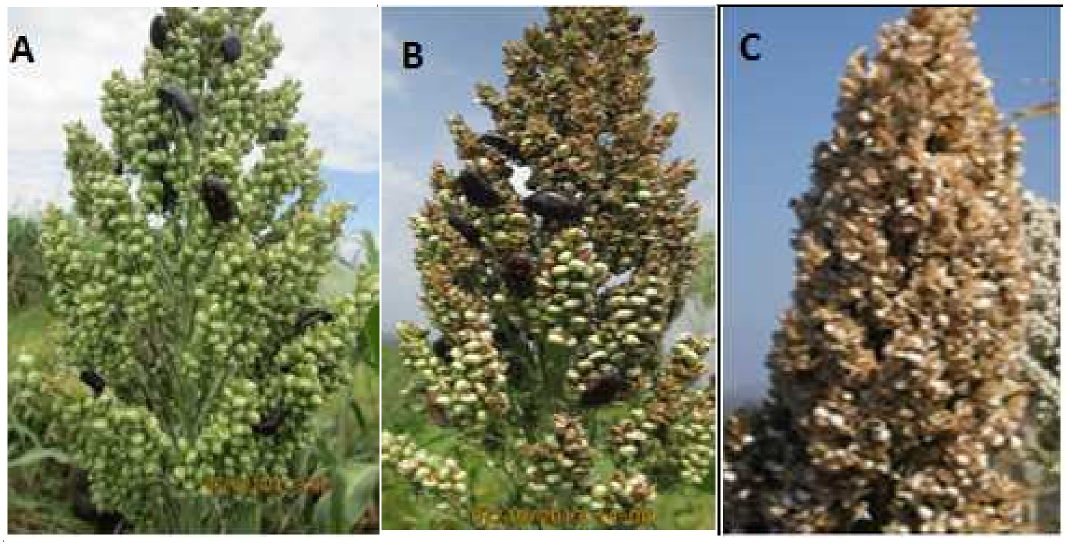


Figure 1. Example of how Sorghum chafers, *P. interrupta* feed on pre-ripening stages of sorghum seeds (A). Feeding starts from the top part of the sorghum panicle and proceeds downwards (B). Complete damage of the sorghum panicle by the adult beetles (C).

Although sorghum is the main host plant among cultivated crops, *P. interrupta* also attack over 35 crop types (Clark and Crowe, 1978; Hiwot Lemma, 2000; Yitbarek Wolde-Hawariat, 2008), of which a few examples are indicated (Figure 2).

Figure 2. *P. interrupta* adults aggregating and feeding on maize cob (a) *Acacia* flowers (b Abutilon (c) Sunflower (d).

Since the big outbreak (1993) of *P. interrupta*, farmers have been trying to control the pest population using different methods such as bending stalks of sorghum and hand picking adult beetles, smoking, burning compost heaps to kill the larvae and use of chemical insecticides (carbaryl) (Hiwot Lemma, 2000). However, in most cases, these methods have had limited success in reducing beetle populations. Reasons for these failures include continuous re-infestation, the height of the local sorghum varieties that reached 2.5 m above ground, and lack of appropriate spraying equipment (Yitbark Woldehawariat and Hiwot Lemma, 2000; Yitbarek Wolde-Hawariat *et al.,* 2007; Bengtsson *et al*., 2009).

According to Yeraswork Yilma (2000), more than 20,000 liters of pesticides were used on sorghum fields during the 1994 – 1999 periods in the Amhara region of Ethiopia alone. During the present study (2011-2013), farmers used insecticides (Sevin®, a.i. carbaryl) along with other traditional control measures against *P. interrupta* in their sorghum fields. The large amount of pesticides used for control of the beetle incurs higher costs to the majority of the small holder farmers. On the other hand, chemical pesticides affect none target insects like bees. Escalating concern on the adverse effects of pesticides on the environment and health related problems has initiated a strong commitment towards limited and discriminating use of pesticides. This has led to the development of integrated pest management strategies based on sound biological principles in sorghum cultivation to meet the expanding demand of food production for the people (Yitbarek Wolde-Hawariat *et al.*, 2007; Bengtsson *et al*., 2009).

The combination of fruits like banana and guava with carbaryl (lure and kill technique) developed by farmers has long been widely practiced in *P. interrupta*-infested areas without understanding the components of the attractants found in fruits. It has been evident that the volatiles emanating from the fruits like banana (*Musa* spp.) were responsible for attracting the sorghum chafers. Pedigo (1996) also reported that food lures and baits treated with a poison have been used for more than a century to control household pests. Even though the attract-and-kill method works well, the lack of fruits in some regions limit the wide-spread use of the method (Yitbarek Wolde-Hawariat *et al.*, 2007). A more practical and innovative approach for the variability of odor from fruit sources could be the application of standardized synthetic volatile compound baits using locally affordable traps. Yet, this method also has to be confirmed against field data and has to be validated under different environmental conditions.

Semiochemicals that mediate interactions between organisms, including olfactory cues to locate host plants, mates etc., are used widely for insect pest management as it is environmentally sound methods (Gregg *et al*., 2010; Del Socorro *et al.*, 2010). Recent studies reported by Yitbarek Wolde-Hawariat *et al*. (2007) and Bengtsson *et al*. (2009)

have investigated different host volatile attractants and pheromones as attractants for future control measures against *P. interrupta* using attract – and– kill methods. Identified compounds were tested in the field and showed novel possibilities for direct control measures by means of mass trapping of the adult beetle. However, different blends of compounds were not tested in order to increase attraction. In addition, many low-molecular-weight volatiles from fermentation processes comprise entirely new classes of compounds that would have potential to synergize the attraction to currently known attractants, as in other insects attracted to fermenting fruit (Becher *et al.*, 2010). Therefore, volatiles emanating from fermented banana should also receive attention for their potential to attract *P. interrupta*. In order to specifically evaluate the physiological effect of fermentation-related compounds of lower molecular weight in fermented banana, Solid-Phase Micro-Extraction (SPME) techniques has been used for collection of volatile compounds in the present study. It is a dynamic headspace-sampling method without use of solvents that could interfere with physiological and analytical processes (Musteata and Pawliszyn, 2007).

For a compound to be considered as a natural behavior-modifying chemical, its synthetic release rate and elicitation of behavioral response in insects should correspond to levels to the effects of natural biological conditions on the targeted insects (Byers, 1988, Heuskin *et al.*, 2011). Laboratory bioassays using olfactometers, wind tunnels and electroantennograms are the main techniques for the characterization of behavior-modifying compounds in eliciting responses of an insect, including scarab beetles towards volatile compounds (Stensmyr *et al.*, 2001; Larsson *et al.*, 2003). After laboratory bioassays, field studies on the behavior of an insect towards lure-baited traps are very important in semiochemical-based insect control methods (Hansson, 1999; Cork, 2004; Rasmy, 2006).

Yitbarek Wolde-Hawariat *et al*. (2007) evaluated the efficiency of one locally affordable trap against the commercially available Japanese beetle trap (JBT) and found that the JBT caught significantly more *P. interrupta* than the locally produced trap. However, more locally made trap designs different in shape, color and dimensions need to be tested to obtain efficient, cheap and affordable traps.

1.2. Objectives of the study

1.2.1. General objective

To develop and apply standard lures and local trap technologies for the management of Sorghum chafer, *P. interrupta* in field conditions for yield increment in sorghum

1.2.2. Specific objectives

- To evaluate release rate, dispenser formulation and field catch efficiency of putative pheromone and certain host plant volatiles in trapping of *P. interrupta*
- To evaluate the performance of affordable local traps designs baited with novel attractant compounds for field trapping strategies of *P. interrupta*
- To identify odors from banana and fermentation volatiles using SPME techniques combined with GC-MS analysis for attraction of *P. interrupta*

1.3. General materials and methods

1.3.1. Description of the study sites

Field experiments to evaluate the attractiveness of individual synthetic volatile compounds and blends on *P. interrupta* were conducted over a period of three years, from 2011- 2013 at three different locations in North-eastern Amhara Regional State of

Ethiopia. The three villages were selected from representative sorghum growing districts in the area and field experiments were conducted in cropping and non-cropping seasons of sorghum. The field sites were Rasa village (09°57'N, 040°05'E) (Kewot District), Abuare village (10^{0}57’N, 040^{0}03’E) (Bati District), and Mendubo village (10°50'N, 040°05'E) (Dewe-Harewa district) with altitudes of 1351 m, 1383 m and 1206 m above sea level, respectively (Figure 3).

The experiments were conducted in the farmers’ sorghum fields and *Acacia* trees. The study sites are located in the semi-arid agro-ecological zone. The experimental field sites were not treated with pesticides during the study period. Rasa, Abuare and Mendubo villages are 255, 355 and 340 km away from Addis Ababa, respectively. The distance between Kewot and Dewe Harewa is 150 km, and about 15 km between Bati and Dewe Harewa.

The three study sites have 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 is distributed unevenly and the annual temperature ranges from 20°C to 40°C. Similar to the rest of Amhara, the farming system of the study sites is mixed. Major crops grown in the study sites are sorghum, tef, maize, and cowpea. These three districts (Kewot, Bati and Dewe-Harewa) are the areas where *P. interrupta* infestation is most prevalent.

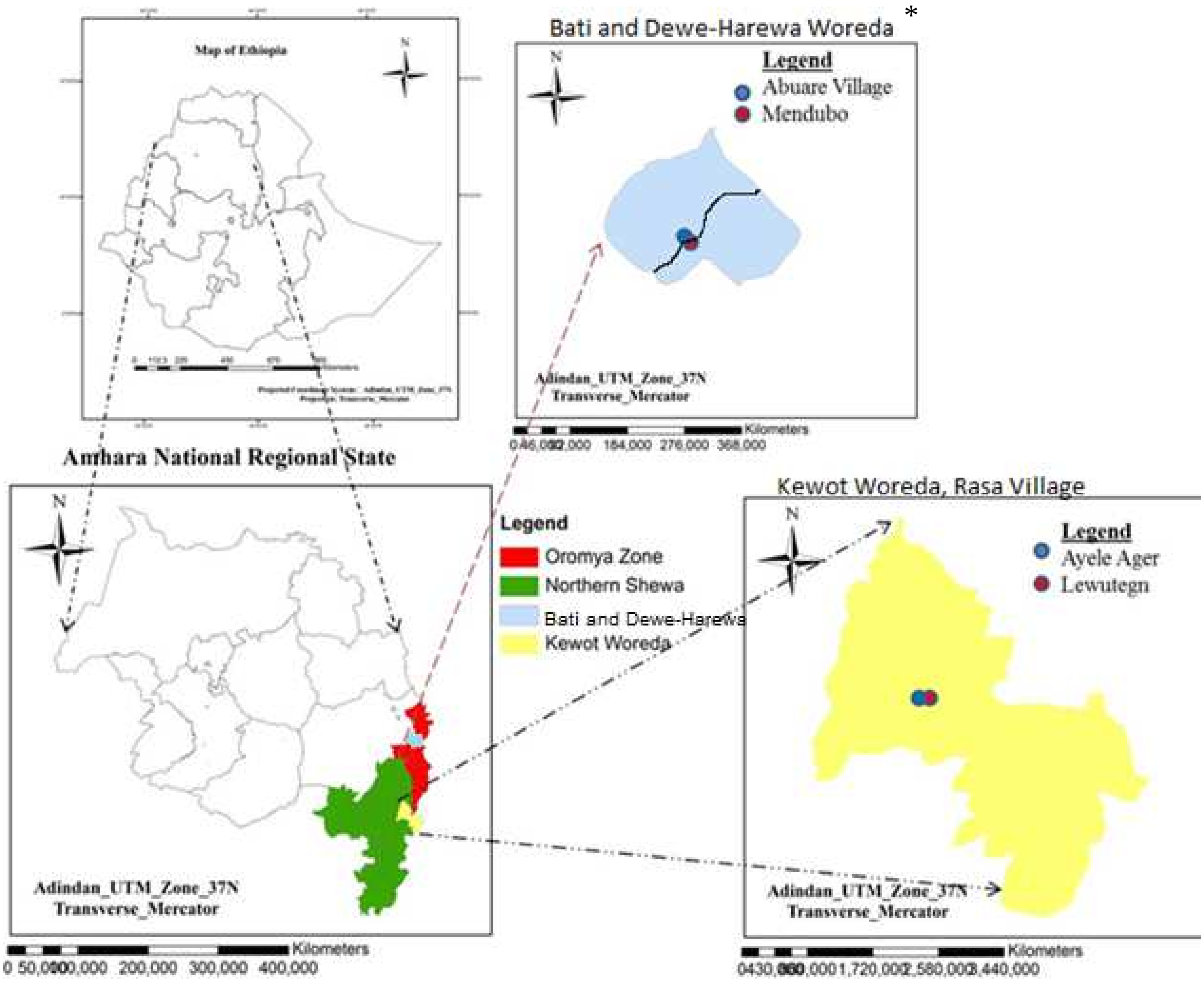


Figure 3. Location of the study villages on the map of Amhara Regional States of Ethiopia. * Woreda = district

1.3.2. Study design and procedures

Natural and synthetic volatile attractant compounds can be used to affect the behavior of *P. interrupta* for mass trapping in attract-and-kill pest management approaches. The main aim of the present thesis was to develop efficient and affordable lures and trap systems for future management studies. This research was initiated to apply the basic findings from previous works (Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009; 2010) on the identification of volatile attractant compounds for the management of the adult beetle. Different locally made and affordable traps were developed and tested for field trapping strategies. Additional compound blends were identified from banana and fermentation volatiles for the attraction of beetles in the laboratory in Alnarp, SLU, Sweden, and tested in the field in Ethiopia. Relevant information on the experimental design, materials used and methodology adopted are described in details in the field and laboratory investigations.

Chapter 1 is a general introduction to the importance and extent of pest problems in sorghum production, significance of *P. interrupta* as a pest of sorghum and available pest management strategies in Ethiopia, and gaps and aims of the present study. Chapter 2 presents an overview of relevant literature with the purpose of consolidating information known about the behavior of the sorghum chafer, applied aspect of the study with generating further information on the topics covered in this research. Chapter 3 presents the release rate of dispensers for tested compounds and evaluation of the catch efficiency of the lures in the field. The effectiveness of individual volatiles and their blends on the attraction of *P. interrupta* under field situations are also elaborated. Chapter 4 deals with evaluation of the performance of different types of locally affordable traps compared with the JBT in the field using the most attractive blend and dispenser identified in chapter 3.

Chapter 5 deals with isolation and identification of odors from overripe banana and fermentation volatiles that mediate attraction of *P. interrupta.* Volatile compounds were collected by SPME methods and identified by GC-MS analysis. Behaviorally active compounds were selected by a behavioral choice bioassay compared with overripe banana and fermentation volatiles that mediate attraction of the beetle. Most of the identified compounds were tested in the form of blends both in the laboratory and in the field. Chapter 6 summarizes the findings and overall conclusions of the PhD study and gives recommendations for future research.

Chapter 2

Literature review

2.1. Biology and ecology of Sorghum chafer, *Pachnoda interrupta* (Olivier)

The Sorghum chafer, *P. interrupta* (Olivier) is a beetle which belongs to the most abundant insect order Coleoptera, family Scarabaeidae and subfamily Cetoniinae (Clark and Crowe, 1978; Grunshaw, 1992). From more than one million species of insects, beetles are the largest order containing of 350 000 species all over the world (Resh and Carde, 2003).

Like other scarab beetles, the genus *Pachnoda* is characterized by its lamellate antennae with asymmetric club-shaped apical antennal segments. *Pachnoda* contains over 130 species world-wide (Krikken, 1984), most of which are found in Africa and a few species in Arabia (Grunshaw, 1992). Nine *Pachnoda* species, one represented by two sub-species have been reported in Ethiopia (Clark and Crowe, 1978). However, the authors have also suggested that there might be more species because Ethiopia is ecologically diverse. Their size range from 12 to 28 mm in length measured from apex of pronotum to apex of elytra and most of them are found in *Acacia* woodlands distributed from 800 to 1800 m.a.s.l. (Clark and Crowe, 1978).

Pachnoda interrupta is a univoltine polyphagous insect pest and exhibits complete metamorphosis between the larval and adult stage (Grunshaw, 1992). According to Yeraswork Yilma (2000), the pest appears two times in a year. The first one occurs following the heavy rains in June and beetles emerge from the breeding sites start to feed on flowers of host plants such as *Acacia* trees and mating, and lay eggs in the rainy season from late June to the end of July depending on the rain situation. The second flight of the pest occurs in September, which coincides with the flowering of the host plants including sorghum and maize (Hiwot Lemma, 2000; Yeraswork Yilma, 2000).

Both sexes of adults have a body length of 12 to 16 mm. The yellow-brown or red-brown spots and stripes on the elytra and pronotum (Figure 4A) of the adult beetles are distinguishing characteristics (Borror *et al.*, 1976). Males can be distinguished from females by the presence of a shallow groove on the underside of the abdomen (Figure 4B), while the abdomen of the female is convex (Clark and Crowe, 1978). The mouthparts of the beetle are small biting mandibles, developed in the front part of the head. They have club-shaped antennae (Grunshaw, 1992; Matthews and Jago, 1993).

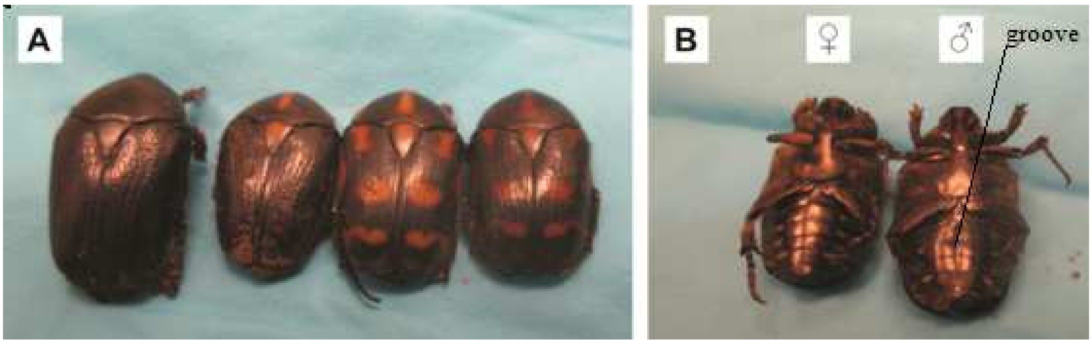


Figure 4. Adult *P. interrupta* color varieties (A), female and male (B)

Both male and female *P. interrupta* feed on flowers of *Acacia* trees and other host plants like abutilon, during the mating season. Mated females lay eggs singly, which become stratified in the oviposition medium where moisture in forest leaf litter and compost heaps is available in the soil (Grunshaw, 1992; Seneshaw Aysheshum and Mulugeta Negeri, 2002). The newly laid eggs are white, ovoid to spherical about 1.40 x 1.23 mm in size and they become brown, swollen and attain a size of 2.0 x 1.77 mm (Grunshaw, 1992).

There are different reports on oviposition rate and duration of egg laying by a single female from studies in the laboratory. A single female lays up to 24 eggs in one night (Grunshaw, 1992) while Seneshaw Aysheshum and Mulugeta Negeri (2002) reported 1.8 eggs per day per beetle; another report by Gebeyehu Feleke (2002) showed that 0.58 eggs were laid per day over 25 days. The diet of the beetles and constant disturbances of egg laying adults have been suggested to determine egg development and rate of oviposition (Giliomee and Donaldson, 1992). Moreover, Asmare Dejen and Yeshitla Merene (2013) reported mean oviposition rate of single females as 1.28 eggs per day for 11 days under field conditions.

Eggs hatch in about two weeks (Yitbarek Wolde-Hawariat, 2008). The newly hatched larvae had a white to yellowish white colour with rows of close setae (Figure 5). The larval body length measures about 30 mm (Grunshaw, 1992; Seneshaw Aysheshum and Mulugeta Negeri, 2002). Larvae consume decaying organic matter in the soil. According to Seneshaw Aysheshum and Mulugeta Negeri (2002), the larval stage lasts between 41 and 71 days with a mean of 55.7 days. Grunshaw (1992) also reported the time taken from egg eclosion to pupation to be 45.3 days. They grow quickly but have low mobility in the first week. As the larvae increase in size, they become active and fast moving on their backs by undulatory locomotion and have well developed mouth parts for chewing. Claws are cylindrical with 6-8 terminal setae. The head width of the first instar larva is 1.17-1.24 mm, the second instar is 2.06 mm and the third instar is 3.05-4.05 mm (Grunshaw, 1992).

When the larva is ready to pupate, it makes an oval cocoon made from the soil cemented with larval saliva (Seneshaw Aysheshum and Mulugeta Negeri, 2002). Pupation takes place within an egg-shaped 13 to 17 mm cocoon. The extent of the pupal stage was reported differently by different scholars. Seneshaw Aysheshum and Mulugeta Negeri (2002) reported as 18.9 days while Gebeyehu Feleke (2002) stated as 24.5 days. Differences have been observed due to variations in temperature regimes used, 28 ± 2 °C (Seneshaw Aysheshum and Mulugeta Negeri, 2002) and 25 °C (Gebeyehu Feleke, 2002), respectively. The adults emerge from their cocoons, make their way to the surface and start to fly off to find food in September-October (Clark and Crowe, 1978). All the developmental stages of *P. interrupta* during its life time are illustrated in figure 5 below.

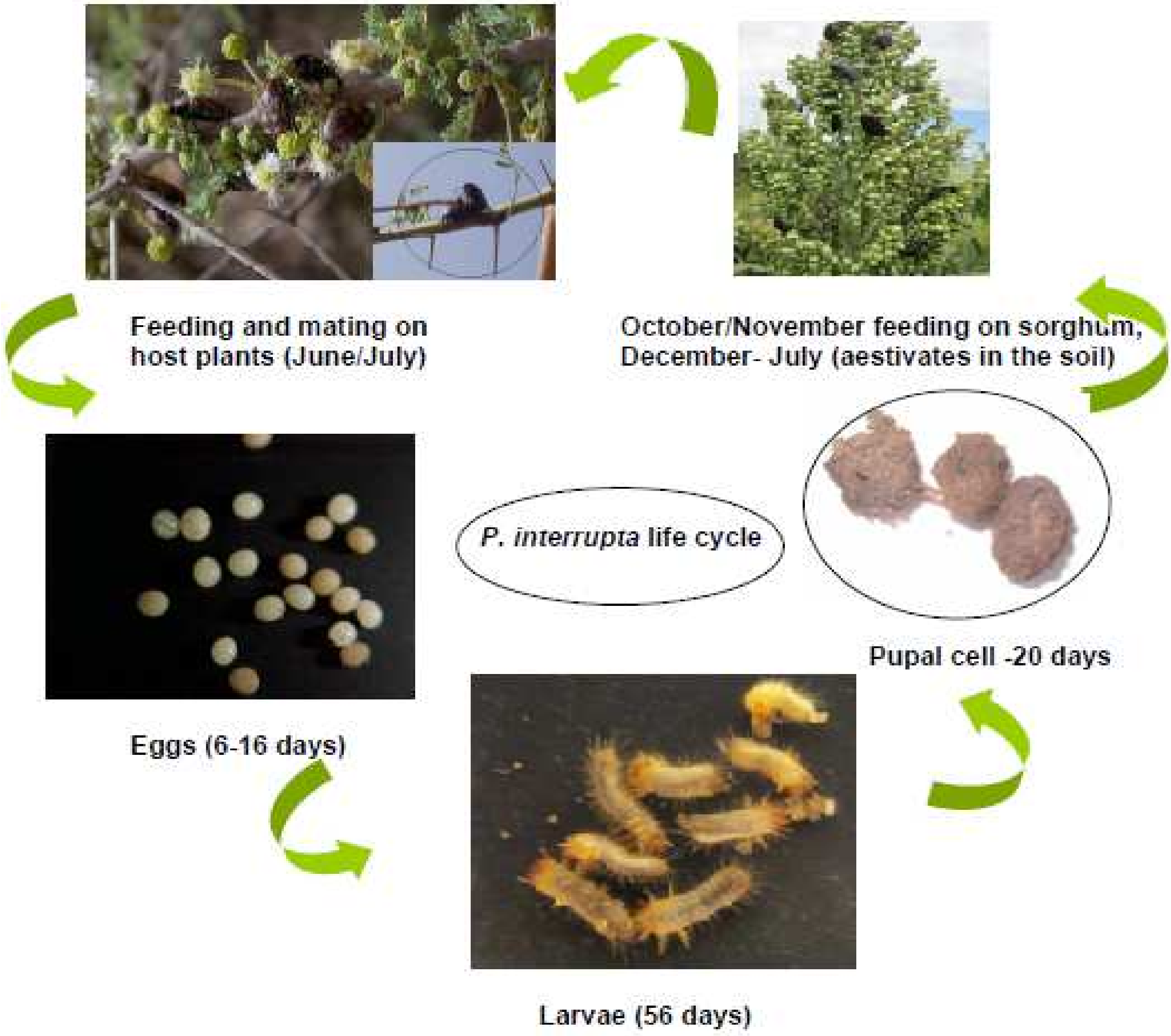


Figure 5. Life cycle of *P. interrupta*. Modified from Yitbarek Wolde-Hawariat, 2008.

2.2. Management options and strategies against *Pachnoda interrupta* (Olivier)

Pachnoda interrupta follows visual cues and odors of specific plant flowers, soft tissue of fruits like banana, guava, and grain seeds (Clark and Crowe, 1978; Jago, 1995). Moreover, MOA and EARO (1999) reported that the beetle is a polyphagous insect which has more than 35 host plant species, feeding on fruits, flowers and seeds, some of which are important crops. Pearl millet is recorded as the main host of *P. interrupta* in Mali, Cameroon, and Nigeria (Grunshaw, 1992; Jago, 1993a, Jago, 1993b; Troure and Yehouienou, 1995; Jago, 1995; Ratnadas and Ajayi, 1995). Moreover, sorghum, maize, sunflower, niger seed, sesame, guava and banana are crop plants on which *P. interrupta* feeds in Ethiopia (MOA and EARO, 1999; Hiwot Lemma, 2000).

September-October is the season for flowering of the host plants of *P. interrupta* including sorghum. Consequently, the beetle starts feeding on the milky stage of sorghum until grains become hard enough to be crushed by the beetle. Adult beetles are diurnal, requiring warm and sunny conditions for their dispersal and searching for suitable hosts. After the feeding season the beetle hides itself in the breeding site soil as a quiescent adult until emerging again as a sexually matured beetle in the coming rainy season (Grunshaw, 1992, Seneshaw Aysheshum and Mulugeta Negeri, 2002).

The different habits of the larvae and adults of *P. interrupta* make it difficult to plan control mechanisms for both life stages where larvae live in the soil and adults feed on different flowering trees and crops. Studies reported by MOA and EARO (1999) show that one or a combination of applied control methods such as lure-and-kill techniques, spraying chemical pesticides, and cultural control measures such as hand picking, smoking have been used by farmers to control the adult beetle.

The lure-and-kill technique developed by farmers is considered to be one of the practically applicable options currently available for reduction of the beetle population. The method involves baiting of locally available materials such as plastic containers or cans with mashed fruits like banana or local brew residue as attractants and a synthetic insecticide (Sevin®, a.i. carbaryl) as a killing agent (MOA and EARO, 1999; Yitbarek Wolde-Hawariat *et al.*, 2007).

Because of the lack of proven alternative control methods, insecticides remain the main control measures for *P. interrupta* in the field. Moreover, the indiscriminate and unscientific use of pesticides has led to associated problems, like pest resurgence, besides environmental and health hazards to man. Pesticides may cause acute and delayed health effects in exposed people. The application of a baited trap for detection and mass trapping would be an alternative method in the monitoring of population size of the beetles and control action (Yitbarek Wolde-Hawariat, *et al.*, 2007; Bengtsson *et al.*, 2009).

2.3. Chemical communication in insects

Chemical signals that are involved in communication, emitted by one individual and trigger specific behavioral responses in another, are referred to as semiochemicals or sometimes called infochemicals. Insects rely on their olfaction as the principal sensory modality for sensing the volatile compounds from the external environment (Hansson, 1999). Detection of infochemicals by insects from plants or other host organisms involves species specific chemicals or specific ratios of chemicals (Bruce *et al.*, 2005). Semiochemicals are further classified based on effect into two mediator groups; intraspecific, mainly the pheromones and interspecific, mainly the allelochemicals (Hansson, 1999; Matthews and Matthews, 2010; Heuskin *et al.*, 2011).

Insect pheromones are the external complements of hormones, which act as internal messengers between organs within the body of an individual organism (Howse *et al.*, 1998; Hansson, 1999; Cork, 2004; Matthews and Matthews, 2010). Insects produce their odors via exocrine glands not just from the abdomen but often from the head or thorax. Sometimes, single chemicals are produced, but more commonly they are complex blends. Insect pheromones, once produced, can be transmitted in the gaseous state, which are perceived by olfactory receptors located primarily on the insect antenna (Kromann, 2012; Leal, 2013).

Allelochemicals, initially defined by Whitaker and Feeny in 1971, are substances that transmit external chemical messages affecting individuals or populations of a species different from their source that is acting between different species. Those compounds are further divided into three classes: allomones, kairomones, and synomones (Howse, *et al*., 1998; Heuskin *et al*., 2011; Matthews and Matthews, 2010).

Allomones are chemical signals that give advantage to the emitter. It is often found in nature as part of a chemical defense, such as toxic insect secretions (Matthews and Matthews, 2010; Heuskin *et al*., 2011). On the other hand, kairomones are compounds produced or acquired by an organism and evokes in the receiver behavior or physiological response but with no advantage to the emitter (Bruce and Pickett, 2011; Heuskin *et al*., 2011). For example, secretions that can be detected by a parasite or predators for location of food sources and sexual mates (Howse, *et al*., 1998, Ruther *et al*., 2002). Synomones are chemical signals that give advantage to both the sender and receiver species. The phenomena are clearly observed in the case of floral scents, which indicate nectar source to insects like bees and ensure pollination of the flowers producing them. Semiochemicals which can harm the emitter or the receiver are termed as antimones (Howse, *et al*., 1998; Matthews and Matthews, 2010; Heuskin *et al*., 2011).

The application of semiochemicals for use in pest management was practiced over half century ago and plant volatiles have been used for trapping of insects for decades. Foliar, flower and fruit scents are often composed of tens to hundreds of compounds, but the insect olfactory systems may typically detect a minority of the components in the blend. To perform these activities, insects are equipped with a multitude of olfactory receptors that are present mainly on their antennae (Hansson, 1995; Jonsson and Anderson, 1999; Zhang *et al*., 1999; de Bruyne *et al*., 2008; Kromann, 2012; Leal, 2013). These host plant compounds include an array of short-chain alcohols, aldehydes and esters, aromatic compounds, and a variety of mono-and sesquiterpenoids (Bernays and Chapman, 1994; Schoonhoven *et al*., 1998). Identifying the range of host volatiles to which insect antennae are most sensitive are important steps to understand the role of chemical composition and communications in insects in modulating their behavior, and for development of pest management strategies (Bruce *et al*., 2005; Bengtsson *et al*., 2009; Del Socorro *et al*., 2010; Bruce and Pickett, 2011; Guerrieri *et al*., 2012).

Another important point for the application of lures is the ability to understand the diffusion speed through dispenser matrix and evaporation speed of molecules in the air that influence the efficiency of insect attractant volatile compounds (Krüger *et al*., 2002; Heuskin *et al*., 2011). When pheromone and host plant volatile lures are used in semiochemical-based insect control, dispensers ideally should release an effective dose of compounds from a sufficient number of point sources at a constant rate. Releasing of optimum amounts of compounds should persist through the whole season to protect the crop where the target insects are active in feeding (Atterholt *et al*., 1999; Yongma *et al*., 2005; Witzgall *et al*., 2010; Heuskin *et al*., 2011).

The emission system of volatile compounds is used for the application of longer acting, less costly, more potent, and easier to release compounds for effective use of insect attractants for pest management systems (Hofmer and Burger, 1995; Howse *et al*., 1998; Hansson, 1999; Cork, 2004; Heuskin *et al*., 2011). However, all these premises have been difficult to fulfill (Howse *et al*., 1998). Several problems occur, such as instability and degradation of the chemicals. It is most preferable to release a constant amount of chemicals over a long period, so called zero order kinetics (Jones, 1998). On the other hand, usually only first order release rates are possible. That is, a large release rate right after field application of the dispensers, with decreasing release over time. The wind speed in the field is also contributing to changes in release rates. Therefore, a dispensing system has to be maintained which actively releases proper amounts of volatile compounds at the proper time of day (Yongma *et al.*, 2005; Kovanci *et al*, 2006; Heuskin *et al.*, 2011; Schmera and Guerin, 2012).

In scarab beetles, chemical attraction is the major means of sexual recruitment, with female normally being the pheromone emitter and males being the receivers. Field observation and experiments on mass trapping have shown that scarab beetles are also attracted to a large number of plant volatile compounds (Donaldson *et al.*, 1986, 1990; Stensmyr *et al.*, 2001; Larsson *et al.*, 2003; Andersson *et al.*, 2009). Odors from plants can lead to the discovery of novel methods for insect pests that are often highly specific and have little harmful effect on other organisms or the environment (Bruce and Pickett, 2011; Guerrieri *et al.*, 2012).

2.4. Trap designing and development

Many types of traps have been designed and developed over the years and their trapping efficiency has been investigated for monitoring and control of insect pests baited with attractant compounds (Howse *et al.*, 1998; Jones, 1998; Cork, 2004; Athanassiou *et al.*, 2007). For example, starting from the discovery of Japanese beetle, *Papillia japonica* in North America in 1916, researchers have been designing and trying many trap types for many years and produced a commercial Japanese beetle trap (JBT) (Klein, 1981; Potter and Held, 2002). Most insect trap designs, therefore, are the results of empirical selection and very few are based on systematic studies of insect behavior and pheromone plume formation with relation to trap design. Clearly, omnidirectional trap such as funnel traps will have a more consistent plume form; irrespective of wind direction compared with others like delta traps (Cork, 2004). Trap efficiency is measured by the proportion of total number of insects captured per trap during the sampling period (Howse *et al*., 1998; Athanassiou *et al*., 2007). Indeed, the processes of catching insects in semiochemical-baited traps involve two stages. These are the entry and retention stages. Therefore, improving the traps based on the behaviors of particular insects, such as a tendency to fly upward or search for protected sites, is central to the effective utilization of lure baited trapping systems (Howse *et al*., 1998; Cork, 2004).

Entrapment systems can be grouped into non-drying adhesives or water to retain the insects caught and those that have one-direction entrance systems with no exit. The one-way entrance may in some case involve a toxicant to immobilize the insect once it has passed through to the interior of the trap. This may not only be to prevent the escape of the insects while the trap is in use but also to prevent escape of live insects when the traps are opened for inspection and counting purposes (Jones, 1998). Some traps like delta traps, wing traps and tent traps have a trapping surface, which is sticky. This type of sticky trap is often used for moths (Hansson, 1999). Other traps like Japanese beetle traps used a kind of flight barrier, such as vanes, or liquid trapping medium such as bucket traps. Trap catch data, collected from the replicated treatments in regular intervals is a means for comparison of trap designs (Cork, 2004; Athanassiou *et al*., 2007). The size of the trap is often related to the size and number of insects that it is intended to catch. A sticky trap, for instance, is often not suitable for large moths or high population density because of the risk of trap saturation (Howse *et al*., 1998; Athanassiou *et al*., 2007; Bouget *et al*., 2009).

There are numerous factors affecting the efficiency of lures in an environment. Temperature, rainfall, and wind speed and direction determine the extent of insect movement from surrounding areas to traps; affect attractant release from dispensers and insect flight (Howse *et al*., 1998; Athanassiou *et al*., 2004). Millar and Sims (1998) reported that temperature and age of the dispensers are the most important factors affecting the release rate of lures during the field application. For all programs that use traps of any type, pest biology, attractant source, trap design and trap placement are important factors (Athanassiou *et al*., 2007; Bouget *et al*., 2009).

The trap plays an important role in the component of any trap-based monitoring and control activities. Trap color may also influence attractiveness and the number of insect caught (Athanassiou *et al*., 2004; El-Sayed *et al*., 2006; Bouget *et al*., 2009; Matthews and Matthews, 2010).

It is a common phenomenon that light attracts many insects, but making use of light and its component colors in visual lures requires considerably more detailed understanding (Athanassiou *et al*., 2007). Utility of visual attractants depends on the behavior of the target insect and on likely population density (Matthews and Matthews, 2010). Visual cues used in insect pest management can be grouped into lights, colored objects and shapes or silhouettes that stand out against a contrasting background (Quartey and Coaker, 1992; Matthews and Matthews, 2010). For example, the work by Quartey and Coaker (1992) has shown the importance of visual cues in entrapment of *Ephestia cautella* in funnel traps. Vertical stripes with 7.5 mm width on the outside of the funnel trap 90% of *E. cautella* and 80% of moths released in a wind tunnel in a moving a still air, captured compared with 70% and 35% for the best available commercial traps respectively. Therefore, specific colors and designs are attractive to most insects. Yellow objects attract many insects and are often used in traps designed to capture insects like winged aphids and adult whiteflies. Combinations of colors can also influence trapping activities (El-Sayed *et al*., 2006; Matthews and Matthews, 2010; Witzgall *et al*., 2010).

Trap catch, in addition to being influenced by the design of the trap and attractant source, is also affected by proper placement of traps (Athanassiou *et al*., 2004; Bouget *et al*., 2009). For trap placement; trap height, time, position with respect to vegetation and trap density are important components for trap placement (Barak *et al.*, 1990; Boucher *et al.*, 2001; Athanassiou *et al*., 2007). Ideally, traps should be placed at optimum height and required number of traps for catching insects, despite the fact that this may not always be practical in plantation crops. In crop fields with a wide area, it is often important to relate the height of the trap to the height of the vegetation (Howse *et al*., 1998; Athanassiou *et al*., 2004; Bouget *et al*., 2009). For example, Mason *et al*. (1997), caught more European corn borer, *Ostrinia nubilalis*, when traps were located 0.1 m below the top of the canopy than when located 0.5 m above. The distance of the traps and wind direction are also important to position traps in such a way that the lure plume emerged passes downwind over the crop. Moreover, the distance between traps should be sufficient to prevent interference of one trap from the plume of another trap (Howse *et al.*, 1998; Bouget *et al.*, 2009).

2.4. Applications of Solid Phase Microextraction (SPME) to insect semiochemical analysis

The common isolation methods of plant volatiles are solvent extraction or distillation and headspace techniques (Millar and Sims, 1998). During solvent extraction, the volatiles present at one time in the plant material are collected together with a variety of non-volatile compounds. The development of static and dynamic techniques for headspace collection of volatiles in combination with gas chromatography–mass spectrometry analysis has significantly improved understanding of the biosynthesis and ecology of plant volatile organic compounds (Pawliszyn, 1997; Agelopoulos and Pickett, 1998). The development of solid phase microextraction (SPME) which is a recent technique; a fast and simple method for collecting volatile organic compounds, was an important advance in static headspace analysis (Pawliszyn, 1997; Flamini *et al.,* 2003; Tholl *et al.,* 2006). This technique does not require the use of solvents for the extraction and it is a field-compatible sample preparation method. The basis for the method is passive diffusion of volatiles onto SPME fiber coated with different types of absorbents and thereby their enrichment during the exposure of the fiber in headspace of the sample (Knudsen *et al.*, 1993; Pawliszyn, 1997; Custodio *et al.*, 2006; Tholl *et al.*, 2006).

The SPME fiber is attached within the needle of a syringe-like device and volatiles can be collected by inserting the needle through a septum of a headspace collection container and pushing the plunger to expose the fiber (Figure 6). After equilibration between the fibre and the volatile sample, the fiber is retracted into the needle and can be transferred immediately to a gas chromatograph for direct thermal desorption. Extraction time depends on the length of time required to obtain precise extractions for the analytes with the highest distribution constants (Pawliszyn, 1997). Thermal desorption of volatile organic compounds from the fiber bypasses the need for solvents that may contain impurities which will interfere with sample analysis. On the other hand, by desorbing the entire sample into the injector of the GC, repeated injections of the sample are not possible (Pawliszyn, 1997; Flamini *et al.*, 2003; Custodio *et al*., 2006).

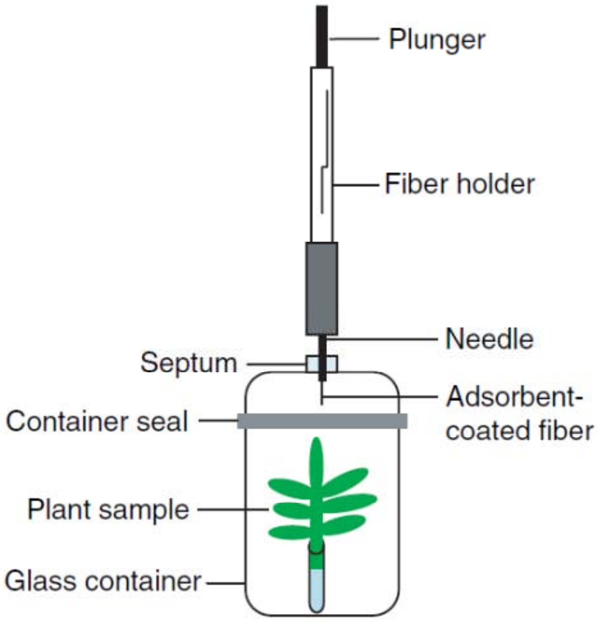


Figure 6. Schematic drawing of static head space sampling techniques of volatiles emitted from plant sample with a solid phase microextraction (SPME) device (source: Tholl *et al*., 2006.

This technology offers several advantages, including simplicity of handling, reduced carryover of impurities and shorter sampling time. One of the limitations of SPME is that it will not allow trapping of sufficient amounts of volatiles for structure elucidation of unknown compounds. This problem can be solved by sampling with iron stir bars, which are coated with the same sorbents as SPME fibers where the bar is placed in the headspace of the plant sample. Trapped volatiles are released by thermal desorption after transfer of the bar into the GC injector liner. Application of iron stir bars techniques for volatile compounds collections were originally developed to extract organic compounds from aqueous samples (Bicchi *et al*., 2000).

Quantification of volatiles by SPME is generally possible by the application of internal or external calibration. To obtain reproducible quantitative results, the fiber and sample should reach equilibrium, at which the amount of analyte removed from the fiber is proportional to the concentration of the compound in the sample. Equilibration time is dependent on the volatility and polarity of the analyte and the properties of the adsorbent (Flamini *et al.,* 2003; Custodio *et al*., 2006). Careful control of sampling parameters (sample volume, temperature, time) and the use of appropriate standard mixtures for calibration are crucial for quantitative analysis. However, quantification by SPME may still be difficult or impractical when dealing with a wide range of compounds with different distribution constants (Pawliszyn, 1997).

Chapter 3

Optimization of release rates, dispenser calibration and field evaluation of putative pheromone and certain host plant volatiles for the management of *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae)

3.1. Introduction

Sorghum (*Sorghum bicolor* L.) is affected by an array of insect pests, including the sorghum chafer, *Pachnoda interrupta* (Hiwot Lemma, 2000; Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009). This insect was recorded as an important pest of sorghum in Ethiopia in the 1970s (Clark and Crowe, 1978). Local farmers had experiences with the beetle since three decades in their crop fields, but damage on sorghum was observed sporadically. Since 1993, *P. interrupta* has been noted to increase both in density and geographic distribution in sorghum growing regions of Ethiopia (MOA and EARO, 1999).

Pachnoda interrupta beetles cause damage on sorghum during the milky stage of the crop. Research reports indicate that during high infestation periods, up to 70% yield reduction was recorded (Yitbarek Wolde-Hawariat and Hiwot Lemma, 2000) and entire fields of sorghum were destroyed during some of these outbreaks (Tsedeke Abate, 1988).

Moreover, the adult stage of this univoltine, polyphagous insect consumes several types of fruits and flowers (Clark and Crowe, 1978; Grunshaw, 1992).

During the pre-adult stages, *P. interrupta* aestivates in the soil from November to May after a feeding period in September-October. Emergence of adult beetles into the fields is triggered by the onset of the rainy season (June-July). When beetles appear in the surrounding vegetation, they begin to search for food and soon are physiologically ready for mating and then oviposition of eggs in favorable breeding sites (Clark and Crowe, 1978). These activities typically take place within about two weeks of emergence. After complete metamorphosis in the soil, the newly emerged adults again start feeding on the developing grains, especially the milky stage seeds of sorghum, during September-October (MOA and EARO, 1999; Hiowt Lemma, 2000).

Despite farmers' attempts to control the adult stage of this devastating pest by traditional practices such as handpicking, smoking and insecticide-mixed baits with fruits like banana, the pest problem could not be solved. Spraying insecticides in the sorghum fields is still another frequently used application by growers, but they have been unsuccessful due to the wide host range of the beetle, lack of appropriate equipment (Yeraswork Yilma, 2000; Seneshaw Aysheshum and Mulugeta Negeri, 2002), and high cost of insecticides. Escalating concern on the adverse effects of pesticides on the environment has led to the attempts to develop environmentally sound control strategies for *P. interrupta*.

Understanding *P. interrupta* evolutionary history and ecological interactions can help us to find alternative control systems. Farmers own observations on ‘attract- and- kill’ method using fruits for attraction of the beetle to the point source of insecticides have led to the development of safe control options (MOA and EARO, 1999; Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009). The method is based on the fact that insects respond to specific volatile odors. Odorant information is fundamental for survival, searching resources, and escape from enemies, mate location and for oviposition site selection of insects (Bruce *et al*., 2005; Van den Berg *et al*., 2008).

Ecologically relevant odorant volatiles are normally mixtures of different compounds. Many studies have shown synergistic effects: that blends of volatiles are more important for attraction than the sum of the effect of the same compounds tested individually (El-Sayed *et al*., 2008; Gregg *et al.*, 2010; Socorro *et al.*, 2010). Responses of insects to odors in the environment have to be evaluated by formulating appropriate dispensers relevant to the target insect. Two strategies are used to test single or blended compounds for the development of an expected behavioral response of insects towards attractants. These are additive and subtractive assays; the former is long established, and adds individual compounds one by one, while in the subtractive assay, first all fractions are tested all together, and compared to the non-fractionated isolate. In the next tests, one fraction is excluded in each bioassay, and finally compared with a blank or control (Byers, 1992). We followed a similar approach with synthetic blends in the current study to identify the best attractant baits on *P. interrupta* in the field to apply environmentally friendly pest control strategies.

There has been a considerable amount of research in identification of volatile attractants for *P. interrupta*. The putative pheromone compound phenylacetaldehyde (Bengtsson *et al*., 2010) and host-related attractants methyl salicylate, eugenol and isoamyl acetate (Yitbarek Wolde-Hawariat *et al*., 2007), and racemic 2, 3-butanediol (Bengtsson *et al*., 2009) have elicited strong behavioral attraction in adult *P. interrupta.* Those volatile compounds detected specifically by the beetles can serve as novel control measures by means of mass trapping and the 'attract-and-kill' system (El-Sayed *et al*., 2006). However, the efficiency of blend/mixture of pheromone component and synthetic host plant volatile compounds with a relatively constant emission rate was not investigated in trapping *P. interrupta* in the field.

Volatile compounds can vary in their release rate over time when applied in the same experimental setup and this can influence the efficiency of attractants against the target insects (Krüger *et al*., 2002; Yongma *et al*., 2005; Heuskin *et al*., 2011). These chemical mixtures tend to release more molecules of attractants when first placed in the field compared with the amount they emit at the end of the useful period of trapping insects (Howse *et al*., 1998; Kovanci *et al*, 2006). To solve this problem, there is a need for a device that can store, protect and moderate release of chemicals in order to efficiently and effectively evoke certain behaviors during the full field season (Cork, 2004; Heuskin *et al*., 2011).

Scientists are interested in simulating naturally related release rates of volatile compounds and quantifying the behavioral reactions. It is emphasized that very pure volatile compounds should remain constant in the field during the trapping time of the target insect (Kovanci *et al*, 2006; Schmera and Guerin, 2012). This can be achieved by incorporating the compound into different dispenser types such as rubber septa, plastic matrix or vials, open ended glass or other inert materials from which the volatile compounds are slowly released at a desired rate (Millar and Haynes, 1998; Atterholt *et al*., 1999; Yongma *et al*., 2005; El-Sayed *et al*., 2008; Heuskin *et al*., 2011). For example, nowadays micro-particle dispensers are broadly applied for the controlled release in mating disruption experiments on insect pests (Stipanovic *et al*., 2004). New release rate strategies are still emerging for the regulated emission of volatile compounds. Cotton rolls alone and cotton roll inserted into 4 ml glass vials have been used recently for dispensing lures in trapping of *P. interrupta* in the field (Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009). Selection of proper amounts of dose may be crucial for determining the trapping efficiency on the target insects (Atterholt *et al*., 1999; Cork, 2004; Yongma *et al*., 2005; El-Sayed *et al*., 2006; Heuskin, *et al*, .2011).

Therefore, the focus of the present study was on determining controlled and constant release rate of synthetic attractant chemicals from dispensers and evaluation of catch performance against *P. interrupta.*

3.2. Materials and Methods

3.2.1. Source of test volatile compounds and dispenser formulation

Experiments were conducted to determine the optimal quantity of the baits needed for the efficient trapping system of *P. interrupta* in the laboratory and evaluate the durability of dispensers in the field. Phenylacetaldehyde, 2, 3-butanediol, methyl salicylate, eugenol, and isoamylacetate with purity levels of 90%, 98%, 98%, 99%, and 95%, respectively were purchased from Sigma-Aldrich (Sweden) for the experiments. Determination of release rates of the test volatile compounds and dispenser formulations were carried out in the Insect Science Laboratory, Addis Ababa University.

3.2.2. Determination of release rate and lure longevity under laboratory condition

Methods and procedures used for dispensing volatile compounds were based on findings reported by Yitbarek Wolde-Hawariat *et al*. (2007) and Bengtsson *et al*. (2009). The mass and volume relationship of the test volatile compounds was determined and it was observed that 100 µl of individual compounds weighed approximately 100 mg. Four ml glass vials (45 × 14.7 mm, clear, Skandinaviska GeneTec AB) and cotton rolls (3.9 cm long and 0.9 cm in diameter, Top Dent®, Dental rolls) were used for dispenser formulations. Individual cotton rolls were pushed into the vials until the exposed surface of the cotton was leveled with the rim of the opening of the vial. The compounds were loaded onto the cotton roll in the vials (Figure 7). The position of loading chemicals in the vials was based on the volatility of the chemicals in order to achieve similar and constant emission rates. The least volatile compounds, 2, 3-butanediol and eugenol, were loaded onto the dispenser deep into the cotton roll. Methyl salicylate was loaded into the bottom part of the cotton roll in the vial. Phenylacetaldehyde was loaded on top centre and isoamyl acetate deep into the edge of the cotton roll. Vials loaded with phenylacetaldehyde and isoamyl acetate were closed with black caps with 5 mm and 1.5 mm diameter holes at the middle, respectively.

Figure 7. Release rate experiments with test volatile compounds loaded in different dispenser vials in the laboratory, April, 2011.

To measure the release rate, each dispenser was weighed in one-hour intervals during a day after loading a dose of 100 mg of each volatile compound, using a scale (LA-114 electronic balance, UK). The weight loss was attributed to the emission of volatile compounds from the dispensers (*N*=3) and was adjusted to the rate of 0.5-1 mg/hr which is equivalent to approximately 25 mg/day, a release rate that has been shown to be attractive in the field (Yitbarek Wolde-Hawariat *et al*., 2007). This volatile compounds emission rate study was followed by loading 1000 mg of each compound into the dispensers. Then we evaluated the longevity of lures that had been loaded once for the consecutive 11 days. Data were recorded (*N*=3) and dispensation rates were calculated. The average temperature and relative humidity were also recorded during the experiment.

3.2.3. Field evaluation of attractant compounds and blends

Field experiments were conducted to evaluate the attractiveness of synthetic volatile compounds on *P. interrupta*. In order to determine the efficiency of lures formulation, the number of beetles captured in each trap was recorded. Experiments at different locations were carried out for a period of three years (2011-2013) during both cropping and non-cropping seasons. The experimental field sites were not treated by pesticides. Temperature and relative humidity of the field was recorded. All the field experiments were done with a randomized complete block design.

For field experiments, different combinations of the test volatile compounds were used to determine the best attractant blend under field conditions in July and October 2011. The commercially available Japanese beetle traps (JBT) (Trécé, Palo Alto, CA, USA) were used as standard traps to deploy test compounds.

3.2.3.1. Experimental procedures design and trap deployment in July and October 2011

In the July 2011 field experiments, the blends of test compounds in separate dispensers were formed by removing one of the component volatile compounds from the blend in the form of a subtractive approach (Byers, 1992). Phenylacetaldehyde, and 2, 3-butanediol were tested individually. Each dispenser was loaded with 100 µl of each volatile compound every morning throughout the experimental period. The dispensers loaded with individual volatile compounds were placed on the cross-veins of Japanese beetle traps in different combinations to produce different volatile blends with ten replications. Details of blend/single attractants used in the field experiments are given in Table 1. Dispensers were attached and fastened with scotch tape on the vane of the JBT. The baited traps were tied and hung from the horizontal arms of wooden poles, at 3 meters above ground during the mating season in *Acacia* forest (ca. 6.05 ha) in Rasa villages. The canopy of the vegetation was about 3 m above the ground. As a positive control that has been reported in previous studies (Yitbarek Wolde-Hawariat *et al*., 2007), mashed banana (about 30 g) was placed inside the canister of the trap per day. An unbaited trap was used as a negative control.

The distance between traps and blocks were 10 m and 50 m respectively. Traps were labeled with the treatment applied. Eleven treatments including the controls were placed in each block. Individual treatments were distributed randomly within each block. Each treatment was replicated ten times. Traps were emptied each morning regularly between 9:00 am to 11:00 am and captured beetles were sorted by sex and collected in transparent labeled plastic boxes with ventilated lids. During the data collection days, flight activity and behavior of the beetles towards the attractant trap was also observed and recorded.

Table 1. Treatment combinations used in the study site for evaluation of blend/single volatile compounds and mashed banana in trapping of *P. interrupta,* in Rasa villages, Ethiopia, July 2011.

Treatment combination #	Composition	Treatments	Number of dispensers/trap	Dose (µl) of each compound/dispenser
T-1	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate	A + B + C + D + E	5 (multiple)	100
T-2	Phenylacetaldehyde + Methyl salicylate + Eugenol + Isoamyl acetate	A + C + D + E	4(multiple)	100
T-3	2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate	B + C + D + E	4 (multiple)	100
T-4	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol	A + B + C + D	4 (multiple)	100
T-5	Phenylacetaldehyde + 2, 3-butanediol + Isoamyl acetate	A + B + E	3 (triple)	100
T-6	Phenylacetaldehyde + 2, 3-butanediol	A + B	2 (dual)	100
T-7	Phenylacetaldehyde	A	1 (single)	100
T-8	2, 3-butanediol	B	1 (single)	100
T-9	Phenylacetaldehyde + mashed banana	A + mashed banana	1 (single)	100 and 30 g banana
T-10	Meshed banana	Mashed banana	none	30 g banana
T-11	Unbaited	Negative Control	none	Unbaited

The letters A, B, C, D, and E refer to the name of compounds as: A = Phenylacetaldehyde, B = 2, 3-butanediol, C = Methyl salicylate, D = Eugenol, E = Isoamyl acetate. The amount of each pure compound was 100 µl in individual dispensers include in each treatment/treatment combination**.**

In order to determine the efficiency of lure formulations, the number of beetles captured in each trap was recorded. The catch efficiency of dispensers was evaluated individually and in blend forms.

The field experiments in October 2011 were conducted in Rasa village at Lewutgn in Kewot District (09°57'N, 040°05'E). The field experiments were based on observations and best findings from the July 2011 field experiment, and were focused on further evaluation of attractant compounds. These field trials aimed to investigate the attractiveness of the five-component blend (100 µl each, multiple dispensers). Based on its efficiency as a single compound in attracting beetles in July 2011 trapping experiment, phenylacetaldehyde (100 µl, single dispenser) was also included as a treatment. In addition, phenylacetaldehyde was also loaded at a dose of 500 µl in a single dispenser and used throughout the duration of the experiments to estimate the longevity of the lures compared with the standard treatment. Each dispenser was loaded with 100 µl of neat compound every morning similar to the July 2011 field experiments. Details of blend/single baits used in the field experiments are given in Table 2.

The baited traps were hung near the panicle of milky stage sorghum. For positive control, about 30 g mashed banana was placed inside the canister of the trap. An unbaited trap was used as a negative control. Traps were labeled with the treatment applied. Each treatment was replicated ten times. Traps were emptied each morning regularly between

9:00 am to 11:00 am and captured beetles were collected in transparent labeled plastic boxes with a ventilated lid for data recording purposes.

Table 2. Treatment combinations used in the study site for evaluation of blend/single compounds in attraction of *P. interrupta*, Rasa village, Kewot District, Ethiopia, October 2011.

Treatment combinations #	Composition	Treatments	Number of dispensers/trap	Dose (µl)
T-1	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate	A + B + C + D + E	5 (multiple)	100 of each compound
T-2	Phenylacetaldehyde	A	single	100
T-3	Phenylacetaldehyde + mashed banana	A + mashed banana	Single + mashed banana	100 + 30g
T-4	Phenylacetaldehyde	A	single	500
T-5	Negative Control	Negative Control	Unbaited (blank)	Unbaited (blank)

The letters A, B, C, D, and E refer to the name of compounds as: A = Phenylacetaldehyde, B = 2, 3-butanediol, C = Methylsalicylate, D = Eugenol, E = Isoamyl acetate. The amount of each compound was 100 µl in every treatment except 500 µl phenylacetaldehyde. The 500 µl phenylacetaldehyde was formulated to estimate how long the compounds will stay in the field without loading every day.

3.2.3.2. Experimental procedures, design, treatments and trap deployment for field trials in July 2012

The field trials in July 2012 were conducted at Rasa village, Kewot District. The five compounds blend in multiple dispensers [phenylacetaldehyde + 2, 3-butanediol + methyl salicylate + eugenol + isoamyl acetate] plus fermentation compounds (ethanol and acetic acid mixture) were used for formulation of baits (Table 3). To avoid re-loading of the compounds everyday onto the dispensers, a dose of 1ml of each compound were loaded once during the period of field experiments. The proportion of ethanol and acetic acid in the mixture was 5:1 and the total amount was 1000 µl. The mixture of fermentation compounds was tested alone and with the combination of the novel five-component blend in trapping *P. interrupta*. Each treatment replicated ten times.

Table 3. Treatment combinations used for evaluation of blend (multiple dispensers) and fermentation compounds in attraction of *P. interrupta*, at Rasa village, Ethiopia, July 2012.

Treatment combinations #	Compositions	Treatments	Number of dispensers	Dose (µl) loaded
T-1	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate	A + B + C + D + E	5 (multiple)	1000 µl/dispenser
T-2	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate + [5:1 ethanol and acetic acid]	[A + B + C + D + E] + [5:1 ethanol and acetic acid]	6 (multiple)	1000 µl/dispenser
T-3	Phenylacetaldehyde + 2, 3-butanediol + Methyl salicylate + Eugenol + Isoamyl acetate + [5:1 ethanol and acetic acid]	Mixture of A + B + C + D + E and [5:1, ethanol and acetic acid]	1 (single)	6000 µl/dispenser
T-4	5:1, ethanol and acetic acid	5:1, ethanol and acetic acid	1 (single)	1000 µl/dispenser
T-5	Control	Control	Unbaited (blank)	Unbaited (blank)

The letters A, B, C, D, and E refer to the name of compounds as: A = Phenylacetaldehyde, B = 2, 3-butanediol, C = Methylsalicylate, D = Eugenol, E = Isoamyl acetate. The amount of each five compound in the blend was 1000 µl in every treatment and the fermentation volatiles mixture was 1000 µl in 5:1 proportion of ethanol and acetic acid.

3.2.3.3. The October 2013 experimental design and procedures for field evaluation of the five compound mixtures in single versus multiple dispensers

The field trial in October 2013 was conducted in Rasa village at a site called Lewutgn, Kewot District. In this experiment, the five novel compounds [Phenylacetaldehyde + 2, 3-butanediol + methyl salicylate + eugenol + isoamyl acetate] mixture was applied in a single dispenser to test the behavioral response of the beetles. The purpose was to formulate lures from those five compounds for easy application in the future. Dispensers were formulated by 1 ml of each compound mixed together in a 6 ml plastic vial and soaked in about 0.5 g cotton wick (Fisherbrand®, large cotton Balls, made in USA). The five multiple dispensers were used for comparison. Vials and cotton rolls, and the methods for loading compounds onto multiple dispensers were the same as in October 2012 field procedures.

The Japanese beetle traps were used as standard traps and dispensers were attached on the vane of the traps. The baited traps were hung near the panicle of milky stage sorghum plants. For positive control, mashed banana (approximately 30 g) was placed inside the canister. Unbaited traps were used as negative control. Traps were labeled with the treatment applied. Randomized completely block design with seven replications was used for deploying traps and treatments for the trapping experiment. The distance between traps and blocks were 10 m and 50 m respectively.

3.3. Data analysis

Data for total catch per trap of *P. interrupta* over the field period was subjected to an analysis of variance (ANOVA) to test whether the treatments and the interaction between them had significant difference in the response variable. SPSS version 17 software was used to analyze the data. Before analysis, field trapping data were transformed using log transformation, log(x+1) to normalized the variance. Microsoft Excel was used for data management and for making the graphs. Untransformed means and standard errors (SE) are presented in the graphs. Whenever there were significant differences, means were separated following the method of Tukey's studentized range test (α =0.05).

3.4. Results

3.4.1. Release rate optimization and evaluation of lure longevity under laboratory conditions

The uniform rate of release was measured after thorough trial and error approaches by loading 100 µl of each compound and the result was between 0.5 to 1.0 mg/hr for all compounds. The longevity of lures that had been loaded with 1000 µl was >11 days (Figure 8). The results indicate that there is a very good match between time of release of compounds and trapping periods of beetles staying in the field. The emission of compounds seems to be relatively constant except for eugenol. There was higher release rate, and considerable fluctuation in the release rate, of eugenol compared with other volatile compounds in the present study. On the other hand, most other compounds were released at similar and relatively uniform rates.

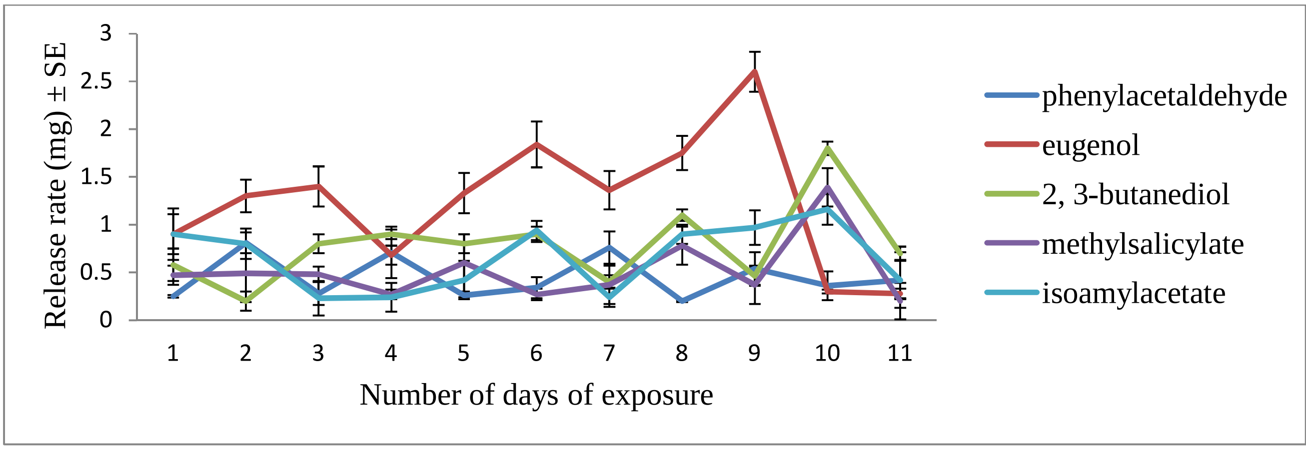


Figure 8. The longevity of phenylacetaldehyde, eugenol, 2,3-butanediol, methyl salicylate and isoamyl acetate released during eleven days after the initial application of 1000 µl of each compounds in their respective dispensers under laboratory conditions. Error bars represent standard error of the mean (*N*=3).

3.4.2. Field evaluation of attractant compounds and blends

Results of the three years of studies carried out on the response of *P. interrupta* on volatile compounds individually as well as in blends executed in the field are presented in each year.

3.4.2.1. The effect of lures on trapping *P. interrupta* in July and October 2011

Results from the evaluation of volatile compounds on the attraction of *P. interrupta* in July and October, 2011 are presented in figures 10 and 11.These results constitute the initial trials for the series of field trapping experiments to evaluate the catch performance of different types of lures. During July, it was found that beetles that could fly in the *Acacia* trees and other host plants were attracted to the flower's scent. During September - October, beetles flew from their breeding sites to the sorghum fields and other host plants. It was observed that beetles in flight were attracted by the baited trap towards the area of the vicinity of the trap, but sometimes flew away from the traps. Sometimes, beetles were found landing on the surface of the vane of the JBT, after which they began searching for the source of the attractant for a period of 2-3 minutes, especially on the blended compounds bait. Some of the beetles flew away from the trap and again flew back to the vicinity of trap and finally collided with the vane of the trap and subsequently fell into the canister of the trap.

Mean trap catch of the July 21-25/2011 field trials (Figure 9) was significantly higher ($P< 0.0001$) in multi-component blends [phenylacetaldehyde + 2, 3-butanediol + methyl salicylate + eugenol + isoamyl acetate] and [phenylacetaldehyde + 2, 3-butanediol + methyl salicylate + eugenol] compared with single compound baits, with no significant differences between multi-component treatments. Combining phenylacetaldehyde, 2, 3-butanediol and isoamyl acetate also led to a significant increase ($P < 0.05$) of trapped beetles compared with the single compound dispensers. Phenylacetaldehyde appears to be the only necessary and indispensable single component for a highly attractive five-compound blend.

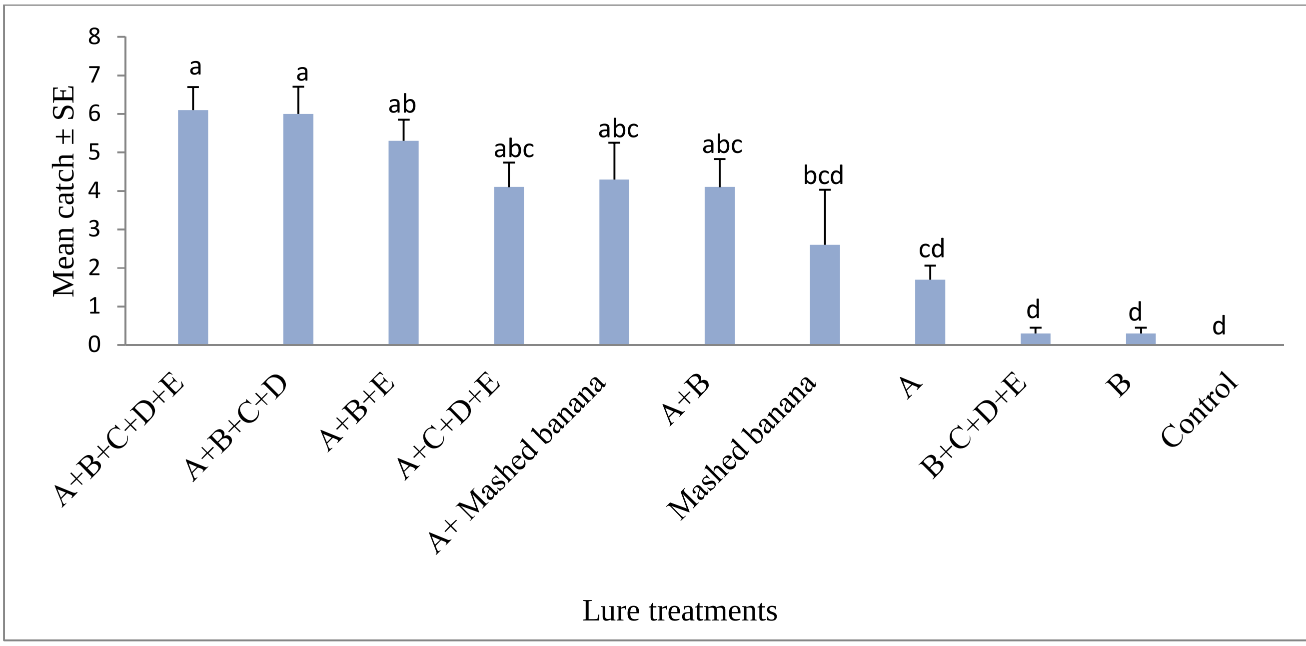


Figure 9. Mean trap catch efficiency of the baits using the standard trap, JBT treatments interactions in the study site at Rasa in July 21-25, 2011. The letters A, B, C, D, and E refer to the name of compounds as: A = Phenylacetaldehyde, B = 2, 3-butanediol, C = Methylsalicylate, D = Eugenol, E = Isoamyl acetate. Error bars show standard error of the mean. Treatments denoted by different letters are significantly different (ANOVA followed by Tukey's Studentized test at α= 0.05).

Results obtained in the October 2011 field experiment demonstrate that the combination of the five-compound blend, phenylacetaldehyde plus banana and the banana alone resulted in the higher catches of the beetles with no statistically significant difference (*P* > 0.05) (Figure 10). The mean trap catch with 500 µl phenylacetaldehyde that has been loaded once was not significantly different from that of baits loaded by 100 µl in every day.

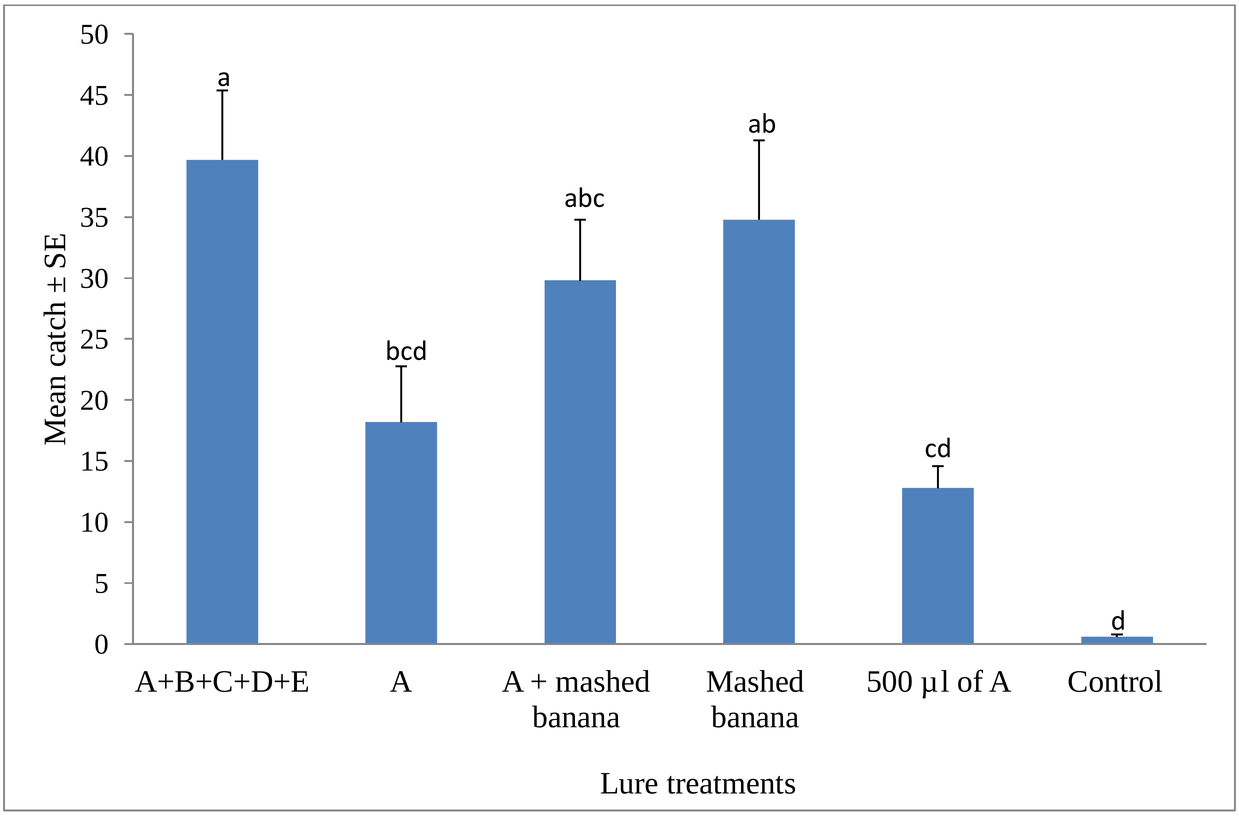


Figure 10. Mean trap catch of *P. interrupta* using the most attractive synthetic single volatile compound (phenylacetaldehyde) singly and in a multi-component blend, and mashed banana with JBT from October 09-11, 2011 at Rasa. The letters A, B, C, D, and E refer to the name of compounds as: A = Phenylacetaldehyde, B = 2, 3-butanediol, C = Methylsalicylate, D = Eugenol, E = Isoamyl acetate. Error bars represent standard error of the means of the treatments. For each treatment, bars followed by the same letter are not significantly different (ANOVA followed by Tukey's Studentized test at α= 0.05).

3.4.2.2. The effect of lures on trapping *P. interrupta* during field trials in July 2012

The July 2012 field experiment was conducted with very low populations of beetles compared with October, 2011 field trials and the result is shown in figure 11 below. The five-compound blend caught significantly more *P. interrupta* than the mixture of the five compounds with fermentation compounds and the fermentation compounds alone (ANOVA, $F= 17.201$, $df = 4$, $P < 0.001$), but no significant difference in trap catch of beetles with that of the five compound blend plus fermentation compounds (not mixed together) ($P > 0.05$).

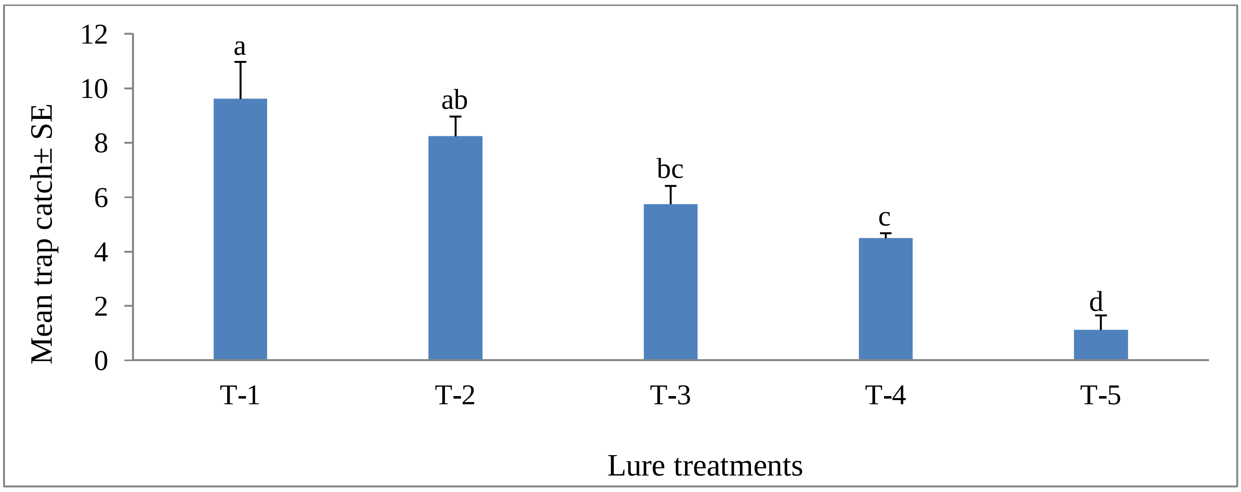


Figure 11. Mean trap catch of *P. interrupta* using the novel synthetic five-compound blend and fermentation compounds (ethanol and acetic acid; 5:1) with JBT from July 14-21, 2012 in Rasa village, Ethiopia. Error bars represent standard error of the means of the treatments. For each treatment, bars followed by the same letter are not significantly different (ANOVA followed by Tukey's Studentized test at α= 0.05).

3.4.2.3. Field evaluation of the five-compound mixture in single versus multiple dispensers in October 2013

Traps baited with the five multiple dispensers blend and the mixture of these five compounds in individual dispenser in October 2013, caught *P. interrupta* with no significant differences; (Figure 12). The sex ratio of the trapped beetles was similar for all treatments.

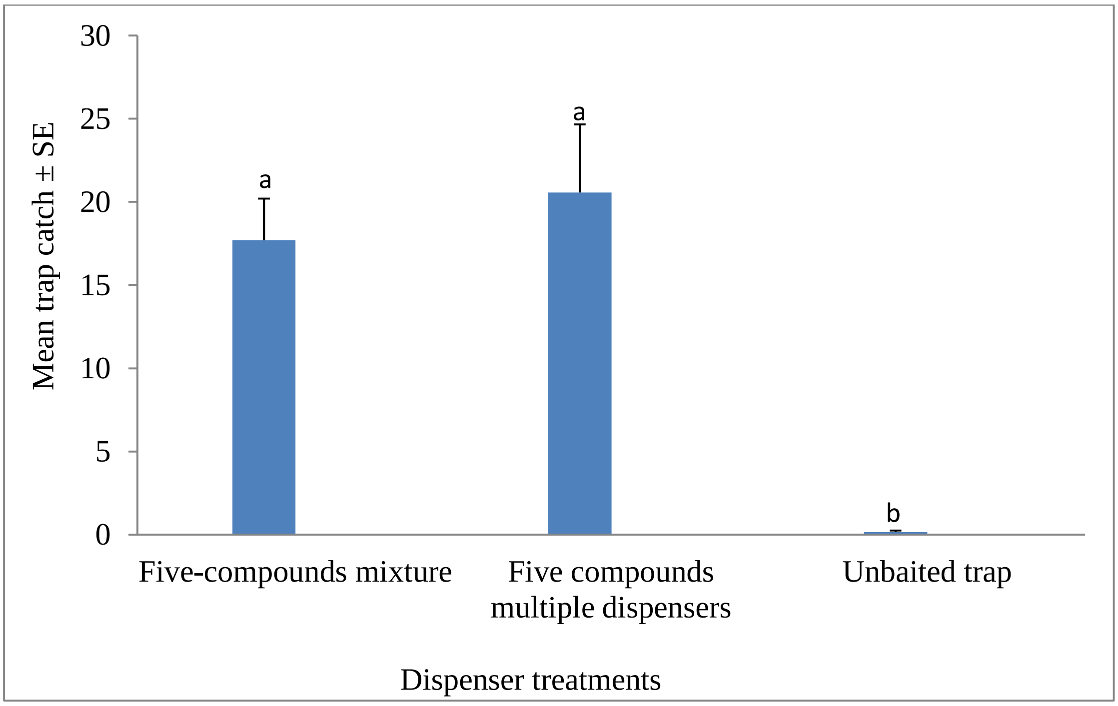


Figure 6. Mean ± SE number of *P. interrupta* captured in the traps baited with the five novel compounds mixture and their five multiple dispensers, October 06-10, 2013, at Rasa village, Ethiopia. Error bars represent standard error of the means of the treatments. Values followed by the same letter are not significantly different (ANOVA followed by Tukey's Studentized test at α= 0.05).

3.5. Discussions

The measurements of the release rates of the five individual compounds gave a good indication that the odors were emitted at similar and relatively constant rates, based on the individualized design of dispensers for each compound. A good estimation of the longevity of the baits is crucial to estimate for how long *P. interrupta* could be captured in the field without reloading baits daily. The good agreement between release rates and the trapping season of beetles in this experiment demonstrated that baits can attract beetles during the full field season, indicating a reasonable expectation for monitoring and control of *P. interrupta* populations. Under the current investigation of attractant chemicals in the study sites, the combination of multiple dispensers attracted more beetles than the individual components. The results of the present study show that selected host plant volatiles, when presented as a blend with phenylacetaldhyde - a putative pheromone of *P. interrupta* (Bengtsson *et al*., 2010) - had a strong effect on the attraction of beetles. That is, combinations made without phenylacetaldehyde showed very little attraction. This result is in line with Bruce and Pickett (2011) that blends of proper volatile compounds can perform better than the sum of individual components for attraction of insects.

From the present study we can observe that phenylacetaldehyde was an essential compound for trapping of *P. interrupta*. The present finding is, therefore, consistent with previous studies (Bengtsson *et al*., 2010) demonstrating the high attractiveness of phenylacetaldehyde to *P. interrupta*. When phenylacetaldehyde was blended with one of the other four compounds, it attracted higher numbers of beetles than phenylacetaldehyde alone. In other words, we found evidence of a synergistic effect of the pheromone and the host plant volatiles on attraction of *P. interrupta*. Combinations of pheromone and host plant kairomones constitute the most efficient blend for trapping the Japanese beetle *Popillia japonica* (Ladd *et al*. 1981). Saïd *et al*., (2011) also reported that synergy to pheromone and plant kairomone has great influence in attraction of American palm weevil, *Rhynchophorus palmarum.* Moreover, during field observations beetles showed specific behavior like hovering near the attractant lure or landing on the blend-baited traps more than the compounds tested individually. The current finding also coincided with the earlier findings of Landolt and Phillips (1997) that a blend of pheromone lures combined with host odor cues improves the efficacy of insect pheromones. Among the host plant volatiles, 2, 3-butanediol attracted more number of beetles when it was combined with phenylacetaldehyde. This indicates the importance of 2, 3-butanediol, which is a fermentation-related compound, for location of food resources for *P. interrupta*. There was no sexual dimorphism in trapped beetles in the field trap catches.

In the present study, inclusion of mashed banana in the baited traps enhanced the attraction of beetles during the field trials. However, the result during the study periods showed that attraction varies from season to season (see also chapter 4 Figure 22, and 23) and there are large differences in numbers of catches among the treatments of field trials. It might be due to the variability of odor composition that would change over time and the stage of banana fruit ripeness. This result can be in line with the report of Karg and Suckling (1999) that insects respond to semiochemicals up to a certain concentration or require exposure to a well-defined blend. As we observed in the field, fruits like banana are scarce in some regions of study sites. Extraction, identification of compounds from banana fruits, and field trials could be necessary to standardize the lures reliable in trapping of *P. interrupta.*

According to MOA and EARO (1999), fermentation products from the residue of local beer are highly attractive to *P. interrupta*. Hypothesis was made test the mixture of ethanol and acetic acid, which are fermentation compounds that might enhance the attraction of *P. interrupta*. But our field experiments showed that the five-compound blend caught significantly more beetles than the mixture of fermentation compounds with the five compounds blend and the mixture of fermentation compound alone. This might be due to the chemistry of the mixtures and high volatility of the fermentation compounds. The blend of the five compounds plus fermentation volatiles caught beetles with no significant difference to that of the five-compound blend alone. From these results we can understand that the addition of the present fermentation volatile compounds does not increase the attraction of *P. interrupta* to the lures.

For proper application of the optimal blend of multiple dispensers, the five compound components have been chosen for the second and third year field experiments. The mixture of the five compounds loaded onto one dispenser showed no significant difference in the mean catch of beetles compared with the multiple-dispenser bait with the same compounds. Hence, we can conclude that the mixture of the five compounds might be a much more practical bait formulation in the future than the five vials bunched together as multiple blend formation. In other words, the mixture of the five novel compounds in one vial reduces the cost of dispenser formulation and labor during trapping of *P. interrupta*.

Chapter 4

Field evaluation of locally affordable beetle traps baited with novel attractant compounds for mass trapping of *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae)

4.1. Introduction

Insect pests are one of the main constraints to small-holder sorghum production in Ethiopia. Sorghum chafer, *P. interrupta* (oliver) (Coleoptera: Scarabaeidae) is one of the most devastating insect pests on sorghum and maize (Clark, and Crowe, 1978; Hiwot Lemma, 2000). The beetles are diurnal and fly and feed during the day and hide in the soil during the night (Gunshaw, 1992; Jago, 1993a and 1993b; Yitbarek Wolde-Hawariat *et al*., 2007). The adult beetles move to the host plants using olfactory and visual cues (Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009). Farmers have tried to trap adults with simple containers baited with fruits such as banana and guava (MOA and EARO, 1999). Traps baited with a combination of insect attractant fruits and synthetic compounds have proved to be efficient for control of several beetle species (Alpizar *et al*., 2002; Oehlschlager *et al*. 2002; Vuts *et al*., 2008).

If an efficient trapping system is available, mass trapping and lure-and-kill of adult beetles can be an alternative control method (Vuts *et al*., 2008). No extensive field studies have been carried out to optimize traps for *P. interrupta.* Attempts were made to test one local trap design for the adult beetles in the field (Yitbarek Wolde-Hawariat *et al*., 2007). There are different locally available, cheap materials to design traps. Traps should be dust resistant, rain resistant, easy to handle and with low capture of non-target beetles and other insects (Cork, 2004; Bouget *et al*., 2009).

In the present study, different locally designed, affordable traps, baited with standard lures were developed in the field and laboratory (as shown in chapter 3 above), are evaluated for their efficiency in trapping the adult beetle, *Pachnoda interrupta* on sorghum crop. The volatile compounds and blends are novel chemicals that were evaluated as lures in standard Japanese beetle traps and gave promising results in attracting *P. interrupta* adults when used as lures in field experiments with standard Japanese beetle traps. Trap designs, including attractant inoculation chambers, were produced from locally available cheap materials.

4.2. Materials and Methods

4.2.1. Field site descriptions and experimental procedures

Field experiments were conducted at three locations: Rasa village, Kewot district (09°57'N, 040°05'E); Abuare village, Bati district ($10^0$57’N, 040^0 03’E) and Mendubo village, Dewe-Harowa district ($10^0$50’N, 040^0 05’E), Northeastern Ethiopia, in July and October in three years (2011, 2012 and 2013) to evaluate locally designed traps in trapping adult *P. interrupta*. These study sites have relatively low rainfall, and sorghum is the predominant crop. The experiments were carried out with no insecticides applied in the experimental sites for the duration of the field trials. Trials were carried out during June–July (mating and feeding season) and September–October (feeding season) for *P. interrupta*. During the mating season, trials were carried out in the breeding sites, which mainly consist of forest with *Acacia* trees, while in the feeding season the experiments were carried out in the sorghum fields.

4.2.2. Design and description of different locally affordable traps for field evaluation

Seventeen different locally affordable traps (LATs) (Table 4) were designed and evaluated for their comparative efficacy in trapping *P. interrupta* under field conditions during the study period (2011-2013). The LATs were designed in Insect Science Research Laboratory, Addis Ababa University. Yellow, white, and green color spray paints (ABRO®, U.S.A) were used for painting traps. Most trap canisters (13) were painted with green color, and cross vanes with their funnels were painted with yellow color except two trap vanes painted white and green color. Cross vane traps had two vanes at right angles to each other fit inside the funnel and most of their parts exposed above the funnel. Canisters and funnels with cross vanes were connected at the bottom of the funnel. Most of the trap designs were slippery on the vanes and beetles attempting to land on the vanes usually fall into the collection chamber. Perforations were made on the canister of LATs to drain water during the field experiment and to imitate the JBT except the open container and calabash traps. The JBT has collection canister and vanes connected to the funnel. The canister of JBT, which is evenly perforated, has a dark green color with the height of 20 cm and diameter of 16.5 cm. Both the vane and funnel have chrome yellow color. The vane has a 20 cm height whereas the funnel has 12 cm and top diameter 18 cm. All traps were baited with the same lure during experimental periods. The experiment was designed in a randomized complete block design in ten replications. The captured beetles were removed from the traps every 24 hrs at regular time (9:00-11:00) in the morning and placed in plastic bags with labels for data recording. Only insects that were actually entrapped in the trap were counted. The method of sex determination was performed with the examination of the abdominal grove on male beetles (Rigout, 1989).

Table 4. Nomenclature and description of LATs

Short name	Top: Vane and funnel	Top Description	Canister	Canister Description	Used in seasons	Figure (#)
JBT	JBT	Chrome yellow commercial model	JBT	Green, perforated bucket	Always: The standard model	14 (1)
YT-Hayat	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Hayat	Hayat oil container, painted green, not perforated	July 2011	14(2)
YT-Jug	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Water Jug	Water Jug, painted green, perforated	July 2011	14(3)
YT-GHWB	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Green Highland Water Bottle	Highland Water Bottle, painted green, perforated	July, October 2011	14(4), 15(3)
TY-MMC	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Merti Metal Container	Cylindrical in Shaped metal container	July 2011	14(5)
YTT-PWB	Yellow Top Tall	JBT mimic: Yellow painted vane and funnel, tall vane	Plastic Water Bucket	Plastic Water Bucket dark green	July 2011	14(6)
OpCont	None	No vane and funnel	Open container	Highland Water Container 5L, top cut off, painted green, non-perforated	October 2011	15(1),18(3)
Calabash	None	No vane and funnel	Open calabash	Calabash, naturally yellowish, top cut off, non-perforated	October 2011	15(2)
YT-TSTJ	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Transparent Screw-top Jar	Cylindrical, transparent plastic jar, screw-top lid, non-perforated	October 2011	15(4)
WT-PPB	White Top Standard	JBT mimic: White painted vane and green funnel, standard size	Plastic Paint Bucket	Plastic paint bucket, top open, painted green, perforated	July and October 2012	16(1)
YT-PPB	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Plastic Paint Bucket	Plastic paint bucket, top open, painted green, perforated	July and October 2012	16(2)
GT-PPB	Green Top Standard	JBT mimic: Green painted vane and funnel, standard size	Plastic Paint Bucket	Plastic paint bucket, top open, painted green, perforated	July and October 2012	16(3)
YT-GPJ	Yellow Top Standard	JBT mimic: Yellow painted vane and funnel, standard size	Green Plastic Jar	White plastic jar, press-on lid, painted green, perforated	July 2013	17(1)
GT-GPJ	Green Top Standard	JBT mimic: Green painted vane and funnel, standard size	Green Plastic Jar	White plastic jar, press-on lid, painted green, perforated	July 2013	17(2)
GLV-THWB	Green Large Vanes	Vanes from 5L Highland Water Container, painted green	Transparent Highland water bottle	Transparent Highland Water container, non-perforated	July 2013	17(3)
PYLV-GHWB	Pale Yellow Large Vanes	Vanes from 5L Highland Water Container, painted pale yellow	Green Highland water bottle	Highland water bottle, painted green, non-perforated	July and October 2013	17(4),18(4)
YTLC-5HWC	Yellow Top Large	5L Highland Water container, Bottom open, painted yellow	5L Highland water container	5L Highland water container, painted green, perforated	October 2013	18(1)
YTSC-2HWC	Yellow Top Small	2L Highland Water container, Bottom open, painted yellow	2L Highland water container	2L Highland water container, painted green, perforated	October 2013	18(2)

4.2.3. Field evaluation of locally affordable traps with phenylacetaldehyde lure in July and October 2011

Field studies were conducted in July and October 2011. The July 21- 26/2011 field experiments were carried out in Rasa village, Kewot District in which five locally made traps (Figure 13) were evaluated. The cross vanes of the experimental local traps (Trap 2-to-5; Figure 13) were similar with the height of 20 cm and 17 cm width whereas the height and width of the cross vane of Trap 6 (Figure 13) was 60 cm and 40 cm, respectively. Traps were hung on wooden poles at a height of 3 m equipped with a horizontal top arm.

All experimental traps were baited with phenylacetaldehyde at a dose of 100 µl for each dispenser. Dispensers were attached on the edge of the cross vane so that the plume dispersed evenly in the vicinity of traps. Each dispenser was reloaded with 100 µl of the volatile compound every morning, just before the active time of the beetles in the field. The baited traps were deployed in a randomized complete block design with 10 replications. The distance between the traps and blocks was 10 m and 50 m, respectively.

1. JBT (std.) 2. YT-Hayat 3. YT-Jug 4. YT-GHWB 5. YT-MMC 6. YTT-PWB

Figure 7. Types of locally affordable traps (LATs) tested for their performance in catching and retaining adult *P. interrupta* in July 21-24, 2011. The arrangement and description of traps are explained in table 4 above.

In the October 2011 field experiments, four LATs (Figure 14) were evaluated against JBT in trapping *P. interrupta*. The experiments were conducted in Rasa village at specific site called Lewutgn (09°57'N, 040°05'E) with altitude of 1351 m.a.s.l. in the sorghum fields. It was the continuation of July 2011 field trials. In the October 2011 field trials, sorghum fields with high beetle densities were identified before the experimental layout for trap deployment was determined. All traps were baited with phenylacetaldehyde following the same procedure as that of July 2011 field trials described in 4.2.3 above. 4 ml glass vials (45 × 14.7 mm, clear, Skandinaviska GeneTec AB) and wicks made of cotton rolls (3.9 cm long and 0.9 cm in diameter, Top Dent®, Dental rolls) were used for dispenser formulation. Dispensers were attached on the rim of the cross vane, the open container trap (15 cm diam. × 21 cm deep) and calabash (12 cm diam. × 16 cm deep) traps.

The open container trap and the calabash were filled with 300 ml tap water plus approximately 5 g powdered soap to drown beetles during the trapping experiment. The same amount of soapy water was loaded every morning in the open container and calabash traps respectively. Individual treatments in each block were different and distributed randomly. In the field, baited traps were hung on the panicle of the milky stage sorghum. Complete randomized block design (N=10) was used. The height of each trap was positioned so that the bait was located about the same height as the top of the sorghum plant canopy (approximately 2.5 meter above the soil). The distance between traps and blocks were 10 m and 50 m respectively in a straight-line arrangement.

1. OpCont 2. Calabash 3. YT-GHWB 4. YT-TSTJ

Figure 8. Different locally made traps (LATs) tested for their efficacy in catching and retaining adult *P. interrupta* in October 13-23, 2011. The arrangement and description of traps are explained in table 4 above.

4.2.4. Field evaluation of locally affordable traps with the five novel volatile compounds in a blend of multiple dispensers in July and October 2012

The July field trial was conducted in Kewot district at Rasa village and the October experiment was carried out in Bati district at Abuare village. Three LATs, prototype mimics of the JBT, were evaluated along with the JBT for trapping *P. interrupta*. The experiment was a continuation of the attempts to get an efficient locally developed trap of the current study. Each of them had an outlet at the lower side of the canister for collecting the captured beetles. The difference between the three LATs was based on the color of their cross vanes, which were painted yellow, white and green, respectively (Figure 15). But their canister was the same in size, shape, and painted green color, and evenly perforated. The blend of the five compounds [phenylacetaldehyde, 2, 3-butanediol, methyl salicylate, eugenol and isoamyl acetate] was chosen for formulation of baits in both seasons. Dispensers were loaded with a dose of 1ml of each compound once during the period of field experiments.

In July, the experiment was conducted in four sub-sites. The distance between each sub-site was 500 m. In every sub-site, five replications from each treatment including the control (JBT) were hung randomly on a 3 m height of *Acacia* trees. In October, the three LATs and the JBT (*N*=10) were hung near the panicle of milky stage sorghum. The distance between traps and blocks was approximately 10 m and 50 m, respectively in side sorghum fields. Traps were emptied each morning and beetles counted based on their sex. During the experimental period, temperature and relative humidity was recorded.

1. WT-PPB 2. YT-PPB 3. GT-PPB

Figure 9.Three different colors of the vane of local traps tested for their efficacy in catching and retaining adult *P. interrupta* in the field, July and October 2012. The arrangement and description of traps are explained in table 4 above.

4.2.5. Field evaluations of locally affordable traps with the five novel volatile compounds in a blend of multiple dispensers in July and October 2013

The 2013, field trials were conducted in two different localities. In July, the experiment was conducted in Dewe-Harewa district at Mendubo village. The number of beetles in July was comparatively high. In October, the beetles had virtually disappeared at Mendubo village (data not shown), and the October, 2013 field experiments were instead carried out at Lewutgn of Rassa village in Kewot district.

Four locally affordable traps (LATs) (Figure 16) were evaluated alongside with the JBT in July. Two of the LATs (YT-GPJ and GT-GPJ) were prototypes of the JBT with color variations. The canister plus funnel and the cross vane of one type was green whereas the other type was green canister and yellow funnel plus cross vane. The other two LATs were improved versions of the open container (Figure 14:1) type trap having the same size vane and funnel (height: 10 cm; top diameter: 15 cm). The canister of trap 3 (GLV-THWB) was transparent and cross vane and funnel was green color. The local trap 4 (PYLV-GHWB) canister and funnel was green and the cross vane was light yellow. The canister of both LAT3 and LAT4 was cylindrical in shape (height: 23 cm, diameter at bottom: 7cm).

Vials and cotton rolls, and the procedures of loading with the same amount of compounds onto dispensers, were similar to the October, 2012 field trials. Dispensers were attached on the cross vane of the traps. The baited traps were hung from *Acacia* trees. Unbaited JBT was used as a negative control. Complete randomized block design was used for the trapping experiment. Treatments were replicated seven times and were moved one position within blocks daily before the active periods of the beetles, to decrease the influence of position effects. The distance between traps and blocks were 10 m and 400 m respectively in July.

1. YT-GPJ 2. GT-GPJ 3. GLV-THWB 4. PYLV-GHWB

Figure 10. Four local traps tested for their performance in catching and retaining adult *P. interrupta* in July16-20, 2013. The arrangement and description of traps are explained in table 4 above.

During October 2013 field trials we evaluated the catch efficiency of the four LATs (Figure 17). All traps were modified from the open canister trap that has been evaluated in October 2011. The effectiveness of LATs was compared with the JBT. Among the four LATs, two of them (1 and 2) were produced from two water containers connected to each other at their open neck parts. The canister of trap YTLC-5HWC (height: 27cm, diameter: 15 cm), and YTSC-2HWC (height: 27 cm, diameter: 9 cm) was painted green color, and well perforated as the JBT. The funnel and the tube shaped top opening LATs were painted by yellow color. Between canister and the funnel, there was an opening (3 cm for trap 1 and 2 cm for trap 2) for beetles to pass down into the canister. The 3rd trap was the green open containers trap from October 2011 (15 cm diam. × 21 cm deep) containing approximately 100 ml tap water in it. The 4th trap was made from light yellow color vane and green funnel and canister (height: 23 cm, diameter at bottom: 7cm) (also tested in July 2013). Vials and cotton rolls, and the procedures of loading compounds onto dispensers were the same as those of July 2013 field trials. Treatments were replicated ten times and distance between traps and blocks were 10 m and 50 m respectively. Traps were hung on the milky stage panicles of sorghum plants. During the experimental periods, temperature and relative humidity was recorded.

1. YTLC-5HWC 2. YTSC-2HWC 3. OpCont 4. PYLV-GHWB

Figure 11. Four locally made traps tested for their effectiveness in catching and retaining adult *P. interrupta* in October 07-09, 2013. Trap 1, 2 and the vane and funnel design of trap 4 were the modification of green open container (trap 3), but different in size. The arrangement and description of traps are explained in table 4 above.

4.3. Data analysis

The field data for total catch per trap of *P. interrupta* was subjected to an analysis of variance (ANOVA) to test whether the treatments and the interaction between them had significant effects on response variables. SPSS version 17 software was used to analyze the data. Microsoft Excel was used for data management and for making the graphs. Whenever there were significant differences, means were separated using *Tukey* studentized rang test at $\alpha = 0.05$.

4.4. Results

4.4.1. Trapping efficiency of locally affordable traps under field conditions in July and October 2011

The mean trap catch results of the mating season are shown in figure 18 below. There was no statistically significant difference ($P > 0.05$) between treatments, although the JBT trap appeared to outperform all the others. During the field trials, there were no rains until July 18, which was unusual in the area. The temperature reached up to 39^{o} C during the day with a relative humidity as low as 38%. When the rains started on July 18, 2011, *P. interrupta* started to appear in the field. Based on field observations, the beetle population was low compared with the previous years.

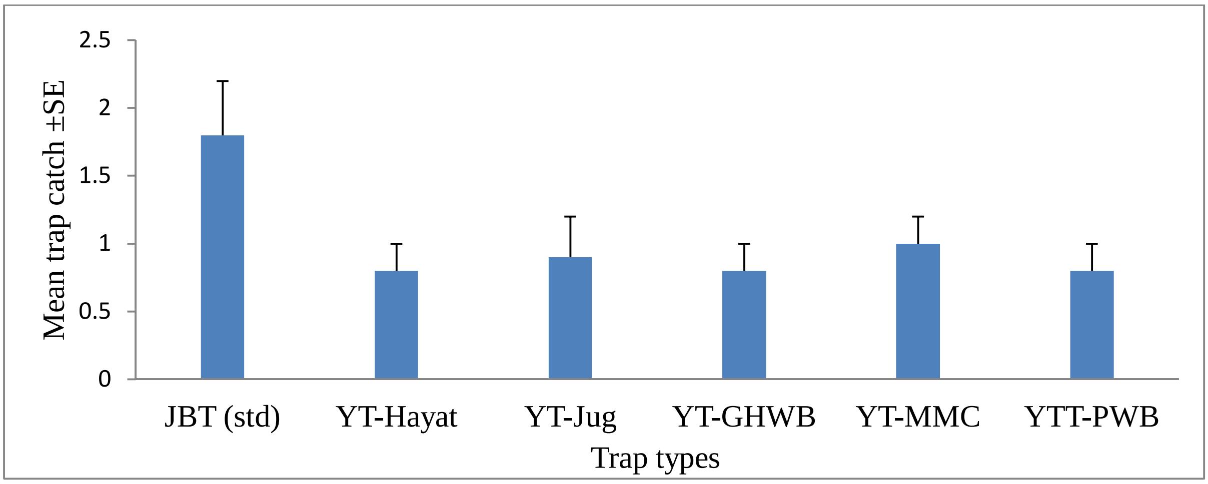


Figure 12. Mean trap catch of adult *P. interrupta* using the JBT and the five locally affordable traps, July 21-26, 2011 in Rasa. The codes of traps in the treatments have shown in table 4.

The October 2011 trap evaluation showed that there was no significant difference ($P > 0.05$) between the open container trap and the JBT (Figure 19). The open container trap caught similar numbers of beetles as the baited JBT, and significantly higher numbers (ANOVA, F=11.1, df =5, $P < 0.0001$) than the other local traps and the unbaited JBT. The average temperature and relative humidity during data collection was 36 °C and 25% respectively.

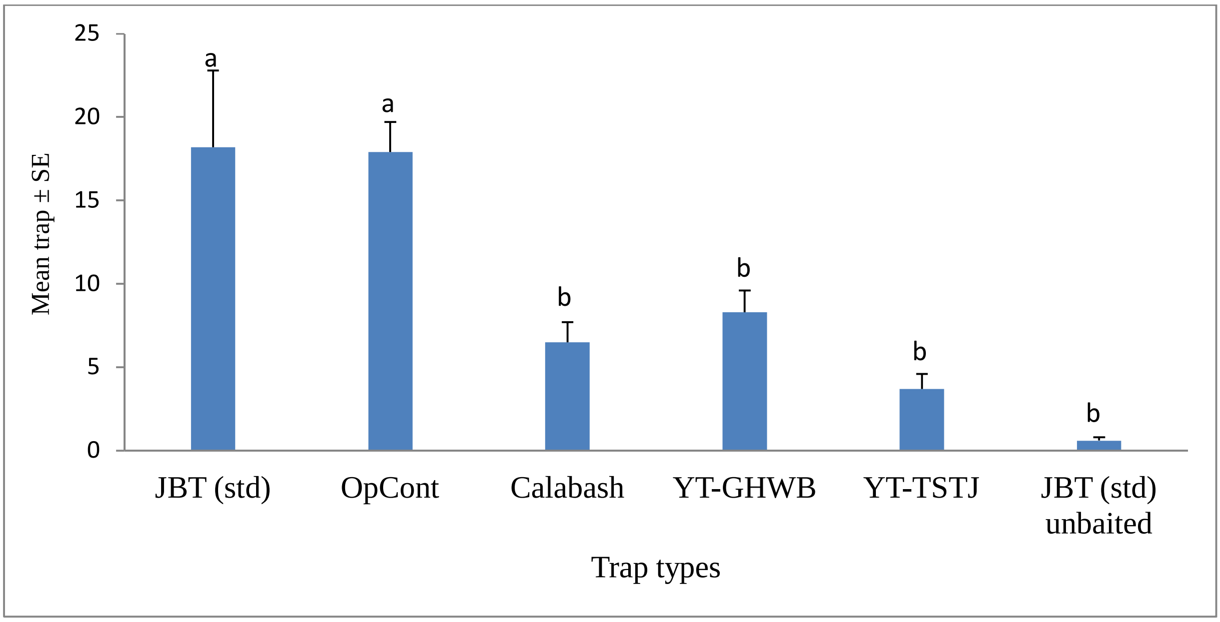


Figure 19. Mean trap catches of *P. interrupta* in JBT (std) (positive control), and LATs baited with phenylacetaldhyde, October 21-23, 2011 in Rasa village Ethiopia. Unbaited JBT was used as negative control. The short names of the locally affordable traps are described in table 4. Error bars represent standard error of the means. Bars followed by different letters are significantly different (ANOVA followed by Tukey's Studentized test, at α = 0.05).

4.4.2. Trapping efficiency of locally affordable traps with the five novel volatile compounds in a blend of multiple dispensers in July and October 2012

The result of the July 2012 field experiment is shown in figure 20. The population of beetles was very low compared with the October, 2011 field trials. The positive control

(JBT (std)) caught significantly higher numbers of beetles than the LATs and the negative control (ANOVA, *F*= 14.1, *df* = 4, *P* < 0.0001). However, there was no significant difference (P> 0.05) in the catch of beetles among the LATs. The average temperature and relative humidity during data collection was 33 °C and 45% respectively.

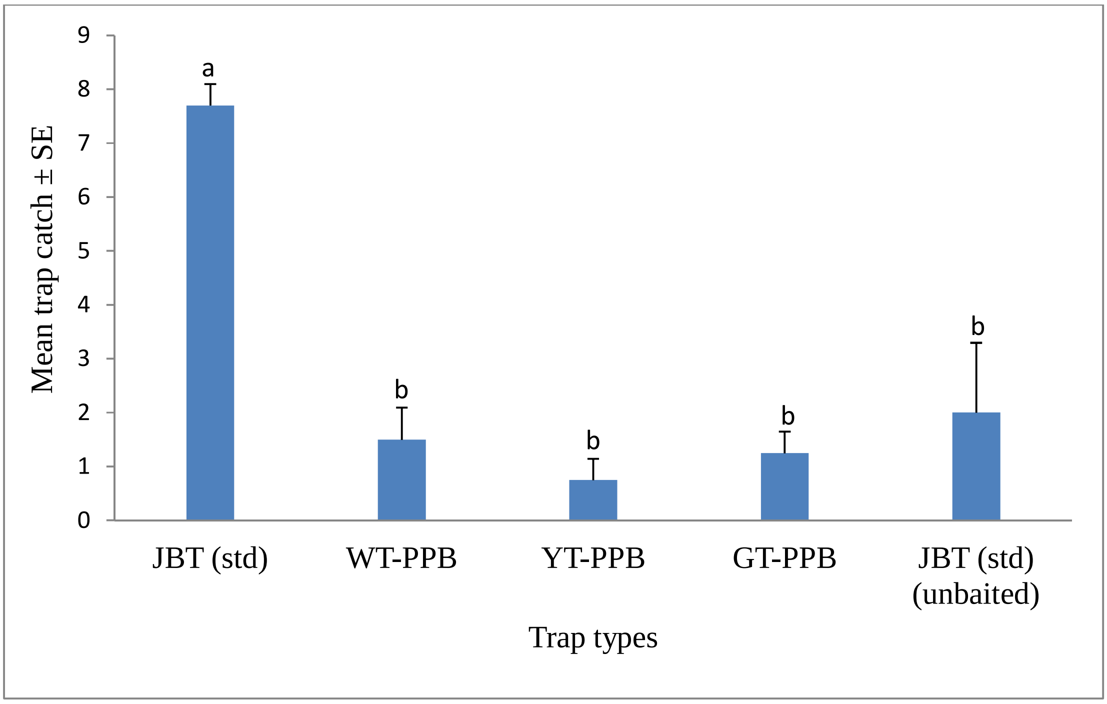


Figure 13. Mean trap catches of *P. interrupta*, from July 14-21, 2012 in Rasa village. The codes for traps in the treatments are described in table 4. Error bars represent standard error of the means. For each treatment, bars followed by the same letter are not significantly different (ANOVA followed by Tukey's Studentized rang test, *at α* = 0.05).

The JBT (positive control) caught significantly higher numbers of beetles than the LATs (ANOVA, *F*= 7.6, df = 3, *P* < 0.005) tested in October, 2012 (Figure 21). However, there was no significant difference (P> 0.05) in caught of beetles among the local traps. Based on the data, the population was almost similar to July, 2012 trapping season.

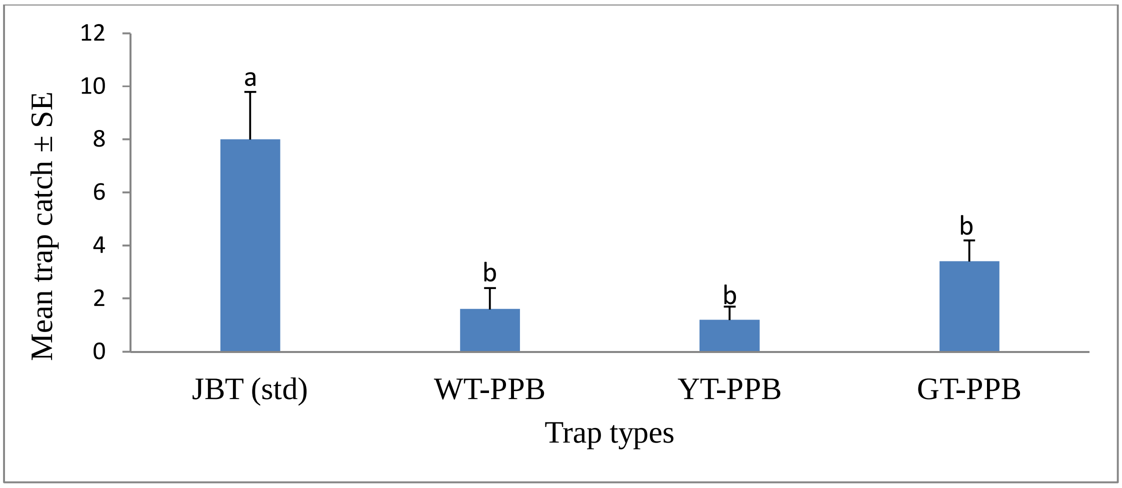


Figure 14. Mean trap catches of P. interrupta, from October 09-11, 2012 in Abuare village, Ethiopia. The canister of LATs (Table 4 above) was the same green color, but the traps have different vane colors. Error bars represent standard error of the means. For each treatment, bars followed by the same letter are not significantly different (ANOVA followed by Tukey's Studentized rang test, *at* $\alpha = 0.05$).

4.3.3. Efficacy of locally affordable traps with the five novel volatile compounds in a blend of multiple dispensers in July and October 2013

The JBT (positive control) caught significantly more *P. interrupta* than the LATs (ANOVA, *F*= 10.06, *df* = 4, *P*< 0.001) (Figure 22). However, there was no significant difference among the LATs in the mean catch performance. The negative control (unbaited JBT), was not included in the statistical analysis due to zero catch during the experiment period.

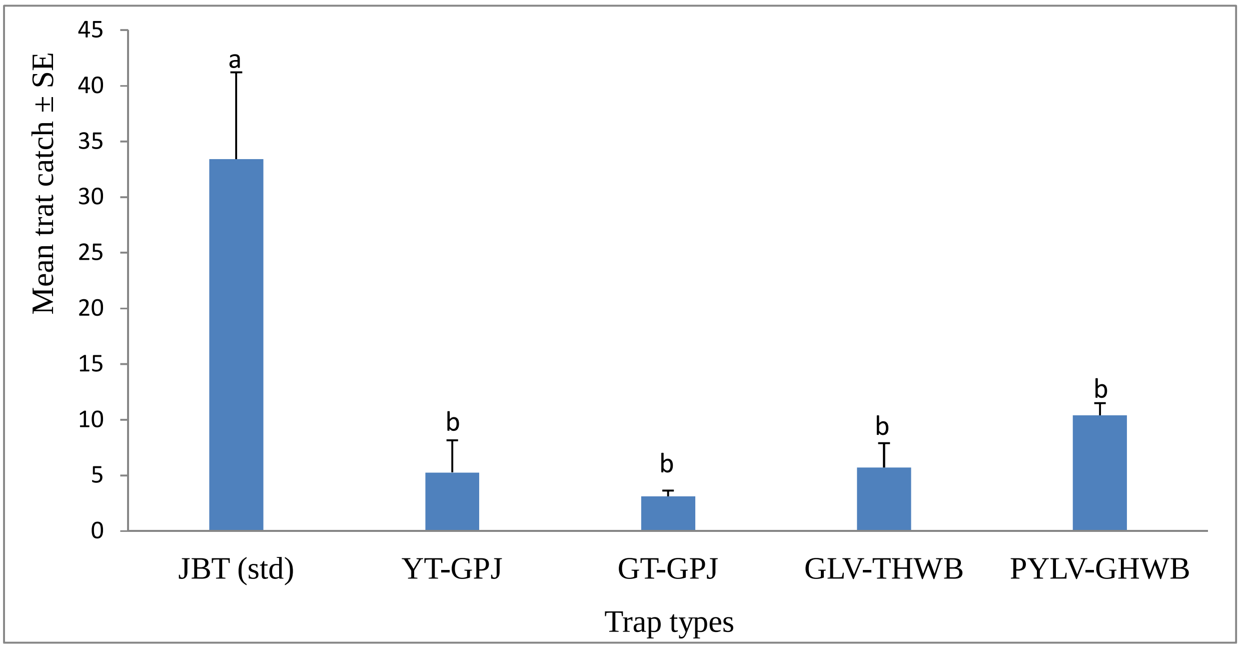


Figure 15. Mean catch of the four LATs the JBT in July 16-20, 2013 in Mendubo village, Ethiopia. The codes of traps in the treatments are described on Table 4 above. Error bars represent standard error of the means. Means with the same letter are not significantly different (ANOVA followed by Tukey's Studentized test, *at α* = 0.05).

The results of the October, 2013 field experiments are presented in Figure 23 below. There was no significant difference among traps in the number of captured beetles. That is, all local traps appeared to have similar efficiency as the JBT.

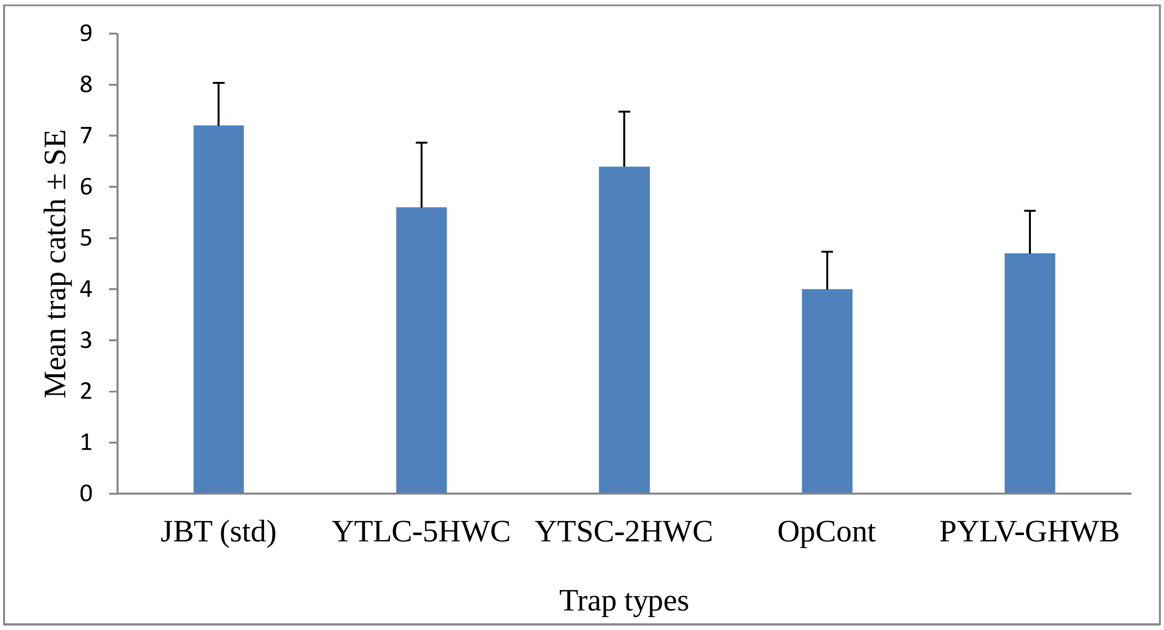


Figure 16. Captures of *P. interrupta* in LATs and JBT baited with the same compounds blend, October 07-09, 2013 in Rasa village. There was no significant difference between treatments $P > 0.05$. Error bars represent standard error of the means.

4.5. Discussions

The commonly used approach for comparing trap designs is insect capture per trap (Dodds *et al*., 2010; Athanassiou *et al*., 2007), to which our procedures conform. Among the fifteen locally affordable trap (LAT) designs evaluated over the three years in the current study, four were significantly more efficient than the others, and could be used as potential trap designs for trapping *P. interrupta*. Both sexes of the beetle were captured by the baited LATs.

We have found some LATs as effective or near as effective as the Japanese beetle trap (JBT) (Trécé, Palo Alto, California, USA) and of course, JBT has proven very efficient in previous studies (Yitbarek Wolde-Hawariat *et al.,* 2007). The baits which have been selected after electrophysiological studies and field-screening performed in previous studies (Yitbarek Wolde-Hawariat *et al*., 2007; Bengtsson *et al.,* 2009, 2010), and in the current experiments described in Chapter 3 and 4, were helpful for identifying better LATs. The population levels of *P. interrupta* during the present study were variable from season to season in the study sites and were low compared with the outbreaks described in earlier studies (Yitbarek Wolde-Hawariat and Hiwot Lemma, 2000). It was common to have large fluctuations in trap counts from one day to another during data collection of the current study. Moreover, it was observed that rain fall resulted in the variation of trap catch.

In most cases, the commercial JBT trap outperformed the locally available designs; even the standard design with yellow painted vanes and funnel made to mimic the important characteristics of the JBT. It is unclear why this would be the case, but all successful locally affordable trap designs were substantially different from the JBT mimic.

As shown in the present study, the green open container trap (OpCont) with 300 ml soapy water is more effective than other local trap designs of the first year experiment in capturing *P. interrupta*. In contrast, the calabash trap with 300 ml soapy water was not strong enough in trapping beetles. Our study indicated that the soapy water as killing agent in the bucket trap has an influence to retain the beetles in the trap and limit the impact of *P. interrupta* on host plants, primarily the milky stage sorghum. As a result, the soapy-water green open container trap along with a lure eliminates the requirement to construct vane traps or escape-proof barriers or the use of insecticide impregnated traps for the beetles. Their general availability and ease of maintenance make locally made traps attractive for large-scale employment in sorghum growing areas. However, the open container trap had the disadvantage of evaporation of water from the reservoir during sunny periods and overflowing during heavy rain events. Therefore, modification of the open container trap is desirable.

There were also other LAT designs, which trapped beetles as efficiently as the JBT in 2013. Long tube-shaped top opening traps with large canisters of the present study had the advantage that they could accommodate more beetles without the need to re-fill or empty the trap. The differences in the number of *P. interrupta* caught in vane traps described in figures 13, 14, 15, 16 and 17 with that of figure 12 might be due to the different designs on top of the funnel that would enhanced exposed portions of the trap through which beetles could gain unhindered access into traps. *P. interrupta* when attempting to land on the trap usually hit the vanes and/or long tube shaped top opening, and fell into the collecting canister. The principle of this mode of action is identical to that of adult Japanese beetle, *P. japonica* trapped by the JBT (Potter and Held, 2002).

Intensive development in trap design, deployment strategy, and lure formulation to overcome problems of low catches are required to achieve sufficient levels of control (Reddy *et al*., 2011). Our initial step was, therefore, to develop effective LATs in capturing *P. interrupta* through successive trials in the field. Data from the current study are useful for establishing the efficiency of various trap designs for capturing *P. interrupta*, because no single design has been superior in capturing beetles. Our findings can provide the basis for mass trapping, 'lure-and-kill', and 'lure-and-infect' approaches for the management of *P. interrupta* using locally developed traps, rather than the unsuccessful traditional methods. It is highly expensive to purchase JBT for sorghum growers in Ethiopia. However, choice of a trap for a particular application like mass trapping or auto-dissemination must also be taken into account. Other considerations such as maintenance, cost, availability, and utility are also vital for sustainable application of baited traps (Cork, 2004; Athanassiou *et al*., 2004; Bouget *et al*., 2009).

In conclusion, based on the results of the present investigation, it is likely that the developed baited local traps can uplift the production of sorghum of the local people and eventually have a multiplier effect on the economy of the country. Baited local traps are also one way to reduce the farmers' dependence on insecticides. Moreover, local traps are useful for monitoring, giving a measurement of the abundance and phenology of *P. interrupta*. The trap designs are such that they can be made in the most economical way possible.

Chapter 5

Isolation and Identification of odors from overripe banana and fermentation volatiles that mediates attraction of sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae)

5.1. Introduction

The adult stage of sorghum chafer, *P. interrupta* is a devastating insect that causes damage on *Sorghum bicolor* (L) seed and also consumes several types of fruits and flowers (Clark and Crowe, 1978; Grunshaw, 1992). Farmers have been applying attract-and-kill methods using fruits like banana and guava for attraction of the adult beetle to the source of insecticides. The method is built on the fact that insect responses to specific olfactory information are fundamental for survival, searching resources, to locate their mate, for reproduction and for selecting their reproductive sites (Bruce *et al*., 2005; Van den Berg *et al*., 2008). However, fruits are scarce in some *P. interrupta*-infested regions. Identification of synthetic standardized attractant compounds from fruits could be a more long-lasting solution. A classical method of extracting insect pheromones/host plant compounds consists of trapping airborne volatile compounds onto an absorbent matrix (activated charcoal; organic polymers) and eluting the trapped chemicals with a solvent (Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2010).

The development of static and dynamic techniques for headspace collection of volatiles in combination with gas chromatography–mass spectrometry analysis has significantly improved understanding of the biosynthesis and ecology of plant volatile organic compounds (Agelopoulos and Pickett, 1998). Adsorbing volatile organic compounds from air has been facilitated by solid phase micro extraction (SPME), which is a solvent-free technique. The odor collection process is not time-consuming. The basis for the method is passive diffusion of volatiles onto a SPME fiber and thereby being enriched during the exposure of the fiber in the closed headspace around the sample (Custodio *et al*., 2006).

Behavioral responses of an insect towards volatile chemicals are usually tested in various laboratory bioassays and in the field by direct observations or catches in odor-baited traps. Plant-derived volatile compounds play a significant role in guiding adult *P. interrupta* to host plants such as banana for feeding (Wolde-Hawariat *et al*., 2007; Bengtsson *et al*., 2009). In the current study, volatile compounds from banana have been collected by SPME. We also included fermentation volatiles, since *P. interrupta* has been reported to be attracted to local beer fermentation residues (MOA, 1999).

Before isolation and identification of volatile compounds from biological material, an experimental set-up has to be designed to study the behavioral response of insects. This behavioral bioassay should also be performed during the later proceedings of this analysis. The aim of the present study, therefore, was to define behaviorally relevant volatile compounds collected from ripe banana for attraction of *P. interrupta* using techniques of SPME couple with GC-MS. During the entire isolation, bioassay procedure and field test, the results were evaluated against behavioral responses at every step.

5.2. Materials and methods

5.2.1. Insect rearing

Adult male and female beetles were collected from Bati district, Abuare village ($10^0$57'.468''N and $040^0$03'.839''E.), and 1383 m above sea level. Ethiopia. Beetles were transported to Alnarp, Sweden and kept in a rearing room in clear transparent plastic boxes with (30 × 12 × 22 cm) at 25°C, RH 60% and 16/8L/D photoperiod. The boxes contained sandy soil brought from under pine and birch trees in Sweden. The soil was first dried in 40°C chamber and cooled before being used for rearing purposes. The soil was moistened through regular watering and beetles were fed organically grown bananas (*Musa* spp.) purchased at local supermarkets. The beetles were sorted by sex and separated in groups of 20 males and females and kept in the rearing cage (22x17x7cm) with 200 g of moist soil for 48 hr to starve them before each bioassay.

5.2.2. Laboratory two-choice bioassays of odors from ripening banana and yeast volatiles

A two-choice laboratory bioassay was designed to assess the response of adult *P. interrupta* to different ripening stages of banana and banana yeast mix bait volatiles. A transparent plastic food container (22x17x7cm) served as a bioassay arena of the central chamber (Figure 24). Air was freely circulated in the chamber. No air flow was drawn through the bioassay chambers; odor transport relied on passive diffusion and air currents. A meshed lid kept beetles from escaping. The 3 cm long and 4.5 cm in diameter gray plastic tube (EN 145-1 Gepruft, 1.8 mm thick) was connected from the two sides (22 cm) of the rectangular plastic arena to the transparent collecting jars. The bioassay design was modified from Larsson *et al.* (2003). The inside part of the tube and the floor of the plastic chamber were covered by a rough paper for easy walking of the beetle during the experiment. The paper was changed after each bioassay session to avoid contamination and cleaned the bioassay setup with ethanol for the next day's experiments. The two transparent collecting plastic jars were 8 cm long and 6.5 cm in diameter. The collection jars were screwed into the plastic lid and were easily removed and switched every data recording time to avoid a position effect.

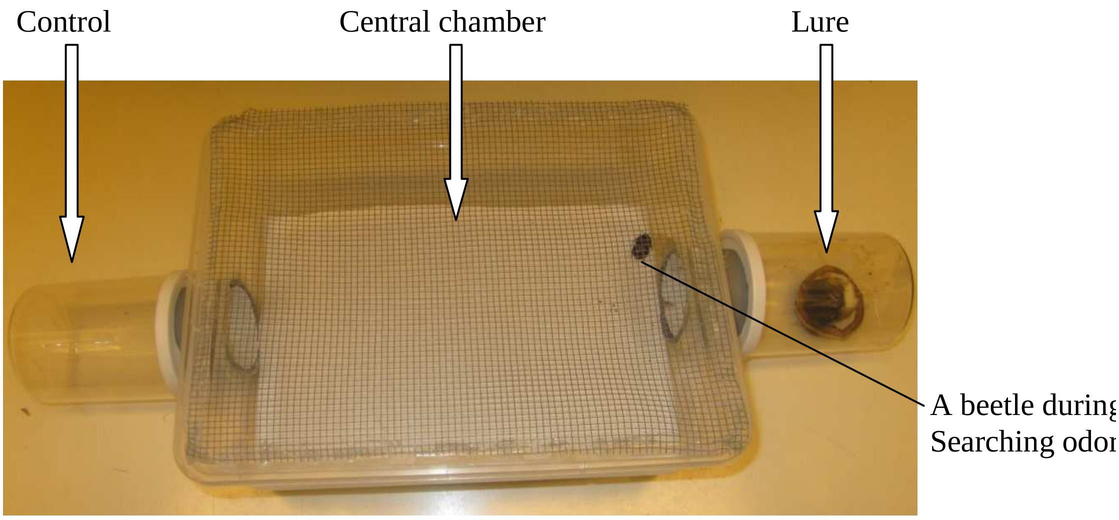


Figure 17. The two-choice laboratory bioassay design used for the behavioral tests on *P. interrupta*. The lid was covered by the mesh so that beetles could not escape and to allow free exchange of air in the central arena of the bioassay. Adult sorghum chafers were placed in the central chamber and could choose either the baited or the control (no bait) sides of the arena over time. Beetles entering each jar were monitored from the time of placing them in the chamber until they made a choice for up to 60 min.

The first step in validating the bioassay was evaluating the behavioral significance of different ripening stages of banana for attraction of *P. interrupta*. During the bioassays, 20 g of sliced banana was placed in one side and with an empty jar on the other side as a control. The experiment was conducted in the rearing room at 25°C; RH 60% during the light time. In each experiment 10 male and 10 female for a total of 20 beetles were used. The beetles were placed one at a time in the central part of the arena to choose freely between the odor and control. Each sex was treated separately and randomly. Time was recorded until the beetles made a choice to enter either of the tubes and crawl into the container, or for up to 60 minutes, after which the trial was considered a non-choice. All test insects were used only once. Male and female responses were recorded separately to see if there was any sexual dimorphism in odor preferences.

5.2.3. Overripe banana and fermentation volatiles collection using SPME

In the present study, volatiles were collected with SPME from overripe banana and fermentation substrates. The degree of ripeness of banana was determined using the color change from greenish color to dark yellow. The banana was washed with tap water to remove debris and rinsed in distilled water for 10 seconds. Approximately 20 g banana was chopped and transferred into pre-cleaned glass vials (24 ml) capped with a 3 mm opening in the center for inserting the SPME needle. Alternative collections for fermentation-related volatiles were prepared by mixing about 20 g of yeast with the banana for 1-24 hours before SPME collection. Before odor collection, the Polydimethylsiloxane (PDMS) SPME fiber was preconditioned at the front inlet of the GC for 1 hr at 200ºC. Then SPME fiber attachment needle was passed into the head space of the vial through the cap opening manually. The fiber coat was pushed by the holder connected to spring tension down the Z slot and positioned in the vial 20 mm from top (Figure 25A). The holder consisted of a stainless steel barrel, a black polymeric plunger, an adjustable depth gauge with needle guide, and a stainless steel retaining nut. The SPME fiber assembly was supported by the metal stand. The fiber was withdrawn into its needle sheath after one hour odor collection (Custodio *et al.*, 2006) and the loaded SPME fiber was ready for desorption in the GC-MS injection port (Figure 25B).

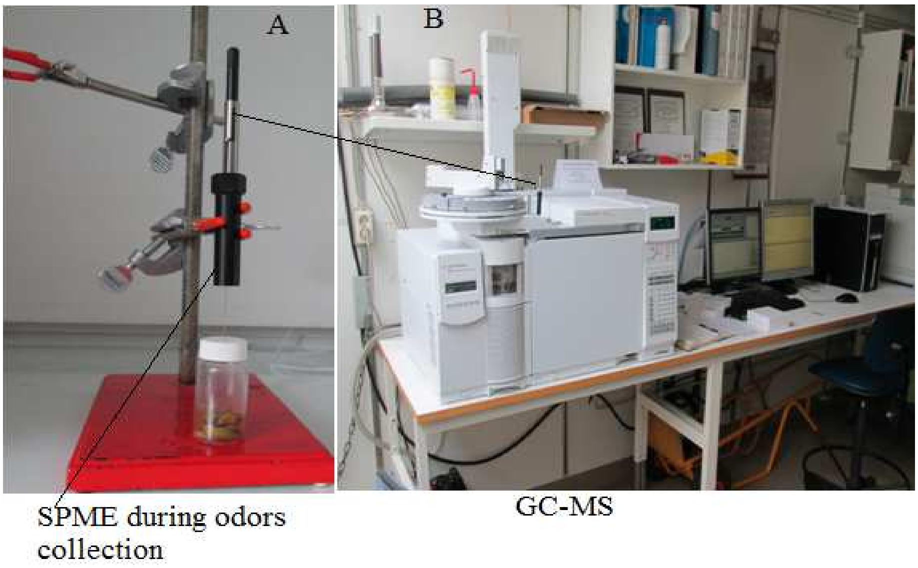


Figure 18 . Odor collection by SPME from overripe banana and fermentation substances (A), and the SPME fiber injected into the injection port of the GC system (B) for analysis in the GC-MS.

5.2.4. GC-MS analysis of volatiles from overripe banana and fermentation volatiles

A wide range of polar and non-polar volatile compounds adsorbed onto the SPME fiber were immediately injected into a splitless mode injection port of the GC-MS (Hewlett Packard 6890 GC and 5975 MS, Agilent Technologies Inc). Analytes are rapidly desorbed into a capillary GC column for analysis. A polar DB-Wax column was used (60 m long with diameter of 0.25 mm). Carrier gas was Helium at a constant flow of 35 cm/second. The temperature program started at 30°C and was held for 2 min. Temperature rose at 8°C/min until 250°C, and was kept at that temperature for the last 5 min. Before odor collection, blank desorption had been made to make sure the fiber and the needle were free of contaminants. Volatile compounds were identified by mass spectral matches to library spectra with the databases customized at SLU, Alnarp and further tentative identification carried out using the libraries NIST 05 and Wiley175. Quantification of the relative ratio of behaviorally active compounds was made from the adsorbent collection based on ion abundances from GC-MS analyses. Re-confirmation was made by re-collecting the synthetic compounds by SPME and injecting into the GC-MS to correlate the peaks produced from the overripe banana with synthetic standards.

5.2.5. Behavioral assays to synthetic compounds versus overripe banana

Among the peaks produced from overripe banana and fermentation volatiles, we made blends of 16 synthetic compounds for approximately matching their relative release ratios to banana, before proceeding to select blends for testing in the behavioral bioassay. Most compounds were quantified from a single canonical collection of headspace from overripe banana. The exceptions to this are acetic acid and 2-phenyl ethanol, which could not be quantified from this collection. Their ratios are added from a collection of banana-yeast fermented for 0.5 h. All the synthetic standards were purchased from Sigma/Aldrich (Sweden) and purities ranged from 99% to 94%. The selection of compounds was based on the information described by Stensmyr *et al*. (2001) and Larsson *et al*. (2003) studied on related species, *Pachnoda marginata* and Becher *et al*. (2012) on *Drosophila melanogaster*. The bioassays were carried out with synthetic volatile compound blends and overripe banana. Different ratios of the identified compounds in the mixture were prepared by varying the quantity of the blended components diluted in paraffin oil to form as single stimulus. 4 ml glass vials (45 × 14.7 mm, clear, Skandinaviska GeneTec

AB) were used for dispenser formulation. In order to formulate appropriate amounts from very small relative abundance found in Z-3-hexenyl acetate and 2-phenyl ethanol, 1 µl neat compound was diluted in paraffin oil and taking 150 and 100 µl from the stock solution respectively and mix with the whole blend. Chemical components tested in blend form were individually mixed in 2.5 ml of paraffin oil using vortex. About 1g of cotton wick was inserted into the vials and soaked with the paraffin blend. Controls contained only 2.5 ml of paraffin oil soaked in about 1g of cotton wick. The synthetic component of the blend was re-collected with SPME and injected in GC-MS to confirm the presence and relative release rates of compounds formulated for the bioassays.

5.2.6. Field evaluation on efficacy of volatile blends

Field trapping experiments were conducted in northeast Ethiopia; in pest regions of Dewe-Harowa district, Mendubo villages (10^{0}50’.468’’N and 040^{0}05’.839’’E) and Kewot district, Rasa village, Lewutegn area (09^{o} 57’ 22.3’’N and 040 ^{o}04 ‘05.8’’E) bordering the Afar region from July 15-23 and October 07-09/2013 respectively. Both districts were found in the Eastern part of Amhara Regional State, Ethiopia. The experiments were carried out on non-pesticide-treated acacia trees and bushy vegetation in July and in sorghum fields during October. During the two seasons of experiments, the weather was characterized by average temperature of 31.5 ºC and average relative humidity of 40%.

For the field trial, 11 and seven components of blends were selected based on the bioassay results. 4 ml, 45×14.7 mm, clear glass vial (Skandinaviska GeneTec AB, Sweden) and cotton wicks (ca. 0.7g) were used for dispensing the volatile compounds. Each compound was 24 -fold stronger compared with the amount tested in the laboratory in order to increase the longevity of the blends in the field. Volatile compounds were loaded in the vials by mixing them with paraffin oil based on their ratios listed in Table 5.

Volatile blends eliciting strong behavioral responses in laboratory studies with minimal numbers of compounds were tested in the field for their ability to attract beetles. The proportion of each neat compound in the blend was formulated based on the information from the behavioral assay findings in the laboratory. The total volume of each bait mixture was 2 ml. Acetic acid (25 µl) mixed in paraffin oil (975µl) was prepared separately from other chemicals in a 4 ml glass vial. Then the mixture of the neat compounds in one vial and acetic acid formulation in another vial were bunched together to form a bait. All the treatments were replicated seven times. Commercially available Japanese beetle trap (JBT) (Trécé, Palo Alto, California, USA) was chosen as standard trap for the field experiment.

Dispensers were attached on the rim of individual traps in upright position. About 25 g of overripe banana was placed inside the JBT's canister as positive control and the blank (paraffin oil soaked into a 0.7g cotton wick) as negative control. Traps were hung from the branch of acacia trees approximately 2 m from the ground in July and on the milky stage sorghum panicle in October. A randomized complete block design was used with seven replications. The distance between the blocks and between the traps in a block was

400 m and 10 m respectively in July and 50 m and 10 m in October. Traps were emptied every day and beetles were counted based on their sex. After each examination every baited trap was shifted its position regularly to minimize impact of trap location on beetle captures. Dispensers were reloaded every two days in July and every day in October field trials.

5.3. Statistical analysis

The two-choice laboratory bioassay data for each blend were pooled as a response category and compared to the assumed 50:50 ratio to find out significant preferences by Chi-square test. There was never any indication that the sexes differed in their preferences. The total number of both sexes that made a choice response for the odors was analyzed to measure attractiveness of the blends. The non-responsive individuals were omitted from the statistical (Chi-square) analysis. For attractive blends, very few individuals remained in the central chamber, however.

The field data for total catch per trap of *P. interrupta* was subjected to an analysis of variance (ANOVA) to test whether the treatments and the interaction between them had significant effects on response variables. SPSS version 17 software was used to analyze the data. Microsoft Excel was used for data management and for making the graphs. Whenever there were significant differences, means were separated using a *Tukey* test. The significance level used in all tests was at the 95% confidence interval.

5.4. Results

5.4.1. Efficacy of volatiles from overripe banana in two-choice bioassays under laboratory conditions

The choice bioassays experiment on overripe banana and the control are represented in figure 26. There was a significant difference between food odors and the control (*N*=20 tested, 15 choosing, $\chi 2 = 6.5$, *df* = 1, $P < 0.01$). No significant difference was found between males and females in the elicited response to overripe banana odor source. The majority of the beetles responded in less than 20 minutes whereas a few of them required as much as 60 minutes reaching to either side of the collection jars.

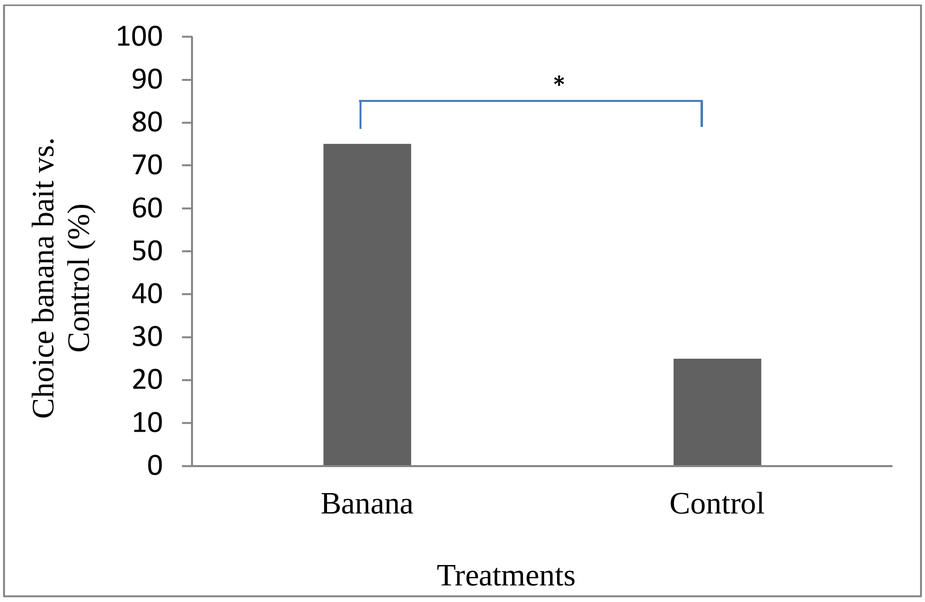


Figure 19. Comparison of responses to overripe banana and responses to the control (empty jar). The Diagram shows relative proportions (%) of beetles (*N*=20 tested, 15 choosing) entering the jar containing the banana or the control (*$P < 0.05$). Results from male and female have been pooled.

5.4.2. Identification of volatile compounds using SPME

The gas chromatography traces of SPME collections of overripe banana (Appendix A) and fermentation volatiles showed at least 26 compounds in the collections, which are listed in table 5. Of these compounds, 20 were identified with some degree of confidence based on mass spectral analysis, of which 16 in turn were further confirmed by means of comparisons with synthetic standards (Appendix B).

Identified compounds were grouped into blends of different chemical class combinations, fruit esters, and fermentation compounds. The number and relative proportion of esters was more than the other components in the blend.

Table 5 Name of compounds after SPME odor collection from overripe banana and fermentation volatiles, and GC-MS analysis. Compounds are listed in ascending order based on their retention time. All compounds except for fermentation compounds acetic acid and 2-phenyl ethanol have been measured from a canonical, representative collection from overripe banana. The three fermentation-related compounds are present based on their ratios from an alternative collection from yeast-fermented overripe banana.

								Laboratory bioassays				Field trial	
Name of compounds	Identification level	RT*	CAS#	** Area (%)	Relative ratios (%) of SyntStd	Dose (µl) of each SyntStd	SPME % of each SyntStd	Blend 1 dose (µl)	Blend 2 dose (µl)	Ester blends dose (µl)	Fermentation blends dose (µl)	Blend 1 dose (µl)	Blend 2 dose (µl)
Ethyl acetate	SyntStd	5.96	141-78-6	3.64	5.23	5	6.59	5	5		5	120	120
Ethanol	SyntStd	6.87	64-17-5	7.06	10.15	22	7.42	22	22		22	528	528
2-pentanone	SyntStd	7.57	107-87-9	0.5	0.71	0.5	2.55						
Isobutyl acetate	SyntStd	8.28	110-19-0	3.85	5.53	3	6.19	3	3	3		72	72
Ethyl butyrate	SyntStd	8.75	105-54-4	3.11	4.47	2	6.37	2		2		48	
Ethyl isovalerate	SyntStd	9.36	108-64-5	0.58	0.83	2	2.20			2			
Butyl acetate	SyntStd	9.46	123-86-4	2.64	3.79	1	7.12			1			
2-methyl-1-propanol	SyntStd	9.91	78-83-1	0.35	0.5	1	4.18	1			1	24	
Isoamyl acetate	SyntStd	10.51	123-92-9	14.7	21.15	7	25.66	7	7	7		168	168
Isobutyl butyrate	MS	11.2	539-90-2	4.93									
Unknown ester 1	Unid.	11.88		2.46									
n-Amyl isobutyrate	MS	11.91	2445-72-9	1.79									
Isoamyl alcohol	SyntStd	12.12	123-51-3	1.35	1.94	1	1.19	1				24	
Butyl butyrate	SyntStd	12.36	109-21-7	2.47	3.55	2	3.53	2	2	2		48	48
Butyl isovalerate	MS	12.94	109-19-3	1.39									
Isoamyl butyrate	MS	13.32	106-27-4	16.19									
Unknown ester 2	Unid.	13.5		1.51									
Isoamyl isovalerate	SyntStd	13.86	659-70-1	15.98	22.99	3	2.98			3			
2-pentyl butyrate	SyntStd	13.64	60415-61-4	10.11	14.54	2	10.11						
Z3-hexenyl acetate	SyntStd	14.65	3681-71-8	0.34	0.48	0.15	1.32	0.15	0.15			4	4
2-phenyl ethanol	SyntStd	15.57	60-12-8	1.98	2.84	0.1	5.26	0.1			0.1	3	3
Unknown ester 3	Unid.	16.4		0.84									
Unknown ester 4	Unid.	17.14		0.74									
Unknown ester 5	Unid.	17.61		1.56									
Acetic acid	SyntStd	20.34	64-19-7	0.84	1.2	1	5.43	1	1		1	24	24
Unknown ester 6	Unid.	21.21		0.15									

SyntStd = Synthetic standard, MS = from Mass spectra, Unid = Unidentified compound, * = the Retention time (RT) was on DB-WAX standard, ** = Data are expressed as percentage of the total peak area of the original SPME collections from overripe banana and fermentation volatiles, (CAS#) = Chemical Abstract Service number

5.4.3. Behavioral response of *P. interrupta* to synthetic compounds under laboratory conditions

The results show that there was no significant difference between the number of beetles being attracted to overripe banana and to the synthetic banana blends 1 and 2 ($P>0.05$) (Figure 27). However, esters and fermentation blends alone did not attract as many beetles as the overripe banana, and were in fact no more attractive than the control. Males and females followed the same pattern of responses to volatile compounds. In other words, no sexual dimorphism was present during attraction of beetles to odor sources.

In bioassays using synthetic standard blends, there was no significant difference between the number of beetles being attracted to blends 1 and 2 ($P > 0.05$) (Figure 27). The two blend components were combinations of esters and fermentation-related products.

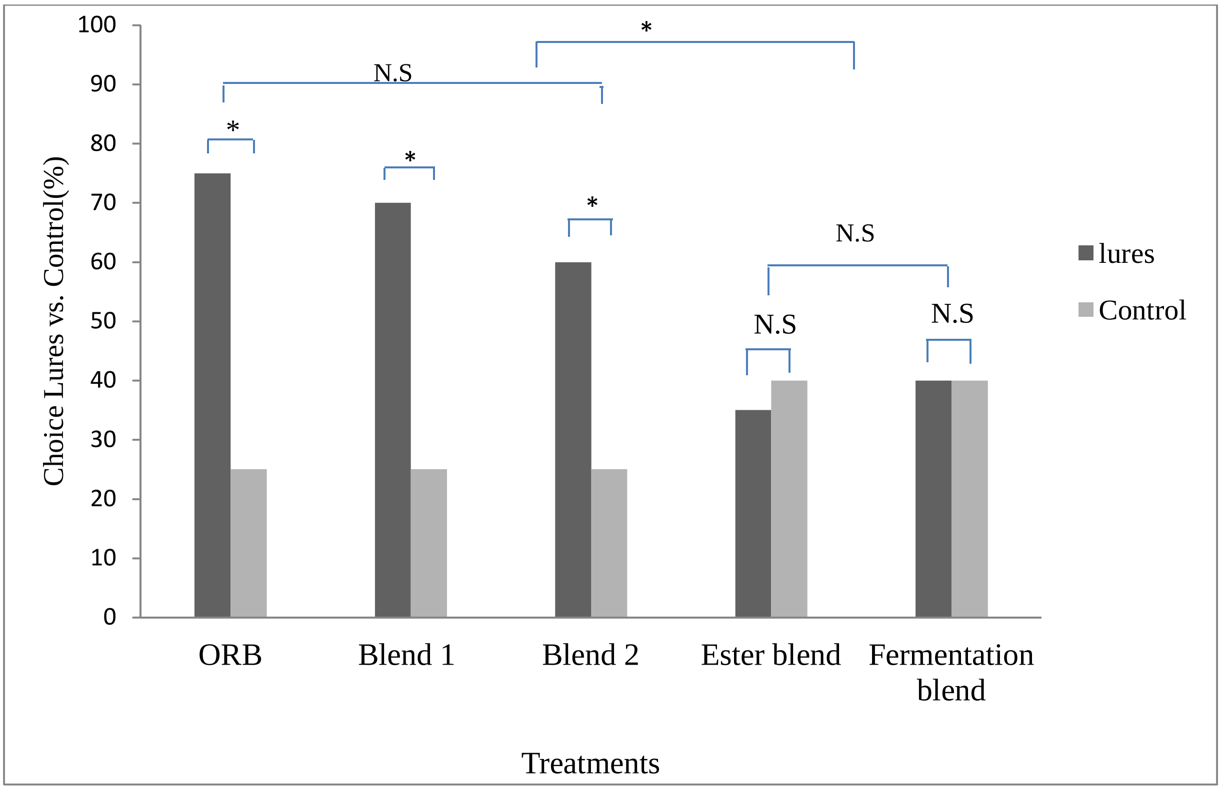


Figure 20. Comparison of responses to overripe banana (ORB) and blends of synthetic compounds. The diagram shows relative proportions of beetles entering the jar containing the lure or the control (paraffin oil), respectively. *N* values are given in table 5. Results from male and female have been pooled. *N.S.* = no significant difference, * $P < 0.05$, according to $\chi 2$ test (1 degree of freedom).

The time taken to respond for the beetles to both overripe banana odor source and synthetic blends was analyzed (Figure 28). Some beetles walked into the collecting jars in less than three minutes. The majority of beetles responded in less than 22 minutes whereas a few others required as much as 60 minutes entering either side of the bioassay arena. There was no significant difference in time to reach into individual odor sources between overripe banana or the two attractive synthetic blends.

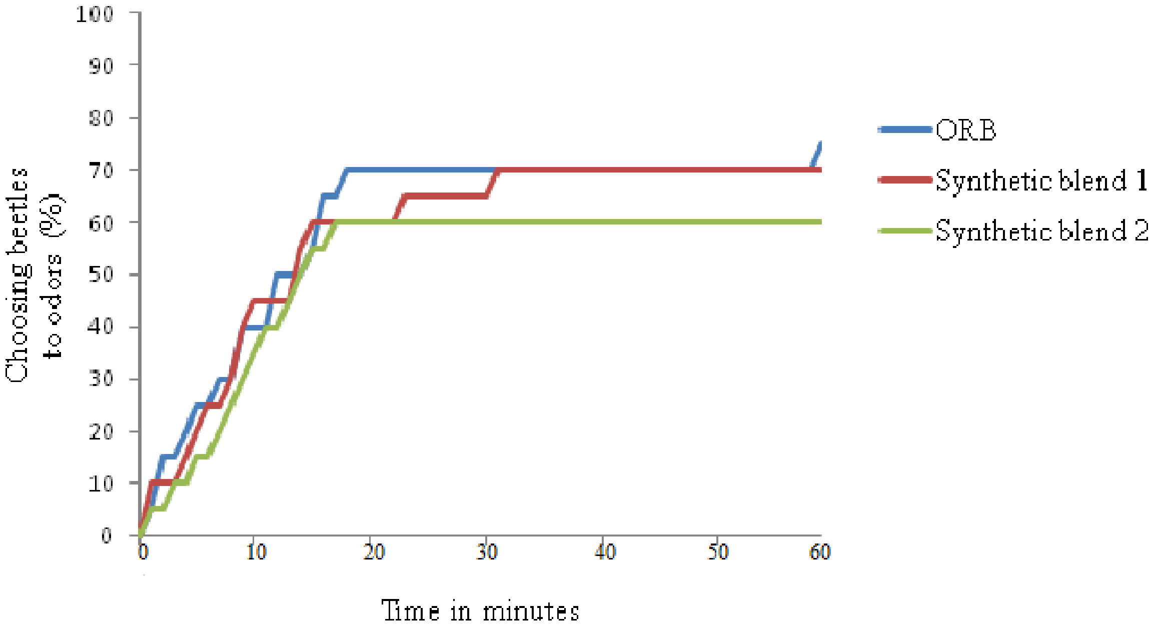


Figure 21. Comparison of time taken for the beetle to respond (cumulative % responding over time) to odor sources of overripe banana (ORB) and synthetic volatile compound blends (blend 1 and 2). All baits elicited virtually identical attraction rates. Results from males and females have been pooled.

5.4.4. Evaluation of banana volatiles and blends as baits for *P. interrupta* in field conditions

There was a significant difference in the mean catch of *P. interrupta* between blend 1 and 2 in both July (*F* = 12.02, *df* = 3, *P* < 0.0001) (Figure 29) and in October (*F* = 38, *df* = 3, *P* < 0.0001) (Figure 30) 2013 with only blend 1 being significantly better than the unbaited control traps. However, there was no significant difference (*P* > 0.05) between banana and blend 1 in either of the two seasons. During both seasons, blend 1 and banana caught significantly more beetles than the unbaited traps.

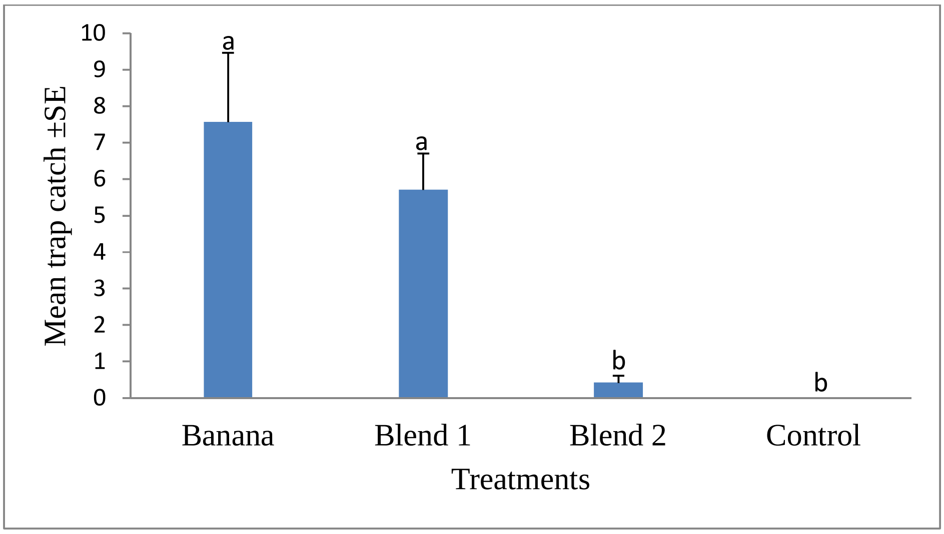


Figure 29. Comparison of captures of *P. interrupta* in JBT traps baited with banana and banana extract blends (N=7) in July 15-22, 2013 in Mendubo villages. Error bars represent standard error of the means. Values followed by the same letter are not significantly different (ANOVA followed by *Tukey* studentized test at α= 0.05).

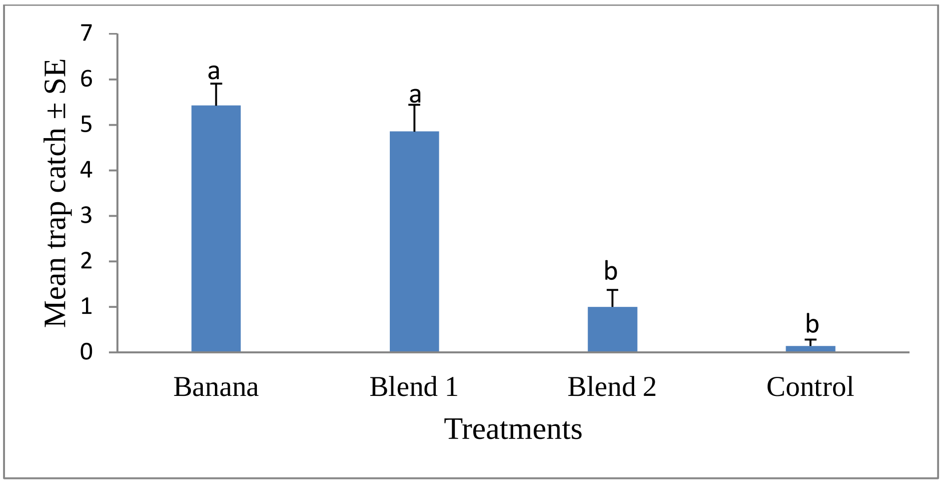


Figure 22. Attraction of *P. interrupta* in the field (October 07-09, 2013) to overripe banana and different ratios of blended compounds in a multiple blend. Error bars represent standard error of the means. Values followed by the same letter are not significantly different (followed by Tukey studentized test at α= 0.05).

5.5. Discussions

Isolation, identification and quantification of the volatile compounds emitted from overripe banana and fermentation volatiles indicated the presence of 11 volatile compounds [ethanol, ethyl acetate, 2-methyl-1-propanol, isobutyl acetate, isoamyl acetate, isoamyl alcohol, ethyl butyrate, Z-3-hexynyl acetate, 2-phenyl ethanol, butyl butyrate, acetic acid] that elicited equivalent behavioral response to the food odor source (banana) on *P. interrupta*.

The second, seven-component blend [ethanol, ethyl acetate, isobutyl acetate, isoamyl acetate, Z-3-hexynyl acetate, acetic acid, and butyl butyrate] of the present study also elicited responses comparable with the 11 component blend in bioassays. Esters blend and fermentation volatile blends alone did not elicit significant behavioral responses (Figure 28), demonstrating that the beetles need the presence of fruit-associated and fermentation-associated compounds together for maximal attraction. This is different from the situation in the fruit fly *Drosophila melanogaster*, which is also attracted to decaying and fermenting fruit. In *D. melanogaster* the fermentation components alone appear to be necessary for attraction (Becher *et al*, 2012). This situation is also different from the situation with five-component bait consisting mainly of flower odors that is currently the standard bait for trapping *P. interrupta*. In that case, addition of fermentation volatiles ethanol and acetic acid did not lead to increased attraction, but possibly even had an adverse effect (Chapter 3, this thesis).

The relative abundance in the peaks of GC-MS of the present study showed that the common fruit esters, isoamyl acetate, isoamyl butyrate and isoamyl isovalerate were higher than the other components of the blend. According to Shiota (1993), 2-pentanone was the principal compound in relative abundance in banana fruit whereas esters and alcohols were present in lower concentrations. However, in our study 2-pentanone displayed the lowest amount next to 2-methyl-1-propanol and Z-3-hexenyl acetate (Table 5).

The behavioral response of *P. interrupta* to food and host plant volatiles appears to be virtually the same in both sexes. This study agrees with the results of Yitbarek Wolde-Hawariat (2008) using the Y-tube olfactometer experiments. This indicates that both sexes of *P. interrupta*, like other phytophagous insects (Bruce and Pickett, 2011), use plant volatiles during the search for food, mating and other behavioral activities. Studies also showed that phytophagous insects rely on volatile attractants in their search for host plants for food, mating, oviposition and avoidance of non-hosts (Sole *et al*., 2010; Bruce and Pickett, 2011). The present study showed that chemical signals from overripe banana may be reliable enough indicators for attraction of *P. interrupta*. This is similar to the results of work done by Yitbarek Wolde-Hawariat *et al*., (2007). Bengtsson *et al*., (2010) also reported that strong increase in attraction of *P. interrupta* was observed in the field by combining the aggregation pheromone and banana compared to pheromone only.

Bruce and Pickett (2011) reported that combinations of appropriate blends are necessary for reproducing attractive host stimuli, but finding the lowest number of truly necessary components can be very complicated. Two-choice laboratory bioassay studies using reduced numbers of components enabled us to narrow down the number of compounds from sixteen to eleven and it was helpful to arrive at a more efficient volatile blend for elicited response of *P. interrupta* in the field. A similar experimental assay was used by Larsson *et al.*, (2003) to screen volatile compounds that could elicit response on *P. marginata*. This proves that although *P. interrupta* is a polyphagous insect, it has the ability to discriminate among the different stimuli and in the presence of these compounds, they showed increased behavioral responses. Stensmyr *et al.* (2001) and Larsson *et al*. (2003) also reported that the related species *Pachnoda marginata* is attracted to a limited number of compounds that represent cues to its potential food sources such as fruits and flowers. The similarity of olfactory system between *P. interrupta* and *P. marginata* was also reported by Bengtsson *et al.*, (2011).

Although both the 11-component and the seven-component blends elicited attraction of the sorghum chafer similar to over ripe banana in the laboratory, the low trap catches of the seven-component blend in the July and October, 2013 field studies clearly demonstrate that its compositions is not optimal for trapping the beetles. The lack of attraction to the seven-component blend in the field demonstrates that, although the laboratory assay appears to be sufficient to screen reasonably attractive blends among many alternatives, it cannot distinguish some of the features necessary for good attraction in the field situation. Conditions in the laboratory do not completely mimic those in nature due to the absence of factors important for natural behavior (Hare, 1998).Our field results demonstrate that some components of the 11-component blend are truly essential for attraction. Some of the subtracted chemicals [2-methyl-1-propanol, isoamyl alcohol, ethyl butyrate, 2-phenyl ethanol] from the 11-component blend are clearly the reason for the lower behavioral response of the beetles in the field. However, there may still be some redundant components remaining in the more complex blend, and we have not performed a complete subtractive field-based trial to determine a true minimal blend for attraction.

Field studies on the behavior of beetles contributed valuable information for efficient trapping of the sorghum chafer. Activity of *P. interrupta* in the field was noticed only during day time. The majority of the beetles observed in the field flew only short distances except in a few cases and these flyers moved to adjoining uninfected milky stage sorghum fields during the feeding season and *Acacia* tree flowers and other host plants in mating seasons. The response of beetles towards over ripe banana observed under laboratory conditions was reflected reasonably well to the behavior under natural conditions. The results from the field-trapping experiment showed no significant differences between males and females captured in traps with synthetic host plant lures. This suggests that both sexes of the beetles respond to the same blend of host-plant volatiles. Donald *et al*. (2012) similarly explained that both male and female weevils, *S. zeamais* in storage houses could modify their behavior based on information from host plant volatiles. Based on the results of the current investigation, therefore, it is likely that the volatile compounds extracted from fermented and/or overripe banana can be applied in *P. interupta* management strategies. Further studies are necessary to demonstrate whether the attractiveness of the banana-derived blend is equal to or better than the current five-component standard blend.

Chapter 6

General Conclusions and Recommendations

6.1. Conclusions

Sorghum (*Sorghum bicolor* (L.)) is a cereal crop that forms one of the staple foods of Ethiopia. The present research has focused on developing tools for *P. interrupta* monitoring and control, to improve sorghum production where this crop is infested. Previous basic studies have identified the first synthetic attractants for attract-and-kill methods to suppress the beetle population. To make this method sustainable and practically applicable, highly attractive synthetic blends and lures, and different varieties of locally affordable traps, were investigated for three consecutive years (2011, 2012 and 2013) in three study site districts (Kewot, Dewe Harawa and Bati), Amhara Regional State of Ethiopia.

Release rates of five previously identified, attractive candidate compounds were tested from dispensers in the lab to ensure a sufficiently high and constant release of volatile chemicals during the flight periods of the beetles in the field. It was also demonstrated that the longevity of the five compounds multiple dispenser blend coincided with the time of beetles staying in the field during the trapping periods. The blend of the putative pheromone and host plant volatiles synergistically attracted more beetles than any of the single volatiles presented alone. Single-dispenser lures with the five compounds released from the same vial proved equally attractive as the multiple-dispenser lure, as there was no significant difference in the mean catch of beetles between the two lures. Our studies have thus led to the development of a practically applicable, cheap and efficient lure formulation that has high potential to reduce cost and labor in future trapping efforts with *P. interrupta*.

Field experiments were performed during both the mating and feeding season in July, and the feeding season in October, to test various blends and single components in the field. Japanese beetle trap (JBT) was used as the standard trap as they have proven attractive in previous studies. We were used a known trap system in order to be able to evaluate the effect of the different volatile chemical blend/single components and dispensers.

Locally affordable yet efficient and practical traps are another cornerstone of locally applicable trapping schemes. Evaluating the performance of the different locally affordable trap designs was carried out during the study periods in parallel with tests of different bait formulations. The field studies were made in close collaboration with farmers. Different local trap types were produced from locally available cheap materials. Their performance was compared with that of the JBT baited with the same lure, which was usually the best confirmed lure available for the present season. The goal was to identify local trap designs that is cheap, easy to produce and use, and still highly effective in catching the beetles. Among the field tested trap designs, we have identified four types that caught beetles with no difference compared to the performance of the JBT. Based on this finding, we can conclude that the current identified local traps showed great promise as an economically viable approach for further trap-based strategies to reduce *P. interrupta* populations.

We have also investigated alternative strategies for designing lures by starting directly from volatile blends of natural stimuli, the banana fruit. A blend of eleven volatile attractant compounds has been identified from banana fruit in the lab extracted by SPME through GC-MS analysis. Behavioral responses tested using the modified two-choice bioassay in the lab and field trials showed no significant different in response between the overripe banana and blend of synthetic compounds. The result indicates that the compounds identified are behaviorally relevant. Field trials showed close agreement with laboratory tests, demonstrating a direct alternative route from attractive substrates via laboratory assays to field trials to identify blends of compounds that can be used as potential candidate for further improvement in trapping of *P. interrupta.*

The current studies have provided the means for further trials to control the sorghum chafer by means of mass trapping, and combined with release of biopesticides that are under way. Further evidence of practical applications to control this beetle in environmentally friendly methods should be obtained from large-scale field trials where farmers would be trained to produce the traps by themselves. We have observed that farmers are highly interested in our study and have an understanding of what happens to the populations of beetles in their sorghum fields. There are several reasons why people accept these beetle control trials. In the first place the expensiveness of the pesticides and the JBT, their awareness of the impact of insecticides on their health and on the environment. Secondly, materials for trap designing are cheap and available. What the farmers were aiming for is how to develop local traps in an effective and inexpensive manner in the future. So this research demonstrates the scientific value for the stakeholders that would apply these environmentally friendly methods, and their potential to mobilize the people in the activities of trapping *P. interrupta* to increase sorghum production with better quality in the regions.

6.2. Recommendations

In view of the basic researches (Yitbarek Wolde-Hawariat, *et al*., 2007; Bengtsson *et al*., 2009; 2010) and no regional and international commercialization (unpublished data), it is fundamental to prompt the locally affordable baited traps to be incorporated into alternative methods for *P. interrupta* control program. Promoting the commercialization of effective local traps might also improve the capability of sorghum growing farmers for the use of affordable local traps for control strategies of the beetle. In order to increase the efficiency of those locally developed traps, it is recommended to conduct more researches to better understand their optimal application in the field.

The mixture of the five-compound dispenser tested in the current study can be used as best attractant in programs to infect *P. interrupta* with entomopathogens. This can be achieved by modifying the locally affordable traps into auto-dissemination system using auto-dissemination devices to infect the population of the beetle with isolated entomopathogens (Klein and Lacey, 1999; Lyons *et al*., 2012). Studies are under way to investigate the effectiveness of trap-based release of inoculated individuals of the adult sorghum chafer beetle in reducing populations.

7. References

Agelopoulos, N. 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.

Alpizar, D., Fallas, M., Oehlschlager, A. C., Gonzalez, L. M., Chinchilla, C. M. and Bulgarelli, J. (2002). Pheromone mass trapping of the West Indian sugarcane weevil and the American palm weevil (Coleoptera: Curculionidae) in palmito palm. *Fla Entomol.* **85**:426–430.

Andersson, M. N., Larsson, M. C. and Schlyter, F. (2009). Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: single-cell responses to ecologically relevant odors. *J. Insect Physiol.* **55**:556-567.

Asmare Dejen and Yeshitila Merene (2013). Ecology and field biology of the sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae) in Ethiopia. *J. Entomol. Nemato*. **5 (5)**:64-69.

Athanassiou, C. G., Kavallieratos, N. G. and Mazomenos, B. E. (2004). Effect of trap type, trap color, trapping location and pheromone dispenser on captures of male *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae). *J. Econ. Entomol*. **97**:321-329.

Athanassiou, C. G., Kavallieratos, N. G., Gakis, S. F. Kyrtsa, L. A., Mazomenos, B. E. and Gravanis, F. T. (2007). Influence of trap type, trap colour, and trapping location on the capture of the pine moth, *Thaumetopoea pityocampa*. *Entomol. Exp. Appl.* **122**:117-123.

Atterholt, C. A., Delwiche, M. J., Rice, R. E., and Krochta, J. M. (1999). Controlled release of insect sex pheromones from paraffin wax and emulsions. *J. Contr. Rel.* **57**: 233-247.

Barak, A., Burkholder, E. R. and Faustini, D. L. (1990). Factors affecting the designs of traps for stored-product insects. *J. Kans. Entomol. Soc.* **63**:466-485.

Becher, P. G., Flick, G., Rozpedowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M. C., Hansson, B. S., Piskur, J., Witzgall, P., Bengtsson, M. (2012). Yeast, not fruit volatiles, mediate fruit fly *Drosophila melanogaster* attraction, ovipositoin and development. *Funct. Ecol.* **26**:822-828.

Becher, P. G., Bengtsson, M., Hansson, B. S. and Witzgall, P. (2010). Flying the fly: long-range flight behavior of *Drosophila melanogaster* to attractive odors. *J. Chem. Ecol.* **36**: 599-607.

Bengtsson, J. M., Yitbarek Wolde-Hawariat, Khbaish, H., Merid Negash., Bekele Jembere, Emiru Seyoum, Hansson, B. S., Larsson, M. C. and Hillbur, Y. (2009). Field attractants for *Pachnoda interrupta* (Coleoptera: Scarabaeidae): Identification by means of GC-EAD and single sensillum screening. *J. Chem. Ecol.* **35**:1063–1076.

Bengtsson, J., Prabhakar Chinta, S., Yitbarek Wolde-Hawariat, Merid Negash., Emiru Seyoum, Hansson, B., Schlyter F, Schulz S. and Hillbur Y. (2010). Pheromone-based mating and aggregation in the sorghum chafer, *Pachnoda interrupta*. *J. Chem. Ecol*. **36**:768–777.

Bengtsson, J. B., Khbaish, H., Yitbarek Wolde-Hawariat, Reinecke, A., Merid Negash., Emiru Seyoum, Hansson, B. S., Hillbur, Y. and Larsson, M. C. (2011). Conserved, highly Conserved, highly specialized olfactory receptor neurons for food compounds in 2 congeneric scarab beetles, *Pachnoda interrupta* and *Pachnoda marginata*. *Chem. Senses* **36**: 499–513.

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

Bicchi, C., Cordero, C., Iori, C., Rubiolo, P. and Sandra, P. (2000). Headspace sorptive extraction (HSSE) in the headspace analysis of aromatic and medicinal plants. *J. High Res. Chromatogr*. **23**: 539–546.

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

Boucher, T. J., Ashley, R. A., Adams, R. G. and Morris, T. R. (2001). Effect of trap position, habitat, and height on the capture of pepper maggot flies (Diptera: Tephritidae). *J. Econ. Entomol*. **94**:455-461.

Bouget, C., Brustel, H, Brin, A. and Valladares, L. (2009). Evaluation of window light traps for effectiveness at monitoring dead wood-associated beetles: the effect of ethanol lure under contrasting environmental conditions. *Agric. For. Entomol*. **11**: 143-152.

Bruce, T. and Pickett, J. A. (2011). Perception of plant volatile blends by herbivorous insects- Finding the right mix. *Phytochemistry.* **72**(13): 1605-1611.

Bruce, T. J. A., Birkett, M. A., Blande, J., Hooper, A. M., Martin, J. L., Khambay, B., Prosser, I.,Smart, L. E. and Wadhams, L. J. (2005). Response of economically important aphids to components of *Hemizygia petiolata* essential oil. *Pest Manag. Sci.* **61:** 1115-1121.

Byers, J. A. (1988). Novel diffusion-dilution method for release of Semiochemicals: testing pheromone component ratios on Western pine beetle. *J. Chem. Ecol*. **14**:199- 212.

Byers, J. A. (1992). Attraction of bark beetles, *Tomicus piniperda, Hylurgops poliatus,* and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. *J. Chem. Ecol.***18**:2385-2402.

Cardé, R. T. and Minks, A. K. (1995). Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol*. **40**:559–585.

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.

Cork, A. (2004). Pheromon manual, natural Resouces Institute Chatham ME4 4TB, UK. pp.73.

CSA (Central statistical Authority). (2013). Agricultural sample survey report 2011/2012 on area and production for major crops (private peasant holdings, Meher season). Statistical Bulletin Volume 1. CSA. Addis Ababa, Ethiopia. pp. 114.

Custódio, L., Serra, H., Nogueira, J. M. F., Gonçalves, S. and Romano, A. (2006). Analysis of the volatiles emitted by whole flowers and isolated flower organs of the carob tree using HSSPME-GC/MS. *J. Chem. Ecol.* **32**: 929-942.

De Bruyne, M. and Baker, T.C. (2008). Odor detection in insects: volatile codes. *J. Chem. Ecol.* **34 (7**): 882-897.

Del Socorro, A. P., Gregg, P. C., Alter, D. and Moore, C. J. (2010). Development of a synthetic plant volatile-based attracticide for female noctuid moths. I. Potential sources of volatiles attractive to *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Aust. J. Entomol.* **49**:10-20.

Dodds, K. J., Dubois, G. D. and Hoebeke, E. R. (2010). Trap type, lure placement, and habitat effects on Cerambycidae and Scolytinae (Coleoptera) catches in the northeastern United States. *J. Econ. Entomol*. **103**: 698-707.

Donald, A. U., Woodcock, C. M., Pickett, J. A. and Birkett, M, A. (2012). Identification of Host Kairomones from Maize, *Zea mays*, for the Maize Weevil, *Sitophilus zeamais*. *J. Chem. Ecol.* **38**:1402–1409.

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. LJ. (1990). Floral Attractants for Cetoniinae and Rutelinae (Coleoptera: Scarabaeidae) *J. Econ. Entomol.* **83**:1298–1305.

El-Sayed, A. M., Byers, J. A., Manning, J. A., Jürgens, M., Mitchell, A. and Suckling, M. V. J. (2008). Floral scent of Canada thistle and its potential as a generic insect attractant. *J. Econ. Entomol*.**101**:720–727.

El-Sayed, A. M., Suckling, D. M., Wearing, C. H. and Byers, J. A. (2006). Potential of mass trapping for long-term pest management and eradication of invasive species. *J. Econ. Entomol.* **99**:1550-1564.

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

Gebeyehu Felek. (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.

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.

Gregg, P. C., Del Socorro, A. P. and Henderson, G. S. (2010). Development of a synthetic plant volatile-based attracticide for female noctuid moths. II. Bioassays of synthetic plant volatiles as attractants for the adults of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Aust. J. Entomol.* **49(1)**: 21-30.

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

Guerrieri, F., Gemeno, C., Monsempes, C., Anton, S., Jacquin-Joly, E., Lucas, P. and Devaud, J. M. (2012). Experience-dependent modulation of antennal sensitivity and input to antennal lobes in male moths (*Spodoptera littoralis*) pre-exposed to sex pheromone. *J. Exper. Biol.* **215(13):** 2334-2341.

Hansson, B. S. (1995). Olfaction in Lepidoptera. *Cell. Mol. Life Sci.* **51(11)**: 1003-1027.

Hansson, B. S. (1999). Insect olfaction. (eds.) Springler Verlag, Berlin Heidelburg. pp.352-449.

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.

Hiwot Lemma. (2000). Historical background on the pest status and control of Sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeidae) in Ethiopia. Proceedings of the Workshop on the Development of Monitoring and Control Strategy against Sorghum Chafer, Pachnoda interrupta (Coleoptera: Scarabaeidae) in Ethiopia, MOA, Addis Ababa, Ethiopia. 9–15.

Heuskin, S., Verheggen, F. J., Haubruge, E., Wathelet, J. P. and Lognay, G. (2011). The use of semiochemical slow-release devices in integrated pest management strategies. *Biotechnol. Agron. Soc. Environ.* **15**(3): 459-470.

Hofmer. H. and Burger, B.V. (1995). Controlled-release pheromone dispenser for use in traps to monitor flight activity of false codling moth. *J. Chem. Ecol.,* **21**(3):355-363.

Howse, P. E., Stevens, I. D. R. and Jones, O. T. (1998). Insect Pheromones and Their Use in Pest Management. Chapman and Hall, London.

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.

Jones, O. T. (1998). Practical applications of pheromones and other semiochemicals (sections 11. Lure and kill), pp. 280-300. **In**: P. Howse, I. Stevens, and O. T. Jones (eds.), Insect pheromones and their use in pest management. Chapman and Hall, London, UK.

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

Karg, G. and Suckling, M. V. J. (1999). Applied aspects of Insect Olfaction. **In**: Insect olfaction. Hansson.B.S. (eds).Springler Verlag, Berline Heidelburg p.449.

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

Klein, M. G. (1981). Mass trapping for suppression of Japanese beetles, pp 183-190. In Mitchell, E. R.(eds.). Management of Insect Pests With Semiochemicals. Plenum Press, New York, NY.

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

Klein, M. O. (1981). Mass trapping for suppression of Japanese beetles, pp 183-190. **In**: Mitchell, E.R. (eds.). Management of Insect Pests with Semiochemicals. Plenum Press, New York, NY.

Klein, M. G., and Lacey, L. A. (1999). An Attractant trap for auto dissemination entomopathogenic fungi in population of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaedae). *Biocon. Sci. Technol.* **9**:151-58.

Kovanci, B. O., Schal, C., James, F. Walgenbach, F. J. and Kennedy, G. G. (2006). Effects of Pheromone Loading, Dispenser Age, and Trap Height on PheromoneTrap Catches of the Oriental Fruit Moth in Apple Orchards. *Phytoparasitica.* **34 (3)**:252-260.

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.

Kromann, A. S. H. (2012). Modulation of olfactory information in the antennal lobe of *Spodoptera littoralis*. Chemica Ecology Unit, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Sweden, PhD-thesis ISBN 978-91-576-7707-5.

Krüger, A. J. and Tolmay, A. T. (2002). Prediction of the release characteristics of alcohols from EVA using a model based on Fick's second law of diffusion. *J. Appl. Polym. Sci.* **84**: 806-813.

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

Landolt, P. J. and Philips, T. W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol.* **42**:371–391.

Larsson, M. C., Stensmyr, M. C., Bice, S. B. and Hansson, B. S. (2003). Attractiveness of fruit and flower odorants detected by olfactory receptor neurons in the fruit chafer *Pachnoda marginata*. *J. Chem. Ecol.* **29**:1253–1268.

Leal, S.W., (2013). Odorant Reception in Insects: Roles of Receptors, Binding Proteins, and Degrading Enzymes. *Annu. Rev. Entomol*. **58**:373–91.

Lomer, C. J., Basteman, R. P., Johnson, D. L., Langewald, J. and Thomas, M. B. (2001). Biological control of locusts and grasshoppers, *Annu. Rev. Entomol*. **46**: 667-702.

Lyons, D. B., Lavallee, R, Kyei-Poku, G., Van Frankenhuyzen, K., Johny, S., Guertin, C., Francese, J. A., Jones, G. C, Blais, M. (2012). Towards the Development of an Autocontamination Trap System to Manage Populations of Emerald Ash Borer (Coleoptera: Buprestidae) With the Native Entomopathogenic Fungus, Beauveria bassiana. *J. Econ. Entomol*. **105**:1929-1939.

Mason ,C. E., Stromdhal, E.Y. and Pesek, Jr. J. D. (1997). Placement of pheromones traps within the vegetation canopy to enhance Capture of male European corn borer, (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **90**:795-800.

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.

Matthews, R. W. and Matthew, R. J. (2010). Insect Behavior, (2nd eds.), Springer Dordrecht Heidelberg London, New York.

Miller, J. G., Haynes, K. F. (1998). Methods in chemical ecology. *Chemical methods*; Kluwer Academic Publishers: Norwell, MA; Vol. 1.

Millar, J. G. and Sims, J. J. (1998). Preparation, clean up, and preliminary fractionation of extracts. **In**:Methods in Chemical Ecology, Vol.1, (eds.) J. G. Millar and K. F. Haynes. New York: Kluwer, pp. 1–37.

Ministry of Agriculture (MOA) and Ethiopian Agricultural Research Organization (1999). Control measures used by farmers and associated problems. Proceedings of a Workshop on the Significance, Distribution and Control of Sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaeidae) in Amhara and Afar regions, Ministry of Agriculture and Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia. 19-23.

Musteata, F. M. and Pawliszyn, J.(2007). Bioanalytical applications of solid-phase microextraction. *Trends Analyt. Chem.* **26:**36–45.

Oehlschlager, A. C., Chinchilla, C., Castillo, G. and Gonzalez, L. (2002). Control of red ring disease by mass trapping of *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *Fla. Entomol.* **85**:507-513.

Paterson, A. H. (2008). Genomics of Sorghum. *Inter. J. Plant Gen.*, Vol. 2008, Article ID 362451. doi:10.1155/2008/362451.

Pawliszyn, J. (1997). Solid Phase Microextraction. Theory and Practice. New York: John Wiley and Sons. Pp. 25–55.

Pedigo, L. P. (1996). Entomology and Pest Management (2nd eds). Prentice-Hall, New Jersey, USA.

Potter, D. A. and Held, D. W. (2002). Biology and Management of the Japanese beetle. *Annu. Rev. Entomol.* **47**:175–205. doi: 10.1146/annurev.ento.47.091201.145153.

Quartey, G. K. and Coaker, T. C. (1992).The development of an improved model trap for monitoring *Ephestia cautella, Entomol. Exp. Appl.* **64**:293-301.

Rasmy, A. (2006). Lure and Kill Strategy: A Promising Safe Approach to Pest Management that Alleviates Synthetic Pesticides Use. *Arab J. Pl. Prot.* **24**:159-161.

Ratnadas, A. and Ajayi, O. (1995). Panicle Insect pests of sorghum in West Africa. Pp 29-38. **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.

Reddy, G. V. P., Balakrishnan, S., Remolona, J. E., Kikuchi, R. and Bamba, J. P. (2011). Influence of Trap Type, Size, Color, and Trapping Location on Capture of *Rhabdoscelus obscurus* (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.* **104**(3):594-603.

Resh, V. H. and Carde,T, R . (2003). Encyclopedia of insects, Academic Press.

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

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

Saïd, I., Kaabi, B. and Rochat, D. (2011). Evaluation and modelling of synergy to pheromone and plant kairomone in American palm weevil. *Chemistry Central Journal* **5**:14 doi: 10.1186/1752-153X-5-14.

Schmera, D. and Guerin, P. M. (2012). Plant volatile compounds shorten reaction time and enhance attraction of the codling moth (Cydia pomonella) to codlemone. *Pest Manag. Sci.***68**:454–461.

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

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

Seneshaw Aysheshum and Mulugeta Negeri (2002). Study on the biology of sorghum chafer, *Pacncnoda intrrupta* (Cleoptera: Scarabaeidae) under laboratory condition. *Pest Manag. J. Ethiop.* **6**:31-36.

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

Sole, J., Sans, A., Riba, M. and Guerrero, A. (2010). Behavioural and electrophysiological response of the European corn borer *Ostrinia nubilalis* to host-plant volatiles and related chemicals. *Physiol. Entomol*. **35**:201–210.

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.

Stipanovic, A. J., Hennessy, P. J., Webster, F. X. and Takahashi, Y. (2004). Microparticle dispensers for the controlled release of insect pheromones. *J. Agric. Food Chem.,* **52**: 2301-2308.

Suckling, D. M. and Karg, G. (1999). Pheromone and Other semiochemicals **In**: Biological and biotechnological control of insects and pests. (eds). Recheigl, J. E., and Recheigl, N. A. Pp: 63-94.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U. S. R., Schnitzler, J-P. (2006). Practical approaches to plant volatile analysis. *Plant J.* **45(4)**: 540-560.

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

Troure, K. and 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. Pp-115.

Van Den Berg, J., Torto, B., Pickett, J. A., Smart, L. E., Wadhams, L. J. and Woodcock, C. M. (2008). Influence of visual and olfactory cues on field trapping of the pollen beetle, *Astylus atromaculatus* (Coleoptera: Melyridae). *J. Appl. Entomol*. **132**:490–496.

Vuts, J. Imrei, Z. and Toth, M. (2008). Development of an Attractant-Baited Trap for *Oxythyrea funesta* Poda (Coleoptera: Scarabaeidae, Cetoniinae). *Z. Naturforsch*. **63**: 761-768.

Witzgall, P., Kirsch, P., and Cork, A. (2010). Sex pheromones and their impact on pest management. *J. Chem. Ecol.* **36**:80–100.

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 Chafer, Pachnoda interrupta* (Olivier), (Coleoptera: Scarabaeidae) in Ethiopia. Addis Ababa. pp 24-35.

Yitbarek Wolde-Hawariat (2008). Electrophysiological and behavioral responses of sorghum chafer, Pachnoda interrupta (Coleoptera: Scarabaeidae) to host plant volatile compounds. PhD Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

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

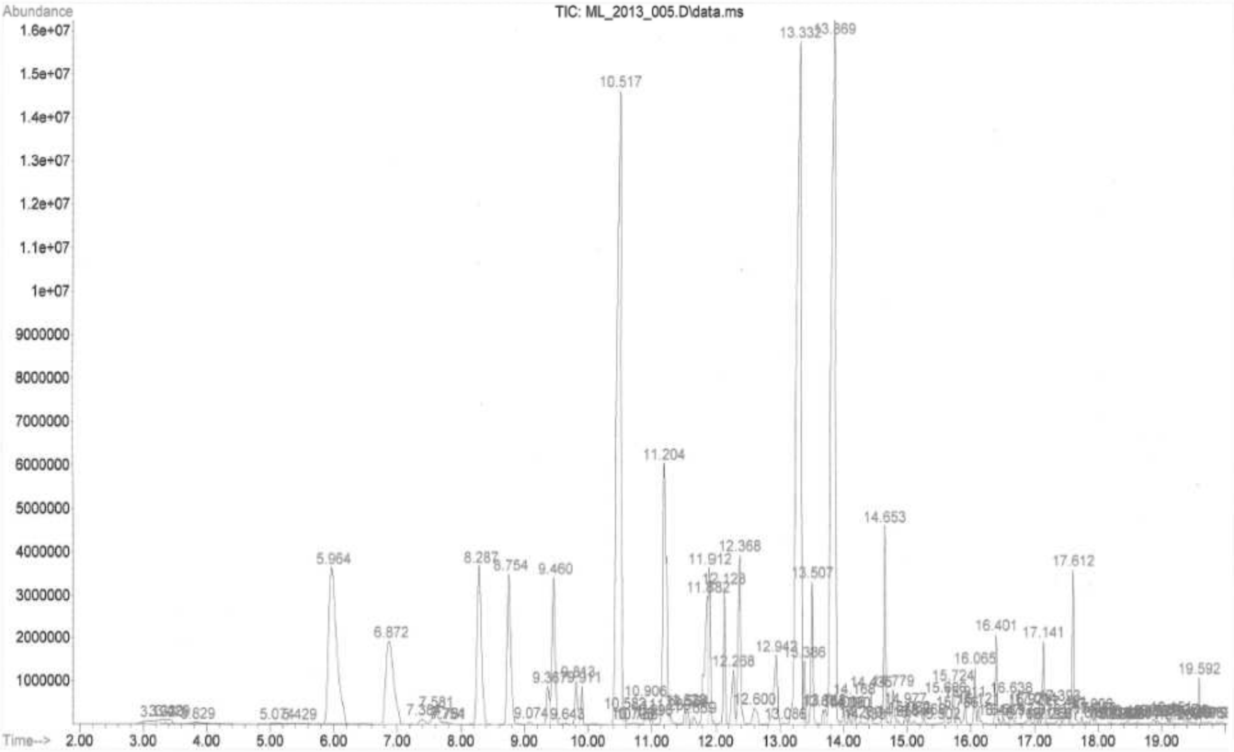
Yitbarek Wolde-Hawariat and Hiwot Lemma (2000). Preliminary yield loss assessment on sorghum due to sorghum chafer, *Pachnoda interrupta* (Olivier) in Amhara Region**. In**: *Proceedings of the Workshop on the Development, Moniotiring and control strategy against sorghum chafer, Pachnoda interrupta*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

Yongma, W., Ge Feng., Xianghui, L., Feng, F. and Lijun, W. (2005). Evaluation of mass trapping for the control of tea tussock moth Euproctis pseudoconspersa (Strand) (Lepidoptera: Lymantriidae) with synthetic sex pheromone in south China. *Intern. J. Pest Manag.* **51**: 289-295.

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.

8. Appendices

Appendix A . Chromatogram from overripe banana generated by GC.



Appendix B . Chromatogram from synthetic standard compounds generated by GC.