Social and Environmental Olfactory Signals Mediate Insect Behavioral Ecology and Evolution

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Cover: *Spodoptera* on a banana
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Abstract

Odors are essential in mediating insect reproductive behavior. Environmental odors help insects locate suitable feeding or egg-laying sites and avoid suboptimal hosts or dangerous habitats. Sex pheromones, on the other hand, are responsible for mate finding and elicit courtship and mating. Although pheromones elicit stereotypical behaviors on their own, they are embedded in a background of environmental odors in nature. Using the cotton leafworm, *Spodoptera littoralis*, and the common fruit fly, *Drosophila melanogaster*, I studied the effect of blending environmentally relevant odors with pheromones on insect behavior.

For the cotton leafworm, we first developed an attractive cotton volatile blend. We next used this blend to determine the physiological effect of DMNT, a strong behavioral antagonist, on the cotton leafworm olfactory system. I then blended the individual volatiles and volatile blends with an incomplete and complete pheromone. The combination of cotton volatiles and the complete pheromone elicits attraction. Deviations from this optimum, either by changing the pheromone composition or the cotton volatile blend strongly reduces male *S. littoralis* attraction.

I then used the fruit fly to study the effect of food (vinegar) and habitat (yeast) volatiles on fly attraction towards pheromones. Starvation affects attraction towards a blend of vinegar and a male produced pheromone in a sexually dimorphic way. We next describe a novel female fruit fly pheromone and the odorant receptor involved in its perception. Finally, we show that vinegar and yeast volatiles interact in a different manner with male and female produced pheromones, suggesting that although vinegar is a good feeding cue, even in the presence of pheromones, it is not an appropriate mate finding cue.

My findings suggest that pheromones and host volatiles function as a single unit that mediates insect behavior, rather than as individual components. As such the olfactory cues that mediate mate finding in insects are under both natural and sexual selection simultaneously, which has strong implications for insect speciation and evolution.

Keywords: Spodoptera littoralis, *Drosophila melanogaster*, pheromones, host volatiles, chemical ecology, olfaction, host plant volatiles, herbivore-induced plant volatiles, sexual selection, natural selection

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Dedication

To those who find my present and future work interesting.

See that the imagination of nature is far, far greater than the imagination of man.

Richard P. Feynman

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 Borrero-Echeverry, F., Becher, P.G., Birgersson, G.Å.O., Bengtsson, M., Witzgall, P., and Saveer, A.M. (2015). Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. *Frontiers in Ecology and Evolution* 3, 56.

II Hatano, E., Saveer, A., Borrero-Echeverry, F., Strauch, M., Zakir, A., Bengtsson, M., Ignell, R., Anderson, P., Becher, P., Witzgall, P., and Dekker, T. (2015). A herbivore-induced plant volatile interferes with host plant and mate location in moths through suppression of olfactory signaling pathways. *BMC Biology* 13**,** 75.

III Borrero-Echeverry, F., Bengtsson, M., Witzgall, P. Blends of host plant volatiles and sex pheromones encode specific mate recognition in moths. (Manuscript).

IV Lebreton, S., Borrero-Echeverry, F., Gonzalez, F., Solum, M., Wallin, E., Trona, F., Grabe, V., Sachse, S., Hedenström, E., Hansson, B.S., Bengtsson, M., Birgersson, G., Walker, W.B., Dweck, H., Witzgall, P., Becher, P. (2015). Feeding regulates sex pheromone attraction and courtship in Drosophila females. *Scientific Reports* 5, 13132.

V Lebreton S., Borrero-Echeverry F., Gonzalez F., Trona F., Solum M., Wallin E., Grabe V., Sachse S., Bengtsson M., Hansson B.S., Hedenström E., Birgersson G., Dweck H., Witzgall P., Becher P.G. 2016. The scent of the fly. (Manuscript).

VI Borrero-Echeverry F., Witzgall, P., Becher, P.G. Drosophila sex pheromones synergize vinegar and yeast attraction. (Manuscript)

The contribution of Felipe Borrero-Echeverry to the papers included in this thesis was as follows:

I Carried out wind tunnel experiments on male moths, analyzed the data and wrote the manuscript with the co-authors.

II Carried out wind tunnel experiments, analyzed wind tunnel data and assisted in writing the manuscript with the co-authors.

III Planned and carried out all experiments and data analysis and wrote the manuscript.

IV Carried out behavioral experiments, analyzed wind tunnel data and assisted in writing the manuscript with the co-authors.

V Carried out wind tunnel experiments, analyzed wind tunnel data and assisted in writing the manuscript with the co-authors.

VI Planned and carried out all experiments and data analysis and wrote the manuscript

Abbreviations

AL	Antennal lobe
EAD	Electroantennographic detection
GC-EAD	Gas chromatography-electroantennographic detection
GC-MS	Gas chromatography-mass spectrometry
GR	Gustatory receptor
HIPV	Herbivore-induced plant volatile
HPV	Host plant volatile
IPM	Integrated pest management
IR	Ionotropic receptor
LH	Lateral horn
LN	Local interneuron
MB	Mushroom bodies
OR	Odorant receptors
OSN	Olfactory sensory neuron
PN	Projection neuron

1 Introduction

1.1 Insect chemical ecology

Chemical Ecology is the discipline that studies interactions between organisms mediated by chemical signals. Chemical communication is the only form of communication found in all living things and is used in communication between organisms, and between organisms and their living environment. Chemical compounds involved in chemical communication are called semiochemicals, and are subdivided into four main types. Pheromones are used in intraspecific communication. The remaining three, allomones, synomones and kairomones are signals that are involved in interspecific communication. Allomones are chemical signals that provide a benefit to the organism that produces them and a cost to the responder. Synomones are those signals that benefit both the organism producing them and the receiver. Lastly, kairomones benefit the receiving organism at a cost to the organism producing them (Eisner & Meinwald, 1995). Since the chemical language is universal and connects all living things, chemical signals have multiple effects on different organisms and can only be defined in a given context.

The importance of chemical communication in herbivorous insects and plants had been recognized in the mid 19th century in the work of Ernst Stahl, Anton Kerner von Marilaun, Léo Herrera and others, which went largely unnoticed until the second half of the 20th century. Advances in plant biochemistry that showed a large discrepancy between the number of secondary metabolites and the number of essential processes in plants, led to the idea that plant secondary metabolites were not by-products of the essential biochemistry, but rather, that biosynthetic pathways had developed under natural selection for discrete purposes. This, along with observations of male moths flying upwind towards pheromone-releasing female moths caused a resurgence of the study of chemical ecology in the 1950's (Hartmann, 2008).

The identification of the *Bombyx mori* pheromone by Adolf Butenandt (1959) gave way to an increased interest in insect pheromone research, which has remained an active field of research ever since. In addition to work on sexual communication, entomologists studying insect pollinators and herbivorous insects observed that insects used volatile plant secondary metabolites to locate their host plants for feeding and oviposition, and that plant secondary metabolites served in defense against herbivorous insects (Harborne, 2001). The 1970's saw the birth of Chemical Ecology as a separate discipline with the publishing of the first treaty on the subject (*Chemical Ecology*, 1970) and the creation of the Journal of Chemical Ecology in 1976 (Hartmann, 2008; Harborne, 2001).

Since then, Chemical Ecology has advanced at a breathtaking pace. Ecological, behavioral and applied studies have been supported by an ever-growing array of biochemical, physiological, anatomical and molecular tools. These new methods have allowed us to better understand the ecological context behind chemical communication as well as the mechanisms behind odor perception processing. The discovery of odorant receptors (ORs) by Linda Buck and Richard Axel (1991) in mammals and the subsequent discovery of similar receptors in the fruit fly, *Drosophila melanogaster* (Clyne *et al.*, 1999; Vosshall *et al.*, 1999) has led to an ever growing understanding of the mechanisms of insect olfaction at the peripheral and central neural level. Although *D. melanogaster* is the main model species in insect olfaction, due to the wide array of available genetic tools, several other species have been thoroughly studied such as the honeybee, *Apis melifera*, due to its learning capacity, and several moths, including the cotton leafworm, *Spodoptera littoralis*. Moths have been a focus of chemical ecology research because of their astounding sensitivity to female sex pheromone and the stereotypical behavior it evokes, and due to their importance as pests in agriculture, horticulture and forestry.

1.2 Model organisms

1.2.1 *Spodoptera littoralis*

The genus *Spodoptera* Guenee (Lepidoptera, Noctuidae) consists of approximately 30 species, half of which are pest insects in different regions of the world. They are commonly referred to as armyworms due to the ability of larvae to migrate in large numbers. Most species within the genus are polyphagous, feeding on hundreds of plant species (for a detailed review of the genus see Pogue, 2002).

Spodoptera littoralis (Boisduval) (Lepidoptera, Noctuidae), the cotton leafworm, is distributed throughout Africa, Mediterranean Europe and the Middle East, through Iran. It is a highly polyphagous species that attacks more than 100 economically important species including cotton, maize and vegetable crops. The cotton leafworm is particularly important as a pest of cotton in Egypt and maize in Africa due to the social importance of these crops. Its geographical distribution overlaps with its sibling species, *S. litura* (Figure 1), in eastern Iran and Pakistan. Due to the lack of clear morphological characters these two species are often confused, although they can be distinguished by larval markings and adult genitalia (CABI, 2015; Pogue, 2002).

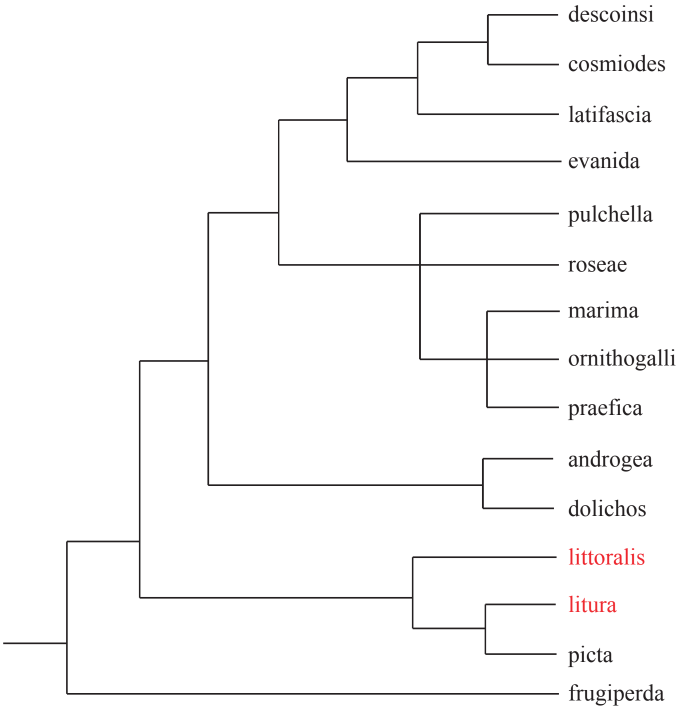


Figure 1. Phylogeny of part of the *Spodoptera* genus. In red, *S. littoralis* and *S. litura*, the species used in this thesis. Based off the phylogeny produced by Pogue (2002).

1.2.2 *Drosophila melanogaster*

The genus *Drosophila* (Fallén) (Diptera, Drosophilidae) comprises approximately 1500 species. They are usually referred to as fruit flies, vinegar flies or wine flies since many species use decaying and fermenting fruit as their hosts (Bächli, 2015). Most species of the genus are only a few millimeters long and only a few are considered to be of economic importance, chief among them *D. suzukii*, the spotted-wing Drosophila, which is a serious invasive pest of stone fruits and berries (Cini *et al.*, 2012).


Image credit: Shutterstock

The common fruit fly, or banana fly, *D. melanogaster* (Meigen), is taxonomically grouped in the melanogaster sub-clade of the *Drosophila* genus, along with *D. simulans*, *D. sechellia* and *D. mauritiana* (Figure 2). It is a model organism in genetics, physiology, neurobiology and developmental biology due to the ease of laboratory rearing, short generation time, high fecundity, easily visible morphological traits, a small, fully sequenced genome, and a wide array of molecular tools which have been developed for it.

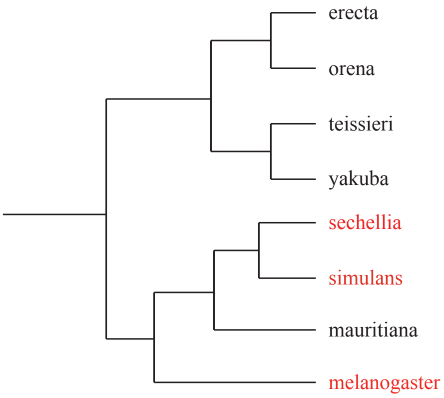


Figure 1. Phylogeny of the melanogaster subclade of the *Drosophila* genus. In red, *D. melanogaster*, *D. simulans* and *D. sechellia*, the species used in this thesis. Based off the phylogeny by van der Linde & Houle (2008).

1.3 Insect olfaction

1.3.1 Overview

The peripheral olfactory system of insects is comprised of the antennae, which are the primary olfactory organs. Insect antennae are covered by different types of sensilla with pores through which odorants enter the sensillar lymph. Each sensillum normally houses between two and three olfactory sensory neurons (OSN) that usually express a single type of odorant receptor (OR), which responds to a limited range of chemical compounds. In the lymph, odorant binding proteins (OBP) bind to odorants and play a role in odor perception. One possible function is the transport of odorant molecules, which tend to be hydrophobic, across the sensillar lymph to the odorant receptors (ORs) (Leal, 2013; Kaissling, 2001). In order for ORs to be capable of responding to new stimuli after interacting with the OBP/odorant complex or odorant molecules, these need to be eliminated to give way to new molecules. This process is carried out by enzymatic degradation in the lymph by odorant and pheromone degrading enzymes (ODE and PDE). ODEs and PDEs both regulate, and terminate signals relayed to the insect brain (Figure 3) (Vogt *et al.*, 1999; Kasang, 1971).

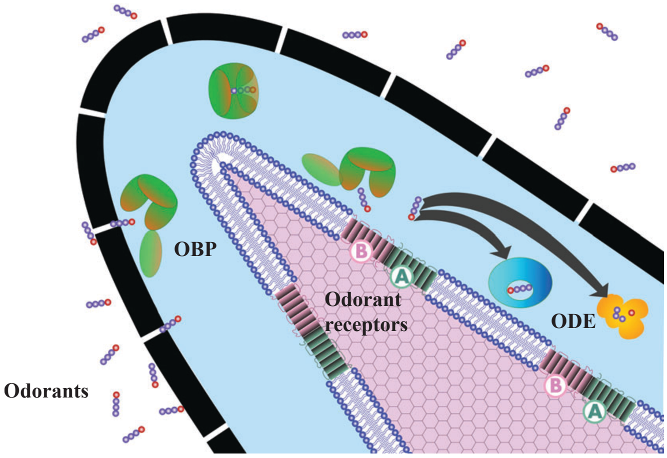


Figure 3. Insect sensilla, showing odorants, pores, odorant binding proteins, odorant receptors and odorant degrading enzymes. Modified from Leal, 2013.

Olfactory sensory neurons transmit information to the primary olfactory center, the antennal lobe (AL). The AL is subdivided into glomeruli which each receive information from all OSNs expressing the same OR. Each glomerulus gathers the information from between 20 and 2000 ORNs. The number of glomeruli in insects varies from 50 to over a thousand, however, it is usually less than 200 (Hansson & Anton, 2000; Anton & Homberg, 1999). Although it was initially believed that the AL functioned as an aggregation center where signals from all OSN with the same receptor combined into one signal, it is now clear that the AL is the first center of integration and processing of information. Glomeruli are interconnected by local interneurons (LN). These LNs may be inhibitory or excitatory and function to integrate the information received by multiple glomeruli (Hansson & Anton, 2000) and in the sharpening, broadening and fine tuning of signals (Martin *et al.*, 2011). The input information reaching each glomerulus from multiple OSNs is reduced to two, or three output, projection neurons (PN), which branch out to higher brain centers (Figure 4).

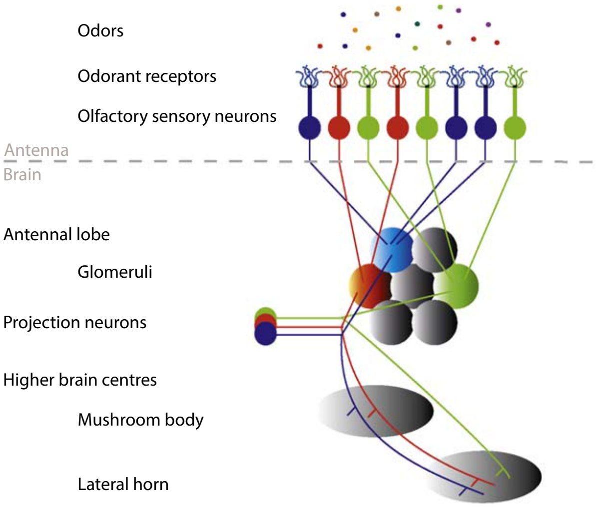


Figure 4. Schematic representation of the insect olfactory system. Modified from (Ramdya & Benton, 2010).

Projection neurons branch out from the ALs to the mushroom bodies (MBs) and the lateral horns (LHs). The MBs are paired regions of the insect protocerebrum that play a key role in integration of information from different senses, decision-making, learning and memory. The MBs are located in the dorso-posterior region of the head capsule. Although their shape and structure varies between different families and genera of insects, they are made up of a cap shaped structure, or calyx, and an elongated pedunculus. (Heisenberg, 2003; Pascual & Préat, 2001). Most of the MB is made up of intrinsic neurons called Kenyon cells (KCs). The calix is made of mainly the dendrites of KCs that supply the pedunculus with branched axon-like structures. The number of KCs ranges from several hundred cells in fruit flies to several hundred thousand cells in hymenopterans (Farris, 2005).

The lateral horn is a aglomerular brain center (Yasuyama *et al.*, 2003). Projection neurons that terminate in a specific region of the LH usually originate in topologically close glomeruli in the antennal lobe. The LH is separated into regions that receive input from food-derived signals and pheromone signals, with some overlap towards the central region (Jefferis *et al.*, 2007; Marin *et al.*, 2002). It is hypothesized that the LH integrate stereotypical information, which is responsible for innate and instinctive behavior.

Little is known of the processing of olfactory information beyond the MB and LH. Neurons branching out from these structures project to diverse regions of the brain where further processing takes place, leading to the generation of behavioral responses (for more information on neural coding and processing see: Galizia, 2014; Martin *et al.*, 2011; Galizia & Rossler, 2010).

1.3.2 Olfaction in *S. littoralis*

The antennae of both female and male *S. littoralis* are filiform. Antennae have two basal segments followed by between 62 and 70 segments (Binyameen *et al.*, 2012). Although antennae of both males and females are morphologically similar, the length and diameter of male antennae is greater (Malo *et al.*, 2004). Antennomeres can be divided into two distinct regions. The dorsal region has two rows of scales and few squamiform sensilla that probably have propio- or mechano-receptive functions, and two gustatory sensilla chaetica (Hix *et al.*, 2003). The ventral region contains most of the olfactory sensilla. Six different types of sensilla have been described on this region of the antennae. Sensilla chaetica, (six per antennomere) are gustatory, sensilla styloconica are believed to be hygro- and thermoreceptors, and coeloconic, auricillic, basiconic and trichoid sensilla (which are subdivided into short and long), serve a chemosensory function (Binyameen *et al.*, 2012). Basiconic, coeloconic, auricilic, and short trichoid sensilla respond to plant volatiles. Long trichoid sensilla house neurons responding to pheromones (Binyameen *et al.*, 2012).

The ORs of *Spodoptera* have received considerable attention. Using transcriptomics a total of 36 candidate ORs, five GRs, and 12 IRs have been identified from the antennae of *S. littoralis* (de Fouchier *et al.*, 2015; Jacquin-Joly *et al.*, 2012; Legeai *et al.*, 2011). However, in view of the fact that 66 ORs have been found in the genome of *Bombyx mori* (Tanaka *et al.*, 2009), and that 63 glomeruli have been identified in the antennal lobe of male *S. littoralis* (Couton *et al.*, 2009) it is likely that nearly 30 candidate ORs have yet have to be identified. More than half of these ORs have been deorphanized, i.e. ligands have been identified for them, using the *Drosophila* empty neuron system (Gonzalez *et al.*, 2016). When challenged with high odorant concentrations, most ORs respond broadly to a variety of different compounds. However, they seem to be narrowly tuned to only one, or a few compounds at low, ecologically relevant concentrations (de Fouchier *et al.,* in prep).

Thirty-five OBPs have been identified in the antennae of female *S. littoralis* (Jacquin-Joly *et al.*, 2012). Additionally, twenty antennal esterases, which may function as odorant degrading enzymes, have also been described (Durand *et al.*, 2010). The protein encoded by the gene SlCXE7 hydrolyzes the two major components (Z9,E11-14Ac and Z9,E12-14Ac) of the *S. littoralis* pheromone and the plant compound, (Z)-3-hexenyl acetate. Degradation of (Z)-3-hexenyl acetate is considerably faster than that of pheromone components, but its affinity to the pheromone components is greater. As such, SlCXE7 may be responsible for the rapid degradation of an abundant and commonly found plant volatile and quick processing of low concentration pheromone signals (Durand *et al.*, 2011).

The female AL of *S littoralis* has only been mapped superficially and 35 glomeruli have been identified (Saveer *et al.*, 2012; Sadek *et al.*, 2002). The male AL, on the other hand, has been completely mapped, revealing between 60 and 63 glomeruli, which are mostly organized in one layer around a fibrous core. Across individuals only 50% of the glomeruli within the AL seem to have a fixed topology (Couton *et al.*, 2009). The macroglomerular complex (MGC) is composed of three glomeruli whose function in pheromone detection has been confirmed through *in vivo* calcium imaging (Carlsson *et al.*, 2002).

Odors are represented by conserved patterns of glomerular activation and this has been demonstrated for both pheromone components and plant volatile compounds in *S. littoralis* (Saveer *et al.*, 2012; Hansson & Anton, 2000; Anton & Hansson, 1994). It is still unclear how odor mixtures interact in the AL. Although in honeybees odor blend representations in the antennal lobe do not correspond to the sum of the individual glomeruli activity, this seems to be the case in *S. littoralis* (Carlsson *et al.*, 2007; Joerges *et al.*, 1997). Whether this is due to taxonomical or functional differences is yet to be determined. Communication between glomeruli via (LNs), leads to asymmetry between input to the AL and output via projection neurons in *S. littoralis* (Sadek *et al.*, 2002). Although the AL appears to be the first center for information processing, further studies are required to understand its role in the interpretation of ecologically relevant odor blends in the cotton leafworm.

Antennal lobe activity in *S. littoralis* has been shown to be correlated with behavior and behavioral modulation. Saveer *et al.* (2012) showed that unmated females are attracted to floral odors and only weakly to host plant odors whereas mated females are strongly attracted to host plant odors and only weakly to floral odors. Activity in the antennal lobe activity was shown to be modulated accordingly. The activity of glomeruli which respond to floral odors is up-regulated before mating and inhibited after mating while those responding to host plant odors show the opposite pattern (Kromann *et al.*, 2015; Saveer *et al.*, 2012). Similarly, males show a strong attraction to host plant odors and females before mating, however, attraction to host plants and females is inhibited after mating. AL activity of the MGC and glomeruli which are activated by host plant volatiles is strong in unmated males while it is strongly inhibited shortly after mating (Kromann *et al.*, 2015).

Projection neurons leaving the antennal lobe branch out into mushroom bodies (MBs) and lateral horn (LH) in *Spodoptera littoralis*. Studies on projections into the LH and the MBs of the cotton leafworm, and the structure and immunochemistry of the later have been carried out (Sinakevitch *et al.*, 2008).

1.3.3 Olfaction in *D. melanogaster*

In contrast to *S. littoralis*, where methodological limitations provide only a blurred picture of its olfactory system and circuitry, the genetic toolbox available for *D. melanogaster* allows us to trace signals from the periphery to higher brain centers at high resolution.

The *Drosophila* maxillary palp and antennae house all sensilla that contain OSNs. The antennae have three segments, the third of which has all of the approximately 410 olfactory sensilla. There are four main types of sensilla on fruit fly antennae: large basiconic sensilla, that are located in the medial-proximal region, trichoid sensilla which cluster on the lateral-distal region, and the small basiconic and coeloconic sensilla which cluster in the medial region of the antennae. There has been recent re-classification of trichoid sensilla which further subdivide them into intermediate sensilla (Lin & Potter, 2015). Males have nearly 30% more trichoid, and 20% less basiconic sensilla than females. There are approximately 1200 OSNs on the antennae which branch into the ALs. The maxillary palps only contain approximately 60 basiconic sensilla which house 120 neurons that also terminate in the ALs (Vosshall & Stocker, 2007; De Bruyne *et al.*, 2001; De Bruyne *et al.*, 1999).

The availability of the *D. melanogaster* genome has made it possible to identify 60 genes that encode 62 ORs. The ORs of the fruit fly and their receptive range have been thoroughly studied. Ligands have been found for most of these ORs, and nearly 700 odorants have been show to activate olfactory circuits (Muench & Galizia, 2015). Most ORs have also been tied to specific OSNs, sensilla, and to their respective glomeruli in the ALs (Couto *et al.*, 2005; Fishilevich & Vosshall, 2005). There is a wealth of information available on AL structure and function of the fly olfactory system, from the periphery via the AL to and higher brain centers. On the other hand, investigations of the behavioral coding and consequences of olfactory stimuli is lagging behind, with the exception of sexual behavior (Auer & Benton, 2016; Martin *et al.*, 2011).

The mushroom bodies of *D. melanogaster* are far more organized than those of moths. Information about the arborisation pattern of PNs from 13 glomeruli into the MBs is available and reveals that PNs from single glomeruli arborize into a stereotypical region of the MBs. Furthermore it seems that PNs from neighboring glomeruli terminate in similar regions of the MB calix (Jefferis *et al.*, 2007; Lin *et al.*, 2007). Projection neurons arborizing into the LHs show a similar pattern, PNs from topologically close glomeruli terminate in similar regions in the LH. Additionally, PNs that receive input from OSNs in the same a sensilla type project to similar areas in the LH (Jefferis *et al.*, 2007).

1.4 Pheromones in insects

1.4.1 Overview

Pheromones are the best-studied semiochemicals in insects. To date tens of thousands of compounds have been identified to function as insect pheromones in nearly 3000 species (El-Sayed, 2014). The composition of pheromones is of fundamental importance. Although some pheromones may be composed of a single chemical component, most of them are multicomponent blends, where the component ratios are critical for signal fidelity. Changes in ratios, or absence of compounds may lead to a breakdown of the signal (Linn *et al.*, 1986). Pheromones are subdivided into sex, aggregation, alarm, trail or recruitment, nest recognition and home range marking pheromones (Yew & Chung, 2015; Vander Meer *et al.*, 1998).

Sexual pheromones are chemical compounds that mediate sexual behavior including species recognition, mate finding, courtship and copulation. Sex attractants, or mate finding cues, are often produced by female insects to attract males. However, they may also play an important role in the sexual arousal of males and in eliciting courtship behavior. Male produced sex pheromones usually serve the function of increasing female receptivity and are often referred to as aphrodisiacs (Jacobson, 2012). Sex pheromones are usually sexually dimorphic. They serve the additional purpose of gender recognition and play a role in intra-gender competition (Vosshall, 2008). Sex pheromones, and their interaction with host volatiles are the focus of this thesis.

Aggregation pheromones also function as attractants. They differ from sexual pheromones in that they attract both male and female insects, although they inherently function as sex pheromones by bringing individuals of both genders together, on a host plant or mating site (Vité & Francke, 1976). Aggregation pheromones are common in the Coleoptera, Blattodea, and Hemiptera and have been best studied in palm weevils and bark beetles due to their economic importance (Symonds & Gitau-Clarke, 2016; Gries *et al.*, 2015; Bell *et al.*, 1972).

Alarm pheromones are difficult to define due to the wide variety of behaviors that they elicit. They have often been subdivided into different categories to better explain their function, such as alerting, anti-aggregation, dispersal or more broadly, agitating pheromones (Vander Meer *et al.*, 1998). Alarm pheromones are common in insects with a varying degree of sociality. Pheromones which serve a dispersal and anti-aggregation purpose are common in the Hemiptera and have been particularly well studied in aphids. In aphids, (*E*)-β-farnesene causes colonies to disperse in the presence of predators, leading to increased predator avoidance (Boullis & Verheggen, 2016). Eusocial insects, such as ants, wasps, bees and termites have a wider array of alarm pheromones which elicit caste-specific behavior including brood guarding, recruiting of soldier castes and stinging behavior (Leonhardt *et al.*, 2016; Delattre *et al.*, 2015; Yew & Chung, 2015; Vander Meer *et al.*, 1998).

The remaining types of pheromones, trail pheromones, nest recognition pheromones and home range marking pheromones and queen pheromones are exclusive to eusocial insects. These pheromones help to differentiate castes, colony members, colony territory, and lead other members of the colony to resources. It is noteworthy that pheromones are the most important form of communication in mediating interaction in social insects (Leonhardt *et al.*, 2016).

1.4.2 Pheromones in *S. littoralis*

The cotton leafworm, like other moths, uses a female-produced sex pheromone for long-range premating communication. The pheromone of *S. littoralis* was first described by Nesbitt *et al.* (1973) as a blend of (*Z*,*E*)-9,11-tetradecadienyl acetate (Z9,E11-14:Ac) (the main pheromone component), (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), (*E*)-11-tetradecadienyl acetate (E11-14:Ac), and tetradecyl acetate (14:Ac). Since then it has been re-described from populations throughout its distribution range and considerable differences may be observed (Table 1) (El-Sayed, 2014; Saveer *et al.*, 2014).

Table 1. Pheromone composition of different populations of S. littoralis and S. litura, based on El-Sayed, 2014 & Saveer et al., 2014. Concentrations of components are expressed as percentages of the total.

Compound	Cyprus	Kenya	Israel	Spain[1]	Spain[2]	Spain[3]	Egypt	S. litura
14:Ac	28	-	-	1	9	1	1	-
Z9-14:Ac	3	-	46	13	14	11	14	14
E11-14:Ac	14	-	9	10	10	11	9	-
Z11-14:Ac	-	-	7	5	9	7	6	-
Z11-16:Ac	-	-	-	-	-	-	4	-
Z9,E11-14:Ac	55	95	33	71	56	57	47	62
Z9,Z11-14:Ac	-	-	4	-	-	-	1	-
E9,Z11-14:Ac	-	-	-	-	-	-	3	-
E9,E11-14:Ac	-	-	-	-	-	-	3	-
Z9,E12-14:Ac	-	5	0.5-1	-	-	-	2	10
E10,E12-14:Ac	-	-	-	-	11	14	10	14

1.4.3 Pheromones in *D. melanogaster*

Sexual communication in *Drosophila melanogaster* is complex, due to the fact that males and females aggregate on decaying fruit to feed, mate and oviposit. The male produced volatile sex pheromone 11-cis-vaccenyl acetate (cVA) is the best-studied fruit fly pheromone. During mating, males release cVA which increases female receptivity, inhibits male-male courtship, and acts as an aggregation pheromone (Greenspan & Ferveur, 2000). Female receptivity is regulated by *doublesex* neurons, while male courtship, on the other hand, is largely determined by the *fruitless* transcription factor, both of which, respond to cVA. The neural circuitry behind cVA elicited behavior is one of the best studied neural pathways in animals (for more on the cVA circuitry see: Auer & Benton, 2016).

In addition to cVA, *D. melanogaster*, uses cuticular hydrocarbons to elicit and modulate courtship and aggregation behaviors. Among these are the female-specific aphrodisiacs (Z,Z)-7,11-heptacosadiene and (Z,Z)-7,11-nonacosadiene, the male specific antiaphrodisiacs (Z)-7-tricosene and the male and female produced compounds methyl laurate, methyl myristate, and methyl palmitate (Dweck *et al.*, 2015; Greenspan & Ferveur, 2000).

1.5 Host volatile use by insects

1.5.1 Overview

Insects use host volatiles to find food sources, mating sites and oviposition sites at a distance (de Bruyne & Baker, 2008; Bruce *et al.*, 2005). Host volatiles provide information about both host identity and quality. The mechanisms insects use to discriminate between hosts in a complex odor space is still poorly understood. One possibility is that insects use species-specific volatile compounds to identify their respective hosts. Although there are examples that suggest that this method of host identification may be used by some insects (Knight & Light, 2001; Hansson *et al.*, 1999; Bjostad & Hibbard, 1992) it is likely that this is only the case for some specialist species. Alternately, insects may use a blend of ubiquitous volatiles, in species specific rations to identify hosts. It is now widely accepted that this is most likely the case for most insect species (Riffell *et al.*, 2014; Xiao *et al.*, 2012; Bruce & Pickett, 2011; de Bruyne & Baker, 2008).

Host volatiles also indicate quality. Plants attacked by herbivorous insects produce volatile defense compounds known as herbivore-induced plant volatiles (HIPVs). Since only plants that have been damaged by herbivorous insects emit HIPVs they provide honest information to conspecifics and other herbivores about host plant quality. HIPVs may reduce attraction to otherwise suitable host plants and reduce oviposition on them (Allmann *et al.*, 2013; Biere & Bennett, 2013; Signoretti *et al.*, 2012; Xiao *et al.*, 2012). Similarly, pathogens and microbes may also produce volatiles that indicate low quality hosts (Biere & Bennett, 2013; Davis *et al.*, 2013; Stensmyr *et al.*, 2012).

1.5.2 Host plant volatiles and *S. littoralis*

As a polyphagous insect, *S. littoralis* is an interesting model for studies on host plant preference and choice. Even though *S. littoralis* is a generalist, adults show a clear innate hierarchy in host plant preference. This innate preference may, however, be modified by larval feeding experience and adult mating experience (Proffit *et al.*, 2015; Thöming *et al.*, 2013). Although it has been shown that odors are responsible for both innate preference and learning, it remains unknown how or on the basis of which volatiles the cotton leafworm discriminates between host plant species.

An equally interesting question is how *S. littoralis* avoids non-hosts or low quality host plants. Although it remains unknown whether specific volatile cues signify a non-host to generalist insects, it is well established that HIPVs function as honest indicators of host quality and deter herbivory and oviposition in *S. littoralis*. Feeding by larvae of *Spodoptera littoralis* on maize changes the odor profile of the plant in a damage dependent manner (Gouinguene *et al.*, 2003). Larvae fed on leaves from damaged plants grow slower, their mortality increases, and take nearly twice as long to pupate than larvae feeding on leaves from undamaged plants (Alborn *et al.*, 1996). Cotton leafworm females reduce oviposition on cotton plants that have been damaged by conspecific larvae and other herbivores (Anderson *et al.*, 2011; Anderson & Alborn, 1999). This reduction in female egg laying may be attributed in great part to *de novo* synthesized HIPVs. Not only do HIPV's confer resistance to the plant emitting them, but also to undamaged neighboring plants (Zakir *et al.*, 2012).

1.5.3 Host volatiles and *D. melanogaster*

Drosophila melanogaster uses over-ripe and decaying fruit as a adult and larval host. The flies feed, court, mate and oviposit on overripe fruit, which serves as a substrate for yeast to grow. Although overripe fruit volatiles and fermentation by-products (wine and vinegar headspace) attract fruit flies (Lebreton *et al.*, 2012; Becher *et al.*, 2010), it is now clear that volatiles produced by fermenting yeasts alone are enough to elicit attraction of male and female fruit flies (Scheidler *et al.*, 2015; Becher *et al.*, 2012).

Since fruit flies live on overripe and decaying fruit they also need to be able to avoid harmful fungi and other microorganisms. It has been shown that microbial produced volatiles, such as geosmin, a common fungal-produced volatile compound elicita avoidance behavior in *D. melanogaster* and that this aversion is controlled by both dedicated channels in the antennal lobe, and a multi-glomerular response (Knaden *et al.*, 2012; Stensmyr *et al.*, 2012).

1.6 Interactions between pheromones and host volatiles

Interactions between host volatiles and pheromones are well established and have received considerable interest (Reddy & Guerrero, 2004; Landolt & Phillips, 1997). Host volatiles affect pheromone communication by modifying insect physiology and behavior when the two signals are combined, and may enhance the effect of both aggregation and sex pheromones. Host volatile synergism with aggregation pheromones is common in coleopterans (Muniappan *et al.*, 2004; Reinecke *et al.*, 2002; Erbilgin & Raffa, 2000; Dowd & Bartelt, 1991) and HPVs have also been shown to synergize with moth pheromones (Trona *et al.*, 2013; Tasin *et al.*, 2007; Yang *et al.*, 2004; Reddy *et al.*, 2002; Light *et al.*, 1993; Dickens *et al.*, 1990). Synergism between pheromones and host volatiles has often been considered beneficial to insects since it may increase the probability of finding mates which may already be on hosts, and since hosts tend to produce far greater amounts of volatile compounds which may persist in the environment over longer distances.

However, HPVs may also have an antagonistic effect on attraction to pheromones (Rouyar *et al.*, 2015; Party *et al.*, 2013; Pregitzer *et al.*, 2012; Byers *et al.*, 2004; Byers *et al.*, 2000; Anton & Hansson, 1995; Anton & Hansson, 1994; Hayes *et al.*, 1994). Negative effects of HPVs on pheromone attraction are far less understood. It is possible that reduced attraction towards pheromones, mediated by HPVs may be a way to avoid mating on low quality hosts or have other effects on mate choice.

1.7 Applied aspects

1.7.1 Overview

Our understanding of insect chemical ecology has widespread application in insect control. One well-known and widely used application is the use of repellent compounds used for protection against blood-feeding insects. Insect repellents are key in the control of insect-vectored diseases and contribute to the wellbeing of millions of people. Hematophagous insects, however, fall outside the scope of this thesis and repellents are, accordingly, not discussed here (for more information see: Maia & Moore, 2011; Katz *et al.*, 2008; Peterson & Coats, 2001).

In agriculture, the most advanced and successful use of semiochemicals for insect management relies on sex pheromones. The high sensitivity of insects to pheromones, and their high specificity, make them a powerful tool in insect management. Pheromones are formulated either in attractant lures for detection and population monitoring, or in reservoir-type dispensers for air permeation and population control through mating disruption (Witzgall *et al.*, 2010). The high selectivity of pheromones makes them ideal tools, not only as early warning systems of specific pest insects or invasive species, but also for sustainable, environmentally safe area-wide management. Pheromones are the most efficient way to monitor the spread of invasive species (Bogich *et al.*, 2008; Tobin *et al.*, 2007) and will play an increasingly important role in a warming climate which will lead to range expansions of pest species. They are also key in early warning systems and in determining pest threshold in integrated pest management (IPM) systems, and may help to better time and to reduce the amount of pesticides or biocontrol angents being used (Cruz *et al.*, 2012; Salas, 2004; Broza *et al.*, 1991).

Pheromones have been used as lures for mass trapping of insects and lure-and-kill techniques (Heuskin *et al.*, 2011). Palm weevils (Soroker *et al.*, 2015; Alpizar *et al.*, 2012; Oehlschlager *et al.*, 2002; Vidyasagar *et al.*, 2000) and the tomato leaf miner *Tuta absoluta* (Choi *et al.*, 2011; Salas, 2004; Michereff Filho *et al.*, 2000) are among the most noteworthy examples. Mass trapping and lure-and-kill techniques work best when both sexes are attracted to the pheromone and when populations have slow reproductive rate and generation turn over.

A third use of pheromones in pest management is known as mating disruption. Mating disruption is carried out by permeating a field or orchard with large amounts of synthetic pheromones, in order to reduce the ability of males to find females and subsequently reduce the number of offspring. Mating disruption probably works either by causing sensorial fatigue in the males, thereby reducing their ability to perceive pheromone signals, or by causing false plume following which leads males towards dispensers rather than females. Mating disruption is widely used in apple orchards in Europe and the United States to control codling moth, *Cydia pomonella,* and in vineyards, mainly against *Lobesia botrana* (Jung *et al.*, 2013; von Arx *et al.*, 2012; Witzgall *et al.*, 2010; Stelinski *et al.*, 2008; Witzgall *et al.*, 2008).

Host volatiles have been far less successful as pest control agents due to their complex chemistry and the fact that insects seem to be far less readily attracted to plant volatiles than they are to pheromones (Cha *et al.*, 2011). Although we now know that insects respond to specific mixes a relatively few of the compounds produced by host plants, the practical application of such knowledge in pest control is still in its early stages. Regardless, some studies have shown that pest control through the use of synthetic host volatiles is feasible (Guerrero *et al.*, 2014; Cork & Hall, 2007; Martel *et al.*, 2005; Khan *et al.*, 2000).

Push-pull systems are another promising type of semiochemical-based control approach. Push-pull systems rely on both attractive and repellent compounds or crops working together. The “push” component of these strategies utilizes repellent crops, or substances within the crop of interest to “push” pest insect populations away from it, while the “pull” component uses attractive substances that may be separate traps (dubbed trap crops), pheromones or mass-trapping lures to pull pests away from the crop of interest (Hassanali *et al.*, 2008). Push-pull strategies are compatible with other IPM methods, including conservation biological control, use of entomopathogens, and traditional biological control and have been proven to be effective in many systems (Khan *et al.*, 2011; Borden *et al.*, 2006; Shelton & Badenes-Perez, 2006; Duraimurugan & Regupathy, 2005). They have been suggested to be an important component in achieving food security in Africa and other developing regions where sustenance farming is the norm (Khan *et al.*, 2014).

A final approach may be to breed for plants which produce semiochemicals that confer defense against herbivorous insects. This could be achieved through breeding for plants that constitutively produce compounds that are normally only produced by plants under herbivore attack in order to make healthy plants less attractive to herbivores, or attract natural enemies to crops. Breeding programs, which target HPVs and HIPVs, have yet to reach application but may help to provide sustainable crop protection and food security in the future (Stenberg *et al.*, 2015).

1.7.2 Semiochemicals in *Spodoptera* control

There have been several attempts to use semiochemicals for armyworm control. Pheromone traps have been developed to monitor *S. littoralis* (Kehat & Dunkelblum, 1993; Kehat *et al.*, 1985; Kehat & Greenberg, 1978) and for lure-and-kill strategies (Downham *et al.*, 1995; De Souza *et al.*, 1992). More recent studies have been carried out in *Spodoptera frugiperda* in the context of IPM strategies to better time the application of biological or synthetic insecticides (Cruz *et al.*, 2012; Meagher, 2001; Broza *et al.*, 1991). Cotton leafworm pheromones have also been tested for mating disruption with good results (Kehat & Dunkelblum, 1993; Kehat *et al.*, 1986; Campion, 1983), however, these developments have not seen widespread application.

There is a lot of on-going work in optimizing pheromone lures in various *Spodoptera* species, and to determine the pheromone composition of local populations (Velasquez-Velez *et al.*, 2011; Acin *et al.*, 2010; Groot *et al.*, 2008; Marques, 2004; Sun *et al.*, 2003; Andrade *et al.*, 2000). *S. frugiperda* has been a focus of these studies, not only because of its economic importance in the neotropics, but because there are distinct ratios difference in the pheromone composition in different host races (Busato *et al.*, 2004; Meagher & Nagoshi, 2004; Murua & Virla, 2004). These differences entail some degree of reproductive isolation between the host races, and lower efficiency of traps (Velasquez-Velez *et al.*, 2011). These results indicate that while pheromones may be useful in the IPM of armyworms, lures need to be developed for local populations rather than species.

Few studies have attempted to use HPVs to control armyworms. Meagher (2001) attempted to optimize pheromone traps by blending the pheromone of *S. frugiperda* with phenyl acetaldehyde, and found that rather than increasing its efficacy, there was a tendency to decrease captures. Traps also captured greater numbers of beneficial insects, reducing its applicability in IPM strategies. In a study by von Merey *et al.* (2011) they attempted to spray green leaf volatiles on corn plantations to induce the emission of HIPVs and attract larger numbers of natural enemies. Although sprayed plants did increase the release of HIPVs, damage by *S. frugiperda* increased in treated plants, and the effect of natural enemies was marginal.

1.7.3 Semiochemicals in *Drosophila* control

The fruit fly *D. melanogaster* oviposits on overripe fruit and may therefore be a nuisance, but causes no damage. However, the taxonomically close and highly invasive spotted wing Drosophila, *D. suzukii* is currently the most serious pest of soft fruit and berries in temperate climates. As opposed to *Spodoptera,* where pheromones are considered to be the best option for semiochemical-based control, fruit fermentation volatiles seem to be the best option for control of spotted wing Drosophila. Several studies have used wine and vinegar as lures in monitoring traps with good success (Cha *et al.*, 2012; Landolt *et al.*, 2012). There has also been progress in the identification and development of synthetic blends based on fermentation and fruit volatiles (Abraham *et al.*, 2015; Cha *et al.*, 2012). Knight *et al* (2015) showed that insecticide efficacy in cherries could by improved by mixing it with yeasts and sugar, and current research investigated whether insecticide-coated cherries become more attractive, due to yeast produced fermentation volatiles, or if flies consume more insecticide due to a phagostimulatory effect of the yeasts and sugars (Mori *et al.*, 2016).

2 Aim and objectives

The aim of this study was to better understand how communication through social and environmental signals are integrated to modulate mate finding behavior in two taxonomically distant insect species with different host associations and larval feeding habits.

The first part of this thesis (Manuscripts I-III) investigates the behavioral effect of cotton leaf volatiles on *Spodoptera littoralis* male moths, alone, and blended with female-produced sex pheromone. The specific objectives were:

- Identify a blend of host plant volatiles that attracts male moths.
- Determine the combined effect of plant volatiles and sex pheromone on male moth attraction.
- Investigate the neural coding of blends of plant volatiles and sex pheromone.

The second part of this thesis studies the behavioral effect of fermentation volatiles in blends with female and male-produced sex pheromones in *Drosophila melanogaster*. Fruit flies use decomposing fruit for adult and larval feeding as well as for aggregation and mate finding. We therefore studied the effect of blending pheromones with food and host cues on fruit fly behavior. The specific objectives were:

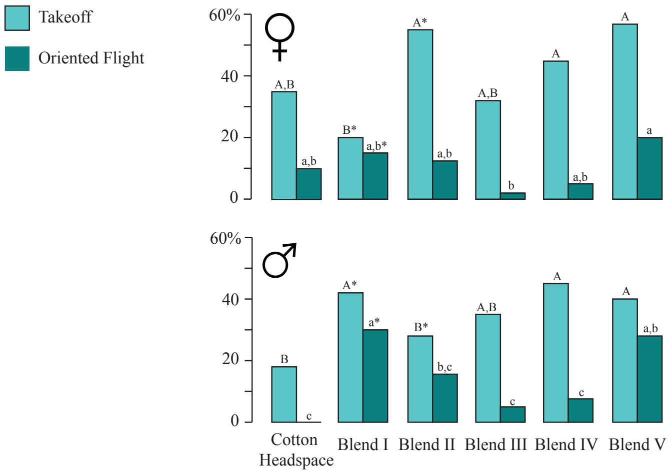
- Establish the effect of feeding status on attraction to vinegar, male produced pheromones (cVA), and their blend on male and female fruit flies.
- Describe the attraction to a novel, female-produced sex pheromone in males and females of *D. melanogaster* and related species.
- Study evolution and function of splice-variants of the novel pheromone's receptor.

- Determine the effect of blending yeast volatiles with male and female sex pheromone on *D. melanogaster* attraction.

3 Summary of results and discussion

3.1 Part I: Interactions between host plant volatiles and pheromones in the cotton leafworm

In order to study interactions between pheromones and plant volatiles we first used behavioral, analytical and electrophysiological techniques to determine antennal-active compounds found in cotton plants, and used them to develop a synthetic kairomone. We found that a blend of nonanal, (*Z*)-3 hexenyl acetate, (*E*)-β-ocimene, and (R)-(+)-limonene was as attractive as cotton headspace to females and more attractive to males (Figure 5). Our results also suggested that (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (R)-(-)-linalool are behavioral antagonists (Chapter I).



		Blend I	Blend II	Blend III	Blend IV	Blend V
DMNT	1800 ng/h	●				
Benzaldehyde	1200	●	●		●	
β-Caryophyllene	850	●				
β-Myrcene	830	●	●	●	●	
Nonanal	650	●	●	●	●	●
(Z)-3-Hexenyl acetate	650	●	●	●	●	●
(E)-β-Ocimene	450	●			●	●
(R)-(+)-Limonene	230	●	●			●
α-Humulene	230	●				
Decanal	90	●	●	●		
(R)-(-)-Linalool	30	●	●	●		

Figure 5. Flight response of *Spodoptera littoralis* females and males to sprayed cotton headspace, and synthetic blends using a piezo-electric sprayer in a wind tunnel. Figure as originally published in Borrero-Echeverry *et al.,* 2015.

Since DMNT is a well-known herbivore-induced volatile, which plays an important role in herbivore deterrence and plant defense, we used it to explore the effect of antagonists on the behavior and neurophysiology of *S. littoralis*. Adding DMNT to our synthetic kairomone strongly suppressed attraction of male and female moths (Figure 6a-b), and suppressed male attraction to pheromone (Figure 6c).

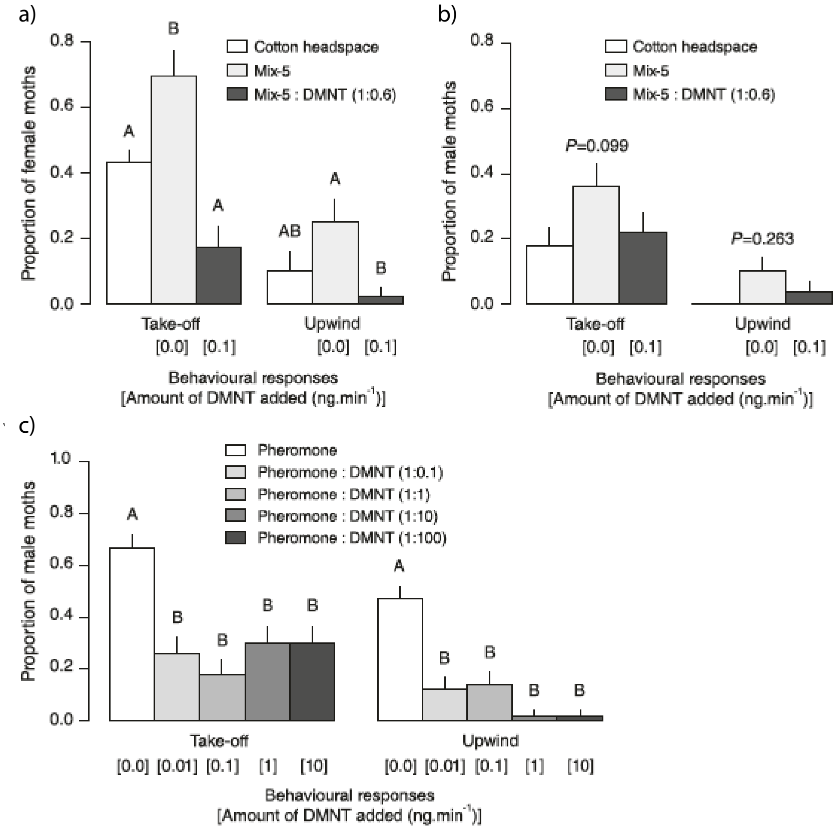


Figure 6. Behavioural responses of male and female S. littoralis to odours from plants or synthetic blends. a) Attraction of mated females to cotton headspace collection, the synthetic kairomone (Mix-5) and the kairomone with DMNT added (Mix-5:DMNT). b) Attraction of unmated males to cotton headspace collection, the synthetic kairomone (Mix-5) and the kairomone with DMNT added (Mix-5:DMNT). c) Attraction of male moths to pheromone, and its blend with DMNT at different proportions. Figure modified from Hatano *et al*., 2015.

We then studied the neurophysiological basis of this interaction. DMNT-responding glomeruli were not exclusively activated by this compound, which suggests that *S. littoralis* doesn't have an olfactory circuit dedicated to DMNT. Although DMNT primarily suppressed the glomeruli that were activated by (Z)-3-hexenyl acetate, a host plant attractant, it appears to have a general inhibitory effect of the antennal lobe at ecologically relevant concentrations in both males and female moths (Figure 7b). In the male AL, DMNT also strongly suppressed the response to the main pheromone component, (Z)-9-(E)-11-tetradecenyl acetate (Figure 7a). HIPVs, such as DMNT, signal unfavorable environments and should be avoided by herbivorous insects. We show that a single HIPV can have a strong antagonistic effect on host and mate finding behavior, and propose a neural mechanism behind the observed behavioral inhibition (Chapter II).

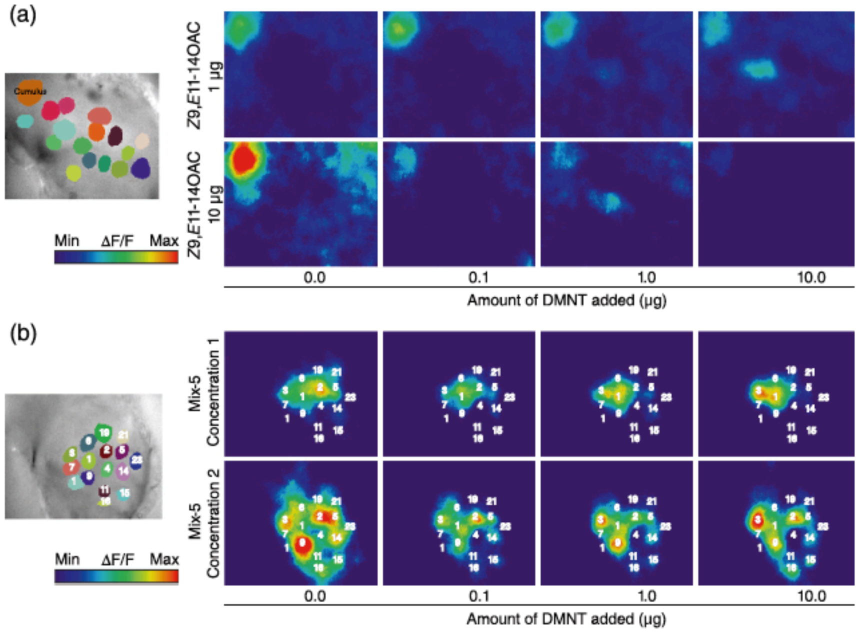


Figure 7. Effect of DMNT on Ca^{2+} responses in male AL to Z9,E11-14:OAc and our synthetic kairomone (Mix-5). (a) Images of maximum Ca^{2+} responses to pheromone (1 and 10 µg) and DMNT (0.1–10 µg). (b) Images of maximum Ca^{2+} responses to Mix-5 at two concentrations mixed with DMNT (0.1–10 µg). Figure modified from Hatano *et al*., 2015.

Lastly, we further studied the behavioral response of male moths towards blends of HPVs and pheromones. Both an incomplete and a heterospecific pheromone elicit male attraction, however, blending it with the previously identified antennally active cotton volatiles, our synthetic kairomone and cotton plants consistently reduced attraction (Figure 8).

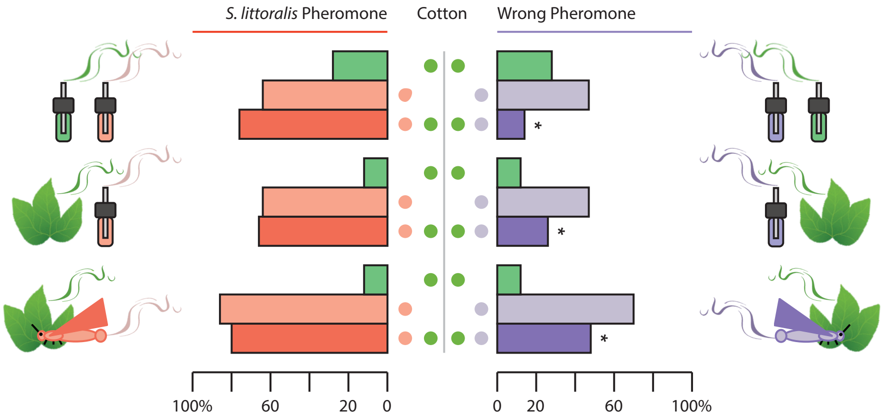


Figure 8. Male *S. littoralis* upwind flight attraction towards blends of the *S. littoralis* pheromone and cotton volatiles and incorrect pheromones and cotton volatiles. Salmon coloured vials represent the four-component pheromone blend, lilac coloured vials represent the main pheromone component, and green vials represent our synthetic cotton volatile blend. Salmon and lilac coloured moths represent calling *S. littoralis* and *S. litura* females, respectively. Cotton leaves represent a healthy cotton plant.

On the contrary, blending the complete S. *littoralis* pheromone with cotton volatiles and plants only reduced attraction of male moths when the volatiles signal a plant damaged by herbivory (Figure 9). Our results demonstrate that pheromones and host volatiles are perceived, and elicit behavior as a single unit, rather than as two separate messages. Mate finding mediate by a unit of pheromones and HPVs not only helps males find females on adequate hosts, but also allows them to avoid mating on sub-optimal plants, which would reduce their offspring's fitness. Pheromones and HPVs working as single unit in mate recognition would make sexual selection and natural selection indistinguishable, which carries strong implications in premating isolation and phylogenetic divergence (Chapter III).

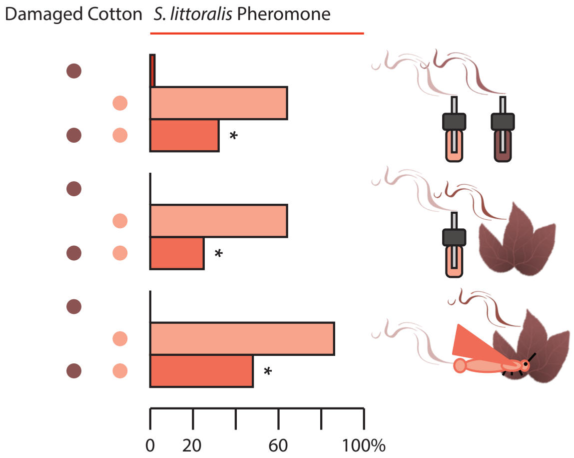


Figure 9. Male *S. littoralis* upwind flight attraction towards blends of the *S. littoralis* pheromone and damaged cotton volatiles. Salmon coloured vials represent the four-component pheromone blend and dark red vials represent our synthetic herbivore damaged cotton volatile blend. Salmon coloured moths represent calling *S. littoralis* females. Red cotton leaves represent cotton plants damaged by conspecific larvae.

3.2 Part II: Interactions between feeding cues, host volatiles and pheromones in the Common Fruit Fly

Since *D. melanogaster* feeds, aggregates, reproduces and develops on overripe fruit, we first studied the effect of starvation on pheromone attraction. We used vinegar as a food odor, cVA, a male produced pheromone, and their blends to test the effect of starvation on male and female behavior and neurophysiology. Starvation increases the attraction to food odor in both sexes. However, adding cVA to vinegar, has a strong synergistic effect on the attraction of fed female flies, while it has no effect in males (Figure 10). Calcium imaging of the AL reveals that activation of the DA1 glomerulus, which responds to cVA, and VM2 glomerulus, which responds to both cVA and vinegar odors, are both modulated by starvation. Our results demonstrate that although sexual behavior mediated by cVA in the fruit fly has been traditionally used to study innate behavior, future research needs to take habitat odors into account since they modulate cVA response (Chapter IV).

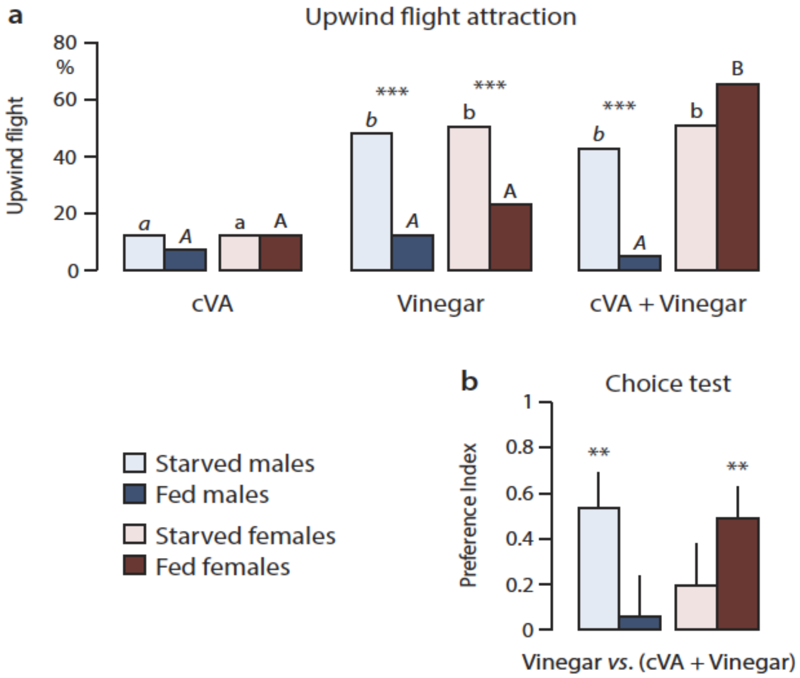


Figure 10. Attraction of starved and fed unmated D. melanogaster males and females to cVA, in a flight tunnel (a) and a y-tube olfactometer (b) bioassay. Wind tunnel: upwind flight attraction to single odor sources (letters show significant differences between insects of same sex and feeding state, in response to different odor sources. Figure modified from Lebreton *et al.* 2015.

Next, we describe a novel fruit fly pheromone, (*Z*)-4-undecenal (Z4-11Al), which is produced by natural oxidation of female cuticular hydrocarbons and strongly modifies the odor of fly infested fruit. We used behavioral experiments to investigate long-range attraction of males and females to Z4-11Al in *D. melanogaster* and the closely related *D. simulans* and *D. sechellia*. Our results show that both male and female *D. melanogaster* are strongly attracted to Z4-11Al, while the two sister species do not react to it al all (Figure 11). We then identified the OR responsible for detection, OR69a. OR69a has two splice-variants, OR69aA and OR69aB, which respond differently to a wide breath of compounds including (R)-linalool, a common yeast volatile. We verified attraction to (R)-linalool in the above-mentioned species and found that while *D. melanogaster* was more strongly attracted, *D. simulans and D. sechellia* are only weakly attracted by it. However, the attraction in these last two species is abolished by blending (R)-linalool with Z4-11Al, while it remains in *D. melanogaster.* Combined, our results verify that Z4-11Al is, in fact, a pheromone that not only attracts flies at a distance, but also modulates courtship and plays a role in avoiding interspecific mating (Chapter V).

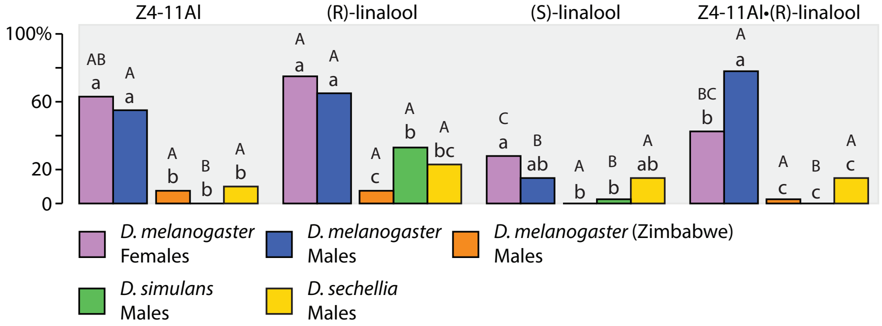


Figure 11. Long distance attraction of *Drosophila* towards Z4-11Al, (R)-linalool, (S)-linalool and the blend between Z4-11Al and (R)-linalool. Lower case letters indicate statistical differences within treatments. Upper case letters indicate statistical differences between treatments for the different *Drosophila* lines.

Lastly, in order to begin to unravel the biological significance of fermentation products on the social behavior of *D. melanogaster*, we studied the effect of blending vinegar and yeast headspace with both cVA and Z4-11Al. While the blend of vinegar and cVA has a strong synergistic effect on female attraction, the blend of yeast and cVA enhances attraction of both sexes. Even though the blend of vinegar with Z4-11Al increases attraction of males to vinegar it reduces attraction of both sexes compared to Z4-11Al alone. Although Z4-11Al makes yeast headspace more attractive to both sexes, it is just as attractive on its own than as part of the blend, suggesting that this compound may mimic yeast odors (Figure 12).

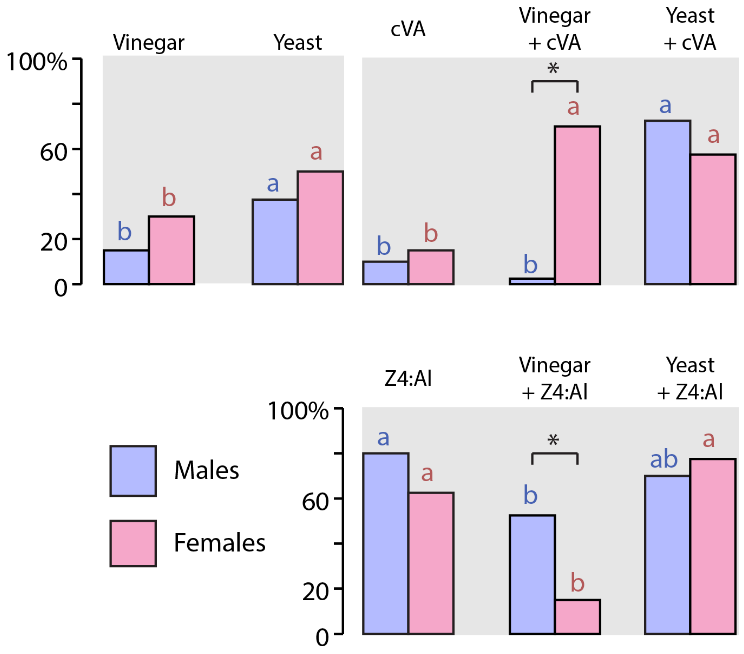


Figure 12. Odour-mediated upwind flight attraction of fruit fly *Drosophila melanogster* males and females vinegar and yeast headspace, male sex pheromone cVA, female sex pheromone Z4-11Al, alone and blended with vinegar or yeast, respectively. Letters of the corresponding colours show differences between treatments.

Our results clearly show that vinegar and yeast odors relay different information to flies. Although yeast headspace is probably synonymous with a substrate that is suitable for larval development and a good aggregation site, the role of vinegar is far less clear. Vinegar might be a good food cue for laboratory assays but it remains an ill-characterized and variable stimulus. The discrepancies between the behavior elicited by the blend of cVA and yeast headspace, and cVA and vinegar, are a strong tool which will allow us to identify chemical compounds responsible for differentiating odors related to feeding behavior and sexual behavior (Chapter VI).

4 Concluding remarks

Insects use host volatiles and pheromones to locate different resources. Pheromones signal the presence of mating partners whereas host volatiles have traditionally been considered to function as either feeding or egg-laying cues. In this thesis I studied the way that host volatiles and pheromones interact in cotton leafworm, *Spodoptera littoralis,* and the fruit fly, *Drosophila melanogaster*. Not only are these species taxonomically distant, but they also use their hosts in very different ways. Cotton leafworm females oviposit on cotton where larvae develop, whereas fruit flies use overripe fruit as an adult and larval feeding substrate, and an aggregation site for mating. Even though the ecology of these two species differs with respect to host association and feeding habits, pheromones and host volatiles interact to mediate mate attraction and generate specific-mate recognition systems.

Although pheromones or host volatiles may be attractive on their own, only a combination of the right pheromone and host volatile blend acts as a unit to produce strong attraction to mating sites and mates. Deviations from this optimal unit, either by changes to the pheromone component, or the host component changes the message. Divergent pheromone or host blends may signify another closely related species or suboptimal habitat for offspring development.

Our results suggest that olfactory cues that mediate mate finding in insects are under both natural and sexual selection simultaneously, since both changes that affect host preference, and pheromone preference will affect specific-mate recognition systems. Populations that either change their host odor preference or pheromone composition should be under strong stabilizing selection, which will accelerate the development of premating isolation barriers.

5 Perspectives

Semiochemicals are essential mediators of insect reproductive behavior. After decades of chemical ecology research we are beginning to understand how insects perceive, decipher and integrate social and environmental olfactory stimuli. A continuing effort in chemical ecology research is to identify the signals that allow insect herbivores to find their host plants, how they are discriminated from other plants in an atmosphere that is filled with odorants, and how they integrate signals from hosts and prospective mates. A better understanding of odor-mediated behavior, and an increased ability to predict key compounds can immediately be brought to practical application for the further development of semiochemical-based insect control methods.

Advances in molecular biology have been fundamental for advances in olfactory research. Combined with better imaging equipment, neurogenetic tools will help us to gain further insight into the brain and the neural basis for innate behavior, learning, and memory formation. To date, molecular tools are available only for *Drosophila*, but advances in CRISPR/Cas technology will allow us to use molecular tools in other species in a near future, leading to new and exciting advances.

The rapid growth in available genomes and transcriptomes in insects, along with OR deorphanization efforts should allow us to better predict OR ligands for orphan receptors in non-model species. Additionally, as soon as the protein structure of ORs has been deciphered, our understanding of ligand-receptor interaction will grow exponentially. Adding bioinformatics, neurogenetics and computational chemistry to the traditional chemical ecology research toolbox will help to predict and identify ligands for odorant receptors, towards a more rapid dissection of behavioral circuits and interpretation of the adaptive significance of semiochemical-mediated behavior.

Biodiversity on the planet has to a great extent been shaped by plant-insect interactions, but the mechanism underlying diversifications are incompletely known. Odor communication with individuals of the same species and with food plants is under sexual and natural selection. Tracing olfactory receptor genes underlying the search for sex and food during past and current phylogenetic development will contribute to answering the question of how this great diversity has been shaped.

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