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# Plume interaction and odour source spacing of pheromone and non-host volatiles: –Behavioural effects on bark beetles and moths

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Front page pictures: 1) Bark beetle, *Ips typographus* 2) Egyptian cotton leafworm, *Spodoptera littoralis* and 3) Tobacco leafworm, *S. litura*

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# ABSTRACT

Insects have a remarkable ability to sense whether odours are released from one point source or from two closely separated ones. Therefore it is of interest to study the interactions of pheromone component odour plumes to affect the insect behaviour when the release points of different components are physically separated. It helps us to understand the mechanism that insects use to find their host or any attractive source. We studied the behavioural response of *Spodoptera littoralis* (Lepidoptera, Noctuidae) males, in a walking bioassay, to two components of the female sex pheromone blend, (9*Z*,11*E*-tetradecadienyl acetate) and (9*Z*,12*E*-tetradecadienyl acetate), released from two separate dispensers at various distances (0-5 cm) from each other. The main pheromone component, 9Z-11E-14:OAc was tested at 1 and 10 ng in combination with the minor component 9Z-12E-14:OAc, at 1 % of the main component. The low dose always showed a lower behavioural response than the high dose at the same separation distances (0-5 cm). When low doses were used, the response was higher at 0 and 3 cm separation than other separations whereas when high doses were used there was not much difference in response at 0, 2, and 3 cm separations. A Photo Ionization Detector (PID) was used to investigate plume width and overlap. The PID showed that 5 cm separation of odour sources was enough to totally separate the odour plumes from each other, whereas at 3 cm separation, plumes partially overlapped. By comparing the behavioural results with PID data it is concluded that insects are much more sensitive than the PID, since they responded at distances where the PID showed zero ppb. Male insects may respond well to doses that are 20 times lower than one female equivalent. A field experiment for *S. litura* was done in Pakistan on cotton crop by spacing sex pheromone components. Pheromone components were spaced horizontally at 0, 3, 5 and 15 cm on plastic moth pheromone traps. The high dose attracted more males than the medium and low dose. Males were highly attracted at 0 cm separation but spacing decreased the catch and at 15 cm spacing there was no catch. The behavioural effect of separating ph. components was further studied on the spruce bark beetle, *Ips typographus* L., in the field. The attraction of *I. typographus* to traps baited with the two aggregation pheromone components (cis-verbenol and 2-metyl-3-buten-2-ol) separated, was investigated in a Norway spruce clear-cut. The pheromone components were separated both vertically (0-112 cm) on extended Lindgren (19 funnel) traps and horizontally (0-80 cm) on modified windvane traps. *I. typographus* was strongly attracted when the two components were released from the same point but spacing between components decreased the trap catch. However, at 16 cm distance, in both the vertical and horizontal test, the trap catch was not much different from the positive control (0 cm separation). The effect of odour source spacing in the field on *I. typographus* was also studied with regards to repellent non-host volatiles (NHV). In this test, the aggregation pheromone were separated from a blend of repellents (*trans*-conopthorin, 1,8-cineol, 3-octanol, 1-octen-3-ol, 1-hexanol and verbenone) using the same experimental design. NHV showed strong inhibitory effect up to 48 cm spacing but there was not much effect at 80 and 112 cm separations.



**Key words:** Odour, plume structure, plume interactions, *Spodoptera littoralis*, *S. litura*, *Ips typographus*, Lepidoptera, Noctuidae, Coleoptera, Scolytidae, PID, orientation, sex pheromone, NHV



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# 1 INTRODUCTION

Odour plumes or olfactory cues are very important for insects in many different ways in their life and ecological interactions, including mate finding, host or food source, escape from their enemies and localizing suitable oviposition sites (Anderson et al., 2003; Riffell et al., 2008). The ability to respond to chemicals exists in all living creatures. How insects respond to odour has helped us to understand the mechanisms that insects use to navigate (Vickers, 2000). To find the position of a sex pheromone source, insects are dependent on blend composition and plume structure (Willis, 1984; Vickers and Baker, 1997). It is of basic interest to determine the physical dimensions of odour source interaction for various species and signal types. The production of sex pheromones in insects is often measured in trace amounts and pheromones are behaviourally active at very low concentrations. But on the other hand, non-host volatiles (NHV) have the potential to inhibit insect response to the pheromone (Byers et al., 1998; Borden et al., 2000; Borden et al., 2001a; Borden et al., 2001b).

How can we analyse a plume structure and how can we measure the overlap or interaction between different plumes? It is not simple to measure plume parameters, but plumes may be visualized by smoke or soap bubbles. Justus et al (2002) measured plumes with a Photo Ionization Detector (PID) and showed that the continuous plume spreads from 30-200 mm in width, downstream of the odour source. However plume pulses expand vertically when moved downstream and also seem merging more with other odour plumes when expanded to 200 mm. In another study, a PID was also used to investigate odour plume structure (Vetter et al., 2006). In this study when the PID was moved perpendicularly from the central line of the plume, the signals decreased quickly. At 10 mm height above the surface at 10 mm distance from the odour source and at 20 mm height above surface with 20 mm distance, no odour detection was observed (Vetter et al., 2006). A PID quantifies the cumulative concentration of all detectable gases and has been used to improve our understanding of the structure of insect pheromone plumes (Justus et al., 2002).

The considerable progress has been made in understanding the fine scale structure of dispersing plumes in the atmosphere (Murlis and Jones., 1981; Jones 1983; Mylne and Mason 1991). However, with respect to animal behaviour, the link between cause and effect is not easy to understand. In work on insect orientation, it is difficult to

correlate directly the fine-scale features of the plume with moment-to-moment behavioural response of insect during experiment and how the distribution of odours influences the navigation of insects to an odour's source. Lewis & Macaulay (1976) described differences in the flight pattern made by moths towards a pheromone source and, using smoke, they described corresponding differences in the shape and structure of a plume issuing from the same source position. With an understanding of the generation and structure of odor plumes, we may now ask what features of the plume are detected and used by insects in walking and flying toward the odor's source. The insect locates the source following walking orientation (Charlton and Carde 1990). This line of inquiry requires knowledge of the mechanisms of insect orientation to odours and the sensory inputs that mediate them (Kennedy 1977). Insects produce and respond to odor plumes that differ markedly. Bark beetles (Scolytidae) can generate a large plume with an effective source, the size of an entire tree trunk with hundreds of odour-emitting beetles. Moths may release pheromone as both a vapor and an aerosol (Krasnoff and Roelofs 1988). Such different plume structures certainly exert different selective pressures. Insects with differing phylogenies and therefore independently evolved orientation mechanisms to assure multiple solutions to the location of odour sources (Murlis 1986).

The noctuid moths, *Spodoptera littoralis* and *S. litura* are well known and serious pests, causing enormous losses to many economically important cultivated crops such as cotton, soybean, groundnut, tobacco and vegetables (Brown and Dewhurst, 1975; Qin et al., 2004). In previous years the pests have mainly been controlled by insecticides. Application of toxic chemicals cause environmental pollution, health hazards, insect resistance and danger for beneficial insects (Ahmad et al., 2008). Now various studies have been performed to control these insect by sex pheromone attraction and mating disruption techniques (Armes et al., 1997). In the two species, the main component of the sex pheromone was found to be ZE-9,11-tetradecadienyl acetate (ZE-9,11-14:OAc) (Nesbitt and Francke, 1973; Campion et al., 1980). In addition, the compound ZE-9,12-tetradecadienyl acetate (ZE-9,12-14:OAc) has been identified from the female gland extract (Dunkelblum et al., 1982). In 2003, a walking bioassay was done in Sweden by using only the main component ZE-9,11-14:OAc to check the attraction of *S. littoralis* males. The study showed that more than 90% of the males reached 2 cm within the source when one female equivalent gland extract or

1000 ng of the pheromone component was used as the odour source (Anderson et al., 2003). The spruce bark beetle, *Ips typographus* (L.), has also long been known as the most destructive pest of Norway spruce, *Picea abies* (L.) (Vité and Francke, 1976). In Europe *I. typographus* regularly mass attacks standing trees (Postner, 1974). In qualitative chemical analyses of *I. typographus*, a blend of (4*S*)-*cis*-verbenol, (R/S)-ipsdienol and 2-methyl-3-buten-2-ol as the aggregation pheromones were found (Bakke et al., 1977; Schlyter et al., 1987a). Birch trees release some volatiles which act as inhibitors to *I. typographus*, called non-host volatiles (NHV). Various studies have been done on *I. typographus* to check the effects of NHV (e.g. *trans*-conophthorin, 3-octanol, 1-octen-3-ol, 1-hexanol, *E*2-hexen-1-ol and *Z*3-hexen-1-ol) and other compounds (e.g. verbenone and 1,8-cineol) on inhibition of attraction (Byers, 1988; Schlyter et al., 1988; Zhang et al., 1999; Zhang et al., 2000; Zhang, 2003; Zhang and Schlyter, 2003; Zhang and Schlyter, 2004).

However, both in the moth and beetle systems there is not much work on the theoretical and biological consequences of physically separating the release points of pheromone components or the release points of attractants and repellents to check the plume interactions. Characterization of the chemical signal environment allows us the determination of when and where olfactory mediated behaviours control ecological interactions (Riffell et al., 2008). Why research on the interactions of odour plumes is of interest to chemical ecology and its application? From a practical point of view it may be that "confusion methods," which disrupt moth pheromone communication, are more effective if synergistic components are released separately in disjointed blends than if full blends are released (Byers, 1987). The proper use of NHV for forest protection is obviously dependent on range of action of NHV blends and components and their active range of inhibition (AIR) (Zhang and Schlyter, 2003). In general the aim of this study was to investigate the mechanism that insect use to locate many resources important to survival by tracking along wind-borne odour plumes to their source but in particular,

I had the following objectives:

(1) To analyse plume structure and interaction and such as width and overlap between two plumes.

(2) To compare the sensitivity (response) of insects at different doses of synthetic sex pheromone components and possible physical interactions of odour plumes and relate the insect response to odour plume structure measured with a Photo Ionization Detector (PID).

(3) To estimate the relationships between the distance of separation (horizontal and vertical) of release points of pheromone components or release points of attractants and repellents, to the attraction or inhibition of insects.

I studied in the laboratory the behavioural response of *S. littoralis* males to the two components of the female sex pheromone blend, released from two separate dispensers at various distances from each other in a walking bioassay. Field study on *S. litura* was done in Pakistan with female sex pheromone components released from separate dispensers. The behavioural effect of separating the aggregation pheromone components and separating the pheromones from a blend of non-host volatiles was also studied on *I. typographus* in the field, using Lindgren traps (Lindgren, 1983) and modified windvane traps with separations both vertically and horizontally.

My behavioural results show that release of individual pheromone components from different release points are more disruptive of olfactory communication than a blend mixture of pheromone components and this method may be helpful for mating disruption to control insects. Field experiments results show that NHV effect insect attraction with an active range of inhibition (AIR).

# 2 MATERIALS AND METHODS

## 2.1 Insect Species

### 2.1.1 The moths (*Spodoptera littoralis* and *S. litura*)

#### 2.1.1.1 Biology

The genus *Spodoptera* (Noctuidae) has 26 known species (Mochida and Okada, 1974). *S. littoralis* is a widely distributed pest on a variety of crops throughout a large part of the warm-temperate and subtropical regions (Brown and Dewhurst, 1975). S. littoralis containing at least 87 species of economic importance including cotton, lucerne, soyabeans, Trifolium and vegetables (Salama et al., 1970)., It is also called Egyptian cotton leafworm and it is considered to be closely related to *S. litura* and some scientist also say that *S. litura* is a sibling species of *S. littoralis*. *S. litura* was first recorded from the Nelson district as a pest of tobacco (Cottier, 1955). In 2003, it had an outbreak in Pakistan throughout the cotton belt and it devastated the cotton crop (Ahmad et al., 2007). Now it has become a serious leafworm of cotton crop in Pakistan and Egypt (Ahmad et al., 2008). *S. litura* has about 150 host species (Rao et al., 1994). These include cotton, cruciferous vegetables, cucurbits, groundnut, maize, potatoes, rice, soybean, tea, tobacco, *Capsicum annum* (hot pepper), *Colocasia esculenta* (L.), schott and *Phaseolus vulgaris* in Southwest Japan and Southeast Asia (Maeda et al., 1990; Qin et al., 2004). Sometimes it has been found to cause 26–100% yield loss, particularly to cotton crop (Dhir et al., 1992).

#### 2.1.1.2 Management

Control has depended mostly on application of various insecticides. An integrated approach is required for the control of this pest because it has developed resistance against a range of insecticides and because other control measures are inadequate when applied alone (Ramakrishna et al., 1984; Armes et al., 1997). Mating disruption, lure and kill, and mass trapping are environment friendly techniques to control the population (McVeigh and Bettany, 1987).

## 2.1.2 Bark beetle (*Ips typographus*)

### 2.1.2.1 Biology

The spruce bark beetle, *Ips typographus* (L.), has long been known as the most important pest of Norway spruce, *Picea abies* (L.) (Vité and Francke, 1976). In Europe, the spruce bark beetle regularly cause mass attacks on standing trees (Postner, 1974). This bark beetle is considered to be one of the most destructive pest of spruce on the continent of Europe and across Russia to Japan (Byers, 1989). In the genus *Ips*, males initiate colonization of the host and produce the pheromone which attracts other males and females (Schlyter and Löfqvist, 1986). Extensive injury to forests resulting from wars, fires and storms, has at numerous times made possible the build-up of high populations of the pest which caused excessive secondary damage (Schlyter et al., 2006). In addition to damaged trees, this species also attacks healthy trees . The ability to breed in very fresh bark, coupled with the habit of continuing to feed in the bark on completion of development, makes the insect a serious pest of spruce forests. (Wermelinger, 2004). This beetle mostly attack Norway spruce, occasionally Scots pine, larches and firs. Adults are 4.0-5.5 mm long, cylindrical and robust, black or brownish-black. Elytral declivity is slightly shiny, with 4 teeth on each margin side. The third tooth is the biggest and club like on its top (Kolk and Starzyk, 1996). The egg is yellowish-white. The larva is white and legless. The pupa is also white. Adults overwinter in litter or under the bark. Occasionally overwintering may occur in larval or pupal stage. Adults of the first generation are active in spring and start colonization of the host tree phloem by dispersing adults that were overwintered in the litter (Byers, 1989). Dependent on weather conditions, this species has one or two generations per year, with one or two sister generations. After mating, 2-3 females tunnel vertical egg galleries from the nuptial chamber, which are not visible on the wood. Eggs are laid every 2 mm. Larvae chew galleries horizontally, and after feeding for 3-4 weeks, they pupate in pupal chambers in the bark. Young adults have maturation feeding under the bark making characteristic horn like tunnels well visible in the wood.

### 2.1.2.2 Management

The aim of managing bark beetles is to minimize attacks on living trees. The measures most commonly applied for this purpose are clearing windthrows, sanitation felling of

infested trees, and the installation of trapping devices (Wermelinger, 2004). Traps are more often used to prevent attacks on living trees than to diminish *I. typographus* populations (Niemeyer et al., 1990). Conventional insecticides are mostly used to protect stored timber. Their application varies according to the legislation in different countries. Systemic chemicals have been reported to protect single susceptible trees (Dedek and Pape, 1990).

## 2.2 Laboratory Experiments

### 2.2.1 Odours and volatile sources

In the behavioural experiment with *S. littoralis* males, female sex pheromone compounds were used. The main component, ZE-9,11-14:OAc (98% purity) and the minor component, ZE-9,12-14:OAc (98 % purity) were diluted in hexane to make two doses, 10 ng and 1 ng of the main component as well as 0.1 ng and 0.01 ng of the minor component for laboratory bioassays. For PID measurements, the aggregation pheromone components of *Ips typographus*, 2-methyl-3-buten-2-ol (MB), as well as *cis*-verbenol (cV), in a ratio of 50:1 were used by diluting cV in MB. The MB/cV mixture was added to 150 µl glass capillaries (Drummond Scientific Co., Broomall PA, USA) for PID (Figure 1) measurements. The capillaries were filled up to 3 cm hight. Capillaries were kept vertically during measurements and they were kept open to allow a constant release of volatiles during measurements. The capillaries were changed when ½ cm solution was left in the capillaries. Capillaries were prepared 5 minutes before measurement.

### 2.2.2 PID (Photo Ionization Detector)

The Photo Ionization Detector (PID) (RAE Systems Inc.) (Figure 1) utilises an ultraviolet light source to detect ionizeable gas molecules, and is commonly deployed in the detection of volatile organic compounds (VOCs). PID sensors are regarded as the preferred choice for the detection of VOCs and they offer very fast response, high accuracy and good sensitivity. A PID measures VOCs and other gases in low concentrations from ppb (parts per billion) up to 10,000 ppm (parts per million or 1% by volume). Ionization occurs when a molecule absorbs high energy UV light, which

excites the molecule, and results in temporary loss of a negatively charged electron and the formation of positively charged ions. The gas becomes electrically charged. A PID is capable of giving instantaneous readings and monitoring continuously (Justus et al., 2002). A PID was used to measure the plumes width and overlap in an open-arena olfactometer described below.

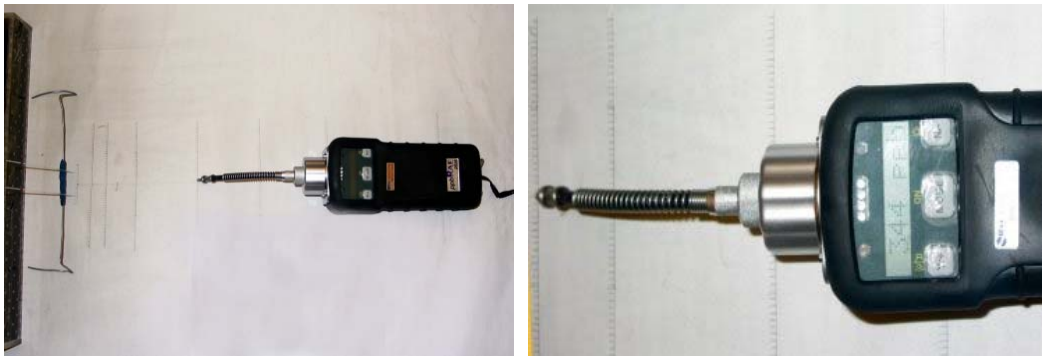

Figure 1. Photo-Ionization Detector on the bioassay surface with pencil marks for positioning. Photo: Muhammad Binyameen

### 2.2.3 PID measurements

PID measurements were done in an open-arena olfactometer measuring 60 × 60 cm (Figure 2). Charcoal filtered air was pushed through a baffle with spaced 2 mm holes generating a constant airflow (0.5 m/s) over the floor of the olfactometer (Schlyter et al., 1994). An exhaust at the other end of the surface sucked out the air from the olfactometer. A central line was marked on the paper sheet by lead pencil from the source and its length was 48 cm. This line was also marked at every cm from the odour source and on every 3$^{rd}$ mm on both sides of the line up to 60 mm. First the measurements were done with one capillary at 2, 4, 8, 16, 24, 32, and 46 cm away (downwind) from the point source and at every 3 mm on both sides of the central line on these points, to measure plume width and concentration. The odour plumes from two capillaries, either placed together or separated by 2, 3 and 5 cm, were measured in the same way. Recordings started 1.5 minutes after placing the PID at every point to stabilize fluctuations, and then five readings were recoded during 10 seconds, every reading after every two seconds and means were calculated in Microsoft Excel 2003. The temperature and humidity were in the range of 22-24 °C and 60-70% respectively. The PID was calibrated daily before experiments using isobutylene gas to reduce variation in readings.

Figure 2. Measurements of plumes with PID in the open-arena olfactometer.

Photo: Muhammad Binyameen

### 2.2.4 Insects

Male *S. littoralis* for the olfactometer experiment were obtained from a laboratory culture reared for many generations on an artificial diet (Hinks and Byers, 1976) at SLU, Alnarp. The culture had been supplemented with moths imported from Egypt. The culture was maintained at 25 °C, 65-70 % RH, and 16:8 h light:dark photoperiod. Males and females were separated at the pupal stage and allowed to emerge in different climatic chambers to exclude any pre-exposure of males to female sex pheromones.

### 2.2.5 Behavioural Bioassay

The behaviour of 2-3 days old adult male *S. littoralis* was studied in the olfactometer described above (Figure 3) (Schlyter et al., 1994). Earlier studies have confirmed that *S. littoralis* males behave normally (i.e. move upwind) to female sex pheromone in the olfactometer (Hartlieb et al., 1999; Anderson et al., 2003). The odour solutions were pipetted on pieces of filter paper (0.5×1 cm), by adding 10 µl on each filter paper, and after solvent evaporation, the stimuli were placed at the upwind end and 3-4 mm above the floor of the olfactometer. The filter papers were replaced after every 20 minutes. Two doses (10 ng, 1 ng and 0.1 ng, 0.01 ng of major and minor compounds, respectively), by placing filter papers together at upwind central point and then separating at 2, 3 and 5 cm were tested. The experiment was carried out about 2 h into the scotophase in red light, at 22-24 °C and 60-70 % RH. Males were transferred individually to glass tubes (80 mm, i.d. 23 mm, one end covered with a plastic net), and transported to the experimental room 30 minutes before the start of bioassay. Males were released individually 46 cm downwind from the odour sources. Three behavioural steps were recorded: orientation (= activation), orientation half

way, and 2 cm from the source. The time for activation and the time it took to reach 2 cm from the source (i.e. major component) were also recorded.

Figure 3. Behavioural bioassay setup for *S. littoralis* in the olfactometer (Schlyter et al., 1994). Photo was taken in white light but assay run in red light only. Photo: Muhammad Binyameen.

## 2.3 Field Experiments

### 2.3.1 Odours and volatile sources

For the *S. litura* field test, pheromone red rubber-septa dispensers were loaded with the same two compounds used in laboratory bioassay diluted in hexane. After solvent evaporation, dispensers were seal packed in ALU/PE bags (Möllestörm, Sweden) to carry for field experiment in Pakistan. Three different doses (1, 10, and 100 μg), of the main component, and for the minor component 1% doses of the main component were used. In the field experiment of *I. typographus*, commercial IT ECOLURE Tubus aggregation pheromone dispensers purchased from Fytofarm Ltd, Bratislava, Slovakia, were used. A combination of NHV (*trans*-conophthorin, 1,8-cineol, 3-octanol, 1-octen-3-ol, 1-hexanol, and verbenone), in separate dispenser vials, was used as repellent stimulus. Synthetic aggregation pheromone (MB: cV, 50:1) dispensers were prepared by ourselves and used in pheromone component spacing. All chemicals, sources, release devices and release rates (Schlyter et al., 1989; Zhang and Schlyter, 2003), are listed in Table 1.

### 2.3.2 Protocol for field trapping experiments of *Ips typographus*

Four field trapping experiments of *Ips typographus* were conducted from 3$^{rd}$ of May to 3$^{rd}$ of June 2008 at Sporrakulla and Parismåla, Sweden. Lindgren multiple funnel traps (Lindgren, 1983) were used both in the vertical and horizontal spacing experiments. In the vertical experiment the 12-unit traps were extended to 19 units (funnels), and in the horizontal experiment the traps were shortened to five units and placed on a windvane attached to a tripod. Dispensers (both pheromone bait and NHV) were either put under an inverted 250 ml plastic cup painted light grey

(attached to Lindgren traps) or hung directly (in case of commercial pheromone dispensers) on the funnel. The position of treatments were initially randomized and then systematically repositioned after each replicate on a procedure of complete randomization, so that each treatment appeared in each location at least once (Byers, 1991). The beetle collection and dispenser rotations were carried out when 100 beetles or more were caught by all traps collectively. Each replicate lasted from one hour to few hours, depending on flight activity. In each experiment, 14 replications were done except in experiment 1 (see below) in which 16 replications were performed.

### 2.3.3 Vertical odour source spacing

#### (a) Expt. 1. Vertical spacing between NHV and pheromone:

In this experiment, commercial IT ECOLURE Tubus aggregation pheromone dispensers of *Ips typographus*, purchased from Fytofarm Ltd, Bratislava, Slovakia, were used versus a combination of repellent non-host volatiles (NHV) (described above). Lindgren 19 funnel traps were used. The pheromone and NHV dispensers were separated vertically at 0, 16, 32, 48, 80 and 112 cm, which corresponded to a separation of 0, 2, 4, 6, 10, and 14 funnels, respectively (distance between funnels: 8 cm). Three traps were considered as control traps: Blank control (no dispenser), positive control with only pheromone on the central funnel ($10^{th}$), and the negative control with 0 cm separation between NHV and pheromone. In all treatments with dispensers separated, both the pheromone and the NHV dispensers were moved equal distances in opposite directions from the central funnel. There was only one trap having 0 cm separation between these two types of dispensers but for the other five separations there were two traps in all replications, i.e. one trap having the pheromone dispenser upward and the second one having the pheromone dispenser downward.

#### (b) Expt. 2. Vertical spacing between pheromone components:

The effects of vertical separation of the aggregation pheromone components (Figure 4) of *I. typographus* (MB and cV) were studied at the same distances as in the experiment of vertical spacing between pheromone and NHV. The experimental procedure was also the same. A total 14 Lindgren traps were used including the four control treatments blank, only MB, only cV and both pheromone components.

TABLE 1. CHEMICALS, SOURCES, PURITIES, RELEASE RATES, AMOUNTS, AND DISPENSERS USED IN FIELD TRAPPING EXPERIMENTS OF PLUMES INTERACTION OF AGGREGATION PHEROMONE COMPONENTS AND NHV IN *Ips typographus*, SWEDEN, 2008 AND PHEROMONE COMPONENT INTERACTIONS IN *Spodoptera litura*, PAKISTAN, 2008

| Attractant/inhibitors | Source | Purity % | Release rate (mg/day) | Amount (µl) | Dispenser (A) |
|---|---|---|---|---|---|
| ZE-9,11-14: OAc | Prof. Charles Decoins, France and Prof. Heinrich Arn, Switzerland | 98 | Diff. doses were used | 1-100 ug | Pippeted on red rubber septa (B) |
| ZE-9,12-14: OAc | | 98 | Diff. doses were used | 0.01-1ug | Pippeted on red rubber septa (B) |
| 2-methyl-3-buten-2-ol | Aldrich, USA | 97 | 57± 0.8 | 200 | 2 mm diameter., 3-ml vial |
| *cis*-verbenol | Borregaard, Norway | 99 | 1± 0.05 | 40 mg | 9 mm diameter.hole,2-ml Vial |
| IT ECOLURE Tubus | Fytofarm Ltd | 98 | 95 ± 0.3 | Commercial | Polyethylene (PE) tube |
| 1-hexanol | Aldrich, USA | 98 | 4.1± 0.13 | 200 | Open #730 PE vial |
| 3-octanol | Acros, USA | 98 | 2-3 | 150 | 8 mm hole#731 PE vial |
| 1-octen-3-ol | Acros, USA | 98 | 2-3 | 150 | 8 mm hole#731 PE vial |
| *trans*-conophthorin | Pherotech, Canada | 98 | 5 | 50 | Closed #730 w. capillary, 1 mm diameter |
| verbenone | Aldrich | 99 | 1.1± 0.03 | 2 x 150 | Open 2 x 730 PE vial |
| 1,8-cineole | Aldrich | 99 | 18 ± 0.76 | 100 | Closed #730 PE vial |

(A)= Dispensers were polyethylene vials that were either closed or closed but with a drilled hole through caps (x-mm-diam.hole) or with a capillary through the lid (w. x-ul cap.) or open. Vials were from Kartell, Italy, with the #730 being a 1-ml vial and with #731 being a 3-ml vial.

(B) Red rubber septa (PheroNet) were pippeted by diluting the compounds in heptane and after evaporation of solvent, were stored in refrigerator until application in the field. Red rubber septa were from Phero. Net AB, Lund, Sweden.

Figure 4. Lindgren 19 funnels trap and changing of dispenser cups in the vertical experiment. Photo: Fredrik Schlyter.

### 2.3.4 Horizontal odour source spacing

#### (a) Expt. 3. Horizontal spacing between NHV and pheromone:

In this experiment, 5 unit Lindgren traps were connected to windvanes that were placed on tripods (Figure 5A). These windwanes were constructed by Jörgen Lantz, a mechanic at Chemical Ecology, Alnarp, Sweden. Windvanes were used in order to ensure a constant distance between the odour plumes despite changes in wind direction. An aluminium pipe of about 1 m length and 8 mm diameter was fixed horizontally at the central funnel (3$^{rd}$), in a right angle compared to the windvane (and thus to wind direction). The same type of dispensers as in vertical spacing between NHV and pheromone were used and they were separated at 0, 16, 32, 48 and 80 cm in one direction from the centre of the central funnel. The attractant was always placed on the central funnel in order to actually catch the attracted beetles. Three traps were considered as control traps, (blank control, only pheromone dispenser as positive control, and 0 cm separation as negative control). The dispensers, except at 0 cm spacing, were hung on the aluminium rod. After hanging the dispenser cups on the aluminium rod, the hangers were fixed between two plastic bands to assure that wind did not change the dispenser position. Seven traps were used in this experiment. The position of treatments were initially randomized and then systematically repositioned after each replicate on a procedure of complete randomization, so that each treatment appeared in each location at least once (Byers, 1991).


**A** **B**

Figure 5. **A.** Modified Lindgren 5 funnel trap used in horizontal spacing between NHV and pheromone. Pheromone dispenser is in the central funnel and NHV under shady hat. **B.** Modified Lindgren trap used in horizontal spacing between pheromone components (placed under shady hats). Photo: Muhammad Binyameen

### (b) Expt. 4. Horizontal spacing between pheromone components:

In this experiment the same dispensers as in experiment 2 were used. The experimental design was similar to the one described in experiment 3. The pheromone components were spaced at same distances as in horizontal spacing between NHV and pheromone dispensers, but in this case both components were moved out from the trap, in opposite directions, when they were separated, as shown in Figure 5B. In this experiment, apart from rotating the treatments between replicates, we also switched sides of the two components to control any potential directional preferences. A total of seven traps were used and three traps were control traps, (only MB, only cV, and 0 cm separation between components as positive control). Treatment randomization was the same as in other experiments.

## 2.3.5 *Spodoptera litura* horizontal pheromone component spacing

A field study on *Spodoptera litura* was conducted from 20$^{th}$ July to 4$^{th}$ August at Vehari District, Punjab, Pakistan with the co-operation of Ayyub Agriculture Research Institute (AARI), Pakistan and Nuclear Institute for Agriculture and Biology (NIAB), Pakistan. Plastic Moth traps (Figure 6) were used in this experiment. Two components of synthetic female sex pheromone of *S. littoralis* were used in this study at three different doses, 1, 10, and 100 μg of the main component (ZE-9,11-14:OAc (98% purity) and the minor component, ZE-9,12-14:OAc (98 % purity) at 1 % of the

main component to check the effect of *S. littoralis* pheromone on the behaviour of *S. litura*. These component dispensers (as discussed above 2.3.1 Odours and volatile sources) were spaced horizontally from the central point of the trap. The distances between dispensers were 0, 3, 5, and 15 cm. There were 2 traps for each separation at 3, 5 and 15 cm distances for every set of 9 traps. Three traps were as control (only main component, only minor component, and 0 cm separated as positive control). Three sets of traps were launched at the same time for each dose. A total of 27 traps were used for each dose and 81 traps for the whole experiment. Three replications were completed for each dose and for each set of traps. The position of treatments were initially randomized and then systematically repositioned after each replicate on a procedure of complete randomization, so that each treatment appeared in each location at least once (Byers, 1991).

Figure 6. Plastic moth field trap with 15 cm separation for *S. litura* in a cotton field. Photo: Muhammad Binyameen

## 2.4 Statistical analysis

The trap catches of field experiments of *I. typographus* were converted into proportions of total number of beetles captured within each replicate. The data was then analysed by statistical software "MINITAB 15 English", ANOVA analyses were done with General Linear Model followed by Tukey's test. The results obtained from the behavioural bioassays of *S. littoralis* were also analyzed by this method. "Effect Sizes" of data from all field and laboratory experiments except PID measurements were calculated with Coe Hyperstat Effect Size Calculator, Microsoft Excel Version 5, following Cohen's equation (Cohen, 1992). Wrong or imprecise conclusions might be drawn from hypothesis testing results if effect sizes are not judged in addition to statistical significance. In particular, *p*-values are insufficient for decision-making; if an experiment includes a sufficient number of subjects. The effect size is a unitless measure of the strength of the relationship between two variables obtained by dividing

the difference between their means with the pooled standard deviation for those means. XY-Scatter graph was plotted with effect sizes and also linear regression lines were added to observe the trend against spacing.

# 3 RESULTS

## 3.1 Olfactometer plume measurements by PID

In the one-plume measurement, the PID showed the highest value of 7,380 ppb at 2 cm from the odour source at central line and the plume was 54 mm wide at this point (Figure 7B). At 46 cm from the source, it showed the lowest value of 235 ppb at the central line and plume width was 36 mm at this point. When two dispensers were placed together (0 cm separation) the resulting plume, at 2 cm distance from the source, gave more than three times higher values (23,580 ppb) than the plume from a single dispenser. Plume width at this point was 114 mm, which was also more than twice the width of the plume from a single dispenser. At the maximum distance of 46 cm from the dispensers, the concentration on the central line was 1,035 ppb and width of the plume was 84 mm (Figure 7C). When two plumes were separated 2 cm from each other (i.e. 1 cm at each side of the central line) and recordings were done from 2-46 cm from the source, it showed very low value at 2 cm (200 ppb), but downwind the concentration increased, and the highest value (3,050 ppb) was observed at 8 cm from the source on the central line (indicating plume overlap). Further downwind, plume concentration decreased and was only 35 ppb at 46 cm distance. The width of the plume at 2, 8 and 46 cm from the odour sources was 66, 72 and 42 mm, respectively (Figure 7D). At 3 cm separation between the dispensers, the PID showed 20 ppb at 2 cm from the source on the central line and the highest value (370 ppb) was found at 16 cm distance on the central line (indicating plume overlap). Again, concentration decreased further downwind and was 160 ppb at 46 cm from the source on the central line. At this separation the width of plumes at 2, 16, and 46 from the central line was 75, 78, and 60 mm, respectively (Figure 7E). When the odour sources were separated 5 cm, the plumes were completely separated and the PID always showed 0 ppb at the central line on each measuring point. The largest plume width (96 mm) was recorded at 8 cm from the odour source and the lowest was 66 mm at 46 cm from the odour source (Figure 7F).

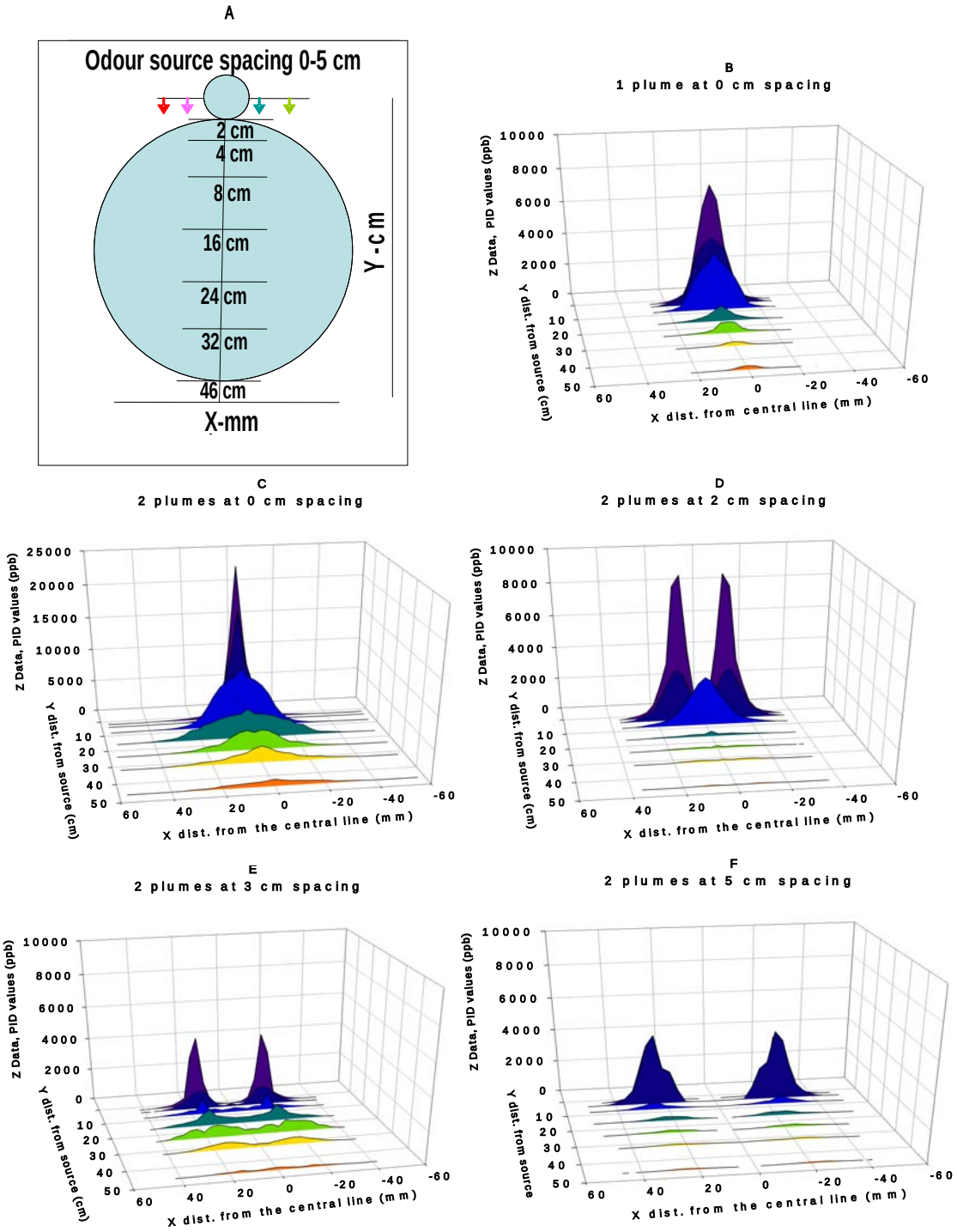


Figure 7. **A**) Schematic drawing of the olfactometer in which measurements were performed. **B**) Measurement of the plume from one odour source. **C-F**) Measurements of the plume(s) from two odour sources, separated by 0, 2, 3, and 5 cm, respectively. Note: In all graphs, the scale on the Y-axis is in cm and the X-axis is in mm, which exaggerates the plume width. The values on the Z-axis are in ppb.

## 3.2 Olfactometer tests with *Spodoptera* pheromone component spacing

A total of 400, 2-3 day old virgin males of *Spodoptera littoralis*, were used to test the interaction of female sex pheromone components in the open arena olfactometer by studying the walking response of males. Throughout the experiment it was observed that the male response was dependent on perception of both synergizing components. When males were released 46 cm downwind from the odour source on the central line, they walked straight upwind but before reaching the source they became confused, probably when the plumes became segregated. At this point, they stopped walking and after some seconds started zigzag walking, they almost always decided to go towards the main component and reached within 2 cm from the odour source.

Almost 100% of the males were activated, ca 85 % walked halfway, and ca 65 % reached within 2 cm of the pheromone source at 0, 2, and 3 cm separations, when the high pheromone dose (10:0.1 ng, major:minor component) was tested. Thus, there was clearly not much difference in response at 0, 2 and 3 cm separations (Figure 8A and A1). At 5 cm separation, 60 % oriented, 46 % walked up half way and 33 % reached within 2 cm from the odour source. At 10 times lower dose, males showed almost 80% orientation, 60% walked halfway and 40-50 % reached within 2 cm from the source at both 0 and 3 cm separation. At 2 cm separation the orientation was about 70 % and 50 % walked halfway, while within 2 cm from the source the result were approximately same as in 0 and 3 cm separations. At 5 cm separation with low dose, 58 % oriented, 37 % walked halfway and 28 % of the males reached within 2 cm from the source (Figure 8B and B1).

In contrast the time taken by the males for orientation showed not much difference in orientation time at any spacing but time to reach within 2 cm from the source was greatly affected. At the low dose, the time to reach the “2 cm” step increased strongly with spacing (Figure 9). Interestingly at the high dose, the trend was opposite.

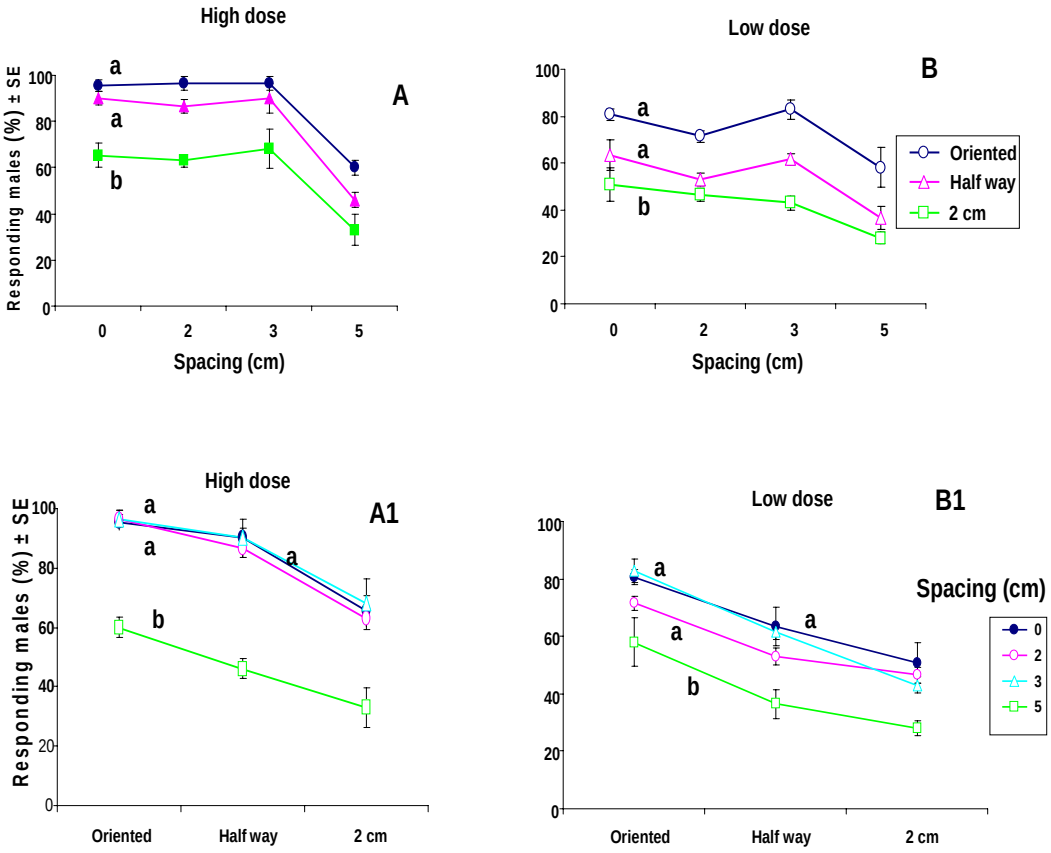


Figure 8. Behavioural response of male *Spodoptera littoralis* in the olfactometer to spacing of pheromone components (ZE-9,11-14:OAc as main component and, ZE-9,12-14:OAc as minor component), two doses (10 ng, 1 ng of main component and 0.1 ng, 0.01 ng of the minor component). Three behavioural steps were recorded: **Orientation)** Males were activated by the odour plumes and started to walk towards the odour source. **Halfway)** males walked halfway up to the odour source, and **2 cm within the source)** males reached within 2 cm from the odour source. 10 males were used as sample size and maximum 7 replications were done. **A)** The three behavioural steps with component spacing at high dose (responding males % plotted against spacing). **B)** The three behavioural steps with component spacing at low dose (responding males % plotted against spacing). **A1)** The three behavioural steps with component spacings at high dose (responding males % plotted against behavioural steps). **B1)** The three behavioural steps with component spacings at low dose (responding males % plotted against behavioural steps). The figures responding males (%) having no letters in common are significantly different at $P < 0.05$ by Tukey's test.

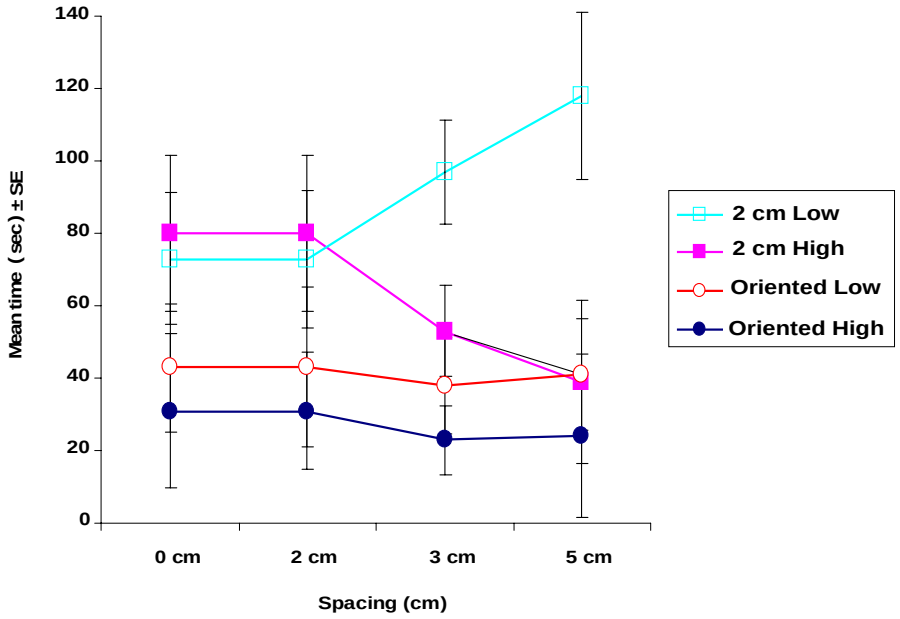


Figure 9. Mean time ± SE taken by each *Spodoptera littoralis* male for orientation and to reach within 2 cm of the source at 4 different spacings of 0, 2, 3 and 5 cm between pheromone components.

## 3.3 Field trapping with *Ips typographus* pheromone and NHV components, spaced horizontally and vertically

### 3.3.1 Interaction between pheromone and NHV

In both vertical and horizontal spacing of pheromone and NHV, trap catch increased with increased distance between NHV and the pheromone (Figure 10 A, B). In vertical spacing, mean proportion of catches (p ± SE) were significantly reduced by NHV at all separations compared to the pheromone catch (Figure 10A). The 0 to 32 cm spacings did not catch significantly more than blank control traps. The catches at vertical spacing from 16 to 112 cm between pheromone and NHV were not significantly different. By the horizontal spacing in a wind-vane trap, there was a more continuous increase of catch from 0 to 80 cm (Figure 10B). In horizontal spacing between pheromone and NHV the catches were not significantly different from 32 to 80 cm separation to the pheromone catch, but catches from 0 to 16 cm

were significantly different to the pheromone catch. 0 cm spacing showed the trap shut-down: it was not different from the blank control.

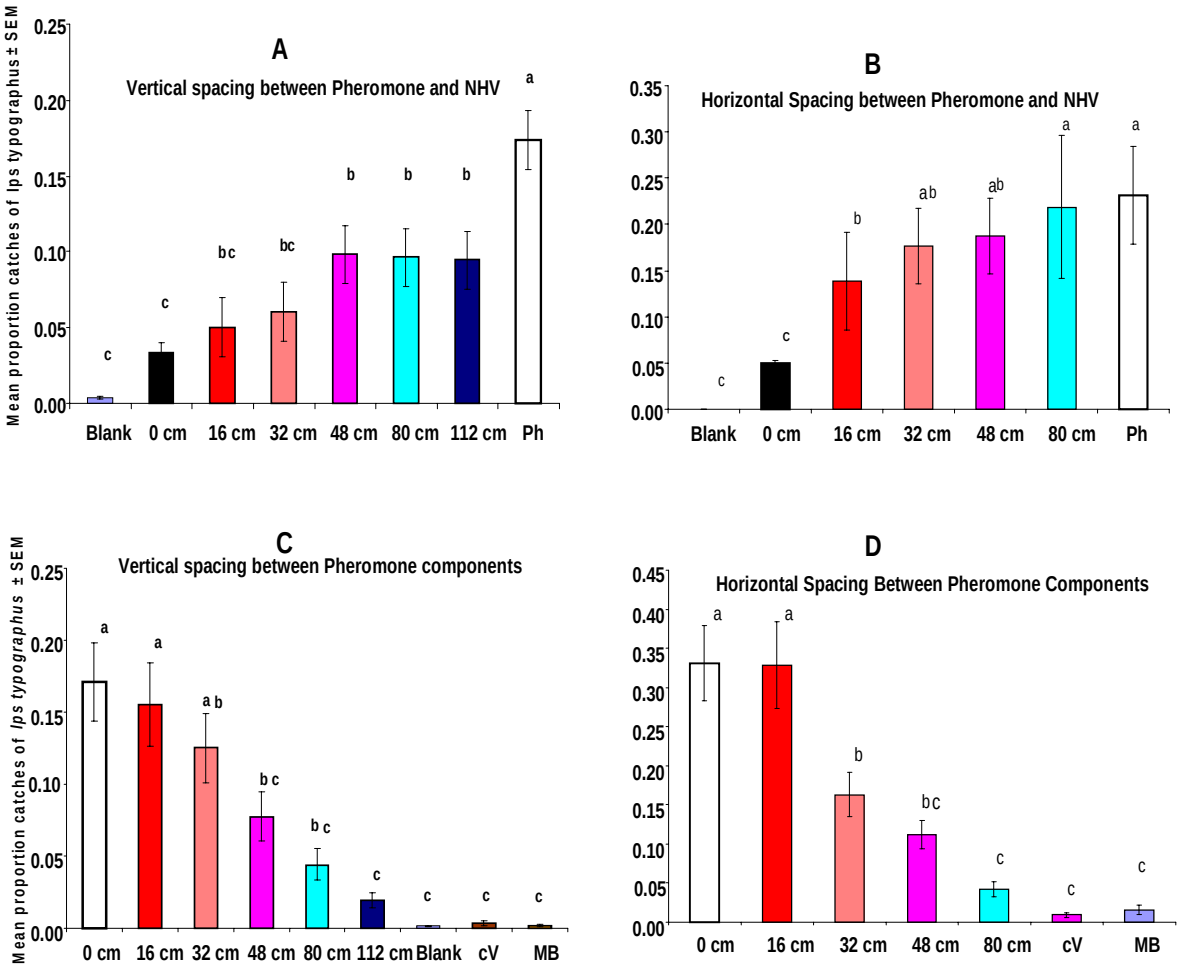


Figure 10. **A-D)** *Ips typographus* catches. **A & B)** Vertical and horizontal spacings between pheromone (MB & cV) and NHV. Blank trap was used as negative control and Pheromone was used as positive control. **C & D)** Vertical and horizontal spacing between pheromone components (MB & cV). Blank, cV, MB were negative controls and Pheromone (0 cm) was positive control but blank was not present in horizontal spacing. The figures mean proportion catches having no letters in common are significantly different at $P < 0.05$ by Tukey's test. In vertical and horizontal spacing 16 and 14 replications were done respectively. Total D.F. for Figures A, B, C and D, were 207, 97, 195 and 97 respectively

### 3.3.2 Interaction between pheromone components

In both modes of pheromone component spacings, there was a close to linear "decrease of catch with increased spacing" (Figure 10 C & D). In vertical spacing between pheromone components (MB, cV) the beetle catches were significantly reduced at 48, 80, and 112 cm spacing (Figure 10C). The closer spacings of 16 and 32 cm were not significantly different from the pheromone catch. The catches at 32, 48 and 80 cm spacing between components were non-significant to each other but 48, 80 and 112 cm spacing showed non-significant difference to the negative controls.

In horizontal spacing between pheromone components, there was no difference between the pheromone trap catch and the 16 cm spacing but all other separation distances were significantly different from 0 and 16 cm separation (Figure 10D). The catches at 48 and 80 cm spacing between pheromone components were non-significant to negative controls of MB and cV.

## 3.4 *Spodoptera litura* pheromone components spacing in field

The *Spodoptera litura* field experiment was done in a cotton field in Pakistan by separating the female pheromone components horizontally on a plastic moth trap. Three different doses were used at different spacings. The high dose caught more *S. litura* males than the medium and low doses at 0 cm spacing, but at 3 and 5 cm spacings the catches were not much different (Figure 11). It shows that spacing had a large effect when a high dose was used or one can say that a high dose confuses the insects more when separated as compared to 0 cm separation. The low dose caught less number of males at 0 and 3 cm separation but higher at 5 cm separation than the medium dose. All three doses caught nothing at 15 cm spacings between pheromone components and also when single components were applied. Effect Sizes for all experiments except PID measurement were calculated by using Cohen's equation as described under the title "Statistical analysis". In all experiments effect size was more than 0.8 except Vertical spacing between pheromone and NHV, which indicates that spacing has strong effect on insect attraction to pheromone sources. Increase in spacing among pheromone components always decreased the catches and increase in spacing between pheromone and NHV always increased the catches (Figure 12A).

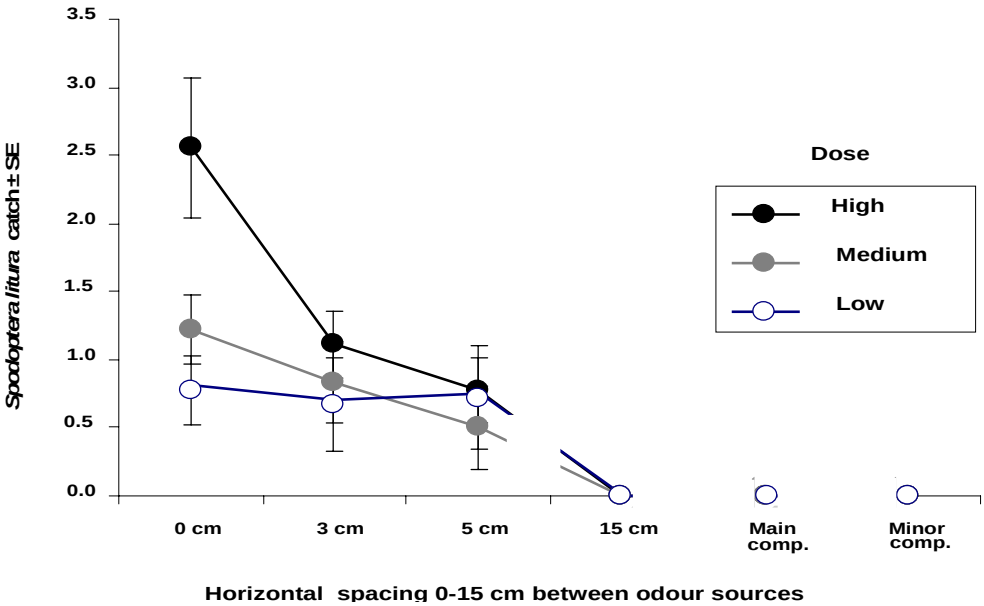


Figure 11. *Spodoptera litura* catches with three (high, medium, and low) doses of two components of female sex pheromone (proportional of components were similar to *S. littoralis* female sex pheromone components), 1, 10 and 100 μg of (ZE-9,11-14:OAc) and 0.01, 0.1 and 1 μg of (ZE-9,12-14:OAc). The components were spaced horizontally at 0, 3, 5, and 15 cm from each other. Two traps having single components were used as control traps. For detail see photo (Figure 6).

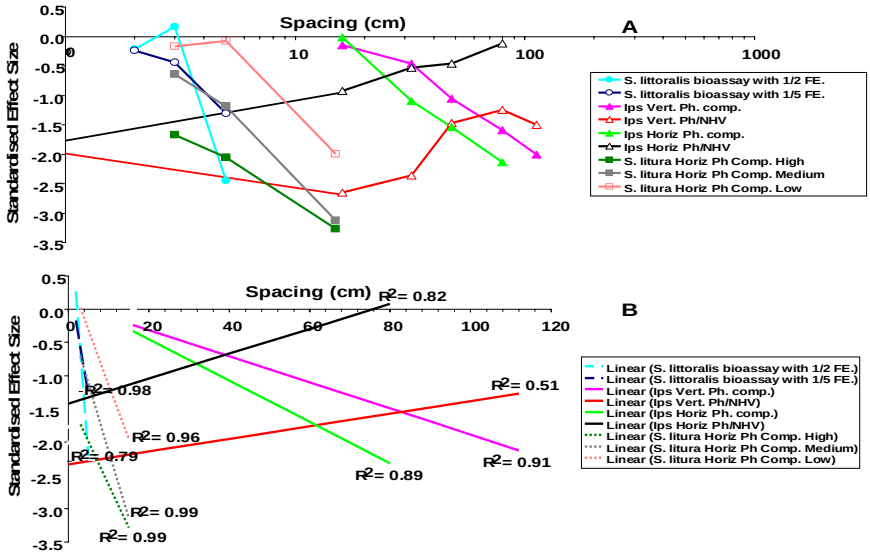


Figure 12. Effect Sizes of *Ips typographus*, *S. litura* field experiments and *S. littoralis* laboratory experiment. **A**) Individual data points for each experiment. **B**) Trendlines with $r^2$ for each experiment (linear scale on X-axis).

# 4 DISCUSSION

My first objective of this study was “To analyse plume structure and interaction and such as width and overlap between two plumes” because measurement of odour plumes will help us to understand the mechanism that insects use to navigate.

A PID can be used to monitor odour concentration at a particular point with high resolution (Justus et al., 2002). I successfully measured the structure of the plumes with a PID. The measurement of plumes with two dispensers at 0 cm spacing PID showed three times higher values at the central line as compared to single dispenser plume and showed that when there were two plumes, horizontal concentration increased and that there may be less volatile loss vertically (perpendicular). The PID measurement demonstrated that by separating the sources 5 cm, both plumes were completely separated from the central line but moths respond well at 46 cm from the source, even though they were released at the central line. The present measurements of plumes are employed in studies of moth orientation to pheromone. During the behavioural bioassay when the pheromone components were separated, the moths first started walk in a straight line but when they approached the odour source, they seemed to be confused, to which side they should go and after some seconds, decided to go toward the main component. This observation clearly shows that activation and halfway walking toward the odour source was not the action of only one component. So, moths oriented towards a blend of both components but 100% of the males that approached 2 cm within the source turned towards the main component.

It has been investigated that plume structure has a significant effect on orientation of *Carda cautella* males (Mafra-Neto and Carde, 1994). It has also been shown that the successful location of a sex pheromone source by a male moth is dependent on blend composition, as well as plume structure (Willis, 1984 ; Vickers and Baker, 1997). In the present study, there was not much difference in response when the high doses of pheromone components were used at 0, 2 and 3 cm spacings between the components. The response to the low dose showed that the wider plume of 3 cm separation has some more response than the narrower plume of 2 cm separation between the compounds.

Linn and Gaston (1981) showed that there was no effect on upwind orientation of cabbage looper moths by separating the pheromone components 12 cm apart and

releasing moths 85 cm downwind from the odour source, but when the separation was only 8 cm between components and moths were released 35 cm downwind, significantly less number of moths approached the sources. However, one of the components was attractive alone while the other was not. The same is true in *Spodoptera littoralis*, where only the main component is active by itself but the minor is not, so the situation is the same in my results, that no difference in orientation and walked up halfway response but less approached the odour source (Figure 8). The male moths were apparently confused when they reached the point where the plumes became separated as if they suddenly passed by a female (blend). It is evident from this behaviour that spacing has a great effect on attraction. Statistical analysis also showed that spacing components up to 3 cm has no strong effect on orientation and walked up half way response but significantly decreased the number of male moths to approach close to the odour source.

My behavioural results thus seem to indicate that male moths are unable to sense that the two pheromone components are released from different sources until the males reach a point where the two plumes no longer overlap. It means, male moths have the ability to resolve closely spaced filaments, which is consistent with other studies (Fadamiro et al., 1999). It has also been shown in the field that the response of beetles was dependent on spacing distance and dose as beetles showed discrimination to different strengths and spacings between pheromone components (Byers, 1987; Schlyter et al., 1987b). My results are similar to earlier findings, where male showed a similar behavioural response (>90 %) to a high dose (1000 ng) of the single component and to 1 fe female extract (Anderson et al., 2003), as in my results high dose (10 ng of the main component in combination with 0.1 ng of the minor component) showed (>90 %) response and it indicates that main component at very high dose of 1000 ng showed the same response as 1 fe female gland extract but in combination with its synergistic component it showed the same response at very low dose of 10 ng only. It also agree with earlier findings of Linn et al. (1986), that synergistic pheromone components do not initiate specific behaviour by themselves, but enhance male response when blended with the main component.

My second aim was “to compare the sensitivity (response) of insects at different doses of pheromone components and possible physical interactions of plume structure”.

Effects of various component ratios on insect behaviour are well known in moths and beetles (Roelofs, 1978). The female *S. littoralis* produce approximately 12.7 ng of the main component and 100 times less of the minor component (Tamaki and Yushima, 1974). So, the low doses of synthetic female sex pheromone components that I used in the bioassay were 0.07 female equivalents. The highest release rate of female *Cydia pomonella*, measured between calling hours was 6.5 ± 3.8 ng/hr (Backman et al., 1997). Earlier findings about release rate of dodecyl acetate from filter papers indicate that the release rate was less than 1 ng/puff or in a second (sec) when 100 μg was applied and also that release rate was proportional to the amount of the stimulus applied on filter paper (Bengtsson et al., 1990). But in the present experiments only 1.01 ng amount of stimulus on filter papers (low dose of both components collectively), was applied, so, here we can say that moths are "much more" sensitive than PID. These insects are also physiologically sensitive to I ng doses of sex pheromone components (Andersson et al., 1993).

In the PID measurements the mixture of MB & cV (50:1) used, was released at a much higher rate than the *S. littoralis* pheromone. The estimated release rate from a capillary was 30 μl/hr. These release rates are very high as compared to the release rates of MB and cV in binary blend measured in windtunnel, that were 57 and 1 mg/day respectively (Zhang and Schlyter, 2003). If we only consider the low release rates (even release rates were very high in PID measurements) of MB & cV, they will be equal to 24,208,333 ng/h but PID showed 0 ppb on central line at 5 cm separation, even the moths are responding well at 5 cm separation between pheromone components with 1 ng of main component and 0.01 ng of the minor component doses. These results indicate that moths are $10^7$ times more sensitive than PID.

My third question was: "what are the relationships between the distance of separation, either horizontal or vertical, of release points of different plumes and how NHV effect the beetle attraction towards pheromone".

In the field experiments with *I. typographus* and *S. litura*, the relationship between catch and pheromone component separation distance or pheromone vs. NHV separation distance (Figure 10 & 11) showed that spacing clearly reduces catches with increased distance between pheromone components but enhances the catch with increased distance between pheromone and NHV. This trend was seen, either if the spacing was horizontal or vertical. The relationship between catch and pheromone

component separation distances have been studied in western pine beetle (Byers, 1987) and it was found that increased distance between pheromone components caused decrease in trap catch. But here I have studied two different systems, one is spacing between pheromone and non-host volatile odours of *I. typographus* and the second one is spacing among aggregation pheromone components of *I. typographus* and spacing among sex pheromone components of *S. litura.*

My study on the relationship between different release points of host and non-host odours is a new interesting insight to determine the active inhibitory range of non-host volatiles horizontally and as well as vertically and also to determine the scale of landscaping of non-host trees to support biodiversity hypothesis or stability hypothesis, which is never done before. It will also be helpful for disruption of olfactory communication in mating disruption as "confusion method". Saint-Germain (2007) showed that prelanding use of host-produced volatiles is scale dependent. An observation on orientation behaviour of *Pityogenes bidentatus* indicated an active inhibitory range of 0.5-1.0 m to a trap baited with pheromone and non-host bark (Byers et al., 2000). In a later study on *I. typographus* showed at least 2 m active inhibitory range (AIR) of NHV plus verbenone blends baited on traps around a central pheromone trap at 1, 2 and 4 m radii (Zhang and Schlyter, 2003). In my study I found that non-host volatiles have inhibitory effect more than 1.0 m in every direction and also upward and downward. The spacing between pheromone components also can modify insect behaviour up to 80 cm in bark beetle, but up to few cm in moths. It was not new that zero spacing between pheromone and NHV reduce the *Ips* catches close to that in blank trap because various studies have demonstrated that NHV act as "shut-off" signals to inhibit pheromone attraction (Schlyter et al., 1988; Schlyter et al., 1989; Schlyter et al., 1994; Byers et al., 1998; Borden et al., 2000; Zhang et al., 2000; Borden et al., 2001b; Zhang and Schlyter, 2004). The result from pheromone component spacing studies could be helpful for mating disruption as "confusion method" to disrupt the pheromonal communication to control insects as it is clear from my results of behavioural bioassay of *Spodoptera littoralis,* where biological responses were significantly reduced at 5 cm spacing between pheromone components (Figure 8). Byers (1987) determined the sex ratio during his study of western pine beetle, that less numbers of males were caught than the number of females. Sex ratio depends on release rate; low release rate caught equal sex ratio but

the highest release rate caught only 30 % males (Schlyter et al, 1987). It was in my plan, also to determine the sex ratio but due to shortage of time, I postponed it and I will do it during further proceeding of my PhD project for scientific publication. The effect size trend lines (Figure 12B) quantify the main conclusion that can be derived from this study, namely that odour source spacing has great impact on attraction and inhibition of insects, and that the effect depend on the distance of odour separation. Intensity and effect of pheromone spacing in moths and bark beetle show similar and very high values of linear fit ($r^2$= 0.79 to 0.99) but different slopes depending on scale.

# 5 CONCLUSIONS

These findings indicate that by using the different release points of pheromone components and inhibitors we can manipulate insect behaviour. The concept of using multi-component lures in attraction or inhibition, released from a single source or many scattered sources for mating disruption and insect control is well established (Linn et al , 1986; Schlyter et al., 1994; Roelofs, 1978). But I think that this study has provided interesting insights into the mechanisms behind odour plume tracking, mate attraction and mating disruption to control insect and will provide a comprehensive understanding of dispersal and host selection at different scales.

# 6 FUTURE RESEARCH

I hope that these experiments of plume interactions and odour source spacing will create interest to find out the physical factors that effect odour information to control insect behaviour and the rapidly evolving technologies that may increase our understanding of these processes. To study the applied aspects of this research for biodiversity hypothesis, further study on effect of plume interaction and odour source spacing, I have planned to do field experiments with background NHV odours on *Ips typographus* and odour sources spacing experiments in windtunnel with Spodoptera spp. My senior colleague Martin Anderson is working on plumes measurement by PID in field and then visual observation of plumes by soap bubbles. We are also working on neuron responses (single-cell recording) in the lab and may also be semifield in near future.

# 7 ACKNOWLEDGEMENTS

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