Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsplanering,
trädgårds– och jordbruksvetenskap

The effects of non-host plant volatiles on the reproductive behaviour of the Egyptian cotton leafworm, *Spodoptera littoralis*

Inverkan av doftdiversitet på fortplantningsbeteendet hos nattflyet Egyptiskt bomullsfly, *Spodoptera littoralis*

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Foreword

This thesis is to be submitted to the Faculty of Landscape Planning, Horticulture and Agricultural Sciences in partial fulfilment of the requirements for the Master's degree in Agroecology at the Swedish University of Agricultural Sciences, Alnarp, Sweden.

I belong to Pakistan, a country where Agriculture sector directly supports three-quarters of the country's population, employs half of the labour force, and contributes a large share in foreign exchange earnings. Pakistan relies on one of the world's largest irrigation systems for agricultural productions. The key to a much-needed sustainable agriculture and to ensure food security lies in a more efficient use of resources, principally land and water.

Innovative farming and plant protection ideas, leading to sustainable crop production have always fascinated me. I was lucky enough to find an opportunity through the Masters programme in Agroecology to acquire knowledge and to contribute to the development of a sustainable Agroecosystem. Due to my strong background in Biology and Agriculture, I chose to work with the Chemical Ecology Group at SLU, Alnarp to investigate the reproductive behaviour of the Egyptian cotton leafworm, focusing on the Egyptian Agroecosystems. Most of my research is aimed at the socioeconomic impact of the pest on the farming communities of Egypt, biological pest control measures and the agroecological implications of the research.

Overall, the degree programme was a nice learning experience. I acquired immense knowledge about sustainable farming practices, resource utilization, ecology, olfaction and plant & insect relationship. My team work skills, independent working abilities, writing and presentation skills have significantly improved. The gained knowledge will surely facilitate me in developing a scientific career, particularly in the fields of Agroecology and Insect Chemical Ecology.

Acknowledgement

It gives me a great pleasure to thank many people who made this project possible. It is hard to overstate my gratitude to my supervisor, Prof. Fredrik Schlyter for his unbroken fervour, sound recommendations, good instructions, great company, and innovative ideas throughout the project. I am also grateful to my assistant supervisor, MSc Muhammad Binyameen for his non-stop support with experimental procedures, statistical analysis, and report writing skills. I wish to express my sincere gratitude to my colleague, Fatemeh Negar Yousefi for equally contributing to first part of the project and generating valuable data for over six months during the behaviour bioassays. Thanks to Elisabeth Marling for rearing the insects and providing the experimental material. I am also very thankful to the course coordinator, Christina Lunner Kolstrup for her support during the entire project and patience while writing this thesis. Many thanks to MSc Saveer Ahmed for his help with the GC-EAD and Muhammad Khallaf for his help in conducting the interviews in Arabic language and the wind tunnel experiments. Last but not least, I thank my family for their love, understanding, patience, endless support and never failing faith in me.

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Abstract

Spodoptera littoralis is a major pest of cotton in Egypt, causing serious economic losses. Egyptian farmers highly appreciate biological control of the pest as synthetic pesticides are very unsustainable. Phytophagous insects rely on plant volatiles to locate oviposition, feeding and, mating sites. We tested newly emerged *S. littoralis* moths by putting them in jars in in-direct contact with leaves of the host plant, *Gossypium hirsutum* (*Gh*) and of non-host plants, *Adhatoda vasica* (*Av*) and *Picea abies* (*Pa*). Combinations of host and non-host plant leaves were also observed. Females kept with *Gh* leaves started calling earlier than the females in all non-host and the control treatments. Moths exhibited delayed mating when *Pa* and *Gh* leaves were offered in combination. Fecundity was reduced with strong to medium effect when *Gh* and *Pa* and, *Gh* and *Av* leaves were offered in combinations, respectively. Pair longevity was decreased in the absence of *Gh* leaves or the presence of *Av* and *Pa* leaves. Gas chromatogram electroantennographic detection (GC-EAD) study on the antennae of female *S. littoralis* moths revealed three bio-active peaks in headspace collections from *Av* and five in *Pa*, which were subsequently identified through gas chromatography mass spectrometry (GC-MS) by a combination of MS library searches, Kovats indices calculating, and matching the retention times with synthetic standards if available. The biological activity of synthetic standards of the identified compounds was further confirmed through electro-antennogram dose-response tests (EAG). A comprehensive knowledge about these inhibitory effects of *Av* and *Pa* leaves or their combinations, or compounds derived from them, could lead to sustainable pest management policies.

Key Words: *Adhatoda vasica*, electrophysiology, *Justicia adhatoda,* Lepidoptera, Noctuidae, non-host plants, reproductive behaviour, semiochemical diversity, socioeconomics, *Spodoptera littoralis*, spruce.

Abbreviations

Av	*Adhatoda vasica*
EAG	Electroantennogram
ha	Hectare
GC	Gas chromatography
GC-EAD	Gas chromatogram electroantennographic detection
GC-MS	Gas chromatogram-mass spectrometry
Gh	*Gossypium hirsutum*
GM	Genetically modified
IPM	Integrated pest management
NHV	Non-host volatile(s)
Pa	*Picea abies*
PRA	Participatory rural appraisal
Rc	*Ricinus communis*
RH	Relative humidity
SDH	Semiochemical diversity hypothesis
SLU	Sveriges Lantbruksuniversitet/ Swedish University of Agricultural Sciences

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1. Introduction

1.1 Background of the Thesis

In September 2011, I and Fatemeh Negar Yousefi asked Prof. Fredrik Schlyter at the Swedish University of Agricultural Sciences, Alnarp if we could work on one of his projects for the completion of our Project Based Research Training Course "LB0067" to fulfil the prerequisites of Master's degree in Agroecology. Considering our objectives and the agroecological importance of all his other projects, we selected "The observation of sexual behaviour in *S. littoralis*". He then introduced us to MSc Muhammad Binyameen, who was already working on similar ideas. Modulation by non-host plant volatiles in *S. littoralis* is a project under Insect Chemical Ecology, Ethology and Evolution (IC-E^3) project funded by the Swedish Research Council for Agricultural Sciences and Spatial Planning (FORMAS) and the Swedish University of Agricultural Sciences (SLU). The calling behaviour was specifically observed in the first experiment, when the females were observed alone. For the mating behaviour and fitness measures, the second experiment was performed in which moth pairs were observed. While Mrs Yousefi left the project after successfully completing her course, I continued with the project during my thesis as well. Based on our findings during the behaviour bioassays, I supplemented the study with odour collections, chemical identification, and electrophysiological work. To assess the socioeconomic impact of the pest and the scope of IPM in the affected areas, I conducted telephonic interviews of the Egyptian cotton growers in the Asyut valley of Egypt.

1.2 Literature review

1.2.1 Cotton production in Egypt

Cotton is a shrub or subshrub, less than two meters tall and refers to four domesticated species in the genus *Gossypium* (Malvaceae). The Egyptian cotton belongs to the species *G. barbadense* L. Cotton is cultivated in the tropical and subtropical parts of the world (Smith and Cothren, 1999). Egypt produces 65 % of the world's total long staple cotton cultivars (Mesbah *et al.,* 2007). In Egypt, cotton cultivation dates back to B.C. 200 (Balls, 1912). Although the Egyptian cotton is known for its fineness, cotton production in Egypt has gradually decreased over the past two decades. Nine major varieties of cotton are cultivated in Egypt: four extra-long staple (ELS) and five long staple (LS) varieties. Yields are generally high in the middle delta Governorates compared to the upper and lower parts of the country (Gillham, 1995). Egypt ranked seventh amongst cotton, yarn and fabric exporting countries during 1980-1984 when production had reached its height and a maximum of 456,000 ha were cultivated with a total production of 782,000 international bales. Cotton has been the most important cash crop and an export commodity for Egypt for many years.

Many industries such as weaving and spinning, ginning, oil and fodder industries, and many other related services and activities rely on raw cotton and cotton by-products. The industry employs about 386,000 or about 3% of the total Egyptian labour force (Goueli and El Miniawy, 1993). Abdel-Salam and El-Sayed Negm (2009) reported that the productivity has remained stagnant for the past three decades but by the end of the first decade of the 21st century, the cultivated area was reduced to 125, 000 ha. Although Egypt is still a major cotton producing nation in Africa, cotton production has significantly decreased in Egypt in the past two decades (Figure 1). In the Sudan and Ivory Coast, cotton production has declined to a negligible amount due to civil wars. In Mali, Tanzania and Zimbabwe, the introduction of genetically modified (GM) cotton and supportive sectoral and institutional reforms in Burkina Faso during the mid-1990s has resulted in an increase in production in these countries (UNCTAD, 2011).

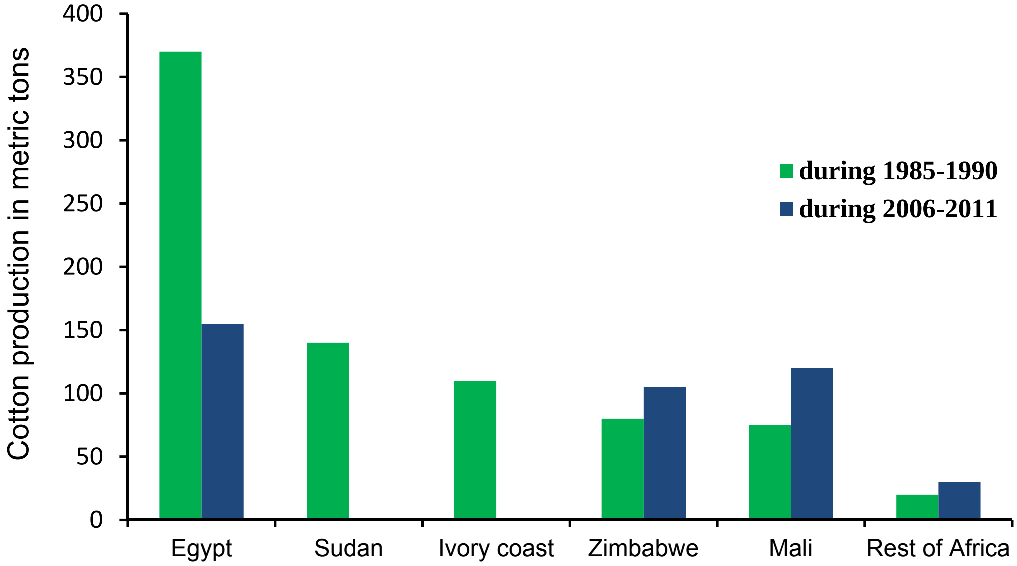

Figure 1: Cotton production in Africa, based on International Cotton Advisory Committee (ICAC) statistics presented at UN Conference on Trade and Development (UNCTAD), Cotonou (Benin) July, 2011.

An approximate 53% of the global cotton fields are irrigated, producing 73% of the global cotton productions. Most of these cotton fields are located in warm climatic regions of the Middle East, Asia and Africa (Soth *et al.,* 1999). The Egyptian agricultural sector is totally reliant on irrigation from the Nile river. The government has established an extensive network of canals to bring public irrigation water to every village and town. Nevertheless, the entire irrigation system is desperately in need of renovation, maintenance and the introduction of modern irrigation techniques (Goueli and El Miniawy, 1993).

1.2.2 **Spodoptera littoralis** ***taxonomy and morphology***

Egyptian cotton leafworm, *S. littoralis* (Boisduval, 1833) is a member of the cutworm family, Noctuidae. It is a serious pest in north eastern Africa, Asia and the Mediterranean Europe (Azab *et al.,* 2001).

S. littoralis is polyphagous in nature and an adult moth is up to 2 cm long with a wingspan of approximately 4 cm. A fully developed larva is 35 to 45 mm long. Its colour varies from light to dark brown and the body is strongly speckled with tiny white spots (Figure 2).

Figure 2. Caterpillar of *S. littoralis* (actual length 35–40 mm)   Source: DEFRA, UK

1.2.3 The biology of Spodoptera littoralis

Depending on the relative humidity and temperature, a cotton leafworm develops in two to seven weeks (Nasr *et al.,* 1984; Harakly and Bishara, 1974). A female moth lays prominent masses of eggs (20 to 1000 eggs), preferably on the underside of the cotton leaf, 50 cm above the ground (El-Saadany and Abdel-Fattah, 1976). The larval stage of *S. littoralis* is the longest and the only destructive stage in terms of physical damage to plant tissues. As the temperature rises, the young larvae hide under the leaves while the older ones move to the soil (Abdel-Megeed and Iss-Hak, 1975; Gawaad and El-Gayar, 1974). In the pupal stage, the insect remains 3-5 cm below the soil surface. The adult moth is very active and agile, capable of longer flights (Salama, 1972). In Egypt, there are usually three population peaks of *S. littoralis* each year. The first peak of egg masses appears in the beginning of June followed by two others in the end of July and September, respectively (Nasr *et al.,* 1980). The largest peak of egg masses appears in late spring, resulting in the production of more larvae in the first generation (Clapham Jr, 1979). Many insect predators e.g., *Coccinella undecimpunctata, Paederus alfierli, Chrysopa vulgaris, Orius laevigatus, Scymnus synacus*, and true spiders are known natural enemies of the Egyptian cotton leafworm (Mesbah *et al.,* 2007). Owing to the socioeconomic importance of the Egyptian cotton leafworm, the insect is subject to extensive research, much of which is envisioned to finding new ways to control it as a pest and to improve the effects of known pest control methods.

1.2.4 Damage caused by Spodoptera littoralis

Egypt is a developing country characterized by 1.75 % annual population growth rate. Agriculture has always been a key segment of the Egyptian economy and it employs 34 % of the Egyptian workforce, which in turn supports 55 % of the entire population. Agriculture also contributes 17 % to the Egyptian GDP (Hatab *et al.,* 2010). Like many other developing countries, Egypt has a lingering balance of payments deficit. *S. littoralis* is one of the major economic pests of cotton in Egypt that causes considerable damage to many other vegetables and crops (Dahi *et al.,* 2009; Hosny, 1980; Hafez and Hassan, 1969). As *S. littoralis* attacks all major cash crops in Egypt, including cotton, clover, corn, cabbage, cowpea, castor bean, sweet potato, lettuce, tomato, pepper, okra, mulberry, soybeans etc., its control is of significant importance. Like many other phytophagous insects, *S. littoralis* larvae defoliate the host plant by chewing the green leaves and buds (Hosny, 1980; Clapham Jr, 1979). The pest has developed resistance to various insecticides commonly used in Egypt (Rashwan *et al.,* 1991). Although some farmers in Egypt laboriously hand pick the egg batches to control cotton leafworm population, most farmers prefer using chemical pesticides, which are detrimental to natural enemies, pollinators and all other non-target insects. The environmental impact of these chemicals can't be neglected either. Some leafworm populations develop resistance against many broad-spectrum insecticides, making their control even more difficult (Miles and Lysandrou, 2002; Smagghe *et al.,* 1999; Ishaaya *et al.,* 1995). Consequently, alternative ways of controlling *S. littoralis* are very important.

1.2.5 The role of volatile semiochemicals in mediating host location and host choice by S. littoralis

Phytophagous insects are predominantly pre-adapted to exploiting specific host plant species, generally belonging to the same family (New, 1988). Insects commonly rely on olfaction to detect mates, food sources and potential larval host plants for oviposition. Insects are masters in identifying the odorants with great accuracy, enabling them to either go towards resources or away from danger (De Bruyne and Baker, 2008). The semiochemical based relationships among plants and insects could be manipulated to regulate insect abundance and diversity. This "push-pull" tactic is an important contribution to modern pest management practices in agriculture and forestry (Schlyter, 2012; Schiebe *et al.,* 2011; Cook *et al.,* 2007).

The physiology of both insects and plants plays a pivotal role in consolidating host plant utilization. However, the chemical communication between insects of the same species, and insects and plants may be vulnerable to malfunction in the presence of non-host plant volatiles (Byers *et al.,* 2004; Tumlinson, 1988). Non-host plant volatiles through their masking effects may disrupt the olfactory host finding behaviour of phytophagous insects, thereby hindering their oviposition behaviour to a greater extent (Thiery and Visser, 1986). Depending on the odour concentration; fecundity and mating durations are also subject to non-host plant odours (Gabel and Thiéry, 1994), resulting in reduced pest infestation levels of the host plants (Hambäck *et al.,* 2003; Van den Berg *et al.,* 2001). Conversely, host plants may increase the rate of sex pheromone production in insects, leading to better reproductive outputs (Landolt and Phillips, 1997; Dicke and Sabelis, 1992).

The semiochemical diversity hypothesis (SDH), suggested by Zhang and Schlyter (2003) suggests that the semiochemical interference with host-selection from NHVs is an important mechanism for associational resistance (Jactel *et al.,* 2011). The presence of other plant species causes associational resistance in the host plant as the herbivore damage is reduced by: 1) an increased habitat diversity which may favour polyphagous insect predators, resulting in a reduced abundance of herbivores 2) deterring the ability of herbivores to find their host plants, and 3) by reducing the time herbivores remain on their host plants (Hambäck *et al.,* 2000). Sexual behaviour of *S. littoralis,* including calling, mating, fecundity and egg viability in the presence of non-host plant, *Av* was only studied by Sadek and Andersson (2007) using direct contact method. All the above mentioned behavioural features in their study showed positive effects in the presence of the host-plant, *Ricinus communis,* except longevity which was affected negatively.

1.2.6 Adhatoda vasica

Adhatoda vasica Nees (Syn. *Justicia adhatoda* L.) is a stiff, evergreen, gregarious, and perennial shrub of the family Acanthaceae, and is used as herbal medicine in treating a wide range of diseases in the Indian subcontinent (Figure 3). Mostly the leaves of the plant are used in drug preparation. A number of chemical compounds including, alkaloids: vasicine, vasicinone, vasinol, essential oil: betane, vitamins: vitamin C, b-carotene, a non-crystalline steroid: vasakin and a mixture of fatty acids have been identified as contributing to the observed medicinal effects of the plant (Kumar *et al.,* 2005). Previous studies like (Sadek, 2003) have shown that *Av* has antifeedent and toxic effects against *S. littoralis*. In terms of reproductive behaviour, the only study by Sadek and Andersson (2007), using direct contact method, has shown that different features of reproductive behaviour of *S. littoralis,* including calling, mating, fecundity and egg viability are affected by the presence of *Av*.

Figure 3. *Adhatoda vasica* plant

1.2.7 Spruce

Norway spruce or European Spruce (*Picea abies* Karst.), is a large evergreen coniferous tree in the genus *Picea* and family Pinaceae. The species is native to the Boreal conditions of northern Europe. It grows 35-55 meters tall and with a trunk diameter of up to 1-1.5 meter. The leaves are sharp and needle like and dark green in colour (Figure 4) (DeGraaf and Sendak, 2006). Sitka spruce (*Picea sitchensis*) is known to delay or reduce ovary development in insects, affecting their mating behaviour, besides host selection (Robert and Bohlmann, 2010). Spruce species are rich in monoterpenes. Kumbaşlı *et al.,* (2011) demonstrated that mortality of spruce budworm larvae (*Choristoneura fumiferana*) reared on diet with a higher concentration of monoterpenes decreases significantly. Similarly, monoterpenes and tannins present in *Pa* may be toxic for insects. The latter is known to cause necrosis and degeneration of midgut epithelial cells in insects (Cardinal-Aucoin *et al.,* 2009; Bernays *et al.,* 1980). Behavioural studies of the spruce bark beetle, *Ips typographus*, have shown the importance of semiochemicals in both plant-insect and insect-insect interactions (Sun *et al.,* 2006; Wermelinger, 2004).

Figure 4. Spruce foliage

1.2.8 Participatory Rural Appraisal (PRA)

As described by (Chambers, 1994), participatory rural appraisal (PRA) is used to portray a wide range of approaches and methods to enable or facilitate rural people to share, enhance, and analyse their knowledge of life and conditions, to plan and to act. In other words, PRA is a tool which is meant to develop and ensure stakeholders' participation in planning, monitoring, and evaluation of a project (Aune, 2000). The strengths of PRA tools reside in their abilities to facilitate discussions, rather than being simply tools to collect data (Richards *et al.,* 1999). People draw boundaries about what they consider PRA in a variety of ways. Generally, PRA is identified with distinctive methods or tools e.g. interviews, maps, calendars, focused group discussions; transect walk and matrices etc. (Milligan, 2003).

Interviews provide rich and valuable research data within a short period of time. The key to a successful interview is the personal interaction between the interviewer and interviewee. Therefore, face-to-face interviews are always more reliable. The extended means of communications, like telephone, video calls, and the internet have broadened the possibilities for a researcher. These technology led sources of verbal communications generate important data without the need to travel longer distances and to conduct face-to-face interviews (Bingham and Moore, 1959).

1.3 Hypothesis

Av and *Pa* are non-host plants for *S. littoralis* and both of these plants release semiochemicals (NHVs), thereby making the insect to avoid these two plants. This relationship could be used to biologically control *S. littoralis* population and to increase plant biodiversity within an agroecosystem.

1.4 Aims of the thesis

The aims of the thesis are:

1) To investigate the socioeconomic impact of *S. littoralis* on the Egyptian cotton growers.
2) To understand the reproductive behaviour of the moth in relation to host and non-host plant volatiles.
3) To investigate the potential role of olfaction and agroforestry in ecologically regulating *S. littoralis* populations.

1.5 Limitations

The thesis is a combination of both qualitative and quantitative studies. Time is the biggest limiting factor in insects' behavioural studies. Most of the laboratory based work was done under strictly controlled environment as minor errors were enough to elicit undesired behavioural responses in insects. The limited number of insects and the availability of the host and non-host plants of the desired age were always an issue during the project. It is worth mentioning here that only physically fit plants had to be used in the experiments as plants damaged either by pests or handling release induced volatiles, making them more variable and unsuitable for behaviour related experiments. For the electrophysiological experiments, usage license, availability, and working condition of the GC-EAD setup were the major issues. As a result of this, some parts of the experiments were performed at night, and some other parts, e.g. wind tunnel bioassays were simply skipped due to limited time. Moreover, synthetic standards of two tentatively identified compounds: n-Butyl ether and the 10µg/µl concentration of Sabinene were not available at our laboratory; hence I could not try them in the dose-response tests. Due to SLU's previous collaboration with Egypt on *S. littoralis* and most importantly, due to the socioeconomic aspects of the pest, the author had to limit the topic to Egypt alone. Nonetheless, *S. littoralis* is not the only threat to Egyptian cotton production and other problems, like lack of irrigation water and poor government legislations do exist. During the interviews, the farmers could not speak English and therefore, an Egyptian researcher helped me to interpret all questions from English language to Arabic language and vice versa for the answers. This may have affected the author's understanding to evaluate some information. Although reactions of the respondents in the telephonic interviews were inferred from their tone of voice, speech style and the speed of their response, reactions from facial expressions, posture, and body language were missed.

2. Materials and Methods

2.1 Insects

S. littoralis pupae (field collected) were imported from Egypt to establish a culture at our laboratory. The culture had been supplemented annually with field collected pupae (Figure 5). Larvae were reared on an artificial diet using potatoes instead of beans (Hinks and Byers, 1976). Larvae were allowed to pupate in the diet. Pupae were sexed under a microscope and shifted to sex specific rearing chambers. All stages of the insects were maintained under (16:8 L: D) photoperiod at 23 ± 1°C and 50-60% RH until emergence. Newly emerged adult moths were used in the experiments.

Figure 5: Field collected female *S. littoralis* pupae.   Photo: Altaf Hussain

2.2 Plants

Freshly detached leaves from three different plant species: cotton, *Pa* and *Av* were used in the experiments. Cotton was the only host plant and *Av* and *Pa* were the non-host plants. Twigs of *Av* were imported from its natural habitat, Egypt, and were re-grown in plastic pots (3.5 L) in a greenhouse for 12 months prior to use. For *Pa*, in the calling behaviour experiment, apical shoot tips (up to 10cm long) with foliage were clipped from 3-4 years old trees grown from commercially available seedlings in (1.5 L) plastic pots. However, in the mating behaviour and fitness measures related experiment, apical shoot tips (up to 10cm long) from 3-4 years old *Pa* trees, grown on experimental land at SLU, Alnarp, were used. Cotton plants were grown individually in pots (1.5 L) in a growth chamber at 25 ± 5°C and70 ± 5% RH. Leaves from the upper third of the plants and almost similar in size were used in the experiments.

2.3 Experimental setup for *S. littoralis* sexual behaviour observation

To understand the role of plant volatiles released by host and non-host plants on the reproductive and sexual performance of *S. littoralis* during the insect's entire reproductive age, we extended our work, using similar experimental conditions as reported by Sadek and Andersson (2007), but we restricted the insects from having a direct contact with the leaves, i.e. effects via volatiles alone. Combinations of host and non-host plant leaves were also observed to see if we could make their future intercropping a possibility. In general, observations on six treatments were performed, namely: cotton (positive control), blank (negative control), *Pa*, *Av*, cotton & *Pa*, and cotton & *Av*. Newly emerged female moths or both male and female moths (mating experiment) were kept inside 250 ml transparent and clean plastic jars with perforated lids. Leaves of the host and non-host plants individually or in combinations were offered to the moths inside the jars. To avoid a direct contact with the leaves, insects were confined to the upper half of the jar with the help of a metallic mesh. No leaves were offered in blank control treatment. Insects in all treatments were given access to little cotton swabs soaked in 16% sucrose solution as a food source.

To avoid any contamination among different treatments, volatile plant odours and insect pheromones escaping through the perforated lids and accumulating inside the enclosing containers were ventilated out through suction tubing, inserted through holes in the containers' roofs. Such contaminations could have behaviourally induced insects or plants in the nearby treatments. Small head mounted (TF Gear Force 2 LED) night vision lamps were used to observe the calling and mating behaviours during the eight hours of scotophase. The experiments were performed at 25°C and 70% RH in a specially designed insect behaviour chamber at SLU, Alnarp (Figure 6).

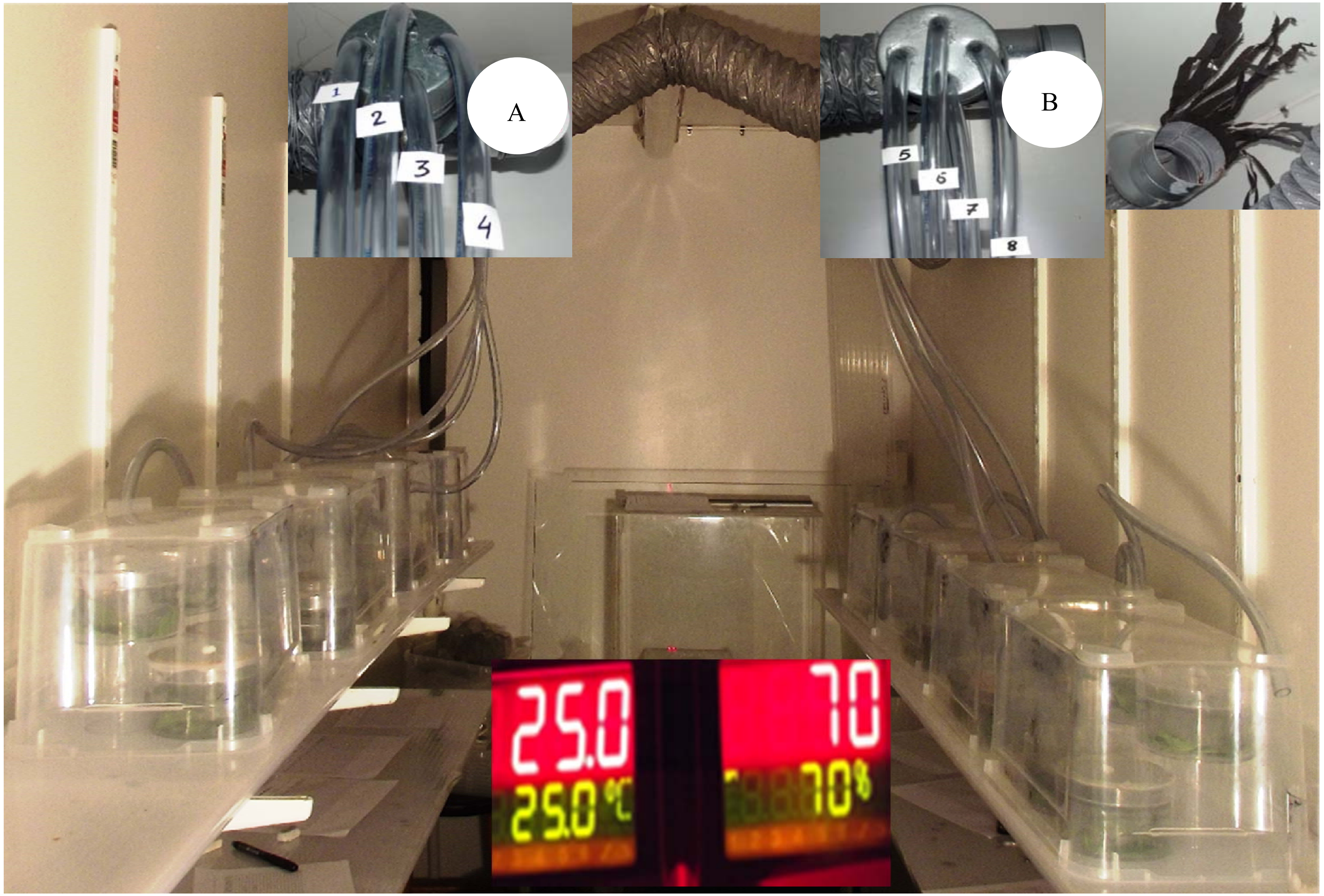

Figure 6. Experimental setup in the insect behaviour observation chamber at SLU, Alnarp. Containers with small jars can be seen on the shelves. Four ventilating tubes are connected to each of the two ducts, A and B. Temperature and RH settings and an in inflow of humidified fresh air blowing into the chamber can be observed in the image. (Photo: Altaf Hussain, 2011).

2.4 Calling behaviour

Calling behaviour is an important factor in understanding the sexual behaviour of insects. In *S. littoralis*, it is known to be characterized by slightly lifting the wings followed by a protrusion of the pheromone gland (Ellis *et al.,* 1980), (Figure 7)**.** A pause of more than ten minutes between two consecutive callings was considered to be the end of any previous calling. However, a frequent interruption of 1-5 minutes in calling was considered normal.

Figure 7. Lateral view of the extended abdominal tip of a female *S. littoralis* moth. The pheromone gland protruded by the moth can be distinguished by its greenish yellow colour (Photo: Altaf Hussain, 2011)

Observations were repeated at least once every ten minutes and the times of onset and end of calling were written on a chart. In the calling behaviour experiments, female moths singly and in the mating behaviour and fitness measures related experiments, male and female moths in pairs were restricted through a very closely meshed metallic net to the upper halves of the jars and they were not allowed to have direct access to the leaves at any stage of the observation. The insect keeping jars were enclosed by ventilated plastic containers (25 × 35 × 20 cm). Plant odours escaping through the perforated lids and accumulating inside the enclosing containers were ventilated out through suction tubing, inserted through holes in the containers' roofs to avoid any contamination among different treatments.

2.5 Mating behaviour

The mating behaviour experiment (2nd experiment) was performed to observe the influence of NHVs on the mating behaviour of the moths. Pairs of newly emerged male and female moths of the same age were placed in the upper halves of large sized 12 × 6 cm Petri dishes whereas the plant leaves in the lower halves. The insects could not physically contact the leaves at the bottom of the Petri dishes as they were enclosed by closely meshed metallic nets.

Plant odours escaping through the perforated lids and accumulating inside the enclosing containers were ventilated out through suction tubing inserted through holes in the containers' roofs to avoid any contamination among different treatments. In addition to onset and ending time of first calling, onset and ending time of matings were also observed throughout the eight hours of scotophases for the first three days. Oviposition and longevity were observed for ten days consecutively. Insects that survived for at least three days were included in calculating mating durations, pair longevity, and fecundity. For fecundity, eggs laid by each female during the scotophase or rarely during the photophase were weighed each day and the number of eggs was calculated according to a previously used "weight vs. number" standard relation (1mg = 20eggs) by Sadek *et al.,* (2010).

2.6 Plant headspace collections

a) Adhatoda vasica

Due to the larger size of the *Av* plants, odours were collected using plastic cooking bags (Meny, Toppits, 45 × 55 cm) and adsorbent (10-15mg Porapak Q 80) filters made of Teflon tubing, 55-60 mm long and 3 mm wide. 2 mm polypropylene wool and 3 mm Teflon stop plugs were used on both ends. The filters or the columns were calibrated at 80 ml/min. The filters were then rinsed with 2 ml ethanol and re-distilled n-hexane (LabScan, Malmö, Sweden) before use. The plants were enclosed with cooking bags and then sealed with plastic wire around the stem. Activated carbon filters were fitted in the air inlets. The filters were placed in the top of the bags and were connected to a 12 V diaphragm aquarium pump (Birgersson and Bergström, 1989). Volatiles were collected continuously for eight hours at 3–4 lux, 22±1°C. The adsorbed volatiles were then desorbed by eluting all the filters with 500µl re-distilled n-hexane. For chemical analyses and electrophysiological recordings, the collected odours were pooled and then condensed to a concentration of (10×) by a stream of nitrogen. Collected volatiles were stored in air tight vials at −20 °C or below.

b) Spruce

Since *Pa* plants were smaller in size (3-4 years old), plant odours were collected using dynamic headspace apparatus of glass as practiced by Saveer *et al.,* (2012). The filters were made of glass tubes, 40mm long and 4 mm wide. The filters were holding 50 mg Super Q adsorbent (80/100 mesh, Altech, Deerfield, IL, USA) between glass wool plugs. The filters were then rinsed with 2ml re-distilled ethanol and n-hexane (LabScan, Malmö, Sweden) before use. The *Pa* plants were enclosed in a 2 l glass jar, which was closed with a grounded glass fitting to avoid contamination by the compounds released by soil. The specially designed filters were then fitted in the top of the apparatus before introducing a continuous charcoal-filtered air stream (150 ml air min¬¹).

2.7 Electrophysiology

a) GC-EAD

Antennal responses of female *S. littoralis* to the volatiles collected from the foliage of non-host plants, *Av* and *Pa*, were studied using gas chromatograph (GC) coupled electroantennographic detection (EAD) as previously described by Saveer *et al.,* (2012). Antennae of two to three days old female *S. littoralis* were amputated at the base (one or two segments) and inserted into the recording glass electrode filled with Beadle Ephrussi Ringer and then connected to a pre-amplifier probe connected to a high impedance DC amplifier interface box (IDAC-2; Syntech). The reference electrode was connected to the tip of the antenna after cutting the distal segment. The antennae were placed 0.5 cm from the GC effluent capillary (Figure 8) and 2µl of *Av* and *Pa* extracts, concentrated to 1µl = 10 min, were individually injected into the GC manually. The GC was equipped with an HP-5 capillary column. Chromatograms and antennal recordings from *Av* headspace *(n* = 5) and *Pa* headspace (*n* = 3) were superimposed and averaged using Syntech GC-EAD-1.2.3 software.

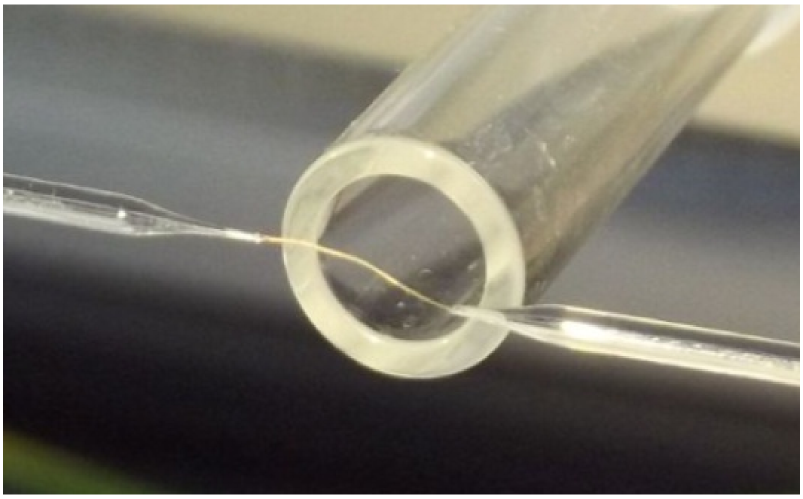

Figure 8: Antenna of a virgin female *S. littoralis* moth mounted on the two glass electrodes connected to the olfactory probe from IDAC-2, Syntech. The GC effluent capillary for the EAD can be seen in the background (Photo: Altaf Hussain, 2012).

b) Chemical analysis

Analyses of the plant headspace collections were made using a coupled gas chromatography–mass spectrometer (GC–MS; 6890 GC and 5975 MS; Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5 capillary column, as previously described by Saveer *et al.,* (2012). Two µl of each sample were injected into the GC with the help of an auto sampler. The GC was programmed from 80 °C (2 min hold) at 10 °C min^{-1} to 110 °C. Compounds were identified according to their retention times as well as Kovats indices and mass spectra, in comparison to the MS library (Agilent) and then matching the retention times with the antennal active peaks. Synthetic standards were used in GC-EAD and GC-MS to confirm the biological activity and chemical identification of antennal active peaks, respectively. A further confirmation of the synthetic standards to be the antennal active compounds was made through dose-response sensitivity tests.

c) EAG, Dose-response tests

Evaluation of biological activity of the identified compounds was carried out by dose-response tests, using a stimulus controller (CS-55; Syntech). A dilution series (100 pg-10 µg/µl) of the identified compounds were prepared using redistilled n-Hexane, except Propanoic acid, which was diluted in water. To produce odour stimuli, filter papers (1 × 1 cm) were loaded with 10 µl of dilutions and inserted in glass Pasteur pipettes and then kept for 5-10 min in a fume hood to let the solvent evaporate. However, since Toluene is a highly volatile compound, pipettes containing toluene laden filter papers were instantly used. To avoid contamination, the wider ends of the pipettes were covered with 1 ml plastic tips and then fitted into a silicon tube, emanating from the stimulus generator. Stimuli were delivered by inserting the pipette tip in a hole in the GC effluent capillary for the EAD and then puffing an air flow of 10 ml s^{-1} for a period of 0.5 s through the pipette. Compounds that had shown minimum antennal response in GC-EAD were tested first and the doses were applied in an ascending order. A new antenna was used each time for each replication and 5 replications were completed. A blank puff of pure air was insufflated before and after every stimulus pipette. A Pasteur pipette containing only blank filter paper was used as the blank control.

2.8 Participatory Rural Appraisal

We decided to do telephonic interviews because it was easier to communicate with the farmers through speech. Also, it was difficult for us to translate certain scientific terms into Arabic. With the help of Mr. Muhammad Khallaf, we conducted the interviews of five farmers in the Asyut valley of Egypt. The semi structured telephonic interviews were meant to gather qualitative information, covering all aspects of cotton production in Egypt, e.g. the socioeconomic impact of cotton production on the living standards of the growers, the prominence of *S. littoralis* as a major limiting factor and the scope of mixed cropping/IPM in Egypt. The growers had been randomly selected for the interviews. Each farmer was asked the same 18 questions. The interviews started with a soft tone and no leading questions were asked. A cordial atmosphere was maintained throughout the interview.

2.9 Data analysis

Data parameters are presented as the mean and standard error of mean (SEM). Hedges’ standardized unbiased effect sizes (Hedges *et al.,* 1985) were calculated for delay in first calling, calling duration, mating duration, fecundity and longevity in “Microsoft Excel” using “Robert Coe calculator” which is a spreadsheet and calculates the effect size by entering the mean, number of values and standard deviation for the groups being compared e.g. experimental treatments vs. control treatments (Coe, 2000). Effect size is of particular importance as it lets the researcher to see the strength of the biological relationship rather than mere statistical significance. Effect size heavily relies on sample sizes and an Effect size above 0.8 is generally considered as a large effect (Nakagawa and Cuthill, 2007). Non-parametric test for Independent-samples (Independent-samples Mann-Whitney U Test), that compares the mean scores of two groups on a given variable as described by Samuels *et al.,* (1989), was applied using IBM SPSS 19 at $\alpha = 0.01$ after Bonferroni Corrections to analyse the effects caused by non-host plant volatiles in relation to fecundity (number of eggs per female) and longevity.

3. Results

3.1 Interviews

3.1.1 Importance of cotton production for the Egyptian cotton growers

Four of the interviewed farmers reported that they grow cotton every year and were totally reliant on this crop for their livelihood. All the farmers were absolutely sure that cotton production could be increased. Despite all the difficulties in growing cotton these days, only two farmers were thinking of switching to other crops. All farmers reported that their expenditures on health care and on the education of their children have increased in the past ten years. Sales opportunities are really high for the Egyptian cotton as all the farmers agreed that they have access to all the local and overseas markets. It was seen that only one farmer was getting subsidies from different sources and had limited crop insurance as well, (Figure 9).

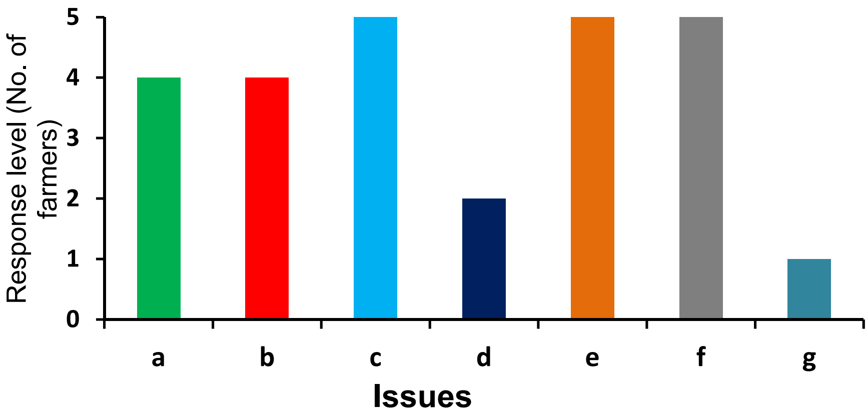

Figure 9. Perception of farmers on the importance of cotton production among the Egyptian cotton growers: **a)** Regularity in cotton cultivation, **b)** Total reliance on cotton production, **c)** Potentials to increase cotton production, **d)** Farmers' opinion about switching to other crops, **e)** Farmers' opinion about an increase in their expenditures on health and education, **f)** Sales opportunities for the Egyptian cotton, **g)** Availability of subsidies and insurance.

3.1.2 Perception of farmers on **Spodoptera littoralis** *as a limiting factor*

According to all the interviewed farmers, the Egyptian cotton leafworm is a serious threat to cotton production. All the farmers currently use only synthetic insecticides to eradicate the pest. Although four farmers were well trained pesticide users, only two of them had access to advisory services. All farmers were very careful in selecting and applying the insecticides, however they were still worried that they themselves or their families could be exposed to these toxic compounds. Four farmers had even noticed resistance in the Egyptian cotton leafworm to synthetic pesticides, (Figure 10).

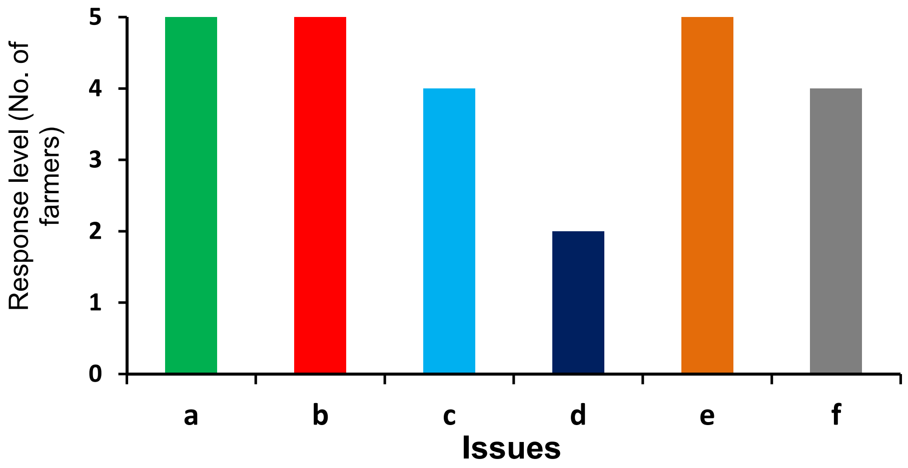

Figure 10. Perception of the interviewed Egyptian farmers on the importance and *S. littoralis* as a threat to cotton production: **a)** Farmers' perception on *S. littoralis* as a threat, **b)** Number of farmers relying on synthetic pesticides to control *S. littoralis*, **c)** Number of trained farmers to use synthetic pesticides, **d)** Access to advisory services, **e)** Awareness about the risks involved in using pesticides, **f)** Number of observations of resistance to synthetic pesticides by *S. littoralis*.

3.1.3 Perception of farmers on the importance of mixed cropping in Egypt

Four of the interviewed farmers were already growing mixed crops (varietal or species mixtures). Their awareness about the benefits of mixed cropping was high. Three farmers were thinking that mixed cropping is environmentally friendly, helps in achieving sustainable cropping systems and makes resource management easy. However, their biggest worry in mixed cropping was harvesting and intercultural operations. Four farmers believed that mixed cropping controls pest infestations and stabilizes yield. It was interesting to know that all farmers (100%) were committed to grow *Av* in their cotton fields if it was proved to be effective against the Egyptian cotton leafworm, (Figure 11).

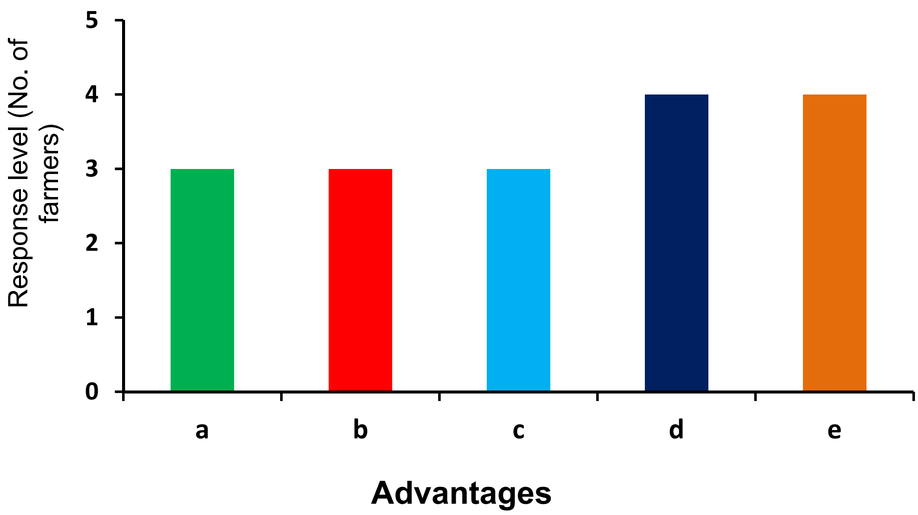

Figure 11. Perception of farmers on the importance and advantages of mixed crop based crop diversification: **a)** Mixed cropping is environmentally friendly, **b)** Mixed cropping helps in achieving sustainable cropping systems, **c)** Mixed cropping helps in resource management, **d)** Mixed cropping helps in controlling pests, **e)** Mixed cropping helps in stabilizing yield.

3.2 Sexual behaviour observations

3.2.1 The role of NHVs in delaying the first calling for mating in female S. littoralis *moths*

The female moths that were restricted from having a physical contact with the leaves of the host plant or in the blank treatment called earlier than the females in all other treatments (Figure 12). Compared to cotton, *Pa* alone and in combination with cotton in both the experiments, whereas *Av* alone and in combination with cotton only in the second experiment exhibited strong effects, |effect size| > 0.8. Blank in the second experiment showed medium difference, |effect size| > 0.5 and in the first experiment blank as well as *Pa* were the least effective treatments |effect size| < 0.5.

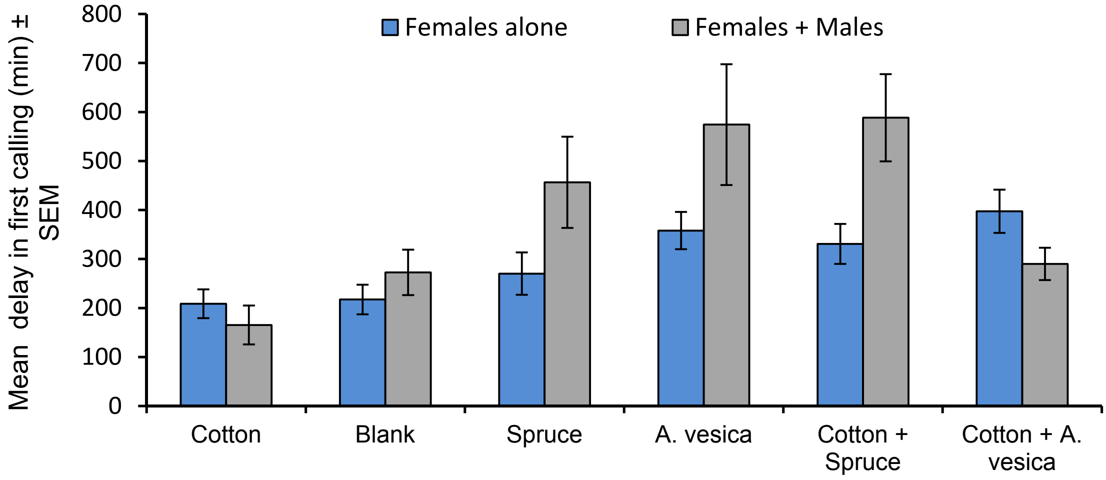

Figure 12. Mean delay in the first calling of *S. littoralis* females when they did not have direct access to the leaves. In all treatments, *n* = 20, except in the second experiment, where blank had 12, cotton & *Pa* had 13, and cotton & *Av* had 18 insects.

3.2.2 The role of NHVs in delaying the first mating in S. littoralis *moth pairs*

Pairs of moths that were offered only cotton leaves started mating earlier than all other treatments, (Figure 13). Significant delay in mating was seen in moths that were offered *Pa* and cotton leaves together, |effect size| > 0.8. Blank, *Pa* and *Av* showed medium difference, |Effect size| > 0.5. However, cotton and *Av* combination was less effective, |effect size < 0.5|.

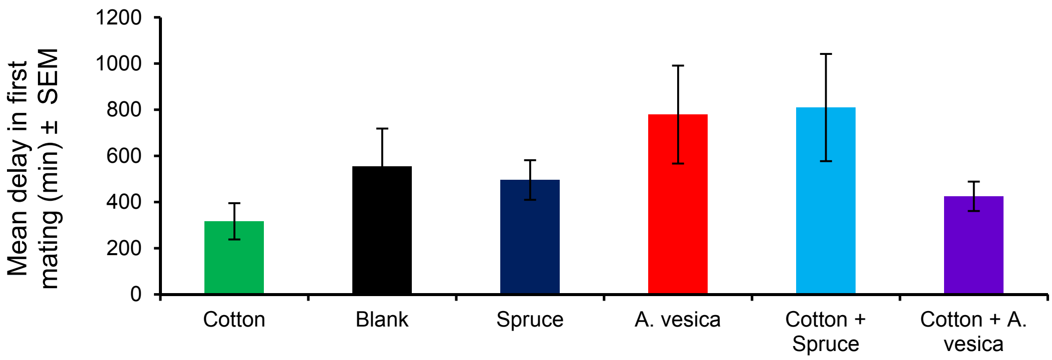

Figure 13. Mean delay in first mating of *S. littoralis* pairs when they were exposed to plant volatiles from different plant leaves: cotton (n=20), *Pa* (n=20), *Av* (n=20) or combinations of host and not host plant leaves, cotton and *Pa* (n=15) and cotton and *Av* (n=18). In blank (n=15), no leaves were offered to the pairs.

3.2.3 Calling durations

Compared to cotton, calling duration was not strongly affected in any treatment, except *Av*, which showed a medium difference when the females were alone. The experiment in which both the sexes had the opportunity to mate, the calling durations were much shorter as compared to the other experiment, where females were kept without any males in the jars (Figure 14).

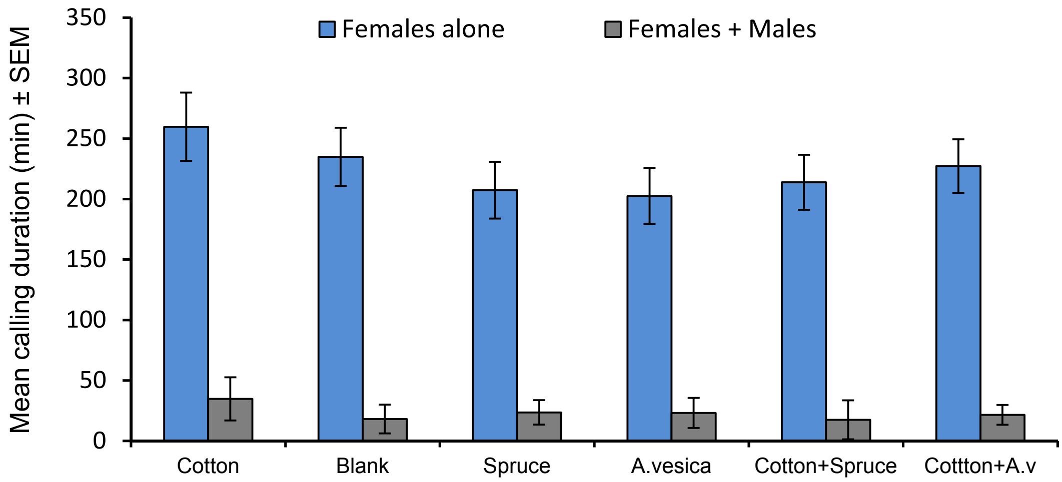

Figure 14. Mean calling duration of *S. littoralis* females when they were restricted from having direct contact with the leaves of host and non-host plant leaves. In all treatments, n=20 except in exp. 2 where blank had 12, cotton & *Pa* had 13, and cotton & *Av* had 18 insects.

The effects in the first experiment were stronger compared to the second experiment as indicated by the effect size calculations (Table 1).

Table 1. The effect size* of blank, host and non-host plant leaves on the mean calling duration of *S. littoralis,* compared to cotton.

Experiment No.	Blank	*Pa*	*Av*	Cotton+ *Pa*	Cotton+*Av*
1) Female	–0.21	–0.45	–0.50	–0.40	–0.29
2) Female+Male	–0.24	–0.17	–0.17	–0.24	–0.21

*) Effect size is measures the strength of a phenomenon and lets the researcher to see the strength of the biological relationship(s) rather than mere statistical significance (Nakagawa and Cuthill, 2007).

3.2.4 Mating duration

Moths in all treatments, compared to the host, cotton, showed a trend of reduced mating duration. Effect sizes (Table 2) show that all treatments had very little effect on the mating duration of the moths, |effect sizes| < 0.5.

Table 2. The effect size of blank, host and non-host plant leaves on the mating duration of *S. littoralis,* compared to cotton.

Treatment	Blank	*Pa*	*Av*	Cotton+ *Pa*	Cotton+*Av*
Effect size	–0.05	0.10	–0.41	–0.47	0.11

3.3 Fitness measures

3.3.1 Fecundity

Fecundity was higher in the presence of cotton leaves and blank (Figure 15). Independent-Samples Mann-Whitney U Test was conducted to statistically analyse the fecundity of female moths, (Table 2). Compared to cotton, only cotton and *Pa* combination showed a significant decrease in fecundity, $p < 0.01$ after Bonferroni corrections. Effect size calculation also showed that the presence of cotton and *Pa* has a strong effect on fecundity, |effect size| > 0.8. Cotton and *Av* showed a medium reduction in fecundity |effect size| > 0.5 and $p = 0.061$.

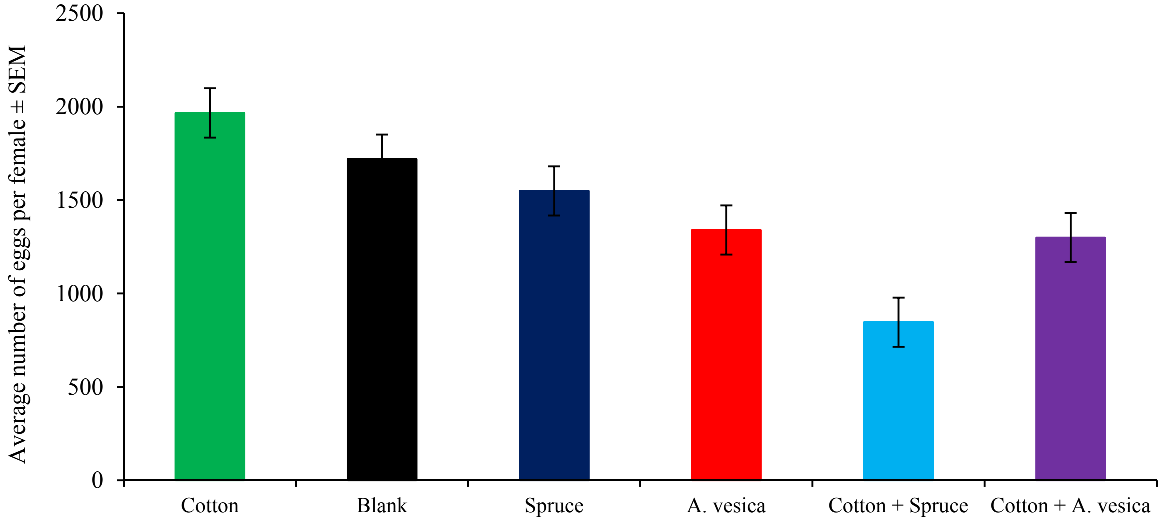

Figure 15. Mean number of eggs laid by *S. littoralis* females when they were exposed to plant volatiles from different plant leaves: cotton (*n*=20), *Pa* (*n*=20), *Av* (*n*=20) or combinations of host and not host plant leaves, cotton and *Pa* (*n*=15) and cotton and *Av* (*n*=18). In blank (n=15), no leaves were offered to the pairs.

Table 3. Sig. (2-tailed) and effect size for *S. littoralis* female fecundity as compared to cotton, the host plant. Non-host plant leaves, *Av* and *Pa* were observed alone and in combination with cotton. Blank was used as a negative control.

Comparison	Cotton vs. Blank	Cotton vs. *Pa*	Cotton vs. *Av*	Cotton vs. Cotton+ *Pa*	Cotton vs. Cotton+*Av*
Sig.(2-tailed)	0.612	0.207	0.085	0.003*	0.061
Effect size	−0.18	−0.41	−0.56	−1.02	−0.61

3.3.2 Longevity

The standard observation time for longevity was ten consecutive days, although some insects lived longer. Insects that died before the third scotophase were excluded in calculating pair longevity (Figure 16). An independent-samples Mann-Whitney U Test was conducted to compare pair longevity (Table 3). Compared to cotton, a highly significant decrease in longevity was seen in all treatments, including blank, $p < 0.01$ after Bonferroni corrections. Effect size also showed that the presence of *Pa* or *Av* leaves have a strong negative effect on insect longevity, |effect size| > 0.8 or the absence of host plant leaves, i.e. blank, |effect size| > 0.5.

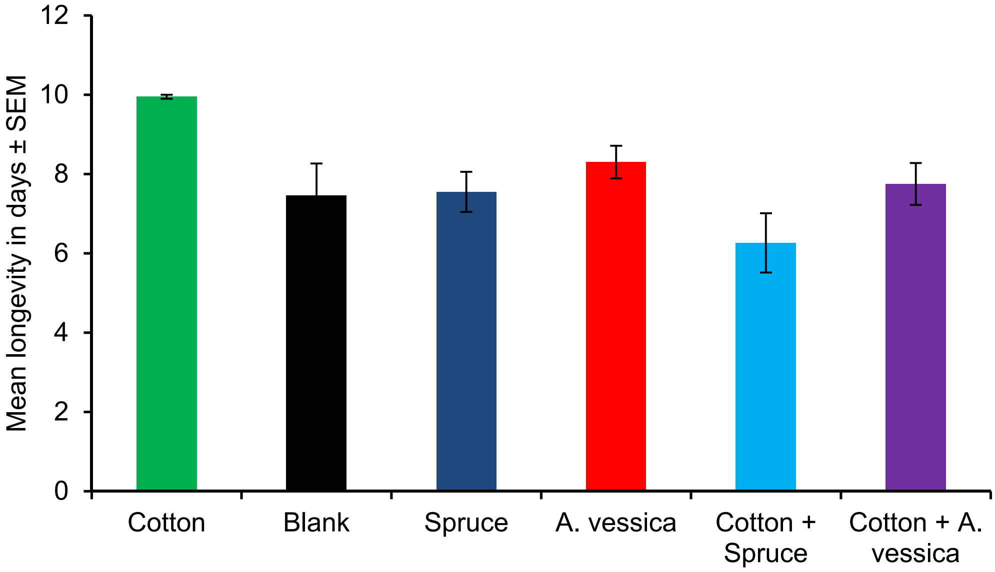

Figure 16. Mean pair longevity of *S. littoralis* moths when they were exposed to volatiles from different plant leaves. Cotton (*n*=20), *Pa* (*n*=20), *Av* (*n*=20) or a combination of host and not-host plant leaves, cotton and *Pa* (*n*=20), cotton and *Av* (*n*=20), and blank control (*n*=18).

Table 4 Sig. (2-tailed) and effect size for *S. littoralis* pair longevity as compared to cotton, the host plant. Non-host plant leaves, *Av* and *Pa* were observed alone and in combination with cotton. Blank was used as a negative control.

Comparison	Cotton vs. Blank	Cotton vs. *Pa*	Cotton vs. *Av*	Cotton vs. Cotton+ *Pa*	Cotton vs. Cotton+*Av*
Sig.(2tailed)	0.007*	0.000***	0.001**	0.000***	0.001**
Effect size	–0.76	–1.47	–1.23	–1.52	–1.29

3.4 Electrophysiology

3.4.1 GC-EAD and identification of active compounds

The antennal responses of unmated female *S. littoralis* moths to the compounds present in *Pa* and *Av* volatiles were studied through GC-EAD. Analyses of the plant headspace collections were made with GC-MS. The retention times and Kovats indices of the candidate compounds in the plant headspace collections were matched with those of the synthetic standards and then confirmed through the MS library. Propanoic acid, n-Butyl ether and Toluene in *Av*, whereas β-Myrcene, Δ-3-Carene, p-Cymene, Sabinene and Terpinolene were identified in *Pa* (Figure 17). The identity of n-Butyl ether was not confirmed by matching with the synthetic standard due to the unavailability of the compound, but it could easily be confirmed later on.

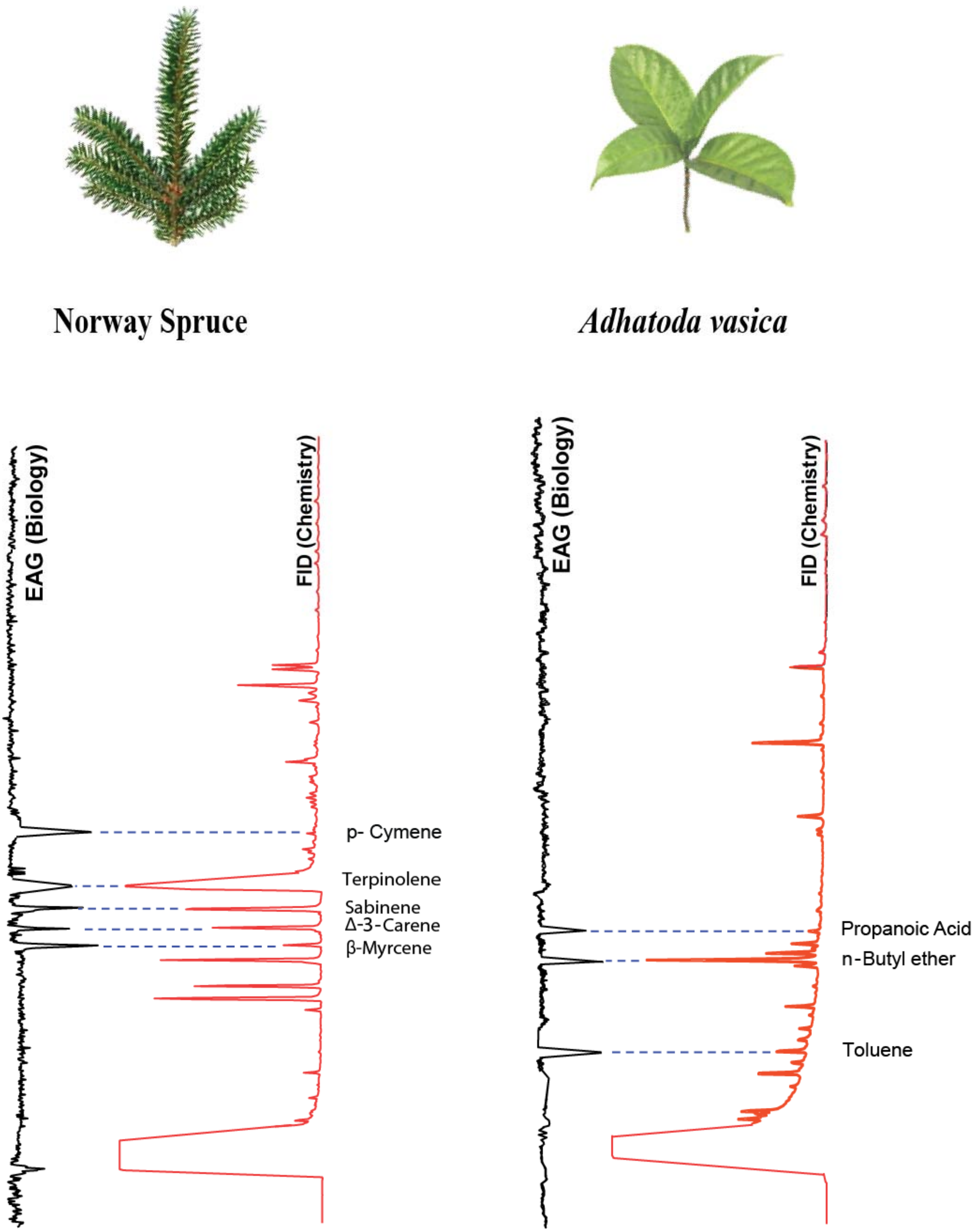

Figure 17. Averaged GC–EAD signals from 2-3 days old, unmated *S. littoralis* female antennae to headspace samples of *Pa* (n=3) and *Av* (n=4) volatiles. Volatile compounds eluting from an HP-5 capillary column and eliciting antennal responses are named.

3.4.2 Dose-response tests

Different doses of synthetic standards of the identified compounds applied with Pasteur pipettes in a short period of time generated a sequence of repeated responses (dose-response curve). The responses to all the identified compounds were dose dependent, with an affinity to increase with increasing dose, (Figure 18). Compared to blank (filter paper), all compounds elicited responses in the antennae with varied amplitudes, even at the lowest dose of 1ng.

In *Av* extracts, Toluene elicited dose-dependent responses: 0.37 mV at 1 ng which increased to 0.57 mV at 100 µg dose compared to Propanoic acid: 0.23 mV at 1 ng and 0.50 mV at 100 µg. In *Pa* extracts, Sabinene elicited dose-dependent responses: 0.39 mV at 1 ng followed by Δ3-Carene and Terpinolene with 0.26 mV at 1 ng. Although all the *Pa* compounds elicited dose-dependent responses, β-Myrcene was found to be more active at higher doses, as weak responses increased to 0.6 and 0.7 mV at 10 µg and 100 µg doses respectively.

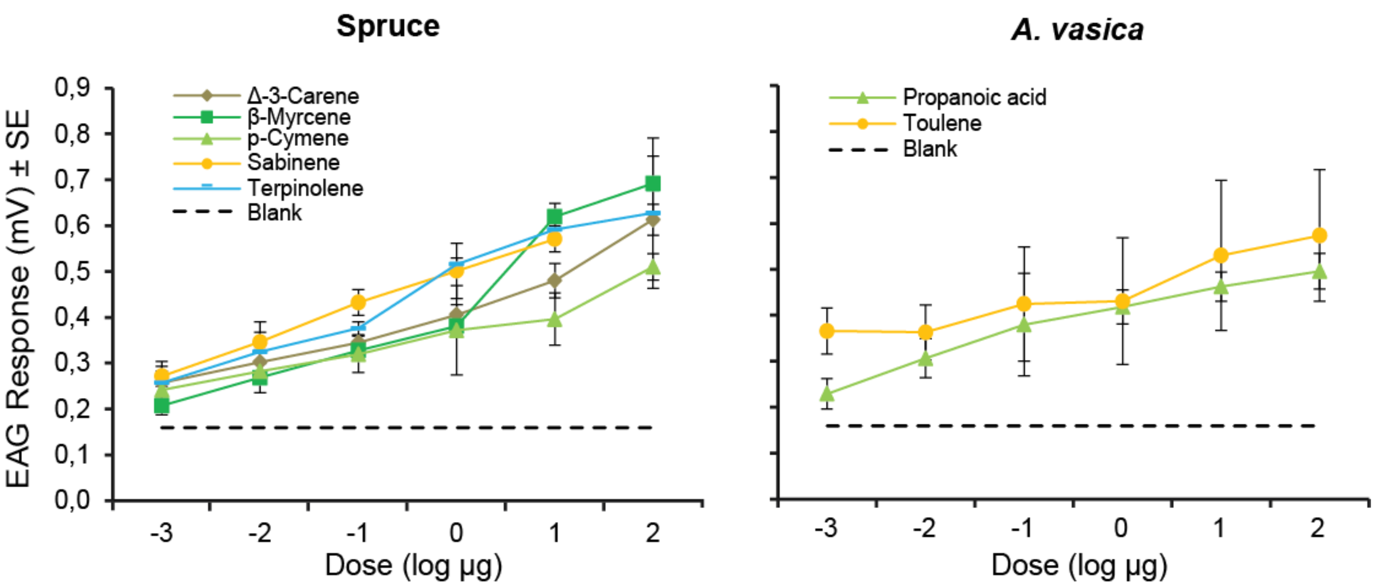

Figure 18. Dose-response curves of the antennal responses of unmated female *S. littoralis* moths to doses in the range of 1 ng -100 µg for each compound (± SEM, *n* =5) applied on filter paper. Compounds were identified in *Pa* and *Av* headspace volatiles that elicited reliable GC-EAD responses.

4. Discussion

Cotton production in Egypt is an important socioeconomic activity, directly or indirectly affecting the living standards of the growers. Being the only source of revenue for most farmers, a decrease in cotton production in recent years has put tremendous pressure on their economic status. Most farmers reported an increase in their expenditures on health and education. The negative impact of the Egyptian cotton leafworm on cotton production and the overall failure of various control measures undertaken by farmers have threatened production (Hosny, 1980). The interviewed farmers reported mixed cropping and encouraged such practices for various benefits, e.g. pest control, resource management, yield stabilization, crop diversification and environmentally friendly etc. Therefore, as hypothesized in the current study, a further investigation of agroforestry, olfaction and the reproductive behaviour of *S. littoralis* could lead to an alternative control strategy for the pest.

Our study provides both behavioural and electrophysiological evidence that the modulation of the reproductive behaviour in *S. littoralis* by host and non-host plant leaves is the result of plant-insect chemical communication.

Successful mating is very important in a female moth's life and delay in mating is known to reduce reproductive output (Torres-Vila *et al.,* 2002). Male moths also heavily compete for female moths during the scotophase and males arriving first at the calling females normally achieve mating success (von Arx *et al.,* 2012). The initiation of mating is subject to the readiness of female moths, manifested in calling. However, the responsiveness of male moths is equally important (Sadek and Anderson 2007). Compared to cotton, female moths exposed to the NHVs of *Pa* or *Av* foliage exhibited delayed calling. Calling and mating durations were not affected by the presence of non-host plant leaves in our study but the combination of cotton and *Pa* leaves as well as *Av* leaves alone delayed mating. It was interesting to find that cotton and *Pa* leaves in combination were more effective in delaying mating than the previously reported *Av* leaves by Sadek and Anderson (2007). *Av* showed a medium difference in reducing calling duration when the females were alone and in delaying mating when males and females were together. The repellence of NHVs may have stressed the insects, causing unusual and prolonged delays in calling and mating behaviour. Since the moths were reared on an artificial diet, they did not have any previous experience either with the host or non-host plants. The jars used to observe the mating behaviour of insects were small enough, and even a little response by both sexes was enough to initiate mating. To avoid habituation and induction of preference, insects were only exposed to the NHVs during the scotophase and not in the photophase period. Therefore, I presume that the differences in sexual behaviour observed during the experiments were innate and without any learned associations.

Calling durations were much shorter when both the sexes were together, possibly as a tactic to ensure further matings. In natural environment, *S. littoralis* female moths mate repeatedly, as a mechanism to increase reproductive capacity and to ensure ultimate mating success, (Sadek, 2001; Kehat and Gordon, 1975). The opportunity of multiple matings increases the chances of finding a suitable host and of mating with newly emerged younger males that have relatively larger spermatophores (Sadek and Andersson, 2007; Svensson, 1996). Therefore, a continuous presence of non-host plants or other sources of NHVs would lower the possibility of repeated matings.

We also observed that every mating was followed by oviposition (Gillott, 2003); however, female moths exposed to cotton leaf volatiles always laid more eggs. The delay in mating caused by the combination of *Pa* with cotton leaves, ultimately resulted in a highly significant decrease in fecundity. As a survival strategy, decrease in oviposition rate leads female moths to avoid plants on which predators are likely to be present or are poor sources of food for their progeny (Zhang and Schlyter, 2004; Paré and Tumlinson, 1999; Jaenike 1990). Similar effects of non-host plants were also reported by Rembold and Sieber (1981) in Indian lilac or Neem (*Azadirachta indica*) which inhibits both oogenesis and ovarian ecdystereroid synthesis in *Locusta migratoria*, averting oviposition. Therefore, the reduction in oviposition during our experiments could be attributed to the NHVs, resulting in decreased oogenesis and hindered oviposition behaviour as reported by Thiery and Visser (1986). The large amounts of monoterpenes, mainly hydrocarbons, present in *Pa* volatiles may be toxic for the moths, as we observed failed post mating separation in some moth pairs, exposed to *Pa* volatiles.

Some combinations of volatiles from host and non-host plants showed a strong effect of decrease in fecundity, a character closely related to fitness. An adaptive behaviour towards non-host plants may be behind such a strong influence on fitness and consequently on evolution. The antennae of *S. littoralis* have two different types of sensilla, long trichodea and basiconica, distinguished by their lengths and are equipped with olfactory receptor neurons (ORNs) that respond to sex pheromones and plant semiochemicals, respectively (Binyameen *et al.,* 2012; Anderson *et al.,* 1995; Ljungberg *et al.,* 1993; Visser, 1986). Host plant cues may influence the release of sex pheromones that interactively boost the response of the opposite sex to the pheromones, resulting in higher calling and mating activities. Some plant volatiles like, β-Farnesene are known to amplify weak pheromone signals in *Cydia pomonella*, thereby increasing the range of communication distance, as described by Yang *et al.,* (2004). In contrast, NHVs through their masking effects may disrupt the olfactory host finding and oviposition behaviour of phytophagous insects to a greater extent (Thiery and Visser, 1986). Thus, mixed cropping that increases semiochemical diversity compared to pure host stands, may disturb olfactory guided host choice in insects and reduce the possibility of pest infestation by associational resistance (Schlyter, 2012; Zhang and Schlyter, 2003), while widespread monocultures expedite pest outbreaks (Jactel and Brockerhoff, 2007; Altieri and Letourneau, 1982).

Sadek and Andersson (2007) reported longevity of *S. littoralis* to be increased by the presence of *Av*. In contrast, we found a significant decrease in longevity of insects in control and all non-host treatments. Some insects exhibited sustained locomotor activity during the observations. As non-host plant volatiles could be toxic or repellent in nature, their perception by the insect could have resulted in sustained locomotor activity, causing insect mortality. Sustained locomotor activity could also interfere with the mating behaviour of the moths, leading to a reduction in mating activity. The longevity of insects was also decreased in control treatment; therefore, an increase in female mortality could be due to host deprivation resulting in abnormal, forced or prolonged egg retention and not directly due to non-host plant volatiles (Gabel and Thiéry, 1994).

Plants release a very large number of volatile compounds, some of which are known to act on the nervous and hormonal system of female moths. The identification of the biologically active plant volatile compounds for insect behaviour tests is commonly done through EAG or more precisely through GC-EAD recordings. For EAG, compounds or headspace collections are formulated on filter paper. For GC-EAD, compounds or headspace collections are injected into the GC that elute onto the antenna according to a programmed GC method. To confirm the identity of antenally active peaks, Kovat indices for the bioactive peaks were calculated based on their retention times and matched with Pherobase Kovat index. Finally, the mass spectra of the active peaks were compared with synthetic standards in the MS libraries according to standard procedures (Anonymous, 2008; Birgersson, oral commun.). Due to constraint of time, the data here falls short of a full identification at present standards. For the three NHVs from *Av*, their precise identification is of special interest as they are not among the more common plant constituents. However, the dose response experiments showed clear dose-dependent and rather strong responses also at low doses for NHVs from both *Pa* and *Av*, lending biological support to the chemical identification of *Av* NHVs. Wind tunnel bioassays, part of the overall project, will confirm the attractant or anti-attractant nature of the non-host plants and the identified electrophysiologically active compounds.

The adaptive behaviour of *S. littoralis* moths towards both non-host plants used in this study may have interesting prospects to be used efficiently against this serious pest. As both *Av* and *Pa* discourage calling, mating, fecundity and longevity in *S. littoralis*, the overall reproductive behaviour is deleteriously affected by the presence of anyone of these two non-host plants. Although non-host plants may affect reproduction in insects, a complete “wipe-out” as achieved temporarily by direct pest control methods may not be possible (Schlyter, 2012). Nevertheless, as reported by Ellis and Steele (1982), a delay in mating for one week is sufficient to bring down the fertility of the moth to almost zero. However, the effects of the two non-host plants on the natural enemies of *S. littoralis* and other pest are unclear, which paves way for further investigations.

5. Conclusion:

Cotton production in Egypt is an important socioeconomic activity. Farmers involved in cotton production are directly affected by cotton leafworm infestation. Manipulating the olfactory guided complementary interactions between insects and plants within an agroecosystem requires an understanding of the entire mechanism. Modern monoculture crop production systems need an addition of selective plant bio-diversity to optimize pest control. Compared to a diverse cropping system, the deficit of semiochemical diversity in monocultures makes it easy for herbivore insects to find suitable host plants by their sensitive antennae. *Av* and *Pa* have been proved in the present study to be effective against *S. littoralis* which provides a basis for their integration through agroforestry. *Av* could be planted distantly in the field or used as a fence crop to control the Egyptian cotton leafworm infestation. *Av* plant extracts could also be utilized in many ways to control insect pests. Since cotton and *Pa* belong to different biomes and are less adaptable to each other’s natural habitats, their mixed-cropping seems difficult. As a potential agroecological implication of the ability of *Pa* to delay calling and mating and decrease fecundity and longevity, plant extracts from *Pa* could be utilized to elicit such behavioural disruptions in *S. littoralis.* Further research on these chemical interactions should provide basis for a self-sustainable, diverse and integrated pest management practices in an agroecosystem.

6. Personal reflection on master thesis

I believe this master thesis is a true representation of my entire academic achievements. The topic of my research was chosen after consulting many of my learned friends. My enthusiasm and devotion for a diverse and sustainable agroecosystem finally made me choose the current topic. The research experience that lasted for almost one year, beginning with the Project Based Research Training was a nice opportunity to learn and understand the role of olfaction in insect and plant interactions. The research also enlightened me with the knowledge about analytical chemistry, electrophysiology, reproductive behaviour of insects, semiochemical diversity, plant biodiversity, interviewing skills and statistics.

Considering all the limitations as previously stated, the results achieved in the specified time are quite convincing. However, being the only study of its type on *S. littoralis* and the two non-host plants, some insect and plant parameters were speculated, based on literature references from other related studies, involving other insects and plants. Although the monoterpenes identified in *Pa* were very similar in volatility, the airborne amounts emitted from the stimulus pipettes at room temperature in the dose-response tests may have varied greatly, possibly affecting the results. Some results were quite contradictory to the results of closely related previous studies, e.g. Sadek and Andersson (2007), which could be the subject of future studies.

The interviews about the future implications of the studies were also quite interesting. Although the number of participating farmers was quite low, I believe the information gathered from the interviews truthfully reflects farmers' perception on the socioeconomic impact of *S. littoralis* and the passion for biological control of the pest. The interviews also revealed some of the problems faced by the farmers related to mixed-cropping. Quite remarkably, awareness about the risks involved in conventional pest management, mixed-cropping, and environmental issues was very high. To conclude, I want to reiterate the need of similar studies as the method not only safeguards human health and environment, but the economic benefits could also be very high.

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