THE CHEMICAL ECOLOGY OF ARMYWORMS

FROM ECOLOGY TO MOLECULES

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Agricultural Science 2013:1
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Alnarp, February 2013

ISSN 1654-3580

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Summary

Moths of the genus *Spodoptera* are economically important pest insects. The necessity to develop novel control strategies which may be included in integrated pest management schemes has led to the study of chemical communication in several species within the genus. The polyphagous nature of most *Spodoptera* species makes it an interesting model to study the way in which different odor profiles are processed and interpreted by the insect brain and how this reflects upon the behavior and ecological interactions which may be of importance in agricultural systems. As such, armyworms have become a model organism in olfactory insect chemical ecology. Here, I attempt to give an overview of what is known about *Spodptera* chemical ecology to date and present perspectives and directions for future research.

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1. Introduction

1.1 *Insect Chemical Ecology*

Chemical Ecology concerns the interactions mediated by chemical signals between organisms. Chemical communication is found in all taxa of living organisms and is the oldest form of communication. Chemical compounds, or blends of chemical compounds used in chemical communication are known as semiochemicals, which can be subdivided into further categories depending on their ecological role. Pheromones are chemical signals used in intraspecies communication. Allomones, synomones and kairomones are signals that are involved in interspecies communication. Allomones are signals which benefit the organism which produces them, to the detriment of the organism which receives them, synomones are those signals which benefit both the organism producing them and the one receiving them and kairomones benefit the receiving organism at a cost to that which produces them. After six decades of chemical ecology research, we are beginning to understand the significance and widespread occurrence of chemical communication between organisms.

Although the importance of chemical communication, particularly amongst herbivores and plants was evident in the mid 19th century through the work of Ernst Stahl and others, the concept was set-aside until the second half of the 20th century. The idea that plant secondary metabolites were by-products of the essential biochemistry of plants and not biosynthetic pathways and end products on which natural selection could act upon was commonplace until the 1950's when several lines of research brought the notion of chemical ecology back (Hartmann, 2008). Observations of male moths flying upwind towards female moths in the absence of visual or auditory queues further supported this idea. The identification of the first insect pheromone, Bombykol, produced by the silk moth *Bombyx mori* by Adolf Butenandt in

1959 paved the ground for insect pheromone research, which has been a major field of study since. Entomologists studying insect pollinators and herbivorous insects made observations which indicated that insects used plant secondary metabolites to find host plants for feeding and oviposition and of plants using secondary metabolites in defense against insect herbivores (Harborne, 2001). Further evidence came from biochemists showing a large discrepancy between the number of secondary metabolites and the number of essential processes in plants. The 1970's saw the birth of Chemical Ecology as a separate discipline with the publishing of the first treaty on the subject in 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. Studies on ecology and ethology have been supported by an ever-growing array of biochemical, physiological, anatomical and molecular techniques allowing us to understand not only the ecological context behind these signals, but also the mechanisms behind odor perception and information processing. After the discovery of Odorant Receptors (ORs) by Linda Buck and Richard Axel in 1991 in mammals and the subsequent confirmation that similar receptors exist in the olfactory system of Drosophila (Clyne *et al.*, 1999; Vosshall *et al.*, 1999) the study behind the mechanisms of insect olfaction at both the peripheral and neural level has skyrocketed. Several insect species have become model organisms in the study of insect chemical ecology and olfaction. Amongst them are the fruit fly *Drosophila melanogaster*, one of the most powerful tools due to the wide array of molecular techniques available for this model organism and the fact that the genome is known, the honeybee, *Apis melifera*, due to its learning plasticity, the common cockroach, and several species of moths such as *Spodoptera littoralis, Heliothis virescens, Manduca sexta* and *Agrotis ipsilon*. Moths were originally studied in the context of pheromone research due to their incredible sensitivity to the female pheromone and stereotypical behavior, however, work in many species, havs been fueled by their importance as pests in agriculture, horticulture and forestry.

1.2 Chemical Ecology in Insect Pest Management

Methods based on chemical ecology have been used for insect control in several ways. The most developed, and successful techniques are those based on insect pheromones. The high sensitivity of insects to pheromones and the amazing specificity of them makes them a powerful tool in insect management. Pheromones may be used in two different ways: as attractants, or in mating disruption.

The high selectivity of pheromones makes them ideal as early warning systems. Pheromone traps are by far the most effective way to determine when populations of a given insect are present in an area. They have an enormous potential in the monitoring of invasive species and in insect pest threshold determination under integrated pest management systems. Early detection of an insect pest may help to better time and to reduce the amount of pesticides being used (Cruz *et al.*, 2012; Broza *et al.*, 1991).

Pheromones have been used successfully as lures in mass trapping of insects and attract-and-kill techniques in many species (Heuskin *et al.*, 2011). Of note among these are palm weevils of the genus *Rhynchophorus* and the tomato leaf miner *Tuta absoluta*. Pheromone based trap and kill or mass trapping techniques work best under two conditions. The first of these is that both sexes are attracted as is the case with aggregation pheromones and the second condition is that populations have a slow reproductive rate and generation turn over is slow. The case of *Tuta absoluta*, a lepidopteran is of particular importance because although only males are attracted by the pheromone and they have an enormous reproductive potential, pheromones have proven to be the best control method for this invasive species (Choi *et al.*, 2011; Salas, 2004; Michereff Filho *et al.*, 2000).

A third use of pheromones in pest management is known as mating disruption. Mating disruption is carried out by releasing large amount of synthetic pheromones into the environment that reduce the capacity of males to find females. Although the exact method in which mating disruption works is not yet known it probably causes sensorial fatigue in the males, reducing their ability to perceive pheromone signals, or leads males towards false pheromone plumes released by dispensers. Regardless of which mechanism is at play, the ultimate effect of mating disruption is that fewer females mate, and hence, there are fewer larvae (which usually cause crop damage) in the crop. Mating disruption has proven to be successful primarily in orchards and vineyards against lepidopteran species such as *Cydia pomonella* in apple orchards in Europe and the United States and *Lobesia botrana* in vineyards across Europe (Witzgall *et al.*, 2010; Witzgall *et al.*, 2008; Gronning, 1994).

On the contrary, host plant volatiles have been far less successful in pest control than pheromones due to much more complex chemistry, a seemingly endless amount and combination of plant produced volatile signals, the fact that plant volatiles cause less stereotypical behavior (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 (Cork *et al.*, 2008; Cork *et al.*, 2005; Khan *et al.*, 2000).

1.3 The genus Spodoptera (Guenee 1852)

The genus *Spodoptera* consists of ca. 30 species of which nearly half are considered to be pest insects in different regions of the world (for a detailed review of the genus including identification keys see Pogue, 2002). The term armyworm refers to the ability of larvae to move in large numbers from plant to plant looking for an adequate host. Most species within the genus are known to be highly polyphagous, feeding on hundreds of plants. Grasses (Poaceae) seem to be preferred host plants for many *Spodoptera* species, which suggests that they may be the ancestral host plants of the genus. Four *Spodoptera* species, *S. exigua*, *S. litura*, *S. frugiperda*, and *S. littoralis* are considered to be among the most important pest insects worldwide due to the wide variety of economically important plants they consume, their migratory capacity and their status as invasive species. Although the pheromones for all four species have been studied and identified, most chemical ecology research in the genus concerns *S. littoralis* and *S. frugiperda* (Acin *et al.*, 2010; Batista-Pereira *et al.*, 2006; Sun *et al.*, 2003; Malo *et al.*, 2000).

2. Ecology and behavior

2.1 Host plant interactions

The polyphagous nature of most *Spodoptera* species makes them interesting models for olfaction studies. The ability of both adults and larvae to select amongst different suitable host plants allows us to ask many questions such as: Do different hosts emit similar signals, which are interpreted as a larval host plant? If so, what differences enable to insect to distinguish between host species and of different quality? Contrarily, does each host plant have a specific chemical signal that the insect can differentiate from other species? Although this approach would facilitate host discrimination, it would be complex from a neural coding and neural capacity point of view and a contradiction with the existence of taxonomically related host plants and speciation events. We are just beginning to find the answers to these questions.

Surprisingly little work has been carried out in terms of host choice preference. In *S. frugiperda* it is well know that there are two ecotypes, the rice strain, and the host strain. Saldamando & Velez-Arango (2010) showed that both strains have a preference for the host but at the same time that the two strains are not equally selective. The corn strains seem to be more tightly associated with corn than the rice strain is with rice. In contrast with this, Meagher et al (2011) found that when both strains were given the choice between corn plants and pasture grass females of the corn stain laid as many egg batches on corn plants and grasses whereas the Rice strain laid more egg batches on grasses than on corn. Field studies carried out by Juárez et al (2012) in Argentina, Brazil and Paraguay do not show the same results and suggest that host plant choice seems to be linked closer to crop history in the area and host availability even though there are genetic differences between the two strains (Nagoshi & Meagher, 2008). Several studies on the temporal dynamics of both strains, genetic differences and population ecology might help to account for the contrasting differences found in different locations and populations (Velasquez-Velez *et al.*, 2011; Wyckhuys & O'Neil, 2006; Meagher & Nagoshi, 2004).

Several studies have focused on life history traits of different *Spodoptera* species on different known host plants. Saeed et al (2010) found that *S. exigua* developed better on cauliflower than on peas and wheat, all known host plants. They also found that fitness decreased with generation number on peas and wheat which suggests that some hosts may be unsuitable over a long period of time, but suitable as substitutions when better that the main host plants are not available. In *S. frugiperda* it was observed that the insect preformed just as well on leaves of four hosts, corn, cotton, millet and soybean under both laboratory and field conditions which suggests that crop rotation needs to be carefully planned to avoid maintaining suitable hosts year round (Barros *et al.*, 2010a; Barros *et al.*, 2010b).

There is growing evidence for a hardwired preference scheme in *S. littoralis* in which certain host plants are usually chosen over others. However it seems that although innate preference may be modified by experience. Larvae feeding on a host plant might be more prone to selecting the same plant over a potentially better host plant due to prior experience. In addition, adults may choose host plants they were fed on as oviposition and mating sites disregarding their innate wiring (Anderson *pers comm.)*. The mechanisms underlying these observations have yet to be studied, but odor or gustatory learning is an exciting field that is just beginning to be understood.

Another key aspect of chemical interactions between host plants and herbivorous insects is the effect of plant defenses on insect behavior. Plants attacked by herbivorous insects produce volatile defense compounds known as herbivore-induced plant volatiles or HIPV's. HIPV's are known to serve two distinct ecological functions. Since only plants that have been damaged by herbivorous insects emit these they provide honest information to other herbivores about host plant quality and competition. Alternately, they may serve as a signal to announce the presence of herbivores to predators and parasitoids (Carroll *et al.*, 2006). Feeding by larvae of *Spodoptera littoralis* on maize has shown that the odor profile of the plant does indeed change in the presence of mechanical damage and larval regurgitate. Gouinguene et al. (2003) showed that feeding by second, third and fourth instar larvae elicited a similar blend of volatiles to be emitted by corn leaves. The amount of volatiles produced was dependent on the amount of damage. This information may therefore work as an honest signal of plant quality to moths seeking host plants since the amount of HIP's released is differential. The authors suggest that since very few differences were found between HIPV's evoked by different instar regurgitate and larvae, the signal is not a reliable indicator of larval development, and may not be providing predators and parasitoids with all the information they require. Alborn et al. (1996) showed that HIPV's are an honest signal which may be of great importance to females by exploring the effect of systemic defenses on larvae feeding off previously damaged plants. Third instar larvae of *S. littoralis* fed on undamaged leaves from damaged plants showed a reduced weight gain and high mortality within a week whereas larvae fed on leaves from an undamaged plant gained weight and pupated within eleven days. Sixth instar larvae took almost twice as long to pupate when fed on leaves from damaged plants than from undamaged plants. These results highlight the importance of HIPV's in female host choice (Alborn *et al.*, 1996).

The above results have been shown to hold true in both *S. frugiperda* and *S. littoralis*. Gravid females of *S. frugiperda* show no difference in preference between undamaged plant volatiles and those of plants with mechanical damage. Although they will prefer volatiles from damaged plants over a blank; this is not the case when confronted with undamaged and damaged plants. Five hours after being treated with mechanical damage and larval regurgitate gravid females preferred undamaged plants in olfactometer bioassays (Signoretti *et al.*, 2012). Similarly, studies on *S. littoralis* have shown that female moths reduce oviposition on cotton plants which have been damaged by conspecific larvae and root feeding herbivores (Anderson *et al.*, 2011; Anderson & Alborn, 1999). Zakir *et al.* (2012) showed that *de novo* synthesized volatiles reduce egg laying on cotton plants. This study showed that in a dual choice test, females oviposited more on undamaged plants than on undamaged plants where volatiles from damaged plants were released along the stem. Additionally when only the *de novo* synthesized volatiles were released along the stem of an undamaged plant the reduction in egg laying was the same as that seen when the entire headspace of a damage plant was being released along an undamaged stem. His results clearly show that it is the volatile chemicals synthesized after damage and not gustatory cues that are involved in female preference for undamaged plants. Taking this study one step further it was also shown that not only do HIPV's confer resistance to the plant emitting them but also to undamaged neighboring plants. In both laboratory and field experiments gravid females oviposited more on undamaged plants with an undamaged neighbor than on undamaged plants with a damaged neighbor in two choice experiments. This effect has a distance related effect and oviposition increase in a linear function with distance from damaged plants (Zakir *et al.*, 2012).

2.2 Mate finding and mating behavior

Mate finding behavior in armyworms is, to the best of our knowledge mediated exclusively by pheromones and host plant volatiles, although the importance of close range optical cues and gustatory signals cannot be ruled out, as they have yet to be studied. Traditionally mate finding behavior in moths has been studied in the context of male attraction to different blends of the pheromone compounds emitted by the female, however it is evident that male moths never encounter the female pheromone in a odor-free background under natural conditions. Studies are now focusing on the interaction between plant volatiles and pheromones. The behavior of both females and males is affected by background odors (Party *et al.*, 2013; Sadek *et al.*, 2012).

Female *S. littoralis* show plasticity in their calling behavior when they are in the presence of host and non-host plant volatiles. Females in the presence of host plants call at an earlier in the scotophase and for a longer time than females in the presence of non-host plants. Mating duration was also found to be affected by the presence of host plant leaves with mating lasting for a longer time in the presence of host plants than in the presence of non-hosts (Sadek & Anderson, 2007). The authors suggest that delayed calling, shorter periods of calling and a reduced mating duration may serve to reduce the amount of energy females use in the presence of unsuitable hosts. Such behavioral changes could be important to balance the need to produce some offspring with the necessity of finding the most suitable host possible to oviposit on.

Similar effects have been observed when females are in the presence of damaged plants releasing HIPV's. Both the number of females calling throughout the scotophase and the duration of calling were reduced when females were compared to females in the presence of undamaged plants. Females also called at a later time when in the presence of HIPV's (Zakir Ali, 2012). Male behavior also changes when presented with pheromone in a background of damaged plant volatiles over undamaged plant volatiles. Males are significantly less attracted to pheromone in wind tunnel assays in the presence of damaged plant volatiles than in the presence of undamaged plant volatiles. Mating success was also reduced under these conditions (Zakir Ali, 2012).

The above results show the importance of host plant volatiles in mating behavior in *S. littoralis*. Differences in background odors can significantly affect mating success and fitness of moths. Both males and females are less likely to mate and invest energy in the presence of unsuitable host plant. Considering that *Spodoptera* moths may live for over a week as adults it is not surprising that they have mechanisms to maximize reproductive output and larval survival. It is still unknown how different host plant background odors interact with pheromone signals and adult behavior when presented in tandem. Such studies could provide a framework in the development of push-pull strategies and more efficient pheromone lures.

2.3 Larval behavior

There are but few studies of the chemical ecology of *Spodoptera* larvae. Curiously in two-way olfactometer experiments, sixth instar larvae of *S. frugiperda* show a preference for the volatiles of damaged maize plants over that of undamaged maize plants.

When exposed to one of the *de novo* synthesized compound, linalool, larvae chose linalool over pure air in a dose dependent manner and as may be expected, from the above results, chose the volatiles from undamaged plants, supplemented with linalool than those unsupplemented undamaged plants (Carroll *et al.*, 2006). A follow up study on cowpea shows that neonate larvae were more attracted to conspecific damaged plants and inceptin-treated (an elicitor found in larval regurgitate) plants than undamaged plants if larvae had previously fed on leaf material. In this study, DMNT was shown to be the most abundant HIPV and accordingly, neonate larvae were more attracted to DMNT than to pure air, and to DMNT-supplemented undamaged plant volatiles than to control plant volatiles (Carroll *et al.*, 2008). Although these results seem counterintuitive it is possible that dispersing towards HIPV's may be useful to larvae due to the fact that developing leaf tissue (the preferred type of *S. frugiperda* larvae) emits higher levels of HIPV's than older tissue (Carroll *et al.*, 2008; Carroll *et al.*, 2006; Hoballah *et al.*, 2004). In the case of a polyphagous insect, homing in to common HIPV's found in developing leaves may help it find adequate feeding sites in a variety of plants. Alternately, *S. frugiperda* can be cannibalistic and a predator of smaller larvae of other herbivore species. As such, attraction to damaged plants might lead to an additional food source. The remarkably low amount of work being carried out in larvae limits our understanding of a crucial part of the S*podoptera* life history. It has often been argued that since larvae are not mobile, adult host choice should be the most important behavior in larval survivability, however, considering that armyworms are known for their migratory behavior as larvae, and host choice in this life stage, further research should be carried out to obtain a better understanding of volatile use by these insects and perhaps, improve upon control schemes which are based upon ingested biopesticides or contact chemical, and biopesticides.

2.4 Behavioral plasticity

Different moments in the life of an insect have different physiological requirements. Adult insects needs to feed, find a mate, and in the case of females, find a suitable place to lay eggs. In the case of *Spodoptera,* males need to balance between feeding and mate finding while females need to feed, produce and release pheromone, and seek out suitable host plants. All of these activities are affected by odors. Taking into account that these behaviors are mutually exclusive, insects need to be able chose one behavior over the other. One possible way in which this may be accomplished is to modulate the olfactory information in such a way that only that which is most important to the physiological state of an individual is processed as the most important, leading to the appropriate behavior. Few studies have attempted to study the role of physiological factors on behaviors mediated by chemical signals. The best example of behavioral plasticity in the genus comes from the work of Saveer et al. (2012) in *Spodoptera littoralis* (Saveer *et al.*, 2012). The behavior of female *S. littoralis* changes drastically after mating. Before mating females are highly attracted to floral volatiles which may be interpreted feeding cues and significantly less attracted to cotton leaf volatiles. After mating, females become attracted to host plant volatiles and attraction to floral volatiles is practically suppressed. These results suggest that there are interactions between the neural circuitry controlling these two distinct behaviors. A follow-up study (Saveer et al. unpublished manuscript) shows that males exhibit a different behavioral model. Virgin males show attraction to host plant volatiles, floral volatiles and calling females, however 3 hours after mating the response to pheromones and host plant volatiles is suppressed while the response to floral volatiles remains the same. 24 hours after mating, host plant attraction remains suppressed but attraction to calling females is restores to pre-mating levels and attraction to floral volatile remains unchanged. These results suggest that the neural circuitry, which is responsible for feeding behavior, is independent of the circuitry behind reproductive behavior. Both of these results seem to agree with ecological predictions for the two sexes. Females need to feed before mating to maximize their egg load and but after mating they have an urgent need to find a suitable host plant. As such, suppressing the stimuli which control feeding behavior is a important to the insect. Males on the other hand feed to have the energy necessary to produce spermatophores and maintaining responsiveness to feeding stimuli is ecologically sound since males need to create a new spermatophore in order to mate a second time (Gadenne *et al.*, 2001). As such, inhibiting the response to pheromone while maintaining the response to food odors would allow the male time to replenish his spermatophore before reacting to mating stimuli once again.

Further behavioral studies are necessary to fully understand how physiological changes affect insect behavior and what the mechanism of this modulation type of modulation is. The neural basis for this switch in behavior will be discussed in the following sections.

3. The peripheral system

3.1 The Antennae

The antennae of insects are the primary olfactory organs. Unlike other moth species, the antennae of both female and male *Spodoptera* are filliform. Antennae have two basal segments followed by a series of antennomeres. In *S. frugiperda* there are between 64 and 71 antennomeres in males and between 56 and 71 in females (Malo *et al.*, 2004) and between 62 and 70 have been found in females of *S. littoralis* (Binyameen *et al.*, 2012). The consistency in the number of antennomeres across species non-closely related species within the genus (Pogue, 2002) suggests that similar numbers might be found in other species of the genus. Although antennae of both males and females are morphologically similar, the length of the male antennae and the diameter of antennomeres in males are greater than in females (Malo *et al.*, 2004). Antennomeres can be divided into two regions: the dorsal region, which has two rows of scales and few squamiform sensilla, who's function is probably propio or mechano receptive (Hix *et al.*, 2003) and two gustatory sensilla chaetica, and the ventral region where most of the sensilla are found (Malo *et al.*, 2004). Six different types of sensilla were found on this region of the antennomeres of the antenna of *S. frugiperda*. Amongst these were sensilla chaetica, typically six per antennomere which serve a gustatory function, sensilla styloconica, found in the upper-middle area of each antennomere and which are believed to be hygro and thermoreceptors, coeloconic sensillas, auricillic sensillas, found amongst the scales, basiconic sensillas and trichoid sensillas, which are by far the most numerous of them all. The last four of these serve a chemosensory function (Malo *et al.*, 2004).The same olfactory sensillas were found on the antennae of *S. littoralis* save for the fact that in this species the trichoid sensilla could be categorized into long trichoid and short trichoid (Figure 1) (Binyameen *et al.*, 2012). A similar situation has been observed in *S. latifascia* and *S. descoinsi* and three classes of trichoid sensillas have described in *S. exigua* and *S. ornithogalli* (Monti *et al.*, 1995; Ljungberg *et al.*, 1993; Jefferson *et al.*, 1970).

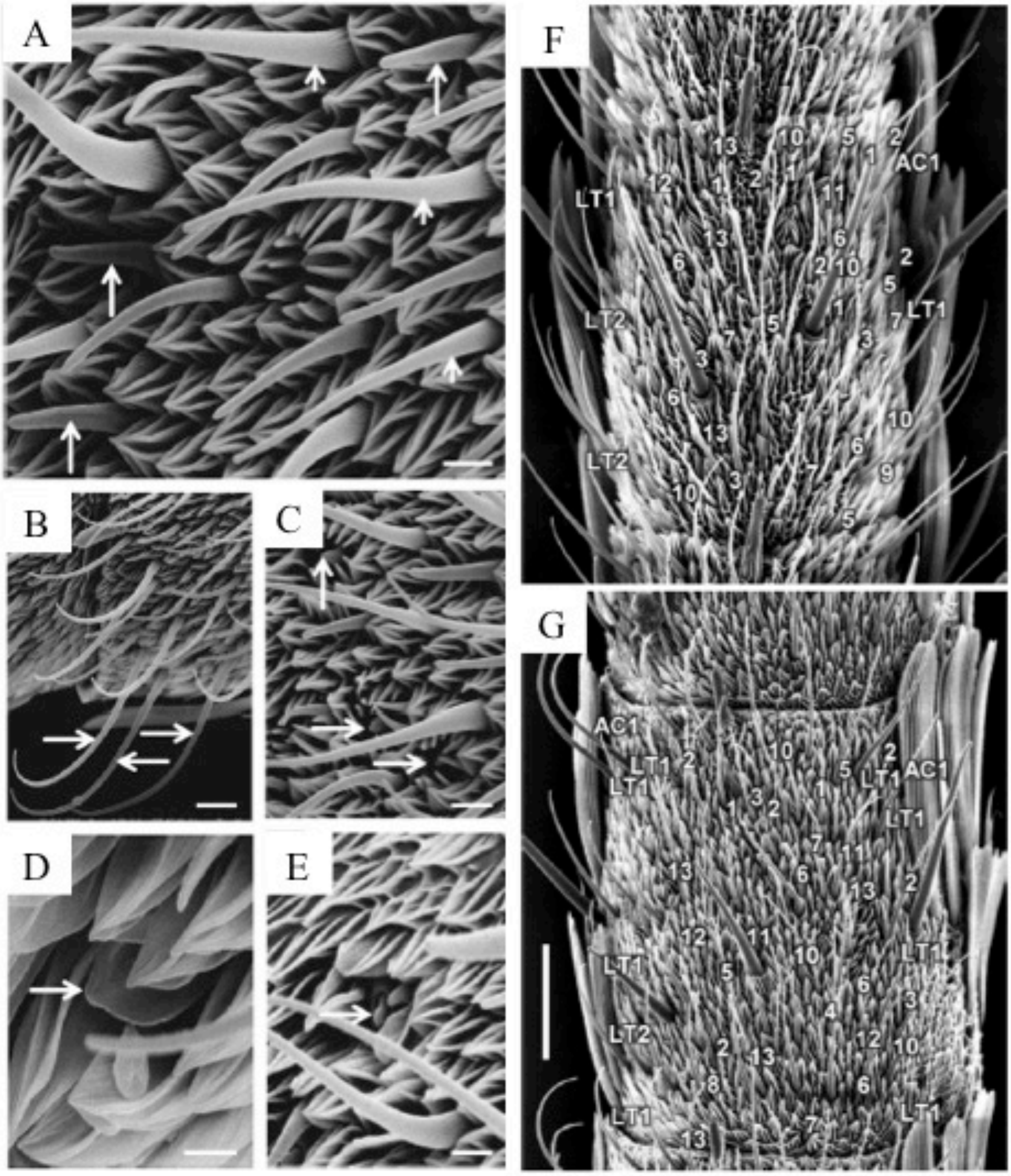

Figure 1. Sensillum types and organization on the antennae of *S. littoralis* females: A. Short trichoid (short arrows) and basiconic (BC) sensilla (long arrows); B. Long trichoid sensilla (arrows) on the lateral surfaces; C. Coeloconic sensilla (arrows); D. Auricilic sensilla (arrow); E. Grooved peg sensilla (arrow); F-G. Maps of sensilla of functional ORN types Bars represent a scale of 5 µm except in (D) where the bar represents a scale of 2 µm and in F-G where they represent 50µm. Modified from Binyameen *et al.* (2012).

3.2 Olfactory Receptor Neurons and Odorant Receptors

Antennal sensilla typically house between two and three olfactory receptor neurons (ORNs) (Figure 2}. Each ORN has, in turn only one type or odorant receptor (OR) on its surface, which suggests that each neuron only responds to a certain type of chemical stimuli. In both *S. frugiperda* and *S. littoralis* the OSNs in basiconic, coeloconic and auricilic sensillas have been shown to respond to plant volatiles (Binyameen *et al.*, 2012; Malo *et al.*, 2004). Neurons in trichoid sensilla in *S. frugiperda* respond to both pheromone components and plant volatiles (Malo *et al.*, 2004) and long trichoid sensillas house neurons which respond to pheromones in *S. littoralis*, however, neither long trichoid, nor short trichoid sensilla have been found to respond to plant volatiles in this species even though it is the most numerous type (Binyameen *et al.*, 2012). Interestingly, the trichoid sensilla of *S. exigua* have not been found to respond to pheromone components (Dickens *et al.*, 1993).

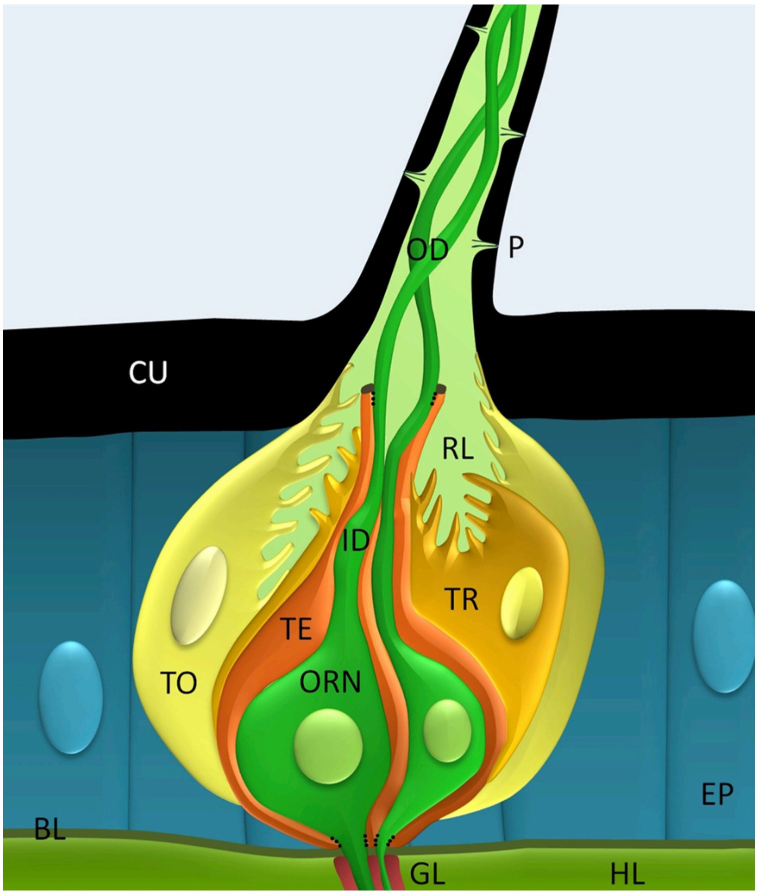

Figure 2. Diagram of the cross section of an insect sensilla showing the non-neuronal

tormogen (TO) and trichogen cells (TR) which build the hollow hair shaft of the long sensory hair. Pores (P) in the cuticle (CU) of the hair allow odorant molecules to enter the receptor, or sensillar lymph (RL). The ORNs (green) subdivided into an outer dendrite (OD), an inner dendrite (ID) and soma, both covered by a thecogen cell (TE), and a glia-wrapped axon (GL). Also showing the hemolymph (HL); basal lamina (BL) and epithelial cells (EP). Modified from Stengl (2010).

The OR's of *Spodoptera* are just beginning to be studied. Using transcriptomics a total of 36 candidate ORs and 5 GRs (gustatory receptors) have been identified from the antennae of *S. littoralis* (Jacquin-Joly *et al.*, 2012; Legeai *et al.*, 2011). Expression studies in antennae of *Bombyx mori* revealed 35 candidate ORs of a total of 66 which were found from the genome analysis (Tanaka *et al.*, 2009) which indicates that there are still many candidates which have yet to be detected in *S. littoralis*. Considering that 63 glomeruli have been identified in the antennal lobe of male *S. littoralis* (Couton *et al.*, 2009) it is safe to assume that there are still nearly 30 candidate ORs which have yet to identified. Additionally, 12 candidate ionotropic receptors (IRs) have been identified in the antennae of male *S. littoralis* of which seven are present in females (Jacquin-Joly *et al.*, 2012; Olivier *et al.*, 2011). Work is currently underway to determine the ligands (deorphanize) of the 36 candidate ORs using the *Drosophila* empty neuron system and the *Xenopus* oocyte system.

3.3 Odorant Binding Proteins and Odorant Degrading Enzymes

Odorant binding proteins (OBP) are proteins found in the sensillar lymph and are known to play a role in odor perception, although the precise mechanism is still unknown. It is thought that OBPs bind odorant molecules and transport them across the lymph to the ORs (Kaissling, 2001). A total of 35 sequences which are similar to known Lepidopteran OBP's have been identified in the antennae of female *S. littoralis*. These sequences share the characteristics of the OBP gene family and although the secondary structure of the proteins is conserved are highly divergent (Jacquin-Joly *et al.*, 2012). Unfortunately little work has been done in determining the ligands of these proteins of the exact mechanism through which they work. Hopefully the ongoing deorphanization work on *S.* littoralis ORs will facilitate future work on OBPs.

In order for ORs to be able to respond to new stimuli after interacting with the OBP/odorant complex or the odorant molecules need to be eliminated rapidly from the system. It has been suggested that this process is carried out by enzymatic degradation within the lymph by odorant and pheromone degrading enzymes (ODE and PDE) in order to both regulate and terminate signals (Vogt *et al.*, 1999; Kasang, 1971). Twenty antennal esterases have been identified in *S.* littoralis adults (Durand *et al.*, 2010). In *S. littoralis* an intracellular antennal esterase has been shown to degrade the plant compound (Z)-3-hexenyl acetate in both adults and larvae (Durand *et al.*, 2010). The protein encoded by the gene SlCXE7 has been found to be able to hydrolyze the two major components (Z9E11-14Ac and Z9E12-14Ac) of the *S. littoralis* pheromone, along with a plant compound, (Z)-3-hexenyl acetate. SlCXE7 was found to be over-expressed in males and during the latter part of the scotophase which is when males are most active which is consistent with tentative role as a PDE. Degradation of (Z)-3-hexenyl acetate was found to be considerably faster than that of pheromone components, but the affinity to both pheromone components was greater than its affinity to (Z)-3-hexenyl acetate. This suggests that this enzyme may be responsible for both the rapid degradation of an abundant and possibly interfering plant volatile and the slow processing, but quick termination of low concentration pheromone signals (Durand *et al.*, 2011). Further study on antennal esterases will surely lead to exciting new developments in the understanding of the regulation and termination of olfactory information at the peripheral level.

4. The Antennal Lobe

4.1 Structure

The antennal lobe (AL) is the primary olfactory center in insects. Like the olfactory bulb in vertebrates the AL is subdivided into glomeruli which receive information from all ORNs of a certain type. Each glomerulus gathers the information from all of the ORNs with the same OR (which may be between 20 and 2000), leading to the one OR one glomerulus hypothesis. Glomeruli are interconnected by local neurons (LN) and reduce the input signal to two or three output, projection neurons (PN). The number of glomeruli in insects varies from 50 to over a thousand but generally falls under 200 (Anton & Homberg, 1999). There are currently three maps of the AL of *S. littoralis* published (Figure 3). The Al of females has only been mapped superficially and between 22 and 35 glomeruli were found (Saveer *et al.*, 2012; Sadek *et al.*, 2002). The male AL, on the contrary has been mapped completely. This map reveals there are a total of between 60 and 63 glomeruli organized in mostly one layer around a fibrous core (Couton *et al.*, 2009). The number of glomeruli is thus close to that found in other lepidopteran species such as *Heliothis virescens, Helicoverpa armigera, Agrotis ipsilon, Bomby mori,* and *Manduca sexta*, where the number of glomeruli varies betweem 60 and 70 (Couton *et al.*, 2009; Kazawa *et al.*, 2009; Skiri *et al.*, 2005; Greiner *et al.*, 2004). Three glomeruli, which are found near the the region where the antennal nerve meets the AL, were found to belong to the macroglomerular complex (MGC), the center specialized center where neurons detecting pheromone compounds converge, in *S.littoralis*. The function of these three glomeruli as MGC subunits had been confirmed via optical imaging (Carlsson *et al.*, 2002). It is interesting that across individuals only 51% of the glomeruli within the AL seem to have fixed positions, a number higher than in other species where similar studies have been carried out (Couton *et al.*, 2009). This result suggests that caution must be taken when undertaking physiological studies.

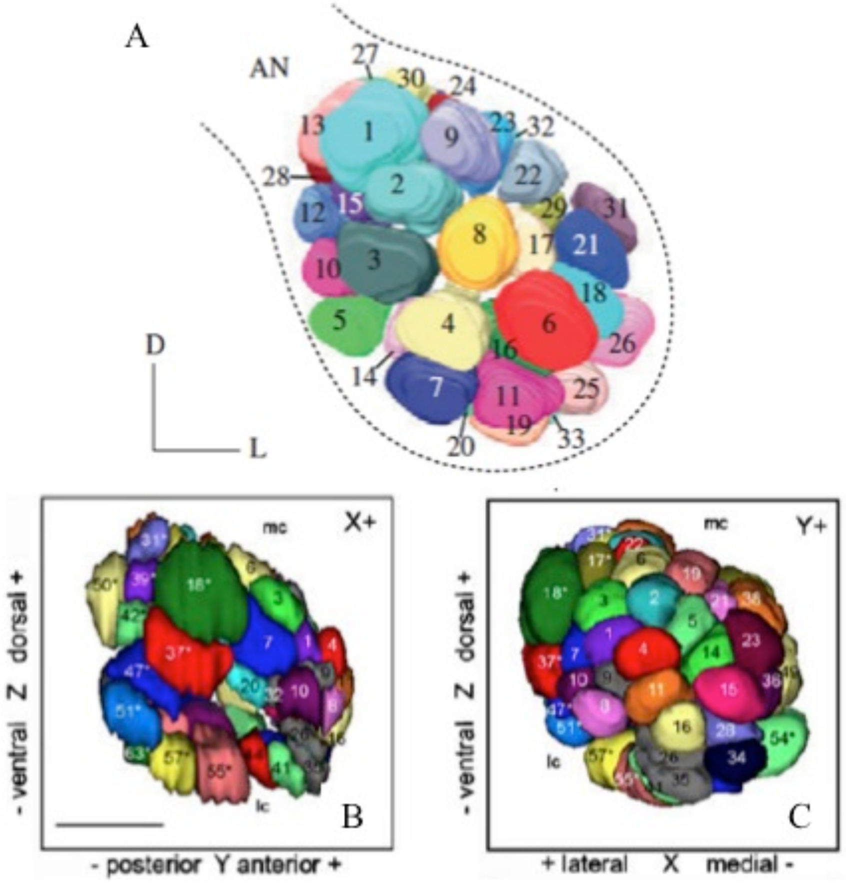

Figure 3. Maps of the AL of *S. littoralis*: A. Surface map of the AL of females. Modified from Saveer *et al.* (2012). B. Two different views of the reconstructed map of the male AL. Glomeruli marked 18 (green) and 37 (red) are part of the MGC. Modified from Couton *et al.* (2009).

4.2 Odor Processing in the Antennal Lobe

Signals sent from the antennae to the AL are distributed throughout the various glomeruli where information is processed and set downstream to higher brain centers. Whereas it was once believed that the AL worked as an aggregation center where the information from all OSN with the same receptor was brought together into one signal, it is now clear that the AL is actually the first center of integration and processing of information

(Hansson & Anton, 2000). Odors in the AL are not represented by the activation of single glomeruli, but rather by a conserved pattern of glomerular activation. This has been studied and shown to hold true in *S. littoralis* for both pheromone components and plant volatile compounds (Saveer *et al.*, 2012; Hansson & Anton, 2000; Anton & Hansson, 1994). However, it is still unclear how odor mixtures interact at the AL level. Further studies have shown that contradicting results with studies in honeybees showing that blend representations in the antennal lobe do not correspond to the simple sum of the representation of the parts whereas in *S. littoralis* it seems to hold true (Carlsson *et al.*, 2007; Joerges *et al.*, 1997). Further studies are necessary to determine is this is due to taxonomical and functional differences or if said contradiction is an experimental artifact. There is mounting evidence that there is communication between glomeruli via local interneurons (LNs), which leads to the signal leaving the AL via projection neurons to not mirror that of glomerular activation in *S. littoralis* (Sadek *et al.*, 2002). Although these results point to the AL as the first center for information processing further studies are required to fully understand is role in the interpretation of ecologically important odor blends in *Spodoptera* and other moths.

4.3 Signal Modulation in the Antennal Lobe and the role of Hormones

Perhaps the most exciting developments with regards to the role of the antennal lobe in olfactory processing has been the discovery of its role in the modulation of olfactory information and its correlation with behavior. Saveer *et al.* (2012) showed that in *S. littoralis* virgin females are attracted to floral odors and only weakly attracted to host plant odors while mated females are strongly attracted to host plant odors and only weakly attracted to floral odors. This shift in behavior was found to match the up and down regulation of glomeruli activity within the AL. Glomeruli which respond to floral odors are up regulated before mating and inhibited after mating while the reverse holds true for host plant odors (Saveer *et* *al.*, 2012). A similar and even more interesting shift was seen in males of *S. littoralis.* Males show a strong attraction to host plant odors and females before mating and 24 hours after mating, however, three hours after mating attraction to both of these stimuli is strongly inhibited. As is the case in females, AL activity of glomeruli which are activated by host plant volatiles and pheromone is very strong in virgin males and 24 hour post mated males while it is strongly inhibited three hours after mating. Interestingly male attraction and glomerular activity in response to floral odors is unaffected by mating, suggesting that males and females may have different olfactory pathways or regulatory pathways for feeding stimuli (Saveer, 2012).

It is well known that juvenile hormone and ecdysteroids regulate reproductory behavior in insects (Simonet *et al.*, 2004). As such, it is possible that these hormones are playing a role in the modulation of olfactory processing. Females of *S. littoralis* show significantly higher levels of dopamine in the AL three hours after mating and 24 hours after mating, levels similar to those of unmated females. Virgin females injected with L-DOPA show attraction to host plant odors and a reduction in the attraction to floral odors similar to that of mated females. AL activity in females treated with L-DOPA is also similar to that of mated females (Kromann, 2012). These results suggest that a dopamine-mediated pathway may indeed play a role in up and down regulating signals in the AL, prior to its output towards higher brain centers.

5. The Mushroom Bodies

5.1 Structure and Immunochemistry

The mushroom bodies (MB) are paired, regions of insect protocerebrum that play a role in sensory integration, decision-making, learning and memory. Although the shape and structure of MBs varies between families and genera of insects some structural similarities remain. A typical insect MB is composed of a cap shaped structure, or calyx, and a pedunculus. These structures are located in the dorso-posterior region of the head capsule, with the pedunculus usually projecting towards the anterior region (Heisenberg, 2003; Pascual & Préat, 2001). The bulk of the MBs are composed of intrinsic neurons known as Kenyon cells (KC) that may be subdivided into three types (I-III). The calix is compromised mainly dendrites of KCs which supply the pedunculus with branched axon like structures. In the number, size, and organization of KCs are where the main differences between taxa are found. The number of can range from a few hundred cells in fruit flies to several hundred thousand in hymenopterans (Farris, 2005).

A brief description of the MBs of *Spodoptera littoralis*, the only species of the genus in which they have been studied is given below based on Sjöholm *et* al, 2005. Approximately 4,000 KCs make up the MB in this species. The calyx is made up of two fused cups. Each cup has a thick wall of KCs lined by a thin layer that receives an afferent supply from the visual system. Projection neurons from the antennal lobes are the main type of input that enters the calyx. The neurons form the inner antennocerebral tract form collateral which penetrate the calyx before terminating in the lateral horn while those from the outer antennocerebral tract first have collateral terminals in the lateral protocerebrum and then converge on the calyx. The calyx has thousands of microglomeruli where afferent neurons synapse with KC dendrites. Projections of these cells penetrate the calyx and form dense fibers that exit each cup and merge to form the pedunculus (Figure 4).

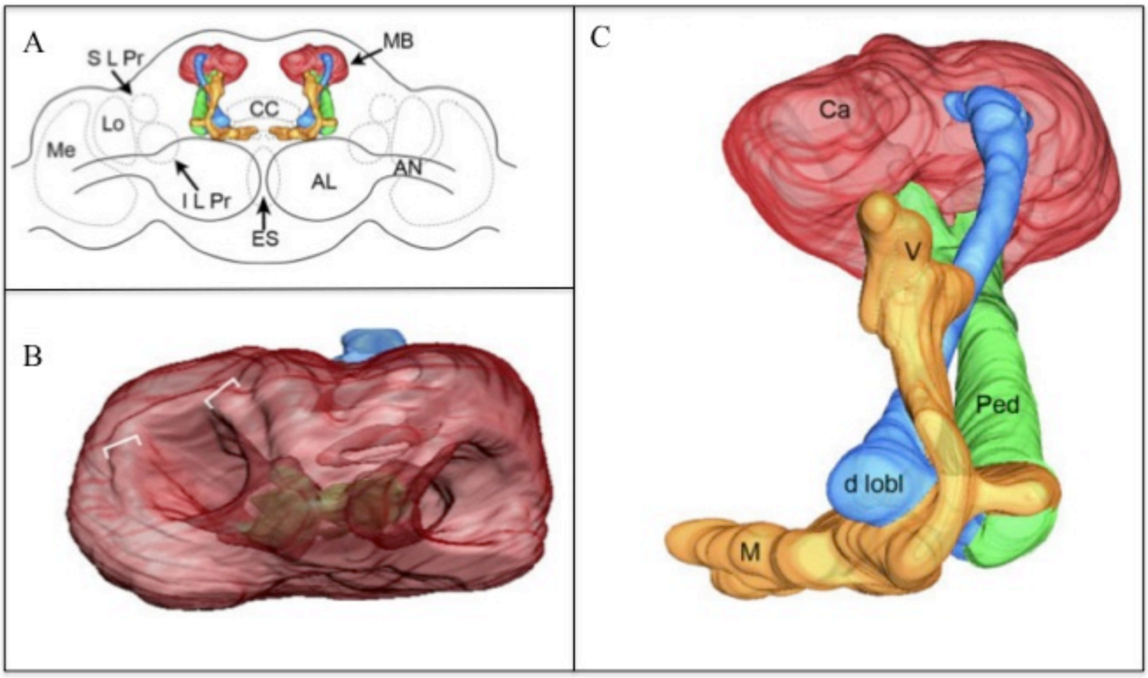

Figure 4. Computer reconstruction of the mushroom body of *S. littoralis.* A: Frontal view of the brain of *S. littoralis*: mushroom bodies (MB); the antennal lobes (AL); antennal nerves (AN); central body complex (CC); lobula (Lo); superior and inferior lateral protocerebrum (S L Pr, I L Pr); medulla (Me); esophageal foramen (ES). B: Top-down view of the calyx (red) showing the fused cups and exit regions of the pedunculus (green) and Y tract (blue). C: Frontal view of the MB showing the calyx (Ca; red), pedunculus (Ped; green), Y tract (blue), and the dorsal lobelet (d lobl), and the vertical (V) and medial (M) lobes (yellow). Modified from Sjoholm *et al.* (2005).

The pedunculus extends for approximately 300 μm before dividing into the vertical lobe that curves back towards the calyx and the medial lobe extends towards the midline of the brain. These two lobes are each divided further in three to form the α, β and γ lobes. The γ lobe extends into both the vertical and medial lobes where as the α lobe subdivides into the α and α’ lobelets in the vertical lobe and the β and β’ lobelets in the medial lobe. An additional stalk, the Y tract originates in the dorsolateral region from the calyx. Like the pedunculus it also has two roots originating in each one of the cups and extends downwards and towards the anterior midline of the brain. In accordance with MB morphology, four main types of KCs have been identified which make up the Y tract, γ, α and β, and α’ and β’ lobelets. Although these cells may be differentiated in the different lobes there is no apparent organization in the pedunculus (Figure 5).

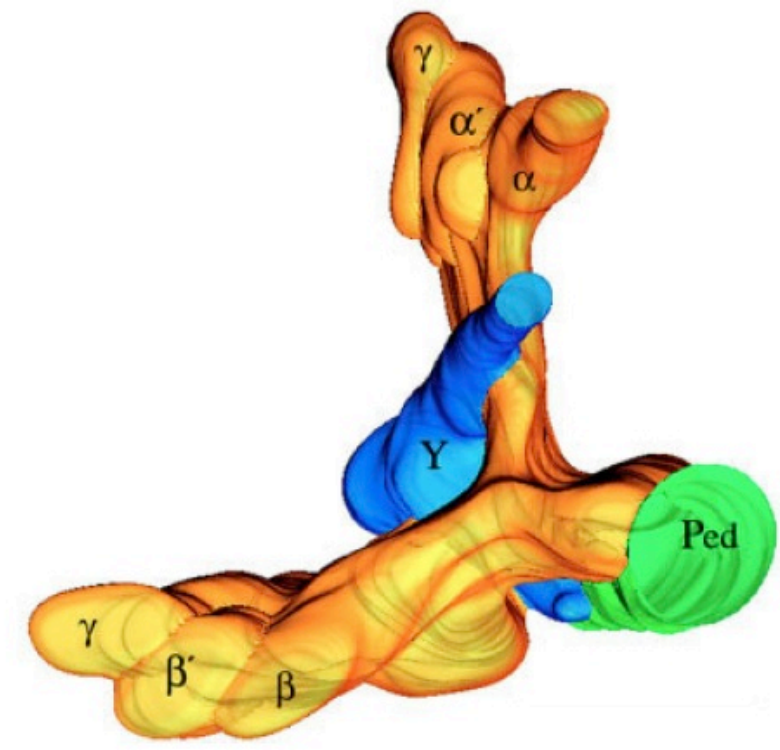

Figure 5. Computer reconstruction of the mushroom body of *S. littoralis* showing its subdivisions and the pedunculus (green) and the Y tract (blue). Modified from Sjöholm *et al* 2005.

Further studies into the organization of KCs within the mushroom bodies have been carried out using antisera against taurine, aspartate, the neuropeptides FMRF-amide, Mas-allatotropin, glutamate and γ-animobutyric acid, and three substances which are believed to be neuromodulators: serotonin, A-type allatostatin (which has been shown to colocalize with GABA (Schachtner *et al.*, 2005)) and techykinin-related peptides (TKRP) (Sinakevitch *et al.*, 2008; Sjoholm *et al.*, 2006). Immunolabeling generally confirmed the morphological and anatomical segmentation of the MB. Anti-taurine sera label the Y tract and the γ lobelets. FMRF-amide labeling is pronounced in the γ lobelets and the anterior areas of the α, β, α' and β' lobelets. Mas-allatotropin labeling showed the opposite pattern, strongly labeling the α, β, α' and β' lobelets but not the Y tract or the γ lobelets (Sjoholm *et al.*, 2006). Glutamate and aspartate immunoreactive KCs are distributed along both the calyx and the lobes, which possibly indicates that these neurons correspond to global processes. GABA immunoreactivity has been shown to be found in systems belonging to both the calyx and the pedunculus. GABA-immunoreactive neurons innervate the calyx from various locations such as the lateral antennoglomerular tract, the Y tract, the MB lobes and the pedunculus. Serotonin immunoreactivity was only seen on the outer regions of the calyx and in the vertical and medial lobes. TKRP-like labeling is evenly distributed along the entire calyx and in some cell bodies around the calyx. There are four or five large TKRP immunoreactive cells medial to the calyx which project their neuritis along the surface of the calyx and then parallel to the pedunculus. Additionally, there are eight large cells located in the pars intercerebralis that supply the MBs with information from each side of the brain. It is believed that these cells are neurosecretory cells (Sinakevitch *et al.*, 2008).

The role of these neurons is still unknown. It has been suggested that GABA immunoreactive cells are responsible for intrinsic modulation of calyx activity by the lobes or lateral horn. However, the presence of GABA immunoreactive cells which are not part of the calyx suggest that other independent inhibitory elements exist. Similarly, the fact that two independent serotonin immunoreactive systems exist strengthens the hypothesis that the calyx and the lobes may be modulated independently (Sinakevitch *et al.*, 2008). TKRP-like immunoreactivity is colocalized with GABA in calyx, but not in other parts of the MB. The fact that TKRP reactive KCs are located in the same area as the arborizations of antennal lobe projections neurons suggests that they may be involved in modulation of olfactory information. Since GABA and TKRP immunoreactive cells penetrate into the inner calyx, it is also possible that they play a role modulating input to this region (Sinakevitch *et al.*, 2008; Sjoholm *et al.*, 2006). Although we know that the MBs receive information from the antennal lobes via the inner and outer antennocerebral tract, it’s role in olfaction is not clear. The role of the MB in sensory integration, modulation and learning has not been studied in any *Spodoptera* species.

6. Applied Aspects

6.1. Pheromones in armyworm control

There have been few attempts to use chemical baits to trap armyworms in an applied context. Most cases found in the literature are about using pheromone traps to monitor *Spodoptera* species, both in the context of invasive species and integrated pest management (IPM) schemes. Pheromone traps are currently being used in the United States to track the arrival of invasive species such as *S. littoralis, S. exempta* and *S. exigua* (Meagher *pers comm.*). Most of the work being done in IPM using pheromones has been in planning the use of chemical pesticides more appropriately. Cruz et al. (2012) found that the application of pesticides based on pheromone trap captures was more efficient than spraying by calendar or by pinhole counts. Similarly Broza et al. (1991) found that timing applications of *Bacillus thuringiensis* based on trap captures of *S. exempta* maximized its effect. The main problem with this approach is the fact that lures require the appropriate pheromone blend. In the case of *S. frugiperda* this has been a mayor challenge due to differences in the composition of pheromones between populations and strains. The pheromone composition of the two host strains of *S. frugiperda*, the rice strain and the corn strain, are known to be different (Velasquez-Velez *et al.*, 2011; Saldamando & Velez-Arango, 2010; Groot *et al.*, 2008). Although the compounds are the same, the ratios between them are distinct (Busato *et al.*, 2004; Meagher & Nagoshi, 2004; Murua & Virla, 2004) and some degree of reproductive isolation has been shown (Velasquez-Velez *et al.*, 2011). These results indicate that while pheromones may be useful in IPM of armyworms, they must be approached with caution and lures need to be independently tested in the area where they with to be used to verify their effect.

6.2 Plant volatiles in armyworm control

Few studied have attempted to use plant volatiles in armyworm control. Meagher (2001) attempted to increase pheromone trap capture by blending the pheromone of *S. frugiperda* with a common floral volatile, phenyl acetaldehyde, and found that there was not a positive effect, but rather, a tendency to have less captures than with the pheromone lure alone. The amount of hymenopterans caught in traps increased with the addition of phenyl acetaldehyde which is an important issue in IPM strategies. In another study von Merey et al. (2011) attempted to spray green leaf volatiles on corn plantations to increase the emission of plant defense compounds that have been known to repel herbivorous insects and attract natural enemies. Their results showed that although sprayed plants did increase their emission of sesquiterpenes, damage by *S. frugiperda* actually increased in treated plants and the effect of parasitoids was only marginal. Keeping in mind that pheromone lures only attract males and not females, lures based on plant volatiles are still promising in trap and kill and mass trapping strategies, however, we still require further understanding about which volatile queues actually are attracting both males and females. Work in this direction is currently underway. Saveer (2012) has developed a five-component blend, based on volatile compounds found in cotton headspace that attracts females of *S. littoralis*. It still remains to be seen if the same blend is also attractive to females and if it attracts females under field conditions and particularly, with a background of different economically important host plant odors.

7. Perspectives and Concluding Remarks

Considering the amount of knowledge we now have on the physiology, behavior and biochemistry of *S. littoralis and S. frugiperda* and some of their most economically important host plants it is remarkable how little information actually exists about them under field conditions. Although there are lists reporting hundreds of plants as host species, not a lot is known about which of these are actually used under natural conditions and for what purpose. Further studies into the behavior and ecology of *Spodoptera* species under cropping conditions and in ecosystems around cropping systems could help to both better understand the information that is currently available, as well as delve deeper into the functional aspects of armyworm chemical ecology.

Traditionally plant volatiles have been studied as oviposition cues and stimuli; however plant volatiles as feeding stimuli have been greatly ignored. Adult armyworms readily feed on floral nectar, extra-floral nectar and there are observations of them feeding on decomposing fruit baits. These observations leave many questions open with regards to the chemical cues involved in feeding. It is unknown whether all food sources produce similar chemical cues or if there are different odor templates which are translated into the same message at higher at the antennal lobe level or at higher computation centers. Additionally, the role of microorganisms and the volatiles produced by their metabolism have yet to be studied independently and in depth.

Armyworms also present a unique opportunity due to the mobility and apparent choice making capacity of larvae. Olfaction has been studied in adult lepidopterans but since larvae are considered to depend upon adult host plant choice, little work has been carried out with regards to their own olfactory systems. *Spodoptera* larvae are a good model system for this research since they naturally orient towards hosts and have the ability to choose between host plant quality and olfactory stimuli. As such it is possible to corroborate physiological observations with behavior and interpret it in an ecological and evolutionary context, but more importantly could provide insight into the functional maturation and development of olfactory systems in insects.

With regards to the olfactory system of insects the current developments are quite promising. To date, the majority of olfactory studies in insects are limited to few species, with *Drosophila melanogaster* being the most important due to the large amount of genetic and physiological tools available. Although future research in mechanisms of olfaction will most likely continue to center around *D. melanogaster* for this reason, *Spodoptera* could become a promising model in understanding complex ecological interactions and olfactory discrimination because of its polyphagous nature and interactions with various hosts and odor profiles.

Current methods allow a deeper understanding about the mechanisms of olfaction and olfactory coding, but if this information was combined with a deeper understanding of behavior and ecology under natural conditions more effective control strategies could be developed. In this regard researchers should take a few steps backwards in order to better understand the information being produced by looking at it from an evolutionary and realistic environmental point of view.

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