Study on the Electrophysiological and Behavioral Responses of *Bactrocera dorsalis*, *B. zonata*, *B. cucurbitae*, *B. oleae* and *C. capitata* to protein lures

A Thesis presented to the School of Graduate Studies of Addis Ababa University in partial Fulfilment of the Degree of Masters of Science in Biology

By: Haimanot T/ Mariam

DEPARTMENT OF ZOOLOGICAL SCIENCES
INSECT SCIENCE STREAM

March, 2015
Declaration by candidate

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere. Where other sources of information have been used, they have been acknowledged.

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Date: .............................................................
Certificate of approval

I hereby declare that this thesis is from the student’s own work and effort, and all other source of information used have been acknowledge. This thesis has been submitted with my approval.

Supervisor: Dr. Habte Tekie

Signature: ................................................

Date: ....................................................
Acknowledgement

I would like to express my deeply appreciation and gratitude to my advisors the late Dr. Emiru Seyoum, Dr. Habte Tekie, and Dr. Teun Dekker for helping me in planning the experiments and for their excellent guidance, follow-up from the beginning to the end of the work. I am also very grateful to Dr. Miriam Karlsson for helping me at the onset of insect rearing and for processing and arranging the exchange programme. I would also like to take this opportunity to thank Proff. Marie Bengtsson for the chemical analysis.

My greatest appreciation also goes to Tibebe Dejene for his technical assistance and support from the beginning to the end of the work.

I wish to thank Swedish University of Agriculture (SLU) for my stay in Sweden and the chemical ecology group staff specially Thomas.

Last but not list I would like to thank my beloved husband Tewodros Mulugeta and my friend Mengistu Dawit for supporting me throughout my work.
# Table of content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgment</td>
<td>i</td>
</tr>
<tr>
<td>Table of contents</td>
<td>ii</td>
</tr>
<tr>
<td>List of tables</td>
<td>iv</td>
</tr>
<tr>
<td>List of figures</td>
<td>v</td>
</tr>
<tr>
<td>List of annexes</td>
<td>vi</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>vii</td>
</tr>
<tr>
<td>Abstract</td>
<td>viii</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Tephritidae attractant</td>
<td>2</td>
</tr>
<tr>
<td>1.1.1 <em>Anastrepha</em> pheromones</td>
<td>2</td>
</tr>
<tr>
<td>1.1.2 <em>Bactrocera</em> pheromones</td>
<td>3</td>
</tr>
<tr>
<td>1.1.3 <em>Ceratitis</em> Pheromones</td>
<td>5</td>
</tr>
<tr>
<td>1.1.4 Male Lures</td>
<td>6</td>
</tr>
<tr>
<td>1.1.4.1 Methyl Eugenol</td>
<td>6</td>
</tr>
<tr>
<td>1.1.4.2 Zingerone</td>
<td>7</td>
</tr>
<tr>
<td>1.1.4.3 Trimedlure</td>
<td>7</td>
</tr>
<tr>
<td>1.1.5 Food baits</td>
<td>8</td>
</tr>
<tr>
<td>2. Objective</td>
<td>9</td>
</tr>
<tr>
<td>2.1 General objectives</td>
<td>9</td>
</tr>
<tr>
<td>2.2 Specific objectives</td>
<td>9</td>
</tr>
<tr>
<td>3. Materials and methods</td>
<td>10</td>
</tr>
<tr>
<td>3.1 Experimental insects</td>
<td>10</td>
</tr>
<tr>
<td>3.2 Protein and fermentation lure sources</td>
<td>10</td>
</tr>
<tr>
<td>3.3 Volatile collection</td>
<td>11</td>
</tr>
<tr>
<td>3.4 Gas chromatography - electroantennographic detection (GC-EAD)</td>
<td>11</td>
</tr>
<tr>
<td>3.5 GC-Mass spectrometry (GC-MS)</td>
<td>12</td>
</tr>
<tr>
<td>3.6 Synthetic blends</td>
<td>12</td>
</tr>
<tr>
<td>3.7 Behavioral bioassay</td>
<td>13</td>
</tr>
</tbody>
</table>
List of Tables

Table 1: Antennal active compounds identified from mix of lures ........................................15

Table 2: Dispersion of the antennal active compounds in different lures and insect species.........17
List of Figures

Fig. 1 Male releasing pheromone that attracts the female.........................................................2

Fig. 2 Structure of ME, DMP and ECF.................................................................................4

Fig. 3 Stereo enantiomers of (R) - and (S)-Olean found in B. oleae sex pheromone..............5

Fig. 4 Antennal response of B. dorsalis, B. zonata, B. cucurbitae, B. oleae and C. capitata to mixed protein bait (± SE)........................................................................................................18

Fig. 5 EAD chromatogram of B. dorsalis................................................................................19

Fig. 6 EAD chromatogram of B. zonata................................................................................19

Fig. 7 EAD chromatogram of B. cucurbitae.........................................................................20

Fig. 8 EAD chromatogram of B. oleae..................................................................................20

Fig. 9 EAD chromatogram of C. capitata.............................................................................21

Fig. 10 Preference of B. zonata in olfactometer bioassay......................................................22

Fig. 11 Preference of B. dorsalis in olfactometer bioassay....................................................23
# List of Annexes

<table>
<thead>
<tr>
<th>Annex</th>
<th>Description</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annex 1</td>
<td>EAD chromatographs of <em>C. capiata</em> for mixed synthetic blends</td>
<td>35</td>
</tr>
<tr>
<td>Annex 2</td>
<td>EAD chromatographs of <em>C. capiata</em> for 3-methyl 1-butanol</td>
<td>36</td>
</tr>
<tr>
<td>Annex 3</td>
<td>EAD chromatographs of <em>C. capiata</em> for butyl acetate</td>
<td>36</td>
</tr>
<tr>
<td>Annex 4</td>
<td>EAD chromatographs of <em>C. capiata</em> for isoamyl acetate</td>
<td>37</td>
</tr>
<tr>
<td>Annex 5</td>
<td>EAD chromatographs of <em>C. capiata</em> for styrene</td>
<td>37</td>
</tr>
<tr>
<td>Annex 6</td>
<td>EAD chromatographs of <em>C. capiata</em> for (\beta)-Myrcene</td>
<td>38</td>
</tr>
<tr>
<td>Annex 7</td>
<td>EAD chromatographs of <em>C. capiata</em> for ethyl hexanoate</td>
<td>38</td>
</tr>
<tr>
<td>Annex 8</td>
<td>EAD chromatographs of <em>C. capiata</em> for 2-phenyl ethanol</td>
<td>39</td>
</tr>
<tr>
<td>Annex 9</td>
<td>EAD chromatographs of <em>C. capiata</em> for alpha methylbenzyl acetate</td>
<td>39</td>
</tr>
<tr>
<td>Annex 10</td>
<td>Annex 10 GC-MS identification</td>
<td>40</td>
</tr>
</tbody>
</table>
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>Methyl eugenol</td>
</tr>
<tr>
<td>CL</td>
<td>Cue lure</td>
</tr>
<tr>
<td>TML</td>
<td>Trimedlure</td>
</tr>
<tr>
<td>RK</td>
<td>Raspberry ketone</td>
</tr>
<tr>
<td>ECF</td>
<td>E-coniferyl alcohol</td>
</tr>
<tr>
<td>DMP</td>
<td>2-allyl-4,5dimethoxy phenol</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstract Service</td>
</tr>
</tbody>
</table>
Abstract

The family of Tephritidae are economically important agricultural pests. Among them *Bactrocera* is the most devastating genus known that damages fruits and lads to economic losses. To control these pests female targeted attractants are essential. Previous work has shown that females of fruit flies are attracted to protein baits. The objective of the present work was to identify volatiles from a mixture of five different lures (brewery yeast, baker’s yeast, torula yeast, GF-120 and anamed) that elicit antennal response of our experimental species. The experiment was carried out on five species of fruit flies (*Ceratitis capitata, Bactrocera dorsalis, Bactrocera zonata, Bactrocera cucurbitae* and on *Bactrocera oleae*). We scored the similarity and dissimilarity of these species in the antennal response to volatiles from mixed fermentation and protein lures using gas chromatography electorantennography detection (GC-EAD). Nine compounds were identified that elicited an antennal response. Of these nine compounds in *C. capitata*, seven compounds in *B. zonata*, six compounds in *B. dorsalis* and *B. cucurbitae* and two compounds in *B. oleae* gave an antennal response. The active compounds were identified using gas chromatography coupled to mass spectrometry (GC-MS). In olfactometer assay flies were attracted to brewery yeast and a mixture of fermentation and protein lures made. The findings are discussed in the potential of developing a bait that attracts these fruit flies.

**Key words:** protein baits, antennal response, GC-EAD, GC-MS, Bactrocera and Olfactometer
1. **Introduction**

Fruit flies jeopardize production of fruits and vegetables worldwide (Vayssières *et al*., 2009). So fruits and vegetables importing and exporting countries are giving increasing attention to fruit fly management to reduce its effect on quality and quantity, in return can affect the nutritional status of fruits and vegetables (Drew, 1992 and Cugala *et al*., 2009). The family Tephritidae, which cause immense economic losses are very destructive group of insects occur all over the tropics and subtropics of the world (Drew, 1992 and Vayssières *et al*., 2009). Female fruit flies that lay eggs under the skin of fruits and vegetables are responsible for direct damage. The eggs hatch into larvae that feed in the decaying flesh of the fruits (Ekesi and Billah, 2007). Infested fruits and vegetables quickly rot and become inedible or drop to the ground. Most of the fruit fly species are polyphagous and attack several fruits and vegetables.

There are about 950 species and 150 genera of fruit fly (Tephritidae) known in Africa, attacking wild fruits and flowers. The Asian species *Bactrocera cucurbitae* (Diptera:Tephritidae), *Bactrocera zonata* and *Bactrocera invadens* are among the exotic alien invasive fruit fly species in Africa even though Africa is known to be the origin of a number of fruit flies (Lux *et al*., 2003; Drew *et al*., 2005 and Mwatawala *et al*., 2006). The hosts of these flies include crops such as citrus, mango, apples, cucurbits, tomatoes, and many others (Cugala and Mangana, 2009).

*Bactrocera dorsalis, B. cucurbitae, B. olea, B. zonata and Ceratitis capitata* are economically important, Therefore my study focused on the above mentioned five species of fruit flies. In controlling fruit flies different techniques are used. Among the several techniques used for fruit fly control, para pheromones and protein bait sprays are the most commonly used. Protein bait can be sprayed by mixing with insecticides, which is environmentally friendly relative to applying full
dosage of insecticides and also attracts both male and female (Sabine, 1992). Pheromones, in contrast, attract only one sex. They are species or genera specific, volatile semiochemicals and can be applied environmentally friendly, with no effect on beneficial organisms. They function by weakening sexual communication and mate annihilation among pests.

### 1.1 Tephritidae attractant

As a rule of thumb in *Bactrocera*, males released pheromone attracts potential searching females (Fig 1). In addition, recent study has shown that naturally occurring male lures may function as precursors in pheromone synthesis (Tan *et al*., 2011). As mentioned above these pheromones are species or genera specific.

![Figure. 1 Male releasing pheromone that attracts the female (Tan *et al*., 2014)](image)

#### 1.1.1 *Anastrepha* pheromones

Different economically important species of the genus *Anastrepha* males take the lek on leaves and attract females to the area through aural, visual and olfactory signals (Aluja *et al*., 2000). In the case of olfactory signals, males emit volatiles from different parts of the insect, like everted pleural pouches, mouth, and anal membranes, and then scattered in the air with the help of rapid
wing vibration (Nation 1972, 1990). Increasing the evaporative surface area of the volatile components can facilitate pheromone attractiveness to the female (Sivinski et al. 1994).

Under laboratory condition mature and virgin females of *A. fraterculus* and *A. obliqua* were found to be attracted to freshly dissected salivary glands of males (Lima et al., 2001). This pheromone attracts females to the males but not to the exact point sources (Robacker 1988), so after reaching vicinity of the male females need to use other ways to find the male like acoustic and/or visual signals (Webb et al., 1983, Sivinski and Calkins 1986). The effort to develop pheromone-based traps for *Anastrepha* is very little because of the complex nature of the pheromonal blend.

1.1.2 *Bactrocera* pheromones

Males of many *Bactrocera* species are attracted to methyl eugenol (ME), a compound found in a wide diversity of plant species and known to be a pheromonal precursor. *B. cucurbitae* and *B. tryoni* are attracted to cue-lure (CL)/raspberry ketone (RK) and *B. latifrons* are attracted to neither CL/RK nor ME (Tan and Nishida, 2012). Like the most economically important tephritid species, *Bactrocera* males are responsible for sex pheromone emission or “calling” while resting on vegetation and detection and subsequent mate searching is by females.

1.1.2.1 Pheromone of *B. dorsalis* and *B. zonata* species

*B. dorsalis* females are attracted to both live males and male rectal gland extract (Kobayashi et al., 1978). The main components of the glands are E-coniferyl alcohol (ECF) and 2-allyl-4,5dimethoxy phenol (DMP) and Z-3,4-dimethoxycinnamyl alcohol, only in some males (Tan and Nishida, 1996). Feeding ME to different species of *B. dorsalis* complex was shown to increase attractiveness of males by converting the consumed ME to the important components of the male pheromones, ECF and DMP (Nishida et al., 1988a & b, Obra and Resilva, 2013). For example, Wee et al., 2007 has shown that females are more attracted to ME fed males than unfed, not only
by feeding synthetic but also feeding on natural floral or fruit sources of ME (Shelly 2000a, 2001a). ME and flower-fed males were found to provoke more frequently and attract greater total numbers of females as compared to non-fed males.

![Methyl eugenol (ME)](image1)

![DMP](image2)

![ECF](image3)

Figure 2 structure of ME, DMP and ECF

In *B. zonata* ME also acts as a pheromone precursor. Like *B. dorsalis* it is transformed into two male sex pheromonal components, DMP and Zconiferyl alcohol (ZCF) (Tan *et al.*, 2014).

**1.1.2.2 Pheromone of *Bactrocera oleae***

This species is quite different from other *Bactrocera* species in that females attract males for mating (Haniotakis, 1974). The major components of the female sex pheromone are 1,7-dioxaspiro[5.5]undecane (also called olean), o-pinene, n-nonanal, and ethyl dodecanoate (Baker *et al.* 1980, Mazomenos and Haniotakis 1981). Olean was also isolated from males (Mazomenos and Pomonis
1983). Baker et al., (1982b) has also identified two other pheromone components, hydroxyl spiroacetals, from B. oleae females. Apart from the attraction of males to female Carpita et al., (2012) identified a compound, (Z)-9-tricosene, from male rectal gland extracts which was attractive to female. Actually not only the rectal gland extracts, but also whole body extract attract females (Mavraganis et al., 2010). Studies (Haniotakis 1974, Mazomenos and Haniotakis 1981, 1985) have shown male attraction to natural or synthetic components or whole blends of the female pheromone in B. oleae. Laboratory and field investigation demonstrated that olean was more attractive than the remaining three components, o-pinene, n-nonanal, and ethyl dodecanoate, but that the blend of all four components was more attractive than olean alone.

![Stereo enantiomers of (R)- and (S)-Olean](image)

Figure. 3 Stereo enantiomers of (R)- and (S)-Olean found in B. oleae sex pheromone (Tan et al., 2014)

### 1.1.3 Ceratitis Pheromones

The detailed description of emission of volatiles associated with the attraction of females to males was given by Fe’ron at different period, 1959 and 1962 in C. capitata. Female attraction to males was also supported from laboratory work by Jang et al., (1998) and field (Shelly 2000c) studies. For the medfly, Ohinata et al., (1977) and Nakagawa et al., (1981a) provided the first quantitative demonstration of the long-range, female attraction to calling male in the field by recording female
captures in male-baited traps. Methyl (E)-6-nonen-1-ol – were thought to be the components of pheromone females are attracted to (Jacobson et al., 1973). Ohinata et al., (1977, 1979) identified 15 carboxylic acids in addition to the two main components. Later Baker et al. (1985) put the three, ethyl (E)-3-octenoate, geranyl acetate, and (E, E)-a-farnesene, to be the main components of the male pheromones.

1.1.4 Male Lures

Male lures can be two type, manmade and plant borne. The manmade are cue lure (CL), trimedlure (TML), fluorinated methyl eugenol analogs, and raspberry ketone-formate (RKF). α- copaene, ME, RK, and zingerone are plant-borne lures. The chemical structures of certain pheromones are simple. Thus, male lure synthesis is relatively cheap for certain species. Since male lures are very potent attractants in most cases using them for mass trapping program is better than developing male sex pheromone as baits (Vargas et al., 2010a). So surveys and detection of invasive species, delimitation of an infestation, and control or eradication is made frequently using male lures as baits via the male annihilation technique. Below different male lures are discussed.

1.1.4.1 Methyl Eugenol

Nowadays, detection, control and eradication of any pestiferous tephritid species are dependent on the most recognized and powerful male lure called ME. The chemical is found naturally in more than 450 plant species of different families from a trace to over 90 %. It occurs in oil, flower, leaves, roots stem or whole plant extracts (Tan and Nishida 2012). It was first discovered as a fruit fly attractant by Howlett (1912, 1915), who observed males of B. dorsalis and B. zonata responding to ME-containing citronella grass, Cymbopogon nardus (L.) Rendle. Steiner (1952) further documented the strong attraction of B. dorsalis male to ME and noted their vigorous feeding on
the chemical. ME was also found to elicit the greatest feeding response compared to other 34 chemicals analogous to it (Metcalf et al., 1975). Carcinogenicity of ME to mice, rats and microbes is the drawback with the use of ME (Miller et al., 1983, Schiestl et al., 1989). The good thing is ME does not pose a significant cancer risk in humans (Long et al., 1963) rather has some benefits to human health, e.g., reduction of cerebral ischemic injury (Choi et al., 2010) as well as anti-anaphylactic properties (Kim et al., 1997) and it is also component of our diet, e.g., flavoring in baked goods and candy and in most spices (Smith et al., 2002).

1.1.4.2 Zingerone

Zingerone (4-(4-hydroxy-3-methoxy-phenyl)-2-butanone, 4-hydroxy-3 methoxybenzyl- acetone, (vanillylacetone) is a phenyl butanoid and has pungency of ginger due to it. Males of ME and RK responsive Bactrocera species, particularly, B. dorsalis and B. cucurbitae are attracted to Zingerone (Tan and Nishida, 2000&2007). Even though, it is relatively very weak attraction in comparison to ME and RK (Tan and Nishida, 2007). As consumed by B. dorsalis males Zingerone is converted to zingerol, as a component of male sex pheromone (Tan and Nishida, 2007). A recent work has shown zingerone’s potential in attracting mates in B. cucurbitae and B. tryoni (Kumaran et al., 2013).

1.1.4.3 Trimedlure

Since its discovery approximately 50 years ago (Beroza et al., 1961), TML (tertbutyl 4(and 5)-chloro-trans_2-methylcyclohexane-1-carboxylate) has become widely adopted as the chief male attractant used in detection and surveillance programs for C. capitata (Jang et al., 2001). Traps baited with TML captured as many C. capitata males.
1.1.5 Food baits

Protein contained foods are essential for fruit flies egg development and to reach sexual maturity (Mazor et al., 2002). It’s been reported that both sexes of fruit flies are attracted to protein baits (Sabine, 1992). Since females are the main reason for increasing the population number of these pests, attractive baits are required for trapping female fruit flies (Mazor et al., 2002). Yeast protein, yeast outolysate and traps baited with fermented sugar and liquid solutions of protein are among the protein baits used in monitoring and control of fruit flies (Epsky et al., 1999, Sabine, 1992).
2. Objective

2.1 General objective

- The main objective of this study is to contribute in developing lure for fruit flies, *B. dorsalis*, *B. zonata*, and *B. oleae*, *B. cucurbitae*, and *Ceratitis capitata*

2.2 Specific objectives

- To identify volatiles from baits or lures attractive to female fruit flies of various species
- To identify which compounds from the complex mixtures/lures elicit responses on female antenna using GC-EAD
- To identify electrophysiological active compounds using GC-MS from baits which are found to be attracting females
- To identify differences and commonalities in the response of closely related and distantly related species to the complex mixtures
- To study the behavioural response of the flies to different protein baits using an olfactometer
3. Materials and methods

3.1 Experimental insects

The pupae of all flies used in these experiments were brought from International Atomic Energy Agency (IAEA) division of nuclear techniques in food and agriculture, Austria, Vienna. Adult flies were kept in 30x30x30cm cages provided with water and mixture of yeast and sugar (three hand of sugar and one hand of yeast) (Biasazin et al., 2014). The colony was maintained at a temperature of 26-29°C, 50-60% relative humidity, and 12:12 hr. light and dark photoperiod. All the experiments were done in Swedish University of Agricultural Sciences (SLU) Alnarp, Sweden.

3.2 Protein and fermentation lure sources

Saccharomyces cerevisae is an important yeast species which has been a very useful tool in bread, beer and wine making for man kinds. The yeast species is produced in a way which is suitable to make the products. For example, the wine and beer making strain is genetically selected to produce more alcohol and less carbon dioxide since alcohol is important product. The reverse is true for baker’s yeast making strain because carbon dioxide is the important product. We also used products made of this yeast species which are baker’s yeast and brewery yeast. We are not sure which species is used the torula yeast we had used in our experiment.

Two of the fruit fly attractants used (anamed and torula yeast) in this experiment were purchased from ISCA technologies and brewery yeast was brought from St. George brewery factory Addis Ababa, Ethiopia and transported to the Swedish University of Agricultural Sciences (SLU) Alnarp, Sweden. Gf-120 and baker’s yeast was provided by SLU.

For volatile collection of baker’s yeast, 2g of Baker’s yeast and 2g of sugar were mixed with 4ml of water in a glass used for aeration while the other three lures (Gf-120, anamed and brewery yeast)
used directly for odor collection. Since torula yeast is the form of pallets, three torula yeast pallets were dissolved in one liter water for volatile collection.

### 3.3 Volatile collection

Odors were collected from five types of protein baits (Brewery yeast, Baker’s yeast, Torula yeast, Anamed and Gf-120). The aeration was held for three hours for all the baits. The compounds in the baits were trapped by passing charcoal-filtered and humidified air through a Porapak-Q mesh 50-80 adsorbent trap, in each column 0.05 g of adsorbent (Porapak Q) were used. The adsorbent (Porapak Q) were contained inside the Teflon tube by two stoppers, one in each end, made from polypropylene wool and thick walled Teflon tube. The adsorbent were rinsed with 2 ml n-hexane before the experiment and used for aeration from baits contained inside a wash bottle. The collected compounds on the adsorbent were extracted with 1 ml of n-hexane into glass vials (size 1.5 ml). Immediately after collection the samples were stored at -20 °C for gas chromatography coupled with electroantennography detection (GC-EAD) and gas chromatography coupled with -mass spectrometer (GC-MS) analysis. The wash bottle used for aeration was rinsed with acetone and burned in an oven at 350°C for 8 hours before the next aeration. The odor collection protocol were the same for all the lures.

For electrophysiological studies the extracts taken from the baits were pooled (mixed) by taking 500µl of each sample and were kept for 24 hours in a glass vial for thorough mixing.

### 3.4 Gas chromatography - electroantennographic detection (GC-EAD)

GC-EAD was used to detect compounds that elicit antennal response of the flies. The set up was linked to the 6890N gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA) with an HP-5 column (diameter 0.25 and length 30m). The carrier gas was hydrogen (1.5 ml/min). The
oven temperature was held at 40°C for 3 min, then increased at 10°C/min to 280°C and held for 5 min.

For EAD recordings, the insect was mounted in a plastic pipette tip with the head protruding from the aperture. The ground electrode was inserted in the head and the recording electrode was placed on the antenna. Most of the time two positions are preferred for EAD: the tip (distal) and the base (proximo-medial) of the antenna, as positional differences yield differences in EAD amplitudes but in this experiment only distal recordings were covered (Biasazin et al., 2014). The micro-electrodes were filled with saline solution creating a saline bridge between the antenna and the Ag/AgCl electrodes. The preparations were held in a humidified continuous air stream of 1.5 l/min.

3.5 GC-Mass spectrometry (GC-MS)

For identification of the compounds from the mixed lures, a gas chromatography coupled with a mass spectrometer was used. Extracted compounds from mixed lures in a mass spectrum were obtained on a 6890N gas chromatography linked to a 5975 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a HP5 column. The carrier gas was Helium. The temperature was set at 30°C for 2 min and increased with 10°C/min until reaching 300°C, and held for 5 min. 2µl of the sample was injected manually. Peaks were identified using NIST 05 and Wiley 175 library.

3.6 Synthetic blends

After the compounds were identified in GC-MS, synthetic blends of the compounds were tested on the insect antennae for conformation. Dilutions of the blends down to 1µg/µl were prepared in a hexane from the stock solutions.
Butyl acetate, isoamyl acetate, styrene, myrcene and ethyl hexanoate (purity 99.5, 98, 99, ?, 99% respectively) were purchased from Sigma Aldrich, 3 methyl-1 butanol (purity 98.5%) and 2 phenyl ethanol (purity 98 %) were provided by Acros organics and Merck schuchardt.

3.7 Behavioral bioassay

Bioassays were carried out under laboratory conditions at the most active period of the day (9 a.m. - 3 p.m.) at a temperature of 25°C and 43% relative humidity for both male and female insects. A glass Y-tube olfactometer of 3 cm diameter with two arms of 20 cm length each was used. Humidified air was purified with activated charcoal and blown into each lateral arm (1 l/min). The humidified air was blown through odorless teflon tube connected to the two arms of the Y-tube, in between a junction of glass. In one arm a filter paper free of treatment and taken as a control, and in the other, the tested lure was enclosed. In the same set up both arms were enclosed with different lures and preference between the lures was determined. To avoid a directional bias, the odor source were alternated after every five trial and the Y-tube set up was placed inside a box, covered with cartoon and white mesh. The Y- tube was changed after every ten trials.

Adult female and male insects in a laboratory were starved for 24 hours before use. The insects were then tested individually by placing them in the downwind end of the Y-tube. A total of 120 individuals were tested per odor source, 60 males and 60 females. The behavioral choice made by each individual was recorded by distinguishing three modalities: choice for compound in one arm or the other, this is when both arms are enclosed with extracts, choice for control, or no choice. No choice was defined as the insect remaining in the common arm and a choice was defined as the insects walking further than two thirds of one arm and remain in that area for 6 minutes. At the end of each experiment the Y-tubes were cleaned with acetone and burned in an oven at 350°C for 8 hrs. For the behavioral bioassay, *B. zonata* and *B. dorsalis* were used; they were tested for 5μl
of brewery yeast and mixtures of five baits, brewery yeast, baker’s yeast, torula yeast, gf-120 and anamed. For the preparation of the mix 1gm of anamed and 1ml of the rest of the compounds were used.

3.8 Statistical analysis

For all the behavioral bioassay non-parametric, binomial test, of SPSS version 20 were used. The result is considered significant when (P < 0.05) and not significant when (P > 0.05).
4. Result

4.1 EAD identified compounds from mixed lures

Visual observation of EAD responses showed 9 responses to the mixed protein bait (Table 1). The traces of each compound in the mixed bait and to which compounds each species gave response are listed in Table 2. For few of the responses (2-3) we could not find neither a matching MS peak nor a good match (quality) in the libraries. These were excluded from further analysis.

Table 1 Antennal active compound

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS No</th>
<th>Ret time</th>
<th>KI</th>
<th>Area</th>
<th>Amount</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>123-86-4</td>
<td>6.841</td>
<td>814</td>
<td>86025</td>
<td>29.5584</td>
<td></td>
</tr>
<tr>
<td>3-Methyl-1butanol</td>
<td>123-51-3</td>
<td>5.673</td>
<td>46544</td>
<td></td>
<td>15.99263</td>
<td></td>
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<tr>
<td>Substance</td>
<td>CAS NO</td>
<td>KI</td>
<td>Ret time</td>
<td>Mass 1</td>
<td>Mass 2</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>----------</td>
<td>-----</td>
<td>----------</td>
<td>--------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>123-92-2</td>
<td>7.977</td>
<td>876</td>
<td>57400</td>
<td>19.72278</td>
<td></td>
</tr>
<tr>
<td>Styrene</td>
<td>100-42-5</td>
<td>8.364</td>
<td>897</td>
<td>180575</td>
<td>62.04602</td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>123-35-3</td>
<td>10.295</td>
<td>993</td>
<td>157148</td>
<td>53.99644</td>
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</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>123-66-0</td>
<td>10.404</td>
<td>999</td>
<td>557381</td>
<td>191.5175</td>
<td></td>
</tr>
<tr>
<td>2-Phenylethanol</td>
<td>60-12-8</td>
<td>12.822</td>
<td>1120</td>
<td>855</td>
<td>0.29378</td>
<td></td>
</tr>
<tr>
<td>α-Methylbenzyl acetate</td>
<td>93-92-5</td>
<td>14.38</td>
<td>1201</td>
<td>2479014</td>
<td>851.7954</td>
<td></td>
</tr>
</tbody>
</table>

CAS NO= Chemical Abstract Service Number

KI= Kovats retention index

Ret time= Retention time
4.2 Lure sources of the identified compounds

In the experiment each lures (brewery yeast, baker’s yeast, torula yeast, anamed and GF-120) were injected singly in GC-MS to trace the source of the compounds that are identified.

Table 2 Antennal active compounds in different lures and insect species

<table>
<thead>
<tr>
<th>compounds</th>
<th>B. dorsalis</th>
<th>B. zonata</th>
<th>B. oleae</th>
<th>B. cucurbitae</th>
<th>C. capitata</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unknown</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td>1,2,3,4,5</td>
</tr>
<tr>
<td>2. 3-Methyl-1-butanol</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td>2,3</td>
</tr>
<tr>
<td>3. Butyl acetate</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>4. Isoamyl acetate</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>2,5</td>
</tr>
<tr>
<td>5. Styrene</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td>*</td>
<td>5</td>
</tr>
<tr>
<td>6. β-Myrcene</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>2</td>
</tr>
<tr>
<td>7. Ethyl hexanoate</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>2,5</td>
</tr>
<tr>
<td>8. 2-Phenyl ethanol</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td>2,3</td>
</tr>
<tr>
<td>9. α-Methylbenzyl acetate</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>5</td>
</tr>
</tbody>
</table>

*represents antennal active compound that elicit response in the species and the numbers, 1- 5, represent the presence of the compounds in the five protein baits.

1= Gf-120, 2= Brewery yeast, 3= Baker’s yeast, 4= Torula yeast and 5= Anamed
Average mini volt of each compounds that elicited antennal response of the experimental insects.

From the index we can observe ethyl hexanoate induced a higher response.

Figure 4 Antennal response of *B. dorsalis*, *B. zonata*, *B. cucurbitae*, *B. oleae* and *C. capitata* to mixed protein bait (± SE). The numbers correspond to compounds in Table 2.
4.3 Gc-Ead Chromatograph of mixed lures

Figure. 5 EAD chromatograph of female *B. dorsalis*. Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

Figure. 6 EAD chromatograph of female *B. zonata*. Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.
Figure. 7 EAD chromatograph of female *B. cucurbitae*. Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.

Figure. 8 EAD chromatograph of female *B. oleae*. Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.
Figure 9 EAD chromatograph of female *C. capitata*. Top trace (A) represents the FID response, the bottom trace (B) the antennal response. Peak numbers correspond to compounds in Table 2.
4.4 Behavioral bioassay result

*B. zonata* and *B. dorsalis* were used for behavioral bioassay. The result indicated that *B. zonata* was highly attracted to both brewer’s yeast and mix of lures over the control (*P*<0.001 for both against control), but the comparison between mix of lure and brewery were not significant (*P*>0.53 for *B. dorsalis* and *P*>0.07 for *B. zonata*) in both species although there is a slight preference between them. The result from both sexes were pooled.
Figure 10 Preference of *B. zonata* in olfactometer bioassay. (A) Mix of lures over control, (B) brewery over control, (C) mix of lures over brewery. NS represents not significant.

Figure 11 Preference of *B. dorsalis* in olfactometer bioassay. Mix of lures over brewery. NS represents not significant.
5. Discussion

For fruit fly control different pheromones, plant semiochemicals and food type attractant were studied previously (Tan et al., 2014, Light and Jang 1996). However, most of female targeted control methods are restricted to food type attractant (Light and Jang 1996). In the present study a mixture of five different lure were tested (brewery yeast, torula yeast, baker’s yeast, gf-120 and anamed). Although it’s not exactly the same lures, similar kinds like fermented sugar, hydrolyzed protein and yeasts were tested previously (Epsky et al., 1999, Sabine, 1992 and Light and Jang 1996).

The research discovered nine compounds which were detected on GC-EAD and later identified on GC-MS. For the experiment, five important species of fruit flies (B. zonata, B. dorsalis, B. oleae, B. cucurbitae and C. capitata) were tested. The result obtained in these studies has shown that C. capitata was the only species that responded for all the nine compounds (Fig.9). However, on GC-EAD when the synthetic blends of these nine compounds tested on C. capitata, six compounds, Butyl acetate, Isoamyl acetate, Styrene, β-Myrcene, Ethyl hexanoate, and α-Methylbenzyl acetate were found to be responsive and we were able to confirm indeed the compounds were the one elicited the antennae. Recent studies have shown that some of the compounds induced antennal response in other fruit flies. For instance, isoamyl acetate, which is found in wine and vinegar, induced antennal response in Drosophila suzukii (Cha et al., 2012).

3-methyl 1-butanol in the synthetic blend has failed to elicit antennal response. In the present study the unknown compound were identified in each of the individual lures and three of the tested
species had respond to it except *B. cucurbitae* and *B. oleae*. In contrast to our study, 3-methyl 1-
butanol, from mango, it is known to elicit antennal response in *B. dorsalis* (Jayanthi et al., 2012).

2-phenyl ethanol is the other compound that didn’t elicit antennal response on the antennae of
female *C. capitata*. In agreement with our result Siderhurst and Jang (2006) found that ethanol
extracts failed to elicit antennal response in *B. dorsalis*. It might be better to test both compounds
(3-methyl 1-butanol and 2-phenyl ethanol) with the best purity (100%) and verify the result. From
the compounds that were identified, isoamyl acetate and ethyl hexanoate were the only two
compounds detected eliciting antennal response in all five species including the specialist fruit fly
*B. oleae* (Fig. 8). In fact, these are the only two compounds that *B. oleae* responded to.

As mentioned in the result, beyond the nine compounds identified in the mixed lure, a few other
compounds were observed inducing antennal response. The identity of these compounds is not
known and are also not included in our result. No apparent GC peak was seen in the regions where
these EAD responses were observed. However, GC-MS revealed several nitrogen containing
compounds, although not a good match in the libraries was not found yet. Some nitrogen
containing compounds may not give good FID peaks, which would explain the discrepancy
between the FID and MS readings.

In the olfactometer assay the result demonstrated that both males and females were attracted to
odors of both brewery and mix of lures over the blank (Fig. 10). This might be due the occurrence
of compounds, in the brewery and mix of lures, which are important component of fruit flies diet.
This agrees with the work done by Siderhurst and Jang (2006) on *Terminalia catappa*, a tropical
tree, fruit extracts of which have been found to be attractive to both sexes of *B. dorsalis*. In
addition, when mix of lures is paired with brewery in the bioassay, *B. zonata* slightly preferred
mix of lures over brewery but was not significant in the other hand *B. dorsalis* was slightly attracted
to brewery than the mix of lures even though the difference was not significant. For the sake of time and number of insect, *B. zonata* and *B. dorsalis* were chosen among the rest of the species for the behavioral assay, the lures were also mixed for the same reason.

According to the result fruit flies were attracted to mixture of fermentation products, brewery and baker yeast, and protein lures, Torula yeast, GF-120 and anamed. Similarly a previous study had shown that fermentation products and protein lures can be used for *Bactrocera control* and other important fruit flies like *Drosophila* species (Landolt *et al.* 2012). For example volatiles from fermentation process like ethanol, acetic acid, ethyl acetate, and acetaldehyde found to attract *Drosophila melanogaster* (West 1961). Torula yeast and hydrolysate protein are also used in monitoring and population suppression (Episky *et al.*, 1999). Female targeted trapping system, using long lasting synthetic attractants is developed, these synthetic attractants are Ammonium Acetate, Putrescine and Trimethylamine (Episky *et al.*, 1999). Actually these synthetic attractant in combination attract more flies than the protein baits (torula yeast and protein hydrolysate) (Katsoyannos *et al.*, 1999a). The compounds that are identified in this experiment can be tested as an attractant like the above mentioned synthetic food lures. The major importance of synthetic food lure is more target specific compared to food lures which trap other tephritid and non-targeted flies. GF-120 (one of the lure in our mixed protein baits) baited with spinosad is also used in combination with three synthetic attractants (Ammonium Acetate, Putrescine and Trimethylamine) for *Ceratitis capitata control* (Cristofaro *et al.*, 2007). So studying the behavioural response of fruit flies to mixed protein lures and fermentation products can be a step ahead in developing a novel attractant.
6. Conclusion

- Most of the identified compounds that elicited antennal response were sourced from brewer’s yeast and anamed.
- Isoamyl acetate and ethyl hexanoate were the compounds that elicited antennal response in all five species and induced a higher response.
- The olfactometer study demonstrate that *B. zonata* and *B. dorsalis* were attracted to both brewery and mix of lures over the control. However, there were no significant preference when brewery was tested against mix of lures in both species. Although it is not significant, the test showed that *B. zonata* slightly preferred mix of lures but *B. dorsalis* preferred brewer’s yeast.
7. **Recommendation**

This research can be a valuable source for further studies in developing baits that selectively capture these fruit flies. Isoamyl acetate and ethyl hexanoate which were identified in all five species EAD result, can be used as attractant bait in fields to trap and kill as methyl eugenol used with spinosad. Since isoamyl acetate and ethyl hexanoate induced a higher response and found in many fruit odors, it would be much help in constructing these attractant baits. Carrying out behavioural bioassay for each of the lures in each species of the insect and performing GC-EAD for the synthetic blend for each insect species are suggested for further study in order to be accurate in developing super attractant.
8. References


Cugala, D., Santos, L. and Albano, G. (2009). Assessment of the Fruit Fly, Batrocera invadens, Pest Status and the Establishment of the Parasitoid, Fopius arisanus in Mozambique. Faculty of Agronomy and Forest Engineering, Eduardo Mondlane University, Main University, Maputo. pp 313-316.


9. Annexes

Annex 1 EAD chromatograph of female *C. capitata* for mix of synthetic blends
Annex 2 EAD chromatograph of *C. capitata* for 3-methyl 1-butanol

Annex 3 EAD chromatograph of *C. capitata* for butyl acetate
Annex 4 EAD chromatograph of *C. capitata* for isoamyl acetate

Annex 5 EAD chromatograph of *C. capitata* for styrene
Annex 6 EAD chromatograph of *C. capitata* for β-Myrcene

Annex 7 EAD chromatograph of *C. capitata* for ethyl hexanoate
Annex 8 EAD chromatograph of *C. capitata* for 2-phenyl ethanol

![EAD Chromatograph of C. capitata for 2-phenyl ethanol](image)

Annex 9 EAD chromatograph of *C. capitata* for alpha methylbenzyl acetate

![EAD Chromatograph of C. capitata for alpha methylbenzyl acetate](image)
Annex 10 GC-MS identification