REPRODUCTIVE BEHAVIOUR AND PHEROMONE TRAPPING OF THE COMMON ARMYWORM, *MYTHIMNA CONVECTA* (WALKER) (LEPIDOPTERA: NOCTUIDAE)

Volume 2. *Mythimna convecta* Sex Pheromone and Male Responses to Synthetic Pheromones

by

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ABSTRACT

Pheromone components of *Mythimna convecta* (Walker) were collected by gland washing and sampling the air around calling females. Pheromone analyses were done by gas-chromatography using the five compounds identified by T. Bellas and C. Whittle of CSIRO (Z-11-hexadecenal, Z-11-hexadecen-1-ol, n-hexadecanal, Z-9-hexadecenal and Z-9-tetradecenal), and Z-11-hexadecenyl acetate as standards. Z-9-tetradecenal and Z-11-hexadecenyl acetate were not detected in either the gland or air samples, but the other four substances were. Z-11-hexadecenal appeared to be the major component in both samples. In addition, four unknown trace components were found in both the gland and air samples.

Five synthetic pheromone blends were tested in the wind tunnel. Males were scored for take off, upwind flight, approach, landing, clasper extrusion and baulking. Baulking behaviour was not observed with the blank source or live females. This behaviour was characterised by an upwind flight to about 20-30 cm from the source followed by immediate and rapid backing-off from the source. Definitive sexual responses (approach, landing and clasper extrusion) and baulking did not occur in 1- and 2-day-old males. Baulking and only low levels of approach occurred with Blends 1, 2 and 4, and landing and clasper extrusion were not observed with these blends. The blends that were comparable to, if not significantly better than, calling females were Blends 3 and 5, which elicited high levels of approach. Of these two blends, landing and clasper extrusion with copulatory attempt were greater with Blend 5. Consideration of these differences, in relation to the components of the five blends, led to a model of *M. convecta* male responses in which Z-11-hexadecenal was proposed as a long-range attractor, n-hexadecanal and Z-11-hexadecen-1-ol stimulated closer approach, and Z-9-hexadecenal was important in short-range sexual behaviours such as landing and clasper extrusion.

Field trapping experiments were conducted at Armidale, Kootingal and Boggabri in NSW and Swan Hill, Dookie and Rutherglen in Victoria. Blends 1, 3 and 5 were tested at these sites using Texas, AgriSense and Hara traps. Texas traps were more efficient than AgriSense or Hara traps. Blend 1 did not catch any *M. convecta* but caught large numbers of *Helicoverpa punctigera* (Wallengren). Blends 3 and 5 caught *M. convecta,* but also caught *H. puntigera,* particularly when populations of the latter were high. Blend 1 caught larger numbers of *H. punctigera* than either Blends 5 or the currently used *H. punctigera* lure. The potential use of pheromone traps for monitoring both *M. convecta* and *H. punctigera* is discussed.

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CHAPTER 9

INTRODUCTION

Volume 1 of this thesis discussed the nocturnal behaviour, particularly the calling behaviour of *M. convecta* females, the effects of temperature and photoperiod on calling, male responses to calling females and the interactions between sexes in the laboratory and in the field. Volume 2 (this volume) deals with the collection and identification of sex pheromone substances in *M. convecta* females and testing of different blends in the laboratory and in the field. The prospects of developing a pheromone trap for this species are discussed.

Pheromones have three main uses in pest management - mass-trapping, mating disruption and monitoring. For *M. convecta*, the first two uses are not likely to be feasible, for reasons discussed below. However, pheromone traps might be useful as monitoring tools for this species.

For mass-trapping of Lepidopteran pests, traps should have a trapping efficiency of about 80-95% (Knipling & McGuire, 1966 as cited by Campion, 1984). To effectively suppress populations, trap densities were estimated to range from 1 to 700/ha. Pheromone traps for mass-trapping of *Helicoverpa* spp. in the US were shown to have limited efficiencies (range of 5-60%) and were very unlikely to be a successful control method because the traps caught large numbers of males 8-9 days after mating had taken place, thus not preventing mating, oviposition and subsequent larval infestations (Sparks *et al.*, 1982). Successful mass-trapping systems have been obtained in some trials with other species, but none achieved success at an economic cost (Campion, 1984).

M. convecta is a pest of cereals and coarse grain crops. These are low-value crops which are grown over large areas in Australia. Over 11.4 and 4.8 million hectares in Australia are planted to wheat and coarse grains such as barley, sorghum, maize and triticale, respectively (Cribb, 1989). Mass-trapping for suppression of *M. convecta* populations on large areas with low-value crops will not be economically feasible because the cost will be too great to operate large numbers of traps.

Mating disruption uses large amounts of pheromones to disrupt sexual communication, and thus prevent mating (Suckling, 1993). Bartell (1982) proposed mechanisms of disruption as sensory adaptation and habituation, trail masking and false

trailing. Mating disruption with pheromones has been successful in some species. Commercialised disruption systems have been developed in the US, Japan, Europe, and Australia, mostly for horticultural or orchard pests (Suckling, 1993). In Australia, successful mating disruption was shown in the Oriental fruit moth, *Cydia molesta* (Busck) (Rothschild, 1975; Vickers & Rothschild, 1985). In New Zealand, the use of pheromones for mating disruption of the lightbrown apple moth, *E. postvittana* and the codling moth, *C. pomonella* is promising (Suckling & Clearwater, 1990; Clearwater & Muhlbacher, 1993).

Mating disruption works well in insect pests that are less mobile, have limited hosts, and have easily synthesised pheromone components (Suckling, 1993). There are some problems, however, with mating disruption systems. They require maintenance of high levels of pheromone concentration for several weeks and even release and distribution of the pheromones, as well as knowledge of the biology and ecology of the target species (Campion, 1976; Rothschild, 1979; Jutsum & Gordon, 1989). Immigration of mated females to the treated area is likely to be a problem with mating disruption (Rothschild *et al.*, 1982a), as was shown in the initial field trials for *Helicoverpa* spp. in cotton in Australia (Betts *et al.*, 1993). *M. convecta* is a highly migratory and polyphagous species. Hence, mating disruption is unlikely to be successful in this species.

Although mass-trapping and mating disruption are not promising for *M. convecta*, monitoring with pheromone traps might be feasible. *M. convecta* infestations are sporadic and (at present) largely unpredictable. Moths are not readily noticed in the day time because they are nocturnal. Most farmers can not easily identify the larvae. Small larvae are not easily noticed, and hence, by the time damage is seen, substantial losses have already occurred. Armyworm species like *M. convecta* move *en masse* during outbreaks, thus damage can be quick (Broadley, 1979). At present, fermentation (FE) traps, which use port wine/sugar solution as lures (McDonald, 1990), are the most reliable methods for monitoring *M. convecta* populations. These traps, however, are not highly specific because they catch many other species, and require frequent topping up or changing the lure due to evaporation in hot weather or dilution in wet weather. Likewise, *M. convecta* moths are not greatly attracted to light traps (Del Socorro, 1991). Pheromone traps, which are specific to the species, are likely to be useful as monitoring tools for the presence or absence of *M. convecta* in an area. Pheromone traps can provide an early warning of crop infestations as well as oviposition, and thus insecticide application can be timed.

A desirable pheromone trap is one that catches large numbers of *M. convecta*. The synthetic lure should be specific. Sympatric species often have common pheromone components (Roelofs & Cardé, 1974). Byers & Struble (1987) pointed out that the specificity of a pheromone is influenced by the relative population densities of the target species and non-target species with similar pheromone components. If the populations of non-target species are high and that of the target species is low, the proportion of non-target species in pheromone traps will be relatively high and accurate identification of the specimens will be essential. On the other hand, if target population densities are high, there may be competition between the traps and target females which might lower trap catches, giving a misleading estimate of target population density (the "female competition effect"; Gregg & Wilson, 1991). Thus, to be an effective monitoring tool, pheromone traps should be both highly attractive and highly specific for *M. convecta*.

If the trap is to be a reliable predictor of potential damage, catch size should be well correlated with the extent of female oviposition. This is a problem with *Helicoverpa* in cotton. According to Daly & Fitt (1993), pheromone trap catches of *Helicoverpa* spp. were poor indicators of species composition of eggs laid on cotton fields adjacent to traps. Discrimination of the two species, *H. armigera* and *H. punctigera*, is important prior to insecticide application. *H. armigera* is resistant to insecticides, so the choice of insecticide to be used depends on species composition in the field (Forrester *et al.*, 1993). Thus, the results of Daly & Fitt (1993) suggest that pheromone traps, at least in their current state of development, have limited usefulness for choosing insecticides against *Helicoverpa* spp. If a successful pheromone trap for *M. convecta* could be developed, field experiments would then need to be done to study the relationship between trap catch and oviposition. Such studies would provide a method of relating trap catches to subsequent damage, and therefore economic thresholds in terms of trap catches. An economic threshold for this species would help farmers to apply appropriate control methods when needed.

Gregg & Wilson (1991) describe various types of pheromone traps, and discuss the advantages and disadvantages of each type. As monitoring tools, traps need to be serviced regularly, hence pheromone traps should be simple and easy to operate. Traps should also be cheap and durable. The number of traps required in a given area depends on whether quantitative or qualitative data are needed. In the role proposed above for *M. convecta* pheromone traps, it is clear that quantitative data would be needed. Research on the optimum number of traps, their placement, and frequency of servicing would be required.

CHAPTER 10

THE SEX PHEROMONE OF FEMALE *M. CONVECTA*

10.1. Introduction

The sex pheromone of *M. convecta* females has not been reported in the literature. Substances thought to be components of the female sex pheromone have been previously characterised by CSIRO chemists (T. Bellas & C. Whittle, pers. comm., 1990). Initial field trials using a blend of these components, however, were unsuccessful, with virtually no moths being trapped (G. McDonald, pers. comm., 1990). Experiments were conducted to study the reproductive behaviour of *M. convecta* females and males in the laboratory and in the field (Chapters 4-7) to provide an understanding of the pheromone biology including behavioural responses of males to the natural sex pheromone of the females.

One of the objectives of this research project was to test a synthetic pheromone blend for *M. convecta* males in field traps, which might form a basis for the development of a pheromone trap in the future. Based on the five substances previously identified by CSIRO, further attempts to identify pheromone substances from the females were done by means of pheromone gland washing and collection of airborne volatiles from calling females.

10.2. Materials and methods

10.2.1. Collection from pheromone glands

The procedure for extracting pheromone substances from the female glands was modified from the various methods reviewed by Golub & Weatherston (1984). *M. convecta* females used were unmated with ages ranging from 4 to 7 days old. Females held individually in clear plastic cages were allowed to call (fully extrude their ovipositors) for at least 15 minutes. When a female had been calling for this period, she was taken out of the cage and the abdominal tip was carefully squeezed to expose the pheromone gland, which is normally located between the 8th and 9th abdominal segments of female moths (Jefferson *et al.*, 1968; Hollander *et al.*, 1982). The abdominal tip was excised with a pair of spring-loaded fine scissors (Australian Entomological Supplies, Bangalow, NSW, Australia) and placed in the solvent.

A sample consisted of three abdominal tips soaked in 200 µl toluene for 15-20 minutes. Five sample batches were prepared, so the total volume of toluene was 1000 µl. From each 200 µl-batch, 50 µl was transferred to a 12 x 32 mm amber microvial (Alltech #95195, Baulkham Hills, NSW, Australia) sealed with a Teflon-lined septa and screw cap,

to make up the final sample (250 µl) for analysis in the gas chromatograph (GC). The gland sample was kept in a freezer until analysis.

10.2.2. Collection of air from calling females

The method of collecting airborne volatiles from calling females was modified from effluvial collection methods reviewed by Golub & Weatherston (1984). Unmated 4- to 7-day-old females were used. Each female was held in a clear plastic cage with a 50 mm-diameter hole in the side. The collection device was a modified glass pasteur pipette with a layer of glass wool inside (Fig. 10.1). Air from the female cage was pulled by a vacuum pump through the glass wool, and some airborne pheromone components were adsorbed onto it.

When a female began calling, the pipette was carefully inserted through the hole in the cage with its tip directed towards the fully extruded ovipositor. If the female stopped calling, the pipette was transferred to another cage where the female was calling. Air collection was done continuously for 2 hours. During this period, collections from 12 different females were passed through the pipette and accumulated on the glass wool. After this period, the pipette was rinsed once with 1000 µl toluene into a microvial for GC analysis. The final sample was kept in a freezer until analysis.

10.2.3. Gas chromatography

Gland and air samples were analysed by gas chromatography within 24 hours after collection. The gas chromatograph (Varian 3400, French's Forest, NSW, Australia) was equipped with a flame ionisation detector and used helium as the carrier gas. The GC injector was set at 150°C and the detector heater at 265°C. The run time for one sample lasted 30 minutes.

The gas chromatograph used a sample injection volume of 1 µl. The analytical column used was a 30 mm x 0.32 mm ID SE-30 capillary column with 0.25 µm film thickness (Alltech Econo Cap #19651) programmed as follows: initial column temperature at 50°C; 5°C/min to 100°C with hold time of 1 min; 10°C/min to 150°C with hold time of 2 min; 15°C/min to 200°C with hold time of 1 min and 20°C/min to 250°C with a final hold time of 5.17 minutes.

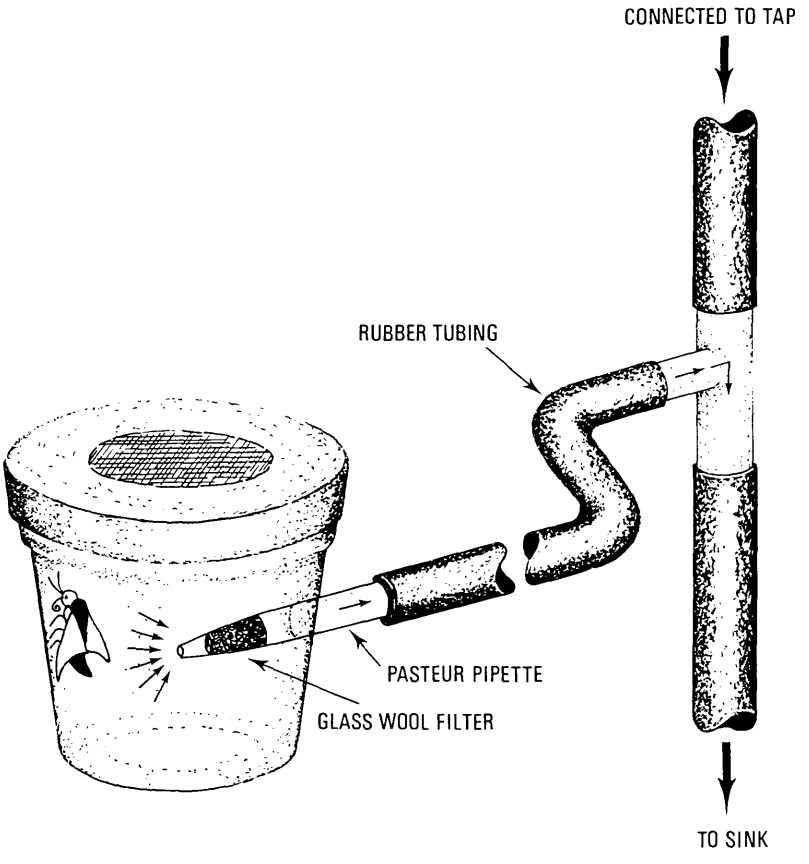


Fig. 10.1. Device used to collect air from calling *M. convecta* females.

The standard components were Z-11-hexadecenal (Z11:16 Ald), Z-11-hexadecen-1-ol (Z11:16 OH), n-hexadecanal (16 Ald), Z-9-hexadecenal (Z9:16 Ald), Z-9-tetradecenal (Z9:14 Ald) and Z-11-hexadecenyl acetate (Z11:16 Ac). The first five components were identified by C. Whittle & T. Bellas (pers. comm., 1990) from pheromone gland extracts of *M. convecta* females (Table 10.1). Z11:16 Ac is the major component of the true armyworm, *P. unipuncta* (McDonough *et al.*, 1980) and has been identified in the oriental armyworm, *M. separata* (Takahashi *et al.*, 1979 as cited by Zhu *et al.*, 1987).

A sample containing 1 µl of each of these six components in 1000 µl of toluene was prepared. The components found in the gland and air samples were quantified by comparing the peak areas and retention times with those of the standard components, which were obtained from Dr. S. Voerman, Research Institute for Plant Protection, Wageningen, The Netherlands and Mr. K. Ogura, Shin-Etsu Co., Tokyo, Japan.

10.3. Results and discussion

The pheromone components of *M. convecta* females from pheromone gland extracts previously identified by the CSIRO chemists, T. Bellas and C. Whittle are shown in Table 10.1.

Component	Ratio	%
Z-11-hexadecenal (Z11:16 Ald)	1.00	72.7
Z-11-hexadecen-1-ol (Z11:16 OH)	0.17	12.4
n-hexadecanal (16 Ald)	0.15	10.9
Z-9-hexadecenal (Z9:16 Ald)	0.05	3.6
Z-9-tetradecenal (Z9:14 Ald)	0.005	0.4

Table 10.1. Pheromone components from gland extracts of *M. convecta* females identified by T. Bellas and C. Whittle, CSIRO.

Fig. 10.2 shows the gas chromatograms of the standard with the six components (a), gland sample (b) and air sample (c). A description of these chromatograms is given in Table 10.2. Fig. 10.2a shows that in the sample of the six components, there was an early peak (Unknown A) with a retention time of 18.623 minutes. It was not one of the standard components, but was identified separately in the samples of five of the six standards (all except Z9:16 Ald) at levels ranging from 1.2 to 13.3%. It was probably a breakdown product from these components, or an impurity in either the pheromones or the solvent. There were also trace amounts of this substance in the gland and air samples.

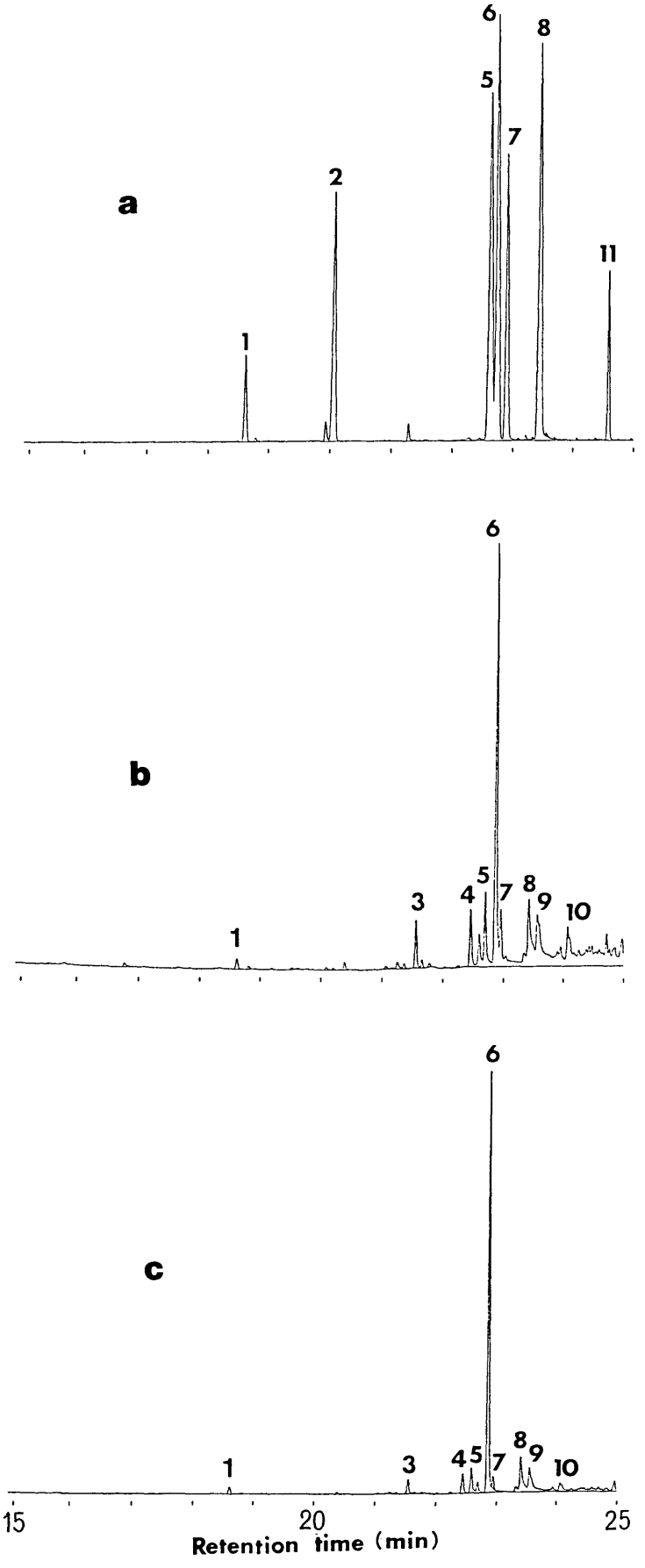


Fig. 10.2. Gas chromatograms of the sample containing the standard components (a), and gland (b) and air samples (c) from *M. convecta* females. The standard components are Z9:14 Ald (2), Z9:16 Ald (5), Z11:16 Ald (6), n-16 Ald (7), Z11:16 OH (8) and Z11:16 Ac (11). There were five unknown peaks - 1, 3, 4, 9 and 10. The vertical axis for the standards (a) is 27x that of the glands (b) and 7x that of the air samples (c).

In the pheromone gland sample, Z9:14 Ald and Z11:16 Ac were not detected (Fig. 10.2b). Z11:16 Ald (6) was the major peak while Z9:16 Ald (5), 16 Ald (7) and Z11:16 OH (8) appeared in smaller peaks. Four other smaller peaks were also detected, of which two (Unknowns B and C) had shorter retention times than Z11:16 Ald and the other two (Unknowns D and E) had longer retention times than Z11:16 Ald. Similar peaks of known and unknown components were present in the chromatogram of the air sample (Fig. 10.2c), though the relative proportions varied.

		Standard			Glands			Air		
Peak	Component	RT	Area	%	RT	Area	%	RT	Area	%
1	Unknown A	18.623	274933	3.3	*	trace	*	*	trace	*
2	Z9:14 Ald	20.081	949468	11.7	*	nil	*	*	nil	*
3	Unknown B	*	nil	*	21.551	4665	3.3	21.544	5446	2.0
4	Unknown C	*	nil	*	22.450	6442	4.6	22.438	8218	3.0
5	Z9:16 Ald	22.652	1681223	20.7	22.590	4181	2.9	22.578	10539	3.9
6	Z11:16 Ald	22.768	1774048	21.9	22.852	41535	29.5	22.682	150014	55.0
7	16 Ald	22.916	1027177	12.6	22.949	6803	4.8	22.940	3843	1.4
8	Z11:16 OH	23.472	1763157	21.7	23.414	12912	9.2	23.399	20759	7.6
9	Unknown D	*	nil	*	23.560	13587	9.6	23.546	18812	6.9
10	Unknown E	*	nil	*	24.069	7780	5.5	24.061	6257	2.3
11	Z11:16 Ac	24.596	426512	5.2	*	nil	*	*	nil	*
	Others**	15-30	234888	2.9	15-30	42899	31.5	15-30	48756	17.9
	Total	15-30	8131406	100.00	15-30	140804	100.00	15-30	272644	100.00

Table 10.2. Retention times (RT, in minutes), area (counts) and percentages (%) for the different peaks in the gas chromatograms of the blend of standards, pheromone gland washings and air collection from calling *M. convecta* females. Peak numbers are shown in Fig. 8.2. **Others represent the series of smaller peaks which might have been due to noise in the instrument, impurities or minor components in the samples.

There were slight variations in the retention times of the peaks between the standard, pheromone gland, and air samples (Table 10.2). However, these variations were rarely large enough to create doubts about the identities of the peaks, and in the few instances where this did occur, sub-samples with "spikes" of the authentic standards were analysed, so that the authenticity of the peaks could be verified.

The percentage of Z11:16 Ald (55.0%) in the air sample was greater than that in the gland sample (29.5%). On the other hand, the percentages of 16 Ald and Z11:16 OH found in the air sample (1.4 and 7.6% respectively) were smaller than those found in the glands (4.8 and 9.2% respectively). The percentages of Z9:16 Ald in both the glands and air were comparable (2.9 vs 3.9%).

These percentages were calculated on the basis of all peaks detected by the GC, including smaller ones which might be due to noise in the instrument, impurities or minor pheromone components. These other peaks were most prominent in the glands, then in the air, and finally in the standards. Another way of expressing the relative amounts is as a percentage of the combined counts for the five major components identified by T. Bellas and C. Whittle. Results from this comparison are given in Table 10.3.

Component	Pheromone glands		Air
	Bellas & Whittle	This study	
Z11:16 Ald	72.7	63.5	81.0
Z11:16 OH	12.4	19.7	11.2
16 Ald	10.9	10.4	2.1
Z9:16 Ald	3.6	6.4	5.7
Z9:14 Ald	0.4	nil	nil

Table 10.3. Comparison of the percentages (%) of the different components identified from the pheromone glands and air of *M. convecta* females.

These results show that the gland samples from this study yielded similar percentages of the first four components to those of the CSIRO study. However, a further four peaks which did not correspond with any of the standards were also detected (Unknowns B, C, D and E). These substances were detected in proportions similar to those of the minor components (Z11:16 OH, 16 Ald and Z9:16 Ald) in the CSIRO study. They may represent additional pheromone components not detected in that work. Further study of these peaks using gas chromatography-mass spectrometry is in progress.

The air sample yielded results similar to those of both gland samples, except that the percentage of the dominant component, Z11:16 Ald, was higher and the percentages of the minor components (especially 16 Ald) were correspondingly lower. The same four unknown compounds present in the glands were also found in the air.

The quantities of the dominant component Z11:16 Ald in the glands and air can be estimated as follows: Since the injection volume was 1 µl and the concentration was 1µg/ml, 1 ng of Z11:16 Ald was present per injection of the standard. This amount yielded an area count of 1774048 and the area counts of Z11:16 Ald peaks in the gland and air samples were 41535 and 150014, respectively. Thus, the amount of Z11:16 Ald in the injections from the gland and air samples was calculated to be 0.0234 ng (41535/1774048) and 0.0846 ng (150014/1774048), respectively. For both the gland and air samples, the total volume was 1000 µl, of which only 1 µl was injected. Thus, the total

amount of Z11:16 Ald present can be obtained by multiplying the above figures by 10^3, yielding results of 23.4 ng for the gland sample and 84.6 ng for the air sample. The gland samples contained the ovipositors of 15 females, thus the average amount of Z11:16 Ald per female was 1.56 ng.

The low level in the glands compared to the air might be because gland samples were taken only 15 minutes after the onset of calling. It is possible that at this early stage of calling the quantity of pheromone being produced was low. Against this theory, however, is the observation that females in individual and mass mating cages and the field cage frequently initiated copulation soon after calling began (Chapter 7). This indicates that the quantity of pheromone produced must have been sufficient to attract males.

An alternative possibility is that the rate of turnover of pheromone components is very high so that at any one time the amount present in the glands represents only a small fraction of the amount released during prolonged calling. In the pyralid *P. interpunctella,* females reared at 27°C and 12:12 light:dark (LD) conditions had a turnover rate of 5 minutes during the scotophase and 440 minutes during the photophase (Nordlund & Brady, 1974). In some species, the release rates of pheromones can be quite high. For example, the noctuid *T. ni* released from 12 to 22 ng per min (Bjostad *et al.*, 1980) and the arctiid *H. lamae* released from 13 to 350 ng in 10-min period (Schal *et al.*, 1987).

A final possibility is that the method of collecting pheromone from the glands was not efficient because only the pheromone that was on the gland surface was collected. In support of this hypothesis is the observation of T. Bellas & C. Whittle (pers. comm., 1990) that up to 100 ng of the major component Z11:16 Ald could be collected from a single female under their conditions. Often, however, much lower levels were obtained. In *P. unipuncta,* McDonough *et al.* (1980) measured an average pheromone content of the major component Z11:16 Ac of 47 ng per female. Delisle & McNeil (1987) obtained a pheromone content of 25 up to 37 ng at 16:8 LD and 19 ng at 12:12 LD after the first night of calling in *P. unipuncta* females. However, there is considerable variation in pheromone contents both within and between Lepidopteran species, and levels of the order of 1 ng are not unusual (Morse *et al.*, 1982; Dunkelblum *et al.*, 1987).

Aldehydes are the major substances of the sex pheromone in the *Mythimna* genus. Renou *et al.* (1991) compared olfactory responses of 24 species of the sub-family Hadeninae to 30 pheromone components by means of electroantennography. Three genera, *Mythimna, Mamestra* and *Orthosia*, were represented by these species. *Mythimna* spp. showed great sensitivity to aldehydes. Of the 9 *Mythimna* spp. tested (not including

M. convecta), Z11:16 Ald was found to be the key compound in six species, Z9:14 Ald in one species, Z9:14 Ald and Z9:16 Ald in one species and Z11:16 Ac in one species. Mayer & McLaughlin (1991) listed the attractants of five *Mythimna* species (again, not including *M. convecta*) which caught males in field traps. These include Z11:16 Ac, Z11:16 Ald, Z7:14 Ac, Z9:12 Ac and E9:12 Ac. In most cases, Z11:16 Ald was the major component.

A species closely related to *M. convecta* is the oriental armyworm, *M. separata.* The sex pheromone of *M. separata* in Japan was identified as a blend of Z11:16 Ac and Z11:16 OH (Takahashi *et al.*, 1979 as cited by Zhu *et al.*, 1987). Sato *et al.* (1980) reported high trap catches using this blend with a ratio of 9:1 and 4:1. In China, Zhu *et al.* (1987) reported that the same blend was not attractive to *M. separata* males. They identified three components from pheromone gland washings of the females, Z11:16 Ald, 16 Ald and Z11:16 OH. The major component Z11:16 Ald alone, or in combination with either or both of 16 Ald and Z11:16 OH attracted males. They suggested that the preferred blend was Z11:16 Ald, 16 Ald and Z11:16 OH with a ratio of 100:10:0.1.

The three components found in Chinese *M. separata* were also found in both the gland and air samples of *M. convecta* females. In both species, Z11:16 Ald is the major component. In *M. convecta*, the amount of Z11:16 OH is greater than 16 Ald, whereas in *M. separata*, the amount of 16 Ald is greater than Z11:16 OH. No studies are found in the literature to differentiate these species, except the taxonomical characters described by Common (1965). These characters include the presence of a distal blackish band on the hindwing of *M. convecta* which is less prominent in *M. separata,* and minor differences in the male and female genitalia. However, the differences are quantitative rather than qualitative, and the two species are easily confused. The morphological similarity of the two species, and the apparent similarity of their pheromone communication systems, raise questions about their reproductive isolation from each other. Electrophoretic studies would be useful to ascertain the extent of genetic differences between them. Attempts to hybridise the two species would also be worthwhile. The unknown substances in *M. convecta* found in this study might be minor components needed in the reproductive isolation of this species, if indeed it is a distinct species.

Another species sometimes thought to be closely related to *M. convecta* is the true armyworm, *P. unipuncta,* which is a major pest in North America. The ecological characteristics of this species are similar to those of *M. convecta.* Some authors, (e.g. Common, 1990; McDonald, 1994, in press) place this species under the genus *Mythimna.* Mayer & McLaughlin (1991) listed six components identified from the glands and effluvium of *P.*

unipuncta by various authors. These are Z11:16 Ac, 16 Ac, Z11:16 OH, Z9:16 Ac, 16 OH and 14 OH. Hill & Roelofs (1980) first identified the primary sex pheromone component, Z11:16 Ac in the abdominal tip extracts and effluvium from females. McDonough *et al.* (1980) reported the presence of Z11:16 Ac, 16 Ac, Z11:16 OH and Z9:16 Ac in pheromone glands. They found that the major component, Z11:16 Ac, alone attracted males in field traps and that the number of males caught was not increased by the addition of any one or a combination of the other three components. Similarly, Kamm *et al.* (1982) found that the addition of Z11:16 OH to Z11:16 Ac did not increase trap catches and that the addition of Z7:12 Ac and Z9:14 Ac decreased trap catches.

The major pheromone component of *P. unipuncta,* Z11:16 Ac, was not found in *M. convecta* gland and air samples. Likewise, T. Bellas and C. Whittle did not identify this component in the gland extracts. Conversely, the major component of *M. convecta* pheromone, Z11:16 Ald, was not found in *P. unipuncta.* Only Z11:16 OH is a common component of these two species, and it is a minor one. Thus, the pheromone communication system in *P. unipuncta* is very distinct from that in *M. convecta.* This supports the idea that, despite their ecological similarities, the placement of these two species in separate genera is probably appropriate.

CHAPTER 11

BEHAVIOUR OF *M. CONVECTA* MALES IN THE PRESENCE OF SYNTHETIC PHEROMONES IN A WIND TUNNEL

11.1. Introduction

M. convecta males exhibited definitive sexual responses such as approach to the source, landing on the female cage, and extrusion of claspers with copulatory attempt, in the presence of calling females in a wind tunnel (Chapter 6). Pheromone gland washing and air collection from calling females indicated the presence of four substances (Z11:16 Ald, Z11:16 OH, 16 Ald and Z9:16 Ald). In addition, four unknown minor substances from both the gland and air samples were also detected.

In this chapter, behavioural responses of *M. convecta* males in the wind tunnel to five different blends of the synthetic pheromone are presented. Male sexual response to these blends, designated by approach behaviour, is compared with the response to calling females described in Chapter 6. These studies were conducted to identify pheromone blends which might be useful in traps for *M. convecta.*

11.2. Materials and methods

Male pupae were kept in a reverse-cycle cabinet at 25 ± 1°C and 16:8 light:dark (LD) conditions with the scotophase at 1000-1800 h AEST. Upon emergence, each moth was placed in a 150-ml plastic container with a piece of dental wick soaked in 5% sucrose (Chapter 3.1). Males of different ages were used. Both fresh and re-used males (Chapter 6.2) were tested. Males were transferred to the wind tunnel room at 1300 h to acclimatise. The wind tunnel room had a temperature of 25 ± 1°C and was dimly lit by red photographic safe light (Chapter 3.3)

Individual males were released at 5-min intervals during the 2nd half of the scotophase (1400-1800 h). The different male behaviours described in Chapter 6.2.1 were recorded on a portable computer using the QuickBASIC program DATA.BAS (Chapter 3.2) and later transcribed for analysis. Behaviours recorded were take off, upwind flight, approach to source, landing and clasper extrusion. Another type of behaviour, termed "baulking", was also recorded. This behaviour was characterised by flight to about 20-30 cm of the source, followed by an immediate and rapid "backing-off" from the source. Unlike approach behaviour, baulking was not accompanied by hovering within the vicinity of the pheromone source. Baulking was never seen in the responses of males to females (whether calling or not) or to blank stands (Chapters 6.3 and 6.4).

11.2.1. Pheromone blends and the rationale for selecting them

The different blends tested in the wind tunnel are given in Table 11.1.

Blend		Z11:16 Ald	Z11:16 OH	16 Ald	Z9:16 Ald	Z9:14 Ald
1	ratio	1.0	0.17	0.15	0.05	0.005
	%	72.7	12.4	10.9	3.6	0.4
2	ratio	1.0	0.17	0.15	0.05	-
	%	73.0	12.4	11.0	3.6	-
3	ratio	1.0	0.001	10.0	-	-
	%	90.8	0.1	9.1	-	-
4	ratio	1.0	0.14	0.025	-	-
	%	85.8	12.0	2.2	-	-
5	ratio	1.0	0.001	10.0	0.03	-
	%	88.4	0.1	8.8	2.7	-

Table 11.1. Ratios and percentages (%) of the different components for each blend tested in the wind tunnel.

Blend 1 is the pheromone blend first identified by T. Bellas and C. Whittle of CSIRO from female gland extracts. In this thesis, it was shown that the amounts of the main components in this blend were similar to those in the pheromone glands (Chapter 10.3). Mimicking the gland components is an accepted strategy for developing pheromones for attracting males to traps (Hummel & Miller, 1984). In spite of unsuccessful results from the initial field trapping experiments (G. McDonald, pers. comm., 1990), it was considered worthwhile to examine male responses to this blend in the wind tunnel.

Blend 2 is similar to the CSIRO blend (Blend 1) but excluding the smallest component, Z9:14 Ald. It might be that Z9:14 Ald is not an attractive component of *M. convecta* pheromone. There are cases where the addition of small quantities of a minor component greatly reduces trap catches (e.g., McDonough *et al.* (1980), Steck *et al.* (1980) and Kamm *et al.* (1982) with *P. unipuncta*; Teal *et al.* (1984) with *H. zea*; Löfstedt *et al.* (1985) with *Agrotis segetum* (Schiff.)). In this study, Z9:14 Ald was not found in either the glands and air samples (Chapter 10.3), in contrast to the CSIRO study. Thus, Blend 2 was tested to see if the performance of the CSIRO blend minus this potential interfering component would improve.

Blend 3 was equivalent to that of Zhu *et al.* (1987) for *M. separata*. It consisted of the first three main components, Z11:16 Ald, Z11:16 OH and 16 Ald. All these components were found both in the glands and air samples (Chapter 10.3). Unlike Blends 1 and 2, the amount of 16 Ald in this blend was greater than Z11:16 OH. It was

considered that because of the similarity between *M. convecta* and *M. separata* in morphology and pheromones (Chapter 10.3), a blend that works for a species closely related to *M. convecta* was worth testing in the wind tunnel.

Blend 4 was a modification from that of Zhu *et al.* (1987). Although the same three components of *M. separata* pheromone were found in the gland and air samples of *M. convecta,* the amounts of the minor components were different. In the *M. separata* blend (Zhu *et al.*, 1987), 16 Ald was greater than Z11:16 OH and in *M. convecta,* vice-versa Sometimes, changing the ratios can affect the attractiveness of a blend (e.g., Zhu *et al.* (1987) with *M. separata;* Quartey & Coaker (1993) with *Ephestia cautella* (Walker)). Thus, a blend similar to Blend 3 in substances but different in the ratios of the minor components was tested. The amount of Z11:16 OH and 16 Ald was based on the amount estimated to be present in the air sample from calling females (Chapter 10.3).

Blend 5 was similar to Blend 3 but with the addition of a fourth component, Z9:16 Ald. This component was not detected from the gland extracts of *M. separata* (Zhu *et al.*, 1987) but was found in *M. convecta* gland and air samples, though in small amounts (Chapter 10.3; T. Bellas & C. Whittle, pers. comm., 1990). In some cases, the addition of a minor component can give specificity (e.g., Stadelbacher *et al.* (1983) with *Heliothis virescens;* Raina *et al.* (1986) with *H. phloxiphaga*; Landolt & Heath (1987) and Linn *et al.* (1988) with *T. ni* and *P. includens).* This blend was tested to see if the performance of Blend 3 might improve with the addition of this component.

These pheromone blends were tested at various dosages (i.e., based on the amount of the major component, Z11:16 Ald). The different dosages tested were the following:

Blend 1 - 0.01 µg, 0.1 µg, 10 µg and 100 µg
Blend 2 - 0.01 µg, 0.1 µg, 10 µg and 100 µg
Blend 3 - 0.01 µg, 0.1 µg, 10 µg and 100 µg
Blend 4 - 0.1 µg
Blend 5 - 0.1 µg and 10 µg

Stock solutions of each component were made up in hexane at 1 µl/ml. The desired blend of components to be tested was prepared by mixing the required amount of each component. The required dosage was made up by dilution with hexane. Stock and blend solutions were kept in the freezer when not in use.

The required amount of the pheromone solution was pipetted on a glass fibre filter disc (Chapter 3.3) using a micro pipette (Volac, John Poulten Ltd., Essex, UK). The pheromone disc was changed every hour during an experiment. The disc was clipped by a 3-cm long copper wire and rested on a 30-cm high wooden stand placed on the upwind end of the wind tunnel. With one of the most attractive blends (Blend 3 at 10 µg; see Fig. 11.4c), the behaviour of males flown to a pheromone disc on the wooden stand was not significantly different from the behaviour of males flown to a disc in a cage. This result means that direct comparisons between the attractiveness of different blends and that of calling females (Chapter 6) can be made.

11.2.2. Statistical analyses

Data on the different male behaviours are provided in the sub-directory A:\CHAP11 of the floppy disk.

Although all the different behaviours were recorded, only the approach category was used as the test variable to compare responses to the different blends and dosages with responses to calling females. There were two reasons for doing this. Firstly, approach was the first level of sexual behaviour observed when males were flown to calling females, but not when flown to a blank source (Table 6.23, Chapter 6). Secondly, in terms of male response to a pheromone trap in the field, approach to 10-15 cm of a source is probably a good indicator of the likelihood that a male will be caught in the trap.

Data for fresh and re-used males were pooled. Behaviours between these groups in the presence of calling females were not significantly different (Chapter 6.4.2.2). Males aged 1 and 2 days were excluded from the analyses because they never exhibited approach behaviour to any blend, probably because they were not reproductively mature (Chapter 7.4).

The percentages of approach by males flown to different synthetic blends at various dosages were compared to the percentage of approach to calling females. Fig. 6.4a (Chapter 6) indicates that the probability of an approach to a calling female depended on the time of the scotophase when the male was tested in the wind tunnel. This might have been due to factors associated with the female (pheromone quantity/quality) or to the presence of a diurnal rhythm of male responsiveness. If the latter interpretation is correct, it would mean accurate comparison of the blends could only be obtained by testing the males at exactly the same time, ideally at the peak time for male approach. However, as each male took 5 minutes to test (and more time was required to

prepare the equipment between each run), this would not have been practical, as insufficient males would have been tested.

The use of synthetic pheromone blends, which remained constant throughout the scotophase, provided a means of distinguishing between the two interpretations. Preliminary analysis of the results for one of the most attractive blends (Blend 3, for which the largest numbers of males were tested; see section 11.3.1) showed a pattern of approaches similar to the pattern of approach to calling females (Fig. 11.1). In both, no approach was observed during the first and the last 30 minutes of the 2nd half of the scotophase. Likewise, the percentages of approach for both were highest between the 6th and 7th hours of the scotophase.

Thus, to enable comparisons between blends, some means of correcting for the time-dependence of male responsiveness was needed. It was decided that the best standard was responsiveness to calling females. The percentage of males approaching to females in each 30-min interval of the 2nd half of the scotophase is shown in Fig. 11.1. The data are from Chapter 6.4.2.2 of Volume 1. A quadratic regression was fitted to these results, which yielded the regression equation:

$$\% \text{ Response} = -6.01 + 0.0341t - 0.000045t^2 \qquad F_{2,5} = 21.30,\ p = 0.004,\ R^2 = 0.90)$$

where t = time of the scotophase (minutes after 1000 h)

The expected number of responders (i.e., approachers) is then given by the equation:

$$E_r = \Sigma p_r$$

where E_r = expected number of approachers

p_r = probability of each male approaching, calculated by the above regression equation ($p_r = 0$, when $t \geq 479$ or $t \leq 278$ min.).

A program in QuickBASIC (CALLTIME.BAS) was used to facilitate calculations. This program can be found in the sub-directory A:\CHAP11 of the floppy disk.

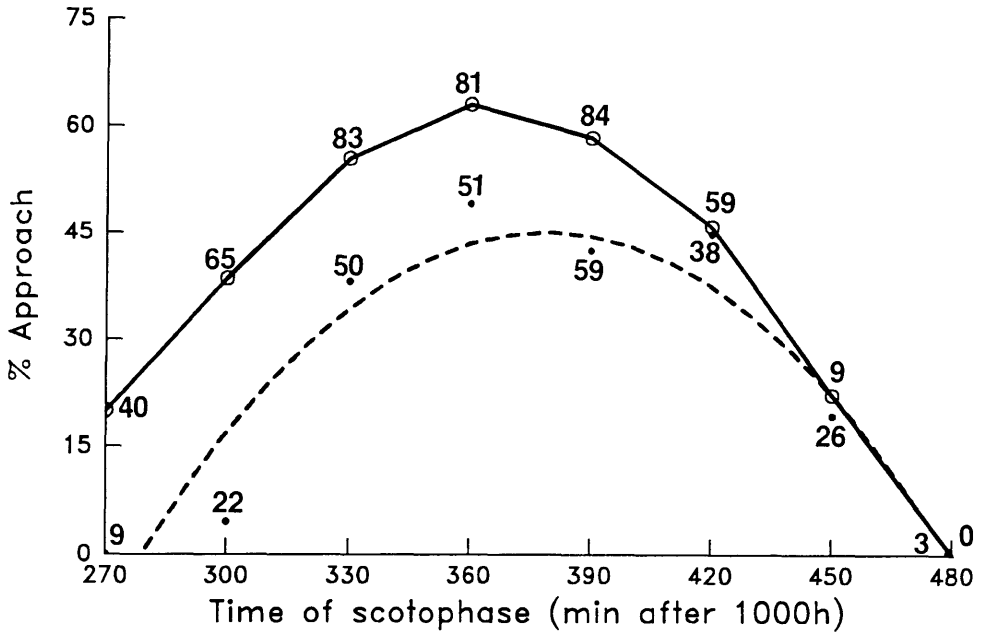


Fig. 11.1. Percentages (%) of approach of *M. convecta* males to Blend 3 (———) compared with the regression line fitted for % approach to calling females (▬ ▬ ▬) at various time of the 2nd half of the scotophase. The number above each point refers to the number of males tested at various times. For Blend 3, data for 0.1, 10 and 10 µg were combined.

A chi-square test using a 2 x 2 contingency table of expected and observed number of approachers and non-approachers was conducted to determine if responses to a given blend and dosage were significantly different from the expected responses to calling females.

11.3. Results and discussion

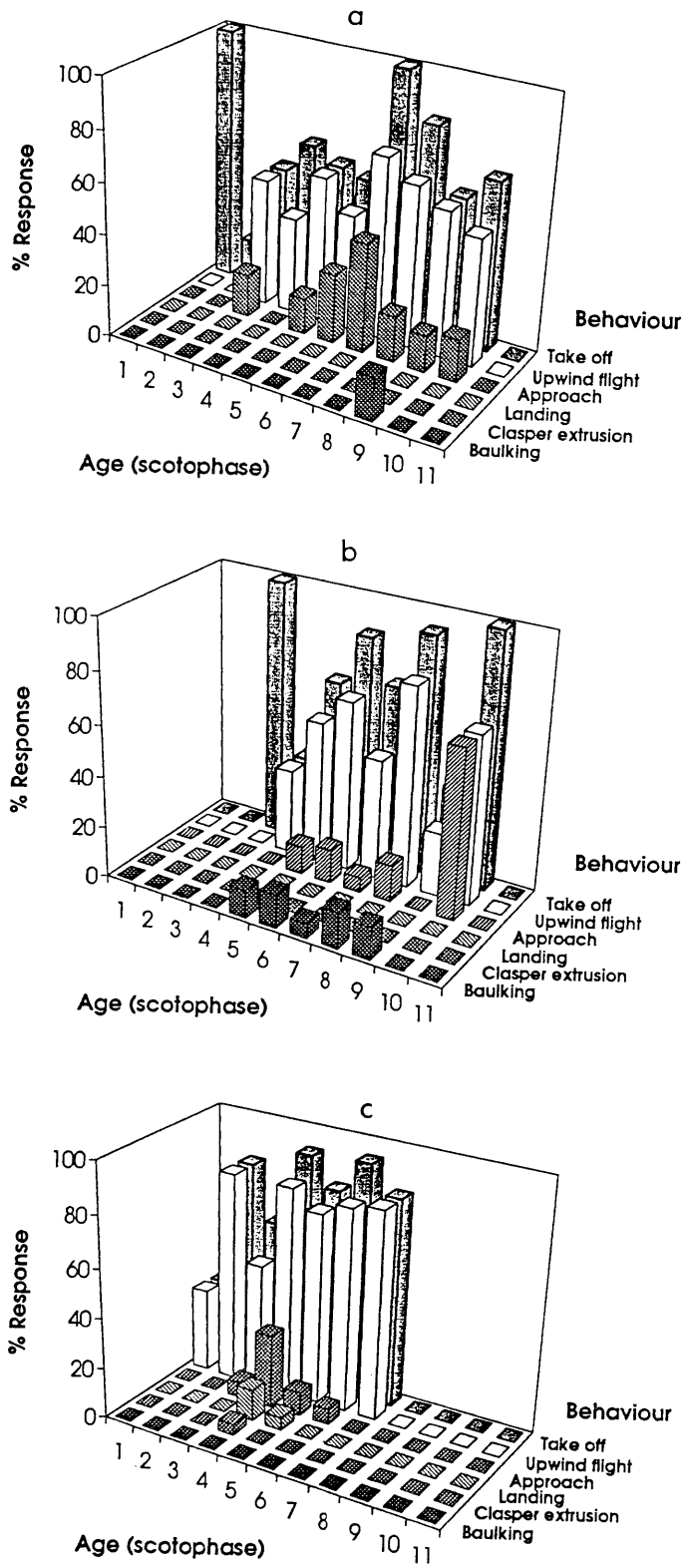
11.3.1. Behaviour of males to different blends

The number of males observed at different ages for the different blends is given in Table 11.2.

Blend and	No. of males observed at ages												
dosage (μg)	1	2	3	4	5	6	7	8	9	10	11	12	Total
Blend 1													
0.01	1	6	6	8	7	11	7	11	7	6	3	-	73
0.1	2	5	8	12	13	14	14	12	10	5	3	-	98
10.0	-	-	-	3	7	10	13	11	7	3	1	-	55
100.0	5	14	21	22	17	10	3	-	-	-	-	-	92
Blend 2													
0.01	-	-	1	3	9	15	17	14	8	3	-	-	70
0.1	-	-	16	21	20	17	7	5	3	2	2	-	93
10.0	2	5	7	11	11	10	8	3	1	-	-	-	58
100.0	-	-	-	11	5	-	-	-	-	-	-	-	16
Blend 3													
0.01	3	6	16	24	21	16	6	-	-	-	-	-	92
0.1	7	10	15	14	13	13	11	11	8	6	-	-	108
10.0	-	-	33	60	48	7	1	-	-	-	-	-	149
100.0	2	5	8	13	19	25	28	30	27	18	13	4	192
Blend 4													
0.1	5	7	19	28	20	14	9	5	1	-	-	-	108
Blend 5													
0.1	-	-	-	1	4	5	9	9	11	9	4	2	54
10.0	2	3	6	10	12	16	19	15	11	3	-	-	97

Table 11.2. Number of *M. convecta* males observed at different ages at various pheromone blends and dosages.

The percentages of males exhibiting the different behaviours at various ages at a dosage of 0.01 μg of Blends 1, 2 and 3 are shown in Fig. 11.2. Blends 4 and 5 were not tested at this dosage. Low levels of approach were recorded. Landing and clasper extrusion were not recorded in Blends 1 and 2 and only occasionally in Blend 3. On the other hand, baulking was recorded in Blends 1 and 2 but not in Blend 3. No approach, landing, clasper extrusion or baulking occurred with any blend if males were less than 3 days old.



d
(not done)

e
(not done)

Fig. 11.2. Percentages (%) of *M. convecta* males exhibiting the different behaviours at 0.01 µg of Blend 1 (a), Blend 2 (b), and Blend 3 (c). Blends 4 (d) and 5 (e) were not tested at this dosage. The number of males observed at each age is given in Table 11.2.

All five blends were tested at 0.1 µg. Baulking was observed with Blends 1, 2 and 4 while landing and clasper extrusion were recorded with Blends 3 and 5 (Fig. 11.3). The percentages of approach were greater with Blends 3 and 5 than with the other three blends. The percentages of approach, landing and clasper extrusion with Blend 3 were greater than those at 0.01 µg. Approach with Blend 3 was greater than that with Blend 5 but this difference was not significant ($\chi^2_1 = 2.08$). Landing with Blend 5 was significantly greater than that with Blend 3 ($\chi^2_1 = 14.90$), whereas clasper extrusion was not significantly different between the two blends ($\chi^2_1 = 2.31$) (Table 11.3).

	@ 0.1 µg			@ 10 µg		
	Yes	No	χ^2_1 value	Yes	No	χ^2_1 value
Approach						
Blend 3	55	36	2.08 n.s.	63	86	8.81**
Blend 5	26	28		57	35	
Landing						
Blend 3	3	88	14.90***	11	138	16.03***
Blend 5	13	41		24	68	
Clasper extrusion						
Blend 3	3	88	2.31 n.s.	7	142	9.24**
Blend 5	5	49		15	77	

Table 11.3. Comparison of approach, landing and clasper extrusion between Blends 3 and 5 at dosages of 0.1 and 10 µg. Yes = males that did behaviour and no = males that did not do behaviour.

At 10 µg, all blends except Blend 4 were tested. At this dosage, the levels of approach with Blends 3 and 5 were greater than those with Blends 1 and 2 (Fig. 11.4). Baulking was recorded with Blends 1 and 2 but not with Blends 3 and 5. Landing and clasper extrusion did not occur with Blends 1 and 2. Approach, landing and clasper extrusion were significantly greater with Blend 5 than those with Blend 3 (χ^2_1 = 8.81,16.03 and 9.24, respectively) (Table 11.3).

At the highest dosage, 100 µg, only Blends 1, 2 and 3 were tested. Baulking was recorded with Blend 1 (Fig. 11.5). This behaviour was not observed with Blend 2, but only 16 males were tested. The percentage of approach with Blend 3 was greater than the other two blends. There was no landing or clasper extrusion with Blends 1 and 2.

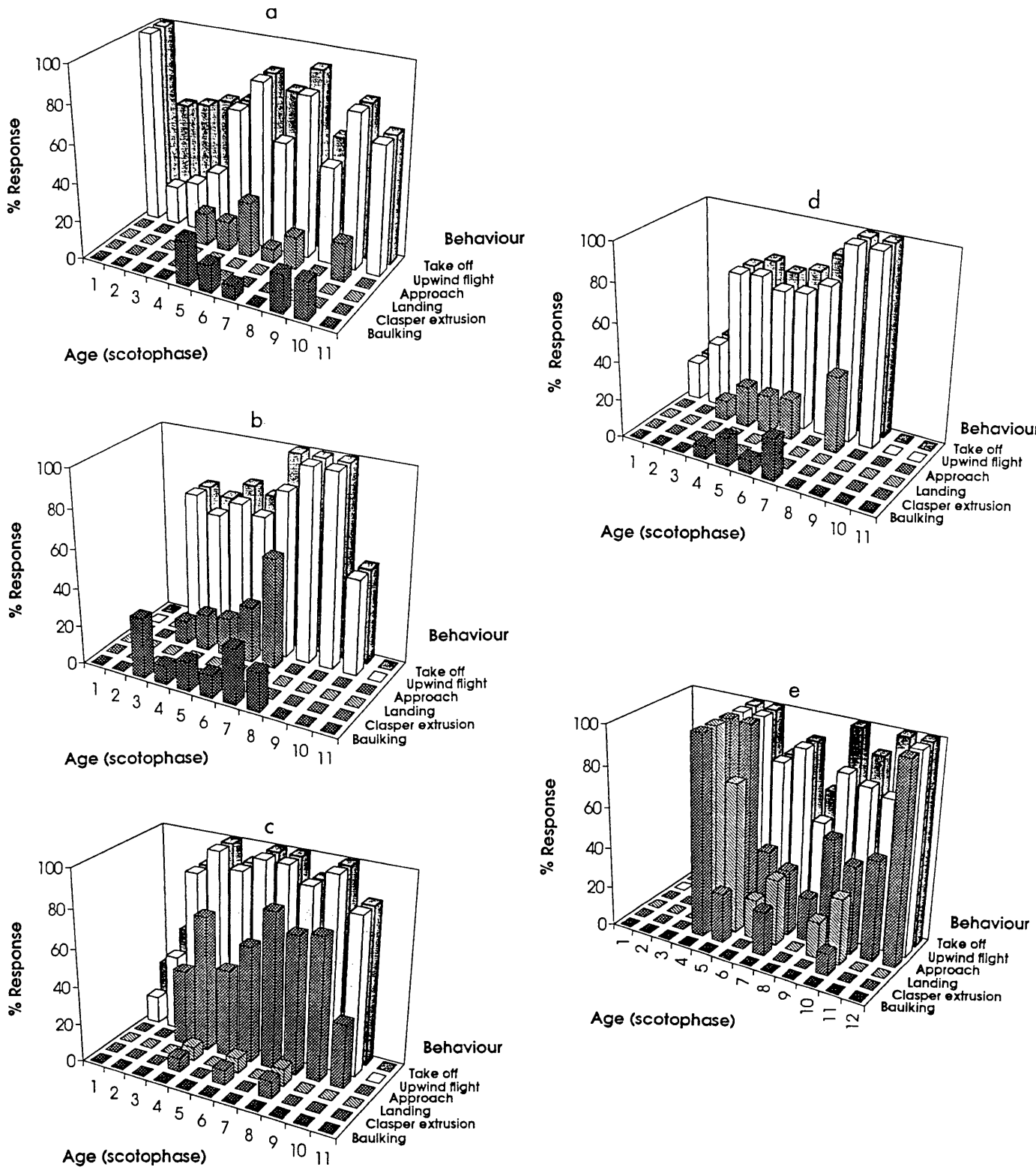


Fig. 11.3. Percentages (%) of *M. convecta* males exhibiting the different behaviours at 0.1 μg of Blend 1 (a), Blend 2 (b), Blend 3 (c), Blend 4 (d), and Blend 5 (e). The number of males observed at each age is given in Table 11.2.

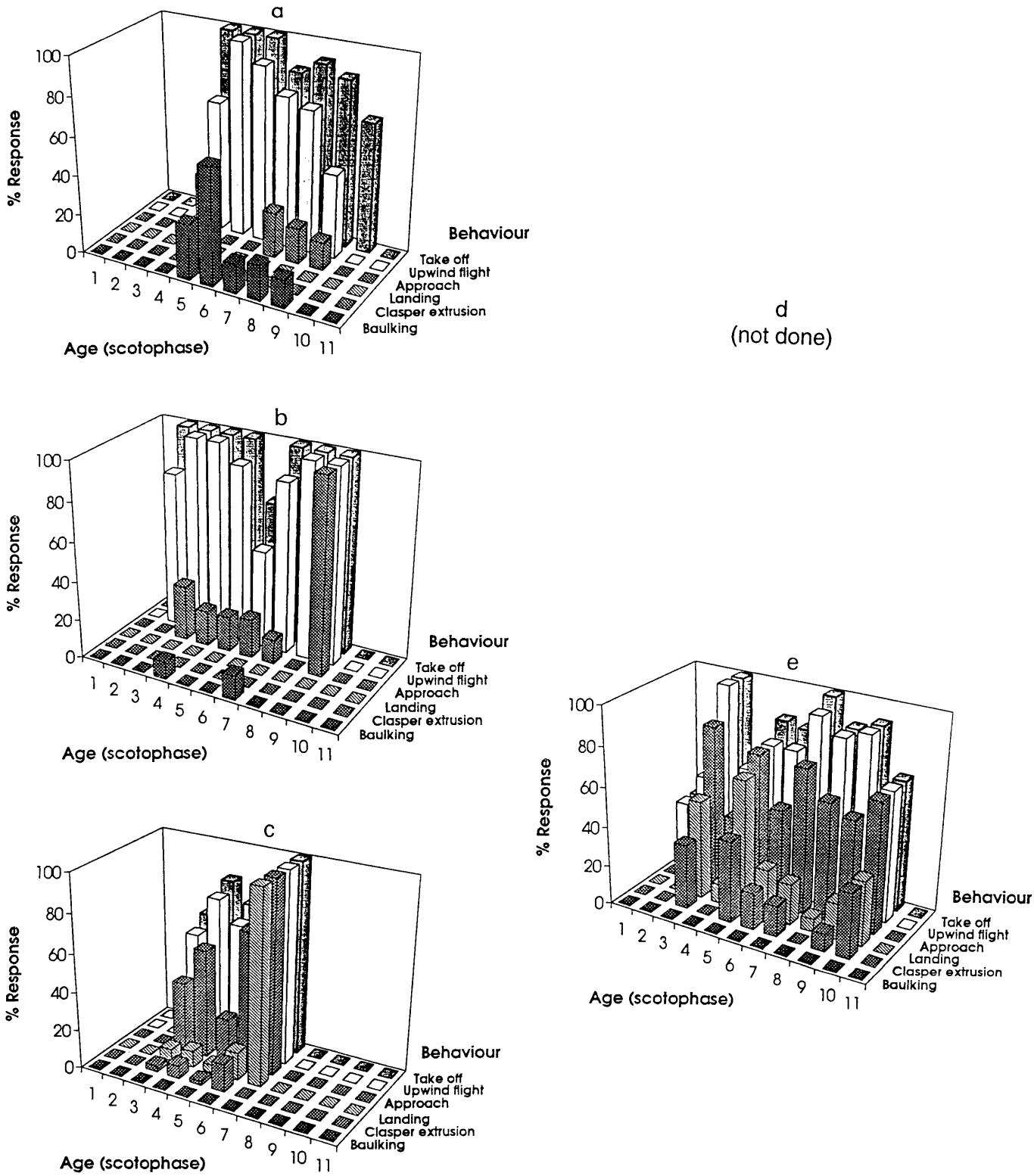
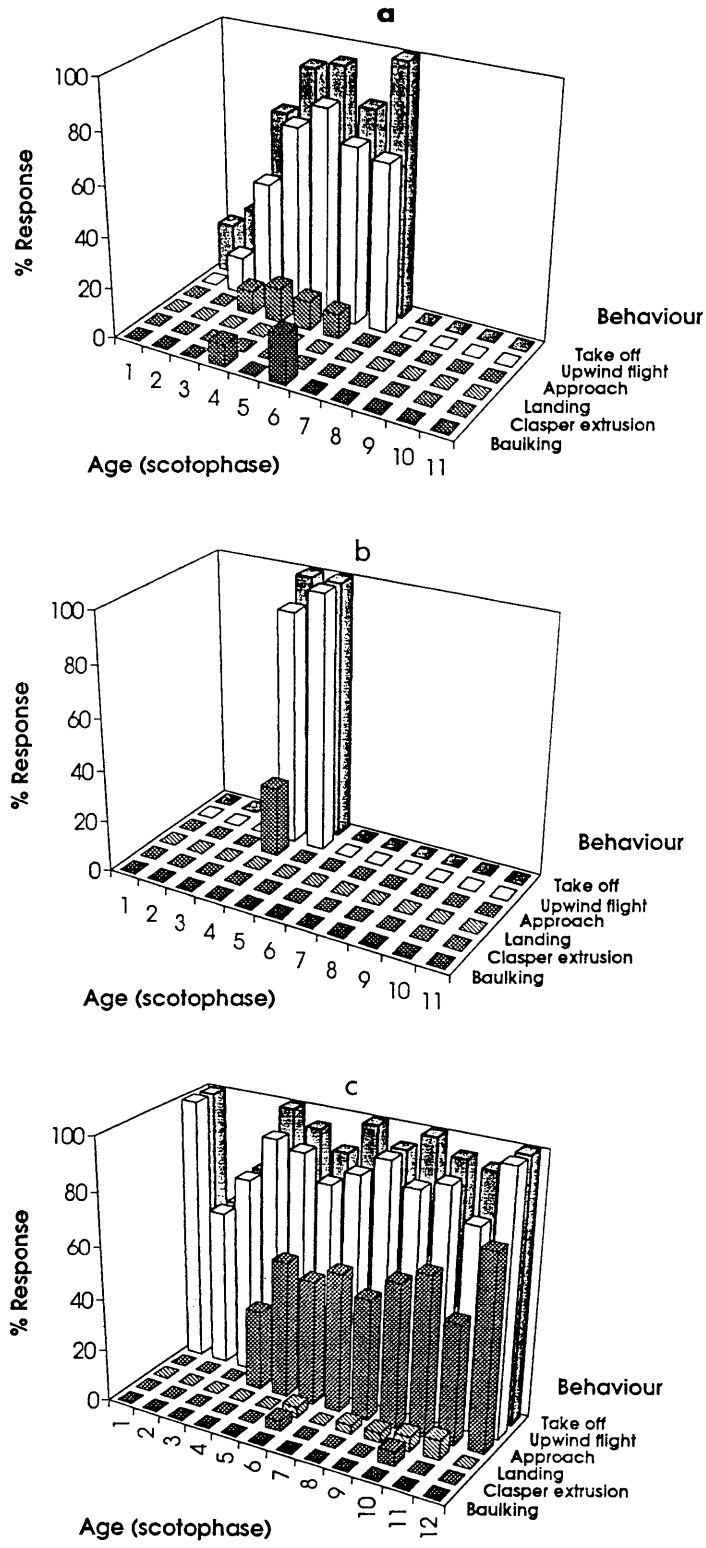


Fig. 11.4. Percentages (%) of *M. convecta* males exhibiting the different behaviours at 10 µg of Blend 1 (a), Blend 2 (b), Blend 3 (c), and Blend 5 (e). Blend 4 (d) was not tested at this dosage. The number of males observed at each age is given in Table 11.2.



d
(not done)

e
(not done)

Fig. 11.5. Percentages (%) of *M. convecta* males exhibiting the different behaviours at 100 µg of Blend 1 (a), Blend 2 (b), and Blend 3 (c). Blends 4 (d) and 5 (e) were not tested at this dosage. The number of males observed at each age is given in Table 11.2.

Baulking was recorded in males flown to various dosages of Blends 1, 2 and 4 but not with Blends 3 and 5. Baulking males flew to within 20-30 cm from the source then rapidly backed-off. The reason why males baulked is not clear. It might be that the presence of one or a combination of the minor components inhibited males from coming closer to the source. Alternatively, the concentration or the ratio of the components might be the inhibiting factor.

The number of males that exhibited baulking behaviour at different blends is given in Table 11.4. Again, males aged 1 and 2 days were excluded because this behaviour did not occur at these ages.

	Number of males	
Blend/dosage (µg)	Baulking	No baulking
Blend 1		
0.01	1	65
0.1	9	82
10.0	13	42
100.0	4	69
Blend 2		
0.01	7	63
0.1	15	78
10.0	2	49
100.0	0	16
Blend 3		
0.01	0	83
0.1	0	91
10.0	0	149
100.0	0	185
Blend 4		
0.1	9	87
Blend 5		
0.1	0	54
10.0	0	92

Table 11.4. Number of *M. convecta* males that did and did not exhibit baulking behaviour at different blends and dosages.

Chi-square tests were conducted to compare baulking between Blends 1, 2 and 4. Within Blend 1, baulking at different dosages was significantly different ($\chi^2_3 = 19.11$, $p < 0.001$) whereas in Blend 2, it was not significantly different between doses ($\chi^2_3 = 7.42$). A chi-square test between Blends 1 and 2 at the four doses was highly significant ($\chi^2_7 = 26.28$, $p < 0.001$). Comparison between Blends 1, 2 and 4 at various doses was also highly significant ($\chi^2_8 = 26.50$, $p < 0.001$). These results indicate that the dosage and blend generally influenced baulking behaviour of males.

11.3.2. Comparison of male response to the different blends with response to calling females

Results from chi-square tests to compare the percentages of approach to different blends and dosages with the percentage approach which would have been expected with calling females (section 11.2.2) are given in Table 11.5.

	Number of males			
Blend and dosage (μg)	No approach	Approach	Expected approach	Difference from calling females (χ^2 value)
Blend 1				
0.01	54	12	22	3.96*
0.1	79	12	28	8.20**
10.0	48	7	20	8.30**
100.0	65	8	27	13.57***
Blend 2				
0.01	62	8	27	13.75***
0.1	74	19	32	4.57*
10.0	41	10	16	1.86 n.s.
100.0	13	3	6	0.28 n.s.
Blend 3				
0.01	72	11	29	10.67**
0.1	36	55	29	14.95***
10.0	86	63	55	0.90 n.s.
100.0	95	90	52	16.50***
Blend 4				
0.1	79	17	34	7.72**
Blend 5				
0.1	28	26	22	0.60 n.s.
10.0	35	57	30	16.86***

Table 11.5. Comparison of observed number of *M. convecta* males that did and did not approach different synthetic pheromone blends and dosages, and expected number of males that would have approached if flown to calling females. Expected approach is the number of males approaching corrected according to the approach pattern with calling females as the pheromone source (refer to section 9.2.2). Difference from calling females refers to the χ^2 values obtained by comparing observed and expected number of approachers and non-approachers for each blend and dosage. The number of * denotes the level of statistical significance.

The blends that were not comparable with calling females were Blends 1, 2 and 4. Approaches with Blend 1 at all four dosages were significantly worse than with calling females. Similar results were obtained with Blend 2. At 0.01 and 0.1 μg, this blend was significantly worse than females. Although approaches with 10 and 100 μg doses were not significantly different from females, the numbers of males tested were relatively low

and the trends were similar to those at the lower dosages, that is, the observed numbers of approaches with this blend were lower than those which would have been expected with females. Blend 4, which was only tested at 0.1 μg, was likewise significantly worse than calling females.

The two blends that elicited higher levels of approaches were Blends 3 and 5. Except at the lowest dosage (0.01 μg), Blend 3 was comparable, if not significantly better than females. Although the difference was not statistically significant at 10 μg, the trend was similar to those at 0.1 and 100 μg, where the observed number was higher than the expected number of approaches. The numbers of observed approaches with Blend 5 at 0.1 and 10 μg were higher than the expected numbers with calling females. The difference was not statistically significant at 0.1 μg but was significant at 10 μg.

The trends in the results can be summarised as follows:

(1) No blend/dosage produced any sexual response (approach, landing, clasper extrusion or baulking) in males aged 1-2 days.

(2) Blends 1, 2 and 4, at all dosages tested, gave low levels of approach, elicited no definitive positive sexual responses (landing and clasper extrusion) and produced variable levels of baulking.

(3) Blend 3 produced higher levels of approach (except at the lowest dosage, 0.01 μg), and some landing and clasper extrusion. There was no baulking with this blend.

(4) Blend 5, at the only doses tested (0.1 and 10 μg) produced high levels of approach and more landing and clasper extrusion than Blend 3. Baulking did not occur with this blend.

Of the five blends tested, Blends 3 (except at the lowest dosage) and 5 elicited responses at least comparable with if not better than, calling females. Both these blends were dominated by Z11:16 Ald. Combined with the results of gland and airborne volatile analyses (Chapter 10), this confirms the importance of this component. Of the 9 *Mythimna* spp. (not including *M. convecta*) tested by Renou *et al.* (1991), Z11:16 Ald was found to be the key compound in six species. Likewise, this compound is the major component of *M. separata* pheromone (Zhu *et al.*, 1987). However, Z11:16 Ald also dominated the unsuccessful blends (1, 2 and 4).

Blends 3 and 5 had a higher amount of 16 Ald than Z11:16 OH. In contrast, with Blends 1, 2 and 4, where there was significantly less approach than there was to females, Z11:16 OH was greater than 16 Ald. These results suggest that in *M. convecta,* 16 Ald might be more important than Z11:16 OH in evoking male response. In the case of *M. separata,* more males were caught in field traps baited with Z11:16 Ald and 16 Ald (total of 676) than in traps baited with Z11:16 Ald and Z11:16 OH (total of 267) (Zhu *et al.*, 1987). In *H. virescens,* Vetter & Baker (1983) reported that the addition of 16 Ald to its pheromone blend of Z11:16 Ald and Z9:14 Ald increased close-range sexual behaviours of males, whereas the addition of Z11:16 OH to this blend did not.

The results of pheromone analyses, however, indicate that in both the glands and air samples of *M. convecta* (Chapter 10), the amount of Z11:16 OH was greater than 16 Ald. It might be that the analyses underestimated the amount of 16 Ald, perhaps because it might be the most unstable of the components and therefore most likely to break down during pheromone collection and GC analysis. If the analyses were accurate, however, it means that what is in the pheromone glands and air emitted by calling females might not always be a good indicator of the best pheromone blend for trapping. There are precedents for this in the literature. For example, Raina *et al.* (1986) reported that positive response from *H. phloxiphaga* males was obtained with virgin females but not with ovipositor extracts or the synthetic blend of Z11:16 Ald, 16 Ald, Z11:16 OH and Z9:16 Ald, in proportions similar to those in the extracts. In *H. zea,* although Z11:16 OH was found in the ovipositor extracts, the addition of this component to a blend of Z11:16 Ald, Z7:16 Ald, Z9:16 Ald and 16 Ald, inhibited upwind flight to the source of wild males, suggesting that it may not be a component of the pheromone being released by the female (Raina *et al.,* 1989).

11.3.3. Sequential responses to pheromone components

An understanding of the role of the components of the female sex pheromone is necessary to explain the behavioural mechanisms involved in the mate-searching activity of males. Some authors believe that, in most species, the full male response sequence to the pheromone is stimulated by the complete blend, rather than individual components of that response being stimulated by partial blends of the female pheromone. Linn *et al.* (1986) found that in three species, *A. velutinana, T. ni* and *G. molesta,* upwind flight was highest when males were flown to the full blends, rather than to partial blends or single components only. They suggested that the complete blend acts as a unit to elicit sequential responses and that the role of the minor components is to enhance male sensitivity to the pheromone rather than to elicit close-range behaviours. Similarly, Quartey & Coaker (1993), reported that in *E. cautella,* the percentages of upwind flight and

source contact were significantly higher when males were flown to the two-component blend than to single components. However, the hypothesis that the major component elicits long-range behaviours and the minor components evoke close-range behaviours has been confirmed in some species. For example, in *Heliothis maritima* Grasl., the addition of 0.1 or 1% Z9:16 Ald to a blend of Z11:16 Ald: Z11:16 OH; 16 Ald (100:6:3) did not significantly increase approaches, but significantly increased source contact and male landing responses at 0.1 and 10 µg doses (Szõcs *et al.*, 1993). Bradshaw *et al.* (1983) proposed a hierarchy of stimuli to the sequential responses of male pine beauty moth, *Panolis flammea* (Schiff.). They suggested that long-range attraction is mediated by the major component, Z9:14 Ac, whereas landing and copulation were elicited by the minor components, Z11:14 Ac and Z11:16 Ac.

The results from this thesis tend to support the sequential response theory. Based on the results from the different blends tested for *M. convecta* males, the sequential responses to the individual components of the female pheromone might be as illustrated in Fig. 11.6. Location of the pheromone plume from a distance is initiated by Z11:16 Ald. This is the major compound in both gland and air samples (Chapter 10) and elicits antennal responses in many *Mythimna* spp. (Renou *et al.*, 1991). These features make it a likely candidate for the role of initial attractor. When a plume is detected, the male proceeds flying upwind towards the source. Upwind flight represents an initial attempt to get closer to the source. Take off and upwind flight were recorded with all the blends tested and all were dominated by Z11:16 Ald.

Once upwind, a male might do either of two behaviours: approach closer to the source or baulk, that is, quickly back-off from the source. The next two main components, Z11:16 OH or 16 Ald, or the ratio between them, might evoke these behaviours. However, one or a combination of the four unknown minor components detected in the gland and air samples (Chapter 10) may also be important to elicit or inhibit approach. Approach behaviour was observed with all the five blends tested. However, the characteristic of this behaviour varied with the different blends. With Blends 1, 2 and 4, approaches were generally brief and males did not exhibit hovering within the source. Hovering when close to the source was observed in males that were sexually responding to calling females (Chapter 6.4.2.2.). This characteristic was observed in males that approached to Blends 3 and 5. Baulking occurred with blends where the amount of Z11:16 OH was greater than 16 Ald (i.e., Blends 1, 2 and 4) while higher levels of close-range approaches were recorded with the blends that had more 16 Ald than Z11:16 OH (i.e., Blends 3 and 5).

When the male gets closer to about 10-15 cm from the source, he may then contact or land on the source with the claspers extruded, followed by a copulatory attempt. Otherwise, he flies a short distance downwind then re-orients and approaches again, or flies further downwind to re-start the sequence from the beginning. Males flown to Blends 3 and 5 exhibited this downwind/upwind flight and repeated approaches.

The close-range behaviours, landing and clasper extrusion including copulatory attempt occurred only with Blends 3 and 5. The levels of approach, landing and clasper extrusion with Blend 5 were greater than those with Blend 3. Three components (Z11:16 Ald, Z11:16 OH and 16 Ald in the same ratios) were common in these blends, but Blend 5 had an additional minor component, Z9:16 Ald. These results suggest that Z9:16 Ald might be important in eliciting these close-range sexual behaviours in *M. convecta*. The unknown minor components might also be involved here.

Studying the behavioural responses of *M. convecta* males to the major component, Z11:16 Ald, in combination with each one or more of the minor components would further elucidate the role of the minor components in the behavioural sequence of males. Electroantennography should be conducted to determine what compounds are detected by males. Likewise, further investigation of the components, blends and ratios of the natural sex pheromone of *M. convecta* and testing synthetic equivalents in a wind tunnel and in the field are important for the development of a pheromone lure for this species in the future. The study showed that of the different blends tested in the wind tunnel, Blends 3 and 5 are potential candidates for this purpose. These blends were tested in the field and the results are discussed in Chapter 12.

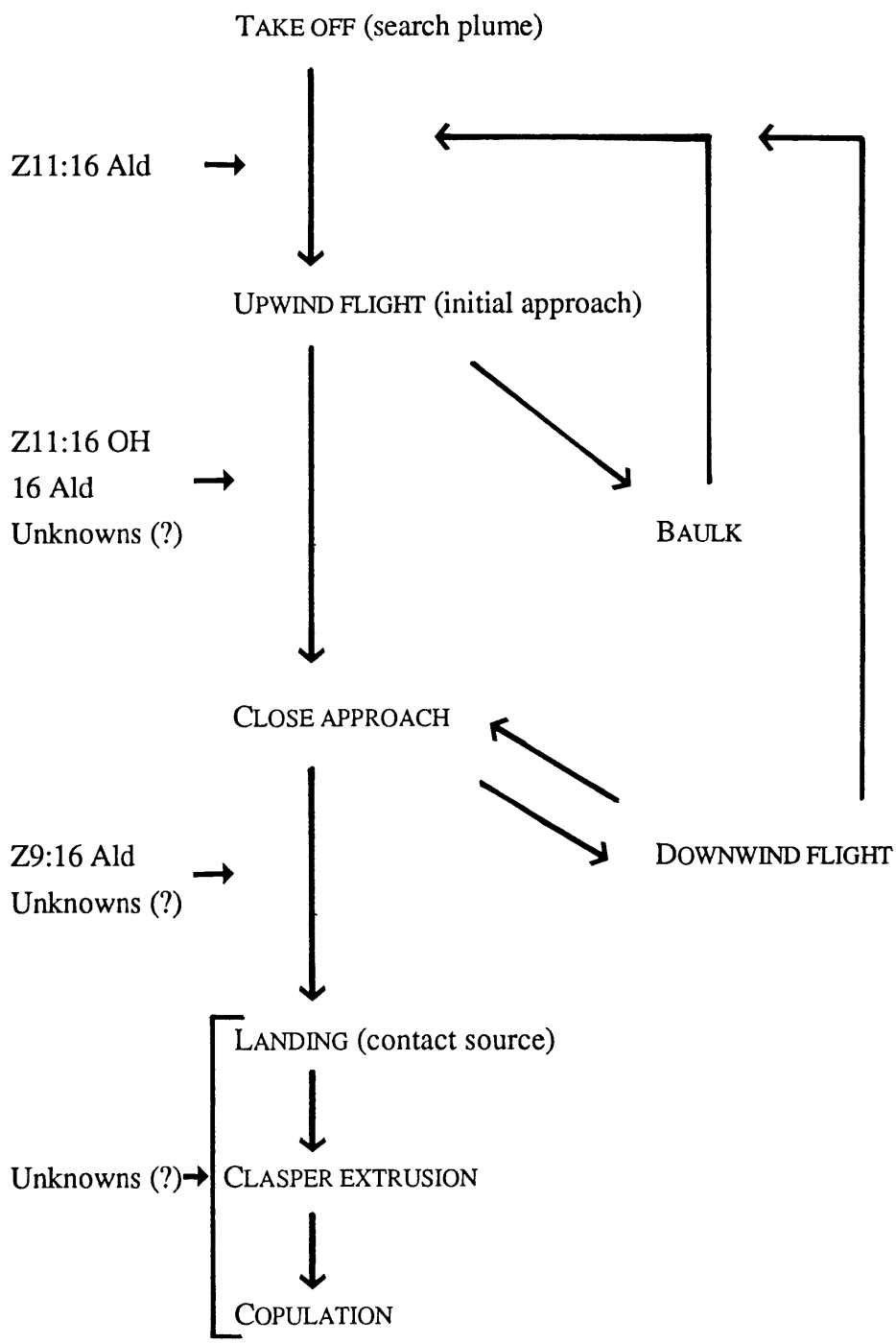


Fig. 11.6. Proposed sequential responses of *M. convecta* males to the individual components of female pheromone.

11.3.4. Relationship between male sexual response and female calling

Sexual response of *M. convecta* males to calling females appeared to be synchronous with the expression of calling behaviour in females (Figs. 6.4a and 6.4b, Chapter 6). Two hypotheses were thought to explain this. The first one was that females that called early in the scotophase might not be releasing sufficient quantity or quality of the pheromone to elicit male response. Investigating the relationship between pheromone titre and the expression of calling behaviour in *M. convecta* females would help elucidate this hypothesis. A second hypothesis was that males had a "response window". In this study, comparing approaches to Blend 3 with approaches to calling females (Fig. 11.1) provided the opportunity to test this hypothesis.

The study showed that sexual response by males depended on a "response window" and not necessarily on the periodicity of female calling. The synchronisation of male responsiveness with the time at which most calling in females occurs would be advantageous under natural conditions. Synchronous reproductive activities of male and female moths would ensure mating success. Likewise, it may be a means by which *M. convecta* can achieve reproductive isolation.

Male responsiveness occurred between the 6th and 8th hours of the scotophase. In comparison with female calling, however, the male window appeared to be slightly narrower. Some calling occurred earlier in the night, at which time males were not yet responsive (compare Fig. 11.1 with Fig. 4.16 from Volume 1). In the laboratory, the highest numbers of copulation were initiated between 5.5 and 6.5 hours of the scotophase, whereas calling started in some moths during the 3rd-4th hours of the scotophase. Similarly, in the field, some calling was observed between 2100 and 2200 h, while copulation was initiated around 2300 h. This fits with the idea that the male response window does not open until some time after the females begin calling. A possible hypothesis as to why the male window is smaller than that of female calling concerns energy expenditure as well as risk of predation on the part of the male. To locate a mate, a male has to fly to find pheromone plumes. Flight is energetically expensive (Rankin & Burchsted, 1992) and might render the male vulnerable to predation by nocturnal birds or bats. It might therefore be adaptive if males did not fly unless they are sure the females will be calling and ready to mate. By contrast, female calling can be done from protected or inconspicuous positions, and probably involves much less energy expenditure.