The chemical ecology of the oriental fruit fly *Bactrocera dorsalis* and the potential for novel odor-based management tools

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Cover: Left: *Bactrocera dorsalis* flies feeding from a SPLAT-ME-spinosad dollop on a leaf of mango tree. Right: *B. dorsalis* hold inside a pippete tip exposing antennae ready for electrophysiological recordings.
(photo: Tibebe Dejene)

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The chemical ecology of the oriental fruit fly *Bactrocera dorsalis* and the potential for novel odor-based management tools

Abstract

Over the last few years, several tephritid species have invaded sub-Saharan Africa, competitively displacing native fruit fly pests, and severely affecting horticulture production. In two different farming scales, small and large, we verified the influence of suppressing the invasive *Bactrocera dorsalis* using the male specific attractant, methyl eugenol (ME), formulated in SPLAT-spinosad. In small-scale farm plots, use of ME did reduce *B. dorsalis* populations, but population levels remained high throughout the study. In mark-release-recapture studies, male flies were found to disperse fast and beyond one km from the release point. In large-scale farm plots, the invasive pest was controlled within eight months of suppression using ME-based suppression in combination with other pest management techniques. However, this was paralleled by a quick resurgence of the native fruit fly *Ceratitis capitata*, likely due to competition release. Targeting female fruit flies using techniques that rely on the olfactory sensitivity of the flies, may support direct and more selective ways to control fly populations. Host fruits as well as proteins produced during fermentation are important resources for tephritid fruit fly nutrition and reproduction. This study used gas chromatography-coupled electoantennography detection (GC-EAD) to test the physiological response of economically important tephritid pest species to host fruits and commercially available food-baits. This was compared to the published database of odorant receptors of *Drosophila melanogaster*. We postulated that volatiles shared across fruits and detected by several fly species may comprise general fruit compounds important in host orientation. Selected blends, composed of 6 or 11 fruit compounds were more attractive to *B. dorsalis* than full fruit odor in a multi-choice olfactometer assay. Species-specific and general blends identified from food-baits were more attractive to *B. dorsalis*. The study underlines the potential of comprehensive database of olfactory sensitivity in the rational design of novel synthetic attractants, or for augmenting existing ones. The study also provides a platform to develop both species-specific attractants and multi-species attractants for tephritid fruit flies.

Keywords: Bactrocera dorsalis, food-baits, fruit volatiles, GC-EAD, male lures, olfactometer, Tephritidae

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የ ምስራቃዊ ኤሺያው የፍራፍሬ ዝንብ “ባክትሮሴራ ዶርሳሊስ” ኬሚካዊ ምሕዳር እና የ ምሕዳሩ የፍራፍሬ ወገ ዝንቦችን አዲስ በሆነ ስልት የመቆጣጠር እምቅ አቅም።

ረቂቅ

ባለፉት ጥቂት አመታት ውስጥ በርካታ መጤ የፍራርፍሬ ወገ ዝንብ ዝርያዎች ከሰሀራ በታች አፍሪካን በመውረር በአትክልትና ፍራፍሬ ምርት ላይ ከፍተኛ ተጽእኖ አሳድረዋል። ከነዚህ የፍራፍሬ ዝንብ አይነቶች እንዱ እና ዋነኛው “ባክትሮሴራ ዶርሳሊስ” በመባል የሚታወቀው ምንጩ ከኢሺያ የሆነ በአጭር ጊዜ ውስጥ ኢትዮጵያን ጨምሮ በመላው አፍሪካ በመሰራጨት በተለያዩ አይነት ፍራፍሬዎች ላይ ጉዳት በማድረስ ላይ ያለ ነፍሳት ነው። በዚህ የከፍተኛ ትምህርት ጥናት ወንዱን የፍራፍሬ ዝንብ ብቻ ለይቶ ወደ መግደያ ወጥመድ በመዐዛው የሚስብ “ሜታይል ኢዩጅኖል” የተባለን ኬሚካዊ ንጥረ ነገር “ስፒኖሳድ” ከተባለ መርዛማ ቅመም እንዲሁም “ስፕላት” ከተባለ የምግብነት ባህሪ ካለው ውህድ ጋር በመደባለቅ፣ የውህዱ ዝንቦቹን የመቆጣጠር ተጽእኖ ኢትዮጵያ ውስጥ በሚገኙ ሰፋፊ እርሻዎች እና አነስተኛ የገበሬ ማሳዎች ላይ ተሞክሯል። በአነስተኛ የገበሬ ማሳዎች ላይ ምንም እንኳን የፍራፍሬ ዝንቦቹ መጠን ቀድሞ ከነበረበት ከፍተኛ መጠን በትንሹ ቢቀንስም በፍራፍሬዎች ላይ የሚታየው ጉዳት ግን ለውጥ አላሳየም። የዝንቦቹ በፍጥነት ካንድ የገበሬ ማሳ ወደ ሌላ ማሳ መብረር መቻል ቁጥጥሩን አስቸጋሪ አድርጎታል። በሰፋፊ እርሻ ላይ በተደረገው ጥናት በ ስምንት ወር ውስጥ መጤውን የፍራፍሬ ዝንብ መቆጣጠር ቢቻልም፣ የመጤው ፍራፍሬ ዝንብ ዝርያ እንደቀነሰ ነባር የፍራፍሬ ዝንቦች ባዲስ መልክ ማሳውን በመውረራቸው በፍራፍሬ ላይ የሚደርሰው ጉዳት አሁንም ቀጥሏል። ለዚህም መፍትሄ ይሆን ዘንድ፣ ብዙ አይነት የፍራፍሬ ዝንቦች ባሉበት ማንኛውም ማሳ ላይ ሌሎችንም አይነት የዝንብ ዝርያዎች በመዐዛቸው ወደ መግደያ ወጥመድ የሚስቡ እና የሚገሉ ውህዶችን መጠቀም እንዲሁም ደሞ ቁጥጥሩን ከተወሰኑ የገበሬ ማሳዎች ከፍ በማድረግ ሀገር አቀፍ እንዲሁም አህጉር አቀፍ የቁጥጥር ዘዴ እንዲመቻች ማድረግ ያስፈልጋል። በተጨማሪም ይህን ጉዳት ቀጥተኛ በሆነ መንገድ ለመቆጣጠር የሴት ፍራፍሬ ወገ ዝንቦችን ዒላማ ማድረግ ጥቅሙ ከፍተኛ ነው። በዚህ ጥናት ሴቶች ዝንቦችን ወደ ፍራፍሬዎች እንዲሁም ወደተለያዩ የ ዝንብ ምግቦች የሚስቧቸውን ከመቶ በላይ የሆኑ መዐዛማ ንጥረ ነገሮች “ጂሲ-ኢኤዲ እና ጂሲ-ኤምኤስ” የተባሉ መሳሪያዎችን እገዛ ተጠቅሜ ለይቻለሁ። ከነዚህም ውስጥ የተወሰኑትን፣ በተለያዩ ፍራፍሬዎች ውስጥ የሚገኙትንና፣ ሌሎች የፍራፍሬ ዝንብ ዝርያዎችም ያሸተቷቸውን መዐዛማ ንጥረ ነገሮች በመጠቀም የሰራሁት ውህድ በቤተ ሙከራ ውስጥ “ባክትሮሴራ ዶርሳሊስን” እንዲሁም ሌሎች ዝርያዎችን ወደ መግደያ ወጥመድ በመሳብ አመርቂ ውጤት አስገኝቷል።

ቁልፍ ቃላት: ባክትሮሴራ ዶርሳሊስ, ሜታይል ኢዩጅኖል, አነስተኛ የገበሬ ማሳ, የፍራፍሬ መዐዛ, ጂሲ-ኢኤዲ, ጂሲ-ኤምኤስ

የጸሐፊው አድራሻ: ጥበበ ደጀኔ ቢያሳዝን, SLU, Department of Plant Protection Biology, P.O. Box 102, 230 53 Alnarp, Sweden.

Dedication

To my family

The fear of the Lord is the beginning of knowledge, but fools despise wisdom and instruction
Proverbs 1:7

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Tibebe Dejene Biasazin**, Tadiwos Woldehana, Sebastian Larsson Herrera, Mattias Larsson, Agenor Mafra-Neto, Teun Dekker. The impact of odor-based management techniques in suppressing the invasive fruit fly pest (*Bactrocera dorsalis*) in large-scale and small-scale orchards in Ethiopia. (Manuscript)

II **Tibebe Dejene Biasazin**, Miriam Frida Karlsson, Ylva Hillbur, Emiru Seyoum, Teun Dekker (2014). Identification of host blends that attract the African invasive fruit fly, *Bactrocera invadens*. *Journal* of *Chemical Ecology* 40, 966-976.

III **Tibebe Dejene Biasazin**, Sebastian Larsson Herrera, Teun Dekker. Fruit-odor olfactomes of four tephritid pests and their use in the rational design of novel female attractants. (Manuscript)

IV **Tibebe Dejene Biasazin**, Haimanot Teklemariam, Sebastian Larsson Herrera, Marie Bengtsson, Miriam Frida Karlsson, Joelle Kristin Lechelt, Teun Dekker. Deconstructing protein-based lures into synthetic compounds to create attractive blends for tephritid fruit flies. (Manuscript)

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The contribution of Tibebe Dejene Biasazin to the papers included in this thesis was as follows:

I Designed, conducted, analysed the data and wrote the manuscript together with the co-authors.

II Planned, carried out all electrophysiological recordings, analysed the data and wrote the manuscripts together with the co-authors.

III Planned, carried out all electrophysiological recordings, analysed the data and wrote the manuscripts together with the co-authors.

IV Planned, carried out all electrophysiological recordings, analysed the data and wrote the manuscripts together with the co-authors.

Abbreviations

CL	Combined Lure
EAD	Electro Antennography Detection
FID	Flame Ionization Detector
FTD	Fruit fly per Trap per Day
GC	Gas Chromatography
ME	Methyl Eugenol
MS	Mass Spectrometer
SPLAT	Specialized Pheromone & Lure Application Technology
TA	Terpinyl Acetate
UAAIE	Upper Awash Agro Industry Enterprise

1 Introduction

The world population is increasing in alarming rate adding 83 million people every year (WPP, 2017) and creating a higher food demand globally (Godfray et al., 2010; Tilman et al., 2011). This is even worse in developing countries like sub-Saharan Africa where the agricultural output may not suffice the increasingly growing demand of the population (Ittersum et al., 2016). This gap can be narrowed down through sustainable intensification of crop production. Horticulture production in sub-Saharan Africa is a potential sector that could be intensified further (Broeck & Maertens, 2016), and because of its higher profit margin offers a key benefits to lift smallholder farmers out of poverty.

The humid and warm temperature of sub-Saharan Africa is a suitable production site for variety of fruit crops. Fruit production in sub-Saharan Africa is directly associated with the livelihood of small-scale farmers (Ekesi et al., 2011). Besides private consumption, small-scale farmers gain some income by selling fruits to local markets or neighbourhoods. In addition, fruits provide indispensable micronutrients that are vital to alleviate the problem of malnutrition in developing countries (Tontisirin et al., 2002). Thus, intensifying the horticulture production in sub-Saharan Africa benefits not only small-scale farmers, but could also improve economic and social development. It could also become an important component to combat the world's main problem of hidden hunger and food security (Joosten et al., 2015; Broeck & Maertens, 2016).

There are many factors that hinder the intensification of horticulture production in developing countries. One of which is the damage caused by Tephritidae fruit fly pests (Ekesi et al., 2011; Badii et al., 2015). In sub-Saharan Africa, alien pests such as *Bactrocera dorsalis*, previously misclassified as the African invasive fruit fly, *B. invadens* (Schutze et al., 2015; Schutze et al., 2017), have aggravated the existing problems caused by native tephritid fruit fly species (Ekesi et al., 2016). The damage caused by Tephritidae fruit flies could be direct due to loss of fruits for consumption and loss of income due to market closure, or it could be indirect when fruits damaged by fruit flies become vulnerable and susceptible to pathogenic microorganisms (Ordax et al., 2015), as well as due to quarantine-measure induced restricted access to high-end markets. An intensified use of pesticides for fruit fly control and associated costs can also be considered indirect losses: the resulting increased use of pesticides not only harms the farmers' health but also disturbs the ecosystem (Aktar et al., 2009). Therefore, an environmentally safe and sustainable fruit fly control system is required.

The use of organic compounds such as pheromones and kairomones in pest control is a novel strategy for sustainable and environmentally sound control system (Pickett et al., 1997). Fruit fly control strategy is mainly based on attract and kill techniques of male fruit flies using male attractants (parapheromones) combined with toxicants (Vargas et al., 2010). However, the damage starts upon oviposition of female fruit flies, for which the control system is not well developed. Fruit flies are attracted to pheromones, parapheromones, host fruits and fermented products for lekking, feeding, mating and ovipositing (Epsky et al., 2014; Quilici et al., 2014; Tan et al., 2014). This attraction is mediated through an insect's olfactory organs, the antennae and the maxillary palp.

In this thesis, we assessed the potential of using parapheromones (male lures), and its effect on local tephritid guild, and the importance of farm scale in suppression of *B. dorsalis* in large-scale and small-scale farming settings in Ethiopia. The study also used gas chromatography-coupled electroantennography, and identified antenna active compounds from host fruits and food-based baits, compared it to olfactory receptors of *Drosophila melanogaster* and developed blends that could be used to attract female fruit flies.

2 Background

2.1 Economic impact of tephritid flies in Africa

Horticultural crop production is a major component for the economic development of most African countries. It is a vastly growing agricultural sector and a well-recognized source of income for smallholder farmers (ISHS, 2015). In rural areas where there is high production of fruits and vegetables, horticulture production offers one of the most important opportunities for employment and revenue, increased access to education, healthcare, food and nutritional security and socio-economic development opportunities particularly for women and children (Weinberger & Lumpkin, 2007). However, the expansion of horticultural production and its international trade are greatly increasing the risk of transferring alien fruit flies within Africa and from & to other regions of the world (Duyck et al., 2004).

Fruit flies are major constraints to the economic development of the horticulture sector (Ekesi et al., 2011; Badii et al., 2015). Several species of fruit flies exist in Africa that are known to attack different types of commercial as well as wild fruits and vegetables, causing considerable damage to the horticulture industry. African fruit production is also vulnerable to invasion by alien fruit flies from other tropical regions, which is exacerbated by the lack of local expertise, affordable technologies and satisfactory quarantine services for the management of fruit flies (Duyck et al., 2004; Ekesi et al., 2009).

Heavy fruit fly infestation seriously reduces the quantity of marketable fruits and vegetables and thus, increases production costs. For example, out of the annual African production of millions of tons of mangoes half is destroyed by fruit flies (Ekesi et al., 2016). In 2009 only in Kenya 80% of all the fruits were infested with *B. dorsalis* that lead to an annual loss of 76000 tons of mangoes. In Benin fruit infestation reached as high as 73%, with sanitary operation leading to extreme measures of uprooting mango trees (De Meyer et al., 2009).

Indirect losses resulting from quarantine restrictions imposed by fruit importing countries to prevent entry and establishment of unwanted fruit fly species can also be enormous. Mozambique lost 10% of its banana export revenue because of *B. dorsalis* infestation and the resulting quarantine measures. Today there is a continuous threat of border closure. In 2008 Kenya lost 1.9 million USD in export of avocado only to South Africa (Ekesi, 2010).

In Ethiopia, Upper Awash Agro-Industry Enterprise (UAAIE), which is the largest producer of a variety of fruits and vegetables for local and export markets, the native fruit fly *Ceratitis capitata* and a species that recently invaded the farm, *B. dorsalis*, are the key pests that cause serious infestations (Dessie, 2014). The native species alone caused annual loss of about 1500 tons of orange and mandarin while, the invasive species caused a complete loss (100%) of guava in 2013, a year after its introduction (Dessie, 2014). The continuous high level of infestation eventually led to complete removal of guava trees, a heavily infested crop, in 2016 (personal observation).

The effect is more severe in smallholder farming communities, where damage translates directly into livelihood, food and nutritional security. For instance, in Arbaminch, Ethiopia, a smallholder fruit production area of natural importance, huge amount of mango alone was lost by *B. dorsalis* infestation since 2007. This has severe consequences, since mango alone generates a significant amount of revenue for the rural households of the area (Piet et al., 2012).

2.2 Management of tephritid fruit flies

The damage caused by tephritid fruit flies may reach up to 100% in unmanaged orchards (Vayssières et al., 2009; Ekesi et al., 2016). Fruit fly management is vastly reliant on cultural methods such as orchard sanitation, use of broad-spectrum protein bait sprays, mass trapping using male lures (male annihilation) and introduction of natural enemies. All these techniques have been implemented effectively either singly or in combination against tephritid fruit flies. Below is a short description of currently available control methods.

2.2.1 Cultural control methods

The most common cultural control method is field sanitation; it is a method by which infested or fallen fruits are removed from the orchard and buried in the soil to a depth where adult flies could not survive or emerge from. This method is laborious and time consuming, but effective when implemented properly and in combination with other control measures (Piñero et al., 2009). The disadvantage of field sanitation is that natural enemies would also be buried together with the infested fruits. However, augmentoria can compensate for this (Deguine et al., 2011). Augmentorium is a tent like structure used to enclose infested fruits. It is made of fine mesh that prevents fruit flies to pass through, but allows the smaller parasitoids to escape.

Other traditional cultural methods involve early harvesting, bagging fruits and soil disturbance (Sarwar, 2015). Wrapping individual fruit requires considerable time and labor but it is relatively inexpensive, easy to implement, prevents physical injuries and it is suitable for smaller orchards or small-scale farmers. In addition, it provides farmers a dependable estimation of anticipated produce. Disturbing the soil through ploughing and flooding exposes pupae for unsuitable climatic conditions leading to higher mortality rate (Verghese et al., 2004).

2.2.2 Classical biological control

Classical biological control remains an important method to mitigate invasive pests. Braconidae parasitoids are the major natural enemies of Tephritidae. The common *Fopius arisanus* is an egg-pupal parasitoid that has been successfully established in Hawaii and French Polynesia to control *B. dorsalis* (Vargas et al., 1993). Following release in several African countries in 2006, *F. arisanus* caused, depending on the fruit, up to 40% parasitization in *B. dorsalis* (Ekesi et al., 2016). Similarly, it caused significant reduction in fruit damage with up to 65% parasitism of *Bactrocera*, *Ceratitis* and *Anastrepha* spp. (Vargas et al., 2013).

Predators can also impact fruit flies. Besides frugivorous vertebrates such as birds and rodents (Drew, 1987), ants such as *Oecophylla longinoda* (the weaver ant) (Van Mele et al., 2007; Van Mele et al., 2009) and wasps such as *Vespula germanica* (Hendrichs et al., 1998) are promising predators of Tephritidae. Weaver ants reduce fruit damage not only by larvae or adult predation but also by causing disturbance and hindrance during adult oviposition. The deterrence could be physical and visual, but also olfactory as lab and field results point to a long-range cue left by weaver ants that deter insects, including flies (Van Mele et al., 2009; Adandonon et al., 2009).

Fruit flies are also susceptible to entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* and their toxic metabolites (Castillo et al., 2000; Ekesi et al., 2002; Dimbi et al., 2003). The fungi can be used in combination with attractants to reduce longevity of flies (Ekesi et al., 2007). However, biopesticides kill over time, providing females with ample opportunity to oviposit their egg load. Formulation also requires considerable input and training of farmers. However, promising results have been reported on the effect of biopesticides on population levels of *B. dorsalis* and other fruit fly species (Vayssières et al., 2009).

2.2.3 Control using male lures

Control with male lures (male annihilation technique) is the most widely used and reliable method in fruit fly control (Vargas et al., 2009; Tan et al., 2014). It is the main component of sterile insect technique and Integrated Pest Management (IPM) programs of fruit fly control (Shelly et al., 2010; Barclay et al., 2014). Although, the basis of attraction and mechanism of detection are not clear, male fruit flies of Tephritidae are highly attracted to different groups of male attractants. For instance, males of *B. dorsalis* and *B. zonata* are highly attracted to methyl eugenol (benzene, 1,2-dimethoxy-4-(2-propenyl)), while *Z. cucurbitae* and *B. tryoni* are attracted to cuelure (4-(p-acetoxyphenyl)-2-butanone) and *C. capitata* and some other *Ceratitis* spp are attracted to trimedlure (cyclohexanecarboxylic acid, 4(or 5)-chloro-2-methyl-, 1,1-dimethylethyl ester).

Male lures combined with different types of toxicants are currently used to mitigate population of tephritid fruit flies in different areas of the world (Christenson et al., 1963; Tan et al., 2014; Ekesi et al., 2016). However, in this strategy only males are affected and male lures other than methyl eugenol (i.e. against other species than *B. dorsalis* and relatives) are much less effective. Additionally, selective suppression of one species may give rise to competition and resurgence of other species that share the same ecosystem. Therefore, alternative approaches that target females and other fruit fly species are much needed.

2.2.4 Control using Protein baits

Soon after emergence, both male and female fruit flies require proteinaceous food for survival and sexual maturation. Protein bait traps capture both sexes, with a bias for female, which need proteins for developing eggs (Drew & Yuval, 1999). Protein / fermentation based attractants combined with toxicants have been used in traps for monitoring, and sprayed on the orchard floor to suppress fruit fly populations (Prokopy et al., 2003). Protein bait traps improve surveillance of tephritid fruit flies that have no known male lures. Yet, compared to male lures, protein baits are non-selective and affect beneficial organisms (Leblanc et al., 2010a; Leblanc et al., 2010b; Leblanc et al., 2010c), less effective, and have a very short field life so that constant replacement is required and are short-range attractants (McQuate & Follett, 2006). Thus, development of inexpensive female lures that are selective, long lasting and attract females of multiple tephritid species could significantly improve intervention.

2.3 Invasion and competitive displacement

When an exotic species invades an area, it can create adverse effects on the habitat, both from the economic and ecologic perspective. It can, threaten ecosystem function and biodiversity (Charles & Dukes, 2008). Invasive species, as indicated by the name, are characterized by a set of life history traits that provides them an advantage to utilize available resources efficiently and outcompete native species (Peacock & Worner, 2008). High dispersal ability, phenotypic plasticity, rapid growth and fast reproductive rates are among the traits that favor an invasive alien species over indigenous guilds (Moran & Alexander, 2014). However, the ability of an invasive species to dominate a niche also depends on the strength and form of its competitive interactions with species in recipient niche, its own competitive ability, climatic factors and availability of natural enemies (Whitney et al., 2008).

Depending on the competitiveness, invasion results in displacement of one species or, a stable coexistent or unstable coexistence of both species occurs. Usually, if the interspecific competition is weaker than intraspecific competition stable equilibrium exists, otherwise the superior competitor forcefully excludes the inferior competitor from the habitat (Duyck et al., 2004). As a result, the inferior competitor differentiates its niche, or becomes extinct from that specific locale. A strong interspecific competition is unlikely to occur between stably coexisting species, although it may initially be observed on arrival or introduction of a new species, which shares resource with indigenous species.

Despite strict quarantine regulations, fruit flies in the family Tephritidae, which include several polyphagous fruit fly pests, have managed to invade and establish in many countries and across continents (Duyck et al., 2004). Human activities and global commercialization of fruits have accelerated the introduction of fruit fly species into new areas. These new areas, however, were not necessarily fruit fly free. Indigenous fruit flies or previous invaders that are established in the area may have already occupied them (Duyck et al., 2004). As a result, any new comer (invader) faces / causes a competitive challenge by / on the recipient community that are already well established, which are either native guilds or previously established exotic pests.

Among Tephritidae, *Bactrocera* spp. and *Ceratitis* spp. are globally frequently recorded invaders, typically the former invades and competitively dominates the latter, whereas the opposite has not been reported yet (Duyck et al., 2004). For instance, the invasion of *B. dorsalis* in Africa and its negative effect on population levels of the native *Ceratitis cosyra* is a recent phenomenon (Ekesi et al., 2009). Much focus was then towards suppression of the population of the invasive species in Africa (Ekesi et al., 2011). However, the effect of targeting a single species is not known, and suppressing the invasive species may lead to resurgence of the native fruit fly guild.

2.4 Biology and ecology of Tephritidae

Gravid female Tephritidae uses its long and sharp ovipositor to penetrate the skin of fruits, bends the ovipositor and deposit elongated eggs within the skin of the fruit (Fig 1), eggs are usually white to creamy yellow in colour. Under optimal conditions, for most tephritids, it takes around two days for larvae to hatch and find their way to the fleshy part of the fruit. The larval stage with its three instars takes approximately two or three weeks to complete, after which the third instar larvae exits the fruit for pupation. Pupation generally occurs in the soil and the pupa stage lasts for about twelve days. Once emerged, to obtain the required nutrients adult flies feeds on a variety of organic matters including honeydew, bird excrement and decaying material which are ideal sources of minerals, vitamins, carbohydrates and proteins, that are fundamental for survival and reproductive maturation. Within a week after emergence adult flies become sexually mature and mating followed by oviposition succeeds (Fletcher 1987).

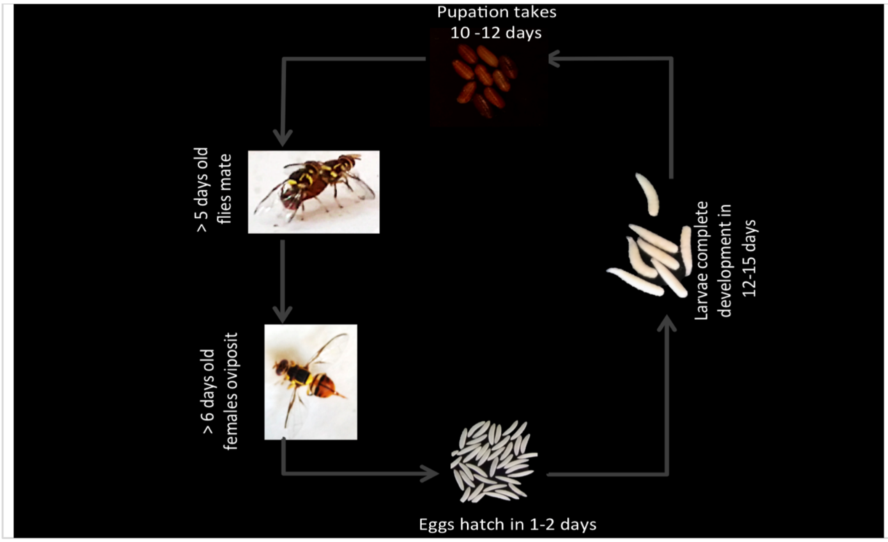

Figure 1. Life cycle of the Oriental fruit fly *Bactrocera dorsalis*: eggs hatch in 1-2 days, larvae development takes for about two weeks, and pupation lasts up to 12 days, adults emerge and mating starts within 5 days.

Factors such as rainfall, temperature, relative humidity, photoperiod and host plant availability affect the distribution and population density of Tephritidae in general (Chen & Ye, 2007). Most Tephritidae flies, including *B. dorsalis*, prefers tropical savanna, humid continental, and subtropical and oceanic climatic zones and tolerate tropical rainforest and monsoon climatic zones. Predictions indicate the current increase in global temperature and the decrease in cold stress puts temperate regions at risk for *B. dorsalis* invasion (Sridhar et al., 2014).

2.5 Gaps in Chemical ecology of Tephritidae

The early discovery of parapheromone (Howlett, 1912) and its efficiency in attracting males fruit flies seems to have to some extent overshadowed progress on finding female attractants. The term parapheromone was coined to describe the very strong attraction by one of the sexes, similar to sex pheromones in insects, although the compound is not produced by either sex (Sivinski & Calkins, 1986).

The study of the chemical ecology of Tephritidae fruit flies started as early as 1912 when Howlett accidentally discovered male *B. zonata* flies attracted to citronella oil. Three years later he identified the active ingredient methyl eugenol

(Howlett, 1912; Howlett, 1915). Other male lures were also discovered and synthesized, such as trimedlure and terpinyl acetate for *Ceratitis* spp. and cuelure for some *Bactrocera, Dacus* and *Zeugodacus* spp. *such as B. tryoni, D. africanus and Z. cucurbitae* respectively. Male Tephritidae are strongly attracted to male lures (Manrakhan et al., 2014). Tephritidae male lures are not produced by either of the sexes, but either they are found naturally from plant sources or some are synthesized in the lab (Sivinski & Calkins, 1986). Of Tephritidae male lures identified so far, methyl eugenol is the most attractive compound that additionally has a phagostimulant effect. Parapheromones are used extensively in control strategies of several Tephritidae pests. In spite of their significance, nothing is known about the detection circuitry that produces sexual dimorphic behavior to these compounds, and differences therein between species. Revealing the mechanism of parapheromones detection is highly relevant, as its understanding may provide additional tools for rationally designing baits that target females (Benelli et al., 2014; Tan et al., 2014).

The chemical ecology of female Tephritidae is not well understood. It is known that female fruit flies manifest some sort of host preference, which may be variable depending on the region or province (Goergen et al., 2011; Rwomushana et al., 2008). Fruit fly species range from specialist to generalist. For example, *B. dorsalis* is generalist, but prefers mango and guava to other fruits, *Z. cucurbitae* has a preference for cucurbitaceous plants, but also oviposits on other fruits, and *B. oleae* is specialist on olives. It is not known if the mode of feeding and host preference is correlated with changes in the olfactory circuitry of Tephritidae. Maybe generalists have conserved olfactory receptor neurons and specialists, as observed in *Drosophila sechellia*, have lost some of these neurons on the expense of others (Dekker et al., 2006; Ibba et al., 2010).

Changes in the olfactory circuitry that leads to preference may occur either in the periphery organs or inside the brain mainly in the antennal lobe. For instance, in *D. sechellia* the sensilla that detects methyl hexanoate, an important *Morinda* fruit odor, are more abundant on the antenna compared to its sibling *D. melanogaster*. Also in the antennal lobe, two of the glomeruli that receive the input from those overrepresented neurons are enlarged compared to *D. melanogaster*. This switch in morphology is correlated to *D. sechellia's* preference to the toxic *Morinda* fruit (Dekker et al., 2006; Ibba et al., 2010). Such glomerular enlargement is also observed in larger sized worker ants of two closely related species *Atta vollenweideri* and *Atta sexdens*, interestingly the location of the enlarged glomeruli is inverted in the two species and small sized ants have similar sizes of glomeruli throughout the antennal lobe (Kleineidam et al., 2005).

In *Rhagoletis pomonella*, variation in sensitivity and temporal firing patterns of olfactory neurons is presumed to have shaped fruit preference and contributed to reproductive isolation and sympatric speciation (Linn et al., 2003; Olsson et al., 2005a; Olsson et al., 2005b). It is not known if such variation exists in other tephritids such as *Bactrocera* species and if fruit preference and attraction to male lures is correlated with any change in the olfactory system of *Bactrocera* flies. Different sexes of most Tephritidae behave differently in their response towards some sexually important odors such as pyrazines and male lures (Benelli et al., 2014). However, it is not clear where this dimorphism lies in the olfactory system. Sexual dimorphism in size of glomeruli has been observed in Hawaiian Drosophilidae, where the volumes of two adjacent glomeruli are 3-6 times larger in males than in females (Kondoh et al., 2003). Also in *Manduca sexta* laterally enlarged glomerulus only in females is associated with processing cues important for host location and oviposition (King et al., 2000).

3 Objectives

The main objectives of the works described in this thesis were

- To investigate the potential of Tephritidae male lures and its effect in intervention at small-scale and large-scale benchmark sites.
- To develop an effective attractive blend from host odors for female *B. dorsalis.*
- To provide a GC-EAD based fruit-odor olfactome of four Tephritidae pest species to four different major and minor host species to be of use in the rational design of novel attractants or for augmentation of existing ones.
- To develop both species-specific attractants and multi-species attractants for tephritid fruit flies from food-baits.

4 Methods

4.1 Experimental insects

4.1.1 *Bactrocera dorsalis* (Hendel)

Bactrocera dorsalis (the oriental fruit fly) is an Asian origin highly invasive, polyphagous pest damaging a wide variety of fruits and vegetables (Duyck et al., 2004; Clark et al., 2005). So far it has been recorded from 478 kinds of host plants (USDA APHIS, 2016). It is now distributed in more than 65 tropical and subtropical regions including sub-Saharan Africa, Oceania and North America, and prefers an optimum temperature that ranges between 25 – 30 °C (Stephens et al., 2007; Wan et al., 2011). These flies have a strong flying capacity, very high dispersal potential (Chen et al., 2015), excessive reproduction ability and high biotic potential with females laying 1200 – 1500 eggs throughout lifetime (Weems et al., 2012).

4.1.2 *Bactrocera zonata* (Saunders)

Bactrocera zonata (the peach fruit fly) is also a polyphagous fruit pest native to South and Southeast Asia. It is a close relative and resource competitor of *B. dorsalis*, which is currently distributed in more than 20 countries including India, Pakistan, Mauritius, Réunion, Arabian Peninsula and North Africa. Its recent observation in Sudan illustrates a southward invasion threat towards sub-Saharan regions of Africa (Satti, 2011), and to subtropical Mediterranean regions (Duyck et al., 2004). Just as *B. dorsalis*, *B. zonata* adults are highly invasive, strong fliers, and have high reproductive potential with females laying up to 564 eggs.

4.1.3 *Bactrocera oleae* (Rossi)

Bactrocera oleae is an African origin (Nardi et al., 2005) monophagous pest, the larvae of which feeds exclusively on wild and cultivated *Oleae* spp. (Daane & Johnson, 2010). It is found in countries where olive fruits are cultivated, such as in Southern Europe and America. Wild relatives are found in some parts of Southern and Eastern Africa and Réunion (Nardi et al., 2005,). Females could lay up to 500 eggs.

4.1.4 *Ceratitis capitata* (Widemann)

The Medfly *C. capitata* is a disastrous, invasive fruit pest endemic to sub-Saharan Africa and found over a large area of Africa and South America. It has been introduced to the Mediterranean region and distributed throughout temperate, subtropical and tropical zones of the world. *Ceratitis captitata* is highly polyphagous that completes development in a wide range of hosts 353 species of plants belonging to 67 families (Copeland et al., 2002; De Meyer et al., 2002; Liquido et al., 2013). *Ceratitis capitata* is able to tolerate colder climates and resist habitat fluctuations.

4.1.5 *Zeugodacus cucurbitae* (Coquillett)

The melon fruit fly, *Zeugodacus cucurbitae* is an agricultural pest that originated from Asia (De Meyer et al., 2015). It is widely distributed throughout the temperate, tropical and subtropical regions of the world in which the optimal temperature ranges between 17 °C - 33 °C (Dhillon et al., 2005; Mir et al., 2014). *Zeugodacus cucurbitae* is an oligophagous pests which has been recorded from more than 125 host species, however, cucumber (*Cucumis sativus*) is one of the most preferred hosts (Dhillon et al., 2005; Piñero et al., 2006). *Zeugodacus cucurbitae* usually rests and mates on non host plants, only gravid females travel to host plants for oviposition. The female can lay as many as 1,000 eggs. Unlike other fruit flies, the larvae could develop on the placenta of the fruit and other plant organs such as stems, leaves, flowers and root nodules (Dhillon et al., 2005).

4.2 Olfactometer bioassays

Two choice and multi-choice behavioural experiments were conducted to assess the behavioural responses of tephritid fruit flies to different odor sources. The two-choice experiment was performed using a Y-tube bioassay apparatus (Fig

2), whereas the multi-choice experiment was performed using custom designed cubic glass cage with six circular holes on the top (Fig 2). In both setups a pump generated air, received through Teflon tubing, was charcoal filtered, humidified and controlled in a flow meter, before reaching the odor sources and carrying the plume towards the point where flies were released.

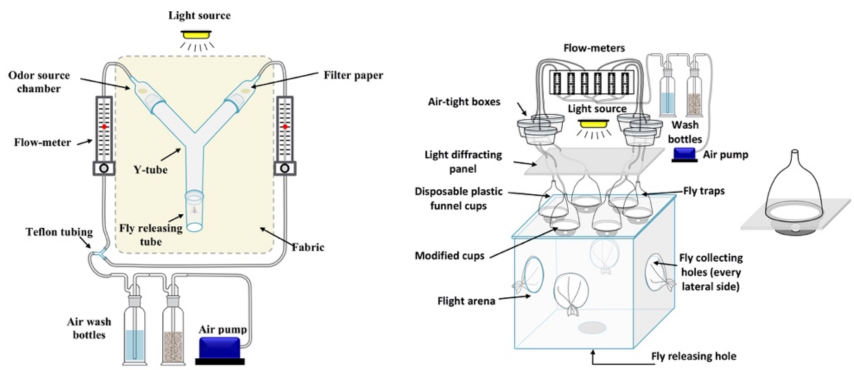

Figure 2. Schematic design of the Y-tube and the multi-choice olfactometer apparatuses used in behavioral bioassays. In both setups a pump generated air, received through Teflon tubing, was charcoal filtered, humidified and controlled in a flow meter, before reaching the odor sources and carrying the plume towards the point where flies were released

4.3 Electrophysiological Experiments

Electrophysiological experiments were conducted using gas chromatography-coupled to electroantennography detection (GC-EAD) apparatus. Flies at the right physiological stages were mounted by immobilizing the fly body in a pipette tip while letting part of the head out and exposing the antennae for recording. Recordings were made using Ag-AgCl glass electrodes filled with ringer solution. The temperature of the GC oven was programmed so that as temperature rose over time, samples injected were separated and split equally to the flame ionization detector (FID) and to the mounted antennae (EAD). Signals from the FID and EAD were amplified converted to a digital signal and displayed on a computer (Fig 3).

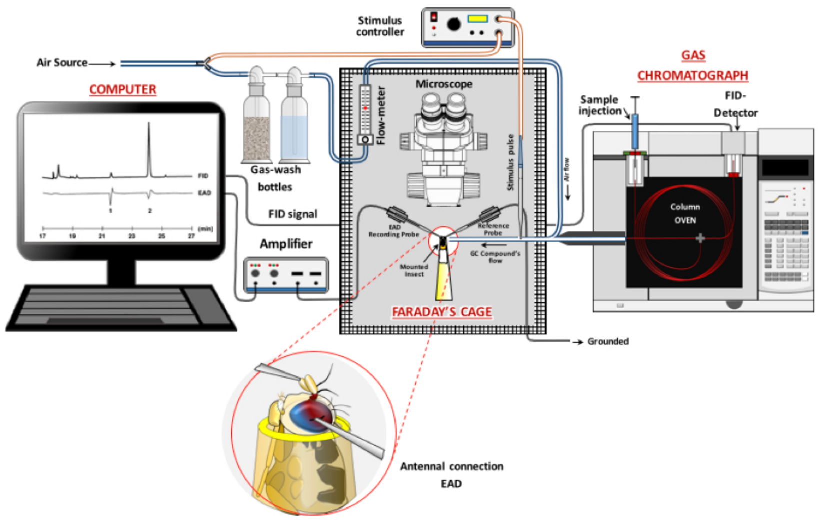

Figure 3. Schematic drawing of Gas chromatography-coupled to electro antennography detection (GC-EAD) apparatus. Samples injected into the injector joins the carrier gas and travels through the column, the effluent is then split into two detectors (FID) and to the fly antennae (EAD). These will be amplified and the output will be displayed on a computer.

5 Results and Discussion

5.1 Part 1: Effect of suppression of the invasive *Bactrocera dorsalis* on the native pest *Ceratitis capitata*

Following rolling out an intervention program in the large commercial guava orchard, we observed a rapid decline of *B. dorsalis* during eight months of applying a combination of various management tools, including the use of parapheromones. Although this illustrates the potential of male-lure-based techniques for suppression of the invasive pest, the selective suppression of *B. dorsalis* caused a resurgence of native tephritid pest populations, highlighting the need for combinatorial tools that target several Tephritidae. We showed that in small scale farming systems, male annihilation alone could reduce the population level of *B. dorsalis*, but population still remains far above economic threshold. We also showed the attractiveness of the combined lure (CL) compared to its individual components (ME, TML, TA) were variable in commercial and smallholder orchards. In commercial orchards, the CL and the individual lures (TML & TA) captured similar numbers of *C. capitata*, which became dominant after suppression of *B. dorsalis*. Meanwhile, the presence of terpinyl acetate (TA) and trimedlure (TML) in the CL didn't affect the capture of *B. dorsalis* (Fig 4). In small-scale orchards, the population of *C. capitata* captured in the CL were significantly lower than *C. capitata* populations captured in TML & TA. This could be due to the very high population of *B. dorsalis* suppressing trap entry of *C. capitata* in the small-scale orchard plots. However, there was no significant difference in the population of *B. dorsalis* captured with CL & ME (Fig 4). In agreement with our finding, traps baited with the combination of ME and cuelure (CUE) attracted similar numbers of *Z. cucurbitae* as CUE alone. However, when *B. dorsalis* populations are very dominant, the use of mixture of ME and CUE is not recommended (Vargas et al., 2000), because of trap entry interference.

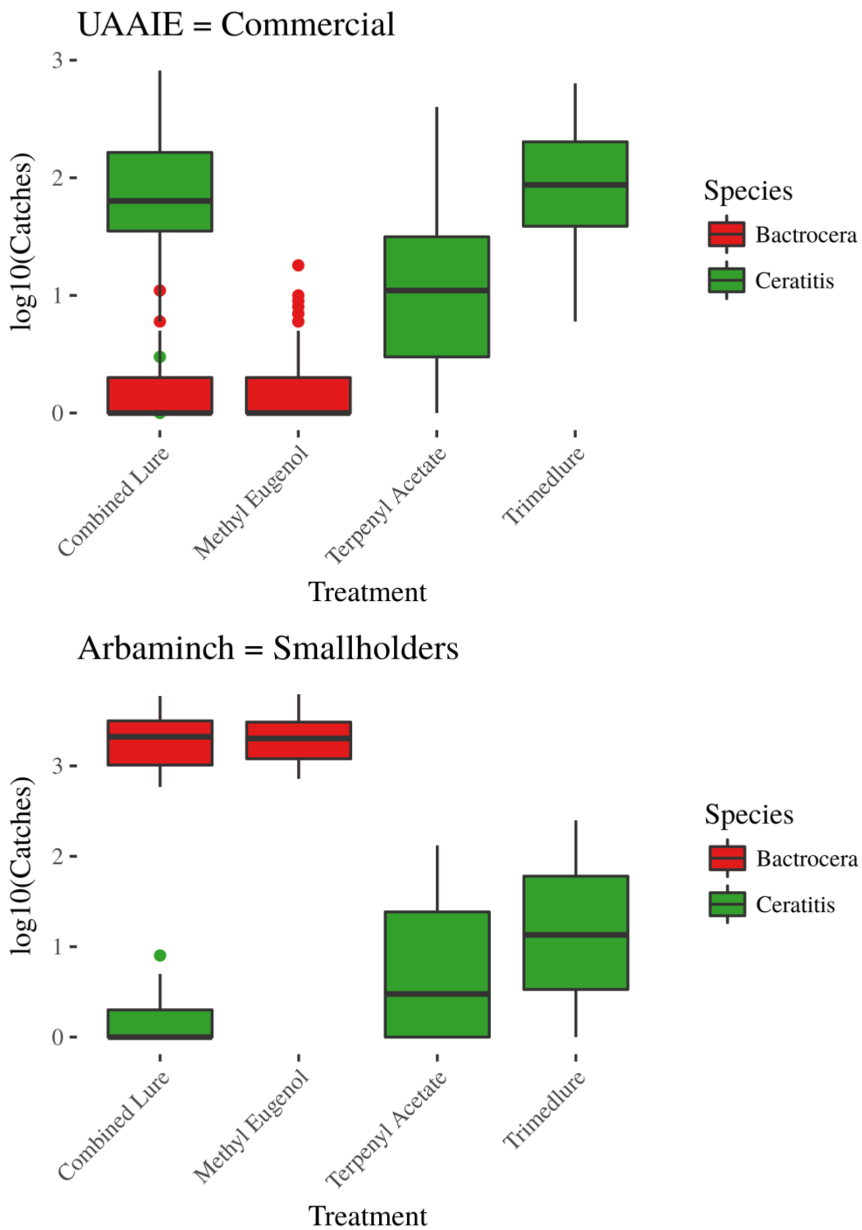

Figure 4. Lure comparison in the commercial orchards (UAAIE, top graph) and smallholder orchards (Arbaminch, bottom graph). Both *C. capitata* and *B. dorsalis* were caught in the trap with the Combined Lure (CL).

Although in the small-scale orchards, application of male lure-based suppression reduced the *B. dorsalis* population, levels remained very high. This is either due to the gradual degradation of the lure and the killing agent (spinosad) or due to the high dispersive ability of the flies beyond several farmers plots or a combination. In mark-release-recapture study, we showed that flies dispersed throughout the study area, despite having ME traps at or nearby the releasing plot (Fig 5). The recapture rate of both green and blue marked flies was generally low, with those released 3 d prior to placement of the traps lower than those released at the same time of trap placement, particularly for traps within a 500 m distance from the release point (Fig 5). This clearly suggests that in smallholders farming areas in Africa, where neighborhood farmers own a small orchard, fly control with male annihilation, and likely with any other technique, requires concerted efforts by the farmers and area-wide management.

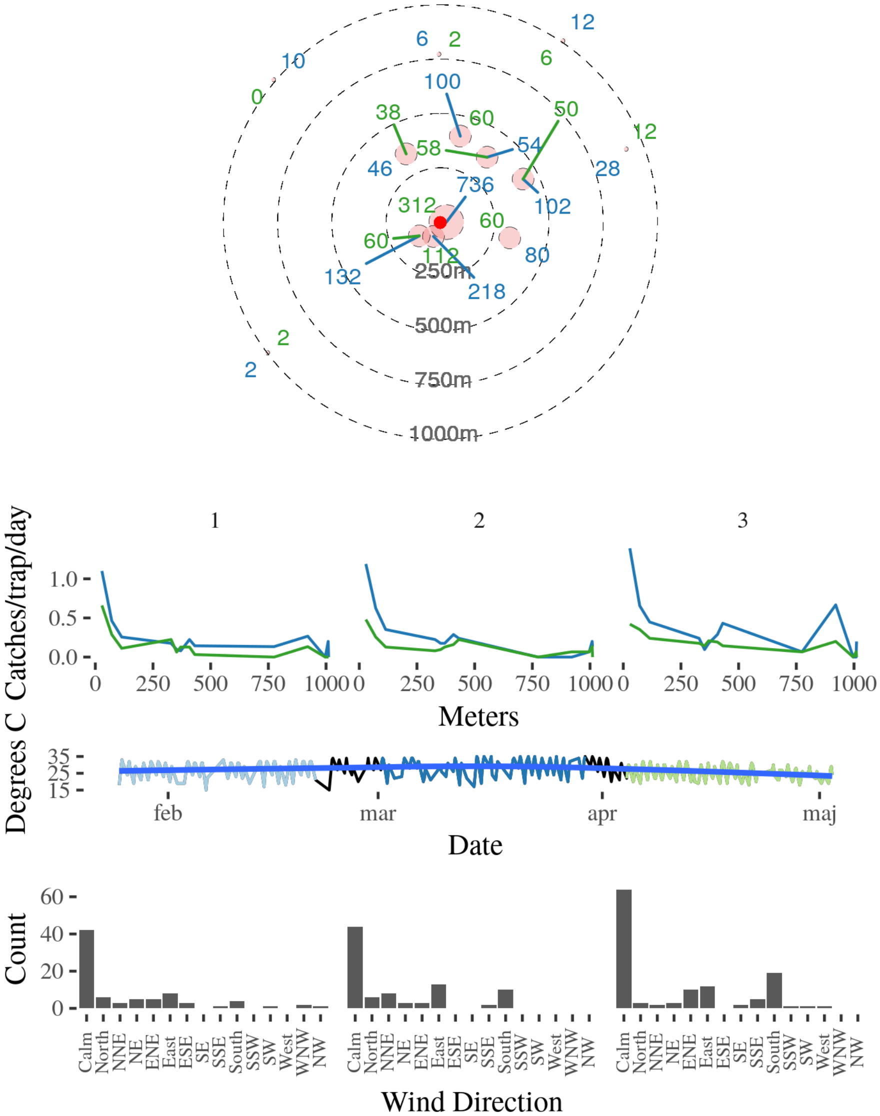

Figure 5. Mark release recapture of blue and green marked *B. dorsalis* fruit flies released before and after trap set up at the small-scale farming setting. Note that a lake was located around 150 m East from the release point. Hence there were no traps in that area. Most fruit-producing farms were situated North and North East from the release plot.

5.2 Part 2: Potential of host fruit volatiles for developing novel female attractants

As a first step to identify odors that attract ovipositing females to potential oviposition sites, we confirmed that intact fruits as well as headspace of fruits were attractive to males and females of *B. dorsalis* in an olfactometer two choice assay. We used GC-EAD to surface which compounds in these blends were detected by the *B. dorsalis* antenna. Recordings from distal and medio-central parts of the antenna showed responses to a number of compounds from each fruit species with a higher depolarization observed from the distal recording. All compounds eliciting a response from the medio-central part of the antenna also elicit responses from the distal part, but not vice-versa (Fig 6).

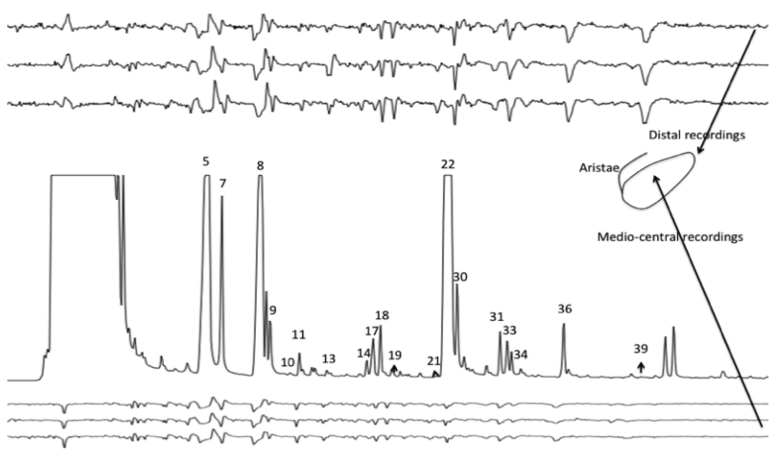

Figure 6. Gas chromatography-coupled electroantennography detection responses of female *Bactrocera dorsalis* to ripe mango volatiles, using distal and medio central recordings.

We constructed a GC-EAD-based fruit-odor olfactome of four Tephritidae pest species to four different major and minor host species. We also showed how these over evolutionary time have been to differing degrees conserved between closely and distantly related Tephritidae pests. Further, we quantified the sensitivities of tephritid antenna by measuring the amplitudes of antennal responses, and compared it with an existing database of odorant receptors of *D. melanogaster*, DoOR (Münch & Galizia, 2016). We found that compounds shared among several fruits tend to be detected by all tephritid fruit flies tested.

On the basis of these shared compounds, we subsequently designed blends that attracted *B. dorsalis* in the laboratory (Fig 7).

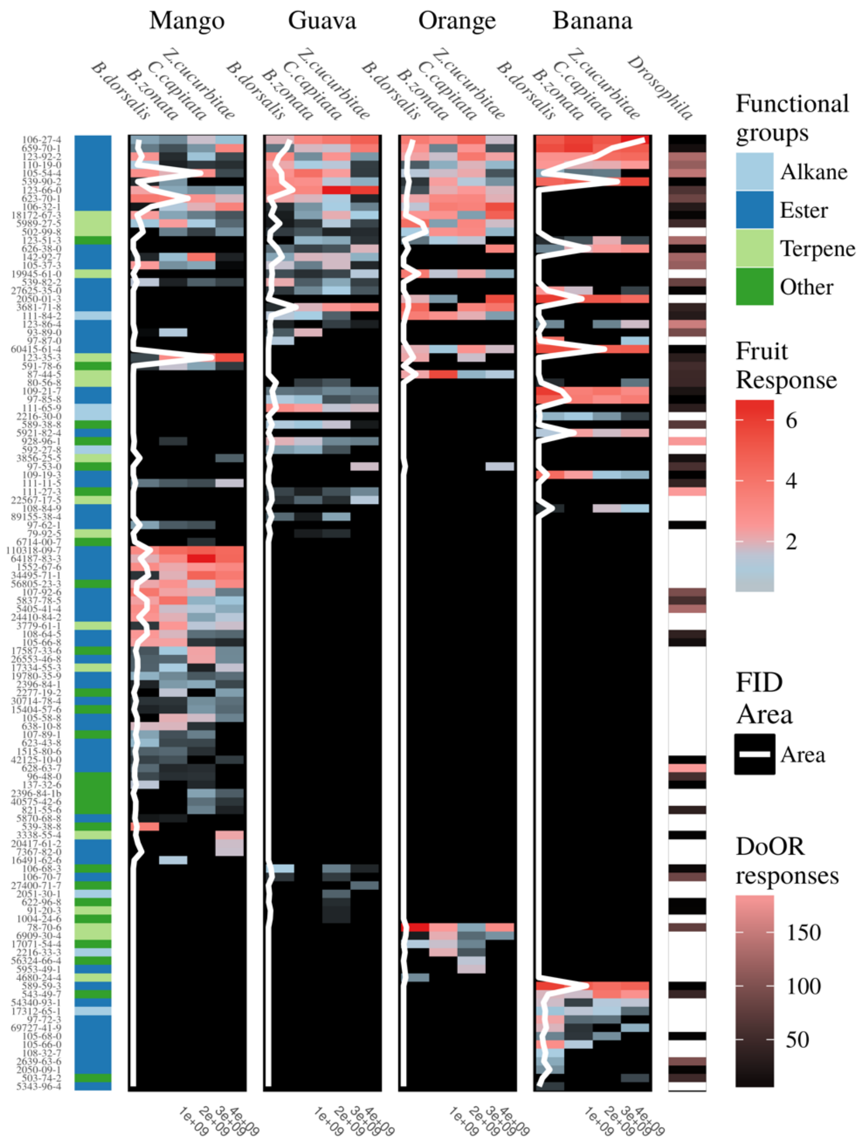

Figure 7. A heat map from left to right shows 1) CAS number to the compounds, 2) functional classes of the compounds 3) sensitivity of the for fruit fly species (*B. dorsalis*, *B. zonata*, *C. capitata* and *Z. cucurbitae*) to compounds in the four fruits (mango, guava, orange and banana) with the FID area in white, and 4)

olfactory response of *D. melanogaster* to the compounds. The chemical groups include Alkane (light blue), Ester (dark blue), Terpenoid (light green) and other (dark green). The average relative sensitivity of the fly's antennae ranges from light gray (0) to dark pink (>6 mv). The olfactory receptors response of *D. melanogaster* increases from dark to pink, white bar indicates compounds not present in the DoOR database.

5.3 Part 3: Potential of food-baits to develop novel female tephritid attractants

Based on antennal responses we provide an approach to the development of protein-based synthetic lures, using five tephritid species to identify 13 physiologically active compounds from food-based fermentation products (Fig 8). Each species tested has a unique response profile to the compounds, and species-specific blends of synthetic compounds were formulated. The specific blend for *B. dorsalis* (dorsablend) was highly attractive in the laboratory, while the other species (*B. zonata*, *C. capitata* and *Z. cucurbitae*) responded variably to their own specific blends. Translation of the dorsablend from laboratory to field trials was not as expected, but traps baited with dorsablend were selective to females of *B. dorsalis*, and it is a promising start to the development of a highly specific bait trap.

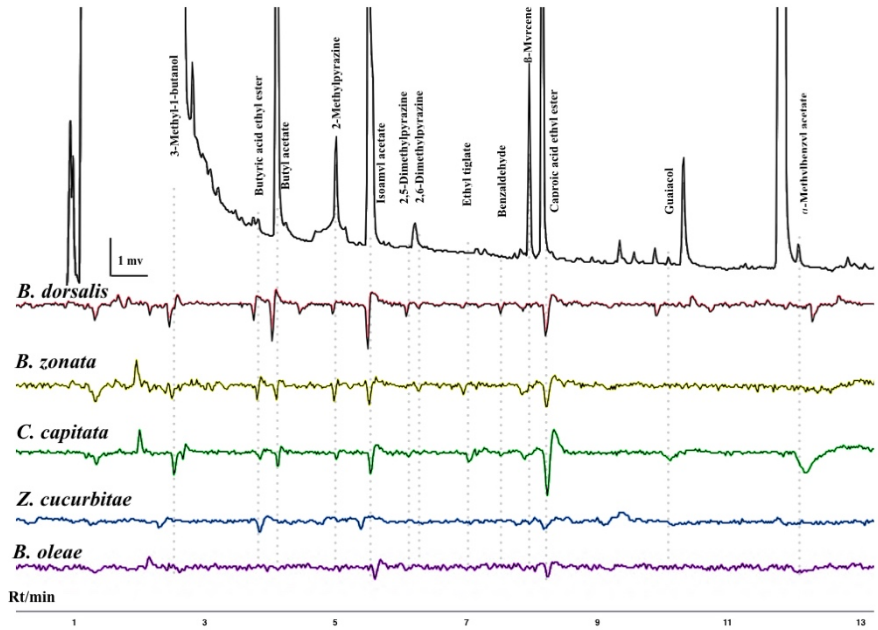

Figure 8. Gas chromatography-coupled electroantennography detection response of the five species of Tephritidae pests tested to the headspace extract of the mixture of food-based lures.

6 Future perspectives

In an ecosystem where more than one species of tephritid fruit fly pests are problems, targeting a single pest unlikely results in fruit protection. This suggests that there is an urgent need to develop a sustainable and environmentally safe method that suppresses multiple tephritid pests from a fruit fly infested area. It has been shown that different male lures could potentially be combined in a single trap and can be used at least in orchards where pests exist at equilibrium (Vargas et al., 2000 and **Chapter I**). Still, for pests whose females initiate the damage, male annihilation alone may not be sufficient. Therefore, future researches should focus on developing a single bait that attracts females of multiple tephritid species. Not only this makes control systems economical, but also avoids competition outbreaks as a result of suppressing a single pest.

What mechanisms are involved in attracting male tephritds to a single compound such as methyl eugenol? And why is there no such compound that attract female fruit flies? These are two very important questions, which, if examined properly, could potentially change current tephritid chemical ecology research approaches. We now know that the maxillary palpae of tephritid fruit flies is very important in detecting pheromones and parapheromones of different types (unpublished data). But, this detection is not solely through the palpae, the antennae also detect parapheromones and pheromones. The search for female attractants should also focus on compounds detected by the palpae, and should include an exploration of functional divergence of the antennal versus palpal detection. The paucity of studies on the palpae may be a reason why tephritid research conducted in the laboratory are not one-to-one translatable to the field (**Chapter III**).

The importance of fruit volatiles or fermented volatiles in attraction of fruit flies is an old discovery, however progress in this area seems to have been slackened due to the discovery of efficient male attractants (parapheromones). In this thesis, based on our results, we speculate that shared volatile compounds of hosts are doorways for polyphagous fruit flies, that is, it may indicate the presence of some sort of substrate for oviposition or lekking, and non-shared or fruit specific volatiles might be used for further fine-tuning the niche of a species. This, however, needs to be studied further and the database and methods produced in this thesis (**Chapters II & III**) could be used as a framework.

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ውዷ እናቴ አየለች ወልደዮሐንስ አንቺን ለማመስገን ቃላቶቸ በቂ አይደሉም። አንድን ልጅ እንኳን በስርዐት ማሳደግ እንዴት ከባድ እንደሆነ ሁሉ ያውቃል። አንቺ ግን 2, 4, 6, 8 ልጆችን እንዲሁም የልጅ ልጆችን በፍቅር አሳድገሽ ለዚህ አብቅተሽናል። ምስጋና ያንስብሻል! እድሜና ጤና ይስጥልኝ!

ጤንነት ደጃኔ (ሚሚ) የሲጃራ ፋብሪካ ድንቸና ቀይስር በዳቦ፣ ይዘሽልኝ የምትመጪው ወተት ከዛም አልፎ ደሞ ለኮካ እፈልጥሽ እንደነበር አልረሳሁትም። እኔ የምልሽ ይሄን ሁላ ግን በዛቸ ደሞዝ እንዴት? አመሰግናለሁ! **ትዕግስት ደጃኔ** (ቲጂ) ያቺን ሱቅ ባትከፍቺያት ኖሮ ልጅነቴ እንዴት ይጥመኝ ነበር። ከ ኮምፕዩተር ጋርም የተዋወቁት አንቺ ከፍለሽልኝ እንደሆነ አልረሳሁትም። አመሰግናለሁ! **መንበረ ደጃኔ** መንቢ አዝለሽ እንዳሳደግሽኝ ሰምቻለው። ልጅ ሆኜ ቤተክርስትያን እየወሰድሽ ስታቆርቢኝ እንደነበርም ትዝ ይለኛል። ከ ጌታችን ስጋና ደም በላይ ምን ስጦታ አለ! አመሰግናለሁ! **ስንቅነሽ ደጃኔ** ስንቄ ከልጅሽ ሳትለዩ ስላለበስሽኝ፣ ባንበሳ ጫማ ስላጫማሽኝ አመሰግናለሁ! **አልማዝ ደጃኔ** ቴቴ ልደታ በመጣች ቁጥር ሳንቲም አስለምደሽኝ የደሞዝሽን ቀን ካንቺ ይልቅ እኔ እናፍቀው ነበር። አመሰግናለሁ! **አስናቀች ደጃኔ** አስኔ የታላላቆቼ አጎት ስላደረግሽኝ አመሰግናለሁ!

ጥላሁን ደጃኔ ሲስ እግዚያብሔር ፈቅዶ ካንተ ጋር ባልኖር ህይወቴ አሁን የያዘውን መስመር ላይዝ ይችል ነበር። ካንተ ብዙ ተምሬያለሁ፣ እምነትን፣ ፍቅርን፣ ጽናትን፣ ጸሎትን እንዲሁም ወጥ መስራትን። በንቅልፋምነቴ አስቸግርህ እንደነበር መቼም አትረሳውም። እንዴት ይረሳል? ከጊዮርጊስ ስትመለስ እንቅልፍ ላይ ሆኜ ስታንኳኳ ስለማልሰማህ ማሪያም ትሄድ ነበር እኮ። አንድ ጊዜ እንደውም ቤቱን ሰብራቸሁ ገብታቸሁ እስክትቀሰቅሱኝ የሞት ያህል ተኝቼ ነበር። ከማስጠናት አልፈህ የትምህርቴንም ክፍያ ባትከፍልልኝኝ እንዴት እዚ እደርስ ነበር? አመሰግናለሁ! **ተሾመ ደጃኔ** ተሼ ለኔ ብቻ ሳይሆን ለቤተሰቡ ሁሉ ምሳሌ ነህ። የመጀመሪያ መሆን ትልቅ ሃላፊነት ነው። ይህን ሃላፊነት በብቃት ስለተወጣሀው ደስ ሊልህ ይገባል። ስራን ሳትንቅ ስራን እንዳልንቅ፣ ታዘህ እንድታዘዝ፣ እረድተህ እንድረዳ፣ ተምረህ እንድማር፣ ወርቅ ተሸልመህ ወርቅ እንድሸለም ሌላም ብዙ ብዙ ስላስተማርከኝ፣ አርአያ ስለሆንከኝ አመሰግናለሁ!

ፍቅርርርር የማደርግሽ ውድ ሚስቴ **ቤተልሔም ወንድወሰን** እኔን ማስደሰት የማይታክተው አምላኬ የሰጠኝ ማራኪ ስጦታዬ አመሰግንሻለሁ!

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ለባርነት አሳልፋቸሁ ያልሰጣቸሁኝ አባቶቸና እናቶቸ፣ በነፃነት ከልቤ አመሰግንበተ ዘንድ ይህን ፊደል የሰጣቸሁኝ ልሂቃን፣ ያስተማራቸሁኝ መምህሮቸ፣ ሳልጸልይ ብተኛም ያለዕንቅልፍ የምትጸልዩልኝ ሃይማኖተኞቸ፣ ለየሰከንድ ሃጢያቴ በንስሃ ጸሎት የምታስቡኝ ቅዱሳን .... ሁላቸሁንም አመሰግናለሁ።