# STUDIES ON THE NYMPHAL AGGREGATION PHEROMONE OF MALAGASY MIGRATORY LOCUST, *LOCUSTA MIGRATORIA CAPITO* (SAUSSURE, 1884) AND ITS EFFECTS ON ADULT MATURATION

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# Studies on the nymphal aggregation pheromone of Malagasy Migratory Locust, *Locusta migratoria capito* (Saussure, 1884) and its effects on adult maturation.

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**A thesis submitted in fulfilment for the degree of Doctor of Philosophy in Zoology in the Jomo Kenyatta University of Agriculture and Technology**

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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## DEDICATION

I dedicated this thesis to my late mother, who struggled to offer me the best education and knowledge possible in life; to my late father; to my beloved wife Heliniavo and our babies Soaniavo who, with a lot of understanding and patience, endured my absence during the four years of this study and gladly gave me all the support to the end; and to my brothers and sister for their support.

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## LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| **A** | Anisole |
| **AD** | Area of Multiplication and Densation |
| **AGB** | Anisole, gauiacol, β-ionone combined |
| **B** | Full blend of volatiles of live insect: 2,3-butanediol + PAN + hexanal + benzyl alcohol + nonanal |
| **BCED** | Behavioural and Chemical Ecology Department at ***icipe*** |
| **B-P** | Full blend of volatiles of live insect without PAN |
| **BHN** | 2,3-butanediol, hexanal, nonanal combined |
| **Boh** | Benzyl alcohol |
| **βI** | β-ionone |
| **CAB** | Anti-locust Centre of Betioky Sud |
| **CAN** | National Anti-locust Centre |
| **DF** | Degrees of freedom |
| **EAG** | Electroantennogram |
| **F** | Full blend of faecal volatiles: 2,3-butanediol + pan + anisole + guaiacol + β-ionone |
| **FID** | Flame Ionization Detector |
| **F-A** | Full blend of faecal volatiles without anisole |
| **F-B** | Full blend of faecal volatiles without β-ionone |
| **F-G** | Full blend of faecal volatiles without guaiacol |

| | |
|---|---|
| **F-P** | Full blend of faecal volatiles without PAN |
| **G** | Guaiacol |
| **GC** | Gas chromatography |
| **GC-EAD** | Coupled gas chromatography – electroantennographic detector |
| **GC-MS** | Coupled gas chromatography – mass spectrometry |
| **IAM** | Initial Area of Multiplication |
| ***icipe*** | International Centre of Insect Physiology and Ecology |
| **P / PAN** | Phenylacetonitrile |
| **PG** | PAN and guaiacol combined |
| **RT** | Retention Time |
| **SAS** | Statistical Analysis System |
| **SE** | Standard Error |
| **SNK** | Student-Newman-Keuls test |
| **TAM** | Transitory Area of Multiplication |
| $\chi^2$ | Chi-square test |

## ABSTRACT

The Malagasy migratory locust, *Locusta migratoria capito* (Saussure, 1884), is the most destructive pest in the Malagasy agricultural production system. The recent plague between 1996 and 2000 during which economic losses amounting to *ca*. US$ 50 million mainly in rice fields, were recorded, is an indication that, the locust menaces is far from being resolved in Madagascar. Control operations against locusts have focused entirely on large-scale application of synthetic chemical insecticides. Given that Madagascar has a unique and rich biodiversity; such spraying has negative effects on the environment and non-target beneficiary organisms.

Recent research on the desert locust, *Schistocerca gregaria* (Forskäl) by the ***icipe*** team for over the last fifteen years has revealed the importance of pheromonal mediation in the aggregation behaviour of adult and nymphal stages and in gregarization-solitarization dynamics. The aim of this study was to provide basic information on the role played by volatiles in the aggregation of nymphs of *L. m. capito* and to explore possible applications of the chemical constituents for preventive control.

The aggregation response of fifth instar nymphs to their own volatiles was investigated using a single-chamber olfactometer. When locust nymphs where reared together with their adult conspecifics, they aggregated weakly to their own volatiles. However, when the male and female nymphs were tested separately, nymphs of each sex responded negatively to the volatiles. Nymphs reared separately from adult locusts showed strong

avoidance to their own volatiles. The same pattern of responses was observed for the body extract.

To identify the constituents, volatiles were collected using Super-Q and analysed using Gas Chromatography (GC), coupled Gas Chromatography-Mass Spectrometry (GC-MS), and coupled Gas Chromatography-ElectroAntennographic Detection (GC-EAD). The results revealed the presence of the following electrophysiologically active compounds: 2,3-butanediol, hexanal, benzyl alcohol, nonanal, phenylacetonitrile and two unidentified compounds from live nymphs; 2,3-butanediol, anisole, guaiacol, phenylacetonitrile, beta ionone and one unidentified compound were identified from volatiles derived from the faeces. There were no sexual differences in the production of volatiles, both qualitative and quantitative.

The identified compounds were further used in bioassays to assess the aggregation responses of the nymphs. The results showed that, the synthetic full blend elicited the same response as the crude volatiles from live nymphs and fresh faeces. However, exclusion of each compound at a time from the full blend modified the response of the nymphs from avoidance to attraction.

In another experiment, the effect of the nymphs on the sexual maturation of young adults was evaluated using a double-storey bioassay and aluminium standard cages. The results showed significant delay in both mating and ovipositing time when young adults were

exposed to nymphs in the presence of visual, tactile and olfactory cues. In contrast, the nymphal volatiles alone did not cause retardation of sexual maturation of young adults with regard to the above parameters. The results are discussed in terms of the relative concentration of the compounds in the volatiles.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 General introduction

Locust plagues are a major problem for Malagasy agricultural production. There are two major species: the Malagasy migratory locust, *Locusta migratoria capito* (Saussure) (Orthoptera: Acrididae) and the red locust, *Nomadacris septemfasciata* (Serville) (Orthoptera: Acrididae). These locusts are able to transform reversibly from solitarious to gregarious phases forming hopper bands and subsequent swarms of adults that are very destructive to crops and pasture. *Locusta migratoria capito* is the most destructive because of its high potential of development.

Madagascar has experienced locust plagues for centuries. The first written report was documented in 1617 and has been attributed to R.P d'Azevedo (Randriamanatsoa, 1997). Later on, several authors cited the presence of locusts in different regions of Madagascar. Since 1880, there have been six large invasions and two outbreaks, the latter having been timely controlled (Randriamanantsoa, 1998; Duranton *et al.*, 2000) (Table 1.1). In the history of locust plagues, the ones of 1939-1957 and 1996-2000 are highlighted due to the long plague duration of the former and the extent of crop losses experienced in the latter (Launois and Duranton, 1997). During the invasion of 1939-1957, in 1946, two thirds of the country was infested with locust swarms at densities of more than 10,000 adults/ha (Launois and Duranton, 1997). The highest damage occurred

in Marovoay, one of the biggest grain baskets of Madagascar in the mid-west zone. The Ministry of production at that time estimated paddy losses amounting to 20 billion of Communauté Financière Africaine franc (CFA), which is approximately US$ 36 million at the current exchange rate (Zehrer, 1997a).

Table 1.1: Historical record of locust invasions in Madagascar (Launois and Duranton, 1997).

| **Period** | **Invasion duration (Years)** | **Time between successive invasions (Years)** |
|---|---|---|
| 1880-1888 | 08 | |
| 1895-1904 | 09 | 07 |
| 1909-1915 | 06 | 05 |
| 1921-1929 | 08 | 06 |
| 1939-1957 | 18 | 10 |
| 1960-1962* | 02 | 03 |
| 1991-1994* | 03 | 29 |
| 1996-2000 | 04 | 04 |

* Outbreaks not reaching plague magnitude

After 39 years of recession, the 1996-2000 outbreaks showed that *L. m. capito* remains a major threat to agricultural production in Madagascar. During this period, only the far north of the island was spared (Fig. 1.1). Even the Eastern forest zone, which was not invaded by all previous plagues because of the abundant rainfall and high humidity, was affected (World Bank, 1998; Duranton *et al*., 2000).

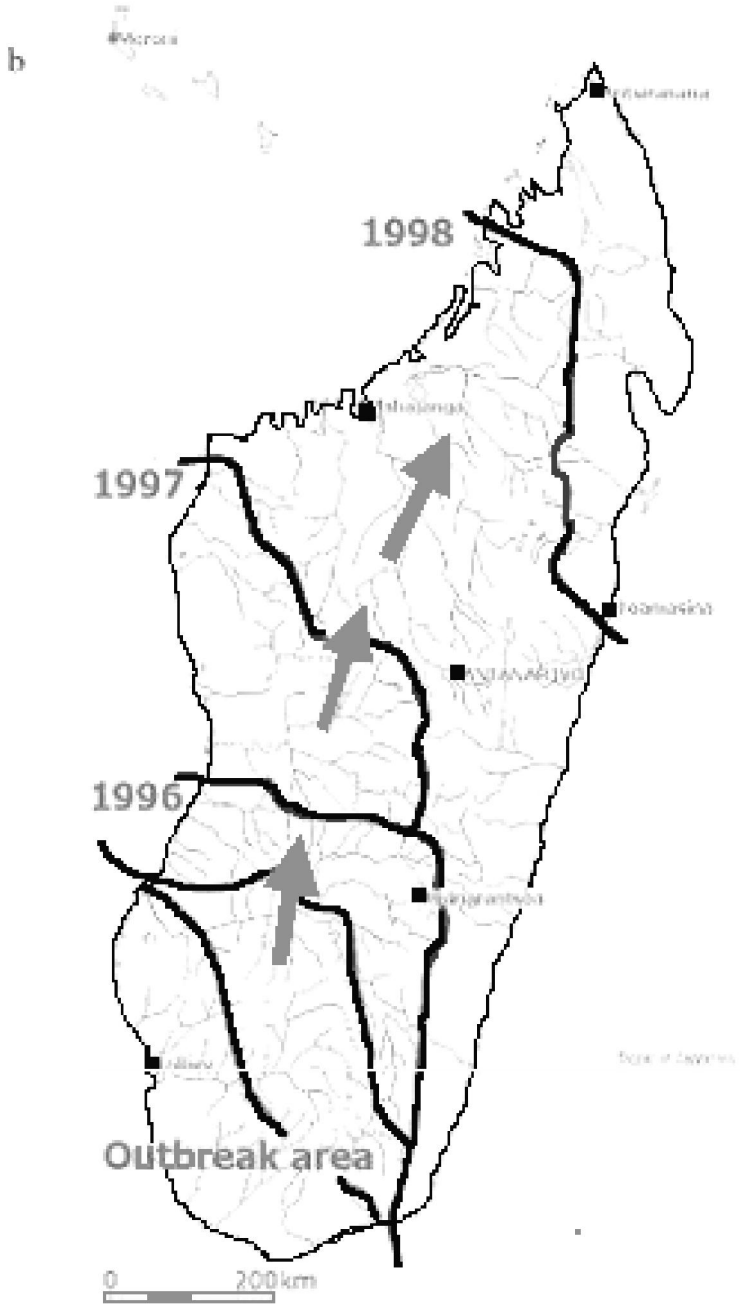



Figure 1.1: Spread of the 1996 – 2000 invasion. The arrows indicate direction of locust invasion from the outbreak area in Southwest Madagascar (Duranton *et al*., 2000).

## 1.2 General plague control strategies

In 1998, at the height of the plague, an area of more than 10 million ha was infested by locusts and the average size of the adult swarm reached 4,000 ha. Large-scale control was carried out for three years using huge quantities of synthetic chemical insecticides. More than 4.2 million ha of infested areas were sprayed, of which 1.2 million ha had full coverage, mostly with deltamethrin, propoxur and fenitrothion. The remaining 3.0 million ha was treated with barrier application using fipronil ® (Lecoq, 2000). Approximately 340,000 litres of insecticides were used and the World Bank estimated the total cost of the campaign at over US$ 50 million (Lecoq, 2001). Several factors contributed to the failure to efficiently control the plague:

1. The government lacked funds in the mid-1990s which led to a breakdown in the operations of the anti-locust centre, thus weakening the control organisation (Dinham, 2000; Lecoq, 2001);
2. The ever escalating degradation of the South-Western forest cover, thus leading to the expansion of the grassy savannah system that is the preferred habitat of the migratory locust (Peveling, 2001; Franc *et al.*, 2003); and
3. Favourable ecological conditions, especially rainfall, which amplified the rapid build up of locust populations during this period (Dinham, 2000).

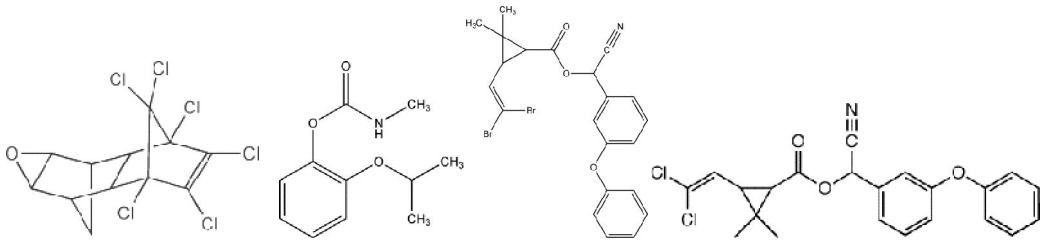
Monitoring and control of locust outbreaks are carried out by the Anti-locust Centre of Betioky Sud (C.A.B.), which was established in 1932 and later (in 2000) became the

National Anti-locust Centre (C.N.A.). It is located in the outbreak area of the locust in the southwest of Madagascar. This area is divided into six zones on the basis of ecological characteristics and practicality of control and comprises of about 30 posts covering about 12.9 million ha (ANAE, 2005). The main role assigned to C.N.A. is to control locusts in their breeding sites, and if necessary, when locust population build up is at the verge of an outbreak, ground-spray or even aerial spray interventions are initiated. Such interventions involve an average coverage of 60,000 ha at a cost of about US$ 0.5 million every year, of which, 80% is spent on purchase of insecticides and contingent expenses (Zehrer, 1997b, 2001).

### 1.3 Chemical control

For locust control, Madagascar has registered a wide range of chemical pesticides consisting of various classes (Fig. 1.2). In the past, dieldrin, an organochlorine was widely used until its restriction in 1989. Organophosphates, especially fenitrothion, have also largely been exploited although propoxur, a carbamate, served as a substitute. The latter was applied in powder formulation for controlling hopper bands. The other insecticides include pyrethroids, of which deltamethrin, cypermethrin and lambda-cyhalothrin were extensively used (Rakotoarimanana, 1997). The phenylpyrazole group, mainly fipronil® was registered and approved for barrier application and spraying against hopper bands and adult swarms, respectively. Its first large-scale application in Madagascar was in 1997 (Dinham, 2000; Zehrer, 2001). According to the U.N. Food

and Agriculture Organisation (FAO) by March 1999, the stock of pesticides for locust control in Madagascar included fipronil®, two Insect Growth Regulators (IGR) *viz* diflubenzuron and triflumuron (alsystin), deltamethrin, chlorpyriphos (organophosphate) and propoxur (carbamate) (Dinham, 2000).



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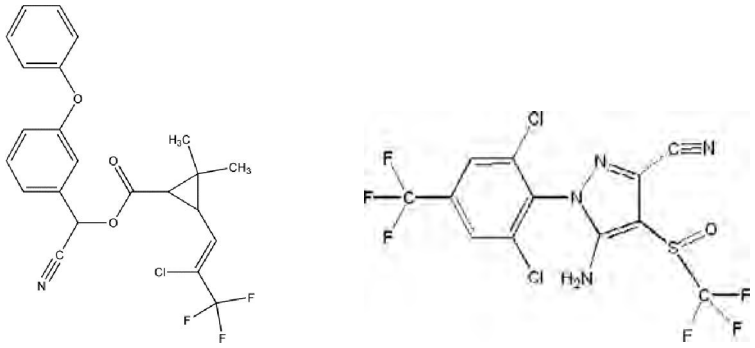


(5) (6)

Figure 1.2: The main chemical insecticides of different classes that are used for locust control in Madagascar. (1) dieldrin, (2) propoxur, (3) deltamethrin, (4) cypermethrin, (5) lambda-cyalothrin, (6) fipronil (source: http://www.pesticideinfo.org/)

## 1.4 Negative effects of chemical control

The widespread use of insecticides has had very negative impact on the environment. The campaign of 1998-2000 was very explicit in terms of environmental damage, and the more so because Madagascar Island has extremely rich and unique native biodiversity, having been declared a "hot spot", being one of the world's "mega diversity" nations (Mc Neely *et al*., 1990). Environmental assessment carried out by Natural Resources Institute (N.R.I., UK), German Development Agency (G.T.Z.) and Malagasy Crop Protection Department (D.P.V.) provided evidence on the high toxicity of Fipronil® on non-target organisms. After 33 months of barrier application at a dose of 7.5 g/ha, non-reconstruction of termite mounds in the treated areas was observed (Zehrer, personal communication). The full treatment at 4 g/ha of Fipronil® elicited high mortality of almost all aquatic invertebrates. This blanket toxicity of Fipronil® led to withdrawal of the authorisation by the government of use of this insecticide on locust swarms in February 1999 (Tingle and McWilliam, 1999; BBC, 2000; Dinham, 2000). This has led to the search for environment-friendly and less toxic alternative control tactics for locusts.

## 1.5 Use of biopesticides

The World Bank Panel (1998) recommended that the broad-spectrum chemical pesticides should be used as a last resort, giving preference to biological control and to chemicals that are highly specific, with minimum or no toxicity to non-target organisms.

The use of mycopesticides has been suggested to be the best solution to replace synthetic insecticides (Prior and Greathead, 1989; Lomer and Prior, 1992; Goettel and Johnson, 1997). The development of effective oil formulations of *Metarhizium anisopliae* spores in Africa, Australia and South America, opened up new possibilities for environmentally safe control operations. At the same time, the formulation based on the local strain SP 9 of *M. anisopliae* was also tested in the field in Madagascar (Delgado *et al*., 1997). *Metarhizium sp.* biopesticide led to mortality of 70-90% of the treated locusts within 14-20 days, with no measurable impact on non-target organisms (Kooyman *et al*., 1997; Lomer *et al*., 2001). These biopesticides are slow acting and inappropriate for emergency situations. However, they have a role to play in an integrated control strategy (Lomer *et al*., 1999).

Research on the pheromones of the desert locust, *Schistocerca gregaria* (Forskäl) has been done at the International Centre of Insect Physiology and Ecology (***icipe***) over the last fifteen years. It has shown that, pre-treatment of hoppers with the major component of the adult aggregation pheromone, phenylacetonitrile (PAN) reduces the amount of insecticides and mycopesticides used in locust control (Bashir *et al*., 2000). Field tests have confirmed that, direct deployment of phenylacetonitrile in the control as an effective solitarizing agent of hopper bands greatly reduced the cost of treatments (Bashir *et al*., 2000).

Despite the long history of the Madagascar locust control (Randriamanantsoa, 1998; Duranton and Lagnaoui, 1999) and research (Lecoq, 1975; Scherer and Fong, 1997), no sustainable control and/or management strategies have been found that are economical and environment-friendly (Peveling, 2001). Therefore, the knowledge being generated on locust behavioural and chemical ecology is important, because it may lead to the development of new management strategies for locust outbreaks. In particular, "preventive" control strategies that may target locusts in the breeding habitats prior to the build up of outbreaks need to be developed. The research carried out in this project identified and characterized the components in volatiles of nymphal locust, *L. m. capito* that may be involved in their aggregation and evaluated their potential, singly or in blends, effects on conspecific adults especially on the sexual maturation.

### 1.6 Statement of the problem

In Madagascar, like in many Sub-Saharan countries, locust menace is constantly present as shown by the recent plague of 1996-2000. The lack of well-organized surveys in the breeding site, broadening of locust habitats due to environment degradation, and many other unpredictable factors due to global warming may lead at anytime to an aggregation, outbreak and plague of *L. m. capito*. Controlling infestations of locusts is always challenging. At present, the current control techniques concentrate on the use of insecticides. However, area-wide spraying of insecticides threatens the biodiversity as well as the agrosystem and human health.

## 1.7 Justification of the study

*Locusta migratoria capito* is a major agricultural pest in Madagascar affecting the production of cereals including rice and maize, and causes serious destruction of pastures. For plague suppression, a wide range of chemical insecticides has been used. For example, during the recent invasion from 1996 to 2000, large quantities of insecticides (ca. 340,000 litres) mainly Fipronil®, a phenylpyrazole, were used as barrier for hoppers and aerial sprays on swarms. More than 4.2 million ha were treated. These insecticides are highly toxic to non-target organisms as well as aquatic or terrestrial fauna. Given the unique but fragile biodiversity of Madagascar, the search for environment-friendly and less toxic alternative control tactics to chemical pesticides for locusts is very urgent. The World Bank Panel (1998) recommended that, long-term control approach for *L. m. capito* should be based on sustainable survey and application of mycopesticides as well as growth regulators. The broad-spectrum chemicals should only be applied as a last resort. On the other hand, research on the behaviour and chemical ecology of the desert locust, *S. gregaria* at ***icipe*** in the last decade has provided clear evidence on the potential of the adult locust pheromone as a tool for disrupting the olfactory system of gregarious hoppers resulting in a series of effects leading to the disruption of their gregarious behaviour. Moreover, treatment of hoppers with the adult pheromone renders them more susceptible to sublethal doses of the pesticidal agents, and minimizes the amount needed for complete control, thus, substantially reducing their hazardous effects on non-target organisms and environment as well as the cost of

control. The nymphal pheromone blend similarly affects the behaviour of the adult locusts, particularly the gravid female, which leads to a random and disruptive deposition of eggs. Hence, understanding the role played by semiochemicals in the behavioural phase dynamics of *L. m. capito* may lead to development of new management strategies that are efficient, cost-effective, environment-friendly and sustainable for control of this locust in Madagascar.

### 1.8 Hypotheses

The following hypotheses were tested:

1 Nymphs of *Locusta migratoria capito* utilise semiochemicals from their body and faecal volatiles for their aggregation and synchronization of sexual maturation of immature adults;

2 Nymphal locust volatiles are compositionally different from those of adult locusts and each stage aggregates to its own volatiles; and

3 Nymphal aggregation pheromone is a blend of distinct chemical components that occur in the volatiles in specific ratios.

## 1.9 Objectives

### 1.9.1 General objective

To characterize the components of the aggregation pheromone of nymphal *L. m. capito* (Saussure, 1884), and to determine its effects on aggregation responses of nymphs and on sexual maturation of gregarious conspecific immature adult locusts.

### 1.9.2 Specific objectives

1. To determine the role of volatiles in the aggregation behaviour of nymphal *L. m. capito*;
2. To characterize the chemical components in the volatiles influencing nymphal behaviour;
3. To evaluate the role of the synthetic blend on the aggregation behaviour of the nymphs; and
4. To evaluate the effects of components of nymphal pheromone and their synthetic blends on sexual maturation of immature conspecific adult locusts.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The Malagasy Migratory Locust, *Locusta migratoria capito* (Saussure)

Locusts occur in distinct gregarious and solitarious phase, as well as in transient forms (Uvarov, 1921; Uvarov and Zolotarevsky, 1929; Kennedy, 1956). Under favourable environmental conditions, mainly the patchy distribution of their host plants, there is rapid increase in the number of locusts that are crowded together. This crowding triggers the inherent ability of these insects to change their physiology, behaviour and morphology to the gregarious form. The threshold density that releases gregarization depends on different factors, which vary depending on different habitats (Randriamanatsoa, 1998). However, a density of 2,000 adults/ha has been taken as reference for *L. m. capito* (Wintrebert, 1970; Launois, 1974; Andrianasolo, 1979). The following changes have been noted when solitarious locusts transform to the gregarious phase.

#### 2.1.1 Morphometrics

In solitarious phase, measurements on the adult locusts have established the average length of male to be 38.5 mm and 51.0 mm for the female (Randriamanantsoa, 1998). Transformation into gregarious phase is characterized by decreasing size of females whereas males increase theirs so that gregarious females and males have the same size (Wintrebert, 1970; Randriamanantsoa, 1998). Calculation of E/F (where E, is the length

of the front wing and F, the length of the femur of the hind leg) has shown a higher ratio in the case of locusts taken from a swarm than those taken from solitarious locusts. The pronotum shape also varies with the phase. Solitarious adult locusts have a convex-shaped pronotum while that of gregarious and transiens are concave or straight, respectively (Pener, 1991) (Fig. 2.1).

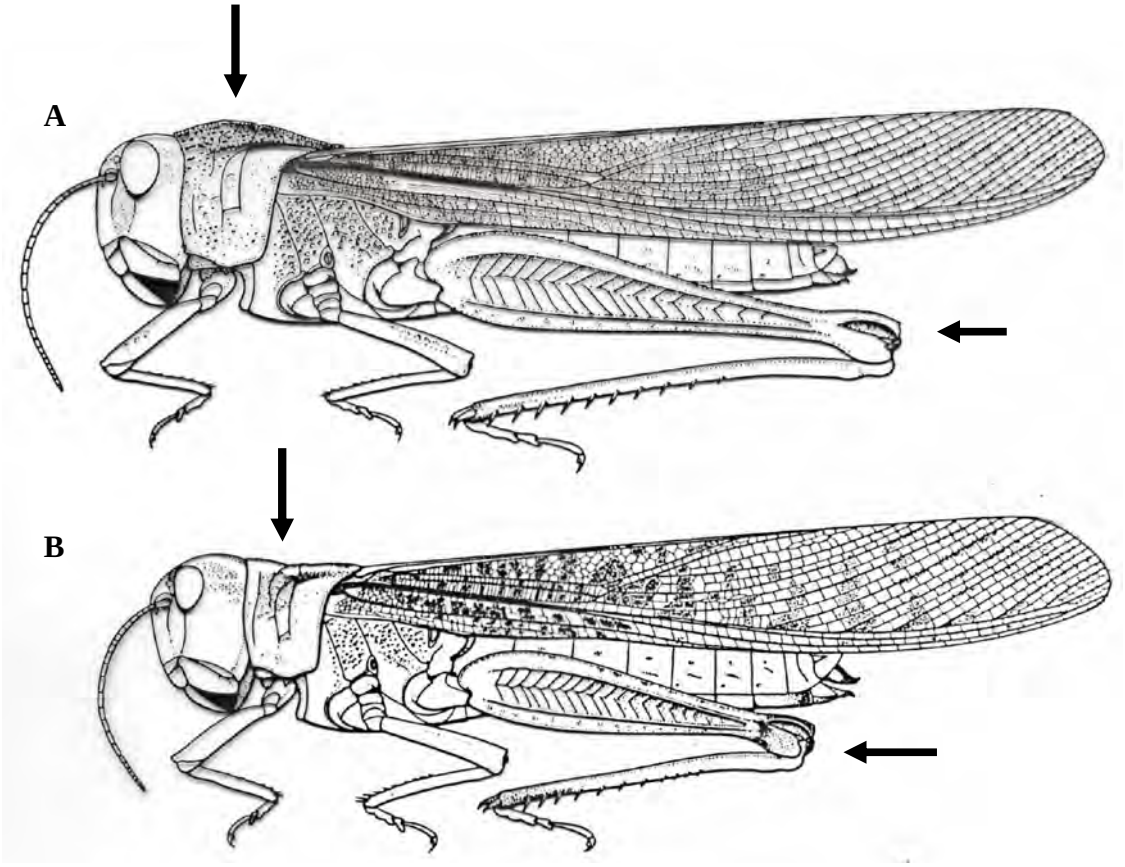



Figure 2.1: Morphological difference between gregarious and solitarious locust. (A) solitarious female showing a convex pronotum (vertical arrow) and long femur (horizontal arrow); (B) gregarious female showing concave pronotum and short femur (Pener, 1991).

### 2.1.2 Body colouration

The general colouration of the solitarious adult is green, light or dark brown; while the gregarious adults are brown and older males are predominantly yellow (Randriamanantsoa, 1998).

### 2.1.3 Anatomy

The gregarious females *L. m. capito* have fewer ovarioles than their solitarious counterparts. Fifteen days of forced grouping are sufficient for the decrease of the ovarioles by 19% (from 106 to 86) from the parental to the immediate descendants (Uvarov, 1966). Among the males, the average number of sperm tubes decreases to 10% (294 to 264) over a similar duration.

### 2.1.3 Physiology

*Gregarious* form have high metabolism. They eat their own weight of food every day. One tonne of locusts, a very small number of an average swarm, eat as much food in one day. In one month, they may consume thirty tonnes (Randriamanantsoa, 1998).

### 2.1.4 Ecology

Gregarious locusts are very resistant to unfavourable ecological conditions. They have a high capacity for dispersion and can occupy various environments. *Locusta migratoria*

*capito* occupies almost the whole of Western side of Madagascar. It has three geographic breeding areas that are ecologically complementary depending on the period of the year. These include the initial area of multiplication (I.A.M.), the transitory area of multiplication (T.A.M.), and the area of multiplication and densation (A.D.). As a result of seasonal wind systems and rainfall range, the solitary adults move from Northeast to Southwest during the rainy season (Lecoq, 1972, 1975; Andrianasolo, 1979; Duranton *et al*., 1979; Scherer, 1997). Through these displacements, they successively colonize zones having temporary favourable conditions for breeding. These zones have monthly rainfall ranges of 50-150 mm (Launois, 1974; Andrianasolo, 1979). In the beginning of the hot rainy season in October, the quantity of rain is sufficient for the first generation of locusts in the I.A.M. However, starting in December, these areas become too humid so that the winged insects migrate further to the Southwest, leading to higher population in the T.A.M. The same phenomena occur in the A.D. and at the height of rainy season, from January to February, the populations are concentrated in a limited space in the extreme Southwest where an ecologically suitable environment still persists. This seasonal migration of solitarious locusts increases the likelihood of gregarization. Locusts from the third generation in the T.A.M. and/or A.D. move again outwards toward the Northeast with the start of the cool dry season and the displacement of the favourable rainy zones. Thus, in one year four generations of locusts can occur, one in the cool dry season and three in the hot rainy season (Table 2.1).

Table 2.1: Reproduction cycle of *Locusta migratoria capito* (Wintrebert, 1970; Scherer, 1997; Randriamanantsoa, 1998).

| **October – November** | **December-January** | **February-April** | **May-September** |
|---|---|---|---|
| 1st generation in hot season | 2nd generation in hot season | 3rd generation in hot season | 4th generation during cool season |
| Rapid increase in density | Multiple gregarization | Possible outbreak | Low density |

### 2.1.5 Life cycle of *L. m. capito*

The life cycle comprises of three stages: eggs, nymphs (five to six instars depending on the phase) and adults. Under hot and rainy conditions, it takes around two months (44 - 66 days) from the time eggs are laid through the nymphal stages to the sexually mature adult locusts (Scherer, 1997). In winter, maturation of the eggs and nymph are much slower and the cycle lasts three to five months (Randriamanantsoa, 1998). Using mark-release and recapture methods, the adult maximum longevity observed in the field is three months for female locusts and ten months for males (Têtefort and Wintrebert, 1963).

#### 2.1.5.1 Oviposition

Selection of oviposition sites is influenced by the presence of conspecific gravid locusts, vegetation and soil conditions. Sufficient water must be available in the soil to ensure both the development of the eggs and the growth of vegetation to sustain hoppers after hatching. Oviposition takes place in soil, which may be burnt patches of grassland, open areas near swamps, bare areas, areas under cultivation, and fallow or forest glades (Wintrebert, 1970). Preference is generally for moist calcareous sand. The number of egg pods laid by one solitarious female locust is about five in the warm season and three in the cool season (Wintrebert, 1970) with each egg pod having an average of 100 eggs. Incubation period lasts 10 - 15 days at 27 - 35$^{o}$C, which is the optimal temperature. According to Wintrebert (1970), the optimal soil humidity ranges between 5 and 25%. Development ceases when temperature falls below 16 $^{o}$C (Tetefort and Wintrebert, 1963).

#### 2.1.5.2 Nymphal stages

Solitarious *L. m. capito* has six nymphal instars while the gregarious has five. The first three instars have their wing buds projected from underneath the pronotum. The first instar is always brown to black and on average 7 - 9 mm long. These nymphs also have a small black pattern on each side of the pronotum. The nymph at second instar is generally brown to black and is on average 10 - 13 mm long. The third instar is green,

brown or blackish and is 15 - 20 mm average length. The third instar “b” exists only in solitarious phase and is characterized by the wing buds orientated to the back of the body (Tetefort and Wintrebert, 1963). The fourth instar is green, brown or blackish, 21 - 25 mm long and has the wing buds shorter than the length of the pronotum. The fifth instar is green, brown or blackish and has an average length of 28 - 35 mm. The wing buds are longer than the pronotum. In gregarious phase, the third to fifth instar nymphs have the dorsal part of the body black and have intense orange colouration on the lateral sides.

#### 2.1.5.3 Sexual maturation

The fifth instar gregarious nymphs of *L. m. capito* fledge into adults that are sexually immature. Sexual maturation depends on environmental conditions and the interaction between the early immature adults and the late fledging fifth instars. The female sexual maturation takes 25 to 45 days in cool season and 12 to 17 days in warm season (Wintrebert, 1970). Mating is observed throughout the year except in winter when it is very rare (Wintrebert, 1970).

### 2.2 Pheromones in locusts

Pheromones are defined as chemical substances produced and released by one individual that affect the physiology or behaviour of another member of the same species (Karlson

and Butenandt, 1959; Karlson and Luscher, 1959). Later, Law and Regnier (1971) used the term “semiochemical” for these substances. When they induce long-term alteration in the physiology of the receiving animals, they are called “primer pheromones” (Wilson and Bossert, 1963), which can be accompanied by morphometric, behavioural and genetic changes (Uvarov, 1966, 1977; Loher, 1990). On the other hand a “releaser pheromone” acts as a signal that is detected and it causes an immediate behavioural response in the receiver (Wilson and Bossert, 1963). In acridids, chemical communication is not well understood. This is in part due to the hypothesis that acridids rely heavily on visual, acoustic and tactile stimuli (Uvarov, 1977; Whitmann, 1990). However, some pheromone systems in a few species have been studied as outlined in the sections below.

### 2.2.1 Gregarization pheromone

The potential role of chemical communication in phase dynamics of locusts was first recognized by Nolte (1963) following observations that isolation of gregarious nymphs of *S. gregaria* (Forskal) or *L. migratoria* (Linné) made them to revert to solitarious phase. When isolated nymphs were kept in the same room with gregarious hoppers, this change did not occur. Gillet (1968; 1975) confirmed Nolte’s observation by using an airborne factor, which significantly influenced the aggregating behaviour of nymphs and adult locusts reared in the absence of any visual and tactile stimuli. The source of the gregarization stimuli was traced to hopper faeces and examination of the constituents led

to the identification of 5-ethylguaiacol referred to as “locustol” (Nolte *et al*., 1970; 1973; Nolte, 1976; 1977) as the principal component of the gregarization pheromone. Subsequently, Fuzeau-Braesch *et al*. (1988) showed that airborne collection of different stages of *S. gregaria* and *L. migratoria* contained varying amounts of four aromatic compounds, three of which were identified as guaiacol, phenol, and veratrole. Behavioural tests indicated that alone or in mixture, these compounds elicited significant increase in grouping behaviour in both species; this was suggested to be the locust cohesion pheromone. Several other laboratories later examined the emissions of gregarious and solitarious adults and nymphs and their faeces and have not found “locustol” (Fuzeau-Braesch *et al*., 1988; Torto *et al*., 1994; 1996; Francke and Schmidt, 1994; Obeng-Ofori *et al*., 1994a; Schmidt and Albütz, 2002).

Research in the 1990s on the gregarious desert locusts, *S. gregaria*, revealed the existence of two sets of releaser pheromones involved in their aggregation behaviour; a juvenile aggregation pheromone produced by both sexes of nymphal stages that is specific to the nymphs and, an adult pheromone produced by older adult males and specific to the adults (Obeng-Ofori *et al*., 1993; 1994b). Moreover, olfactometric bioassays showed that, nymphs aggregated in response to volatiles emitted by their own faeces and of those emitted by the young adult but did not respond to faecal volatiles of the older adult. Young and older adults not only responded to their own faecal volatiles, but also to each other’s and those of the nymphs (Obeng-Ofori *et al*., 1994a). The

aggregation responses were independent of whether the locusts were bioassayed in groups or singly, indicating that non-olfactory cues are not a requisite for pheromonal mediation (Obeng-Ofori, 1993).

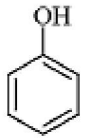
### 2.2.2 Aggregation pheromones of gregarious locusts

#### 2.2.2.1 Pheromone in gregarious nymphs

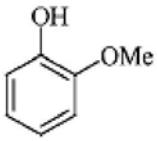
Bioassay-guided characterization of locust volatiles using coupled gas chromatography-mass spectrometric (GC-MS) and coupled gas chromatography-electroantennographic detector (GC-EAD) analyses led to the identification of components of these volatiles that had been collected on charcoal traps. The aggregation pheromone of gregarious fifth instar nymphs of *S. gregaria* comprises of four aldehydes (hexanal, octanal, nonanal, decanal), the corresponding acids (hexanoic acid, octanoic acid, nonanoic acid, and decanoic acid) and faecal phenols comprising of phenol, guaiacol and indole as the major constituents (Torto *et al*., 1996; 1999; Hassanali and Torto, 1999) (Fig. 2.2). In laboratory assays, synthetics of these eight aliphatic compounds and the two phenolic compounds evoked strong aggregation in nymphs, similar to the natural crude volatiles from live nymphs (Torto *et al*., 1996).

$CH_3(CH_2)_4CHO$ $CH_3(CH_2)_{6-8}CHO$ $CH_3(CH_2)_4COOH$ $CH_3(CH_2)_{6-8}COOH$

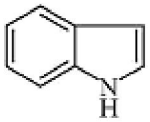
(a) (b) (c) (d)




(e)




(f)




(g)

Figure 2.2: Major components of gregarious nymphal locust pheromone. (a) hexanal; (b) octanal, nonanal, decanal; (c) hexanoic acid; (d) octanoic, nonanoic, decanoic acids; (e) phenol; (f) guaiacol; (g) indole (Hassanali *et al.,* 2005)

#### 2.2.2.2 Pheromone in gregarious adult locust

The aggregation pheromone of older adult of *S. gregaria* comprises of six aromatic compounds namely: benzene, anisole, benzaldehyde, veratrole, guaiacol, phenol and phenylacetonitrile (PAN) (Fig. 2.3). Phenylacetonitrile, guaiacol, phenol and benzaldehyde elicited significant aggregation responses in the adults (Torto *et al*., 1994). In addition, PAN constitutes 70-80 % of the volatiles emitted by older males and is the most active in eliciting aggregation, either on its own or in combination with the others (Torto *et al*., 1994; Hassanali *et al*., 1999).

Faecal volatile components *viz.* guaiacol and phenol are also components of the aggregation pheromone of older adults. The latter are present in relatively lower

proportion when compared to their amounts in nymphal faeces (Obeng-Ofori *et al.*, 1994a). Phenylacetonitrile is also present in faecal volatiles of adult locusts in smaller amounts.

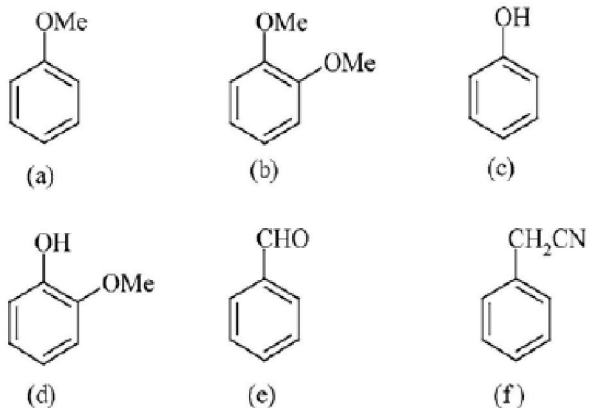



Figure 2.3: Major components of gregarious adult locust pheromone. (a) anisole, (b) veratrole, (c) phenol, (d) guaiacol, (e) benzaldehyde, (f) phenylacetonitrile (Hassanali *et al.*, 2005).

### 2.2.3 Pheromonal effects on the biology of *Schistocerca gregaria*

#### 2.2.3.1 Pheromonal effect on sexual maturation of immature adult locusts

##### 2.2.3.1.1 Maturation-accelerating effect of adult pheromone on immature adult locusts

The experimental work of Norris (1954, 1962) showed that, the presence of mature males accelerated the sexual maturation of young immature male and female adults. Maturation was monitored by yellowing, copulation activity and oviposition time. Loher

(1960) proposed that the pheromone was produced and released from the glandular abdominal epidermis found only in mature males and played a role in maturation acceleration. The exact chemical nature of components of the volatiles remained unknown despite various attempts on their characterization (Blight *et al*., 1969).

More recent studies confirmed the accelerating effects of mature males of the desert locust on the maturation of immature males and females (Mahamat *et al*., 1993; Schmidt and Albütz, 2002). Mahamat *et al.* (1993) using a two-chamber bioassay system as an experimental device without visual and tactile contact, concluded that hexane extracts of body washes of mature males and airborne volatiles trapped from mature male produced the same effect as the volatiles emanating from live mature males on the immature males. The older adult stimuli were not significantly effective when immature adults were exposed to it less than one week (Mahamat *et al.,* 1993). The major active components were anisole, veratrole, phenylacetonitrile, 4-vinyl veratrole and benzaldehyde. Phenylacetonitrile represented 75 - 85 % of the blend (Mahamat *et al*., 1993). In fact, the adult volatiles have dual roles, as an adult aggregant and as a maturation accelerant (Mahamat *et al*., 1993).

#### 2.2.3.1.2 Maturation-retarding effect of nymphal pheromone on immature adult locusts

The volatiles of fifth instar nymphs of *S. gregaria* inhibit sexual maturation of conspecific immature adult males and females (Assad *et al*., 1997). Newly moulted adult *S. gregaria* kept together with fifth instar nymphs without visual or tactile contact had their maturation delayed significantly. Nymphs of both sexes were equally effective. Volatiles trapped from nymphs and the synthetic blend of nymphal aggregation pheromone elicited the same effects on the immature adults. This provides evidence for the dual role of the nymphal volatiles, as a nymphal aggregant and an adult maturation retardant (Assad *et al*., 1997).

In dense locust populations in the field, it is evident that the two pheromonal systems operate together and synchronize the maturation of the entire population: the inhibitory effects tend to delay the maturation of the earlier fledging individuals until the majority of nymphs have moulted into adults (Norris, 1964; Uvarov, 1966; Richard and El Mangoury, 1968).

#### 2.2.3.1.3 Pheromonal effects on oviposition of gravid female locusts

Several observations showed that, gravid female locusts are strongly attracted to common egg-laying sites by a pheromone emitted in volatiles released by ovipositing

gregarious females (Saini *et al.*, 1995). They preferred to oviposit in moist sand contaminated with froth of egg pods. Two compounds, acetophenone and veratrole were identified, each of which induced oviposition (Rai *et al*., 1997). Torto *et al*., (1999) identified additional three EAG-active unsaturated ketones from the sand previously used for oviposition: (*Z*)-6-octen-2-one, (*E,E*)-3,5-octadien-2-one, and (*E,Z*)-3,5-octadien-2-one (Fig. 2.4). On the other hand nymphal pheromones elicited random distribution of egg pods by inhibiting the detection of the oviposition pheromones by gravid females (Kane, 2004).

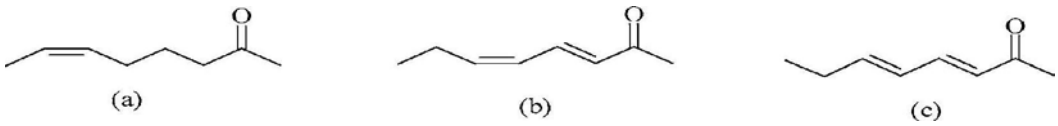



Figure 2.4: Ketones secreted into sand by gregarious female *S. gregaria* during oviposition: (a) (Z)-6-octen-2-one, (b)(E,Z)-3,5-octadien-2-one, (c)(E,E)-3,5octadien-2-one (Torto *et al*., 1999).

#### 2.2.3.2 Pheromones as solitarization factor

Test for grouping behaviour of gregarious nymphs undertaken by Gillet (1983) and by Gillet and Phillip (1977) showed that faeces from adults of *S. gregaria* had a dispersive effect on conspecific hopper stages. Treatment of hopper bands in the field with the adult pheromone leads to disorientation and gradual dispersal of the insects. Bashir *et al.* (2000) and Ochieng (1997) demonstrated that, phenylacetonitrile inhibits nymphs from detecting their own aggregation pheromone.

### 2.2.4 Aggregation pheromone of *Locusta spp*

In the African migratory locust, *Locusta migratoria migratorioides*, unlike in the desert locust, *S. gregaria*, stage and sex differentiation in its aggregation pheromones are not well-defined (Niassy *et al*., 1999). In this locust species, although fifth instar nymphs did not respond significantly to the adult volatiles, adults responded significantly to the nymphal volatiles. On the other hand, both sexes of *L. m migratorioides* adults produced an aggregation stimulus. However, the female locusts responded more strongly to the male volatiles than the females to their own volatiles and *vice versa* (Niassy *et al*., 1999). Analyses of *L. m. migratorioides* nymphal volatiles showed three compounds: hexanoic acid, phenylacetonitrile, and an additional unidentified compound. Hexanoic acid and phenylacetonitrile evoked the strongest EAG responses from the male antennae. Of the volatiles from adults, 14 compounds were identified of which nine were

E-2-pentenal, Z-2-pentenal, Z-2-hexenal, Z-2-penten-1-ol, Z-2-hexen-1-ol, veratrole, hexanoic acid, guaiacol, nonanoic acid. The other five were aliphatic components that were not fully characterized though they evoked EAGs from male antennae. Faecal volatiles contained phenol and guaiacol.

Recently Yu *et al.*, (2007) collected faecal volatiles from 2-4 day-old gregarious adults of the oriental migratory locust, *L. m. manilensis* and reported 11 compounds that elicited electrophysiological responses in ther antennae. Nine of the 11 compounds were identified to be 2-hexenal, 2,5-dimethyl-pyrazine, cyclohexanol, hexanal, benzaldehyde, benzyl alcohol, nonanal, 2,6,6-trimethylcyclohex-2-en-1,4-dione and beta ionone. 2-Hexenal was reported to be the most active.

# CHAPTER THREE

## 3.0 GENERAL MATERIALS AND METHODS

### 3.1 Experimental locusts

*Locusta migratoria capito* (Saussure, 1884) (Orthoptera: Acrididae) that were reared from egg pods originating from Betioky Sud (23° 40' 0" South, 44° 23' 0" East), in the Southwest of Madagascar were used in this study. They were bred and reared under crowded conditions in aluminium cages (50 x 50 x 50cm) in the quarantine unit at ***icipe***. The bottom and two sides of the cage were made from wire mesh and the front side had a sliding glass door. At the back of the cage, a 40-W electric light bulb was mounted to provide light and heat to the insects inside. The rearing room was aerated by a duct system that maintains a negative pressure with a nycthemeral temperature of 30 ± 2.0$^{o}$C. The light-dark cycle was 12: 12 hours and the ambient relative humidity (40 - 50 %) (Ochieng-Odero *et al.,* 1994). Each cage contained 150 to 200 of both female and male locusts. Locusts were fed on wheat bran and 2 week-old wheat shoots which were provided daily. Locusts used in the experiments ranged from 3$^{rd}$ to 15$^{th}$ generations.

### 3.2 Olfactometer for bioassay

#### 3.2.1 Single-chamber olfactometer

The olfactometer (Fig. 3.1) was a glass chamber (60 x 30 x 30cm), the top of which had a removable wire mesh and the bottom was a galvanised iron plate drilled with 2-mm-

diameter holes with a spacing of 1 cm (Obeng-Ofori *et al*., 1993). Each half of the floor of the chamber was attached to a square pyramidal aluminium funnel, each connected by Teflon tubing to a 2-litre round-bottomed flask. During experiments, one side of the chamber was permeated with air from the cylinder enriched with volatiles from a volatile source and the other side only with clean air. Either live nymphs, nymphal faeces, solvent extract from nymphs or synthetic compounds were used as sources of volatiles. To avoid mixing of volatiles in the arena, the olfactometer was placed under an extraction hood which sucked air out of the system. Uniform illumination was provided by two 60W fluorescent tubes fitted above the olfactometer. In all tests, room temperature was maintained at 30 ± 2.0 ºC, and locusts were used once and then discarded.

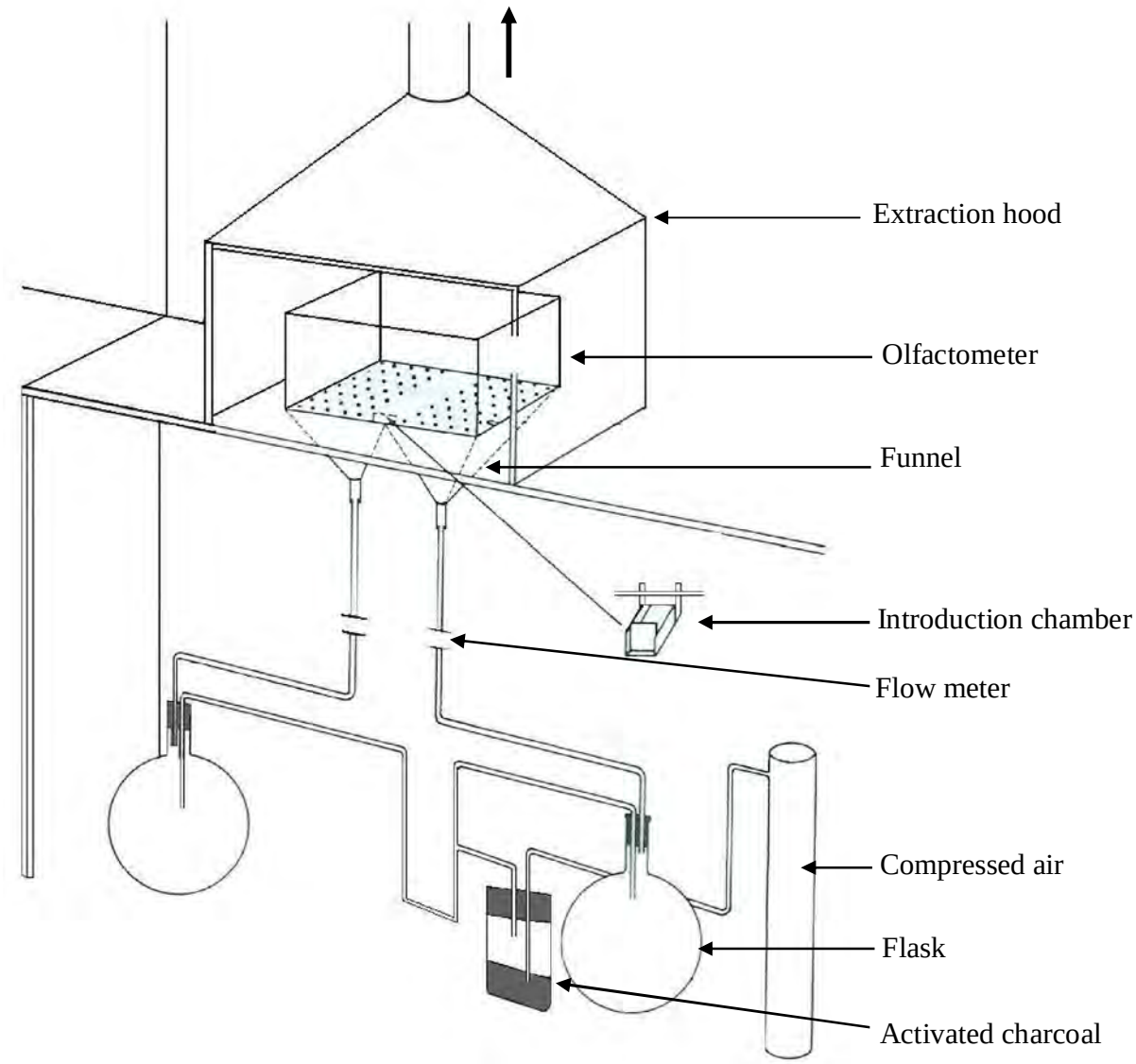



Figure 3.1: Diagram of the single-chamber olfactometer for studying aggregation responses of locusts (Obeng-Ofori *et al.*, 1993).

### 3.2.2 Cage assays for studying sexual maturation of immature adult locusts

Two types of cages were used for testing the effect of nymphal stimuli on sexual maturation of the immature adults, the standard aluminium cage and a double storey aluminium cage.

#### 3.2.2.1 Standard aluminium cage

This cage was used to test the effect of live nymphs on the maturation of immature adults. It allowed olfactory, tactile and visual contact between test locusts (immature adults) and signal source (fifth instar nymphs). The cage measured 20 x 20 x 20cm with sliding Perspex® front and wire gauze on the top side, bottom and the two other sides. In addition, the front bottom plate had two holes to hold oviposition cups (Plate 3.1).

#### 3.2.2.2 Double-storey cage

For bioassays in which the effects of volatiles alone on the maturation were tested, two-chamber aluminium cages (15 x 15 x 30cm) (Plate 3.2) as described by Mahamat *et al.*, (1993) were used. Each cage had two compartments separated in the middle by wire gauze, which allowed the recipient locusts to detect the volatiles emitted by the source locusts. The front end of the cage was a sliding Perspex® plate and the other two sides were made of wire gauze.

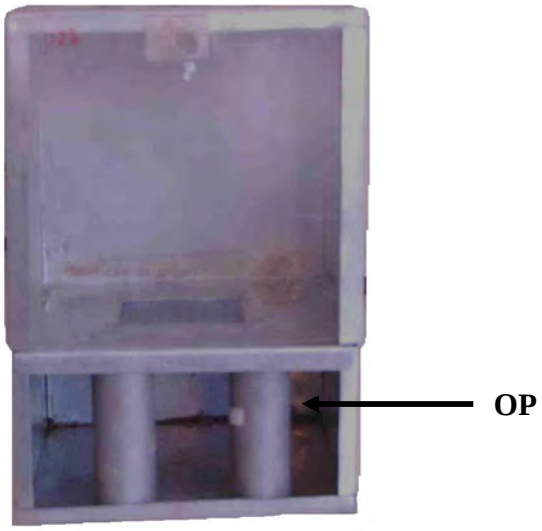



Plate 3.1: Standard aluminium cage (20 x 20 x 20cm) for testing the effect of nymphal stimuli on sexual maturation of immature adults. OP, oviposition cup.


Plate 3.2: Double storey cage (30 x 15 x 15cm) for testing the effect of nymphal volatiles on sexual maturation of immature adults. LC, Lower compartment; UC, Upper compartment.

## 3.3 Collection of volatiles

### 3.3.1 Adsorbent for trapping volatiles

A 30 mg of prepacked Super-Q (80-100 mesh; Alltech, IC, USA) served as adsorbent for trapping volatiles from both live nymphs and nymphal faeces. Before use, each trap was cleaned by flushing 1ml of dichloromethane (HPLC grade, Fluka 99.9%) through the adsorbent and then dried by passing purified nitrogen.

### 3.3.2 Collection of volatiles from live nymphs

Volatiles were collected from fifty, 3-5 day-old fifth instar female and male nymph locusts. The locusts were placed in a cylindrical glass tube (50cm long x 14cm ID), with a port at each end. The glass tube was then connected to the air-entrainment system with Teflon® tubes and then clean humidified air from a cylinder (BOC Kenya Ltd.) was pumped in via one port over the insects through a Super-Q filter trap fitted in the other port (Plate 3.3). The latter was connected to a diaphragm vacuum pump (Wertheim, Germany) and air was sucked at a flow rate of 106ml/min. The trapping of volatiles was done for 8 to 10 hours.

### 3.3.3 Collection of volatiles from nymphal faeces

Faecal volatiles were collected using the same protocol as above from 4 - 6g of fresh faeces obtained after feeding 40 fifth instar nymph overnight on fresh wheat shoots. The

faecal pellets were held in quick-fit glass tubes (12cm long X 2.5cm wide) that were connected to the air-entrainment system and volatiles collected as above.

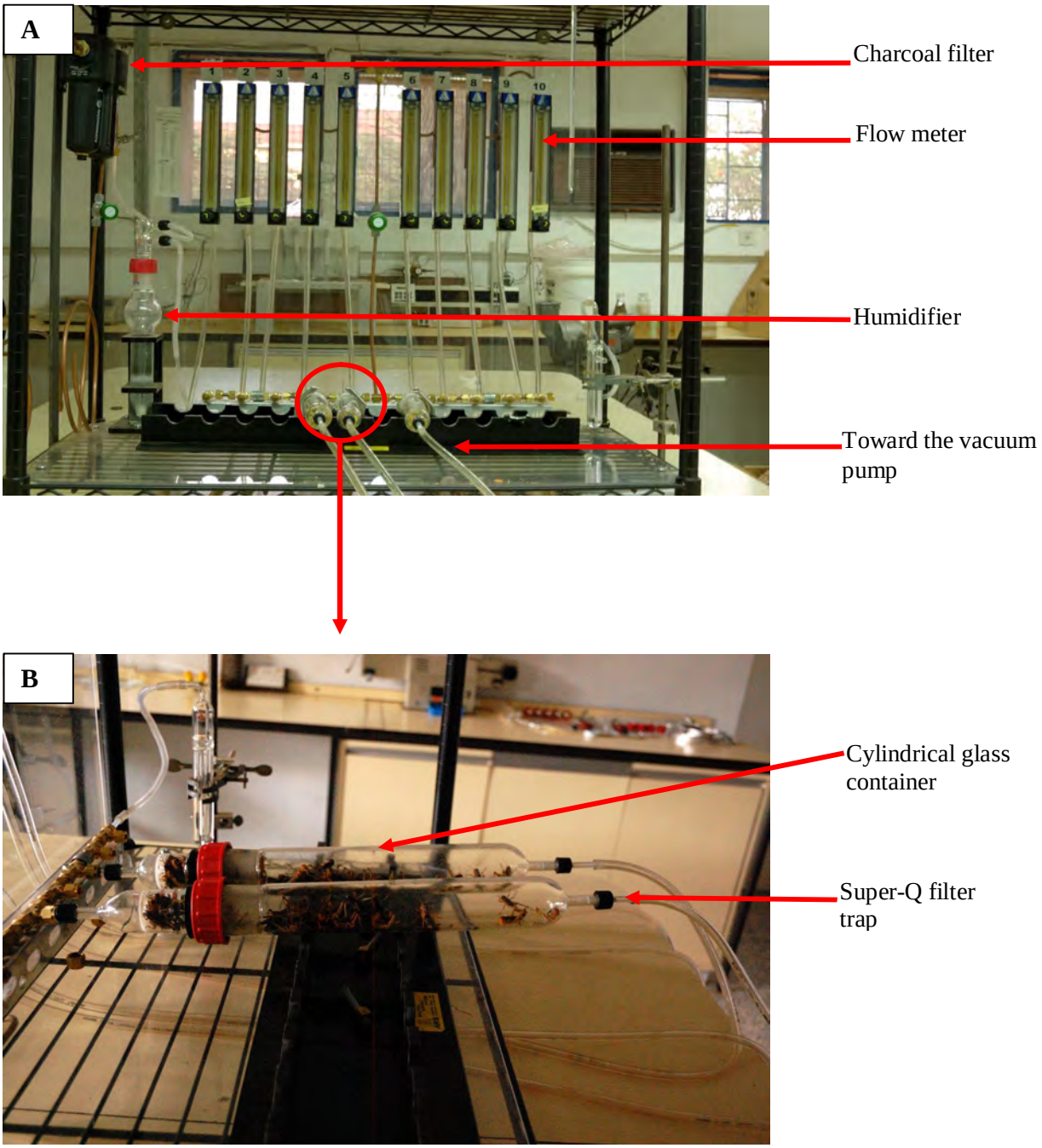



Plate 3.3: The air-entrainment system for collecting volatiles showing (A) the ten channels, (B) a close up photograph of nymphs in two quick fit tubes, each connected to the volatile connection channel on the system.

### 3.3.4 Elution of trapped volatiles

The volatiles were eluted from the trap into 2ml-vials (Sigma, Aldrich) with 200µl of high purity dichloromethane (HPLC grade, Fluka 99.9%) under a stream of high purity nitrogen (BOC Kenya Ltd). To minimise loss of volatile compounds, the vial was immersed in an ice bucket during the elution and thereafter immediately closed with their cap, then tightly sealed with Teflon® tape and stored at -20ºC until use. For GC analyses, an equivalent volume of 1.174µg of methyl salicylate (Sigma, USA) was added to 40µl of the sample and used as the internal standard.

## 3.4 Analysis of the eluted volatiles

### 3.4.1 Gas chromatographic (GC) analysis

Gas chromatographic analyses were performed on HP 5890 A Series II GC equipped with a Flame Ionization Detector (FID), an autosampler injector HP 7673, HP capillary column (HP1 methyl silicone non-polar capillary column; 30m x 0.25m ID x 0.20µm film thickness). The GC was configured to run in splitless mode, and monitored from a computer (Dell Optiplex X 520), which displayed chromatograms of analysed volatiles using ChromPerfect software (Version 5.5.5 Copyright © 2004, 2005 Justice Laboratory Software).

Sample analyses were carried out by injecting 1µl of the eluate into the GC using the following oven temperature programme: initial oven temperature set at 60$^{o}$C for 3min, followed by an increase at 10$^{o}$C/min to 250$^{o}$C, then held for 8min. Nitrogen was used as the carrier gas at a flow rate of 1.20ml/min. The FID had a mixture of medical air and hydrogen gas from a hydrogen generator (Domnick Hunter, USA) at flow rates of 405ml/min and 31ml/min, respectively. A delay of 0.5min before injection purging was maintained. The identification of the individual compounds was done by coupled gas chromatograph-mass spectrometric (GC-MS) analysis (section 3.4.3).

### 3.4.2 GC-Electroantennographic Detector (GC-EAD) analysis

GC-EAD analysis was done to detect the biologically active compounds in the volatiles. Preparation of the locust antennae was as described by Torto *et al.* (1994). Antenna was pulled off the head capsule, the scape and pedicel were cut off and the flagellum inserted into a glass micropipette containing locust saline solution. Electrodes were connected to a universal AC/DC amplifier. GC-EAD tests were recorded for antenna from male and female fifth instar nymphs.

The same column and conditions as for GC analyses of volatiles were used. However, the column effluent was split 1:1 into two 50-cm-long deactivated fused silica capillary column lines connected to the FID and to the stimulus delivery tube over the antenna, respectively. FID and EAD signals were acquired and monitored synchronously using a

computer program via a GC/EAD interface card (Synthech) installed in a PC (Harvard Professional Computer, American Megatrends Inc.) (Plate 3.4).

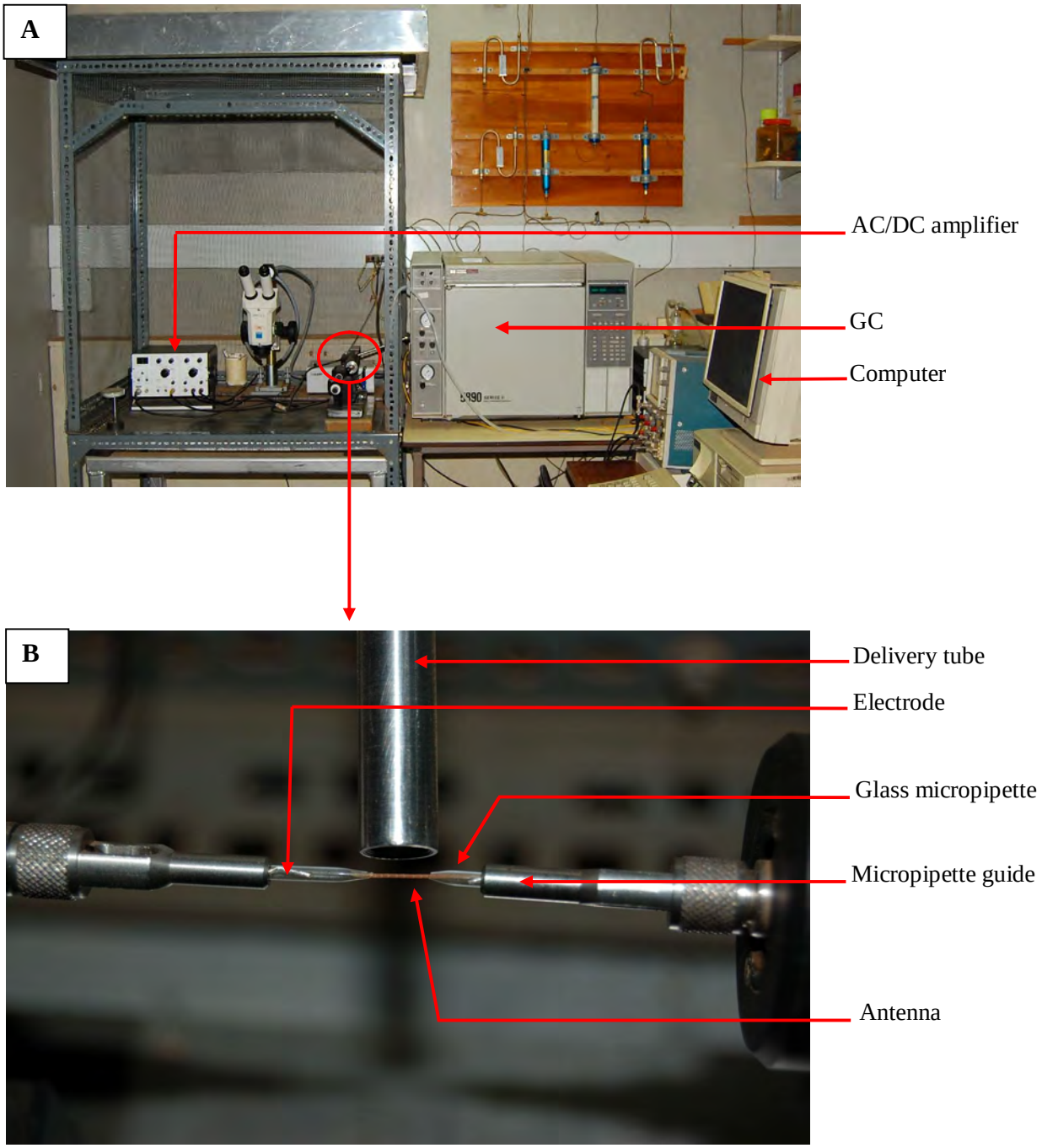



Plate 3.4: The GC-EAD system showing (A) the various instruments, (B) a close up photograph of a detached locust antenna mounted in two electrodes, placed close to the end of the stimulus delivery tube.

### 3.4.3 GC-Mass spectrometric (GC-MS) analysis

GC-MS analysis of eluted samples was carried out on HP 7890 A Series GC (Agilent technologies, Wilmington, DE, USA) coupled to a 5975C Series Tripple Axis Quadruple mass spectrometer operating in the Electron Impact (EI) mode at 70eV. The GC was equipped with a 7683 B Series autosampler, HP capillary column (30m x 0.25m ID x 0.25µm film thickness). The system was monitored from a computer (HP L1710) and data were acquired by MSD Chemstation software (G1701EA E.02.00.493). One µl of the eluate was analysed using the following oven programme: initial oven temperature set at 35$^{o}$C for 5min, followed by a rise at 10$^{o}$C/min to 280$^{o}$C, held for 10.5min. The chemical identity of the peaks was based on the analysis of the spectral fragmentation patterns obtained from the NIST MS data libraries (HP, USA). The identity of the peak then was confirmed by comparison with the retention times and mass spectra of authentic synthetic standards (Sigma Aldrich).

# CHAPTER FOUR

## 4.0 ROLE OF VOLATILES IN THE AGGREGATION BEHAVIOUR OF NYMPHS OF *LOCUSTA MIGRATORIA CAPITO*

### 4.1 Introduction

Locusts use pheromones to aggregate and to gregarize. Several studies have shown the importance of pheromones in the aggregation behaviour and on the cohesiveness of hoppers in bands for different species of locusts. Fuzeau-Braesch *et al*. (1988) found that a chemical blend identified from volatiles collected from air surrounding crowd-reared *S. gregaria, L. m. migratorioides* and *L. m. cinerascens* promoted group formation among gregarious locusts. This was considered to be a cohesion pheromone. However it did not attract locusts in an olfactometer test. Work on *S. gregaria*, by Obeng-Ofori *et al.* (1993) showed that, gregarious locusts aggregated in response to their own volatiles in a single chamber olfactometer. Further studies have shown that nymphs only responded to nymphal volatiles while a separate system operates for adults (Obeng-Ofori *et al.,* 1994b). These authors reported that responses to pheromone for each of two stages were the same in both sexes of nymphs and in adult locusts.

Male and female fifth instar nymphs of *L. m. migratorioides* responded highly to their own volatiles, however, there was no sex preference to the volatiles (Niassy *et al.*, 1999). On the other hand, mature adult locusts were not responsive to nymphal volatiles,

but responded to their own volatiles to which fifth instar nymphs, young and older adult locusts were also responsive (Niassy *et al*., 1999). Studying the behavioural response to faecal volatiles, Obeng-Ofori *et al*. (1994a) found that nymphs of both sexes responded to nymphal and young adult's faecal odours but were not responsive to volatiles from faeces of mature adult locusts.

Similar chemical ecological work has not been carried out on the Malagasy migratory locust, *L. m. capito*. In this chapter, responses of fifth instar nymphs to their own volatiles were assessed. The volatiles included direct emissions from live nymphs, their faeces and the body extract.

## 4.2 Materials and methods

### 4.2.1 Olfactometer assay

The aggregation responses of fifth instar nymphs to their body and faecal volatiles were determined using a single-chamber arena olfactometer (section 3.2.1). Charcoal-filtered air from a cylinder was split into two streams; each was passed into a round bottomed flask, and into either side of the arena at a flow rate of 120ml/min/side regulated by flow meters. One of the flasks contained either ten 3- 4 day-old fifth instar nymphs or 4 - 6 g of fresh faeces, while the other one acted as a control. Faeces were obtained after feeding 40 nymphs overnight on fresh wheat shoots.

To determine the aggregartion responses of locust nymphs to the volatiles, test locusts were released either singly or in group of three or five into the olfactometer chamber through a small door on the front of the chamber between the funnels. Testing single nymphs eliminated possible effects of visual and tactile stimuli due to the presence of other locusts. The number of locusts in each part of the arena was then counted every five minutes. Uncommitted insects in the middle part of the arena were treated as non-responders. The aggregation index (*AI*) was calculated as 100 $(T - C)/N$ where $T$ represents the number of locusts found in the treated compartment, $C$ the number of locust in the control compartment and $N$ the total number of locusts tested.

Treatments are shown in Table 4.1. For single nymph locusts, tests were replicated 50 to 100 times while those involving nymphs tested in groups were replicated 3 to 10 times. Between experiments, the olfactometer was cleaned with acetone and then flushed with clean air to remove any volatile residues. The control and source flasks were systematically switched throughout the experiments to minimize any positional bias. A blank test was run before each experiment to ensure there was no positional bias in the responses of locust nymphs to either side of the arena.

Table 4.1: Treatments carried out to determine the responses of nymphs of *Locusta migratoria capito* to volatiles from conspecific nymphs

| **Source of volatiles** | **Recipient (test) insects** |
|---|---|
| Either Ten 5th instar nymphs mixed sex or faeces | 5th instar mixed sex A [a] |
| | 5th instar mixed sex B [b] |
| | Single 5th instar female |
| | Single 5th instar male |
| *****Either ten females or ten males 5th instar instar nymphs | Ten 5th instar females |
| | Single 5th instar female |
| | Ten 5th instar males |
| | Single 5th instar male |

***** Cross tests

[a] Group of five fifth instar males + five fifth instar females

[b] Group of three fifth instar males + three fifth instar females

For each treatment, two sets of nymphs were tested: the first set comprised of fifth instar nymphs that had been reared together with adult conspecific locusts. The second set consisted of fifth instar nymphs that had been reared separately from the adult locusts. The body extract of nymph locusts was tested only on the latter for which, a group of ten fifth instar females and another of ten males were tested separately. The extract was prepared as outlined below.

### 4.2.2 Solvent extract from *Locusta migratoria capito* nymphs

Tests using live nymphs allowed only highly volatile compounds to reach the recipient locusts in the arena. To have the whole range of compounds including the ones of higher molecular weight, a body extract was necessary. Ten 3 - 5 day-old fifth instar nymphs (5 females and 5 males) were immersed into 20ml of hexane (Sigma, Aldrich) in a glass vial for two hours. Nymphs were then removed and the extract concentrated by flushing with purified nitrogen to 1ml and then applied onto eight filter paper discs (each 2cm diameter; Whatman No. 10). In the bioassay, the hexane extract acted as test stimulus while for the control, similar filter paper discs were impregnated with 1 ml of hexane. The filter paper discs were distributed on the floor of the arena and then covered with another wire mesh to prevent direct contact by the test nymphs. Air was passed directly to the square pyramidal aluminium funnel. Both the test and control filter paper discs were left for one hour to allow hexane and the highly volatile compounds to evaporate before the commencement of the tests. Ten fifth instar nymphs from the group that was

reared separate from their adult conspecifics were used in each replicate and tests were replicated three times.

### 4.2.3 Data analysis

Data on the aggregation responses were subjected to Chi-square ($\chi^2$) goodness of fit test using SAS (SAS Institute Inc., Cary, NC, USA. 2002-2003) for comparison between controls and tests.

## 4.3 Results

For the blank tests, there was no significant difference in the distribution of the nymphs in the two sides of the chamber in the absence of an odour source meaning that, there was no bias to either side and no other factors influenced the olfactory choice of the nymphs.

### 4.3.1 Responses of fifth instar nymphs to volatiles of conspecific nymphs when they were reared together with conspecific adult locusts

Aggregation responses of fifth instar nymphs to their own volatiles are shown in Fig. 4.1. The nymphs had similar aggregation responses to volatile emissions from live nymphs and to those from faeces. They responded positively but weakly to the volatiles. There was no significant difference between behavioural responses of male and female nymphs to the two volatiles tested ($\chi^2$ = 3.7216, df = 1, $P$ = 0.0537) and ($\chi^2$ = 3.3750,

df = 1, $P$ = 0.0662) for single male and single female against fifth instar mixed sex respectively; ($\chi^2$ = 0.5765, df = 1, $P$ = 0.4477) and ($\chi^2$ = 3.3750, df = 1, $P$ = 0.0662) for single male and single female against the faecal volatiles respectively. The number of recipient locusts did not influence the aggregation response ($\chi^2$ = 0.0051, df = 1, $P$ = 0.9429, and $\chi^2$ = 0.2807, df = 1, $P$ = 0.5962) of fifth instar mixed sex A (10 nymphs) and B (6 nymphs), respectively, to fifth instar mixed sex nymphs; ($\chi^2$ = 0.1517, df = 1, $P$ = 0.6969 and $\chi^2$ = 1.0384, df = 1, $P$ = 0.3082), of fifth instar mixed sex A and B respectively, to the faecal volatiles.

With regard to the responses of male and female fifth instar nymphs the trend was the same for both sexes as they avoided the airborne volatiles (Fig. 4.2). This was more evident when fifth instar males were tested singly, ($\chi^2$ = 15.68, df = 1, $P$ = 0.0001 and ($\chi^2$ = 6.75, df = 1, $P$ = 0.0094) when tested against volatiles from a group of ten fifth instar males and ten fifth instar females respectively. Single fifth instar female nymphs responded weakly to either volatile sources ($\chi^2$ = 0.0213, df = 1, $P$ = 0.8840) and to the group of ten fifth instar males and fifth instar females ($\chi^2$ = 1.0426, df = 1, $P$ = 0.3072). When the nymphs were tested in groups of males or females, neither sex had responded significantly different from the control ($\chi^2$ = 0.3507, df = 1, $P$ = 0.5537 for males, and $\chi^2$ = 0.0084, df = 1, $P$ = 0.9271 for females to volatiles from fifth instar males; $\chi^2$ = 0.0014, df = 1, $P$ = 0.9703 and $\chi^2$ = 0.0548, df = 1, $P$ = 0.8149, respectively, for males and females to volatiles from fifth instar female.

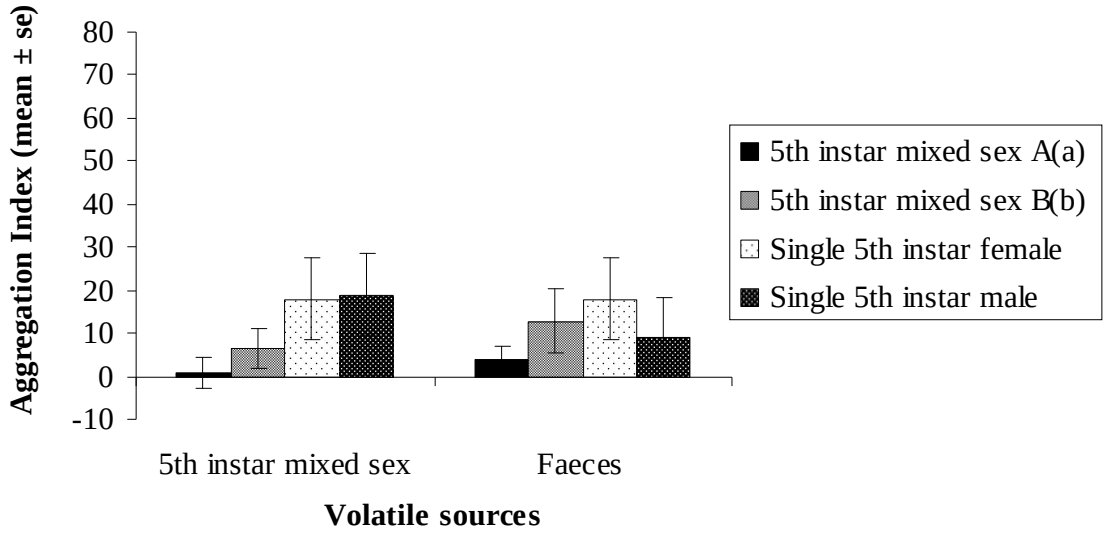



Figure 4.1: Responses of fifth instar nymphs of *Locusta migratoria capito* to volatiles of conspecific nymphs when they were reared together with conspecific adults. $\chi^2$ test was not significant, $P > 0.05$.

[a] Groups of five fifth instar males + five 5th instar females

[b] Groups of three fifth instar males + three 5th instar females

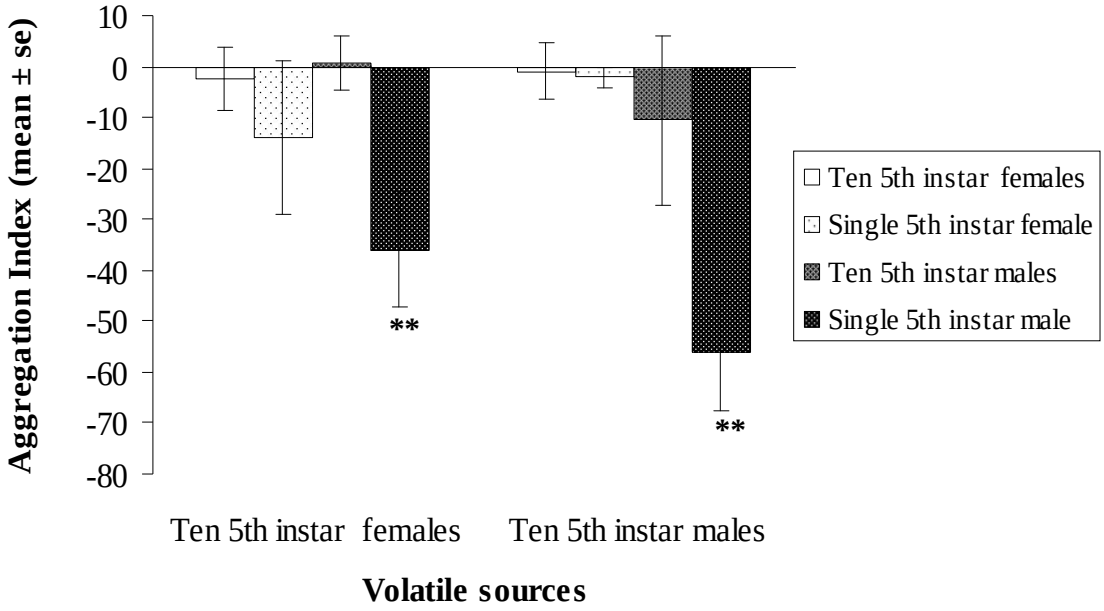



Figure 4.2: Responses of male and female fifth instar nymphs of *Locusta migratoria capito* to volatiles from conspecific nymphs that had been reared with conspecific adults. $\chi^2$ test significant, $^{**}P < 0.01$.

### 4.3.2 Response of nymphs to their volatiles when they were reared separately from conspecific adult locusts

The fifth instar females and males responded to volatiles released from live fifth instar nymphs similarly to those from faeces. Both sexes avoided the side of the arena treated (or enriched) with the volatiles (Fig. 4.3). The fifth instar males were significantly repelled by volatiles from the live fifth instar nymphs ($\chi^2$ = 31.36, df = 1, $P$ = 0.0001) and those from faeces ($\chi^2$ = 8.22, df = 1, $P$ = 0.0041). A similar trend was observed for the fifth instar females to live nymphs ($\chi^2$ = 17.64, df = 1, $P$ = 0.0001), and faecal volatiles ($\chi^2$ = 13.13, df = 1, $P$ = 0.0003).

In cross tests in which volatiles from groups of ten nymphs of one sex were tested against nymphs of the other sex, single male or female nymphs responded negatively to volatiles of nymphs of the other sex ($\chi^2$ = 9.68, df = 1, $P$ = 0.0019 for males, and $\chi^2$ = 27.04, df = 1, $P$ = 0.0001 for females). Exposure of single male or female nymphs to volatiles from a group of ten fifth instar male nymphs had the same trend of responses ($\chi^2$ = 12.96, df = 1, $P$ = 0.0003 for male, and $\chi^2$ = 6.31, df = 1, $P$ = 0.012 for females). On the other hand, when groups of ten nymphs were exposed to the volatiles from nymphs of the other sex, there were no significant differences between their responses to the volatiles and those to the controls ($\chi^2$ = 1.6531, df = 1, $P$ = 0.1985 for groups of 10 males, and $\chi^2$ = 0.4111, df = 1, $P$ = 0.5214 for groups of ten females to male volatiles;

and $\chi^2$ = 1.6243, df = 1, $P$ = 0.2025 for groups of 10 males, and $\chi^2$ = 1.0232, df = 1, $P$ = 0.3118 for groups of 10 females).

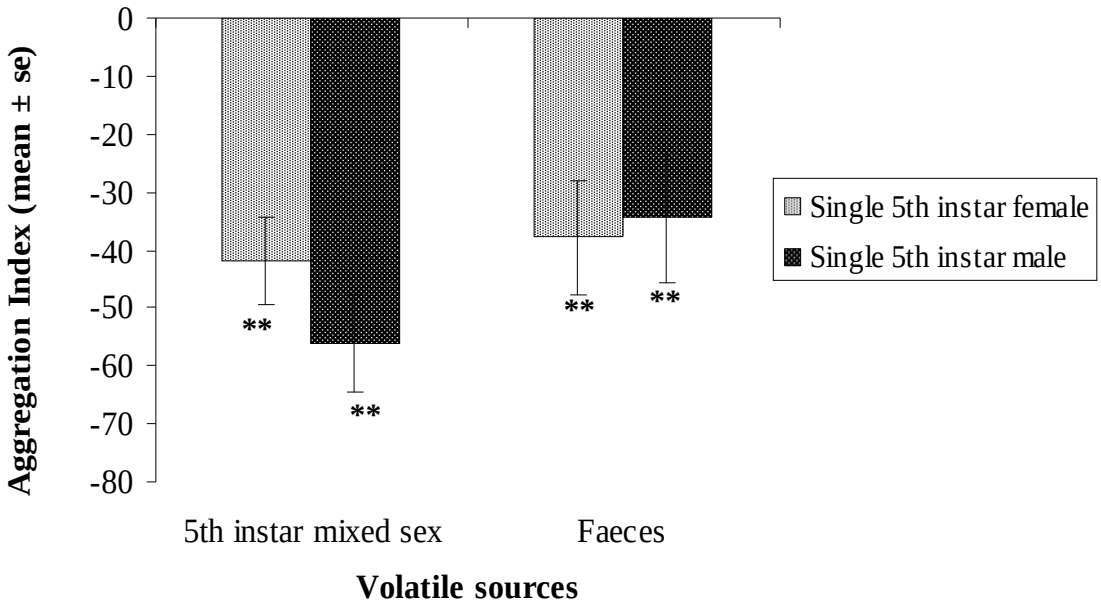



Figure 4.3: Responses of fifth instar nymphs of *Locusta migratoria capito* to volatiles of conspecific nymphs that had been reared separately from conspecific adults. $\chi^2$ test significant, ** $P < 0.01$.

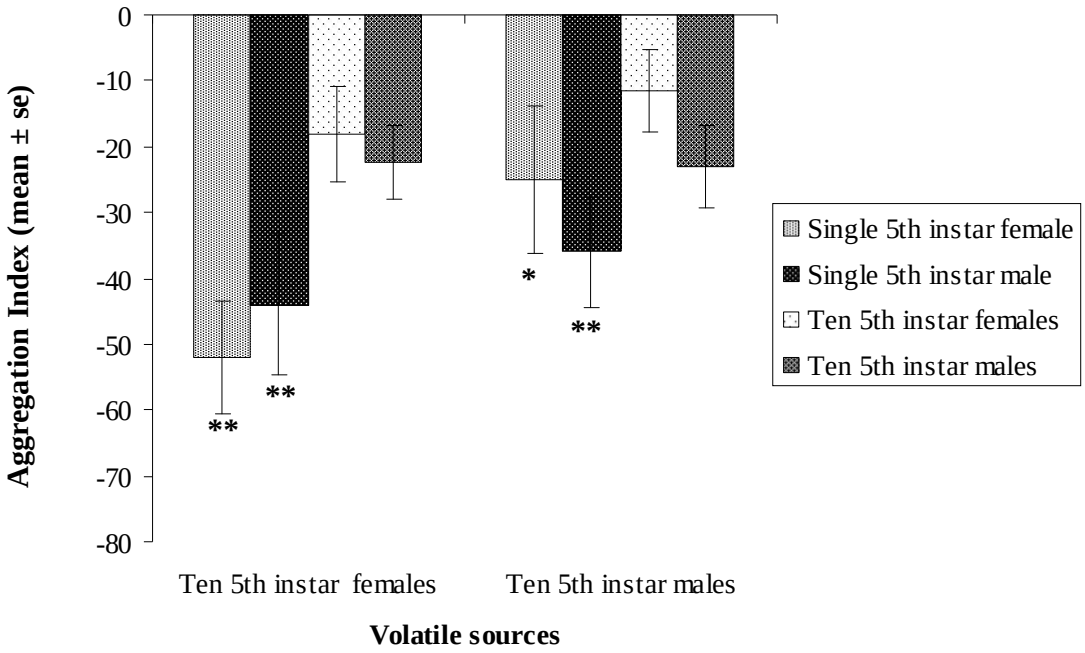



Figure 4.4: Responses of male and female fifth instar nymphs of *Locusta migratoria capito* to volatiles from conspecific nymphs that were reared separately from conspecific adults. $\chi^2$ test significant, $*P < 0.05$; $** P < 0.01$.

### 4.3.3 Response of fifth instar nymphs of *Locusta migratoria capito* to body extracts of conspecific nymphs

Fifth instar nymphs were not responsive to volatiles from body extract of conspecific nymphs (Fig. 4.5). $\chi^2$ = 0.0221, df = 1, $P$ = 0.8819 for males, and $\chi^2$ = 1.2, df = 1, $P$ = 0.2733 for females.

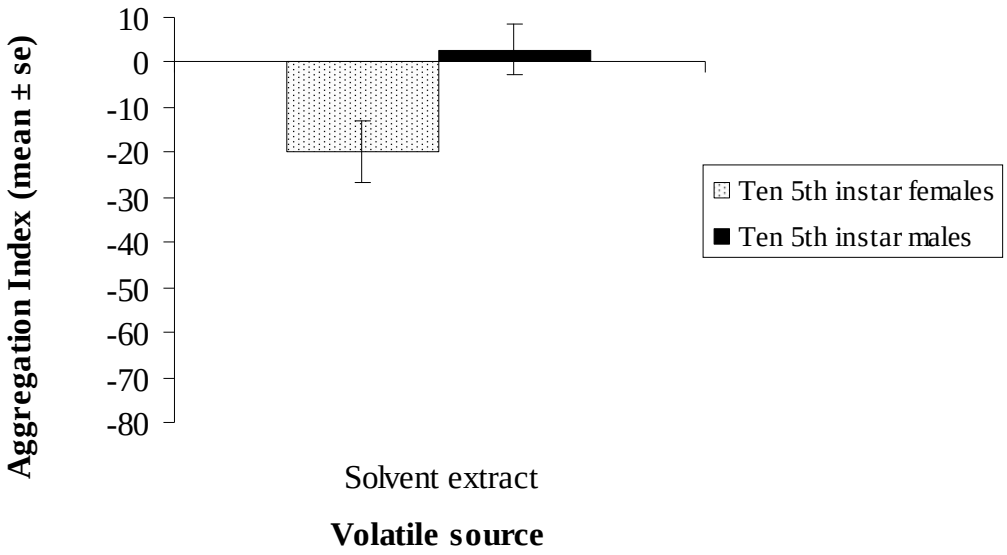



Figure 4.5: Aggregation response of fifth instar nymphs of *Locusta migratoria capito* to body extract of conpecific nymphs. Ten nymphs were used per replicate and tests were replicated three times. $\chi^2$ test not significant, $P > 0.05$.

## 4.4 Discussion

The results showed that, fifth instar nymphs responded differently to their own volatiles depending on whether they were reared together with conspecific adult locusts or separately. Some inconsistencies were observed when the nymphs were reared together with conspecific adults. In the initial tests, nymphs showed positive aggregation indices to the volatiles though these were not statistically significant. However in subsequent cross tests, nymphs of a given sex that were similarly reared and tested against their own volatiles and to those from nymphs of the other sex predominantly chose the control side of the arena, away from air permeated with the volatiles from conspecifics.

The pattern of the responses from the cross tests suggest that there were no differences between the volatiles that were emitted by fifth instar female and male nymphs. Comparison of the responses when fifth instar male nymphs were tested singly and in groups of ten against volatiles from both fifth instar female and male nymphs indicated that other cues might be involved eliciting nymphal aggregation behaviour. However, there were no significant differences in the aggregation responses in the olfactometric tests when live nymphs and faeces were used as sources of volatiles. The cross tests also revealed a behavioural difference between fifth instar male nymhs and their female counterparts. This was in contrast to the responses that were observed when nymphs were exposed to volatiles from live nymphs or nymphal faeces.

The foregoing inconsistencies necessitated a change in the rearing of nymphs. In their natural habitat, locusts develop sequentially and synchronously through the nymphal stages to the adult without lengthy coexistence of any of the developmental stages that follow one another (COPR, 1982). It was thus suspected that volatiles produced by the adult locusts within the rearing cages may have affected the physiology and behaviour of the nymphs. Unlike when locust nymphs were reared together with their adult conspecifics, nymphs that were reared separately from the adult locusts had consistent responses to their volatiles. The results showed clear avoidance by the fifth instar nymphs to volatiles from live fifth instar conspecifics or from their faeces. This may be interpreted as non-existence or non-detection of any kind of pheromone mediating aggregation behaviour in the laboratory mass-reared nymphs of *L. m. capito*. The cross-sex tests showed no sex difference in the emission of volatiles on the basis of the responsiveness of the nymphs to their volatiles.

The solvent body extract from the nymphs, that supposedly contained few highly volatile components that act at short range, did not elicit any significant response from the nymphs. This may either be due to the fact that the nymphs did not detect any volatile compounds emanating from the extract, or, any compounds that were released had no effect on the aggregation behaviour of the nymphs.

In different species of locusts, the gregarious and solitarious locusts differ substantially in their behaviour, in particular, their responses towards their conspecifics (Uvarov, 1966; Pener, 1991; Pener and Yerushalmi, 1998; Simpson *et al.,* 1999). Solitarious locusts have a tendency to actively avoid one another (Ellis, 1959; Roffey and Popov, 1968; Roessingh *et al.,* 1993). The avoidance behaviour of the nymphs to the volatiles may have been an indication of nymphs undergoing solitarization during rearing. However, a preliminary experiment using an aluminium circular arena (Wiesel *et al.*, 1996; Malual *et al.*, 2001) had confirmed that the nymphs were gregarious.

On the other hand, the concentration of the volatiles may have triggered the avoidance by the nymphs as demonstrated in the desert locust *S. gregaria* by Rono *et al.* (2008). At a low concentration of PAN, the major component in the aggregation pheromone of adult desert locust, locusts stayed close to the release point while at high concentration, the locusts moved away from the source. To overcome this, a lower number of locusts (maximum of three nymphs) was used as a source of volatiles. However, the results showed that nymphs behaved like those in the control.

In the field, when environmental conditions are suitable, gregarious nymphal locusts form dense aggregations of marching hopper bands that eventually develop into swarms of flying adult locusts (Rainey, 1962; Roffey and Popov, 1968; Despland et *al*., 2000; Despland and Simpson, 2000). Under laboratory conditions, Roessingh *et al.* (1993)

showed that in a rectangular arena, crowd-reared *S.gregaria* nymphs moved toward the side of the arena where a group of *S. gregaria* nymphs were concealed. Hoste *et al.*, (2002) reported similar findings for the African migratory locust, *L. m. migratorioides*. Crowd-reared nymphs of *S. gregaria* were shown to strongly aggregate to their volatiles and to those from their faeces (Obeng-Ofori *et al.*, 1993; 1994). Further, Niassy *et al.*, (1999) found similar results for the nymphs of *L. m. migratorioides.* In the present study, the role played by volatiles in the aggregation behaviour of nymphal Malagasy migratory locust, *L. m. capito* was investigated for the first time. The results have shown a very different pattern of responses of the nymphs to their volatiles compared to nymphs of the locust species cited above. This may be attributed to the isolation of this locust on the island of Madagascar since it drifted away from mainland Africa 160 million years ago (Jacobs, 1997). For survival *L. m. capito* had to adapt to the different habitat and may thus have evolved to respond to a different set of specific stimuli in that environment, consequently making its behaviour different from that of other locusts.

In conclusion, the results of he behavioural tests showed that under laboratory conditions, although nymphs of *L. m. capito* detected their volatiles, they did not aggregate in response to them. Contrary, the nymphs preferred the side of the arena without the volatiles in the two-choice olfactometer. In view of these results, characterization and identification of chemical components in the volatiles from live

nymphs and their faeces was carried out in order to investigate the detailed responses of the nymphs to the synthetic blend and the constituent individual compounds. This was expected to help in determining which components elicited the avoidance behaviour in nymph locusts, which is the subject matter of the following Chapter.

# CHAPTER FIVE

# 5.0 IDENTIFICATION OF CHEMICAL COMPONENTS IN NYMPHAL VOLATILES

## 5.1 Introduction

Volatile emissions of locusts are complex and have been the target of research over the years. Due to its economic importance, *S. gregaria* was the subject of considerable interest and is so far the most studied locust species. Nymphs of *S. gregaria* have been reported to emit eight EAG-active compounds: hexanal, octanal, nonanal, decanal, hexanoic acid, octanoic acid, nonanoic acid and decanoic acid (Torto *et al.,* 1996; Niassy *et al.,* 1999). Weakly active compounds include undecanal, dodecanal, pentanoic acid, heptanoic acid, undecanoic acid and dodecanoic acid. Trace amounts of heptanal, anisole, benzaldehyde, acetophenone, veratrole were also recorded (Torto *et al.,* 1996). In contrast, nymphs of *L. m. migratorioides* were found to produce three EAG-active compounds, namely hexanoic acid, phenylacetonitrile (PAN) and an unidentified compound (Niassy *et al.,* 1999). Faecal volatiles of both species included guaiacol, phenol and indole, with the first two compounds being the predominant ones (Niassy *et al.,* 1999). Volatile emissions from *L. m. capito* have not been studied; hence, the role they may play in the chemical ecology of this locust remains unknown.

In this Chapter volatile emissions from live nymphs and from faeces were collected, analyzed and identified using GC and combined GC-MS. Coupled GC-EAD was used

to establish which compounds were detected by the sensory system of the nymphs. Retention time and EAG activity of each active compound was confirmed using authentic synthetic standards.

## 5.2 Materials and methods

### 5.2.1 Collection, analysis and identification of volatiles of *Locusta migratoria capito* nymphs

Airborne volatiles were collected following the procedure described in sections 3.3.2 and 3.3.3; then eluted as in section 3.3.4. One µl of the eluate containing 29.35ng of the internal standard was analyzed by capillary GC and GC-MS as in section 3.4.2, and 3.4.4 respectively. Electroantennographic detector analysis was conducted on antennae of three to four day-old fifth instar female and male nymphs. The protocol is described in section 3.4.3.

Each EAG-active peak was quantified on GC by comparing its percentage peak area to the internal standard.

### 5.2.2 Quantification of components in the nymphal volatiles

Only EAD-active compounds were quantified on GC using methyl salicylate as the internal standard as described in section 3.4.1. Amounts of volatiles from live nymphs were calculated from seven replicates while those from faeces were calculated from 4

replicates.

### 5.2.3 Data analysis

Means of the amounts of EAG-active components from fifth instar male and female nymphs were compared using independent group *t*-test on SAS (SAS Institute Inc., Cary, NC, USA. 2002-2003).

## 5.3 Results

### 5.3.1 GC analyses of volatiles from fifth instar nymphs

Chromatographic profile of the trapped volatiles indicated no discernable qualitative differences between fifth instar male and female nymphs (Fig. 5.1). The quantities of the various compounds were estimated in section 5.3.4. Each peak in the chromatogram indicates the presence of a distinct chemical compound in the volatile. The profile comprised of a series of peaks with the compound at a retention time of 13.4 min being the dominant one for both sexes.

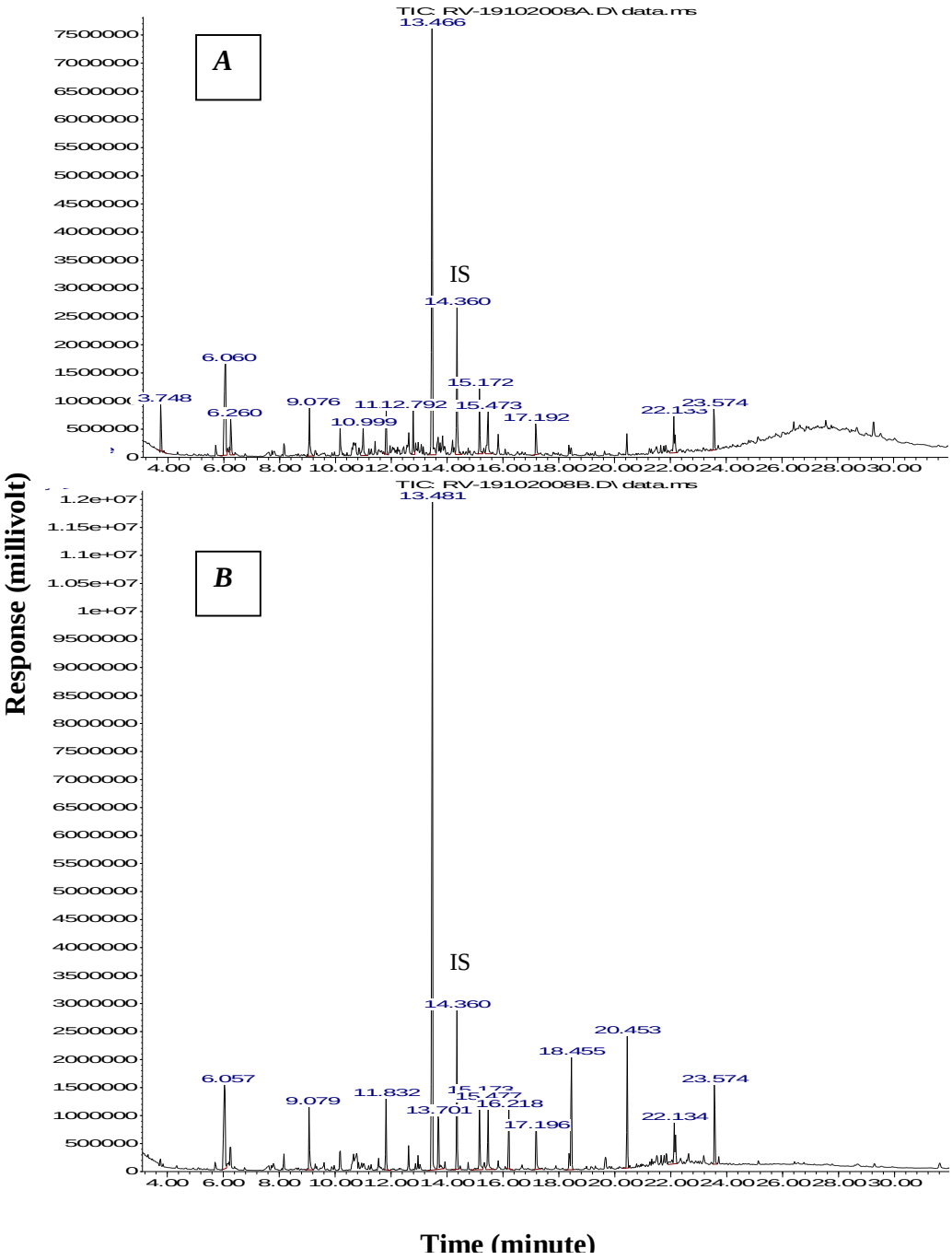



Figure 5.1: Representative chromatographic profiles of volatiles trapped from live fifth instar female (A) and male (B) nymphs. IS: internal standard.

### 5.3.2 GC-EAD analyses of the nymphal volatiles

Volatile collections from live fifth instar nymphs (Fig. 5.2) and faeces (Fig. 5.3) revealed a bouquet of seven and six EAG-active compounds respectively. Compounds represented by peaks 1, 7, and 9 were common to both live nymphs and faeces (Fig. 5.2, and 5.3). Compound peaks 2, 4, 6, 10 were specific to volatiles from live nymphs (Fig. 5.2), while peaks 3, 5, 8 were either found only in faecal volatiles or present in very low amounts in those from live nymphs (Fig. 5.2, and 5.3).

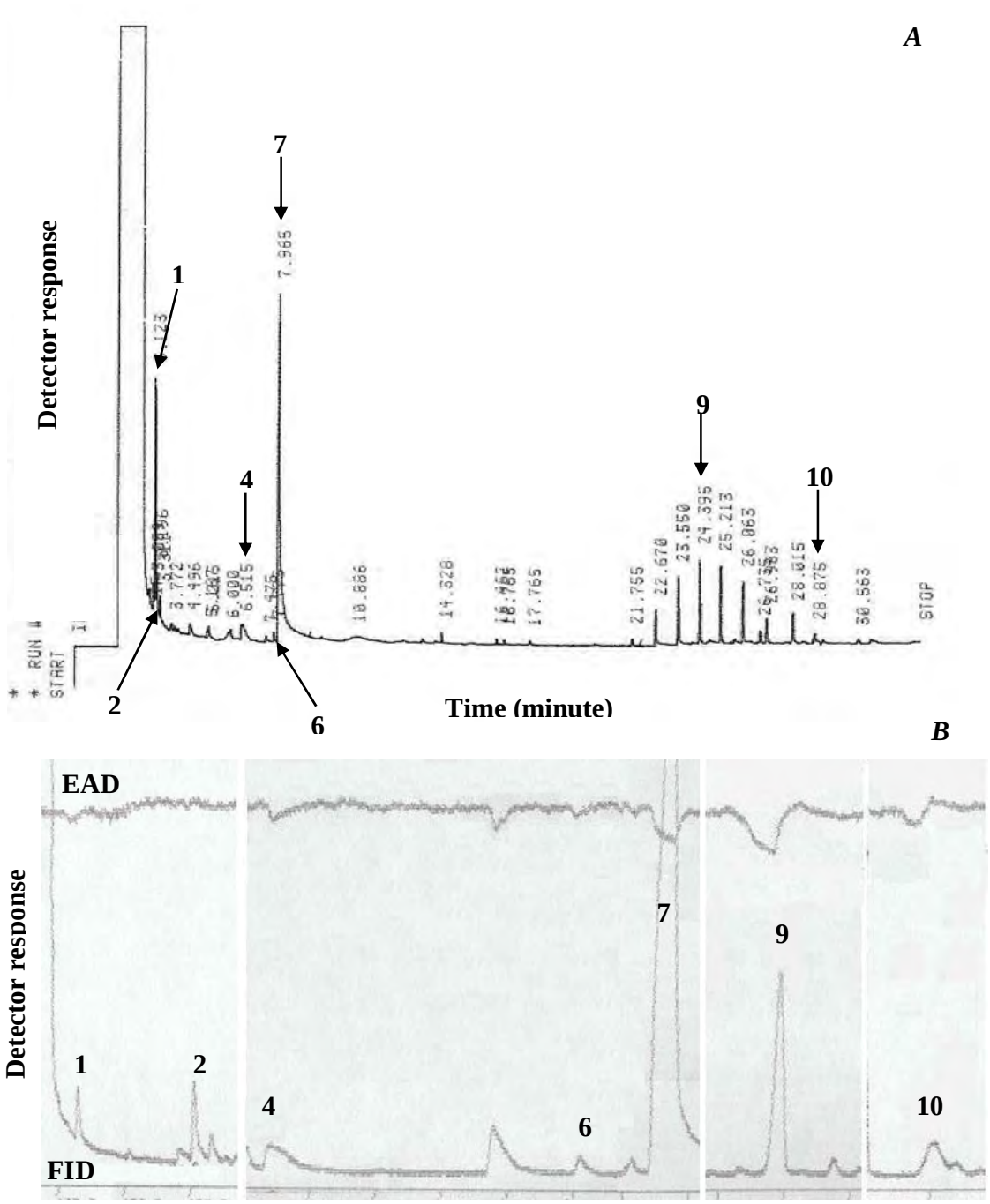



Figure 5.2: *A*, Representative GC profile showing EAG-active compound peaks (1-10) in volatiles from live fifth instar nymphs; *B*, EAD and FID signals in a GC-EAD recording.

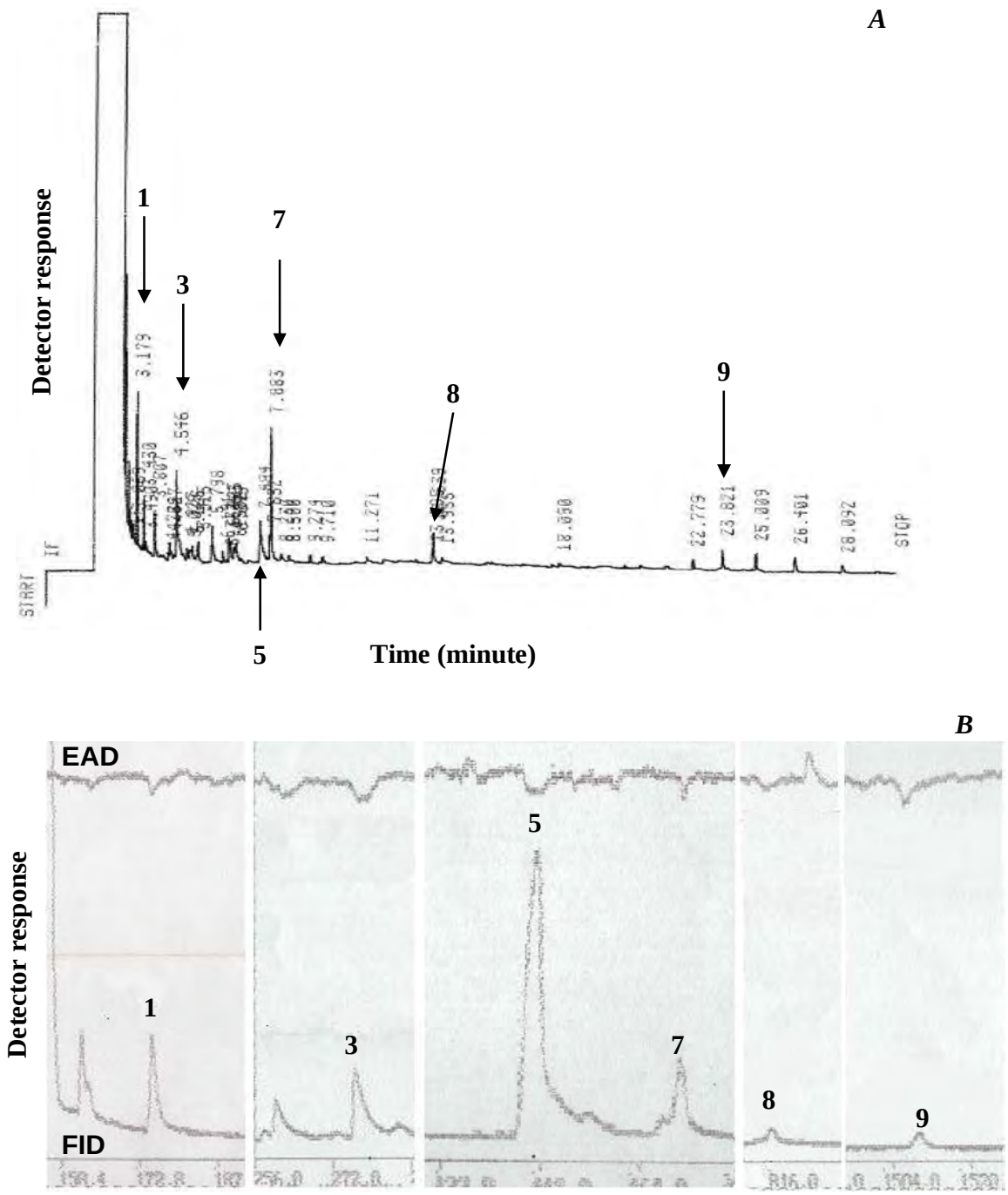



Figure 5.3: *A*, Representative GC profile showing EAG-active compound peaks (1-9) from fifth instar fresh faeces (3-5g); *B*, EAD and FID signals in GC-EAD recording.

### 5.3.3 GC-MS analysis of the nymphal volatiles

The GC-MS identification of EAG-active compounds in nymphal body and faecal volatiles labelled 1 to 8 gave mass spectra characteristic of the following:

1)-2,3-butanediol, m/z (rel, intensity) at 45.10 (100.00), 43.10 (13.58), 57.10 (12.59), 47.10 (6.64), 75.05 (4.61);

2)-Hexanal, m/z (rel, intensity) at 56.10 (100.00), 44.10 (94.55), 41.10 (81.47), 45.10 (80.32), 57.10 (72.83); 43.10 (61.94);

3)-Anisole, m/z (rel, intensity) at 108.00 (100.00), 78.00 (83.41), 65.00 (80.15), 39.10 (37.43), 51.00 (19.09), 93.10 (16.83),

4)-Benzylalcohol, m/z (rel, intensity) at 108.10 (100.00), 79.10 (98.92), 67.10 (70.04), 107.10 (69.87), 77.10 (58.77);

5)-Guaiacol, m/z (rel, intensity) at 109.00 (100.00), 124.10 (95.19), 81.10 (68.98), 53.10 (14.96), 52.10 (8.27);

6)-Nonanal, m/z (rel, intensity) at 57.10 (100.00), 41.10 (72.29), 43.10 (66.71), 56.10 (62.79), 55.10 (58.62);

7)-Phenylacetonitrile, m/z (rel. intensity) at 117.10 (100.00), 90.10 (40.89), 116 (40.41), 89.10 (24.34), 118.10 (8.6);

8)-Beta-ionone, m/z (rel, intensity) at 177.10 (100.00); 123.10 (40.68), 43.10 (26.65), 135.10 (14.82), 178.10 (13.04).

Compounds 9 and 10 were not resolved sufficiently for characterization and could not be identified. Fig. 5.4 represents the structure of the identified compounds.

Combined GC-EAD recording using synthetic compounds confirmed the retention times and the EAG activities observed with the components in the crude volatile collection.

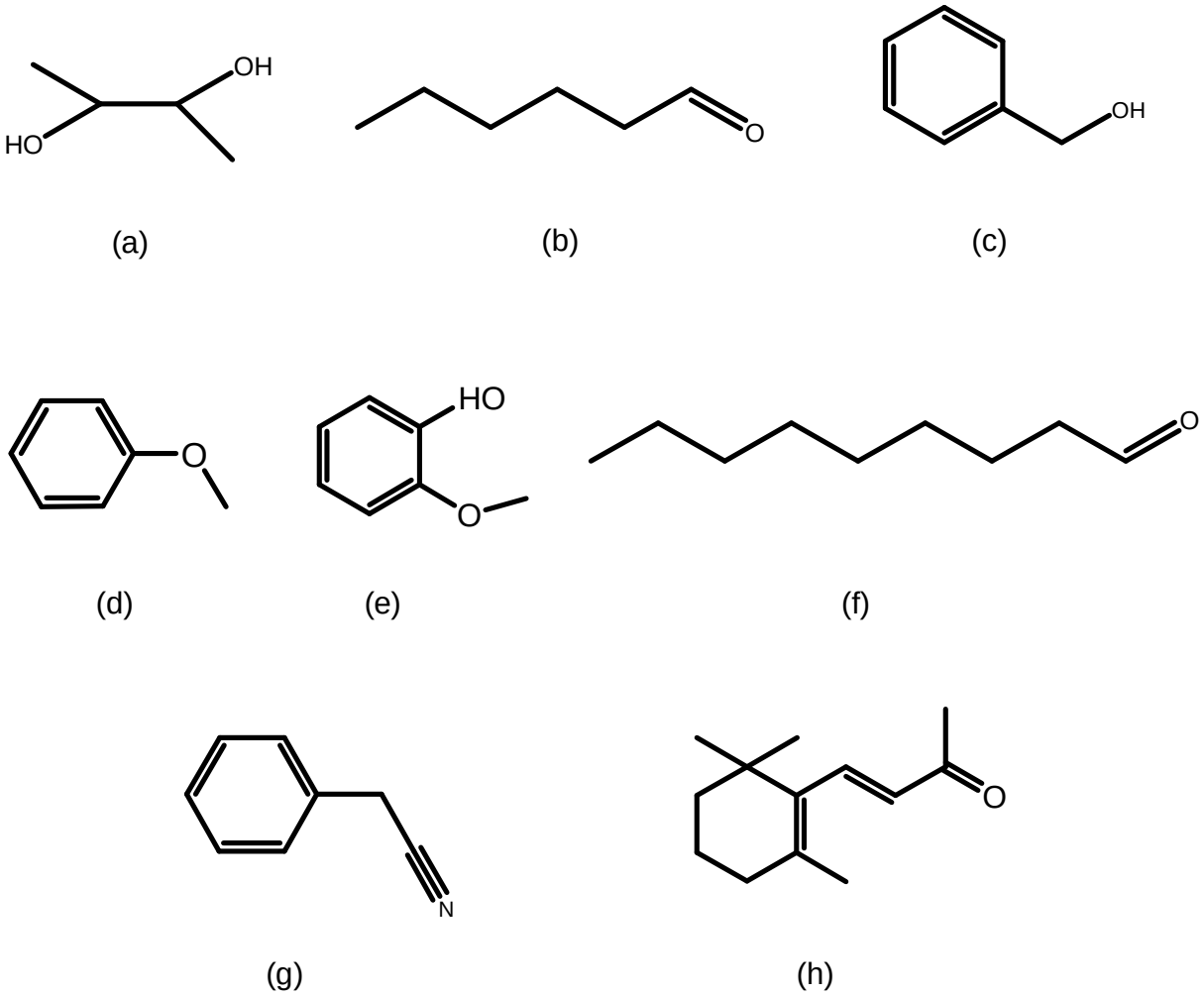



Figure 5.4: Structures of the EAG-active compounds in the volatile emission of the fifth instar nymphs and nymphal faeces of *Locusta migratoria capito*: (a) 2,3-butanediol, (b) hexanal, (c) benzyl alcohol, (d) anisole, (e) guaiacol, (f) nonanal, (g) phenylacetonitrile, (h) β-ionone.

### 5.3.4 Quantification of the nymphal volatiles

It was noticed that the relative amount and the occurrence or absence of compounds in the volatiles vary more or less with the density of nymphs in the rearing cage from which the trappings were done. Of the eight compounds, 2,3-butanediol, and PAN were common to live nymphs and faeces; hexanal, benzyl alcohol and nonanal were found only from live nymphs while anisole and guaiacol were specific to the faecal volatiles.

With regard to the volatiles from live nymphs, no quantitative difference was found for the volatiles collected from the fifth instar male and female nymphs ($t$ = 0.23, df = 6, $P$ = 0.8243; $t$ = -0.39, df = 8, $P$ = 0.7083; $t$ = -0.95, df = 12, $P$ = 0.3618; $t$ = -0.35, df = 5, $P$ = 0.7387; $t$ = -0.61, df = 12 $P$ = 0.5508 for 2,3-butanediol, hexanal, benzyl alcohol, nonanal and PAN respectively). PAN constituted the major component of the volatiles from the live nymphs. Its amount was at least two times higher than the other compounds whereas guaiacol was the major component in the faecal volatiles, followed by PAN and β-ionone (Table 5.1).

Table 5.1: Mean (±se) of amount of each compound identified in the crude volatiles of fifth instar nymphs of *Locusta migratoria capito*.

| **Name of the compound** | **Amount/insect/hr (ng)** | | **Amount from 3g to 5g of faeces/hr (mean ±se) (ng)** |
|---|---|---|---|
| | **Males (mean ±se)** | **Females (mean ±se)** | |
| 2,3-butanediol ***BF*** | 0.03 ± 0.02 (a) | 0.040 ± 0.028 (a) | 0.419 ± 0.282 |
| Hexanal ***B*** | 0.04 ± 0.03 (a) | 0.025 ± 0.013 (a) | |
| Anisole ; methoxybenzene ***F*** | | | 0.541 ± 0.166 |
| Benzyl alcohol ***B*** | 0.04 ± 0.02 (a) | 0.022 ± 0.01 (a) | |
| Guaiacol : 2-methoxyphenol ***F*** | | | 8.145 ± 2.967 |
| Nonanal ***B*** | 0.01 ± 0.01 (a) | 0.011 ± 0.005 (a) | |
| PAN *BF* | 0.09 ± 0.03 (a) | 0.067 ± 0.025 (a) | 1.134 ± 0.466 |
| Beta ionone ***F*** | | | 1.072 ± 0.282 |

For males and females, means in the same row followed by the same letter are not significantly different. Student's *t*-test; $\alpha = 0.05$.

***BF*** Compounds common to live nymphs and faeces

***B*** Compounds from live nymphs alone

*F* Compounds from faeces alone

## 5.4 Discussion

Both male and female nymphs of *L. m. capito* produce varying amounts of 2,3-butanediol, hexanal, benzyl alcohol, nonanal and PAN in their volatiles. The results showed no sexual differences in the production and composition of volatiles. This is the main reason why fifth instar nymphs had the same level of responsiveness when they were exposed to volatiles from either male or female nymph conspecifics (Figs. 4.2 and 4.4). The results also imply that, from the biological point of view, there was no behavioural difference between male and female nymphs. Thus, any changes that may lead to sexual differentiation in the behavioural repertoire of adults of *L. m. capito* occur during or after fledging of nymphs into adult locusts.

Among the constituent compounds in the volatiles of *L. m. capito* nymphs, 2,3-butanediol has not been reported previously in the volatile collections of gregarious locusts. Phenylacetonitrile (PAN) had previously been found to occur in volatiles of nymphs of the African migratory locust, *L. m. migratotrioides* (Niassy *et al.*, 1999). In addition, PAN is the major component in the volatiles of crowd-reared mature adult males of the desert locust *S. gregaria* and it is also present in substantial amounts in their faecal odours (Obeng-Ofori *et al*., 1994a, Torto *et al*., 1994; Deng *et al*., 1995; Mahamat *et al*., 2000; Seidelmann *et al*., 2000). Hexanal and nonanal were reported to be present in volatiles of nymphal *S. gregaria* while anisole is a component from the volatiles of both sexes of adults of this locust (Torto *et al*., 1994; 1996). The other components, *viz.*

hexanal, nonanal, benzyl alcohol and beta ionone were reported to occur in faecal volatiles of adults of the oriental migratory locust, *L. m. manilensis* (Yu *et al.,* 2007). Guaiacol is a major component of faecal volatiles of all developmental stages of *L. m. migratorioides* and *S. gregaria* (Torto *et al.,* 1994, 1996; Niassy *et al*., 1999).

It is noteworthy that in *S. gregaria,* anisole was produced by nymphs and adults of both sexes (Torto *et al.,* 1994; 1996) whereas in *L. m. capito,* it was only found in the volatiles from nymphal faeces. In addition, in *L. m. capito,* benzyl alcohol was found in volatiles from the live nymphs, while in *L. m. manilensis,* it was collected from faeces (Yu *et al.,* 2007).

These differences imply that, in the different species of locusts, anisole and benzyl alcohol may be produced using the same metabolic pathway, however they are excreted through different sites. Hence, these two compounds may play very different roles in the biology and behaviour of *L. m. capito* and the other two species of locusts.

The presence of PAN in the faecal volatiles has already raised some questions since the work of Seidelmann *et al.* (2003) who found that, in mature *S. gregaria* adult male, the major release sites of PAN were on the tibia and tarsi of hind legs and the fore and hind wings. The wings were reported not only to be the site of release but also the site of production (Seidelmann *et al.,* 2003). Pener and Simpson (2009) suggested that,

cannibalism may be a possible cause of the presence of PAN in the faeces. However, in *L. m. capito*, PAN may play a totally different role and the mechanism of production and emission might be different from those in the desert locust. This remains a subject of further investigation.

Chemical compounds that evoke electroantennographic (EAG) activity in the antennae of insects in general may be involved in different biological process in the life of a given insect. In the locust, such compounds may be involved in different kinds of communication between nymphs, play a role outwardly as a defence mechanism or in the immune system, or in any other biological processes in the insect. Thus, it was necessary to evaluate the role played by the blend of synthetics of the identified constituent compounds and the single compounds in the aggregation behaviour of fifth instar nymphs, which was the objective of the next Chapter.

# CHAPTER SIX

## 6.0 EFFECT OF THE SYNTHETICS OF THE IDENTIFIED COMPOUNDS ON THE AGGREGATION BEHAVIOUR OF FIFTH INSTAR NYMPHS

### 6.1 Introduction

The EAG-active compounds may play different roles in the biology of the locust and not all of them may play a role in medition of aggregation. For example, in adult desert locust *S. gregaria*, veratrole and acetophenone were behaviourally active volatiles produced by mature females and were responsible for their oviposition aggregation (Rai *et al.*, 1997). A blend of the constituents in volatiles of mature male (phenylacetonitrile (PAN), benzaldehyde, veratrole, anisole, guaiacol and phenol) elicited high aggregation responses in both sexes of young and mature adults but not in nymphs (Torto *et al.*, 1994). When chemicals were presented singly, PAN evoked aggregation responses from the locusts almost comparable to those of the blend of six electrophysiologically active components (Torto *et al.*, 1994). Guaiacol, phenol and benzaldehyde were moderately effective whereas anisole and veratrole were inactive. Similar observation has been reported for the nymphs of *S. gregaria*. Eight electrophysiologically active compounds were identified consisting of hexanal, octanal nonanal, decanal and their corresponding acids. Although nymphs responded strongly to the full synthetic blend, individual aldehydes did not elicit any significant activity (Torto *et al.*, 1996). However, addition of guaiacol and phenol to a blend of four acids and four aldehydes substantially enhanced the aggregation responses of the nymphs (Torto *et al.*, 1996).

In this Chapter, the release rates of the synthetic-equivalents of the active compounds found in the previous Chapter were first calculated. Then, the synthetic blend was tested in the bioassay to determine the role of the identified compounds on the aggregation behaviour of *L. m. capito* nymphs.

## 6.2 Materials and methods

### 6.2.1 Calibration of the dispenser and calculation of the release rate for each synthetic compound

Four different volumes of each synthetic compound were used to calculate the release rate: 1µl, 2µl, 4µl, and 8µl; each was dissolved in a 0.5ml of light paraffin oil (Merck) held in a 1ml U-bottomed microcuve (KART 730 VWR International).The latter was the dispenser and was used only once. Each dispenser was put inside a 50 cm quick fit glass tube and connected to the trapping system (Plate 3.3). Compounds released from the dispenser were collected for 4 hours using the same adsorbent as in section 3.3.1, and then eluted (section 3.3.4), analysed and quantified on GC.

### 6.2.2 Effect of synthetic blend on the aggregation response of fifth instar nymphs of *Locusta migratoria capito*

The test was carried out in the single-chamber olfactometer previously used for testing crude volatiles from live nymphs and faeces (section 3.2.1). Locusts were provided with

a choice between an odour-free control air column and an air column permeated with volatiles. The control was comprised of 0.5ml of paraffin oil in a 1ml dispenser while a synthetic compound provided the volatiles. Instead of the round-bottomed flasks for holding sources of volatiles (Fig. 3.1), quick-fit glass tubes (50 cm long) contained the stimuli. Five sets of 10 fifth instar females and 10 fifth instar males were separately tested with each of the treatments in table 6.1.

Table 6.1 Treatments carried out to determine the responses of fifth instar nymphs of *Locusta migratoria capito* to different blends of synthetic compounds. Treatment (A): equivalent synthetic blend of volatiles from live fifth instar nymphs. Treatment (B): equivalent synthetic blend of faecal volatiles

| **Treatments (A)** | **Composition of the blend** | **Release rate (ng/h)** |
|---|---|---|
| 1 | 2,3-butanediol, hexanal, benzyl alcohol, nonanal and PAN *(B)* | 0.33: 0.31: 0.39: 0.13: 0.80 |
| 2 | 2,3-butanediol, hexanal, benzyl alcohol, nonanal *(B-P)* | 0.29: 0.40: 0.30: 0.21 |
| 3 | 2,3-butanediol, hexanal, nonanal *(BHN)* | 0.1: 0.4: 0.2 |
| 4 | Benzyl alcohol *(Boh)* | 0.41 |

| Treatments (B) | Composition of the blend | Release rate (ng/h) |
|---|---|---|
| 5 | 2,3-butanediol, anisole, guaiacol, PAN, β-ionone *(F)* | 0.64: 0.73: 7.67: 1.36: 0.9 |
| 6 | 2,3-butanediol, anisole, guaiacol, β-ionone *(F-P)* | 0.41: 0.7: 7.98: 1.4 |
| 7 | 2,3-butanediol, guaiacol, PAN, β-ionone *(F-A)* | 0.7: 9.72: 2.5: 1.4 |
| 8 | 2,3-butanediol, anisole, PAN, β-ionone *(F-G)* | 0.34: 0.7: 2.01: 1.0 |
| 9 | Anisole, guaiacol, β-ionone (*F-B)* | 0.35: 0.8: 9.10: 1.10 |
| 10 | Anisole, guaiacol, β-ionone (*AGB)* | 0.7: 7.01: 0.8 |
| 11 | β-ionone (*βI)* | 1.3 |
| 12 | Guaiacol *(G)* | 8.02 |
| 13 | Anisole (*A)* | 0.6 |
| 14 | PAN *(P)* | 1.32 |
| 15 | Guaiacol and PAN *(PG)* | 8.10: 1.34 |

The release rate ratios in treatments 1) to 4) and 5) to 15) corresponded approximately to the relative amount of volatiles released by ten locusts (ng/hour), and to the relative amount of volatiles released from 3 to 5g (ng/hour) of fresh faeces respectively.

Treatment 1) was the equivalent to the crude volatiles from 10 live locusts. Treatment 2) corresponded to the crude volatiles of live locusts from which PAN was not included. Treatment 3) corresponded to the crude volatiles of live nymphs from which PAN and benzyl alcohol were not included.

Treatment 5) was the equivalent of the crude volatiles from faeces. Treatments 6) to 9) corresponded to the faecal volatiles from which PAN, anisole, guaiacol, and β-ionone were not included in the blend, respectively.

The number of insects in each side of the chamber was counted after every five minutes and the aggregation index calculated as in section 4.2.1. Each test was replicated ten times. A blank control (paraffin oil against empty quick-fit) was run every day to check the reliability of the system.

### 6.3.3 Data analysis

For calibration of the dispenser, the mean release rate was calculated using an equation generated from the correlation between the volume of synthetic compound and the amount of volatiles emitted.

Data on the aggregation responses of fifth instar nymphs over the sources of volatiles were subjected to SNK test ( $\alpha$= 0.05) using SAS (SAS Institute Inc., Cary, NC, USA. 2002-2003).

## 6.3 Results

### 6.3.1 Release rate of the standards

The correlation volume and volatiles emitted showed that the synthetic compounds can be classified into two groups according to their release rates: high volatility compounds including PAN, guaiacol, benzyl alcohol and anisole (Fig. 6.1 A) and relatively low volatility compounds including 2,3-butanediol, hexanal, nonanal, and β-ionone (Fig. 6.1 B). Equations generated from that correlation were used to calculate the amounts of synthetic compounds needed in the bioassay.

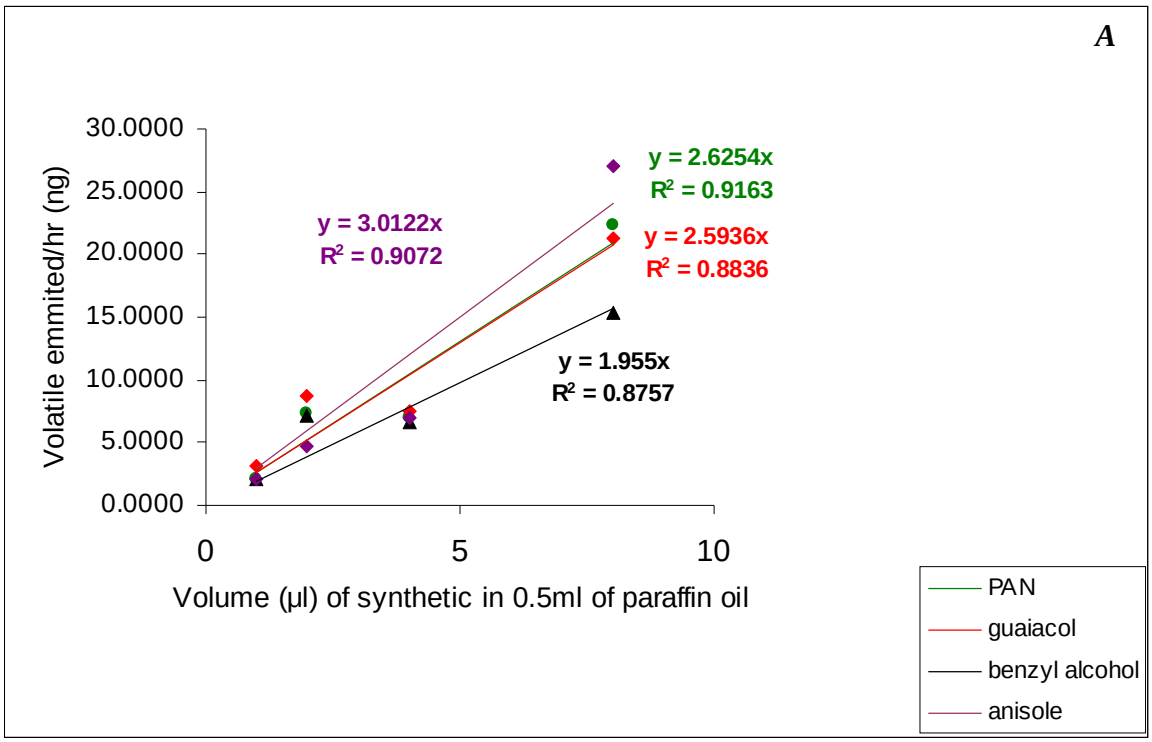



Figure 6.1 (A): Correlation plots of volume vs. amount released by each of the synthetic standards of high volatility compounds tested.

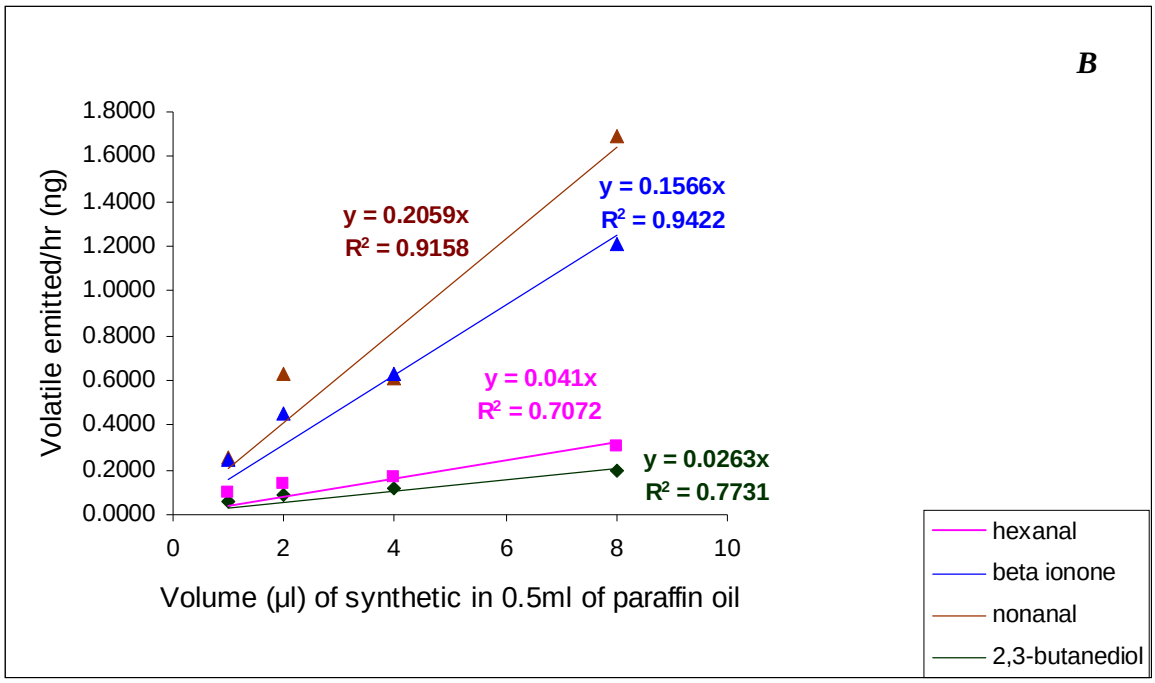



Figure 6.1 (B): Correlation plots volume vs. amount released by each of the synthetic standards of low volatility compounds tested.

### 6.3.2 Responses of fifth instar nymphs to the equivalent synthetic blend of live fifth instar nymph volatiles

Aggregation responses of fifth instar male and female nymphs to the different sources of volatiles were significantly different ($F_{(3,14)}$ = 6.93, $P$ = 0.0043 and $F_{(3,13)}$ = 26.87, $P$ = 0.0001 respectively). Fifth instar male and female nymphs had similar aggregation responses to the synthetic blend (Fig. 6.2). Both sexes avoided the side of the chamber that was permeated the full blend of the components, which was in agreement with the

pattern of responses previously reported in section 4.3.2. When PAN was excluded from the blend, a weak aggregation response was observed from both male and female nymphs. Benzyl alcohol tested singly elicited the strongest aggregation from fifth instar nymphs. While the pattern of the response from fifth instar male and female nymphs was similar, a slight variation was observed on the response to the blend of 2,3-butanediol, hexanal and nonanal. The blend of the three compounds had no effect on the fifth instar males whereas it repelled the fifth instar females, though the response was not comparable to that elicited by the full blend.

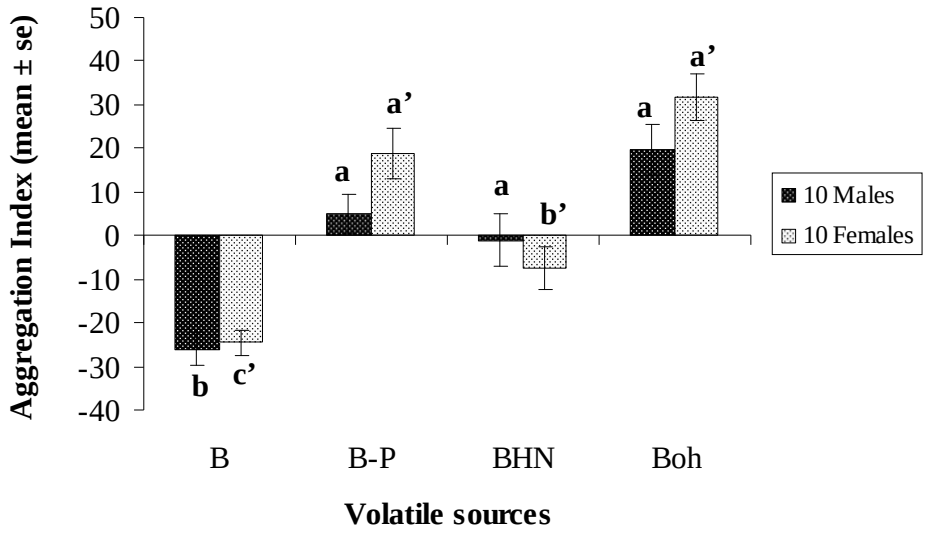



Figure 6.2: Aggregation responses of fifth instar *Locusta migratoria capito* nymphs to the equivalent synthetic blend of nymphal volatiles. Bars with different letters for the same sex were significantly different at $\alpha = 0.05$ (SNK test).

B: Full blend of volatiles from live insect: 2,3-butanediol + PAN + hexanal + benzyl alcohol + nonanal;

B-P: Full blend without PAN;

BHN: 2,3-butanediol, hexanal, nonanal combined;

Boh: Benzyl alcohol.

### 6.3.3 Responses of fifth instar nymphs to the equivalent synthetic blend of faecal volatiles.

Like the previous results, responses of the nymphs to different combinations of the synthetic compounds were significantly different ($F_{(10,39)} = 6.61$, $P = 0.0001$ and $F_{(10,39)} = 14.07$, $P = 0.0001$ respectively for male and female). The results also confirmed that, there were slight variations in the levels of responsiveness to the various compounds between fifth instar males and their female conspecifics. This was the case when both sexes were exposed to guaiacol. The pattern of the response seems to indicate that, fifth instar males are more sensitive to the volatiles compared to the fifth instar females. The fifth instar males detected five different compounds in the volatiles among the eleven tested, whereas the females detected only four.

Sequential exclusion of PAN, anisole, guaiacol and beta ionone from the blend, one compound at a time led to an increase in responsiveness of the locusts. Nymphs had a weak response to a blend of anisole, guaiacol and beta ionone. Of the individual compounds, the nymphs responded to beta ionone and anisole similar to the blend of the two and gauaicol. Response of the nymphs to guaiacol was the weakest. Aggregation responses by both male and female nymphs to PAN were relatively strong. However, a blend of PAN and guaiacol significantly repelled the nymphs, in particular, the females.

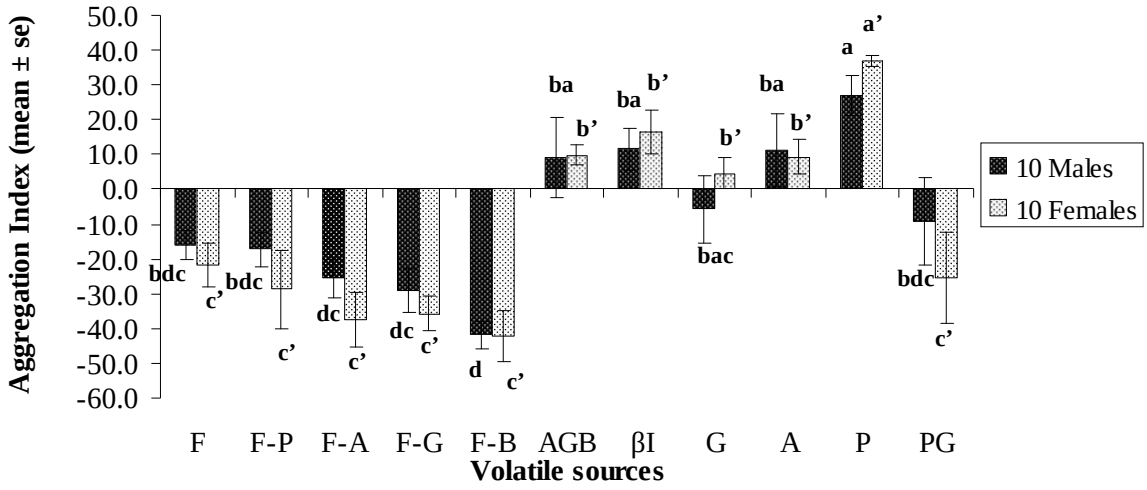



Figure 6.3: Aggregation responses of fifth instar *Locusta migratoria capito* nymphs to the equivalent synthetic blend of compounds in faecal volatiles. Bars with different letters for the same sex were significantly different at $\alpha = 0.05$ (SNK test).

F: Full blend from faeces: 2,3-butanediol + pan + anisole + guaiacol + β-ionone;

F-P: Full blend without PAN;

F-A: Full blend without anisole;

F-G: Full blend without guaiacol;

F-B: Full blend without β-ionone;

AGB: Anisole, gauiacol, β-ionone combined;

βI: β-ionone;

G: Guaiacol;

A: Anisole;

P: PAN;

PG: Blend of PAN and guaiacol.

## 6.4 Discussion

In all the olfactometric tests, there was no sexual difference in the response of fifth instar nymphs to the synthetic blends. The responses of fifth instar nymphs to the equivalent synthetic blend of nymphal volatiles seem to indicate that volatiles of live nymphs comprised of two groups of compounds which play antagonistic roles in their aggregation behaviour. When PAN was not included in the blend, nymphs aggregated weakly to the blend. This indicates that PAN might act as a repellent at the concentration tested. Response of fifth instar nymphs to the blend without PAN was similar to that obtained for the nymphs to their own volatiles when they were reared together with adults. This may suggest a variation in the composition of volatiles from the nymphs reared together with or separate from their adult locust conspecifics. The nymphs avoided the side of the arena permeated with the blend when PAN and benzyl alcohol were not included suggesting that 2,3-butanediol, hexanal and nonanal might also play a role in repelling the nymphs and that benzyl alcohol countered the repellent effect. The level of the response when PAN was in the blend suggested a possible additive effect of the repellents. Of the five components identified to be part of the volatiles of live nymphs, benzyl alcohol was the most efficient in aggregating the nymphs in the olfactometer.

The second set of tests using the equivalent faecal volatiles confirmed the previous results. Single compounds and their blends influenced the behaviour of the nymphs

differently. The similarity as stated above can be highlighted concerning the response of fifth instar nymphs to the blend of anisole, guaiacol and beta-ionone and the response of fifth instar to volatiles from fresh faeces. In particular, PAN elicited a weak aggregation response when it was tested alone while its blend with guaiacol was repellent and fifth instar nymphs hardly responded to the latter. This result seems to indicate that the presence of other compounds in the blend weaken the aggregation response to PAN. Therefore, increasing concentration of PAN in the blend might completely change the nature of the response. The same observation was evident when comparing the response of fifth instar nymphs to other blends and the individual compounds excluded. The highest repellency was obtained when anisole, guaiacol and beta ionone were each excluded from the blend. Consequently, while it may have been expected that the combination of these three compounds would have elicited strong aggregation in the nymphs, this was not the case. These results suggest that the nymphs of *L. m. capito* detected the blend as a single unique cue. Hence, it is possible that any variation in the composition of the volatiles shifted the response of the nymphs from one extreme to another. Therefore, the discrepancies in the results reported in Chapter four of this thesis in which live nymphs were used as the source of volatiles, when the adults and nymphs were reared together and the difference of the response to that of the nymphs reared separated from adults locusts can be attributed to a possible variation in the production of volatiles. In such a case, volatiles produced by the adult locusts may influence the amounts of volatiles produced by the nymphs by inhibiting or enhancing the secretion

and/or the release of certain compounds.

While *L. m. capito* and *S. gregaria* share some of the components in their volatiles, the aggregation responses of these two locusts to those components contrast in many ways. The lack of responsiveness of nymphs of both sexes of *L. m. capito* to guaiacol contrasts to the fact that this compound is a strong component of the aggregation pheromone of *S. gregaria* nymphs (Obeng-Ofori *et al.*, 1994a; Torto *et al.*, 1996). In *L. m. capito*, guaiacol on its own had no effect on the behaviour of the nymphs. This suggests a different role for this compound in the biology of the nymphs of *L. m. capito* as it is the dominant compound in their faecal volatiles. Further, while nymphs of *L. m. capito* showed positive responsiveness to PAN, those nymphs of *S. gregaria* did not aggregate to it (Obeng-Ofori *et al.*, 1994a; Torto *et al.*, 1994; Ochieng, 1997) and PAN is a strong dispersant of *S. gregaria* hopper bands in the field (Kane, 2004). This difference in behaviour in the two species of locusts may be due to the fact that PAN is the key component of the aggregation pheromone of adult *S. gregaria* that is not produced by their nymphal conspecifics.

# CHAPTER SEVEN

# 7.0 EFFECTS OF THE NYMPHAL PHEROMONE ON SEXUAL MATURATION OF IMMATURE CONSPECIFIC *LOCUSTA MIGRATORIA CAPITO* ADULTS

## 7.1 Introduction

Sexual maturation time is defined as the developmental time elapsing between the final moult (fledging) in the locust to the sexually mature adult stage, first copulation and first oviposition in the female (Pener and Simpson, 2009). Various experimental studies have indicated that pheromones are also involved in the sexual maturation of locusts. In *S. gregaria* sexual maturation has been shown to be accelerated in the presence of volatiles from conspecific sexually mature adults (Norris, 1954; Loher, 1960; Richard and El Mangouri, 1968; Amerasinghe, 1978; Mahamat *et al.,* 1997). In contrast, nymphal volatiles have been reported to delay the maturation of young adults (Assad *et al.,* 1997). Under field conditions, it was interpreted that these counteracting effects resulted in synchronization of sexual maturation of the gregarious adults. The inhibitory effect of the nymphal pheromone tends to delay maturation of the newly fledged adult until the majority of the fifth instar nymphs have moulted into adult and then, the entire group mature together.

Different observations have been reported for the African migratory locust, *L. m. migratorioides*. Norris (1950) and Pener (1976) noted that, solitarious locusts mature

faster than those in the gregarious phase. Loher (1990) reported that, young males normally copulated with females of their own age 17 - 25 days after fledging and that it took 13 - 14 days in the presence of mature males. The objective of this study was to determine the possible factors, if any, that may influence the sexual maturation of young adults of *L. m. capito*. Of particular interest was whether the nymphal volatiles play a role in this regard.

## 7.2 Materials and methods

Sexual maturation was monitored by recording the time immature adults started mating and the time gravid females started laying egg pods.

Two types of experiments were carried out. The first experiment involved exposure of the immature adult locusts to fifth instar nymphs while in the second experiment, the influence of volatiles from mature adults on the maturation of immature adults was investigated.

### 7.2.1 Effect of fifth instar nymphs on sexual maturation of immature adults

Male and female fifth instar nymphs were used as stimuli source while newly moulted immature adult males and females were the recipients (test). Treatments are shown in Table 7.1 below. Standard aluminium cages (Plate 1.1) were used for tests involving olfactory, tactile and visual contact between recipient locusts and signal sources. The effect of nymphal volatiles alone was tested using the double-storey cage (Plate 1.2). For

the latter, locusts used as source of volatiles were placed in either compartment.

Table 7.1: Treatments used to test the effect of nymphs on sexual maturation of conspecific immature adult locusts.

| | **Recipient (test) locusts** | **Signal Sources** |
|---|---|---|
| **Control** | 2 immature males<br>+<br>2 immature females | 2 immature males<br>+<br>2 immature females |
| **Test** | 2 immature males<br>+<br>2 immature females | 2 fifth instar males<br>+<br>2 fifth instar females |

For each treatment, the number of mating pairs and the age at which gravid females started ovipositing were recorded daily. The number of egg pods laid was counted and the laying cups changed every day. Each pair of recipient insects was considered as a replicate and all tests were replicated fifty times.

### 7.2.2 Data analysis

Data were subjected to Student's *t*-test for the comparison of means using SAS (SAS Institute Inc., Cary, NC, USA. 2002-2003). Percentages were arcsine-transformed prior to analysis.

## 7.3 Results

Preliminary tests with *L. m. capito* had shown that, more than eight insects in the cage (four/four for the double storey cage) increased their mortality.

### 7.3.1 Effect of *Locusta migratoria capito* nymphs on sexual maturation of fledgling conspecifics

In the presence of contact, visual and olfactory stimuli originating from fifth instar nymphs, significant delays were observed in sexual maturation of the immature adults. The copulation started 24 – 26 days after fledging (Fig. 7.1); which was about six days after the control ($t$ = -3.13; df = 87; $P$ = 0.0024). On the other hand, the first oviposition of the females was delayed by about four days. However, it was not significantly different from the control ($t$ = -1.42; df = 34; $P$ = 0.1643). The percentage of mating and ovipositing females (Fig. 7.2) were significantly reduced by 40 % ($t$ = 2.34; df = 52.6; $P$ = 0.0233) and 44 % ($t$ = 2.18; df = 29; $P$ = 0.0374) respectively.

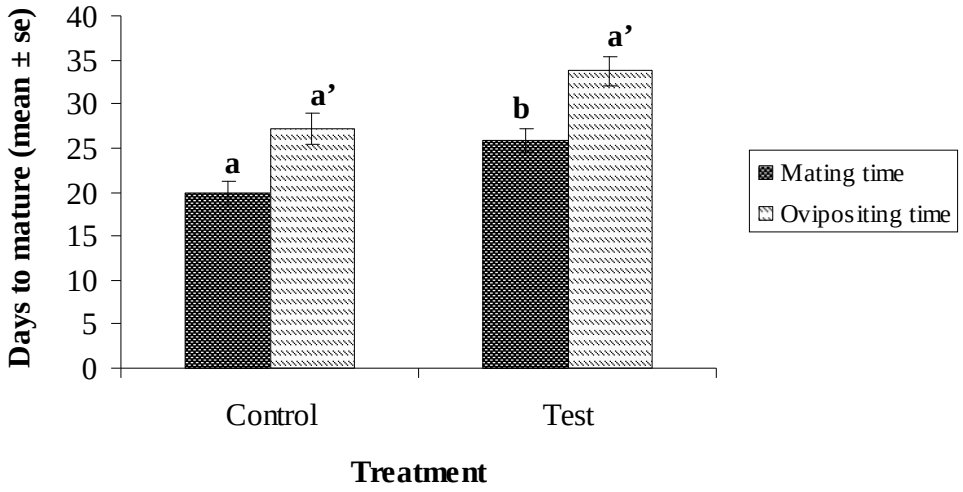



Figure 7.1: Time taken by immature adults of *Locusta migratoria capito* exposed to fifth instar nymphs to mature as assessed by copulation and oviposition. Bars with same letters for the same category are not significantly different. Student's *t*-test; $\alpha = 0.05$.

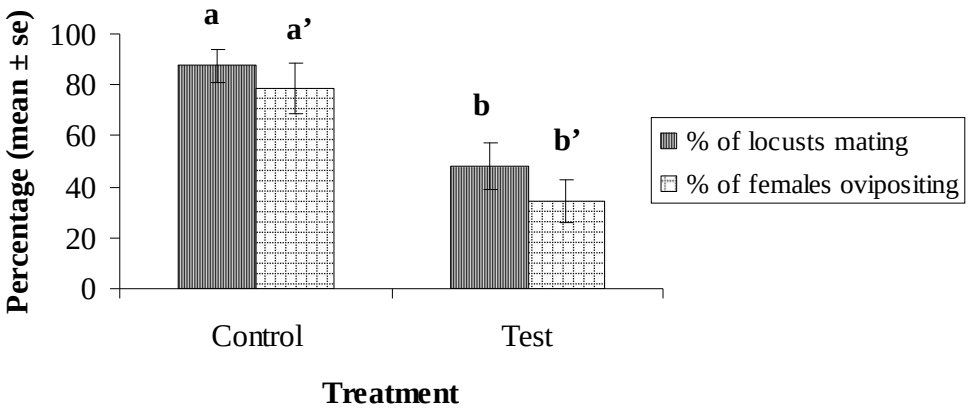



Figure 7.2: Percentage of new fledglings of *Locusta migratoria capito* that matured when exposed to fifth instar nymphs. Bars with same letters for the same category are not significantly different. Student's *t*-test; $\alpha = 0.05$.

### 7.3.2 Effect of nymphal volatiles of *Locusta migratoria capito* on sexual maturation of conspecific fledglings

Volatiles of fith-instar nymphs alone did not influence the maturation of immature adults (Fig. 7.3). Time taken for the immature adults to start copulating when exposed to volatiles was not significantly different from that of the non-exposed ones ($t$ = -0.28; df = 52; $P$ = 0.7837). Both sets of locusts took twenty one days after the final moult to start copulating. However, the oviposition of the exposed immature adult locusts was delayed by two days, and was not significant from that of the non-exposed ones ($t$ = -0.74; df = 26; $P$ = 0.4638) (Fig. 7.3). Unlike the previous experiments, percentages of mature insects were almost the same in the control and test, a dropped by 2% in the number of copulating pairs ($t$ = 0.25; df = 33; $P$ = 0.8028) and 1% of those ovipositing ($t$ = -0.05; df = 21; $P$ = 0.9613) was observed in the insects exposed to volatiles.

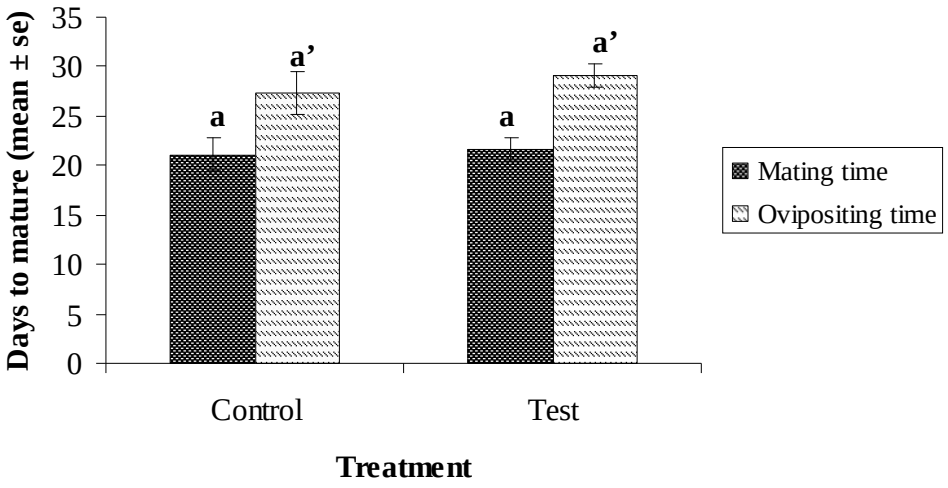



Figure 7.3: Time taken by immature adults exposed to nymphal volatiles to mature as assessed by copulation and oviposition time. Bars with same letters for the same category are not significantly different. Student's *t*-test; $\alpha = 0.05$.

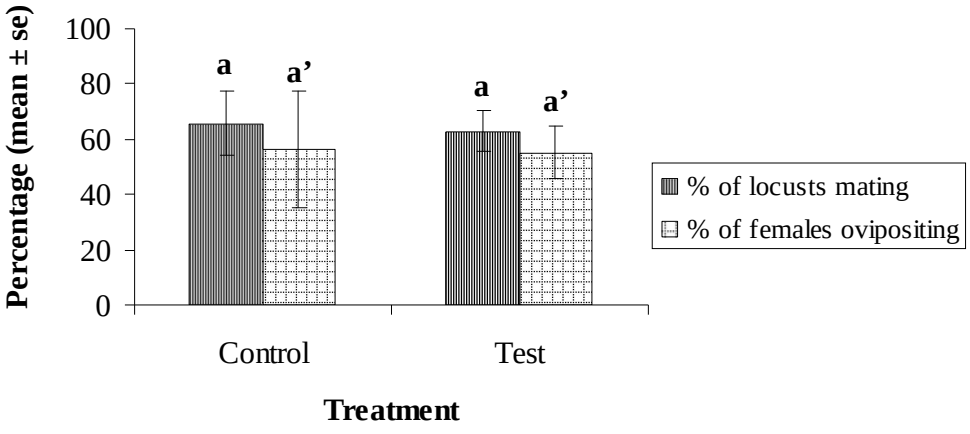



Figure 7.4: Percentage of new fledglings that matured while exposed to fifth instar nymph volatiles. Bars with same letters for the same category are not significantly different. Student's *t*-test; $\alpha = 0.05$.

## 7.4 Discussion

The results of this chapter show that, gregarious fifth instar nymphs of *L. m. capito* had a retarding effect on the sexual maturation of conspecific immature adults with regard to the number of days taken by the immature adults to attain reproductive age. The presence of nymphs in the same cage with immature locusts also significantly reduced the number of young adults reaching sexual maturity. However, in contrast to *S. gregaria*, volatiles of nymphal *L. m. capito* on their own were not effective in significantly delaying sexual maturation in the immatures. In desert locust, the nymphal stages have a different aggregation pheromone from that of the adults and both nymphal crude volatiles and the synthetic blend of the identified compounds were shown to effectively retard maturation (Assad *et al.*, 1997). Lack of such an effect of nymphal volatiles on maturation in young adults of *L. m. capito* may largely be due to the fact that both the adult and nymphal stages of this locust produce similar volatile blends that have the same components (Razafindraleva, personal communication). Thus exposure of the immature adult locusts to nymphal volatiles did not play a significant role in changing the physiological state of young adults.

The overall objective of the study reported in this thesis was to investigate the role played by nymphal body and faecal volatiles produced in their aggregation behaviour. Thus, since no tests were carried out on role of other cues *e.g*. the visual and tactile signals, it is not possible to pinpoint the factors that delayed the maturation of the

immature locusts when they were in contact with their nymphal connspecifics. However, under field conditions, nymphal and adult stages develop sequentially such that they have their separate ecological niches (COPR, 1982). Therefore, confining them together in the same cage in a laboratory assay might have stressed the immature adults and generated a succession of physiological reactions leading to a delay in their maturation. As volatiles from the fifth instar exerted a maturation-delaying effect even though not significant, it may be possible that an overproduction of certain volatile compounds led to such stress causing the maturation delay. This may in turn explain the increasing mortality observed during the preliminary tests when eight or more locusts were held in the same cage.

The possibility of the existence of contact chemicals such as cuticular hydrocarbons that the immature adults could pick when they are together with the nymphs may also be associated with the delay in maturation. In the desert locust, *S. gregaria*, cuticular hydrocarbon extracts have been shown to significantly behavioural phase change in nymphal solitarious locusts (Heifetz *et al.*, 1996; 1997). These compounds may play albeit a different role in the biology of *L. m. capito*, which remains to be investigated.

With regard to the role played by volatile semiochemials in retardation of sexual maturation in *S. gregaria,* Richard and El Mangoury (1968) found that, not only fifth instar nymphs, but also young adults exerted a retarding influence on maturation of

immature adults. Assad *et al.* (1997) confirmed that, sexual maturation delay of immature adults was due to the conspecific nymphal aggregation pheromone. The nymphal faeces were found to be ineffective. At present, the factors in young adults that retard maturation of other young adults remain unknown since guaiacol and phenol have been found to be the major components of the nymphal volatiles and young adults and the nymphal faeces were ineffective on delaying such maturation in desert locust. In *L. m. capito*, no in-depth research has been done and further investigations are necessary.

# CHAPTER EIGHT

## 8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### 8.1 General discussion

The results of the work reported here confirmed that, semiochemicals are involved to an extent, in the aggregation behaviour of gregarious *L. m. capito* nymphs. However, volatiles produced by the laboratory mass-reared *L. m. capito* nymphs did not stimulate their grouping behaviour and this represents an exception since nymphs respond strongly to their volatiles in other locusts such as *S. gregaria* (Fuzeau-Braesch *et al.,* 1988; Obeng-Ofori *et al.,* 1994a; Torto *et al.,* 1996; Niassy *et al.,* 1999), *L. m. migratorioides* (Niassy *et al.,* 1999), and *L. m. manilensis* (Yu *et al.,* 2007). The avoidance reaction of fifth instar nymphs of *L. m. capito* to their volatiles raises a number of questions: How do hopper bands maintain their cohesiveness in the field if they avoid their own volatiles? What other stimuli are involved in their cohesiveness and how does it operate together with the volatiles? Two possibilities can be considered.

The first possibility could be that these volatiles are involved in the marching behaviour of the hopper bands and the nymphs use the visual and tactile stimuli to recognize each other and stay together. According to Ellis and Ashall (1957) and Stower (1963), in dense populations of hoppers, at any given time about 50% of individuals were observed to be marching while the others were pausing. Buhl *et al.* (2006), using an automated digital tracking system, showed that coordinated marching behaviour depended strongly

on density. At low density (≤ 7 locusts in the arena), there was a low incidence of alignment among the individuals while at intermediate density (10-25 locusts in the arena), there were long periods of collective rotational motion with spontaneous change in direction. However, at high densities (≥30 locusts in the arena), hoppers adopted a common and persistent rotational direction. Optomotors response was suggested by Uvarov (1966) as the factor involved in such alignment. However, Ellis (1951, 1962) reported that rather than seeing others marching, physical contact was necessary to evoke full marching behaviour. Recently, Bazazi *et al.* (2008) explained that coordinated mass migration in juvenile desert locust was influenced by cannibalistic interactions since individuals in marching bands tend to bite others but risk being bitten themselves. The reduction of the capacity of individuals to detect the approach of others from behind by abdominal denervation decreased their probability to start moving and reduced the proportion of moving individuals in the group. In this respect, the repellent compounds might be used by individuals in a band to "push" the other members to move ahead. It can also be interpreted as a feeding strategy by enabling the creation of a minimum space between individuals and, therefore, a better distribution of the available resources.

Secondly, although the micro-environmental pressures imposed under the mass-rearing conditions of the colony may to an extent affect the biology and behaviour of locusts, this was minimised by reproducing as best as possible field conditions (temperature, humidity and photoperiod) in the laboratory. Various species of insects have manifested

different behavioural effects when reared in the laboratory. For example, in *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), a major horticultural pest in eastern Australia, mating behaviour of the mass-reared males was different from that of the wild males (Weldon, 2005). In the malaria mosquito *Anopheles gambiae s.l.* (Diptera: Culicidae), insects reared in the laboratory have been shown to be less sensitive to olfactory cues when compared to the wild ones (Hassanali, personal communication). With regard to the aggregation response of *L. m. capito* nymphs over time, the first tests showed positive aggregation index when the nymphs and the adult were kept together. However, the nymphs did not respond to their volatiles in cross tests carried out later. As previously discussed, the composition of the volatiles and the ratios in which the compounds occurred could have undergone slight variations leading to different responses from the nymphs.

With regard to the volatile emission of the nymphs, the following approach was adopted to investigate the aggregation behaviour of *L. m. capito* nymphs to the synthetic compounds. The first step was to trap volatiles emissions from both nymphs and faeces, and then, characterize and identify the chemical constituents of the trapped volatiles using Gas Chromatography and combined Gas Chromatography-Mass Spectrometry. The second step was to establish which volatile compounds were detected by the sensory system of the locust using GC-EAD. The last step was to determine the biological activities of the active compounds in bioassays. The results showed that both fifth instar

male and females produce 2,3-butanediol, hexanal, benzyl alcohol, nonanal and PAN in varying amounts. In addition, 2,3-butanediol, PAN, anisole and guaiacol were identified from faecal volatiles. The composition of the volatiles appears to be specific to *L. m. capito* nymphs and so were their roles. As expected, fifth instar nymphs did not aggregate to either the equivalent synthetic blend of nymphal volatiles or the equivalent synthetic blend of faecal volatiles confirming the aggregation response of the nymphs to their own volatiles. However, details of the response of the nymphs to different combinations of individual synthetic compounds revealed that, aggregation responses were elicited. This was the case when the nymphs were exposed to a blend of 2,3-butanediol, hexanal, benzyl alcohol and nonanal; a blend of anisole, guaiacol and beta ionone. This aggregation response was maximal with PAN, benzyl alcohol, anisole and beta ionone tested individually. In contrast, a strong avoidance was observed with a combination of 2,3-butanediol, PAN, anisole, guaiacol. This result confirm that locust perceive the volatiles as one cue and not a combination of different individual compounds. Of the individual compounds, beta-ionone has been reported from floral scent of plants in several families such as Cactaceae (Kaiser and Tollsten, 1995), Fabaceae (Kaiser, 1997), Oleaceae (Mookherjee *et al.*, 1990). In nature, since flowering time of these plants occurs during the rainy season, this could be a contributing factor in aggregating nymphs by attracting them to the plants in bloom.

If these results are confirmed under field conditions, the avoidance effects of certain

combinations of compounds described in this work could be useful for protecting crops against locust invasion. In addition, the attraction effect of the blend could serve as a lure to trap locust with the condition that these substances are not toxic to the environment and the human health.

In Chapter seven of this thesis, fifth instar nymphs of *L. m. capito* were shown to cause a delay in the sexual maturation of conspecific immature adults. The nymphal volatiles alone were not effective in triggering this delay. Different factors have been reported to affect the sexual maturation of immature adult locusts. Norris and Pener (1965) found that juvenile hormone was responsible for the maturation-accelerating effect on crowded adults of *S. gregaria*. Absence of juvenile hormone by allatectomy retarded maturation of immature adults. Senescent plant diet may also delay the sexual maturation of locusts (Carlisle *et al.*, 1965; Assad *et al.*, 1997). However the mechanism underlaying the inhibition is still unknown. It is not impossible that the delay in sexual maturation of *L. m. capito* immature adults exposed to fifth instar nymphs observed in the present work has similar underlying mechanisms as in *S. gregaria*.

### 8.2 Conclusions

In conclusion, the cohesiveness of the hopper bands in the field may be as a result of a different composition and concentration of the compounds in the volatiles. Blends with the same constituents occurring in different ratios and varying concentrations are

detected by nymphs as different volatile cues and these may elicit different behavioural responses, in that may manifest either aggregation or avoidance. In the investigation on effects of nymphal volatiles on sexual maturation of immature *L. m. capito* adults, contact with fifth instar nymphs significantly delayed adult maturation. However, further studies are necessary to identify the factors emanating from their conspecific nymphs that affect sexual maturation.

### 8.3 Recommendations

In the course of the above study and based on the proceeding discussion, various questions arose and are suggested as topics for further research in the following recommendations:

1. It is necessary to trap volatiles released by gregarious fifth instar nymphs in the field and carry out chromatographic analysis on these to confirm their composition and evaluate the effect of these volatiles on their aggregation behaviour. This will provide comprehensive understanding of the processes underlying the aggregation behaviour of the nymphs;

2. It is important to carry out a comparative study on the volatiles that are produced by solitarious nymphs of *L. m. capito*. This will partly help in understanding the processes involved in phase shift in this locust; and

3. Investigations on possible physiological and neurophysiological effects of host plants on the chemical ecology of *L. m. capito* may provide data in understanding the role of locust-plant interactions in the population dynamics of this locust in their breeding and outbreak areas in southwest Madagascar.

These areas of research will certainly provide a better understanding of the biology and chemical ecology of *L. m. capito* and fill gaps in the knowledge that is necessary for the development of an effective preventive control strategy.

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## APPENDICES

Appendix 1: Details of the response of the fifth instar nymphs to their own volatiles when they were reared with adults.

| Volatiles source | Recipient insects | Number in stimulus | Number in control | Total tested | Aggregation Index (%) [a] |
|---|---|---|---|---|---|
| 5th instar (5females + 5 males) | 5th instar mixed sex A [b] | 48.10 | 47.40 | 100 | 0.70 NS |
| | 5th instar mixed sex B [c] | 30.50 | 26.50 | 60 | 6.67 NS |
| | Single 5th instar female | 57.00 | 39.00 | 100 | 18.00 NS |
| | Single 5th instar male | 58.00 | 39.00 | 100 | 19.00 NS |
| Faeces | 5th instar mixed sex A [b] | 49.50 | 45.70 | 100 | 3.80 NS |
| | 5th instar mixed sex B [c] | 32.40 | 24.70 | 60 | 12.83 NS |
| | Single 5th instar female | 57.00 | 39.00 | 100 | 18.00 NS |
| | Single 5th instar male | 46.00 | 39.00 | 90 | 7.78 NS |

[a] Difference from control ($\chi^2$ test) indicated by NS = not significant

[b] Five fifth instar males + five fifth instar females

[c] Three fifth instar males + three fifth instar females

Appendix 2: Details of the cross-test responses of the fifth instar nymphs when they were reared together with adult.

| Volatiles source | Recipient insects | Number in stimulus | Number in control | Total tested | Aggregation Index (%) |
|---|---|---|---|---|---|
| 10 fifth instar females | Ten 5th instarfemale | 47.10 | 49.40 | 100 | -2.30 NS |
| | Single 5th instar female | 20.00 | 27.00 | 50 | -14.00 NS |
| | Ten 5th instar male | 14.50 | 14.30 | 30 | 0.67 NS |
| | Single 5th instar male | 15.00 | 33.00 | 50 | -36.00 ** |
| 10 fifth instar males | Ten 5th instarfemale | 47.90 | 48.80 | 100 | -0.90 NS |
| | Single 5th instar female | 23.00 | 24.00 | 50 | -2.00 NS |
| | Ten 5th instar male | 13.00 | 16.2 | 30 | -10.67 NS |
| | Single 5th instar male | 11.00 | 39.00 | 50 | -56.00 ** |

Difference from control ($\chi^2$ test) indicated by NS = not significant; significant $^{**}P < 0.01$

Appendix 3: Details of the responses of the fifth instar nymphs to their own volatiles when they were separately reared from adults

| **Volatiles source** | **Recipient insects** | **Number in stimulus** | **Number in control** | **Total tested** | **Aggregation Index (%)** |
|---|---|---|---|---|---|
| 10 fifth instar (5females + 5 males) | Single 5th instar female | 29.00 | 71.00 | 100 | -42.00 ** |
| | Single 5th instar male | 22.00 | 78.00 | 100 | -56.00 ** |
| Faeces | Single 5th instar female | 27.00 | 61.00 | 90 | -37.78 ** |
| | Single 5th instar male | 23.00 | 47.00 | 70 | -34.29 ** |

Difference from control ($\chi^2$ test) significant ** $P < 0.01$

Appendix 4: Details of the cross test response of the fifth instar nymphs when they were reared separately from adult.

| Volatiles source | Recipient insects | Number in stimulus | Number in control | Total tested | Aggregation Index (%) |
|---|---|---|---|---|---|
| 10 fifth instar females | 10 fifth instar female | 11.55 | 16.95 | 30 | -18.00 NS |
| | Single 5th instar female | 24.00 | 76.00 | 100 | -52.00 ** |
| | 10 5th instar male | 10.65 | 17.4 | 30 | -22.50 NS |
| | Single 5th instar male | 14.00 | 36.00 | 50 | -44.00 ** |
| 10 fifth instar males | 10 5th instar female | 12.75 | 16.2 | 30 | -11.50 NS |
| | Single 5th instar female | 37.00 | 62.00 | 100 | -25.00 * |
| | 10 5th instar male | 10.95 | 17.85 | 30 | -23.00 NS |
| | Single 5th instar male | 32.00 | 68.00 | 100 | -36.00 ** |

Difference from control ($\chi^2$ test) indicated by NS = not significant; significant, *$P < 0.05$ **$P < 0.01$.

Appendix 5: Details of the aggregation response of fifth instar nymphs to their body extract

| Volatiles source | Recipient insects | Number in stimulus | Number in control | Total tested | Aggregation Index (%) |
|---|---|---|---|---|---|
| Body extract | 10 fifth instar female | 12 | 18 | 30 | -20 NS |
| | 10 5th instar male | 15 | 14 | 30 | 2.75 NS |

Difference from control ($\chi^2$ test) indicated by NS = not significant

Appendix 6: Top view diagram of the aluminium circular arena used for studying the grouping behaviour of the nymphs (Malual *et al*., 2001, Wiesel *et al*., 1996).