CHAPTER 1

INTRODUCTION

Armyworms, so named because of the characteristic movement *en masse* of their larvae, are important pests of cereals, pastures and forage crops in many parts of the world. In North America, the true armyworm, *Pseudaletia unipuncta* (Haworth) is a sporadic pest of agricultural crops (Guppy, 1961; Beirne, 1971). The African armyworm, *Spodoptera exempta* (Walker) is a pest of graminaceous crops and pasture grasses, with larval outbreaks occurring throughout eastern, central and southern Africa (Haggis, 1986; Wilson & Gatehouse, 1993). The oriental armyworm, *Mythimna separata* (Walker) is an important pest of cereals and pasture grasses throughout its range in eastern Asia, Oceania and Australasia (Sharma & Davies, 1983).

Several armyworm species are found in Australia. These are the common armyworm, *Mythimna convecta* (Walker), the northern armyworm, *Mythimna separata* (Walker), the sugarcane armyworm, *Mythimna loreyimima* (Rungs), the southern armyworm, *Persectania ewingii* (Westwood), the inland armyworm, *Persectania dyscrita* Common, the lawn armyworm, *Spodoptera mauritia* (Boisduval), the day-feeding armyworm, *Spodoptera exempta* (Walker), the cluster catterpillar *Spodoptera litura* (Fabricius), the lesser armyworm, *Spodoptera exigua* (Hübner) and *Spodoptera umbraculata* (Walker). The common armyworm, *M. convecta* is an endemic species which is widely distributed throughout Australia (Common, 1965; Woods *et al.*, 1980; McDonald & Smith, 1986). The moth has a wing span of 3-5 cm, with yellow brown or red brown forewings speckled with black dots and with a small white spot near the centre, and with grey hindwings (Plate 1.1). A fully fed larva is about 4 cm long, smooth, green brown, red brown or purplish brown with three prominent lateral body stripes of similar width (Plate 1.2).

Armyworm larvae can cause severe damage in cereals, with as high as 80% crop loss (Strickland & Rimes, 1976). In terms of insecticide use, about $1 million per year was spent for *M. convecta* in the 1970's (Marsden *et al.*, 1980). There are no recent figures, but the amount is likely to have increased. The larvae cause damage through leaf feeding and head cutting (Broadley, 1979). Strickland & Rimes (1976) describe armyworm damage occurring in two phases associated with the different stages of the crop. In the first phase, the larvae eat the lower flag leaves and sheaths of the tillers and in the second or "head lopping" phase, they climb the heads and chew

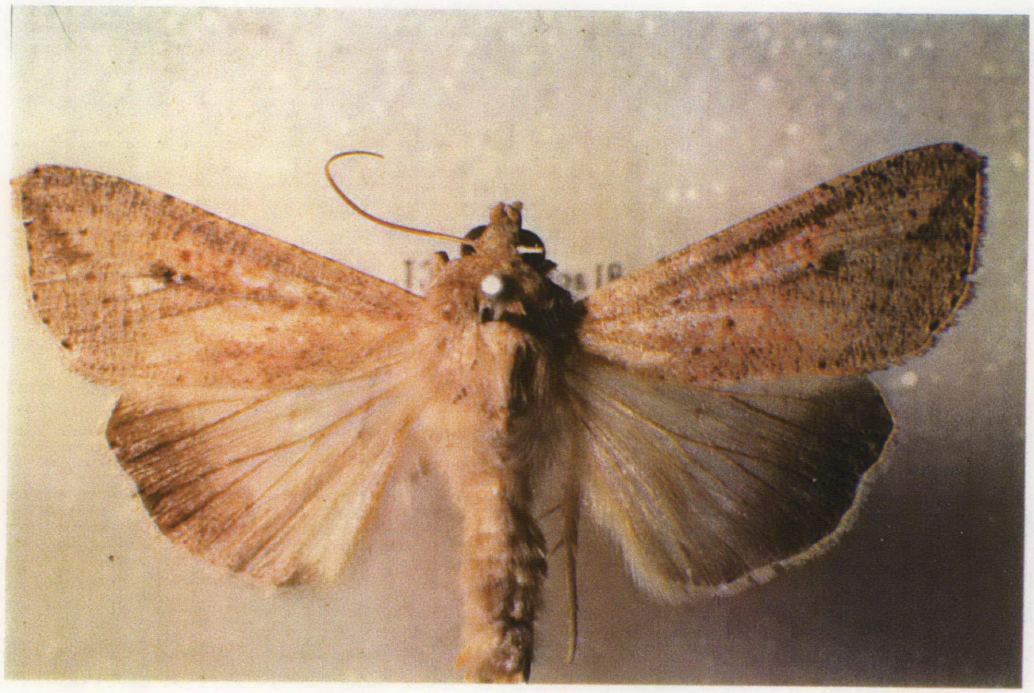

Plate 1.1. Adult of the common armyworm, *Mythimna convecta* (Walker).

Plate 2.2. Larva of the common armyworm, *Mythimna convecta* (Walker).

through the green material of the tillers, cutting off the seed heads. It is during the second phase when up to 80% crop loss may occur over a period of four or five days.

M. convecta is one of the highly migratory noctuid species in Australia (Farrow & McDonald, 1987; McDonald, 1991; Gregg *et al.*, 1993). When conditions are favourable in autumn and winter, it can breed in native grasses and pastures in the semi-arid inland areas of Australia, particularly in northern New South Wales and Queensland. When large populations are produced in these areas, they migrate to the southern areas in spring, usually on warm northerly airflows ahead of cold fronts (Farrow & McDonald, 1987; McDonald, 1991).

Outbreaks of the common armyworm are sporadic and largely unpredictable. Pheromone traps might be useful for pest management by providing an early warning of armyworm incidence and hence, better prediction of outbreaks. Little is known of the pheromone biology of this species. Substances which were thought to be the components of the female sex pheromone have been characterised (C. Whittle & T. Bellas, pers. comm., 1990). However, initial field trapping tests showed that the synthetic pheromone lures were not successful in attracting males (G. McDonald, pers. comm., 1990). An understanding of the reproductive biology of this species might help resolve this problem. Studies on the female behaviour, particularly calling behaviour, were therefore conducted. Likewise, male behaviour in the presence of the natural and synthetic female sex pheromone was investigated to provide insights for possible development of a pheromone lure for this species.

Specifically, the study aimed to:

(1) determine the nocturnal behaviour, particularly the calling behaviour, of virgin females;
(2) determine at what age following emergence virgin females start calling;
(3) investigate if calling behaviour changes with moth age and time of the night;
(4) determine the effects of environmental conditions, particularly temperature and photoperiod, on calling behaviour;
(5) study the behaviour of males in the presence of calling females in a wind tunnel;
(6) study the copulatory behaviour of males and females;
(7) identify the components of the female sex pheromone from pheromone glands and airborne volatiles and prepare pheromone blends based on the identified components;

(8) study male behaviour in the presence of different blends of the synthetic sex pheromone in a wind tunnel and;
(9) test different synthetic pheromone blends under field conditions using different trap designs.

The study consists of two main parts, presented in Volume 1 and Volume 2 of the thesis. This volume (1) discusses the behaviour of females and males, and the interactions between sexes. It includes laboratory experiments on the nocturnal behaviour of females, with emphasis on calling behaviour, the effects of temperature and photoperiod on calling, male behaviour in the presence of conspecific females in a wind tunnel, and copulatory behaviour in laboratory and field cages.

CHAPTER 2
REVIEW OF THE LITERATURE

2.1. Sexual communication in insects

Reproductive interactions between insect species depend largely on ecological factors, geographical distribution and pheromonal communication systems (Roelofs & Cardé, 1974a). These communication systems vary in different insect groups, resulting in attraction, aggregation or arrestment. In the orders Lepidoptera, Coleoptera, Hymenoptera, Orthoptera, Diptera and Hemiptera, sex pheromones are employed by many species for mating, with the females as the primary releasers and the males the receivers (Cardé & Baker, 1984).

Sex pheromones are "chemicals that are secreted by animals of one sex and cause behavioural reactions in the opposite sex that facilitate mating" (Shorey, 1976). They are "odourant molecules which are transmitted in the gaseous state and perceived by olfactory receptors found on the antennae of insects" (Seabrook, 1978). In female Lepidoptera, pheromone chemicals are generally unbranched chains of 10 to 18 carbon atoms with one or two points of unsaturation and terminated by a functional group which may be an acetate, aldehyde, alcohol or acid (Shorey, 1976).

Sex pheromone blends consist of two or more related chemicals and the optimum blend of components that elicits sexual responses from males usually is one that most closely approximates the natural ratio emitted by females (Cardé & Baker, 1984). Reproductive isolation in insects is achieved by having different pheromone compounds (Roelofs & Cardé, 1974a). Related species having similar pheromone components can achieve reproductive isolation by having a concentration or release rate that is species-specific (Roelofs & Cardé, 1974a), by varying the ratios of the components or by having an additional component to elicit male attraction (Cardé & Baker, 1984).

A flying insect approaches a chemical source through a sequence of behavioural events - initiation of flight, directed movement along or through the chemical trail, arrestment of locomotion and landing (Farkas & Shorey, 1974). Baker (1989) reviewed two mechanisms of pheromone-mediated flight in insects, optomotor anemotaxis and self-steered counterturning system. With optomotor anemotaxis, a flying insect locates the source by tracking the direction of the wind, usually with zigzags or lateral reversals. Self-steered counterturning or across-wind reversals occur not only as a response to losing the pheromone plume, but rather as a

"programmed" response to the pheromone which might be modulated by pheromone concentration or quality. Further reviews on mechanisms of orientation of insects to olfactory stimuli can be found in Cardé (1984) and Payne *et al.* (1986).

Wall & Perry (1987) pointed out that it is difficult to determine at what distance an insect which arrives at a sex-attractant source actually started to respond to it. They defined three terminologies - "sampling range, range of stimulation and range of attraction" - to describe a continuum prior to stimulation through to attraction of an insect to the source. The sampling range is "the maximum distance from which insects can be shown to reach the source in a given time period. It comprises the radial distance from the source travelled both before stimulation and during attraction." The range of stimulation is "the maximum distance at which an attractive source can be shown to elicit a response." The range of attraction is "the maximum distance over which insects can be shown to direct their movement to the source." Their studies with the pea moth, *Cydia nigricana* (F.) showed that its ranges of attraction, stimulation and the daily sampling range are about 200, 500 and 500 m, respectively.

2.2. Sexual behaviour in Lepidoptera: females

Calling behaviour is the term used to describe a female moth releasing sex pheromone to attract males for mating. The sex pheromone glands in several noctuid species are situated dorsally or ventrally in the intersegmental membrane between the 8th and 9th abdominal segments (Jefferson *et al.*, 1966, 1968). In the geometrid, *Lambdina fiscellaria lugubrosa* (Hulst), paired glands located ventro-laterally in the 8th terminal abdominal segment appeared to be the sex pheromone glands (Ostaff *et al.*, 1974). A typical calling posture in female moths is characterised by the full protrusion of the ovipositor. The calling posture in several moth species is described below.

Noctuidae: The true armyworm, *P. unipuncta* fully extrudes its ovipositor and raises the wings, orienting vertically with the head uppermost (Turgeon & McNeil, 1982). The bertha armyworm, *Mamestra configurata* Walker, moves its ovipositor from side to side or rotates it accompanied by rapid protrusion and retraction (Howlader & Gerber, 1986a). During full calling, its ovipositor is extruded and curved downward at about 45° and the wings are raised above the abdomen. The cabbage moth, *Mamestra brassicae* L. starts with a rhythmic protrusion and retraction of the ovipositor and when fully calling, the ovipositor is extruded and pointing downward, and the wings are also raised (Noldus & Potting, 1990). In the potato stem borer, *Hydraecia micacea* Esper, the wings are either fully raised to about 45°

from the body or held close to the body during calling (West *et al.*, 1984). Wing vibration during calling occurs frequently when the wings are raised.

Sphingidae: Calling behaviour in the tobacco hornworm moth, *Manduca sexta* (L.) is described as the extrusion of the 8th or 9th abdominal segments (Itagaki & Conner, 1988).

Tortricidae: The complete extrusion of the sex pheromone gland in the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) is accompanied by a slight raising of the body from the substrate and slight bending of the abdomen ventrally, away from the wings (Sanders, 1969).

Geometridae: Calling in *L. f. lugubrosa* is characterised by the full protraction of the 8th to 10th terminal abdominal segments, with a slight rhythmical protraction and retraction (Ostaff *et al.*, 1974).

Gelechiidae: Leppla (1972) described two phases in the calling behaviour in the pink bollworm, *Pectinophora gossypiella* (Saunders). During the first phase or initial calling, the wings are extended, raised and separated, the ovipositor is projected posteriorly and curled downward, and the ovipositor and the pheromone gland contact the substrate in short circular motions. During the second phase or overt calling, the wings are raised to about 45°, more widely separated and tensely extended, the ovipositor vibrates dorso-ventrally with rhythmic lateral movements producing a circular motion about 1 mm in diameter and the pheromone gland is about 2 mm above and parallel to the substrate.

Calling behaviours in some species are categorised as weak or strong calling. In the black cutworm, *Agrotis ipsilon* (Hufnagel) and the tropical warehouse moth, *Ephestia cautella* (Walker), the ovipositors of weakly calling females are only partially extruded while those of strongly calling females are fully extruded (Swier *et al.*, 1977; Barrer & Hill, 1977). Nordlund & Brady (1974) also defined weak calling and strong calling in the pyralid *Plodia interpunctella* (Hübner) based on the position of the abdomen. During strong calling, the abdomen is extended high above the top of the wings and the abdominal tip generally fully extruded while in weak calling, the abdomen is only slightly above the top of the wings and the abdominal tip only partially extruded.

2.2.1. Factors affecting calling behaviour

Shorey (1974) and McNeil (1991) review the various endogenous and exogenous factors that can modify the production of pheromone in female moths and its subsequent release during calling.

2.2.1.1. Moth age and physiological development

The age at first calling in female moths varies from one to several days after emergence. Examples of moths that were observed to start calling on the first day after emergence are the tortricids, *Epiphyas postvittana* (Walker), *Choristoneura fumiferana* and *C. rosaceana, Grapholitha molesta* (Busck), *Cydia pomonella* (L.) and *Platynota stultana* (Walsingham), the pyralid, *Chilo suppressalis* (Walker), the sphingid, *M. sexta,* the lymantrid, *Portheria (=Lymantria) dispar* (L.) and the noctuids, *H. micacea* and *Sesamia nonagrioides* (Lef.) (Lawrence & Bartell, 1972; Sanders & Lucuik, 1972; Delisle, 1992; Doane, 1968; Baker & Cardé, 1979; Webster & Cardé, 1982; Kanno, 1979; Itagaki & Conner, 1988; Castrovillo & Cardé, 1979; West *et al.*, 1984; Babilis & Mazomenos, 1992).

P. unipuncta females called for the first time between 2 and 10 days after emergence (Turgeon & McNeil, 1982). *M. configurata* females started their first calling on the 2nd and 3rd nights after emergence (Howlader & Gerber, 1986a). First calling in *Helicoverpa (=Heliothis) armigera* (Hübner) was between 2 and 5 days after emergence (Kou & Chow, 1987). In *Helicoverpa zea* (Boddie), first calling occurred in the 3rd scotophase (Raina *et al.*, 1986a). *Heliothis peltigera* (Denis & Schiffermüller) started calling in the 2nd night (Dunkelblum & Kehat, 1992). *M. separata* females were inactive on the 1st and 2nd nights after emergence except for flight with feeding, and started to call on the 3rd night in the latter half of an 8-hour scotophase (Kanda & Naito, 1979).

Several parameters of calling behaviour change with moth age. These include the onset time of calling, the number and duration of calling bouts and the total time spent calling. The mean onset time of calling in *P. unipuncta* females was advanced and the total time spent calling increased in older females (Turgeon & McNeil, 1982). Similar changes were observed in *A. ipsilon, M. separata, C. suppressalis, M. configurata, H. armigera, S. nonagrioides* and *C. rosaceana* (Swier *et al.*, 1977; Kanda & Naito, 1979; Kanno, 1979; Howlader & Gerber, 1986b; Kou & Chow, 1987; Babilis & Mazomenos, 1992; Delisle, 1992). In *P. unipuncta*, younger moths generally had more calling bouts with shorter duration while older moths called less frequently but with longer bout duration (Turgeon & McNeil, 1982). The mean

number of calling bouts in *H. armigera* increased significantly from 4.0 ± 2.4 bouts on calling day 1 to 8.9 ± 3.2 and 8.1 ± 3.1 bouts on calling days 2 and 3, respectively, then decreased significantly to 5.8 ± 1.7 bouts by calling day 4 (Kou & Chow, 1987). Babilis & Mazomenos (1992) reported that majority of 1- and 2-day-old *S. nonagrioides* females called continuously, whereas 3-and 4-day-old females exhibited periodic calling.

2.2.1.2. Neural/hormonal factors

Hollander & Yin (1982) reported that the rhythmic extrusion and retraction of the terminal abdominal segments in the gypsy moth, *L. dispar* was curtailed by removing the terminal abdominal ganglion or severing the nerves leading posteriorly from it. Pheromone release was stopped by removing the terminal abdominal ganglion and the brain. A hormonal substance, a peptide, produced in the brain regulates sex pheromone production in *H. zea* (Raina & Klun, 1984). This substance is stored in the brain during the photophase but is released only in the scotophase.

Rafaeli & Soroker (1989) suggested that a brain hormone induces pheromone production in *H. armigera* and *Spodoptera littoralis* (Boisduval). Normal sex pheromone production in these species was inhibited when females were ligated between the head and the thorax. When their abdomens were injected with brain homogenates (i.e., brains, suboesophageal ganglia, *corpora allata* and *corpora cardiaca*) pheromone production was restored.

The presence of the *corpora allata*, the source of juvenile hormone, is important in the pheromone biosynthesis and the expression of calling behaviour in *P. unipuncta* virgin females (Cusson & McNeil, 1989a). Females without *corpora allata* did not produce pheromone and did not call, but when juvenile hormone (JH II) was injected to allatectomized females, these activities were restored. Age at first calling in this species is likely to be determined by the brain through the release of neurohormones, such as allatotropin or allatostatin, which stimulate or inhibit the production of juvenile hormone by the *corpora allata*. Cusson *et al.* (1993) reported that age and rearing conditions (temperature and photoperiod) influenced the amount of juvenile hormones (JH) and JH acid (JHA) released by the *corpora allata* of *P. unipuncta* females and males, respectively. At 25°C and 16:8 light:dark (LD) regime, JH I and II and JHA I and II increased from the 1st to 5th days, whereas JH III and JHA III generally remained constant. At 10°C and 12:12 LD regime, JH II increased up to 25th day and JHA I and II peaked on the 10th day then declined with age. They suggested that

the patterns of these release products were correlated with the pheromonal activities in both sexes, except in the males reared at 10°C and 12:12 LD.

Cusson *et al.* (1990) confirmed that the initiation of pheromone production and calling behaviour in *P. unipuncta* is associated with an increase in the production of juvenile hormone *in vitro*. They found that the rate of juvenile hormone biosynthesis was greater in 2-day-old females that called for the first time than in non-calling females of the same age, while newly-emerged and 1-day-old females had lower juvenile hormone biosynthesis than older females. Delays in the initiation of calling in females corresponded to similar delays in the activation of the *corpora allata* and females with basal oocyte diameter of < 0.33 mm had lower juvenile hormone production than females with larger basal oocytes. Cusson & McNeil (1989b) showed that *P. unipuncta* females with basal oocyte width < 0.33 mm did not call.

The effects of decapitation and application of an exogenous juvenile hormone and a juvenile hormone analogue on calling and pheromone production in *P. stultana*, a species that mates only once, has been investigated by Webster & Cardé (1984). Pheromone titre and calling in this species is likely to be controlled from the head. After decapitation, pheromone titre in virgin females declined and decapitated females did not call. Webster & Cardé (1984) suggested that the decline of titre and prevention of calling may be due to enzymatic degradation of pheromone and neural, neural-humoral or neuro-hormonal regulation of pheromone biosynthesis. They also observed that pheromone disappeared from the pheromone glands of decapitated virgin females, with or without the application of the exogenous juvenile hormone analogue, ZR-512 at 0 or 12 hours after decapitation. The same analogue did not prevent the decline in pheromone titre in mated females and the application of juvenile hormone I, II, III and ZR-512 resulted in a significant reduction in the proportion of virgin females that called.

Gadenne (1993) studied the role of juvenile hormone in the regulation of reproductive activities in *A. ipsilon* by means of topical application of fenoxycarb, a juvenile hormone mimic. Fenoxycarb induced earlier ovarian maturation and resulted in earlier calling, mating and oviposition. Allatectomized females treated with fenoxycarb re-initiated calling and mating and ovarian development resumed in decapitated females treated with the JH mimic.

In *M. sexta*, Itagaki & Conner (1986) did not find evidence for a hormone that controls calling. Neither the removal of the *corpora allata* and the *corpora cardiaca*

in the pupae nor blood transfers from calling to non-calling females affected calling behaviour in this species. They reported that a neural output from the brain and/or the suboesophageal ganglion triggers calling in this species.

2.2.1.3. Ovarian development

Variability in the initiation of calling in female moths is associated with the degree of ovarian development. Cusson & McNeil (1989b) suggested that a certain level of ovarian development was required for calling to be initiated in *P. unipuncta*. Females of this species with mean basal oocyte widths below 0.33 mm did not call and those calling for the first time had larger basal oocytes and contained more eggs than noncallers of the same age. The number of eggs and the proportion of females with eggs increased with age although the presence of eggs was not necessary for calling to occur.

Howlader & Gerber (1986b) reported that the time of first calling in *M. configurata* females coincided with the appearance of the first chorionated eggs in their ovaries. In both photophase- and scotophase-emerged females, first calling was delayed until the next scotophase if there were no chorionated eggs in the ovaries at the beginning of a scotophase.

The highest number of mature eggs in the ovarioles of *A. ipsilon* at 4 days old coincided with female peak attractancy (Swier *et al.*, 1976). In the lightbrown apple moth, *E. postvittana* calling peaked when they were 2 days old then declined on the 3rd to 5th days. Reduced calling in older moths may be related to either the presence of large number of mature eggs in the ovarioles or the act of oviposition itself (Lawrence & Bartell, 1972).

2.2.1.4. Mating

According to Raina *et al.* (1986a), females of many moth species are generally less receptive to male courtship for a period of time after mating. They suggested that the loss of pheromone production may be related to the loss of readiness to engage in sexual activity after females have been mated.

Some female moths had non-calling periods while in others, calling was terminated after mating. In *M. configurata,* where first mating occurred during the 2nd to 5th scotophases after emergence, mated females resumed calling between 1 and 4 scotophases after the first mating and had shorter calling periods during each night compared to virgin females of the same age (Howlader & Gerber, 1986b). *H. zea*

females did not call on the same night after mating (Raina *et al.*, 1986a). Mated females of the gypsy moth, *P. dispar,* did not call and avoided males attempting to copulate (Doane, 1968). After copulation, they exhibited negative phototropism or moving away from light and positive geotropism or moving downwards, where they began laying eggs once they found suitable sites for oviposition. Although mating in *M. sexta* females resulted in the cessation of calling behaviour, mated females occasionally resumed calling after 7-9 days (Sasaki & Riddiford, 1984). In *P. stultana,* females terminated calling after being mated (Webster & Cardé, 1984).

Sperm content in mated females can also affect female attraction to males (Raulston *et al.*, 1975). In field-cage tests using sticky traps, virgin females of *Heliothis virescens* (F.) caught significantly more males than mated females containing sperm. Females that previously mated but contained no sperm caught as many males as the virgin females did.

Mating did not have a significant effect on the calling behaviour of *C. fumiferana* (Sanders & Lucuik, 1972). Mated females called each night like the virgin females of the same age, although in the former calling was more intermittent and a smaller proportion was calling at any one time compared with the latter.

2.2.1.5. Conspecific sex pheromone

The presence of conspecific synthetic sex pheromone of *C. fumiferana* altered the time of calling and the proportion of females calling. Calling occurred earlier and the proportion of females that called was higher in females exposed to the pheromone compared with the unexposed females. Under high population densities in the field, the time of calling and hence, mating, is likely to be before dusk and the number of moths calling and mating is also likely to increase (Palaniswamy & Seabrook, 1985).

Conspecific synthetic sex pheromone in the tortricids, *Adoxophyes* sp. and *Homona magnanima* Diakonoff, delayed the onset time of calling by 20-40 minutes (Noguchi & Tamaki, 1985). Calling of upwind conspecific females in a wind tunnel also suppressed the calling of downwind females of both species during the initial 140 minutes. In contrast, the presence of conspecific synthetic pheromone did not affect calling in *S. littoralis* females but caused a significant reduction in mating (Dunkelblum *et al.*, 1987).

Conspecific female pheromone may also influence behaviour of females other than calling. Sanders (1987) reported that flight activity of both virgin and mated *C.*

fumiferana females was higher in pheromone-permeated air than in clean air. Palaniswamy & Seabrook (1978) suggested that conspecific pheromone may induce oviposition in *C. fumiferana* while Birch (1977) suggested that it may influence aggregation, resulting in increased chances of mating in *Trichoplusia ni* (Hübner).

2.2.1.6. Temperature

The temperature limits for the expression of calling behaviour vary in different species. Sower *et al.* (1971) reported that sex pheromone release in the noctuid *T. ni* occurred between 12 and 36°C. In the tortricid *P. stultana*, the lower temperature limit for calling is about 13°C and the upper limit is within a few degrees above 35°C (Webster, 1988). The optimal temperature range for calling in *M. configurata* is between 10 and 25°C with the threshold at < 5°C and the upper limit at about 35°C (Gerber & Howlader, 1987). Calling in *C. fumiferana* was observed between 3.5 and 25°C (Sanders & Lucuik, 1972). In *C. suppressalis*, the low threshold temperature for calling is 5°C and the high threshold is 41°C, with the optimum temperature for calling at near 20°C (Kanno & Sato, 1979).

The length of the pre-calling period in female moths is influenced by temperature conditions. The initiation of calling in *P. unipuncta* females varied with temperature during the pupal and adult stages (Turgeon & McNeil, 1983). The age at first calling was earlier at higher ambient temperatures (20-25°C) than at lower temperatures (10-15°C) during the adult stage. Females that experienced higher temperature as pupae started to call for the first time earlier than those held under lower temperature during the pupal stage. When both the pupal and adult stages were under the same temperature conditions, first calling in females at 15°C occurred significantly later that those at 20 or 25°C. In another study, under either 16:8 or 12:12 LD periods, *P. unipuncta* started calling 11 days later at 10°C than at 25°C (Delisle & McNeil, 1987a). The age at first calling in *M. configurata* females was significantly different at six different temperatures (Gerber & Howlader, 1987). First calling occurred during the 5th to 6th scotophases at 10°C, 3rd to 4th scotophases at 15°C, 2nd to 3rd scotophases at 20-30°C and 4th to 5th scotophases at 35°C. In the silver Y moth, *Autographa gamma* (Linnaeus), the pre-calling period ranged from 3 to 11 days (median of 6 days) at 19°C, and from 3 to 9 days (median of 4-5 days) at 22-25°C (Hill & Gatehouse, 1992).

The onset and the duration of calling may also vary with temperature. In *M. configurata*, calling started earlier during the night at 15-25°C than at 30-35°C and the calling periods were longer at 10-25°C than at 30-35°C (Gerber & Howlader, 1987).

Calling in *P. unipuncta* occurred earlier in the scotophase at 10°C than at 25°C (Delisle & McNeil, 1987a). The time spent calling increased as the females got older but was not significantly different between the two temperatures. Earlier calling at lower temperatures was also reported for *P. stultana* (Webster, 1988). Calling in this species was initiated and terminated earlier at 10-18°C than at 20-35°C. *C. fumiferana* called earlier in the photophase at 3.5-15°C than at 18-25°C (Sanders & Lucuik, 1972). The onset of calling in *C. rosaceana* was significantly earlier at 15 than at 20°C, but calling durations under both temperatures were not significantly different (Delisle, 1992). Raina *et al.* (1991) reported that under 16:8 LD period, calling in 3-day-old *H. zea* did not occur at 14°C. At 24°C, *H. zea* females started calling in the 1st hour and peaked in the 5th hour while at 34°C, females did not call until the 3rd hour and peak calling was in the 7th hour.

Conner *et al.* (1985) measured the frequency of ovipositor extrusion (number/ min) in 15 arctiid species at 15, 20 and 25°C. They found that the frequency of ovipositor extrusions was positively related to the ambient temperature. Similar observations have been reported for another arctiid moth, *Holomelina immaculata* (Reakirt), where the rate of ovipositor protrusion was higher at 24°C than at 15°C (Cardé & Roelofs, 1973).

Webster (1988) pointed out that temperature influences the time of onset and end of calling in *P. stultana* in two ways. First, at each temperature, there is a different period or "calling window" and calling occurs at any combination of time and temperature within the calling window, but not outside the window. An increase or decrease in temperature into the calling window can initiate calling while an increase or decrease out of the window can terminate calling. Second, the time of calling is also influenced by the temperature moths experience before that calling period, but this does not affect the time of calling on the following day. He hypothesised that the conditioning temperature, before a temperature increase or decrease, influences the rate of accumulation of a substance that regulates the initiation of calling.

Calling behaviour can be modified by either an increase or decrease in the prevailing temperature. Under a 16:8 LD condition, the pre-calling period of *P. unipuncta* females transferred from 10 to 25°C at 10 and 15 days of age was significantly shorter than in those females subjected to a similar temperature increase at 5 days of age (Delisle & McNeil, 1987a). The mean onset time of calling on the first day following transfer from 10 to 25°C was similar to that in females maintained at 25°C. In the tiger moth, *Holomelina lamae* (Freeman), a species that starts calling during the

photophase, the effect of a temperature change depends on the hour of the photophase (16:8 LD) it occurs (Schal & Cardé, 1986). In 2-day-old moths, a decrease from 24 to 14°C at lights-on resulted in a significant delay in the onset of calling and an advance in the peak time of calling compared with those maintained at 24°C. The same decrease at 4 hours after lights-on resulted in a greater advance in the peak time of calling while a decrease at 8 hours after lights-on stimulated calling immediately. An increase from 24 to 34°C at lights-on did not significantly advance either the mean onset time of calling or the peak calling time but the same increase at 4 and 8 hours after lights-on significantly delayed the onset and peak calling times.

Delisle & McNeil (1987b) suggested that the expression of calling is related not only to the prevailing ambient temperature but also to the temperature moths experience during the previous calling period. *P. unipuncta* females subjected to 20, 15 and 10°C after their 1st night of calling at 25°C significantly advanced their mean onset time of calling compared with those maintained at 25°C. Females transferred from 10 to 25°C at 2 and 4 hours after the onset of the 2nd scotophase called earlier than those maintained at 25°C. A decrease of 10 or 15°C from 25°C also resulted in a significant increase in the time spent calling, while a decrease of 5°C had no effect.

2.2.1.7. Photoperiod

Another important environmental factor influencing calling behaviour in female moths is the light:dark cycle. The endogenous periodicity of calling may be entrained by photoperiod (Sower *et al.*, 1970; Cardé & Roelofs, 1973). Either the "lights-off" or the "lights-on" signal has been demonstrated to be responsible for setting the timing of the diel periodicity of calling in female Lepidoptera (e.g., Cardé & Roelofs, 1973; Delisle & McNeil, 1986; Gerber & Howlader, 1987; Sanders & Lucuik, 1972; Schal & Cardé, 1986; Sower *et al.*, 1971; Webster & Conner, 1986).

The age at first calling in *P. unipuncta* varied with different photoperiodic conditions (Delisle & McNeil, 1986). Under five photoperiodic conditions at 25°C - 18:6, 16:8, 14:10, 12:12 and 10:14 LD periods, the age at first calling increased with longer scotophases, except at 18:6 LD. On the other hand, in some species, the age at first calling did not vary with photoperiod. *M. configurata* females called for the first time during the 2nd scotophase following emergence under three different photoperiodic regimes - 16:8, 12:12 and 8:16 LD (Gerber & Howlader, 1987). The majority of *M. brassicae* females called for the first time during the 2nd or 3rd scotophase following emergence under either 16:8 LD or 18:6 LD regimes (Noldus & Potting, 1990).

The onset time of calling and the length of the calling period varied with photoperiodic regimes in *M. brassicae* (Noldus & Potting, 1990). Under 16:8 and 18:6 LD conditions, calling did not occur in the 1st scotophase. Under 16:8 LD period, the mean onset time of calling decreased from scotophases 2 to 3, and stabilised at about 260 minutes after lights-off while at 18:6 LD, the onset calling time decreased until scotophase 4 and stabilised at around 130 minutes after lights-off.

The expression of calling behaviour is modified when the entraining light:dark cycle is altered. In *P. unipuncta,* a reduction of either 4 or 6 hours in the length of the scotophase after the 1st night of calling resulted in a small proportion of females (11-21%) calling on the following night (Delisle & McNeil, 1987a). According to Sanders & Lucuik (1972), lights-on appeared to be the critical cue determining the timing of calling in *C. fumiferana.* Under a normal 17:7 LD cycle, there was a lag of 13.5 hours between lights-on and the start of calling. Calling advanced by 2 hours on the following day when the time of lights-on was made 3 hours earlier while a 3-hour-delay in the time of lights-off did not alter it. Calling began with the onset of darkness under photophases of 4, 8, 10 or 12 hours. Under photophases of 14, 16, 18 or 20 hours, calling started before the scotophase.

A study on the combined effect of temperature and length of the scotophase on the calling behaviour in *P. unipuncta* showed that under either 10 or 25°C, there was a delay of 3-4 days in the initiation of calling under a short-day condition (12:12 LD) compared with a long-day (16:8 LD) condition. The mean onset time of calling occurred earlier at lower temperature and under long days. Females subjected to a temperature change only immediately adjusted their calling periodicity to the prevailing conditions while those subjected to both temperature and photoperiod changes required several days to adjust their calling periodicity to the new regime (Delisle & McNeil, 1987a).

2.2.1.8. Light intensity

The occurrence of calling is inversely proportional to light intensity during the scotophase in two species, *T. ni* and *C. suppressalis.* Under a 12-hour-dark period, increasing light intensities from 0.3 to 300 lux inhibited calling in *T. ni* (Sower *et al.,* 1970). In *C. suppressalis,* the critical illumination intensity shifted lower with increasing temperature (Kanno, 1981a). The critical light intensity at 30°C was ca. 40 lux, ca. 150 lux at 25°C, ca. 500 lux at 20°C, and ca. 1200 lux at 15°C. On the other hand,

calling in the Mediterranean flour moth, *Anagasta kühniella* (Zell.) was not affected by varying light intensities during the photophase (Traynier, 1970).

2.2.1.9. Wind speed

Wind velocity had a significant effect on the frequency of ovipositor extrusion in the arctiid *Utethesia ornatrix* (L.) but not in five other arctiid species, *Pyrrarctia isabella* (J.E. Smith), *Estigmene acrea* (Drury), *Estigmene scribonia* (Stoll), *Spilosoma congrua* Walker and *Apantesis nais* (Drury) (Conner *et al.*, 1985). The frequency of extrusion in *U. ornatrix* increased with increasing wind speed up to 1.2 m/s.

Kaae & Shorey (1972) reported that the time *T. ni* females spent releasing sex pheromone per night was longer when exposed to wind speed ranging from 0.3 to 1.0 m/s than at lower or higher velocities. At a wind speed of 4.0 m/s females did not extrude their pheromone glands.

2.2.1.10. Relative humidity

Relative humidity influenced the onset of calling and the proportion of females calling in the European corn borer, *Ostrinia nubilalis* (Hübner) (Webster & Cardé, 1982). Calling started later in the scotophase under dry conditions and the proportion of females calling decreased when they were transferred to low humidity conditions on the 2nd or 3rd day after emergence. The number of females calling was significantly lower at 25 than at 100% r.h.

In another study with *O. nubilalis*, Royer & McNeil (1991) reported that the age at first calling was not significantly different under three humidity conditions - 53, 62 and 82%. Most females called for the first time within the first 3 days although some females called on the 4th to 6th days under 53% r.h. The mean onset time of calling significantly advanced at 82% r.h. during the first 4 nights of calling compared with the lower humidity conditions. The mean number and duration of calling bouts as well as the time spent calling generally increased over time under the three conditions but was more pronounced at 82% than at 53 and 62% r.h..

2.2.1.11. Host plants

McNeil & Delisle (1989a) reviewed the influence of plant volatiles on pheromone production and release in female moths. In the sunflower moth, *Homoeosoma electellum* (Hulst.), females had earlier calling, longer calling duration, and increased ovarian development rate in the presence of pollen or pollen extract from sunflower, compared with those without pollen (McNeil & Delisle, 1989b). Similar results were

reported for the diamondback moth, *Plutella xylostella* (L.). The presence of *Brassica juncea* (L.) seedlings resulted in earlier calling and longer calling period, and accelerated egg maturation in this species. Pittendrigh & Pivnick (1993) suggested that the effect of the host plant on *P. xylostella* might be a kairomonal or signal effect rather than a direct nutritional effect. In *Yponomeuta* spp., the proportion of calling was higher with host plant foliage (Hendrikse & Vos-Bunnemeyer, 1987). Raina *et al.* (1992) reported that volatile chemicals from corn silk, including the plant hormone ethylene, induced pheromone production in *H. zea*. The presence of corn silk resulted in a 20- to 30-fold increase in the production of Z11:16 Ald.

2.2.2. Relationship between pheromone production, calling and mating

The expression of calling behaviour in female moths is related to the production of sex pheromone in their pheromone glands. In some species, peak pheromone titre coincides with peak calling while in others, there is a time-lag between titre and calling behaviour.

Ramaswamy *et al.* (1988) studied the calling behaviour in two populations of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), the Honduras and Mississippi females. In both populations, peak calling during the 2nd scotophase coincided with their peak pheromone titre. Peak calling in the Honduras females occurred between 0200-0400 h while the pheromone titre peaked at 0300 h. In the Mississippi females, calling peaked at 2300 h while titre peaked at 2400 h. In the tomato looper, *Plusia chalcites* (Esp.), maximal calling occurred on the 4th night following emergence while the pheromone content reached the maximum on the 4th to 5th nights (Snir *et al.*, 1986). Calling in *S. littoralis* was maximal during the 2nd to 3rd nights and the quantity of the main pheromone component, (Z,E)-9,11-tetradecadienyl acetate (Z9, E11:14 Ac) reached a maximum in 1-to 3-day-old females (Dunkelblum *et al.*, 1987). The amount of Z9, E11:14 Ac in 1-2-day-old females was highest during the first 2-3 hours after the onset of darkness and the proportion of calling females increased after the 3rd hour of the night.

A time-lag of about 5 hours between sex pheromone production and calling behaviour was observed in the yellow peach moth, *Conogethes punctiferalis* (Guenée) under 23°C and 15:9 LD conditions (Konno, 1986). The amount of (E)-10 hexadecenal (E10:16 Ald) increased soon after lights-off, reached a maximum at 5 hours after lights-off and thereafter decreased. On the other hand, calling was initiated at 5 hours and peaked at 7.5 hours after lights-off. He proposed two theories to explain this time-lag between pheromone quantity and calling behaviour: that the stimulation of

the sex pheromone stored in the pheromone gland induces calling behaviour and that the 5-hour time lag between the two rhythms is predetermined by certain organs such as the cephalic organs.

The pheromone content (Z9, E11:14 Ac) in *S. littoralis* females declined after mating and oviposition (Dunkelblum *et al.*, 1987). Virgin females had an average of 3.40 ± 0.53 ng. At 3 hours after mating, pheromone content decreased to 0.17 ± 0.06 ng. At 24 hours after mating before egg laying occurred, it increased significantly (2.39 ± 0.45 ng) then declined again at 24 hours after egg laying (0.24 ± 0.08 ng). These results indicate that the pheromone may be lost before or during the process of oviposition.

The proportion of mating pairs in *H. virescens* was highest between 51 and 54 hours after eclosion when pheromone content in the females was also highest (Mbata & Ramaswamy, 1990). The pheromone content was based on the total amount of the six components, Z-11-hexadecenal (Z11:16 Ald), hexadecanal (16 Ald), tetradecanal (14 Ald), Z-11-hexadecenol (Z11:16 OH), Z-9-tetradecenal (Z9:14 Ald) and Z-7/9-hexadecenal (Z7/9:16 Ald). Immediately after mating, pheromone content was high but it dropped off sharply within a few hours. Mated females never attained the pheromone levels found in virgin females. The pheromone content of *H. virescens* females mated to previously mated males and males of different ages was also investigated. It was found that pheromone quantity in females mated to previously mated males decreased within the next 3 hours after mating except in females paired with males that mated thrice previously. Pheromone production in females mated to 6-day-old males was significantly greater than in those mated to younger males within the next 3 and 24 hours after uncoupling. Mbata & Ramaswamy (1990) suggested that a pheromonostatic factor transferred from the male during mating suppresses pheromone production in the females and that this suppressive activity depends on the previous mating history and age of the males.

The quantity of the major sex pheromone component of the lightbrown apple moth, *E. postvittana*, E-11-tetradecenyl acetate, was significantly less in mated females compared with that in virgin females of the same ages (Foster, 1993). In contrast, mating did not affect pheromone production in *P. chalcites* (Snir *et al.*, 1986). The quantity of Z-7-dodecenyl acetate (Z7:12 Ac) and Z-9-tetradecenyl acetate (Z9:14 Ac), measured one night after mating did not change significantly. Similarly, the pheromone quantity in virgin and mated females of the same age did not differ significantly.

Pheromone titre is associated with moth age. The production of E,Z-11-tetradecenyl acetate (E,Z11:14 Ac) in the redbanded leafroller moth, *Argyrotaenia velutinana* (Walker) was detected in pupae (1 day pre-eclosion) and reached a peak of 80 ng/tip in 4- to 6-day-old females and declined thereafter (Miller & Roelofs, 1977). In *H. peltigera*, the amount of Z11:16 Ald ranged between 10-50 ng/gland in the first 4 days, and between 30-130 ng/gland from 5th to 10th days (Dunkelblum & Kehat 1992).

The rate of sex pheromone release may also be related to moth age and time of the scotophase. The average pheromone release rate of Z-7-dodecenyl acetate (Z7:12 Ac) in *T. ni* significantly increased but the time spent releasing pheromone per night significantly decreased as the females got older (Bjostad *et al.*, 1980). The emission rate of Z-11-hexadecenal (Z11:16 Ald) in *Platyptilia carduidactyla* (Riley) reached a maximum between the 2nd and 6th hours after the onset of the scotophase, when more than 50% of the females were calling (Haynes *et al.*, 1983). In the potato tuberworm moth, *Phthorimaea operculella* (Zeller), the production of E,Z-4,7-tridecadien-1-yl acetate (diene) and E,Z,Z-4,7,10-tridecatrien-1-yl acetate (triene) peaked during lights-off, coinciding with the beginning of the calling period (Ono *et al.*, 1990). Pheromone content was high on day 1 then declined as the females got older.

Schal *et al.* (1987) suggested that there is rapid biosynthesis of the sex pheromone during calling in *H. lamae*. The emission rate of its sex pheromone, consisting of six hydrocarbons (2-methylhexadecane, *n*-heptadecane, 2-methylheptadecane, 2-methyloctadecane, *n*-nonadecane, and 2-methlynonadecane), was high at the onset of calling. The total pheromone quantity increased over the first 4 days after emergence and subsequently decreased or remained unchanged, and there were no significant differences in the diel fluctuations in 1-, 2- and 4-day-old females.

In *H. zea*, pheromone titre was positively correlated with calling (Raina *et al.*, 1991). Calling in the 3rd scotophase started at 20 minutes into the scotophase peaking at 35 minutes then remained constant until 60 minutes. On the other hand, pheromone was detected during the first 15 minutes, increasing until 60 minutes. These authors, however, suggested that high pheromone production alone does not ensure calling. When they injected homogenates of female brain-suboesophageal ganglion to females during the photophase, *H. zea* females did not exhibit calling behaviour, indicating that other factors such as the lights-off signal that entrains the circadian rhythm of calling might be responsible.

2.3. Sexual behaviour in Lepidoptera: males

Sexual behaviour of male moths has been characterised in many species through behavioural studies in flight wind tunnels. In general, male responsiveness to a sex pheromone source begins with an activation upon release in the wind tunnel, upwind flight towards the source, then landing on the source followed by attempt to copulate. According to Ono & Ito (1989), male behaviour of *P. operculella* approaching a female can be divided into two stages: flight from a distance and close-range behaviour. The first stage allows the male to approach the vicinity of the female while the second stage enables him to pinpoint the female.

Male behavioural responses to a female sex pheromone source in several species are described below.

Burns & Teal (1989) described the behavioural responses of *H. micacea* males to calling females as activation, random flight, upwind orientation, hovering, landing, searching, attempted copulation, casting downwind, reorientation and stopping. They characterised these behaviours as follows: Activation included ambulation, wing fanning and antennal grooming. Random flight involved movement through the entire volume of the tunnel and included short bouts (< 1 minute) of sitting or walking on the tunnel walls. Orientation involved wide horizontal and vertical sweeps in the down-wind half of the tunnel with slow upwind movement, and narrower sweeps closer to the source. Hovering was stationary flight within 10 cm downwind of the holding cage, and landing indicated that the male had contacted the holding cage. Searching consisted of a combination of behaviours performed while on the holding cage that included walking vertically and horizontally and turning clockwise or counterclockwise, and was always accompanied by rapid wing-fanning. Attempted copulation was a lateral curving of the abdomen. Casting was direct downwind flight, after the male had oriented to the lure. Reorientation was upwind flight to the pheromone source after having oriented previously. Males were regarded as stopped when they sat on a wall for at least 1 minute.

Turgeon *et al.* (1983) scored behaviour of *P. unipuncta* males to the female synthetic sex pheromone using the following sequence - wing fanning, antennal erection, orientation toward the pheromone source while still inside the cage, initiation of flight, upwind flight up to 50 cm, upwind flight between 50 and 100 cm, upwind flight greater than 100 but less than 150 cm and reaching the pheromone source with claspers extruded.

The following behavioural responses to the female sex pheromone were described for *H. virescens* males: *UpW*- moth flies upwind in the pheromone plume from the release point; *Pl* - moth flies within 10 cm of platform edge; *S* - moth lands on source; *Hp* - after landing on the source, moth everts his hairpencils; and *C* - after landing on the source, moth exhibits the copulatory response, i.e., with full hairpencil eversion and curling of the abdomen (Vetter & Baker, 1983).

Traynier (1968) characterised the behaviour in *A. kühniella* males detecting a female sex pheromone as follows: waving of each alternate antenna, walking at random with waving antennae, wing vibration while walking at random or while walking upwind, flight, and copulatory movements towards the source after landing. He described male flight to be upwind in the presence of sex pheromone, crosswind in casts if the scent is momentarily lost and downwind, sometimes with crosswind casts if the scent was not recovered.

2.3.1. Male response to calling females

The degree of male response to sex pheromone may vary with the type of the pheromone source, i.e, live calling females or synthetic sex pheromone. Vetter & Baker (1983) reported that a greater number of *H. virescens* males exhibited eversion of hairpencils and copulatory attempt when exposed to calling females than when they were exposed to a 7-compound synthetic pheromone. They suggested that the greater responsiveness elicited by females was most likely due to better chemical cues, that is, the components or the blend quantity or quality in the synthetic pheromone was not comparable to actual female emissions. Cardé *et al.* (1974) also reported that calling *P. dispar* females were more attractive to males than various formulations of the gypsy moth synthetic pheromone, disparlure. They suggested that a key factor in the periodicity of male attraction to females is the rhythm of male response rather than the rhythm of female calling.

In *G. molesta,* male response did not vary with the type of pheromone source (Rothschild & Minks, 1974). The time of male activity in traps containing live virgin females was similar to those baited with the synthetic sex pheromone. Male activity did not begin earlier at synthetic sources where pheromone is emitted continuously compared with female sources which emitted pheromone during a particular period.

The periodicity of male response in some species coincides with that of female calling. In *Spodoptera litura* (F.), the timing of male response coincided with female

calling, with the first peak at 3 hours after the onset of the scotophase and the second peak at 1 hour before lights-on (Ohbayashi *et al.*, 1973). The periodicity of behavioural response of *P. carduidactyla* males also parallels the periodicity of female calling (Haynes & Birch, 1984).

The periodicity of male attraction to a pheromone trap baited with the synthetic female sex pheromone differed from the periodicity of female calling in the arctiid, *Holomelina aurantiaca* (Hübner) (Cardé, 1974). Maximal calling in the females occurred within the first 3 hours of an 8-h-scotophase at 24°C in the laboratory. In the field, where the temperature ranged from about 14-27°C, the highest number of males were caught in the pheromone trap about 4-5 hours before sunset.

2.3.2. Male response to synthetic sex pheromones

Synthetic pheromone blends usually elicit the normal sequence of male responses when the ratios and release rates of the components approximate those of pheromone-releasing females (Roelofs & Cardé, 1974b). Male sensitivity to synthetic sex pheromones depends on the quality as well as the quantity of the pheromone blend.

Linn *et al.* (1986) suggested that the minor components enhance male sensitivity to the pheromone and that the active space of the pheromone is determined by the upper and lower thresholds of the appropriate blend of the components and not by the major component alone. Males of *A. velutinana, T. ni* and *G. molesta* were tested in a flight tunnel to various dosages of the major component, partial blends and the female-released blends. It was found that the number of males that initiated upwind flight was significantly higher when complete blends of the pheromones were used than with single components or partial blends only.

Specificity of male response in species possessing a common major component is achieved by the presence of minor components. Linn *et al.* (1988a) tested *T. ni* and *Pseudoplusia includens* (Walker) males to the 6-component blend of *T. ni*, the 5-component blend of *P. includens* and Z7:12 OAc, which is the major component of the two species. They found that the level of cross-attraction between *T. ni* and *P. includens* males was lower compared with their responses to conspecific blends. They suggested that discrimination of the pheromone blend was due to the effects of the minor components and that the threshold for male response to the complete blend was significantly lower compared to the response threshold to the major component alone. When males detect a component that is not in the species blend but is present in that of the related species, this results in a significant arrestment of upwind flight.

In *P. operculella*, male response to the major component alone was comparable to that with a blend of the pheromone components. Ono & Ito (1989) reported that the behavioural responses of males to (E,Z,Z)-4,7,10-tridecatrienyl acetate alone were similar to those with a blend of this substance and (E,Z)-4,7-tridecadienyl acetate. Linn & Gaston (1981) tested *T. ni* males to the single components, Z7:12 Ac and 12:Ac and to two ratios of these components, 93:7 and 50:50, respectively, at doses ranging from 10^{-7} to 10^{-1} μg. They observed that activation and initiation of upwind oriented flight was mainly due to the primary component, Z7:12 Ac and there were no behavioural responses with 12:Ac alone. Of the two ratios, the 93:7 ratio elicited the greatest upwind oriented flight. They suggested that 12:Ac functions in combination with Z7:12 Ac to sustain upwind flight and close-range orientation to the source.

Components that have been identified from ovipositor extracts might not be all necessary to elicit significant behavioural responses. In *Heliothis phloxiphaga* Grote & Robinson, although four components (Z11:16 Ald, Z9:16 Ald, 16:Ald and Z11:16 OH) have been identified from ovipositor extracts, the blend that elicited responses equivalent to those with calling females consisted of three components only (Z11:16 Ald, Z9:16 Ald and Z11:16 OH), with a ratio of 96.4: 0.6: 3.0 (Raina *et al.*, 1986b). The behavioural responses of laboratory-reared and wild males of *H. zea* to 4-component (Z11:16 Ald, Z7:16 Ald, Z9:16 Ald and 16:Ald) and 5-component (above + Z11:16 OH) synthetic blends were compared by Raina *et al.* (1989). There were no significant differences in the proportion of laboratory-reared and wild females that flew upwind to the source, landed on source and attempted copulation when exposed to the 4-component-blend. With the 5-component-blend, however, the proportion of laboratory-reared males that did these behaviours was significantly lower than that with the 4-component blend and none of the wild males completed the flight. Raina *et al.* (1989) concluded that although Z11:16 OH is present in the female ovipositor extract, it may not be a component of the pheromone that is released by the female and that prolonged rearing appeared to have affected the ability of laboratory-reared males to discriminate the presence of Z11:16 OH in the 5-component blend.

2.3.3. Factors influencing sexual behaviour in males

Similar factors affecting female behaviour can influence sexual behaviour in male moths.

2.3.3.1. Moth age

Behavioural responses of *P. unipuncta* males to the female synthetic sex pheromone in a wind tunnel at 25°C varied with moth age (Turgeon *et al.*, 1983a). The proportion of males responding to three pheromone doses increased up to day 5 then declined on day 7. One- and 2-day-old males at 30 and 60 µg doses did not reach the pheromone source while those at 100 µg dose did. They suggested that age-specific responsiveness of male moths may be associated with their sexual maturation.

The proportion of *S. litura* males caught in a pheromone-baited trap in a wind tunnel was highest on the 4th day after emergence (Kawasaki, 1985). *P. chalcites* males observed in a wind tunnel started sexual activity on the 5th day following emergence and peaked on the 6th night and then gradually declined till the 15th night (Snir *et al.*, 1986).

2.3.3.2. Temperature

The level of response of *P. unipuncta* males to the female synthetic sex pheromone differed at 15°C and at 25°C (Turgeon *et al.*, 1983). A very small proportion of the males reached the source with their claspers extruded at 15°C compared with those at 25°C. Upwind flight was initiated in some males on the 5th day at 15°C at either 30, 60 or 100 µg doses while at 25°C, some males did this behaviour as early as the 1st day.

Under either 16:8 or 12:12 LD regimes, responsiveness of *P. unipuncta* males to the sex pheromone was significantly lower at 10°C than at 25°C (Dumont & McNeil, 1992). Under 16:8 LD, the level of responsiveness of males held at 10°C for 10 or 20 days then transferred to 25°C after five days was similar to 5-day-old males held at constant 25°C.

Cardé & Hagaman (1983) reported that ambient and thoracic temperatures influenced sexual behaviour in the gypsy moth, *L. dispar*. In the field where the ambient temperature ranged from 18-28°C, males caught at flight had thoracic temperatures from 21-36.5°C. In the wind tunnel at 16°C ambient temperature, take off occurred only when the thoracic temperature reached a minimum of 24°C. At 24 and 28°C ambient temperatures, take off occurred when the thoracic temperatures

were 28 and 31°C, respectively. After 1 minute of flight at 16, 20, 24 and 28°C ambient temperatures, the thoracic temperature increased by 1-3°C above take off temperature. Pre-flight behaviours, categorised as antennal twitch, body jerk, step and wing tremor, were more frequent at ambients of 16 and 20°C than at 24 and 28°C.

The responsiveness of *T. ni* males to the synthetic female sex pheromone, Z-7-dodecenyl acetate (Z7:12 Ac), under a 14:10 LD regime varied with temperature (Bollinger *et al.*, 1977). At 25:25°C day:night temperature, it was maximal on the 3rd to 5th days after emergence and between the 5th and 7th hours of the scotophase. At 25:15°C, they were maximally responsive also on the 3rd to 5th days, but during the 3rd hour of the scotophase. Moths maintained at 15:15°C responded maximally on the 7th to 9th days and during the 1st hour of the scotophase. Bollinger *et al.* (1977) suggested that males held at fluctuating temperatures may have lower threshold of response to pheromone than those held at constant temperatures.

Flight activity of *G. molesta* males to pheromone traps in the field did not occur below 15°C (Rothschild & Minks, 1974). Trap catches did not seem to be dependent on temperature above this lower threshold up to a maximum of 35°C. In the Australian *Helicoverpa* spp., Rothschild *et al.* (1982) found no evidence of a lower temperature threshold for pheromone trap catches. Baker & Roelofs (1981) reported that *G. molesta* males did not initiate flight during days when temperatures were below 16°C and flight in a wind tunnel was significantly less frequent at 14.5 and 16°C than at 18°C.

Linn *et al.* (1988b) reported that response specificity of *G. molesta* and *P. gossypiella* to different blends and doses of the pheromone is altered by temperature. Males of both species exhibit a greater response specificity at 20°C than at 26°C. They suggested that temperature effect on male behaviour is directly on neural pathways involved in the perception of odour and not simply the result of an increase in motor activity or a significant change in the release rate of the pheromone.

2.3.3.3. Photoperiod

In *P. unipuncta*, Dumont & McNeil (1992) reported that at 25°C, the level of male responsiveness to the female sex pheromone was higher at 16:8 than at 12:12 LD periods and there was delay in male receptivity at 12:12 compared with 16:8 LD regimes. At 10°C, however, photoperiod did not have significant effects on responsiveness of males of different ages.

Kanno (1981b) reported that in *C. suppressalis*, the periodicity of male sexual response to the female sex pheromone extracts occurred later in the scotophase under 8:16 LD period than under 16:8 LD condition.

2.3.3.4. Light intensity

The proportion of *S. litura* males captured in a pheromone trap in a wind tunnel varied with the light intensity (Kawasaki, 1985). No males were caught in complete darkness, when most of the moths did not take off from the cage. The number of trapped males was highest under 0.1 lux.

Farkas *et al.* (1974) studied the effects of varying light intensities on the initiation of flight and orientation to a pheromone source of *P. gossypiella* males. Most males under light intensities ranging from 10 to $< 10^{-7}$ lux initiated flight. Directed flight to the pheromone source was observed between 4 and 10^{-4} lux but not at $< 10^{-7}$ lux.

Rothschild & Minks (1974) reported that the onset of flight in *G. molesta* males at pheromone sources in the field appeared to be determined by the sunset time. Flight activity peaked at from 1 hour before to shortly after sunset except in the over-wintering generation when most activity occurred 2-3 hours before sunset. The timing of male flight was not well correlated with light intensity. Light intensity for flight initiation ranged from 3000-100,000 lux, for peak activity 1000-50,000 lux and for flight termination 4-3000 lux.

In the codling moth, *C. pomonella*, little motor activity of the males in the release cage was observed under full lights (1500 lux) but when subjected to "artificial dusk", they started walking with wing fanning or attempting to fly (Preiss & Priesner, 1988).

2.3.3.5. Wind speed

Baker & Roelofs (1981) reported that the proportion of upwind flight in *G. molesta* males in a flight tunnel decreased with increasing wind velocity. Upwind flight was totally suppressed at wind speed of 1.98 m/sec. Kaae & Shorey (1972) also reported that pheromone trap catches of *T. ni* males declined on nights with wind velocity approaching 2-3 m/s.

2.3.3.6. Relative humidity

Royer & McNeil (1993) investigated the effects of relative humidity on *O. nubilalis* male response to female sex pheromone. They found that at three concentrations (30, 100 and 200 µg of 97:3 Z:E 11-tetradecenyl acetate), the proportions of males that took flight and those that reached the source declined, whereas those that exhibited in-flight arrestment of upwind progress increased, with increasing relative humidity.

2.3.3.7. Pheromone concentration

The response level of *P. unipuncta* males to the female synthetic pheromone at 25°C varied with pheromone concentration (Turgeon *et al.*, 1983). At 30 µg, no response was recorded in 1-day-old males and only a small proportion of the males flew upwind to the source. At 60 µg, less than 50% of 1- and 2-day-old males flew upwind. Some males started to reach the source with claspers extruded on the 3rd day under the 30 and 60 µg doses. At a higher pheromone dose of 100 µg, some males reached the source as early as day 1.

Visual orientation by *P. gossypiella* males, described as hovering or landing on a dowel 40 cm downwind from a pheromone source, was significantly greater when the source contained 1 female equivalent than that with 0.01 female equivalent (Farkas *et al.*, 1974). In *G. molesta,* at 10-1000 µg, 83-93% of the males flew upwind while at 1 µg, only 40% did, and the proportion of males terminating upwind flight before reaching the source was lower at 1 and 10 µg (8 and 12%, respectively) compared with those at 100 and 1000 µg (88 and 100%, respectively) (Baker & Roelofs, 1981).

In the turnip moth, *Agrotis segetum* Schiff., the highest number of males that flew upwind and made source contact was obtained with pheromone doses of 3 and 30 µg of Z5:10 OAc (Löfstedt *et al.*, 1985). A dosage of 300 µg resulted in arrestment of upwind flight. The highest percentage of male codling moths with "successful flights" (approach flights terminated by landing at the source) was obtained at doses of 10^{-1} and 10^{-3} µg of codlemone while a dose of 10^{-6} µg did not elicit such response (Preiss & Priesner, 1988).

2.3.3.8. Conspecific males

Several species of male moths have eversible scent structures located on the 8th abdominal sternite (Birch *et al.*, 1990). Volatile chemicals have been identified from the hairpencils of male noctuid moths and some were found to elicit female response. Hirai *et al.* (1978) investigated the behavioural function of a pheromone

released by *P. unipuncta* males which consisted of a benzaldehyde, benzyl alcohol and benzoic acid. They reported that the male pheromone did not affect the calling behaviour of the females but did affect the behaviour of other males in a wind tunnel. The male pheromone inhibited other males from approaching calling females and attempting copulation when near sexually-receptive females. In a later study, however, Fitzpatrick *et al.* (1988) found that *P. unipuncta* males were not inhibited or repelled by conspecific male pheromone. Male responses to either 300 or 30 μg of the female synthetic sex pheromone in the presence of freshly excised male hairpencil, solvent-rinsed hairpencil or a small paintbrush, were not significantly different.

Two chemicals, *trans*-ethyl cinnamate and methyl-2 epijasmonate, were identified from hairpencil extracts of *G. molesta* (Baker *et al.*, 1981). Baker (1983) reported that male competition influenced the courtship patterns in *G. molesta* males in a wind tunnel. He released four males in the tunnel at a time in the presence of a calling female. "First-arrival" males exhibited the typical courtship behaviour, i.e., upwind flight to hairpencil display, then copulation. On the other hand, variations in the courtship patterns of "late-arrival" males were observed, such as copulation without hairpencil display, touching the abdomen of first-arrival male doing a hairpencil display with the female resulting in breaking up the courtship sequence, and simultaneous hairpencil display with the first-arrival male resulting in copulation of the late-arrival male with the female.

2.4. Mating behaviour

Teal *et al.* (1981) categorised the reproductive behaviour in *H. virescens* into precourtship and courtship behaviours. The female precourtship behaviour consisted of short bouts of calling followed by "scent marking" in which females dragged the ventral portion of the ovipositor on the substrate. This was followed by a quiescent calling period. The precourtship behaviour of males that were inactive on release in the flight tunnel began with activation behaviours such as wing elevation and vibration, antennal movement, antennal cleaning with the epiphysis of the foreleg, extension of proboscis and random ambulation during preflight wing fanning. After this, males had either a non-directed or directed flight to calling females. Males that were active on release began with a period of aerial searching marked by erratic flights, bumping into walls and stopping frequently. Upwind flight towards calling females was characterised by a series of zigzags. Courtship interactions started with the male approaching and moving under a female's wing. The female fanned her wings and the male partially exposed the hairpencils. The male then moved parallel to the female and turned to face her, fully exposing the hairpencil and finally clasping the female genitalia.

Similar premating behaviours were observed in *H. zea* but unlike *H. virescens*, *H. zea* males tapped the female ovipositor with their antennae before moving parallel with the females (Agee, 1969). In addition, *H. zea* males did not attempt to copulate with females that were not vibrating their wings or had not extended their ovipositors. Males that were unsuccessful in copulating within 3-5 minutes soon assumed the resting position. Receptive *H. zea* females did not move when their ovipositors were tapped by male antennae while unreceptive ones attempted to escape when males extended their claspers to grasp their genitalia. Male touching of the extruded female ovipositor with the antennae and foreleg has also been observed in *T. ni* (Shorey, 1964). Babilis & Mazomenos (1992) described precopulatory behaviour in *S. nonagrioides* females as extrusion of pheromone glands, and male response as wing fanning, upwind flight, approach to female and copulatory attempts.

The frequency and duration of mating vary in different species. On the average, *T. ni* females mated twice, while males appeared to be capable of releasing spermatophores as long as there were receptive females (Shorey, 1964). Mated pairs of *H. zea* remain in copula from 45 to 90 minutes (Agee, 1969) while the mating period in the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough) averaged 26 minutes (Swaby *et al.*, 1987). Miyashita & Fuwa (1972) reported that the duration of mating in *S. litura* ranged between 30 and 90 minutes. In *H. peltigera*, mating duration lasted between 50 and 180 minutes (Dunkelblum & Kehat, 1992). The fall armyworm, *S. frugiperda* had a mean mating duration of 130 minutes (Simmons & Marti, 1992). The frequency of mating in this species averaged 3.7 times in females and 6.7 times in males.

2.4.1. Factors affecting mating behaviour

Like calling behaviour, mating behaviour is influenced by a number of factors. Agee (1969) summarised the conditions for successful mating in *H. zea* (in order of importance): (1) moths were 30-60 hours old; (2) light level was less than 0.015 mw/cm^2 (quarter moon); (3) pigment of the secondary iris of the compound eye of the moths had migrated from the green to the brown-gold position (dark-adapted); (4) there was a slight breeze or movement of air; (5) contact with the other sex was fresh (moths had not been caged together for long periods); (6) the relative humidity was between 50 and 100% and; (7) the temperature ranged from 21 to 28°C.

Under a 14:10 LD regime, mating in *S. littoralis* varied with moth age and time of the scotophase (Dunkelblum *et al.*, 1987). During the 1st scotophase, mating

occurred between the 6th and 9th hours, while during the 2nd to 4th scotophases, most mating occurred within the first 3 hours. In *T. ni,* first mating occurred mostly during the 2nd or 3rd nights following emergence (Shorey, 1964). *O. pseudotsugata* females were most attractive during the first 3 days after emergence and the proportion of mating was highest in females that were < 1 day old (Swaby *et al.*, 1987). Under natural photoperiodic conditions, mating in *S. litura* occurred in two peaks, with the major peak at 2000 h or 1 hour after sunset and the minor peak at 0300 h or 2 hours before sunrise (Yushima *et al.*, 1973). The age of both males and females also affected mating in this species. Mating did not occur in 1-day-old males and females, and was highest in 3- to 4-day-old pairs. The proportion of mating was higher in 1-day-old females paired with 2- to 4-day-old males than in 2-to 4-day-old females paired with 1-day-old males. In 3-day-old *H. peltigera*, peak mating activity occurred between the 6th and 8th hours in a 10-hour scotophase (Dunkelblum & Kehat, 1992).

Shorey *et al.* (1968) studied the timing of maturity of three reproductive factors - sex pheromone production, mating and ovarian development - in 7 noctuid species. Rapid sex pheromone production in the females generally occurred within 1 day preceding or following adult emergence. Mating and the development of chorionated eggs were maximal during 0 to 1.5 and 0.5 to 2 days following emergence, respectively.

The periodicity of mating in *C. suppressalis* under varying photoperiodic conditions - 20:4, 16:8, 12:12, 8:16 and 4:20 LD periods, at 15 and 25°C was investigated by Kanno (1981b). Mating time shifted later under longer scotophases and this shift was larger with variations in the photoperiod at 25°C compared with 15°C.

In *O. nubilalis,* mating success under high humidity (> 75%) differed from that under low humidity (< 40%) (Royer & McNeil, 1991). Under high humidity, the proportion of mated females was consistently high during the first 5 days while under low humidity, this proportion was lower during the first 2 days and was similar to that under high humidity in the 3rd to 5th days. Delayed mating under low humidity may be partly due to delayed calling in females under lower humidity.

2.5. Uses of pheromones in insect pest management

Campion (1984) summarised the uses of pheromones in the management of cotton, orchard, forest, vegetables and stored product pests, including problems with their usage.

The three main uses of pheromones in pest control are mating disruption, mass-trapping and population monitoring. Mating disruption is achieved by permeating the atmosphere with the pheromone to reduce aggregation or mate finding, resulting in the suppression of mating. The main problem encountered with mating disruption is the immigration of mated females to the treated area. Pest control by mating disruption works well if the target species is of key pest status but is not very migratory and has a narrow range of host plants. An example of a successful mating disruptant is gossyplure, the pheromone of the pink bollworm *P. gossypiella*, a cotton pest. Mass-trapping aims to reduce pest population levels by using large numbers of pheromone traps. Problems associated with this method include trap deployment and maintenance, as well as immigration of mated females to the trapped area. An example of successful control by mass-trapping is with the citrus flower moth, *Prays citri* Mill. in Israel. Pheromone traps are useful in monitoring the presence or absence of certain pest species. They can provide an early warning of pest incidence and thus, application of control methods can be timed. To be reliable monitoring tools, however, pheromone trap catches should be correlated with the extent of female oviposition as a measure of subsequent economic damage.

The use of pheromones in pest management, and particularly as monitoring tools, is further discussed in Volume 2 of this thesis.