INTERACTION BETWEEN PHEROMONS AND HORMONES IN PHASE DYNAMICS OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSKAL)

A THESIS
Submitted for the Degree of Ph. D.
(ENTOMOLOGY)

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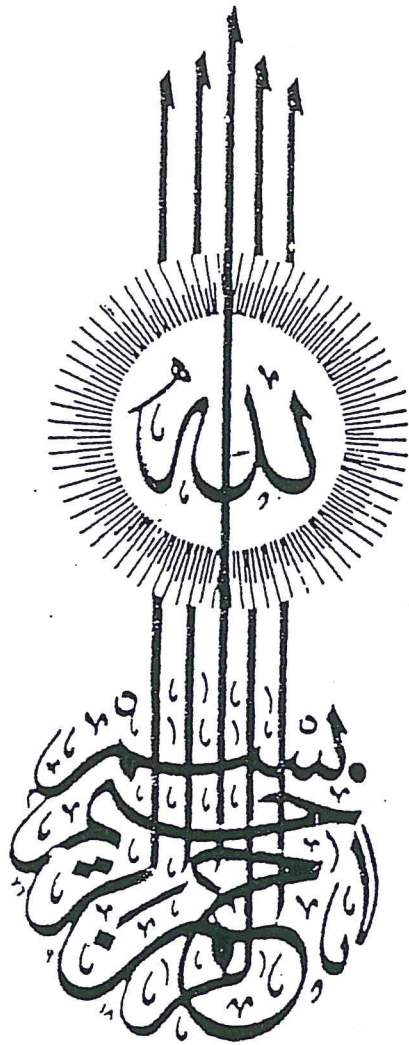ZOOLOGY DEPARTMENT, FACULTY OF SCIENCE, ASSIUT
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1995

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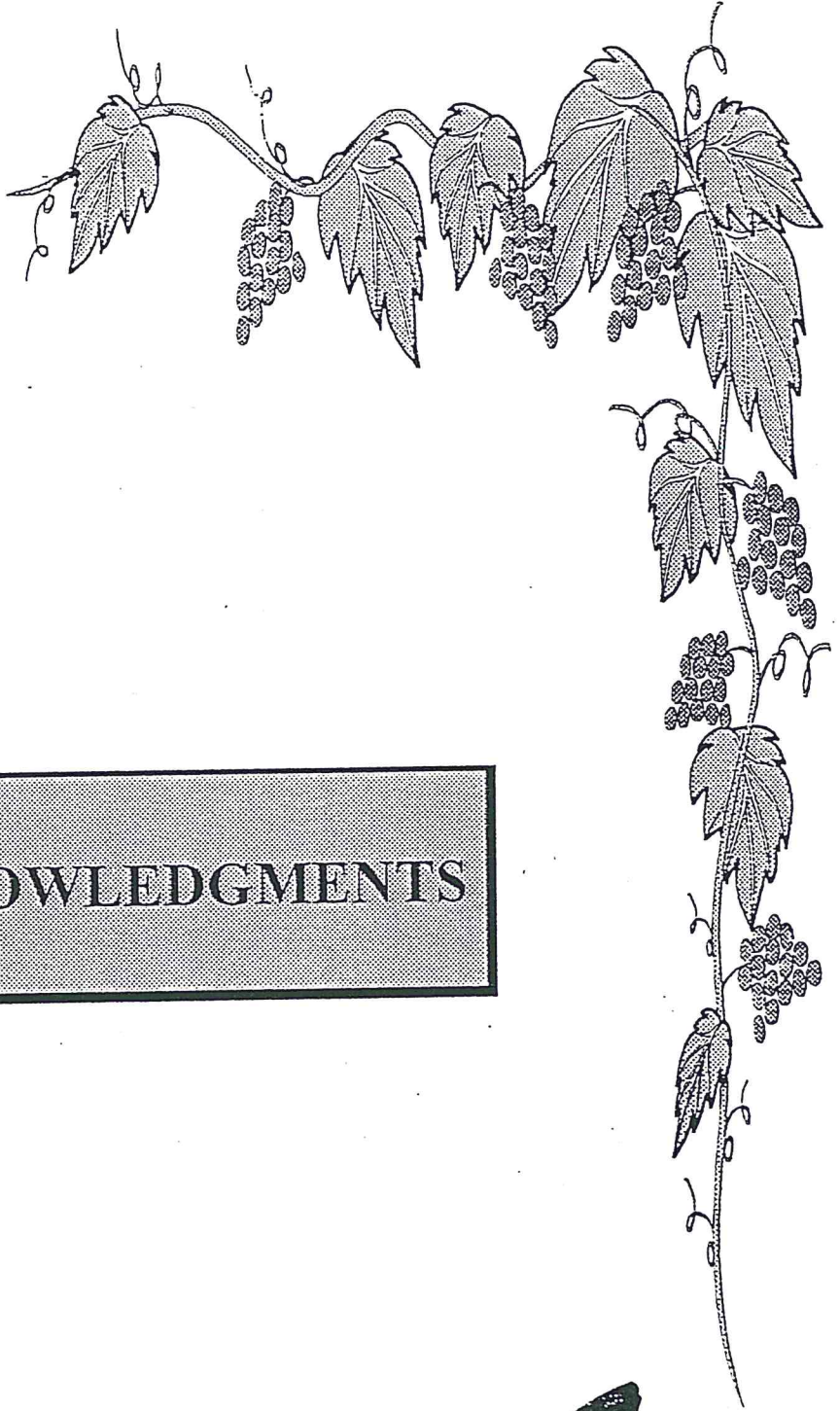
ACKNOWLEDGMENTS

ACKNOWLEDGEMENTS

Finally, my most special gratitude is reserved to my wife, Amal Zakarea, brothers and parents whose continuous encouragement was a driving force without which this study could not have been completed.

AMER I. TAWFIK

ABBREVIATIONS
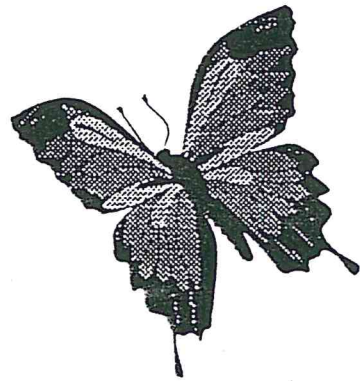
ABBREVIATIONS

List of abbreviations used in the present work:

AFESD	:	Arab Fund for Economic and Social Development
AKH	:	Adipokinetic hormone
AMP	:	Adenosine monophosphate
ANOVA	:	Analysis of variance
CA	:	Corpora allata
CC	:	Corpora cardiaca
CGLR	:	Consultative Group on Locust Research
DLCO-EA	:	Desert Locust Control Organization for Eastern Africa
E	:	Ecdysone
20E	:	20-hydroxyecdysone
26E	:	26-hydroxyecdysone
E/F	:	Length of elytron/length of hind femur
FID	:	Flam ionization detector
Fumig.	:	Fumigation (vapor phase)
G	:	Gregarious
GC	:	Gas chromatography
GLC	:	Gas liquid chromatography
HP	:	Hewlett-Packad
HPLC	:	High pressure liquid chromatography
HPP	:	Highly polar products or metabolites (ecdysteroids)
ICIPE	:	International Center of Insect Physiology and Ecology

IFAD	:	International fund for Agricultural Development
IGRs	:	Insect growth regulators
Inj.	:	Injection
JH	:	Juvenile hormone
JH 0	:	Juvenile hormone 0
JH I	:	Juvenile hormone I
JH II	:	Juvenile hormone II
JH III	:	Juvenile hormone III
JHA	:	Juvenile hormone analogs
LSD	:	Least Significant Difference
MaA	:	Makisterone A
MS	:	Mass spectrometry
NSM	:	Neurosecretory material
P I	:	Precocene I
P II	:	Precocene II
PBAN	:	Pheromone biosynthesis activating neuropeptide
PG	:	Prothoracic gland
PI-NSC	:	Pars intercerebralis neurosecretory cells
RIA	:	Radioimmunoassay
S	:	Solitary
SAREC	:	Swedish Agency for Research Cooperation with Developing Countries
SAS	:	Statistical Analysis System
TA	:	Topical application
TLC	:	Thin layer chromatography
UNDP	:	United Nation Development Programme

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INTRODUCTION

Locusts are a special group of grasshoppers which belong to the family Acrididae. They differ from other grasshoppers by their ability to change behavior and transform, under certain climatic and ecological conditions, from a solitary to a gregarious phase. In the solitary phase, locusts are harmless because they occur in very low number; but as soon as they transform into gregarious phase and develop into swarms which migrate long distances, they pose a serious threat to crops.

The theory of locust phases was formulated by Uvarov (1921) in a taxonomic revision of the genus *Locusta*. He concluded that *L. migratoria* and *L. danica*, previously regarded as two distinct species, are respectively the swarming and solitary forms or phases of the same species; these forms are capable of transforming into one another and are connected by intermediate forms. The phase theory was soon extended to other locust species and phase transformation was verified both experimentally and by field evidence (Faure, 1932). The swarming crowded phase and the more sedentary isolated one were given the latinized names *gregaria* and *solitaria*, respectively, while the intermediates were named as phase *transiens*.

The economically important locust species of Africa are: the red locust, *Nomadacris septemfasciata*; the brown locust, *Locusta pardalina*; the African migratory locust, *Locusta migratoria*; the tree locust, *Anacridium sp.*; and the desert locust, *Schistocerca gregaria* (Schmidt and Osman, 1988). Among these species, the desert locust is

regarded as the single most serious threat to agriculture in Central and North Africa, the Middle East and Southwest Asia. Some statistics concerning the gregarious desert locust are mind boggling. For example:

* The invasion area covers about 29 million sq km affecting 57 countries in Africa and Asia and representing more than one fifth of the total land surface of the world.

* Swarms can measure over 1000 sq km with each sq km having 40 to 80 million locusts; thus a swarm may contain 40 billion; locusts weighing some 80,000 tones. In may 1988, it was reported that a swarm in Mali was three times that size.

* A swarm multiplies 30-fold every time breeding occurs under optimum conditions, this can be 3-4 times in a year.

* Each individual locust consumes its own weight (approximately 2 g) of vegetation every day; thus one million locusts can eat in one day as much food as needed to feed 5000 people.

* Locusts are polyphagous insects (i. e. they can consume different types of vegetation) although they prefer plants belonging to the family Gramineae (grasses) which includes all the major staple food crops in locust-affected countries.

* By partially gliding, locusts can stay airborne for a long time and can travel over 300 km in a day. In October 1988, one swarm was reported to have hopped 5000 km across the Atlantic to the Caribbean, helped by atmospheric winds.

The desert locust, *S. gregaria* is the most widely studied species

among the five different types of locusts. These studies have revealed that its two extreme forms or phases, *solitaria* and *gregaria*, differ from one another morphologically, such as in their color, shape and size, as well as in their behavior, and physiology (Schmidt and Osman, 1988; Dearn, 1990; Pener, 1991). An adult in the solitary phase is often pale grey or bieg when immature, with males becoming pale yellow on maturation. Solitary nymphs are often green or brown. In contrast, the gregarious adult is usually bright pink when immature and bright yellow when mature, with nymphs showing a distinctive black pattern on their bodies (Gunn and Hunter-Jones, 1952; Nickerson, 1956; pener, 1983).

It has been shown in several studies that a pheromone or pheromones produced by desert locust can make *solitaria* phase nymphs show some shift towards the *gregaria* end of their spectrum of polymorphism (Gillett, 1975; 1983). These changes of phase or morph can be measured in the color of the nymphs and in the extent to which the nymphs form social groups. The pheromone believed to cause these changes is called the gregarisation pheromone.

Locust polymorphism (including physiological and behavioral polymorphism) may also be controlled by environment; extrinsic cues may induce sensory and/or nutritional inputs. These are somehow coupled to the mechanisms which prefer a certain morph over the other(s), then substantiate this preference in the course of development. Components of the endocrine system are usually involved in these mechanisms and are often major factors in the control of such

polymorphism (Joly, 1956; 1958; 1962; Staal, 1961; Novak and Ellis, 1967; Ellis and Novak, 1971; Amerasighe, 1978; Wilson and Morgan, 1978; Pener, 1991).

It is noteworthy that the world locust problems would be solved through a better understanding of fundamental locust biology. It has been largely accepted that the factors affecting, and basic processes underlying locust phase transformation may lead to practical control of these insects. The current maturation of insect endocrinology as an established branch of biology opens of new possibilities for understanding phase dynamics.

In fact, the problem of endocrine effects on locust phase changes is far from being solved. Probably we are nearer to the beginning than to the end of the road because till now nothing is known about the possible involvement of endocrine factors in the regulation of pheromone production in locusts.

The main goal of the research is to study the interaction between pheromones and hormones during phase transformation of the desert locust, *S. gregaria*. Pheromone-induced gregarisation will be monitored by JH levels within individual insects. Likewise the effect of exogenous JH on pheromone production will be monitored. Another objective also, is to examine in more detail the differences in the hemolymph ecdysteroids and juvenile hormone between the solitary and gregarious phases of *S. gregaria*.

The long-term objective of the Locust Research Programme at ICIPE, is to develop alternative, environmentally friendly, biorational

strategies for sustainable management of locusts. In order to achieve this goal, research has been focused on two major areas, namely semiochemicals and biological control.

The main objective of the semiochemical approach is to develop a viable preventive control strategy that interferes with the process of gregarisation and swarm formation. This requires a fundamental understanding of the endocrine organs, hormones and their role in phase changes and pheromone production. As part of the Locust Research Programme activities at ICIPE, this work has been initiated to investigate the interaction between pheromones and hormones in phase dynamics of the desert locust, *Schistocerca gregaria* (Forskal).
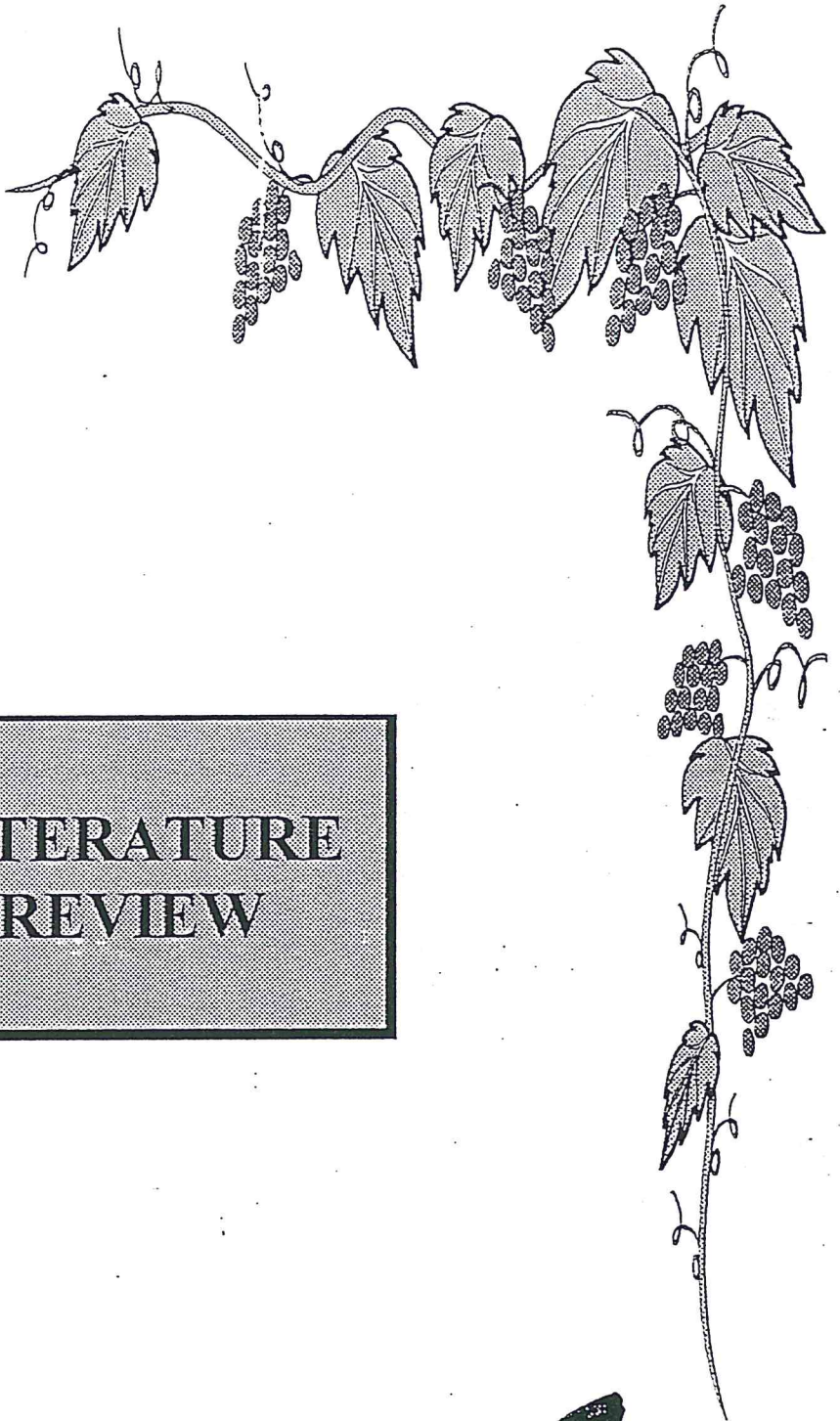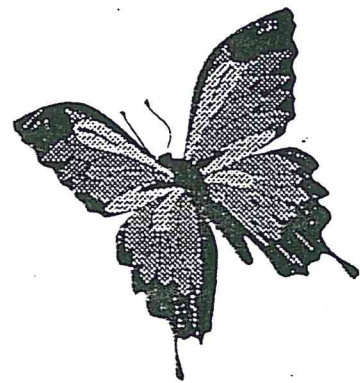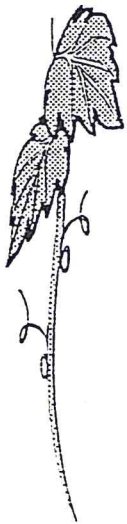
LITERATURE REVIEW

LITERATURE REVIEW

1. POLYMORPHISM AND ENDOCRINE FACTORS

The first studies on hormonal effects on insect polymorphism were carried out on locusts by Joly (1949; 1951). From the 1950s onward, publications on endocrine effects on insect polymorphism become more frequent. Up to the early 1960s most of them were devoted to locust phase polymorphism, culminating in the comprehensive experimental works of Joly (1960) and Staal (1961). Even in this early period, however, some studies already dealt with endocrine aspects of other kinds of polymorphism. For example, color polymorphism in the grasshopper, *Acrida turrita* (Joly, 1952); wing polymorphism in the cricket, *Gryllus campestris* (Sellier, 1955); cast polymorphism in lower termites (Luscher, 1961); wing polymorphism in aphids (Lees, 1961; 1983); and cast polymorphism in honey bee (De Wild and Beetsma, 1982).

More recently, endocrine effects on locust polymorphism were reviewed by Nijhout and Wheeler (1982) and Hardie and Lees (1985). Other recent reviews, dealing with more restricted aspects of the subject are those of Dale and Tobe (1990) and Pener (1990; 1991).

2. ENDOCRINE ORGANS, HORMONES AND THEIR ROLE IN PHASE TRANSFORMATION

2.1. THE CORPORA ALLATA AND JUVENILE HORMONE

Staal (1961) reported that the volume of the corpora allata (CA) is larger in isolated than in crowded adults of *Locusta*. However, the

results of Staal (1961) also showed a marked interaction between density and humidity; in crowded locusts, the humidity had little effect in the volume of the CA, whereas in isolated ones high humidity led to a considerable increase in gland volume. In another experiment of the same study, CA volumes measured in the fifth instar were found to be larger in hoppers which had been kept isolated from the later part of the third instar than those which had been maintained continuously under conditions of crowding. Thus, differences in density experienced during one (the fourth) instar were sufficient to affect gland volume.

Highnam and Haskell (1964) studied CA volume and its increase during the sexual maturation of adult female locusts under various experimental conditions. They found that the maximum volume of the CA, as related to oocyte length, was quite similar in isolated flown and unflown and in crowded flown females of *Locusta* kept without males. However, the major increase in gland volume occurred at a smaller oocyte length in the crowded flown females than in the isolated (flown or unflown) ones. The steepest increase in this species was observed in unflown crowded females kept without males, and maximum gland volumes in this group greatly exceeded those in the other three groups. The results obtained by Highnam and Haskell (1964) in *Schistocerca gregaria* were somewhat different. In adult females kept without males, the maximum volumes of the CA were quite similar in unflown isolated, flown isolated and unflown crowded locusts and a little smaller in flown crowded ones, but the increase in gland volume was steeper

in the crowded than the isolated females. The highest gland volumes and steepest increase were found in crowded females kept with mature males producing maturation-accelerating pheromones; such females also showed the shortest period of sexual maturation.

Measuring CA volume in penultimate and last-instar female hoppers and in adult females of *Schistocerca gregaria*, Injeyan and Tobe (1981b) recorded consistently larger volumes in isolated than in crowded locusts. These finding somewhat differ from those of Highnam and Haskell (1964), but direct comparison may not be justified because the isolated locusts of Injeyan and Tobe were reared for two or more generations under strict isolation and all exhibited an extra hopper instar, suggesting that, they were *solitaria* whereas Highnam and Haskell separated their locusts from a crowded stock only at the molt to adult.

Dale and Tobe (1986) found larger CA volumes in isolated than in crowded adult females of *Locusta* during the first 8 days after fledging. Considering that sexual maturation is quicker in isolated than in crowded *Locusta* adults, these results correlated well with density-dependent differences in maturation time.

Implantation of CA into gregarious nymphs of *L. migratoria* (Joly, 1949; 1951; 1954; 1972; Joly and Joly, 1954; 1974; Staal, 1961) and *S. gregaria* (Novak and Ellis, 1967) causes them to assume a green color like that of solitary nymphs, although the effectiveness of the implantation varies according to the instar used. The color change seems definitely to be due to the action of the implanted CA because in

some recipients (Joly, 1954), a localized patch of especially dark green has been observed in the integument above the site of the implanted glands.

Injeyan and Tobe (1981b) reported that juvenile hormone (JH) biosynthesis activity of the CA, assessed by radiochemical assay *in vitro*, was higher in isolated than in crowded penultimate and last-instar female hoppers of *Schistocerca*. In the same study, the activity of the CA was found to be slightly lower in crowded than in isolated adult *Schistocerca* female, but major differences were temporal; the isolated locusts exhibited relatively higher rates of JH synthesis earlier in the first gonotrophic cycle.

Employing the *Galleria* bioassay, Joly and Joly (1974) and Joly *et al.* (1977) found higher hemolymph JH titer in isolated than in crowded fourth- and fifth-instar hopper of *Locusta*. These authors have also observed that in isolated young *Locusta* adults, JH titers increased much more rapidly with age than in crowded ones. Using the more reliable method of gas chromatography-mass spectrometry, Dale and Tobe (1986) found low JH III titers in 1-day-old adult *Locusta* females and no differences between isolated and crowded locusts at this age. The titers were much higher on day 4, and the increase was approximately twice as in isolated than in crowded females.

On the other hand, Fuzeau-Braesch *et al.*(1982) assessed JH titers in last-instar hoppers and adults of *Locusta*, comparing crowded, isolated green, isolated homochrome (light green), and artificially solitarised (by CO_2) locusts. Except for higher JH III titers in the artificially

solitarized ($=CO_2$ treated) locusts, no clear differences were found.

Amerasinghe (1978) studied the effects of JH I and JH II on yellowing, sexual activity and pheromone production in allatectomized male, *S. gregaria* and found that both hormones are capable of stimulating yellowing in crowded allatectomized males; the effects of JH I being far superior to those of JH III. However, the hormones are ineffective in inducing yellowing when the insects are kept isolated. Also she reported that both hormones are active in inducing production of the maturation pheromone in these insects.

In locusts (Pener, 1983) and migratory Lepidoptera (Iwao, 1968; Johnson, 1969), larval color, rate of growth and size are the prominent characters associated with phase variation. Phase variation in migratory insects appears to be a response to population density which is mediated by the neuroendocrine system (Nijhout and Wheeler, 1982; Pener, 1983). This system regulates the release of JH which appears to have an important role in mediating the effect of density on phase variation (Rankin and Rankin, 1979; Nijhout and Wheeler, 1982; Pener, 1991).

Fescemyer and Hammond (1988) studied the relationship between population density, JH, JHE and phase variation in larvae of migrant insect *Anticarsia gemmatalis*; they found that the JH titer of crowded *A. gemmatalis* larvae was lower than uncrowded larvae. Also they reported that the esterase activity was in part, a function of the JH titer.

2.2. ANTI-JUVENILE HORMONE (PRECOCENES)

Pener *et al.* (1978) reported that precocene causes all the effects

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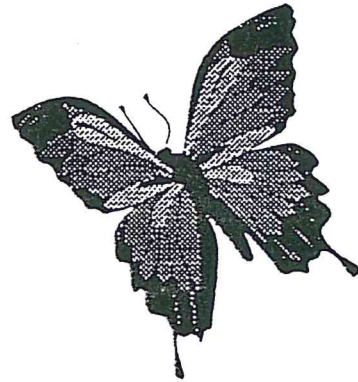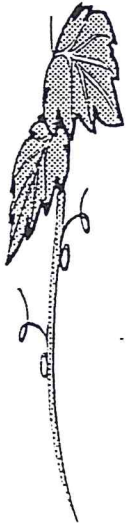
expected in *Locusta*, with atrophy of the CA and a resultant loss of any circulating JH. They found that topical treatment with 100 μg applied in the fourth-instar and within 24 hr of ecdysis was most effective in producing exclusively permanent, precocious fifth-instar adultiforms, and with lowest mortalities. Other effects noticed by Pener *et al.* were delayed moulting and anti-gonotrophic effects in morphologically normal adults.

Furthermore, Pener *et al.* (1981) found that precocenes cause atrophy of the CA in *Locusta*, due to selective cytotoxic action, thus they induce permanent JH deficiency by chemical allatectomy. Also they reported that precocenes can be utilized to obtain third-, fourth- or fifth-instar precocious permanent adultiform, or morphogenetically normal adults with complete JH deficiency.

On the other hand, results by Pedersen (1978) showed that the induction of green pigmentation in *Locusta* migratoria by juvenile hormone injections can be prevented by precocene I. In the same species Miall (1980) reported that the precocious adultiform larvae produced by topical applications of precocene II to early 4th instar were found to be intermediate between normal 5th instar larvae and normal adults on behavioral as well as morphological criteria.

3. PHASE POLYMORPHISM AND ENDOCRINES OTHER THAN JH

The importance of endocrine agents other than JH in the context of phase polymorphism has been studied, but in no cases have the

investigations provided bases for definitive conclusion. For example, according to Ellis and Carlisle (1961), locusts in the solitary phases have larger prothoracic glands than those in the gregarious phase. In addition, the surgical removal of part of the gland appeared to reduce the green color in *Schistocerca* (Carlisle and Ellis, 1959). However, Staal and De Wilde (1962) could not confirm that the prothoracic glands influenced the phase of *Locusta*. Also, a comparison of the ecdysteroid levels in fifth instar nymphal *solitaria* and *gregaria* of *Schistocerca* has not revealed any significant differences (Wilson and Morgan, 1978).

There is evidence of differences between locust phases in the abundance of certain neurochemicals in the nervous system and endocrine glands. A number of studies have shown that the amount of octopamine extractable from solitary *L. migratoria* adults is considerably greater than that extractable from gregarious adults (Fuzeau-Braesch and David, 1978; 1980; Fuzeau-Braesch *et al.*, 1979). Conversely, dopamine is more abundant in gregarious than in solitary *L. migratoria* (Fuzeau-Braesch, 1977a&b). However, at present the functional implications of these data are unclear.

In *Locusta*, Ayali and Pener (1992), and Ayali *et al.* (1994), concluded that solitary locusts have lower hemolymph lipid levels and a less intense response to adipokinetic hormone (AKH) than gregarious locusts.

Highnam and Haskell (1964) studied the amount of neurosecretory material (NSM) in the pars intercerebralis

neurosecretory cells (PI-NSC) and corpora cardiaca (CC) of isolated and crowded adult females in relation to oocyte growth and the effect of flight upon maturation in both *Schistocerca* and *Locusta*. Generally, they found that experimental conditions which led to slow maturation of the oocyte also led to the accumulation of NSM in the PI-NSC and CC system. Conditions which enhanced oocyte maturation also promoted the release of NSM from this system.

On the other hand, using microcautery Girardie (1967; 1970) has shown that the "C" cells of the pars intercerebralis of *Locusta* secrete a neurohormone that acts directly on the epidermis to promote melanization. Whereas, Staal (1961) obtained an increase of the black patterns after implantation of extra CC into hoppers of *Locusta*.

4. PHEROMONES AND THEIR ROLE IN PHASE TRANSFORMATION

Pheromones are often classified as primer and releaser; the later have short-term effects and trigger preprogrammed behavior in the receiving animals, whereas primer pheromones induce long-term effects changing the physiology and/or behavior of the receiving animals, so that they become physiologically different and/or react differently to environmental stimuli from those animals which have not been exposed to the primer (Wilson and Bosset, 1963; Weaver, 1983; Loher, 1990).

Pener (1991) reported that the relationship between locust phases and pheromones may be two-fold; phase may affect pheromone production and/or reception, or (primer) pheromones may affect locust phase changes.

Based on results indicating the promotion of gregarious black hopper coloration (Nolte, 1963) and increase in long-term gregarious behavior (Gillett, 1968; Ellis and Gillett, 1968), a so-called gregarisation pheromone was found to be produced by locusts. This pheromone seems to be a primer (Gillett, 1968), and it was proposed to induce or intensify gregarious phase characteristics.

Nolte *et al.* (1970; 1973) and Nolte (1974; 1976) have concluded that the gregarisation pheromone is produced in the crop of the alimentary tract and is present in the faeces of hoppers; they named it locustol. Nolte (1977) postulated that locustol somehow promotes the production of cyclic AMP, and the latter promotes transformation from solitary to gregarious phase.

On the other hand, Gillett (1975; 1983) reported that a gregarisation pheromone in *Schistocerca* affects some phase characteristics such as color or certain components of behavior. Also Gillett (1983) found that the gregarisation pheromone from faeces of the hoppers is perceived by the antennae, whereas Nolte *et al.* (1970) and Nolte (1974) claimed that the locustol is received and/or perceived through the spiracles.

It has been shown in some insect species that, endogenous regulation of physiology and behavior associated with pheromone production results from the action of nervous, neuroendocrine and/or endocrine stimulation (Barth and Lester, 1973; Blomquist and Dillwith, 1983; Raina and Menn, 1987; Vanderwel and Oehlschlager, 1987).

MATERIALS
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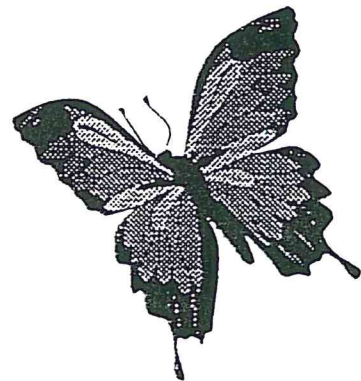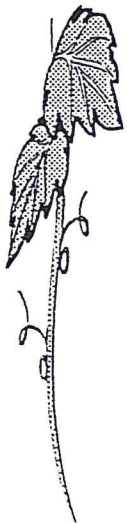
MATERIALS AND METHODS

1. INSECTS

Crowded and isolated desert locusts, *Schistocerca gregaria* (Forskal) were supplied by ICIPE colony. They originated from a stock obtained from the Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Addis Ababa, Ethiopia. They had been reared for more than 35 generations as separate solitary and gregarious lines on a diet of grass and wheat bran at $32 \pm 2°C$, R. H. 60% and light cycle of 12:12 hr light:dark. Insects (300-400) of both sexes were bred under crowded conditions in aluminum cage (50 x 50 x 50 cm). They were reared in a special room (4.4 x 4.5 m) provided with a special duct system for efficient air exchange. Whereas, locusts of solitary phase were reared singly (paired only for mating) in small cages that were kept in another room under the same conditions.

2. COLLECTION AND ANALYSES OF VOLATILES

Collection and analyses of volatiles from treated and control insects were carried out as described in Torto *et al.* (1994). Briefly, air from a compressed air cylinder was passed through a charcoal filter, over locusts contained in a trapping chamber (10 cm long x 3.5 cm-ID) and through a charcoal trap packed between two glass wool plugs in 6 cm long x 8 mm-ID glass tube at 106 ml/min for 18 hrs at $32 \pm 2°C$ (Fig. 1). Collections were done from sets of nine adult males in groups
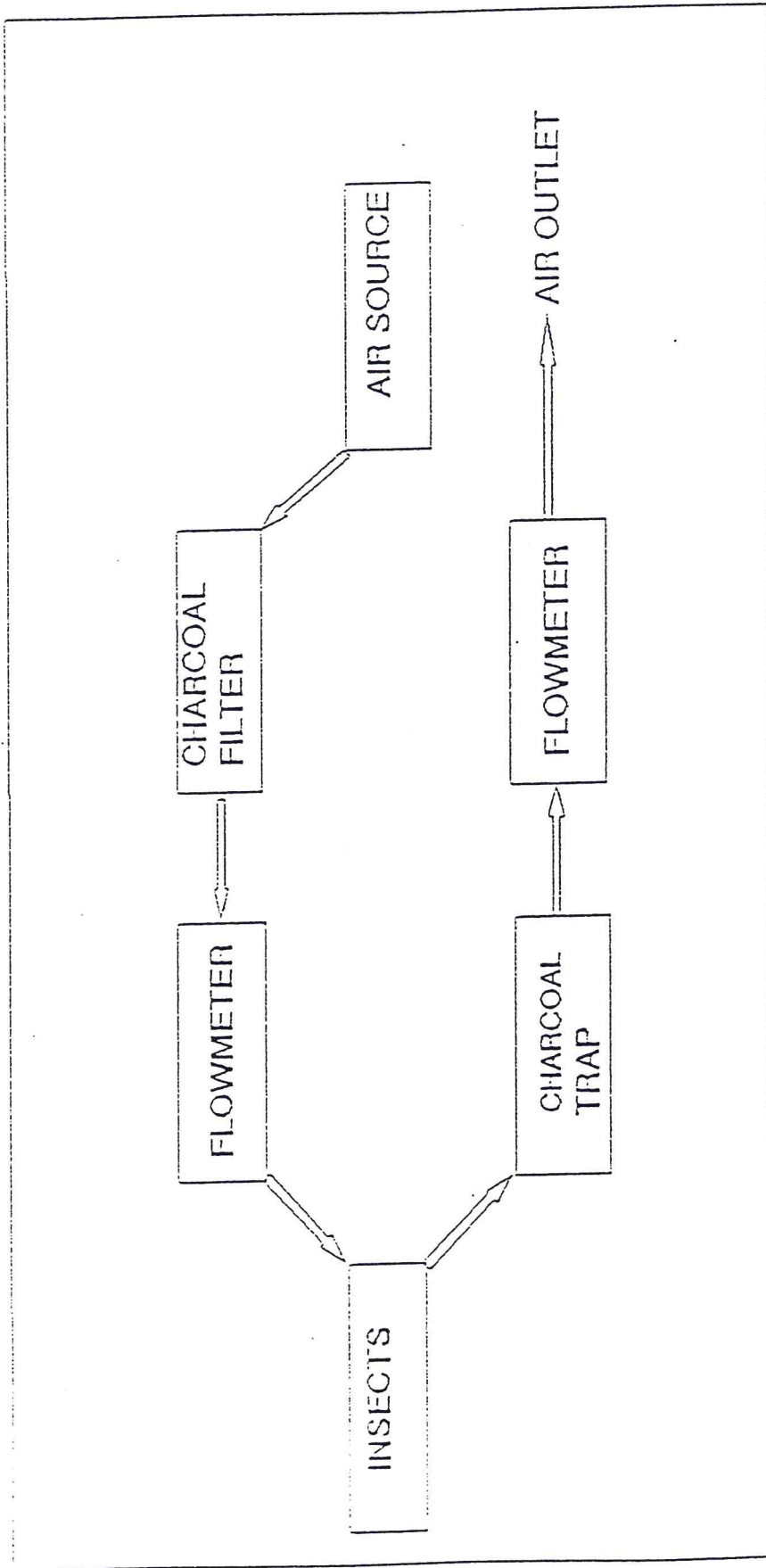
Fig. 1. Diagrammatic representation of volatiles collection assembly.

of three. Volatile extracts were concentrated to 100 μl and then 250 μg of O-methyl acetophenone (internal standard) were added. 2.5 μl aliquot of extract were analyzed by capillary gas chromatography (GC). Analyses were performed on a Hewlett-Packad (HP) 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HD capillary column (Carbowax, 50 m x 0.32 mm ID x 0.3 μm film thickness) using nitrogen as the carrier gas at flow rate of 0.35 ml/min. The oven temperature was intially isothermal at 60°C for 10 min then programmed at 5°C/min to 180°C for 5 min and to a rate of 10°C / min to 220°C for 15 min. Chromatographic peaks including that of phenylacetonitrile were integrated using a HP 3393 integrator.

3. MORPHOMETRIC INVESTIGATIONS OF THE CORPORA ALLATA AND OVARIES

The volume of the corpora allata was determined immediately after dissection using the formula V (mm^3) = 4/3 π ($r_1 + r_2$)^3/2 in which r_1 means the larger and r_2 the smaller radius (Schmidt and Othman, 1993).

In each female dissected in Ringer's solution, 10 terminal oocytes each ovary were measured by means of an ocular micrometer at a suitable magnification of a binocular microscope. In this way 20 terminal oocytes per female were used to calculate the variation in the mean length during the first and second gonotrophic cycles.

4. IDENTIFICATION AND QUANTIFICATION OF JUVENILE HORMONE

Identification and quantification of juvenile hormones were carried out by the method of Rembold and Lackner (1985). This method allows a quantitative and qualitative determination of JHs in the hemolymph by means of combined gas chromatography (GC) and mass spectrometry (MS) after microderivation. Some purification steps, methoxy derivatization, and silyation are necessary and then measurement can be made in the picomole range. An internal standard was used; therefore, loss of JH during the procedure is not important. Each sample contained 1 ml methanol, 5 μl of JH I, and JH III standard, consisting of 1 pmol/μl (5 pmol total concentration) ethyl esters, and the hemolymph sample (10 μl).

5. TREATMENTS BY JUVENILE HORMONE AND PRECOCENE

The insects used for the experiments were adult and fifth (last) nymphal instar males and females (3-days-old) from the same batch to eliminate possible variability in physiological status. For each experiment gregarious males and females in groups of 30 individuals were used under crowded conditions in aluminum cage (20 x 20 x 20 cm) in another room under the same conditions as above. JH III and precocene II (Sigma Chemical Company, U. K.) were dissolved in different concentrations in AnalaR grade acetone for topical application on the dorsal side of the abdomen, and in pure olive oil for injection between the second and third sternites using a microsyringe.

5.1. TOPICAL APPLICATION

Insects received a single dose. 50 μg or 150 μg of JH III or precocene II in 5 μl acetone on the 4th day of the 5th nymphal instar or the adult stage. Three conseculative topical applications, each of 50 μl (a cumulative of 150 μg) of JH III or precocene II per insect, were administered daily for 3 days on the 3rd, 4th, and 5th days of the last nymphal instar or the adult stage. The control insects received 5 μl acetone in each treatment.

5.2. INJECTION

Five μl of olive oil with 50 μg or 150 μg of JH III or precocene II were injected on the 4th day of the last nymphal instar or the adult stage. A cumulative dose of 150 μg (3 x 50 μg) of JH III or precocene II per insect were injected daily for 3 days as described above. Control insects received 5 μl olive oil in each treatment.

5.3. VAPOR PHASE

It was previously observed that the control insects were affected by those that had received JH topically in the same room. Therefore, we investigated the effects of JH in the vapor phase as follow: JH III was tested in the vapor phase by dissolving 200 or 400 μg of JH III in 5 μl acetone, placed in rubber septem until the solvent evaporated, then the rubber septum covered by perforated aluminum foil and placed in the same cage with tested insects during the whole life.

6. OBSERVATIONS ON MALE SEXUAL BEHAVIOR AND MATING

Observations on male sexual behavior and mating were carried out during the adult stage, four times a day for treated and control insects.

7. COLLECTION AND ANALYSIS OF THE HEMOLYMPH PIGMENTS

Collection and analyses of the hemolymph pigments were carried out as described by Mahamat *et al.* (1995). Briefly, hemolymph samples (10 μl) were collected from 10-, 20-, 30-, and 40-days old adults, and drained into an Eppendorf tube containing 0.5 ml mordue buffer. Spectral analyses of the hemolymph samples were carried out on a Beckman DU-50 spectrophotometer at a wavelength range of 300-700 nm. The ratios of absorbances at 460 and 680 nm were calculated for each sample and compared for control and treated insects. Seven to ten samples were analyzed for each age group.

8. HEMOLYMPH COLLECTION FOR ECDYSTEROIDES

From the stock cultures we selected larvae within 4 h after ecdysis into the penultimate (4th in the gregarious and 5th in the solitary phases), last larval instar or adult stage. Analyses were performed at this time (day 0) and in 24 h intervals thereafter. The solitary hoppers and adults were kept individually, those of the gregarious phase were grouped by at least 100 individuals. Under these

conditions, the penultimate and last larval instars lasted in the solitary phase 4 and 8 days, respectively, and in the gregarious phase 4.5 and 8.5 days. Hemolymph was collected into 10 μl capillary tubes from cut antennae or legs and drained into an Eppendorf tube containing 100 μl ice-cold methanol. Samples were held at -15°C until assayed.

8.1. ECDYSTEROID QUANTIFICATION WITH RADIOIMMUNOASSAY

Hemolymph samples were extracted three times with 100% methanol (100 μl each time) at room temperature. Cumulated supernatants were evaporated in vacuum centrifuge, the desiccant was dissolved in 300 μl methanol and divided into two or more aliquotes. After methanol evaporation, the samples were taken for radioimmunoassay (RIA) performed according to Chang and O'Connor (1979) with [23,24-^{3}H]ecdysone (NEN Research Products, Boston, MA) as ligand. Two polyclonal rabbit antisera were used. Antiserum H-22, prepared with 22-hemisuccinate of ecdysone (Warren and Gilbert, 1986) was used in dilution 1:700; standard curve was routinely generated with 0.02-4 ng 20-hydroxyecdysone (20E) and the results expressed in 20E equivalents. Affinity of this antiserum to 20E was in our tests 1.5x, and to makisterone A (MaA) 5x lower than to ecdysone (E). Antiserum 85-B/L2, which had been prepared by Dr. J.-P. Delbecque with ecdysone 2/3 hemisuccinate, was diluted 1:30.000 and used to quantify ecdysteroids in E equivalents using a standard curve prepared with 0.01-2 ng E; antiserum affinity to 20E was 16x, and to MaA > 100x lower than to E.

8.2. ECDYSTEROID SEPARATION WITH TLC AND HPLC

Methanolic extracts of 200 μl hemolymph from the 2nd day larvae of the penultimate, and 6th day larvae of the last instar were taken for ecdysteroid characterization by thin layer chromatography (TLC), and similar extracts from 60-120 μl hemolymph for the separation by high pressure liquid chromatography (HPLC). E, 20E, MaA, 2-deoxyecdysone (2dE), and 26-hydroxyecdysone (26E) were used as standards. Pre-coated TLC silica plates 60F-254, 10x20 cm (Merck, Darmstadt, Germany) were developed twice with chloroform-methanol system (80:20, v/v) and the position of standards was then identified in UV light. Separated ecdysteroids were eluted from 0.5 cm wide bands of silica with methanol and quantified with RIA. Separations by reverse phase HPLC (Merck-Hitachi D-6000 chromatography system) were carried out on the LiChrosphere 100RP-18 column (15cm x 4mm i.d., 4 μm, Merck) and monitored at 242 nm. In pilot experiments we found that all RIA-positive material occurred in 30 fractions eluted with 15% acetonitrile in 0.1% trifluoroacetic acid water solution (flow rate 1 ml/min). This procedure was used routinely and only in some experiments the columns was first eluted for 30 min isocratically with 14% acetonitrile and then for 30 min with linear gradient of 14-80% acetonitrile. All fractions were individually analyzed with RIA: highly polar products and 20E fraction with the H-22 antiserum and 20E as standard; the E fraction with 85-B/L2 antiserum and E as standard; and fraction behaving as MaA was measured with H-22 antiserum and MaA as standard.

8.3. HYDROLYSIS OF ECDYSTEROID CONJUGATES

By TLC and HPLC we found that hemolymoph contains a small fraction of highly polar products (HPP) that are recognized by the H-22 antiserum. A sample of HPP cumulated from several HPLC analyses and corresponding approximatively to 500 μl fresh hemolymph was dried and then dissolved in 1 ml sodium acetate buffer (50mM, pH 5.1) containing 1 mg type H-1 arylsulphatase/ -glucuronidase from *Helix pomatia* (Sigma, St. Louis, MO) and 1 mg type II acid phosphatase from potatoes (Sigma). The sample was incubated at 37°C for 24 h. Hydrolysis was terminated with 1 ml methanol, the methanolic extract was evaporated and taken again for HPLC and RIA.

9. DATA ANALYSIS

The results were analysed using analysis of variance (ANOVA) followed by Least Significant Difference test (LSD) at $P < 0.05$ using SAS (1987).

CHAPTER I
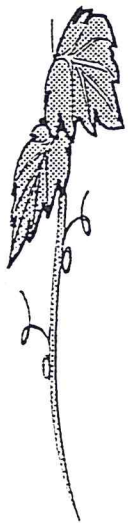
CHAPTER I

CORPUS ALLATUM VOLUME AND JUVENILE HORMONE TITER

INTRODUCTION

Locust show density dependent polymorphism; under crowding, or under isolation, they respectively develop characteristics of the "gregarious" or of the "solitary" phases (Uvarov, 1966). The recent locust plague (Skaf, 1990) rekindled interest in locust phase transformation.

As generally recognized now, the phase mechanism did not provide a key for the solution of problems concerning mass multiplication (Pener, 1991). The conspicuous polymorphism, however, remained with a problem of physiological interest. Since phase transformation affects a number of characters involved in metamorphosis as well as development, e.g. pigmentation and morphometry, the possibility should be invisaged that both phenomena may have a common control by neuroendocrine processes. Also, changes in behavior and pheromone production are the expression of changes in more fundamental physiological processes.

Involvement of endocrine factors in the regulation of locust phase transformation was extensively investigated and reviewed (Pener, 1983; 1990; Hardie and Lees, 1985; Dale and Tobe, 1990). Nevertheless, as has been repeatedly pointed out (Pener, 1991), no fully decisive and

revolutionary results on the endocrine control of phase changes. For example, in locust species which exhibit color changes in relation to density dependent phase polymorphism, implantation of active corpora allata (CA) into crowded gregarious hoppers induces green coloration. This effect had first been demonstrated in *Locusta migratoria* (Joly, 1954; Joly *et al.*, 1956; Joly, 1960; Staal, 1961). The same principal effect was observed in *Schistocerca gregaria* (Novak and Ellis, 1967; Ellis and Novak, 1971). Administration of Juvenile hormone (JH) or its analogs also induced green color in both species (Joly and Meyer, 1970; Nemec *et al.* 1970; Roussel, 1976).

On the other hand, by using a radiochemical assay *in vitro*, Injeyan and Tobe (1981b) reported that JH biosynthetic activity of the CA was higher in isolated than in crowded penultimate and last-instar female hoppers of *S. gregaria*. In the same study, the activity of the CA was found to be slightly lower in crowded than in isolated adult females, but major differences were temporal. Whereas, JH biosynthetic activity of the CA was similar in crowded and isolated *Locusta* females within the first 5-6 days after fledging, but on day 8 gland activity was much higher in isolated locusts (Dale and Tobe, 1986). Furthermore, employing the *Galleria* bioassay, Joly and Joly (1974) and Joly *et al.* (1977) showed that JH titers in the hemolymph of fouth- and fifth-instar hoppers as well as adult females are higher in isolated than in crowded *Locusta*.

It seems, therefore, that in spite of the vast literature we know only some, perhaps just minor aspects of the endocrine events (CA/JH)

which may control locust phase polymorphism. Most information was based on the effect of implantation of the CA, administration of JH, or measuriw the biosynthetic activity of the CA.

In fact the problem of physiological determination of locust phases cannot be explained on the basis only of extirpation or implantation of the CA, or even by measuring the biosynthetic activity of the CA. Therefore, this study has been undertaken to investigate in more detail the differences in the CA volume and the titer of hemolymph JH in the solitary and gregarious phases of the desert locust, *S. gregaria* in relation to phase changes and pheromone production.

RESULTS

CHANGES IN THE CORPUS ALLATUM VOLUME IN THE PENULTIMATE AND LAST LARVAL INSTARS

The CA of the desert locust, *S. gregaria*, are endocrine glands of globular or ellipsoid form. They are found on both sides of the foregut (oesophagus).

In the solitary and gregarious penultimate instar male larvae, the CA volume increased slightly during the first two days after molting and reached a maximum at day 4 (Fig. 2). On the other hand, in last instar larvae of solitary males the CA volume increased slightly, reaching the first peak at day 3 and then the second peak at day 6 after molting. In last instar larvae of gregarious males, on the other hand, the volume of the CA increased gradually, reaching a peak at day 5 after molting (Fig. 2). Generally, there was no significant difference ($P > 0.4$) in the CA volume between the solitary and gregarious males during the last two stadia.

In penultimate instar larvae of gregarious females, the CA volume increased gradually, reaching a maximum at day 3 after molting. Whereas, in the gregarious penultimate instar female larvae, the CA volume reached a peak at day 2 after molting (Fig. 3). The volume of the CA in the last larval instar of the solitary females showed a small peak at day 3 and reached a maximum at day 6 after molting. In gregarious last instar femwe larvae, the CA volume increased gradually reaching a maximum at day 5 after molting (Fig. 3). The CA volumes

of the solitary females were significantly larger ($P < 0.05$) than those of gregarious females during the last two nymphal instars.

CHANGES IN THE CORPUS ALLATUM VOLUME IN ADULT MALES

In gregarious adult males, the CA volume increased after adult emergence, to a maximum at day 12 and then decreased. On the other hand, in solitary adult males, the CA volume slightly increased after emergence, reaching a small peak at day 8 and then decreased. Thereafter, the CA volume increased, reaching a maximum at day 22 after adult emergence (Fig. 4).

As shown in Fig. 4, the CA volumes were larger in gregarious adult males than in solitary males during a period from day 2 to day 18. On the other hand, between days 20-24, the CA volume in solitary males were larger than those of their gregarious counterparts.

CHANGES IN THE CORPUS ALLATUM VOLUME IN ADULT FEMALES DURING THE FIRST AND SECOND GONOTROPHIC CYCLES

An almost identical variation in the volumes of the CA was found in solitary and gregarious adult females. The CA volume of the solitary and gregarious females varied cyclically in relation to the growth of the oocytes. Fig. 5 shows that in both phases during the first gonotrophic cycle, the maximum length of the basal oocyte peaked just after the first peak of the CA volume. However, in gregarious females during

the second gonotrophic cycle the CA volume coincided with the maximum length of the basal oocytes. In contrast, in the solitary females the second peak of the CA volume peaked just before the maximum length of the basal oocytes during the second gonotrophic cycle (Fig. 5). In general, the volumes of the CA were slightly larger in solitary adult females ($P > 0.05$) than those of their gregarious counterparts.

IDENTIFICATION AND QUANTIFICATION OF JUVENILE HORMONE IN THE ADULT STAGE

GCMS analysis confirmed that the JH III was the only JH detected in the hemolymph of the desert locust, *S. gregaria*. In gregarious adult males, the JH titer was very high (197 nmol/ml) at day 10, very low titer (26 nmol/ml) at day 20, and then increased again to 101 nmol/ml at day 30 after fledging. In the solitary adult males, on the other hand, the JH titer was very low (12 nmol/ml) at day 10 and then fluctuated between 46-67 nmol/ml at day 30 and day 20, respectively (Fig. 6).

The JH titer at different ages in adult females for both the solitary and gregarious phases are shown in Fig. 7. The JH titers of gregarious females were generally higher than those of their solitary counterparts.

VOLATILES ANALYSES AND THE RELATIONSHIPS BETWEEN THE CA VOLUME, JH TITER AND PHEROMONE PRODUCTION

Gas chromatographic analysis of trapped volatiles from the different groups of adults confirmed that aged gregarious males (over 10 days after fledging) liberate 6 aromatic compounds, viz., anisole, benzaldehyde, veratrole, guaiacol, phenylacetonitrile and phenol. Maximal production was detected on day 18, with phenilacetonitrile comprising 80-85 % of the total volatile. Listed compounds are liberated only in low and pheromonally inactive amounts by young males of either phases and females of either phases and any age (Figs. 4 and 8). In the gregarious adult males the maximum titer of pheromone production coincides with a specific titer of hemolymph JH (threshold) and with a reduction in the CA volume (Figs. 4 and 6).
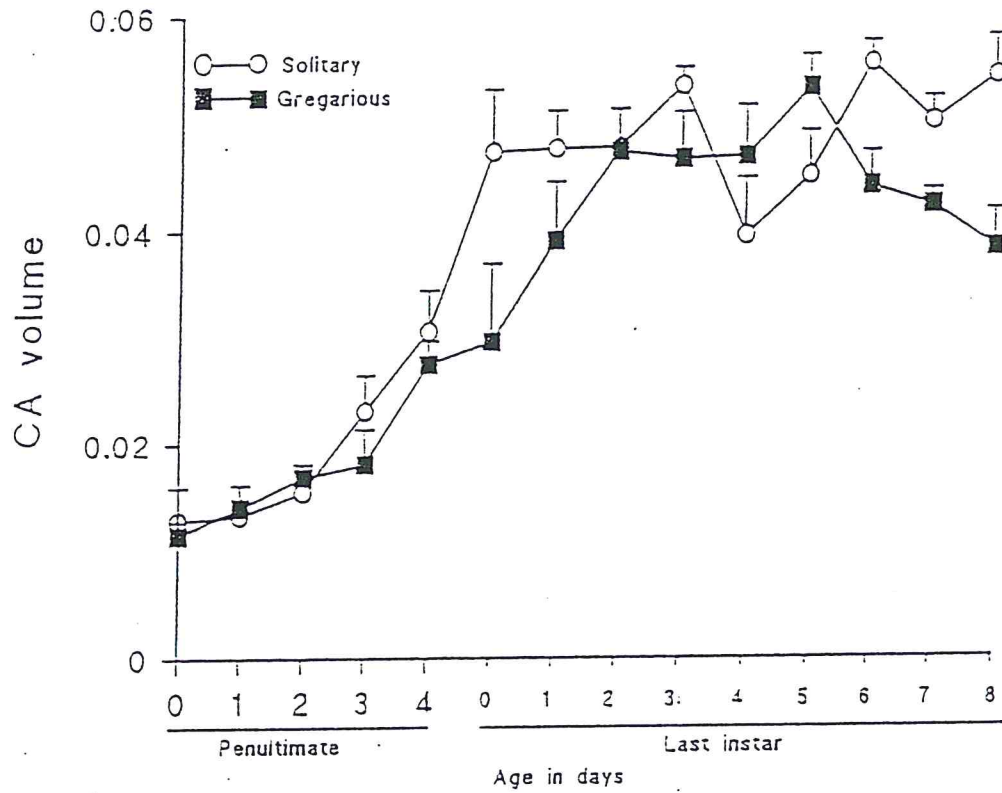
Fig. 2.   Changes in mean volume of the CA of solitary and gregarious males during penultimate and last stadia.
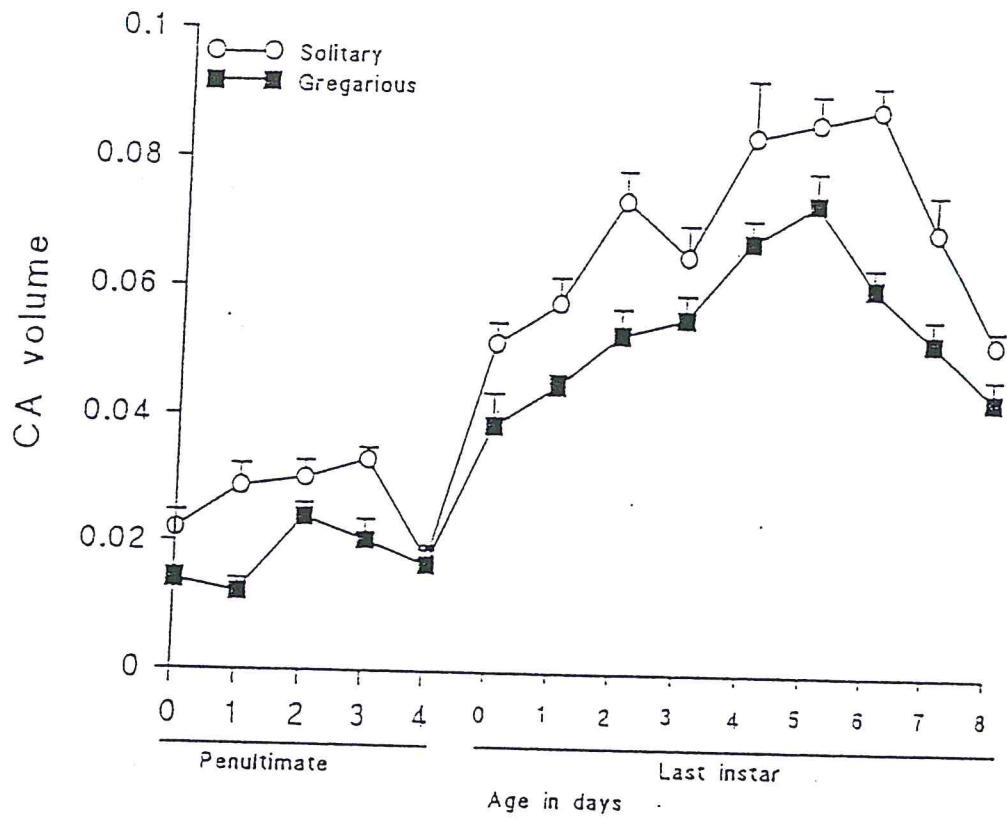
Fig. 3.   Changes in mean volume of the CA of solitary and gregarious females during penultimate and last stadia.
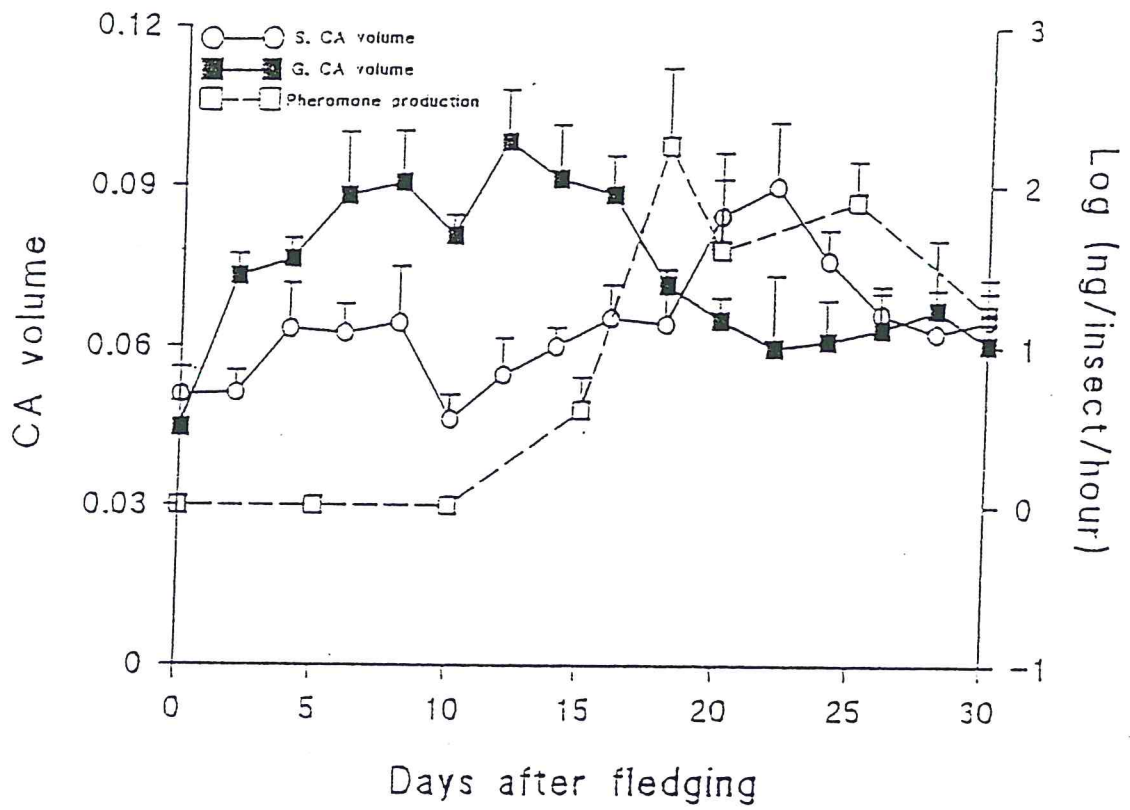
Fig. 4.   Changes in mean volume of the CA of solitary and gregarious adult males, in relation to pheromone production.
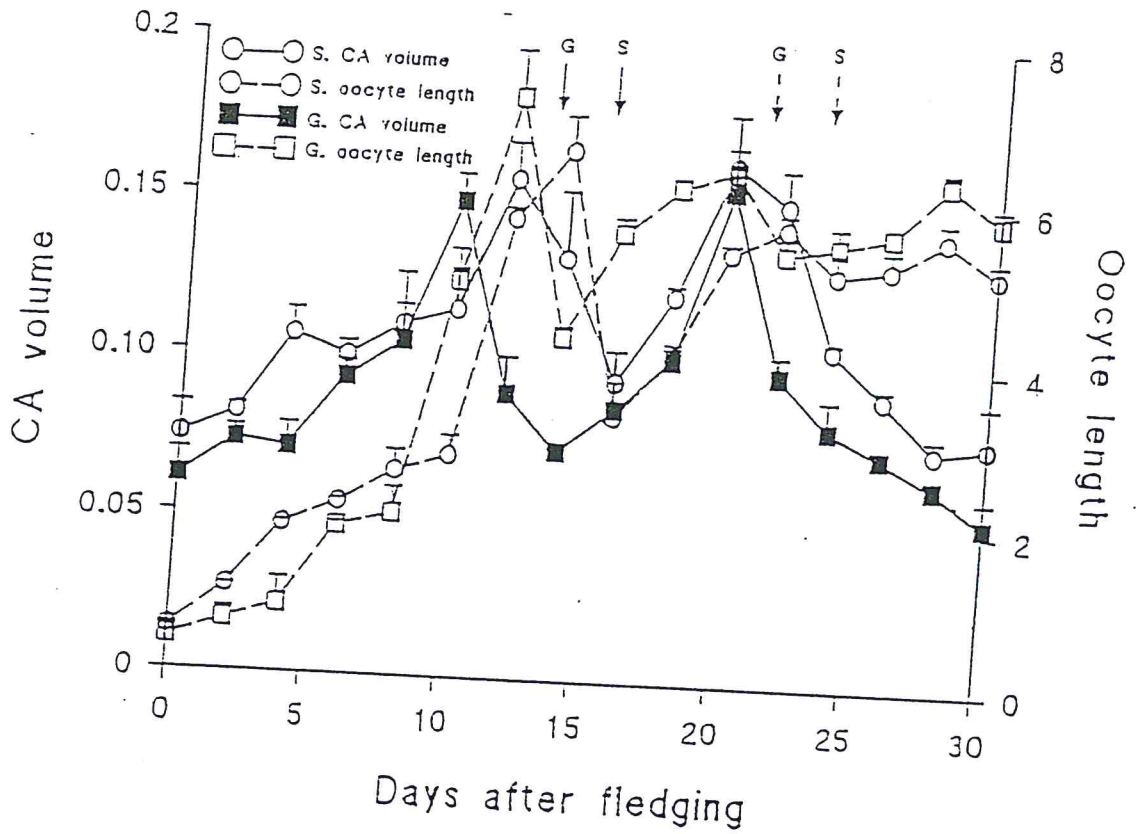
Fig. 5. Changes in mean volume of the CA of solitary and gregarious adult females, in relation to oocyte growth during the first and second gonotrophic cycles. Arrows indicate time of oviposition in the solitary (S) and gregarious (G) females.
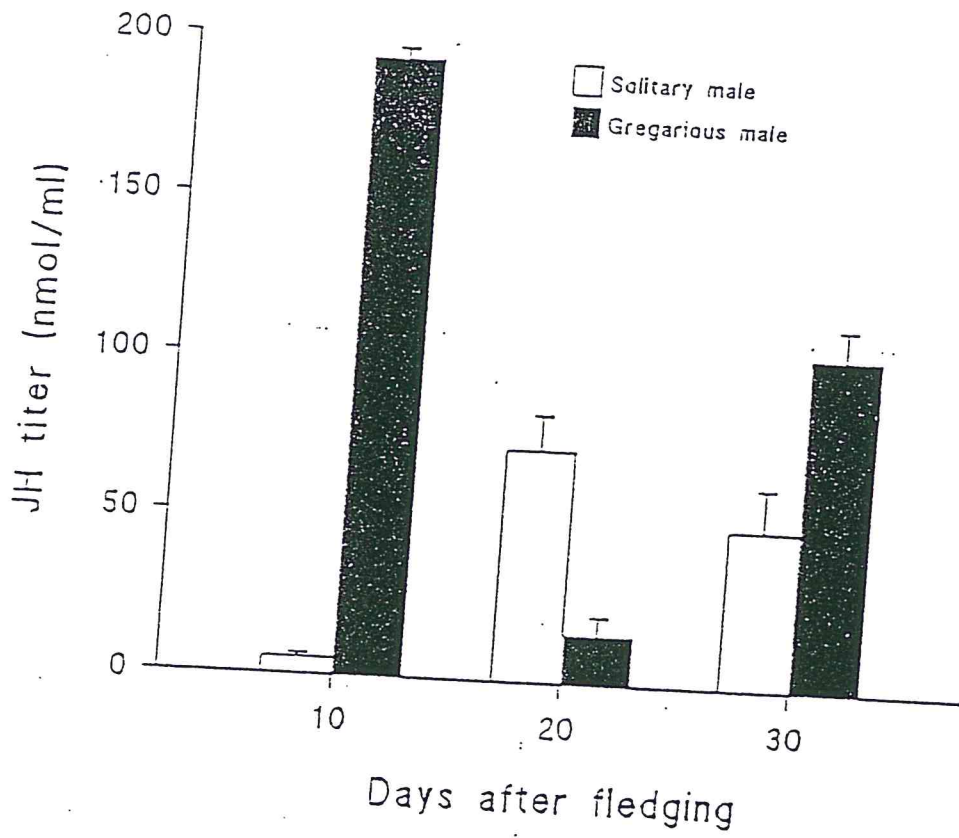
Fig. 6. JH titer in the hemolymph of the solitary and gregarious adult males.
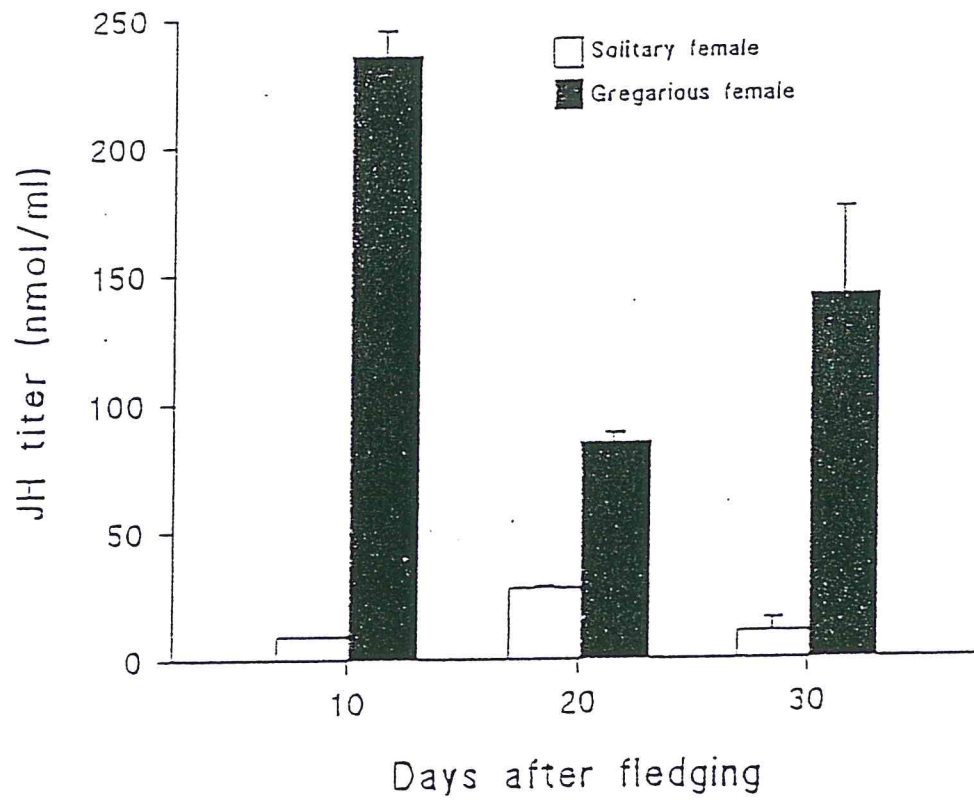
Fig. 7.   JH titer in the hemolymph of the solitary and gregarious adult females.
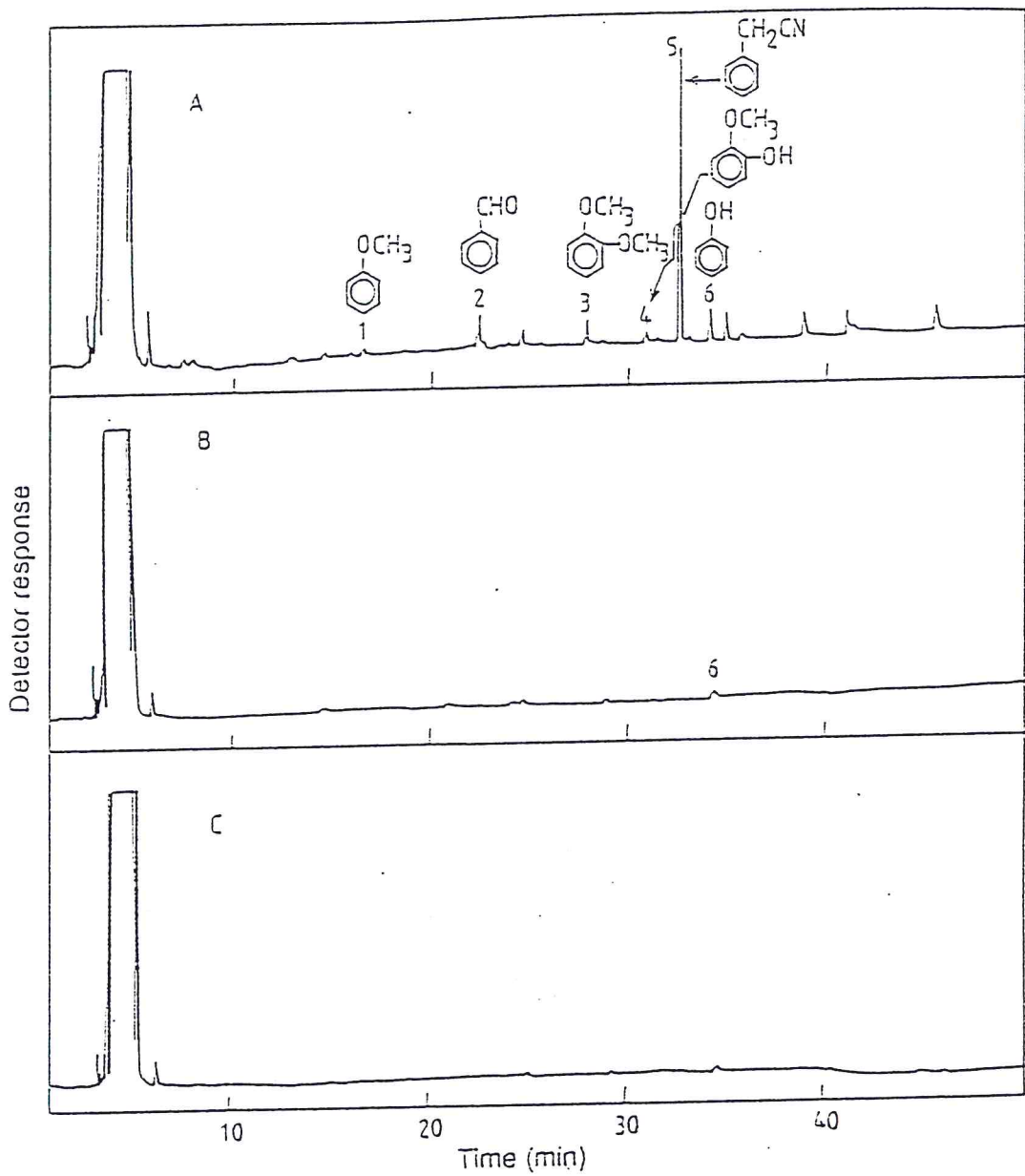
Fig. 8. Gas chromatograms of air-borne volatiles collected from 18-day-old gregarious adult males (A), 10-day-old gregarious adult males (B) and 18-day-old solitary adult males (C).

DISCUSSION

The results of the present study show that the corpora allata (CA) of the solitary females are larger in volume than those of gregarious females, *S. gregaria*. Also, Staal (1961) found that the CA volumes of isolated fifth instar larvae of *L. migratoria* were significantly larger than those of crowded ones under certain experimental conditions. In addition, in the desert locust, *S. gregaria*, Injeyan and Tobe (1981b) reported that the CA volume of solitary females was generally larger than those of gregarious females. This was particularly evident in the first half of the last nymphal instar and throughout the first gonotophic cycle. It could be suggested that the relatively large CA volume of the solitary females is correlated with their characteristically larger body size (Uvarov, 1966).

Our results confirm that cyclic changes in the volume of the CA do occur during the first two gonotrophic cycles. Such changes have been observed previously in female, *S. gregaria* (Highnam, 1962; Highnam *et al.*, 1963; Tobe and Pratt, 1975a&b; Injeyan and Tobe, 1981b), in female *L. migratoria* (Highnam and Haskell, 1964) as well as in other acridids (Schmidt and Othman, 1993) and apparently correlate well with the gonotrophic cycles.

However, it is clear from the present results that at least in adult female *S. gregaria*, the volume of the CA is not a good indicator of the JH titer in the hemolymph. These results confirm and extend the findings of Tobe and Pratt (1975b), and Injeyan and Tobe (1981b), that

volume changes *per se* are not a valid indicator of gland activity in adult female *S. gregaria*. On the other hand, in *L. migratoria*, Rembold (1981), and Dale and Tobe (1986), found that the activity of the CA and their volume are related to the length of the terminal oocytes, and thus to egg production. An increase in the CA size in relation to growth of oocytes and an increase in the JH titer in the hemolymph were reported in *L. migratoria* by Ferenz and Kaufner (1981), in *Aiolopus thalassinus* by Schmidt and Othman (1993), and in *Nauphoeta cinerea* by Lanzrein *et al.* (1985).

There is a definite increase in the CA volume of gregarious adult male *S. gregaria* from the value immediately after adult emergence to a maximum value between the 12th and the 16th days; then there is a tendency to decrease. A similar change in the CA volume has been observed previously in gregarious adult male *S. gregaria* by Odhiambo (1966). On the other hand, Loher (1961) suggested that pheromone production from the vaculated epidermal cells of the desert locust is under the control of corpora allata. Tawfik *et al.* (1994) quantified pheromone production and confirmed that its rate is related to the corpora allata volume. Consistently with these findings, Amerasinghe (1978) showed that exogenous juvenile hormone stimulates yellowing in crowded allatectomized males and apparently also induces production of the maturation pheromone.

The fact that we observed the presence of only JH III in the hemolymph of the solitary and gregarious adult *S. gregaria*, agrees well with previous findings which have shown that when reliable

physicochemical analyses are employed neither JH I nor JH II was detected in *Locusta* (Huibregtse-Minderhoud *et al.*, 1980; Dale and Tobe, 1986), in *Schistocerca* (Blight and Wenham, 1976a&b; Trautmann *et al.*, 1976) or in other orthopteroid insects (Loher *et al.*, 1983; Baker *et al.*, 1984; Strambi *et al.*, 1984; Schmidt and Othman, 1993). In contrast, using radioimmuniassay, Baehr *et al.* (1979), and Fuzeau-Braesch *et al.* (1982), found that the hemolymph of *Locusta* contained JH I, II and III, but these results could not be confirmed either by using our analytical procedure (Rembold and Lackner, 1985), or by using a GLC with electron capture detector (ECD) (Huibregtse-Minderhoud *et al.*, 1980).

The juvenile hormone titer values presented here for gregarious adult female *S. gregaria* are within the same range as that of the values reported by previous investigators using physicochemical methods (Hubregtse-Minderhoud *et al.*, 1980; Bergot *et al.*, 1981b) although these authors assayed titer in crowded adult females 10-11 and 18 days after fledging respectively.

Our results on JH III in *S. gregaria* show that the JH hemolymph titer in gregarious adult females was higher than that of their solitary counterparts. Contrarily, Injeyan and Tobe (1981b) reported that in adult female *S. gregaria* there was a temporal difference between the CA activities of gregarious and solitary locusts, the later exhibiting relatively higher rates of JH synthesis early in the first gonotrophic cycle. In addition, Dale and Tobe (1986) found that no significant difference in the rates of JH biosynthesis by CA from isolated and

crowded adult female *L. migratoria*, from newly molted to 6 days after fledging. On day 8, however, the rates of JH biosynthesis of CA from isolated females were very high and were significantly greater than those of CA from crowded females. As no data were presented for older females, the difference found in the 8-day-old females (Dale and Tobe, 1986) may be related to the shorter maturation time of isolated *L. migratoria* adult female (Norris, 1950; Pener, 1976). Joly and Joly (1974), and Joly *et al.* (1977), have also observed that JH titers increased much more rapidly with age in isolated young *L. migratoria* adult female than in crowded ones. On the other hand, Fuzeau-Braesch *et al.* (1982), reported that no clear differences in the JH titers were found between isolated and crowded adults in this species.

Oocyte development strictly depends on the CA and JH in locusts (Joly, 1960; Highnam *et al.*, 1963; Roussel, 1975; 1976) as well as in other acridids (Engelman, 1983). The period elapsing between fledging and sexual maturation is shorter in crowded than in isolated female *Schistocerca* (Norris, 1952; Papillon, 1968; Injeyan and Tobe, 1981b). Thus, the differences in the JH titer between gregarious and solitary adult female, *S. gregaria* may reflect density-dependent changes in the rate of sexual maturation. It is also possible that such a difference in JH titer may simply be another physiological parameter responding to the conditions of isolation or crowding.

A major behavioral difference between solitary and gregarious adult locusts in the field is that migratory group flights are displayed by the later. On the other hand, laboratory studies of flight performance

of *S. gregaria* confirmed that crowded adults fly much more intensely than isolated ones (Michel, 1970a&b; 1980a&b). Allatectomy of both *Locusta* (Wajc and Pener, 1971) and *S. gregaria* (Odhiambo, 1966; Goldsworthy *et al.*, 1972) caused a decrease in locomotory and flight activity which was reversed by implantation of active CA. Therefore, the difference in the JH titer in the hemolymph between solitary and gregarious phases may play a role in both flight and general activity.

Finally, data presented in this chapter regarding the JH titer in the hemolymph of gregarious adult male, *S. gregaria* strongly suggested that the pheromone biosynthesis and emission are controlled by a specific titer of hemolymph JH (threshold). However, Rankin and Riddiford (1978) concluded that lower JH titres normally stimulate migratory flight in the prereproductive adult *Oncopeltus fasciatus*. Also they reported that the CA in this bug coordinates migration and reproduction in response to the environmental cues of photoperiod, temperature and food quality. Thus, in the desert locust, *S. gregaria* certain stimuli or events (density, environmental factors, food,...) may trigger pheromone production via JH action.

CHAPTER II
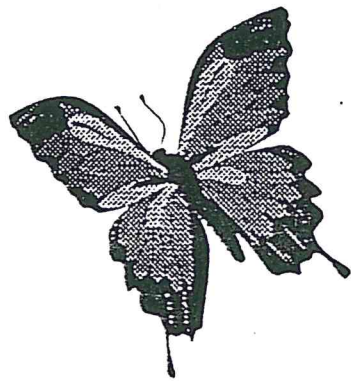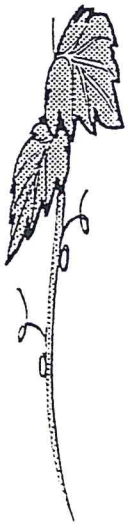
CHAPTER II

THE EFFECTS OF EXOGENOUS JUVENILE HORMONE
(JH III) AND ANTI-JH (PRECOCENE II)

INTRODUCTION

Locusts appear in two forms or "phases", gregarious and solitary, which differ in many features, collectively termed "phase characteristics". Locust phase polymorphism is continuous in a sense that intermediate forms exist between the extreme phases. *Schistocerca gregaria* usually lives in arid habitats (recession areas) where there is little or no farmland or pastures. Under certain conditions, especially increased rainfall and vegetation, a mass multiplication can occur leading to a shift from the solitary to the gregarious phase. In correlation with the population density, color, size, several morphological parameters and behavior change drastically. Whereas, the gregarious *S. gregaria* are considered the world's most destructive insect pest (Walsh, 1988), the non-migratory solitary phase is of no economic importance. Therefore, the internal factors which regulate the transition from a solitary to a gregarious state and then maintain the gregarious phase are of considerable interest.

The involvement of endocrine factors (as an internal factor) in the regulation of locust phase transformation has been extensively investigated and reviewed (Pener, 1983; 1991; Dal and Tobe, 1990). The corpora allata (CA) and their product, the juvenile hormone (JH)

undoubtedly affect some phase characteristics and may play a role in locust phase change. For example, implantation of extra CA or administration of JH or JH analogs, to crowded hoppers induces green color which is concedered as a solitary characteristic. This effect was demonstrated in *Locusta* (Joly, 1960; Staal, 1961; Roussel, 1975; 1976; Couillaud *et al.,* 1987), *Schistocerca* (Novak and Ellis, 1967; Langewald and Schmutterer, 1995), and in other grasshoppers (Rowell, 1967). On the other hand, Joly and Joly (1954) and Joly (1956; 1962) reported that implantation of extra CA into crowded *Locusta* hoppers results in a decrease of the E/F (length of elytron/length of hind femur) ratio. They even obtained "hypersolitary" values. Also, allatectomy of the gregarious adult male results in complete absence of the yellow color, but reimplantation of CA or administration of JH restores yellowing (Loher, 1961; Pener and Lazarovici, 1979). The effects of endocrine factors in locust coloration have usually been investigated only at the visible colors (Nijhout and Wheeler, 1982; Hardie and Lees, 1985; Tanaka, 1993; Tanaka and Pener, 1994), and very little is known about the effects of endocrine factors on the hemolymph pigments. Thus, in the present study we investigate the effects of exogenous JH and precocene on the hemolymph pigments of gregarious desert locust.

The mediation of chemical stimuli in the aggregation of gregarious locusts was first recognized by Nolte (1963) and later by Gillett (1968), who demonstrated that the grouping behavior of nymphs and adults of *S. gregaria* reared in visual and tactile isolation was influenced significantly by the action of an airborne factor. Moreover,

Obeng-Ofori *et al.* (1993) demonstrated the existence of two distinct sets of releaser pheromones that appeared to modulate the aggregation behavior of the two stages of the gregarious desert locust: a juvenile aggregation pheromone produced by nymphs and specific to nymphal stages and adult pheromone produced by older adults and specific to the adult stage. Recently, Torto *et al.* (1994) showed that the aggregation pheromone system of adults consists of phenylacetonitrile (benzyl cyanide), which is the dominant component, present in as much as 75-85 % in the volatile emission of older males along with benzaldehyde, guaiacol, and phenol. In spite of this very important results concerning the identification of the aggregation pheromone systems in the desert locust, nothing is known about the interaction between pheromones and hormones in phase dynamics of the desert locust, *S. gregaria*.

So far, five juvenile hormones (JH 0, JH I, 4-methyl-JH I, JH II and JH III) have been identified in insects. They differ in the number of methyl groups. Results by Baehr *et al.* (1979) showed that the haemolymph of *Locusta migratoria* contain JH I, II and III. Also, Fuzeau-Braesch *et al.* (1982) reported JH I and JH II in *L. migratoria*. However, when more reliable physicochemical analyses were employed, both *Schistocerca and Locusta* had exclusively JH III, and no JH I or JH II can be detected (Huibregts-Minderhoud *et al.*, 1980; Rembold *et al.*, 1980; Bergot *et al.*, 1981a&b; Mauchamp *et al.*, 1985), or in other orthopteroid insects (Loher *et al.*, 1983; Baker *et al.*, 1984). As already discussed by Schooley *et al.* (1984), the

occurrence of JH I, II and 0 has been demonstrawd convincingly only in lepidopteran species. On the other hand, Bowers *et al.* (1976) reported that certain chromene derivatives from the plant *Ageratum houstonianum* induce symptoms of JH-deficiency in the large milkweed bug, *Oncopeltus fasciatus* as well as in acridids and other Hemipteran species. These authors termed the active substances precocene I (PI) and precocene II (PII). Thus, we decided to use JH III and precocene II in the present study.

In locusts, pheromone communication may be regulated by the neuroendocrine system. Thus, hormones may regulate the biosynthesis or release of the pheromone. Nevertheless, no attempt has been made to investigate the effects of exogenous hormones on pheromone production of the desert locust. Therefore, the objective of the experiments described in the present chapter was to examine in more detail the effects of exogenous JH and anti-JH (precocene) on phase changes (external coloration and hemolymph pigments) and pheromone production of the gregarious desert locust, *S. gregaria*.

RESUITS

EFFECTS OF TOPICAL APPLICATION OF JH III

There was no significant difference ($P > 0.7$) in the onset and titer of pheromone emission between the adults treated with a single dose 50 μg of JH on the 4th day after adult emergence, and those of acetone and untreated control. When gregarious adults received a cumulative dose of 150 μg (3 x 50 μg) on the 3rd, 4th, and 5th days per insect, the onset of pheromone emission occurred on day 18 ($P > 0.1$), and the maximum titer was at day 20 after fledging. However, there was no significant difference ($P > 0.2$) in the titer of pheromone, mating and sexual and aggregation behavior between treated and control ones (Fig. 9A).

Similarly, topical application of a single dose of 50 μg of JH on the 4th day of the last nymphal instar had no significant effect ($P > 0.7$) on the onset and titer of pheromone emission, mating and sexual and aggregation behavior during adult stage (Fig. 9B). In contrast, a significant effect ($P > 0.001$) was observed in treated insects with a cumulative dose of 150 μg (3 x 50 μg) on the 3rd, 4th, and 5th days of the last nymphal instar. The onset of pheromone emission was at day 35 and the maximum emission was reached at day 45 after fledging. Also they started exhibiting sexual behavior and mating between 40-45 days after fledging, while normal adult males started exhibiting sexual behavior and mating between 13-18 days after the last molt. Further, a significant reduction of aggregation behavior and activity was

observed in treated insects during the adult stage in comparison to control insects. Treated males also showed a color change and acquired fading yellow color instead of the bright yellow color of normal gregarious adult males (Fig. 10). Generally, the effect of topical application of a single high dose of 150 μg on the 4th day of the last nymphal instar or the adult stage elicited no effect on the onset and titer of pheromone emission, mating and sexual and aggregation behavior in comparison to control ones.

EFFECTS OF TOPICAL APPLICATION OF P II

Figure 11A shows that topical application of a single dose of 50 μg of precocene II on the 4th day or a cumulative dose of 150 μg (3 x 50 μg) on the 3rd, 4th, and 5th days old adult, had no significant effect ($P > 0.7$) on the onset and titer of pheromone emission, mating and sexual and aggregation behavior.

A significant effect ($P > 0.001$) was observed in the onset and titer of pheromone emission in insects treated with a cumulative dose of 150 μg (3 x 50 μg) on the 3rd, 4th, and 5th days of the last nymphal instar; but not in those treated with a single dose of 50 μg on the 4th day. Insects treated with a cumulative dose of 150 μg started pheromone emission at day 20 and the maximum emission occurred 35 days after fledging. They started exhibiting sexual behavior and mating between 25-30 days after the last molt (Fig. 11B).

EFFECTS OF INJECTION OF JH III

Injection of a single dose of 50 μg of JH or even a higher dose of 150 μg to 4-day-old adults had no effect on the onset or titer of pheromone emission, mating and sexual and aggregation behavior. On the other hand, adults injected with a cumulative dose of 150 μg (3 x 50 μg) of JH on the 3rd, 4th and 5th days of adult life started pheromone emission on day 18 (P > 0.1) and reached a maximum on day 25 from fledging. They started exhibiting sexual behavior and mating between 20-25 days after fledging (Fig. 12A).

Injection of a single dose of 50 μg of JH into 4-day-old last instar nymphs resulted in a little delay (P > 0.1) in the onset of pheromone emission (Fig. 12B). In this case the pheromone emission was observed on day 18 and reached a maximum on day 25 after fledging. They started exhibiting sexual behavior and mating between 20-25 days after the last molt. High mortality and deformed adults resulted from a cumulative dose of 150 μg (3 x 50 μg) or a single dose of 150 μg.

EFFECTS OF INJECTION OF P II

There was no significant difference (P > 0.7) in the onset and titer of pheromone emission, mating and sexual and aggregation behavior between locusts given a single dose of 50 or 150 μg of precocene II on the 4th day of adult life, and those of control ones. On the other hand, a cumulative dose of 150 μg (3 x 50 μg) given during the 3rd to 5th days of adult life had a significant effect (P > 0.0001) in the titer of pheromone emission. In this case, pheromone emission

started on day 18 (P > 0.1) and reached a maximum on day 25. Treated insects started exhibiting sexual behavior and mating between 20-25 days after fledging (Fig. 13A).

Injection of a single dose of 50 μg of precocene II to 4-day-old last instar nymphs had no significant effect (P > 0.7) on the onset and titer of pheromone emission, mating and sexual and aggregation behavior (Fig. 13B). However, a cumulative dose of 150 μg (3 x 50 μg) given during the 3rd to 5th days of the last instar nymphs or a single dose of 150 μg on the 4th day resulted in high mortality and deformed adults.

EFFECTS OF JUVENILE HORMONE IN THE VAPOR PHASE

Treatment of adults with 200 μg of JH had no effect (P > 0.8) on the onset and titer of pheromone emission, mating, and sexual and aggregation behavior. However, pheromone emission started on day 18 (P > 0.1) and reached a maximum on day 30 in those insects treated with 400 μg. They started exhibiting sexual behavior and mating between 20-25 days after fledging (Fig. 14A). On the other hand, there was a significant effect on the onset and titer of pheromone emission, mating and in sexual and aggregation behavior during adult stage between treated insects during the last nymphal instar with 200 μg or 400 μg and those of untreated control (Fig. 14B). Insects treated with 200 μg started pheromone emission on day 20 (P > 0.001), and the maximum titer (P > 0.01) occurred at 30 day after fledging. They

started exhibiting sexual behavior and mating between 25-30 days after the last molt. While a dose of 400 μg had the strongest effect, and adult males started emitting pheromone at day 40 (P > 0.0001), and the maximum titer (P > 0.001) observed at day 50. They started exhibiting sexual behavior and mating between 40-45 days after fledging. In treated insects a significant reduction of aggregation behavior and activity was observed during the adult stage in comparison to untreated control. Also, those insects showed a color change; They faded yellow color instead of bright yellow or very bright yellow of normal gregarious adult males.

EFFECTS OF JH AND ANTI-JH (PRECOCENE) ON THE HEMOLYMPH PIGMENT RATIO

There was a significant shift (P > 0.0001) in the hemolymph pigment ratio from gregarious to solitary phase in the last instar nymphs that had received a cumulative dose of 150 μg or 400 μg of JH III in the vapor phase compared to controls (Figs. 15 and 16). No significant effects were observed in the other treatments.
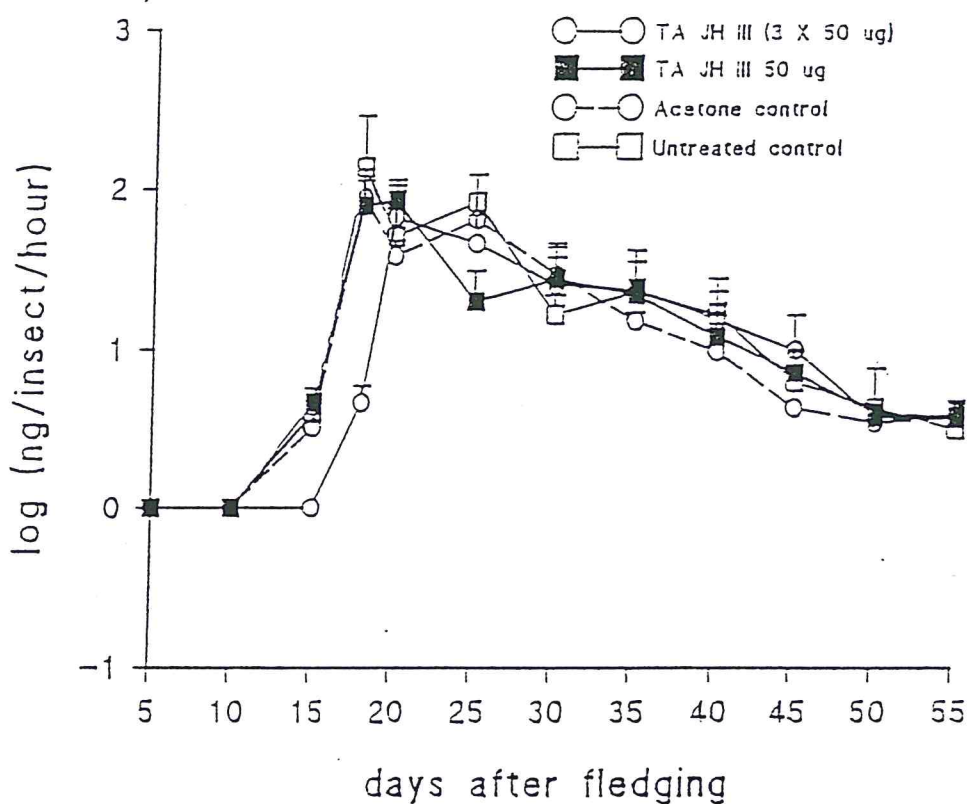
Fig. 9A. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated adults with topical applications of JH III.
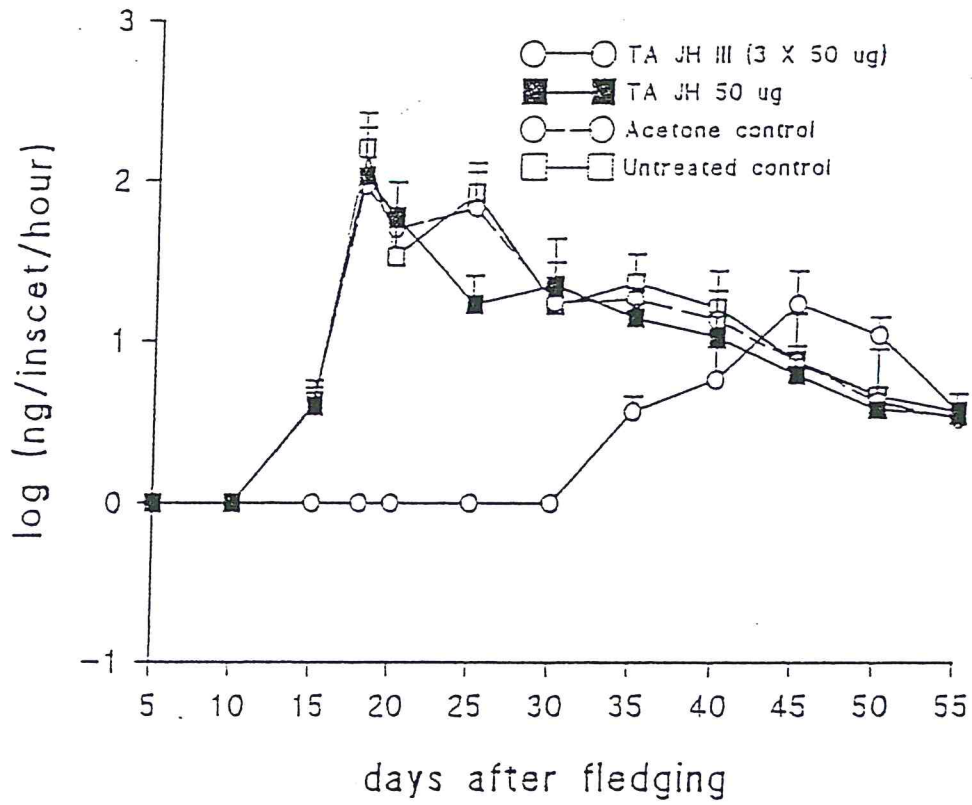
Fig. 9B. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated last instar nymphs with topical applications of JH III.
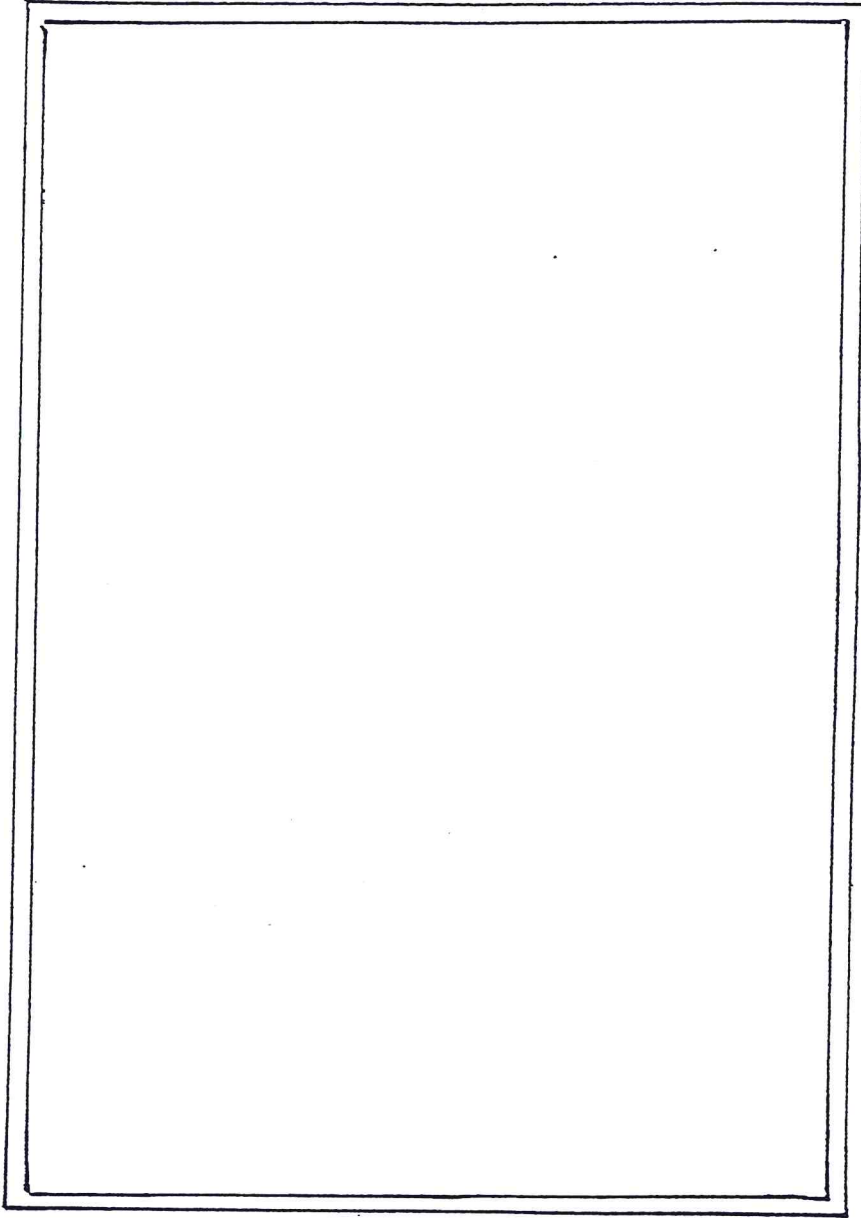
Fig. 10. 30-day-old adult male, gregarious control (A), solitary control (B) and treated with topical application of JH III (C).
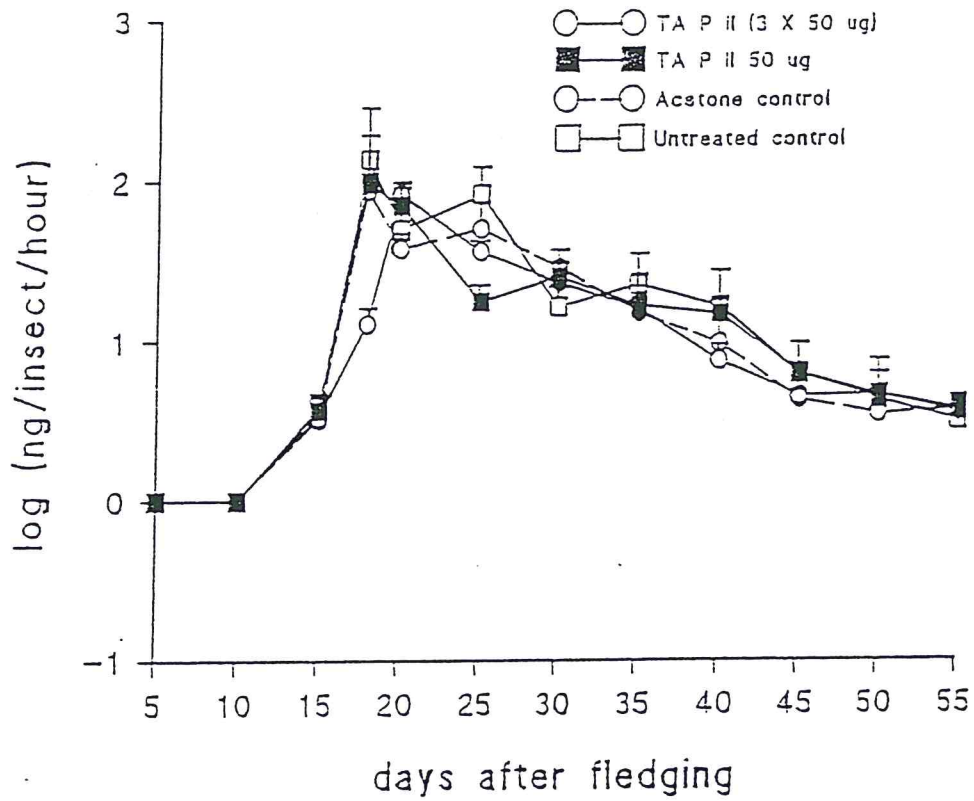
Fig. 11A. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated adults with topical applications of P II.
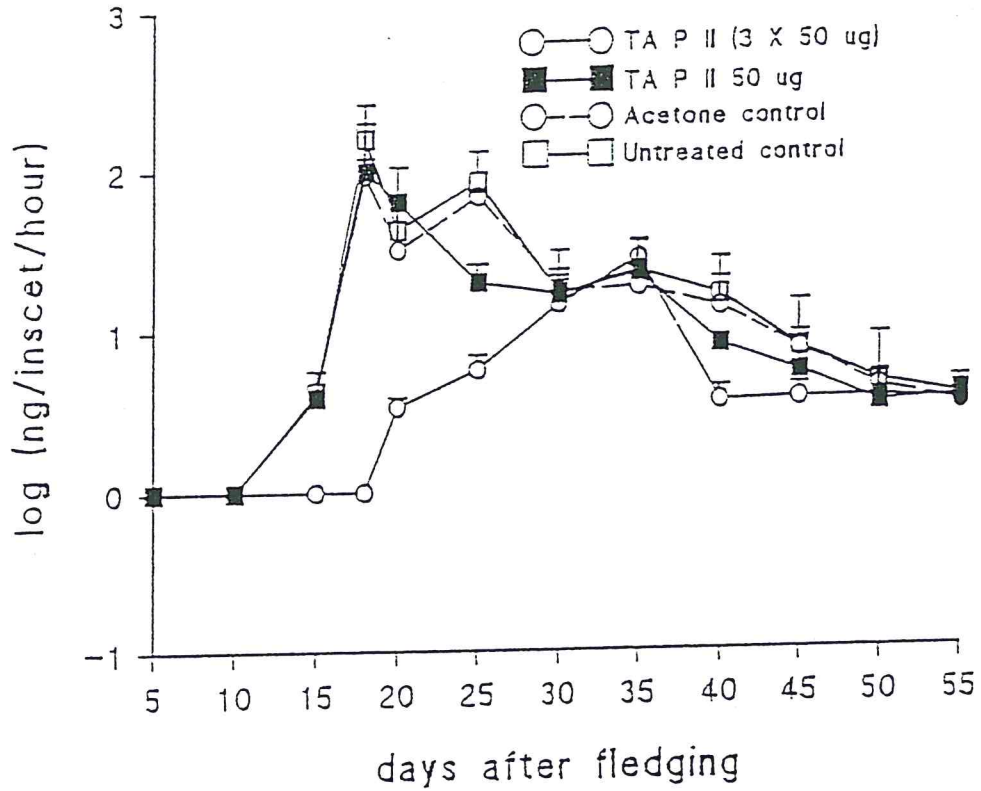
Fig. 11B. Log phenilacetonitrile (mean ± SE) reieased at different ages by adult males, from control and treated last instar nymphs with topical applications of P II.
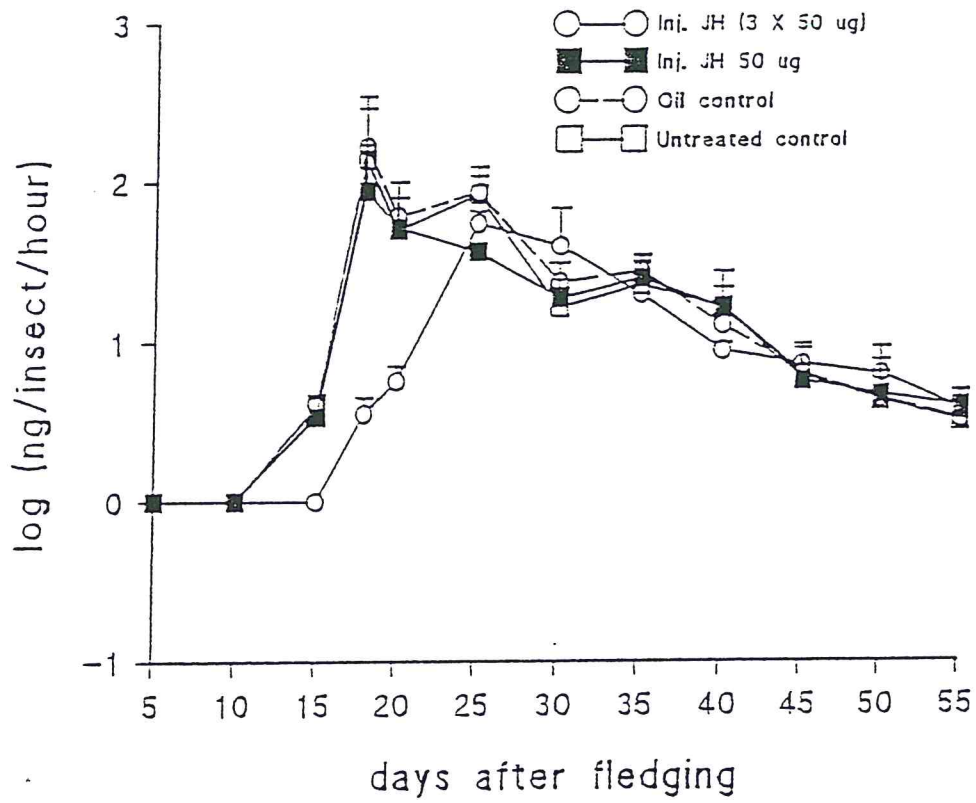
Fig. 12A. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated adults with injections of JH III.
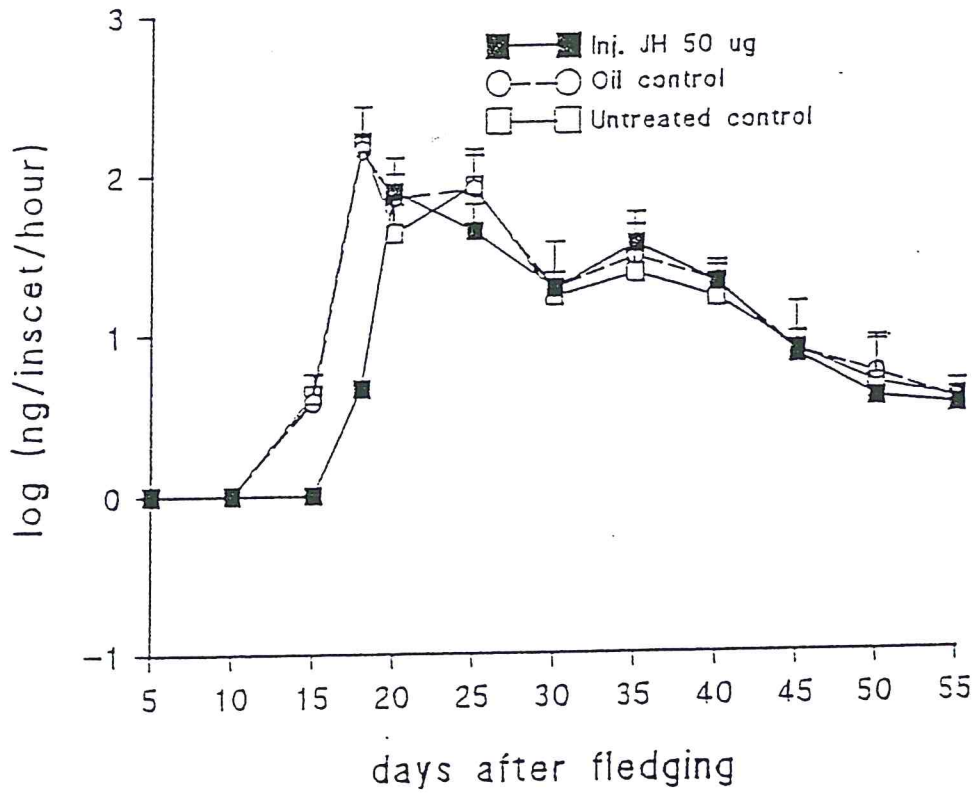
Fig. 12B. Log phenilacetonitrile (mean ± SE) released at different ages oy adult males, from control and treated last instar nymphs with injections of JH III.
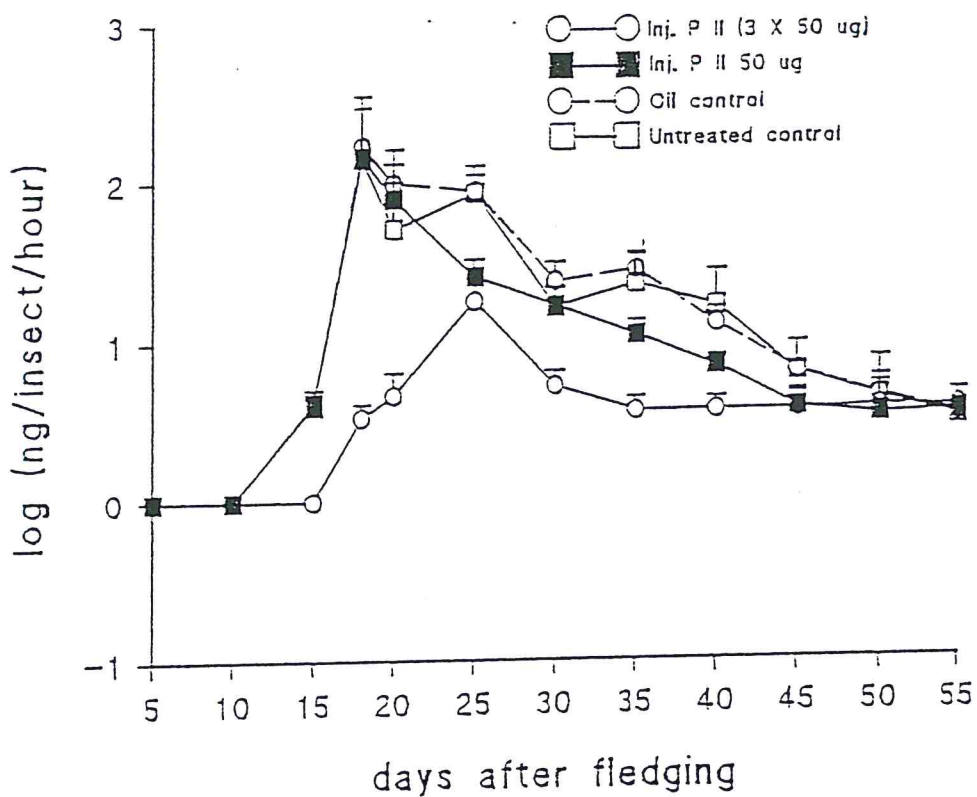
Fig. 13A. Log phenilacetonitrile (mean $\pm$ SE) released at different ages by adult males, from control and treated adults with injections of P II.
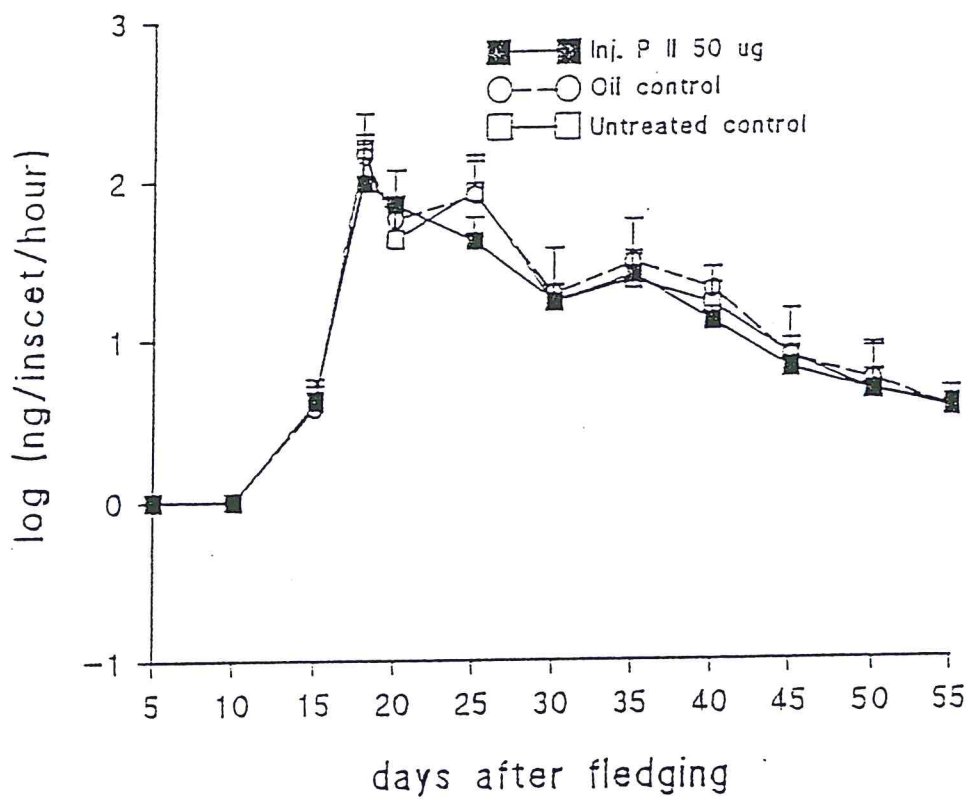
Fig. 13B. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated last instar nymphs with injections of P II.
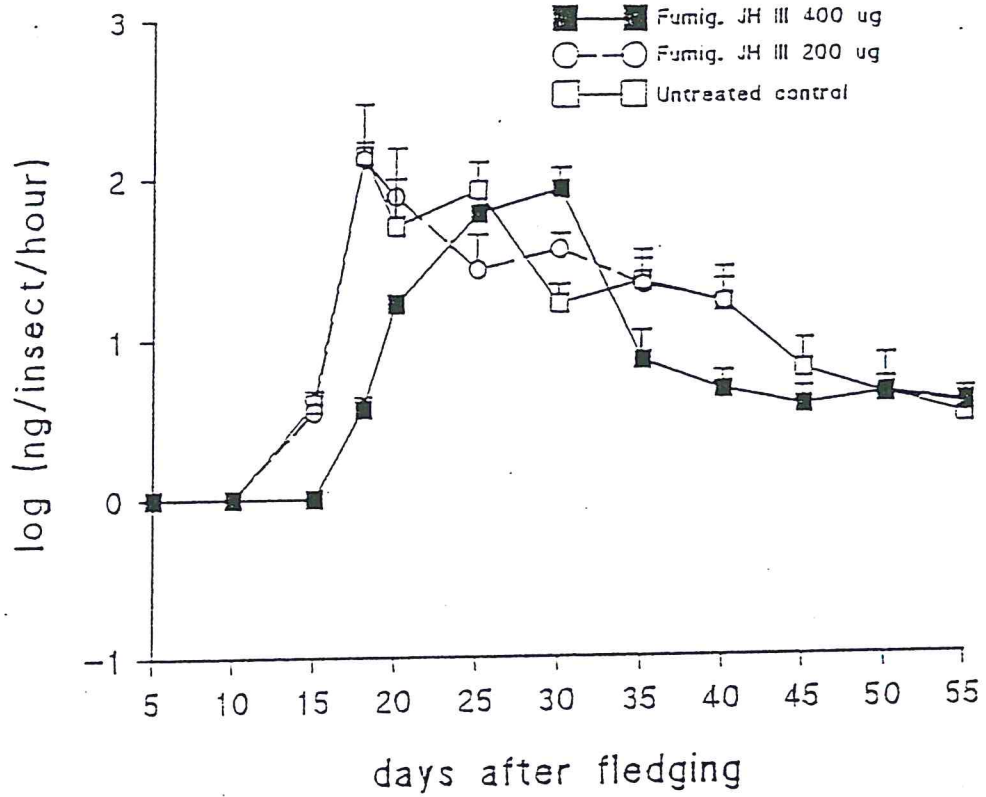
Fig. 14A. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated adults with JH III in the vapor phase.
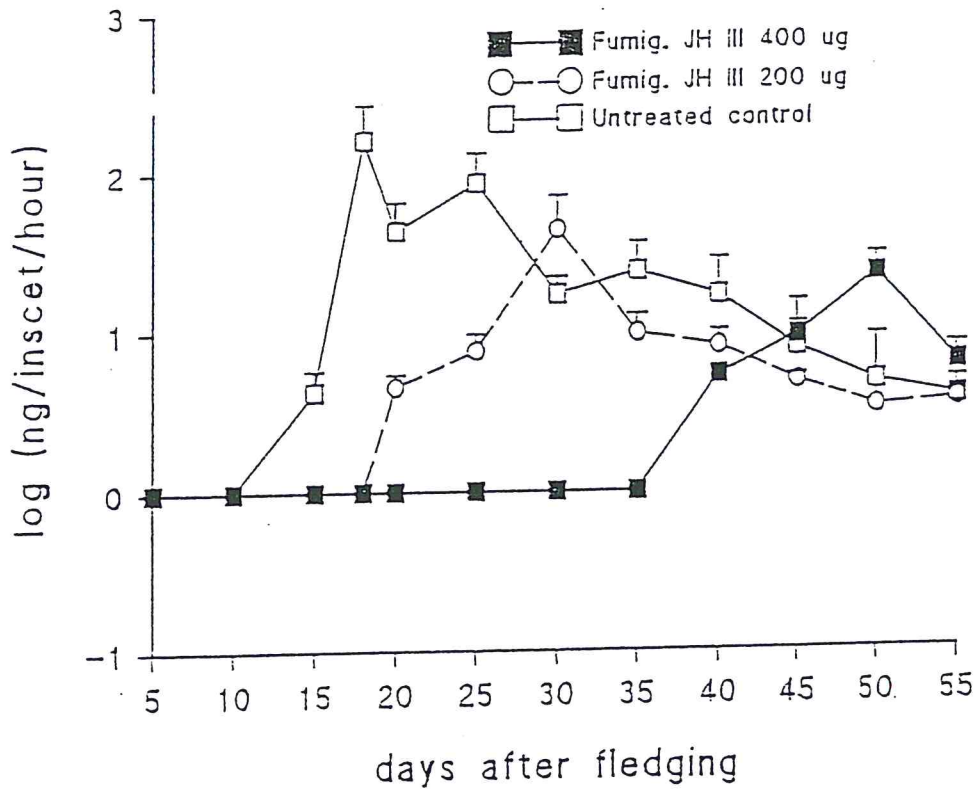
Fig. 14B. Log phenilacetonitrile (mean ± SE) released at different ages by adult males, from control and treated last instar nymphs with JH III in the vapor phase.
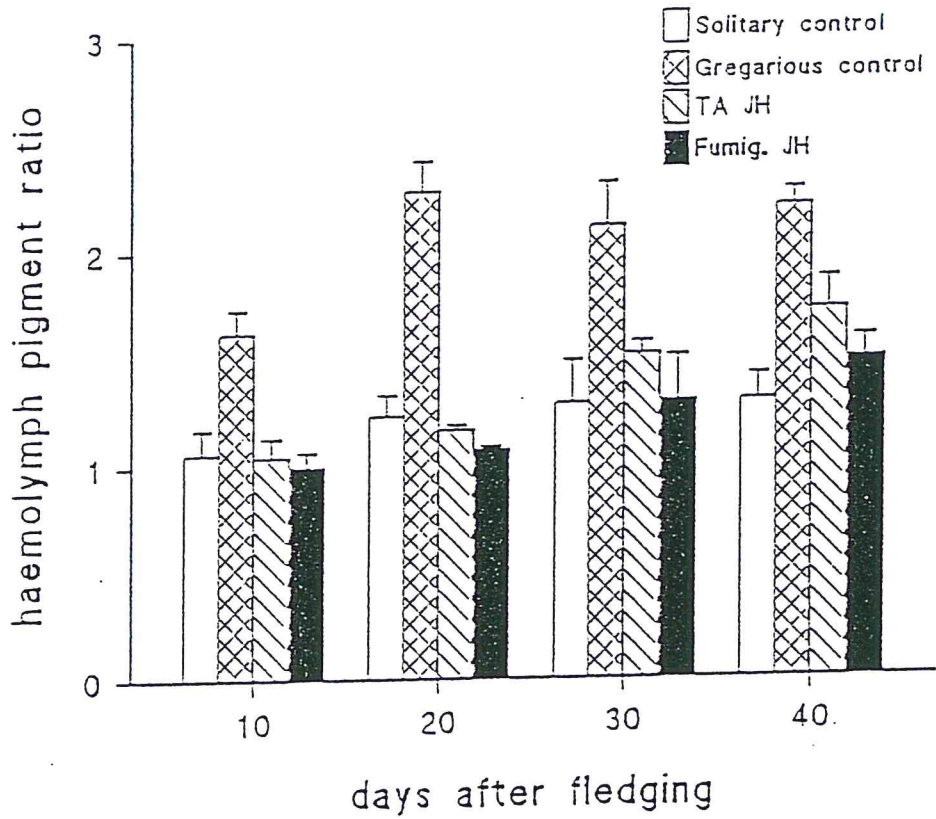
Fig. 15. Hemolymph pigment changes at different ages of adult males (no sex difference) in solitary control, gregarious control and treated insects with JH III.
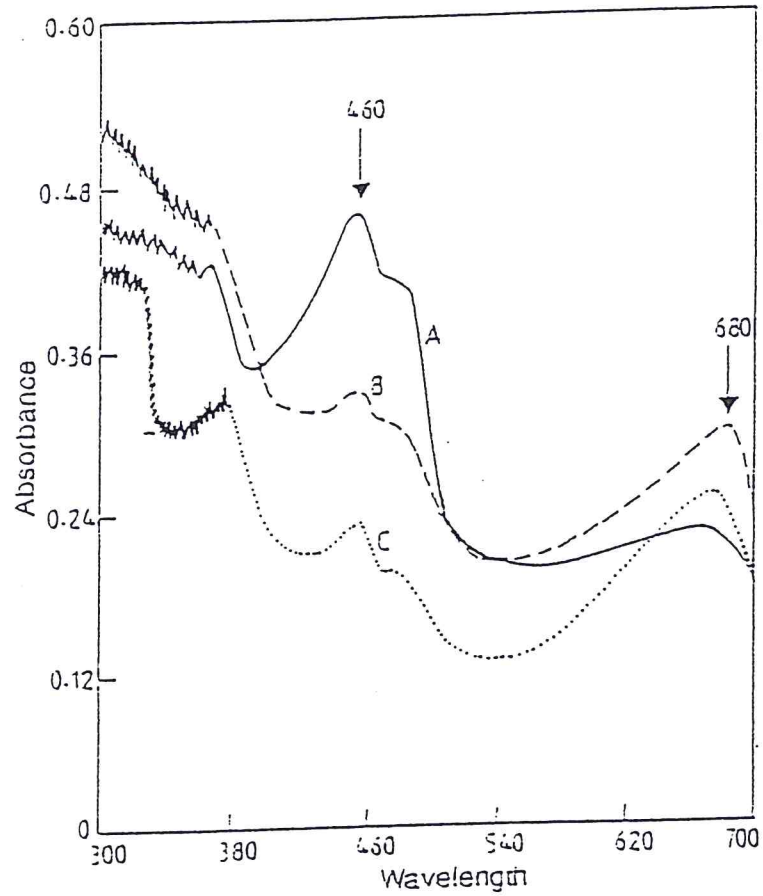
Fig 16.   UV/ visible spectra of the hemolymph from 30-day-old adult males; gregarious control (A), treated with topical applications of JH III (B) and solitary control (C) (no sex difference).

DISCUSSION

The production and/or release of pheromones by insects are influenced by a variety of environmental and physiological factors (Shorey, 1974; Blomquist and Dillwith, 1983). The translation of physiological and environmental signals into the production of pheromone has been shown to be mediated by the endocrine system in a number of insect species (Blomquist *et al.*, 1987; Raina and Menn, 1987; Vanderwel, 1994). Pheromone production is regulated by the corpus allatum-juvenile hormone in a number of insects, including *Leucophaea maderae* (Engelmann, 1960), *Bryostria fumigata* (Barth, 1961; 1962; Barth and Bell, 1970), *Periplaneta americana, Blaberus discoidallis* (Barth, 1965; Barth and Lester, 1973), *S. gregaria* (Loher, 1961; Norris and Pener, 1965), *Tenebrio molitor* (Menon, 1970), *Ips confusus* (Hughes and Renwick, 1977) and *Pityokteines* sp (Harring 1978). Removal of the CA prevented pheromone synthesis in these studies, and its reimplantation of CA or administration of exogenous JH initiated pheromone synthesis. Recent studies on several lepidopteran species have demonstrated that a neuropeptide, the pheromone biosynthesis activating neuropeptide (PBAN), induces sex pheromone production (Raina, 1993).

Gregarious or crowded locusts exhibit a consistent course of color changes during adult life which is strongly associated with sexual maturation. Solitary or isolated adults do not show such changes.

Crowded fledglings of *Schistocerca* are pink, but after a few days, the color turns to pinkish-beige, then to beige or brown. Eventually, females become yellowish and males bright yellow after the onset of full sexual maturation (Norris, 1954; Pener, 1967; 1991). Our results show that application of JH results in the fading of the yellow color of crowded adult males, and shifting the hemolymph pigment ratio from the gregarious to the solitary phase ranges. The yellowing of crowded adult locusts depends completely on the CA and JH. Allatectomy of young adults or last instar hoppers prevents yellowing, whereas reimplantation of CA or administration of JH reinduces it in *Schistocerca* (Loher, 1961; Pener, 1965; 1967; Odhiambo, 1966; Amerasinghe, 1978; Pener and Lazarovici, 1979), *Locusta* (Girardie, 1966; Girardie and Vogel, 1966; Pener *et al.*, 1972; Pener, 1976) and *Nomadacris* (Pener, 1968). Pener (1965) also reported that allatectomy of sexually mature yellow adults of *Schistocerca* results in the fading of yellow color.

Our results reveal that maturation, sexual behavior and mating are affected by exogenous JH and precocene, and coincided with the pheromone production by adult males. These results confirm our previous finding that the pheromone has a releaser and primer effects (Mahamat *et al.*, 1993; Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994). The effect of the CA on mating behavior of adult male locusts and grasshoppers has been investigated by several workers. These glands completely control male sexual behavior in the desert locust, *S.*

gregaria (Loher, 1961; Pener, 1965; 1967; Odhiambo, 1966; Cantacuzene, 1967; Pener and Wajc, 1971; Amerasinghe, 1978), in the migratory locust, *L. migratoria* (Wajc and Pener, 1969) and in the red locust, *Nomadacris sepemfasciata* (Pener, 1968). However, Norris and Pener (1965) reported that the presence of allatectomized males or of allatectomized females inhibit the maturation of young adult males of *S. gregaria*.

The results of the present experiments show that JH plays an important role in the regulation of pheromone production in the desert locust, *S. gregaria*. Pheromone production in males was shown to depend on the presence of the CA (Loher, 1961; Norris and Pener, 1965; Amerasinghe, 1978). Further, Schneider *et al.* (1995) demonstrated that exogenous JH III or JHA applied to gregarious females of *S. gregaria* exerts significant effects on lipid metabolism and egg maturation that can be explained as a shift from the gregarious to the solitary phase. In *Ips paraconfusus*, topical applications of JH analogs (JHA) initiated the pheromone synthesis (Borden *et al.*, 1969; Hughes and Renwick, 1977). However, in *Blattella germanica* (Schal *et al.*, 1990) and *Tenebrio molitor* (Menon, 1970), JH induces pheromone production at both low and high doses. On the other hand, Webster and Carde (1984) found that the JHA treatment blocks the pheromone production and emission in *Platynota stultana*. Ono (1993) also demonstrated that JHA showed a positive effect on pheromone production in the potato tuberworm moth, *Phthorimaea operculella*.

Recently, Ivarsson and Birgersson (1995) found that methoprene (JHA) stimulated pheromone production in the double spined beetle, *Ips doplicatus*.

In another experiment, we showed that precocene II (which can act as a JH antagonists) exerts a somewhat weaker effect on the pheromone production and coloration of the desert locust. It seems likely that precocene II influences the pheromone production indirectly by inhibiting JH production. Topical treatments with precocene II inhibited the pheromone production by *Ips paraconfusus* (Kiehlman *et al.*, 1982). Precocene II also inhibits or delays both pheromone production and oocyte growth in a dose-dependent manner in *Blattella germanica* (Schal *et al.*, 1990). Furthermore, Bowers (1976) reported that topical application of precocene II to virgin female *Priplaneta americana* terminated the pheromone production within 5 days. On the other hand, Fridman-Cohen *et al.* (1984) demonstrated that the anti-allatin effect of precocene II applied to last instar nymphs of *S. gregaria* became overt by sterility and lack of yellow color in the adults.

Injection of JH or precocene has no or only minor influences on phase changes and pheromone production. This might be explained by a faster degradation and higher rate of excretion of injected materials. Erley *et al.* (1975) reported that in locusts the administrated cecropia JH was excreted mainly by the malpighian tubules and a small part through the gut wall. They observed that two thirds of the injected dose

of synthetic cecropia JH was excreted during the first 24 hr after application. The rate of excretion of injected juvenoids in locusts and other insects depends mainly on the dose (Slama *et al.*, 1974). On the other hand, treated adults exhibit resistance against applications in comparison to last instar nymphs. This could be due to better peripheral protecting mechanism(s) against the JH and precocene, such as more efficient metabolic detoxification, higher excretion rate and lower penetration rate through the integument.

On the other hand, last instar nymphs exhibit a higher sensitivity to applications of JH III and precocene II. From the point of view of the effects of JH and JHA, the last nymphal instar of both *Schistocerca* and *Locusta* may be divided into three distinct intervals (Joly, 1954; Staal, 1961; Fuzeau-Braesch, 1968; Nemec *et al.*, 1970). The first one, ranging from the ecdysis to the fourth day of the instar, is the period of the action of JH on morphogenesis. The second interval, which encompassed days 4 and 5, represents the sensitive period for the phase changes. In the third interval from day 6 to the next ecdysis, JHs induce no visible effect. Further important endocrinological relationships exist between the occurrence of sensitive period to JH and the content of ecdysteroids in the body (Slama, 1985). Moreover, Tawfik *et al.* (1995a) found a significant differences in the ecdysteroid titers between solitary and gregarious phases of the desert locust during the last two nymphal instars.

It has been assumed that the differences in the behavior between

gregarious and solitary locusts arise from differences in the central nervous processing of information. Greenwood and Chapman (1984) demonstrated that solitary adults and fifth instar nymphs of *L. migratoria* have more olfactory sensilla on the antennae than conspecific gregarious phase insects. Furthermore, Ramswamy and Gupta (1981) reported that JH treatment of the last instar female nymphs of *Blattella germanica* led to the retention of nymphal sensillar characteristics on both the antennae and the palps of the resulting adults, with a reduced electroantennogram response. Thus, the insect behaves like a nymph and does not mate in spite of its ability to produce pheromone. Therefore, one possible mode of action in JH-treated *Schistocerca* is that this hormone could regulate antennal responses, which in turn might regulate aggregation behavior and pheromone production.

On the other hand, in locusts the pheromone is believed to be produced by vaculated epidermal gland cells and conducted onto the surface of the cuticle through cuticular ducts secreted by specialized duct cells (Loher, 1961; Strong, 1970; 1971; Thomas, 1970; Kendall 1972; Cassier and Delorme-Joulie, 1976; Amerasinghe, 1977; Ali, 1987). Thus, another possible mode of action is that JH (especially in the vapor phase and topical application) act directly at the site of pheromone production.

Locust density is the primary extrinsic factor that affects phase transformation and pheromone production (Gillett, 1988; Roessingh and

Simpson, 1994; Deng *et al.*, 1995), but the intrinsic factors underlying the physiological mechanism(s) of phase changes and pheromone production are not sufficiently known. The present results clearly demonstrate that the CA and their product, JH play a crucial role if not the primary intrinsic factor on phase changes and pheromone production in the desert locust.

It is an interesting and very important fact that JH in the vapor phase is more effective on phase changes and pheromone production of the desert locust. McGovern *et al.* (1971) showed that vapors of some JHA caused juvenilization in the yellow mealworm. Also, Bowers (1969) reported some data on the effect of the vapors of some JHA. In such a method consideration should be given to the application of the JH and JHA in vapor form i.e. as fumigant, to prevent pheromone production and so aggregation of the desert locust.

Several reports have indicated that in locusts, the gregarious phase is characterized by a lower JH content than in the solitary phase (Joly and Joly, 1974; Baehr *et al.*, 1979; Injeyan and Tobe, 1981b; Dale and Tobe, 1986). Thus, an experimental elevation of the JH content in gregarious locusts should induce a solitarization. Contrarily, we found that both JH excess and JH deficiency affect gregarious coloration, maturation and pheromone production in *S. gregaria*. Therefore, phase dynamics of the desert locust could be regulated by threshold of JH at a critical period. Tawfik *et al.* (1995b) found that the pheromone production coincides with a specific titer of JH in gregarious adult males of the desert locust.

CHAPTER III
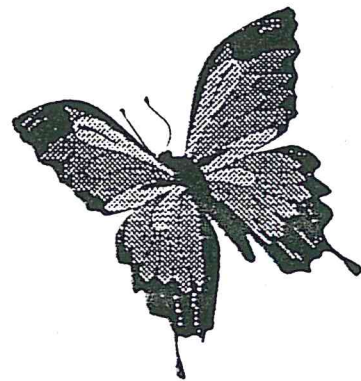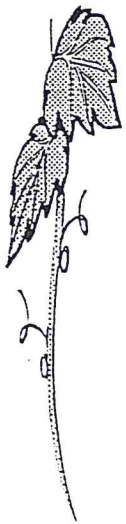
CHAPTER III

COMPOSITION AND TITER OF HEMOLYMPH ECDYSTERODS IN LARVAE

INTRODUCTION

The development of locusts may follow either a solitary or a gregarious pathway, which differ from one another in the developmental rate and in the morphology, behavior, viability, and reproductive potential of the insects (Uvarov, 1966). The choice of pathway depends on environmental factors and population density whose effect is mediated by the nervous and endocrine systems (Hardie and Lees, 1985). Locust populations often persist in semiarid regions for years as the solitary phase, until an environmental cue triggers a switch to the gregarious phase that forms devastating swarms. Since the switch between the two phases is decisive for locust outbreaks, its mechanism has been studied extensively. It has been established that juvenile hormones and apparently also the ecdysteroids are involved (Girardie and Joly, 1967; Pener, 1991) but precise roles of these hormones remain to be elucidated.

The implication of ecdysteroids in the phase dimorphism was indicated by differences in the appearance of the prothoracic (also called ventral) glands (PG) that are the source of larval ecdysteroids. Ellis and Carlisle (1961) observed in the desert locust, *Schistocerca gregaria*, and in the migratory locust, *Locusta migratoria*, that PG are always larger in the solitary than in the gregarious phases. and that

partial ablation of PG causes solitary *S. gregaria* larvae to develop some gregarious features. In the adults of *L. migratoria* and *S. gregaria*, PG persist permanently in the solitary but not in the gregarious phase (Carlisle and Ellis, 1959). However, Staal and De Wilde (1962) could not confirm in surgical experiments that the PG influence the phase characters of *L. migratoria*. In addition, there are indications that PG affect certain developmental processes independently of ecdysteroid levels (Joly *et al.*, 1977). Hence, although the role of PG as the only source of ecdysteroids in locust larvae has been demonstrated (Hoffmann and Koolman, 1974), attribution of PG effects on phase dimorphism to ecdysteroids without a biochemical analysis is questionable.

Major ecdysteroids were identified and their body contents or hemolymph titers ascertained in the gregarious phase of both *S. gregaria* and *L. migratoria* (Morgan *et al.*, 1975; Morgan and Poole, 1976; Gande *et al.*, 1979; Baehr *et al.*, 1979; Hirn *et al.*, 1979) but only Wilson and Morgan (1978) compared solitary and gregarious phases in the last instar larvae of *S. gregaria*. They found no differences in the ecdysteroid body content and concluded that ecdysteroids play no role in the phase dimorphism.

Persisting arguments about the role of ecdysteroids in locust phase dimorphism spurred the present study. Assuming that the involvement of ecdysteroids must be reflected either in different developmental titer profiles or in diverse ecdysteroid composition, we decided to identify ecdysteroids and measure their titer in well defined gregarious and solitary larvae of *S. gregaria*.

RESULTS

IDENTIFICATION OF ECDYSTEROIDS BY HPLC AND TLC

Pilot HPLC (Fig. 17) and TLC analyses of a cumulative hemolymph sample revealed two major RIA-positive fractions that behaved as 20E and E. Both were readily detected with the H-22 antiserum, while the 85-B/L2 antiserum reacted preferentially with the E-like fraction. The 20E-like fraction occasionally overlapped with the 26E standard but in additional analysis with a normal phase column we failed to detect 26E in the hemolymph. Previous identification of 20E and E as major body ecdysteroids of *S. gregaria* (Gande *et al.*, 1979) support our conclusion that two major hemolymph ecdysteroid fractions correspond to 20E and E. A separate fraction eluted between 20E and E in the position of MaA standard and, again similarly as this standard, reacted readily with H-22, but hardly with the 85-B/L2 antiserum. We therefore assume that this fraction represents MaA. We did not find any RIA-positive fraction more apolar than E; putative precursor of MaA, the 20-deoxyMaA, could have escaped detection because its hemolymph concentrations are negligible.

With the H-22 antiserum (reaction was very weak with the 85-B/L2 antiserum) we found several fractions containing highly polar products (HPP) that could have consisted either of very polar ecdysteroids or of ecdysteroid conjugates with polar moieties. To distinguish between these two classes of ecdysteroid metabolites, the HPP fractions were pooled and subjected to enzymatic cleavage and

then again to HPLC. The RIA value of hydrolyzed HPP was lowered only by 5-6%, while fractions eluting in position of 20E and 26E correspondingly increased. This result was obtained repeatedly under incubation conditions that afforded efficient hydrolysis of ecdysteroid conjugates extracted from locust eggs. We conclude that hemolymph contains negligible amounts of ecdysteroid conjugates, but includes polar metabolites that are products of ecdysteroid oxidation. Neither the conjugates nor the polar metabolites were analyzed in this work.

MUTUAL RATIOS OF IDENTIFIED ECDYSTEROIDS IN THE SOLITARY AND GREGARIOUS LARVAE

The contents of identified ecdysteroids were measured at the time of ecdysteroid peaks. 20E clearly dominated in the penultimate and last instar larvae of both sexes (Fig. 18 A&B), making up 63 to 84% of total ecdysteroids (Table 1). In absolute terms, penultimate instar females contained about 30% more 20E than the males, whereas a still lower 20E content in the last instar larvae was similar in both sexes. Differences in 20E between the solitary and gregarious phases were insignificant in both instars and sexes.

Certain discrepancies between the solitary and gregarious phases were found in the contents of minor ecdysteroids (Fig. 18 A&B). Solitary phase differed from the gregarious one by higher representation of E and MaA, and lower proportion of HPP. Phase divergence was particularly obvious in the penultimate instar, when the solitary larvae contained 3-5x more E and MaA than the gregarious ones (Table 1). In

the last instar, phase distinction in respect to HPP, MaA and E ratios was pronounced in the males but virtually absent in the females, in which we also failed to detect the MaA fraction.

ECDYSTEROID TITERS DURING PENULTIMATE AND LAST LARVAL INSTARS

Ecdysteroid titer in the hemolymph was measured with the aid of both H-22 and 85-B/L2 antisera (Fig. 19 A&B). Since the first mentioned antiserum detected all identified ecdysteroids with similar sensitivity, established values reflect changes in the sum of 20E, E, MaA and HPP. On the other hand, with the latter antiserum of low affinity to 20E, very low to HPP, and negligible to MaA, we identified changes that are primarily due to E. Parallel course of the titer curves that were obtained with the two antisera (Fig. 19 A&B) indicates that the ratio of dominating 20E to the other ecdysteroids remains similar throughout the period examined. Changes in total ecdysteroids expressed in 20E equivalents obviously reflect hormone fluctuations that are of physiological importance.

The hemolymph content of 20E equivalents in the penultimate instar larvae of *S. gregaria* never fell below a baseline of about 100 ng/ml hemolymph. From this baseline, the titer grew in the solitary male and female larvae to a single peak of 3.1 and 3.6 μg 20E equiv./ml, respectively, on day 2 of the penultimate instar, declining drastically prior to ecdysis into the last instar (Fig. 19 A&B). In the gregarious larvae, ecdysteroid peak extended over days 2 and 3, falling

to pre-ecdysial level only on day 4. In the females we found a maximal value of 2.4 μg/ml on day 2, and a somewhat lower value (2.1 μg/ml) on day 3; corresponding figures for the males were 2.4 and 2.6 μg/ml, respectively. It is obvious that ecdysteroid titer significantly higher than the baseline is maintained in penultimate instar gregarious larvae about twice longer than in the solitary larvae. Maximal titer reached in the instar seems to be lower in the gregarious larvae but it cannot be excluded that we missed a maximum between days 2 and 3.

Minimal ecdysteroid levels fluctuated in the last instar larvae between 40 and 65 ng 20E equiv./ml. In the solitary larvae they were elevated to small peaks on days 2 and 4, reaching 420 and 420 ng/ml in males, and 275 and 630 ng/ml in females, respectively (Fig. 19 A&B). After a drop on day 5, the titer rose to a sharp peak on day 6 (2.3 and 1.7 μg/ml in males and females, respectively), declining again on day 7, i.e. about one day prior to the last ecdysis. The gregarious larvae exhibited only one small peak located on day 2 in the females (275 ng/ml) and on day 1 in the males (370 ng/ml). The rise to major peak began already after day 3 and values surpassing 1 μg/ml were maintained from day 5 to day 7. Maxima established in the females on day 6 (1.4 μg/ml) and in the males on day 7 (1.6 ng/ml) were somewhat lower than the peaks found in the solitary last instar larvae.

Last larval instar of both males and females differs from the previous one by lower ecdysteroid peak and high complexity of the titer changes. In either instar, maximal titers seem to be reached by the solitary phase, but the period of high ecdysteroid concentration is longer in the gregarious phase.

TABLE 1.   Per cent ratios of ecdysteroid fractions in the solitary and gregarious larvae

Instar/sex	Solitary				Gregarious			
	HPP	20E	MaA	E	HPP	20E	MaA	E
Penultimate male	15.9	64.9	2.5	16.7	30.5	63.4	0.7	5.4
Penultimate female	9.80	73.9	5.4	10.9	13.4	82.9	1.2	2.5
Last/male	8.20	62.9	1.9	26.8	23.2	73.8	0.5	2.5
Last/female	10.8	83.5	n.d.	5.70	17.6	77.3	n.d.	5.1

n.d. = not detected
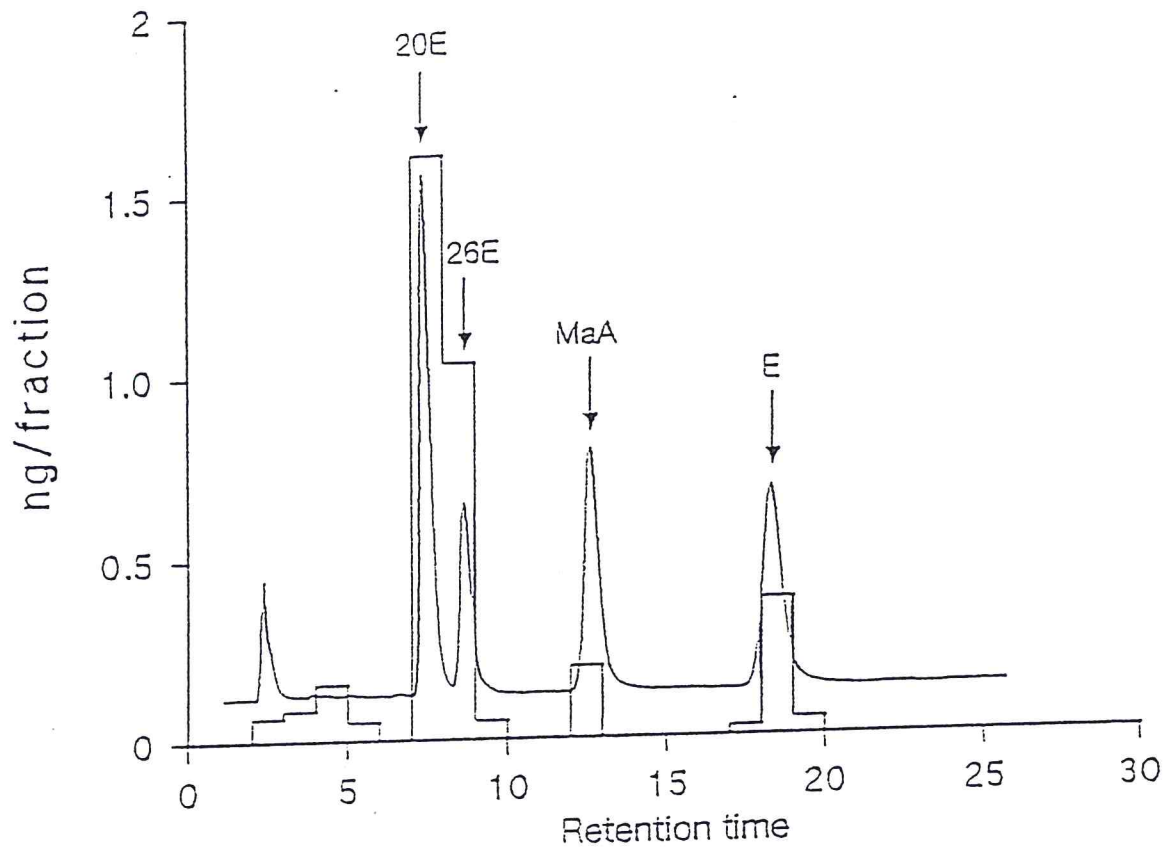
Fig. 17. HPLC elution profile of ecdysteroids extracted from adult's hemolymph in comparison with the standard: 20E, 20-hydroxyecdysone; 26E, 26-hydroxyecdysone; MaA, makisterone A; E, ecdysone.
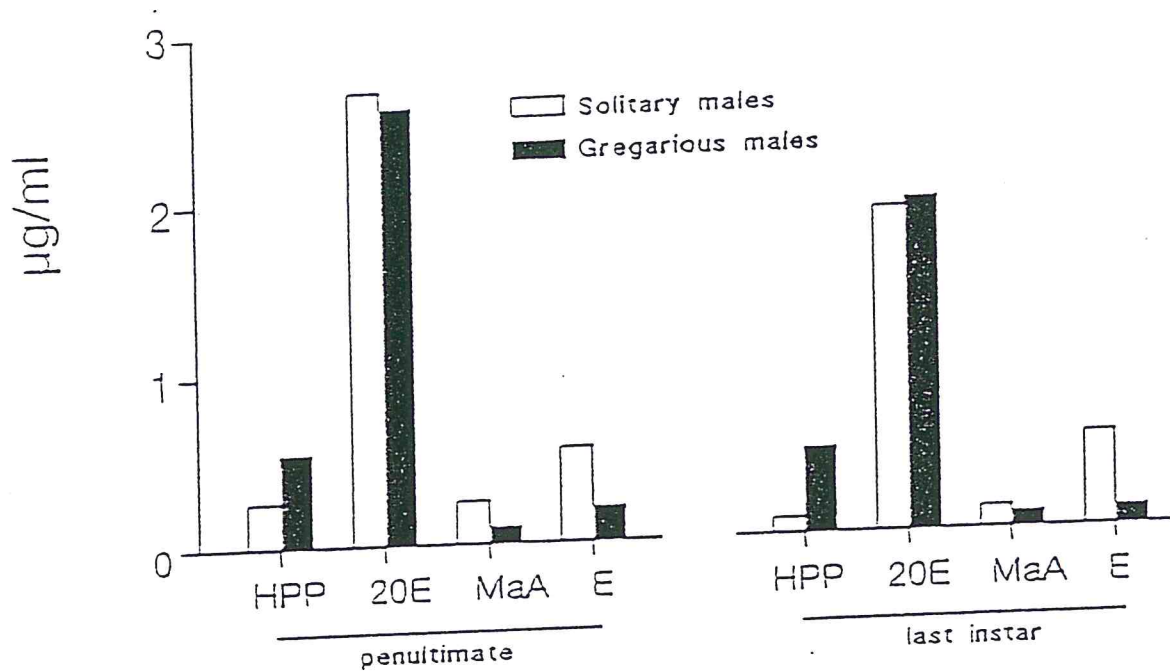
Fig. 18A. Quatification of hemolymph ecdysteroids in the solitary (open columns) and gregarious (solid columns) larvae of the penultimate and last instar male. Identified fractions: HPP, highly polar products; 20E, 20-hydroxyecdysone; MaA, makisterone A; E, ecdysone (expressed in 20E equiv.).
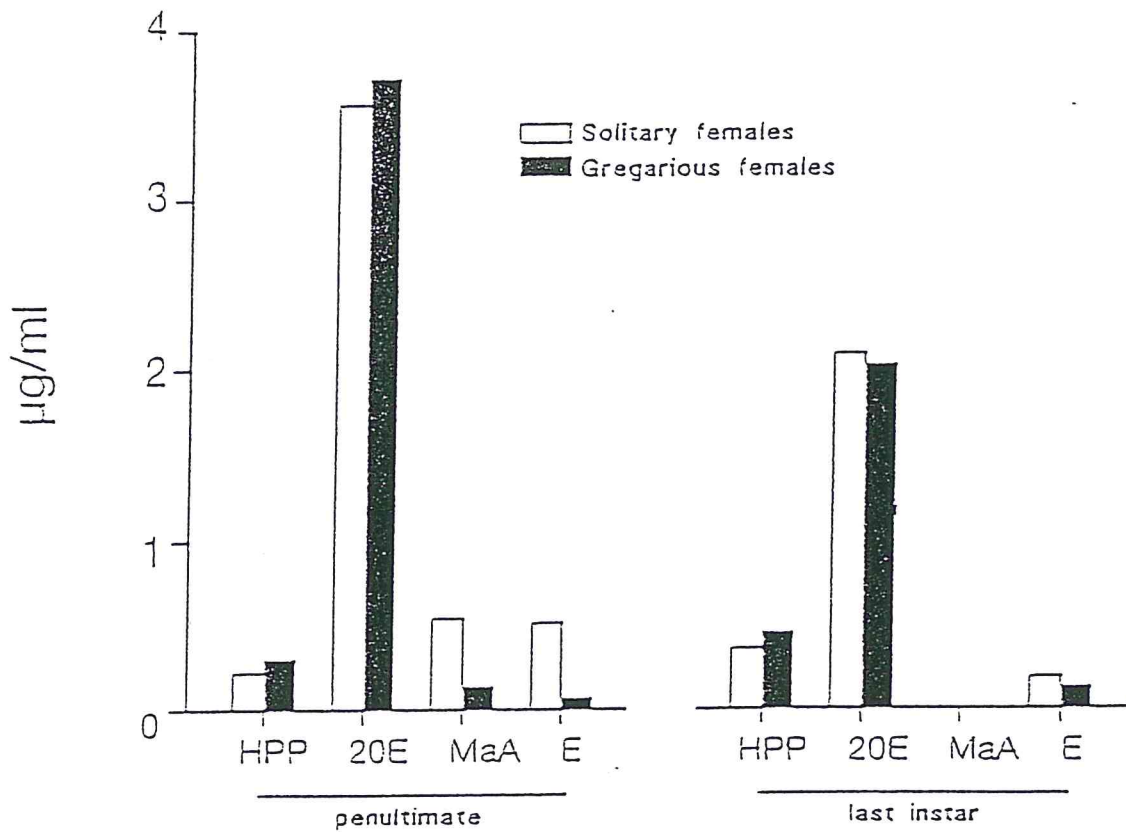
Fig. 18B. Quatification of hemolymph ecdysteroids in the solitary (open columns) and gregarious (solid columns) larvae of the penultimate and last instar female. Identified fractions: HPP, highly polar products; 20E, 20-hydroxyecdysone; MaA, makisterone A; E, ecdysone (expressed in 20E equiv.).
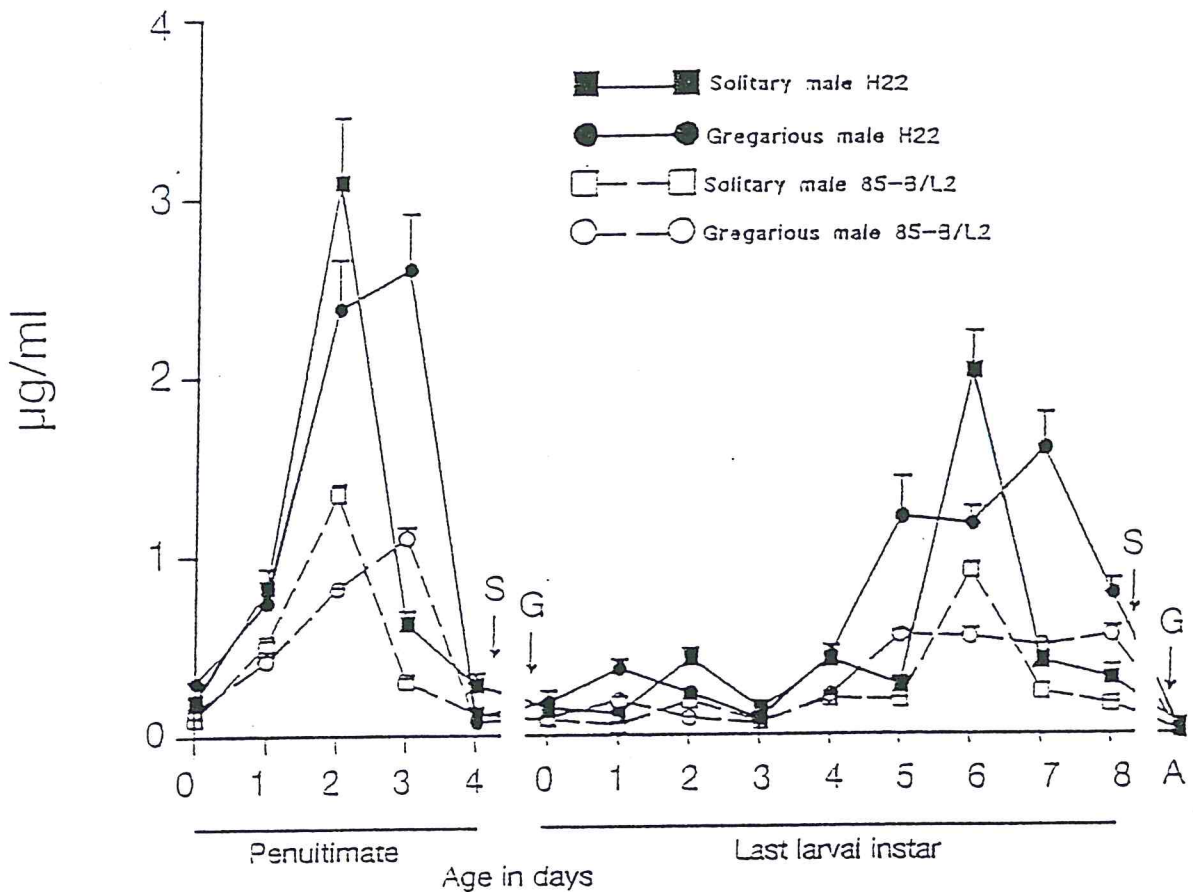
Fig. 19A. Profiles of ecdysteroid titer established in the solitary (square data points) and gregarious (round data points) penultimate and last larval instar male with H-22 antiserum and 20E as standard (solid lines) and with 85-B/L2 antiserum and E as standard (dashed lines). Age is given since ecdysis into the respective instar, A denotes freshly emerged adult. Arrows indicate time of ecdysis in the solitary (S) and gregarious (G) insects. Data established with H-22 antiserum are meane ± SD of three measurements, those obtained with the 85-B/L2 antiserum are mostly results of just two assays.
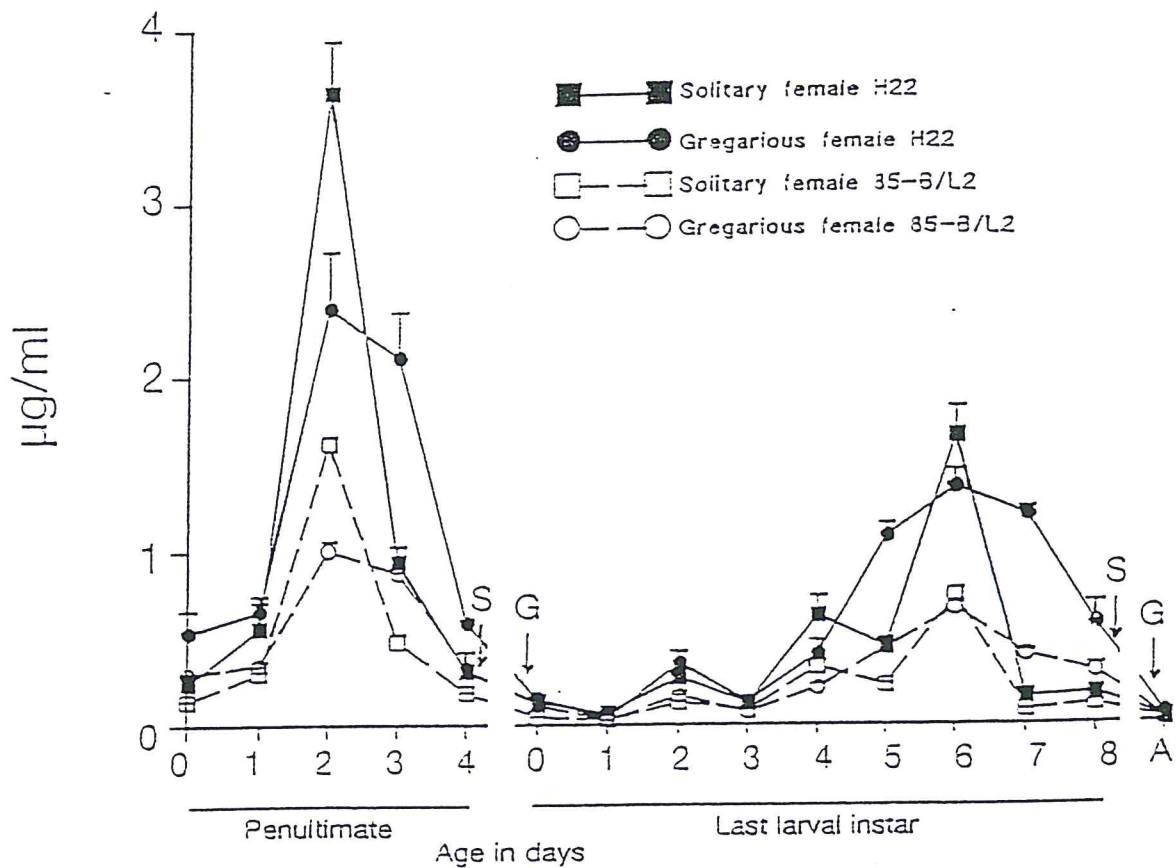
Fig. 19B. Profiles of ecdysteroid titer established in the solitary (square data points) and gregarious (round data points) penultimate and last larval instar female with H-22 antiserum and 20E as standard (solid lines) and with 85-B/L2 antiserum and E as standard (dashed lines). Age is given since ecdysis into the respective instar, A denotes freshly emerged adult. Arrows indicate time of ecdysis in the solitary (S) and gregarious (G) insects. Data established with H-22 antiserum are means ± SD of three measurements, those obtained with the 85-B/L2 antiserum are mostly results of just two assays.

DISCUSSION

In *L. migratoria*, radiolabelled ecdysteroid precursors can be converted by PG into both E and 3dE (Dolle *et al.*, 1991; Roussel, 1992a) but only E was identified as the secretory product of PG in the absence of the radioactive precursors (Hirn *et al.*, 1979). Secreted E is apparently rapidly converted to 20E, which prevails in hemolymph circulation of *L. migratoria* (Hirn *et al.*, 1979) as well as in larval body extracts of *S. gregaria* (Morgan and Poole, 1976; Wilson and Morgan, 1978; Gande *et al.*, 1979). Report on a third, unidentified ecdysteroid (Morgan and Poole, 1976) was later denounced as due to an artefact (Wilson and Morgan, 1978). Our results show, however, that *S. gregaria* hemolymph contains 20E, E, and a third compound that exhibits chromatographic and antigenic properties of MaA (Fig. 17). MaA was identified in some representatives of Hemiptera, Hymenoptera and Diptera (Feldlaufer, 1989). Since its presence in some species depends on the composition of dietary sterols, in *S. gregaria* it may also occur only when the insects are reared on certain diets. This would explain why it was not detected by previous researchers analyzing this species.

The RIA-positive highly polar products (Figs. 17 and 18 A&B) probably represent metabolites generated by ecdysteroid degradation (Gibson *et al.*, 1984; Modde *et al.*, 1984). Enzymatic hydrolysis of this fraction yielded only small amount of free 20E, indicating that most of it consisted of free or conjugated metabolites such as 26E, 2026E, and

26-oic acids, which are common in the degradation pathway of ecdysteroids (Lafont and Connat, 1989). Formation of polar conjugates from exogenous E and its metabolites 20E, 3dE and 3d20E was demonstrated in *L. migratoria* (Hoffmann and Koolman, 1974), but without a radioactive tracer no conjugates could be detected either in the blood (Feyereisen *et al.*, 1976; Gande *et al.*, 1979) or in the whole body extracts (Wilson and Morgan, 1978) of locust larvae.

There is no doubt that 20E is of major physiological significance in *S. gregaria* larvae (Fig. 18 A&B). Its rise around the middle of the penultimate instar in both gregarious and solitary phases (Fig. 19 A&B) is consistent with previous report that maximum ecdysteroid titer in *S. gregaria* is reached 2 days before the next ecdysis (Gande *et al.*, 1979). A similar profile of ecdysteroid changes was found in *L. migratoria* (Baehr *et al.*, 1979) and *Melanoplus sanguinipes* (Ismail and Gillott, 1993). Our data demonstrate that ecdysteroid surge is over within one day in the solitary phase but extends for two days in the gregarious locusts; this difference is reflected in somewhat longer penultimate instar in the gregarious phase.

Hemolymph ecdysteroids in the solitary last instar larvae of *S. gregaria* show two small peaks before the molt-inducing surge occurs, while in the gregarious phase the second small peak fuses with the surge (Fig. 19 A&B). A single small peak was also detected in gregarious *M. sanguinipes* (Ismail and Gillott, 1993). In gregarious *L. migratoria*, several authors reported a single small peak (Bouthier *et al.*, 1975; Baehr *et al.*, 1979), while a study performed in 8 h intervals

on locusts kept under fluctuating temperature regime (25-38°C) revealed that the molt-inducing surge is preceded by two ecdysteroid peaks, the 2nd of them being nearly half as high as the surge peak (Hirn *et al.*, 1979). Timing of the peaks coincided with the periods of highest secretory activity in explanted PG. It cannot be excluded that PG activity and ecdysteroid titer are affected by environmental conditions. Significance of the small peaks is obscure; the middle one in *L. migratoria* reportedly coincides with the period of extensive cell divisions in the epidermis (Hirn *et al.*, 1979).

Similarly as in the penultimate instar, the molt-inducing ecdysteroid surge in the last larval instar of gregarious *S. gregaria* lasts considerably longer than in the solitary hoppers (Fig. 19 A&B). Our titer profiles contrast with the report by Wilson and Morgan (1978) who analyzed whole body contents of 20E titres in *S. gregaria* and found no difference between the solitary and gregarious phases. It is possible that the phase-specific differences are hard to detect in the whole body extracts.

The decline of ecdysteroid titer prior to imaginal ecdysis (Fig. 19 A&B) was reported by most previous authors working with *S. gregaria* (Wilson and Morgan, 1978; Gande *et al.*, 1979; Morgan *et al.*, 1975), *L. migratoria* (Baehr *et al.*, 1979; Hirn *et al.*, 1979) and *M. sanguinipes* (Ismail and Gillot, 1993), and was shown to be due to cessation of ecdysteroid secretion from PG (Hirn *et al.*, 1979; Roussel, 1992b). The drop in ecdysteroid concentration is apparently crucial for the initiation of ecdysial behavior (Sláma, 1980).

In contrast to our results, previous analysis of E and 20E in gregarious *S. gregaria* showed that the body content (Gande *et al.*, 1979) as well as the hemolymph titer (Morgan and Poole, 1976) of ecdysteroids are considerably higher in the last than in the penultimate instars. Morgan and Poole (1976), reported for *S. gregaria* and Ismail and Gillott (1993), for *M. sanguinipenis* that ecdysteroid peak in penultimate instar larvae is below 100 ng/ml and in the last instar larvae below 500 ng/ml. We have no explanation for this discrepance with our data that are closer to ecdysteroid quantification in *L. migratoria* (Baehr *et al.*, 1979). Higher ecdysteroid content in the penultimate than in the last larval instar was demonstrated in the cricket *Gryllus bimaculatus* (Gerstenlauer and Hoffmann, 1995).

According to our results, longer duration of the molt-inducing ecdysteroid surge but higher proportion of HPP and somewhat lower peak concentrations of ecdysteroids distinguish in *S. gregaria* the gregarious from the solitary larvae. Previous experimental findings indicate that differences in the peak concentration may be most significant. It was shown that partial ablation of PG, presumably followed by a reduction of ecdysteroid titer, causes appearance of some gregarious features in the solitary hoppers (Ellis and Carlisle, 1961), and that injection of phytoecdysteroids into gregarious larvae leads to their partial conversion to the solitary phase (El-Ibrashy *et al.*, 1976). Experiments like this must obviously be repeated and actual ecdysteroid titers established in their course. Tentatively we conclude that both the amplitude and frequency of ecdysteroid titer changes are important. A

balance between the height and duration of ecdysteroid peaks was implicated in wing morph determination in the cricket *Gryllus rubens* (Zera *et al.*, 1989). In the penultimate instar of this species, ecdysteroid peak is higher in presumptive macropterous versus presumptive brachypterous males, while longer duration of elevated ecdysteroid titer is associated with the macroptery in females. In the last instar, ecdysteroid titer is higher in presumptive long-winged adults of both sexes.

It is possible that diverse ecdysteroid titers affect the morphological, behavioral, and other phase characters only indirectly, via changes in instar duration. Other effects may be even more subtle and difficult to detect; for example, ecdysteroids could control the production of nymphal aggregation pheromones that were recently identified in the gregarious phase of *S. gregaria* (Obeng-Ofori · *et al.*, 1993). Ecdysteroids stimulate . sex pheromone activity in some arthropods (Adams *et al.*, 1984; Blomquist *et al.*, 1984; Dees *et al.*, 1984; Jaffe *et al.*, 1986). In some insects, control of sex pheromone biosynthesis is expressed via an intermediary, namely 20E, secreted by the ovary when stimulated by gonotrophic hormone or in concert with a neuropeptide (Koeppe *et al.*, 1985).
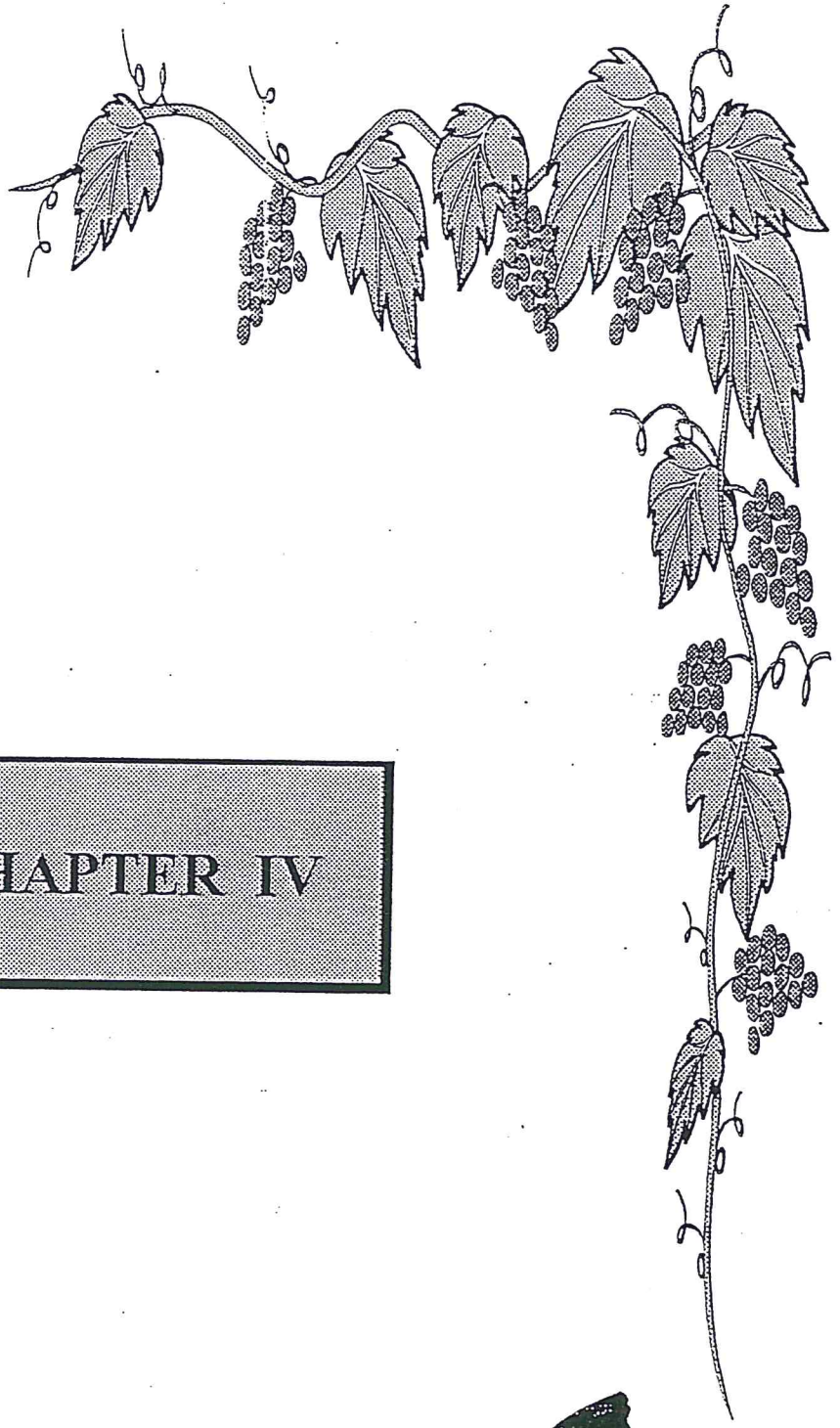
CHAPTER IV
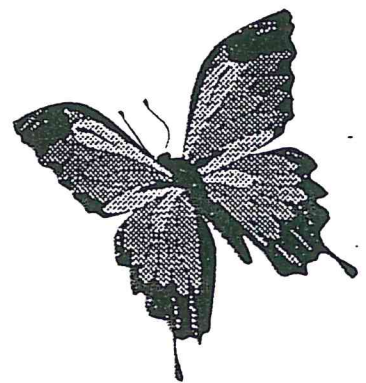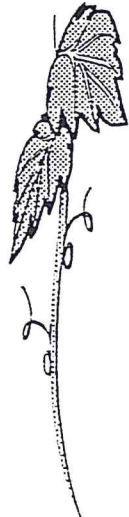
CHAPTER IV

COMPOSITION AND TITER OF HEMOLYMPH ECDYSTEROIDS IN ADULTS

INTRODUCTION

Ecdysteroids had been discovered as the molting hormone in insect larvae and pupae but later they were identified also in the eggs and adults (Hoffmann *et al.*, 1980). Ecdysteroids present in the eggs originate in the ovaries, which in some species also release ecdysteroids into the hemolymph (Hagedorn, 1989). Females of other species, however, contain separate pools of the ovarian and hemolymph ecdysteroids, the latter being derived from various organs (Delbecque *et al.*, 1990). The function of hemolymph ecdysteroids is unclear. An archetype situation is possibly found in firebrat, *Thermobia domestica*, a representative of apterygote Thysanura that retain their prothoracic glands and continue molting in the adult stage. Female firebrat oviposits a batch of eggs in the middle of each interecdysial period. A surge of ecdysteroids is released from the prothoracic glands into hemolymph after the egg laying, causing both the molting and initiation of previtellogenesis in a new set of ovarian follicles (Bitsch and Bitsch, 1988). Fertilization occurs after ecdysis and stimulates production of the juvenile hormone that evokes vitellogenesis: ovaries are brought to a stage of ecdysteroid synthesis and ovarian ecdysteroids are sequestered in the developing oocytes without being liberated into the

hemolymph (Rojo de la Paz *et al.*, 1983).

Stimulation of previtellogenic follicles by hemolymph ecdysteroids was demonstrated also in the bug *Rhodnius prolixius* (Ruegg *et al.*, 1982) and in mosquitoes (Beckemeyer and Lea, 1980; Lea, 1982). It may be characteristic also for some other pterygotes but it is hard to detect in adults with continuous egg production. Other functions of hemolymph ecdysteroids include in various insects stimulation of vitellogenin synthesis, induction of pheromone production, control of corpora allata function, and others (Hagedorn, 1989). The list of known functions is probably incomplete and it cannot be excluded that ecdysteroids play a role also in the phenotypic polymorphism of some insects. If so, one may expect that distinct morphs differ in their ecdysteroid titer or by containing different types of ecdysteroids. Our present study examines this possibility by comparing hemolymph ecdysteroids in the gregarious and solitary adults of the desert locust, *Schistocerca gregaria*. Gregarious and solitary phases of locusts differ morphologically, in coloration and size, by the behavior and physiology, and also by their reproductive capacity (Injeyan and Tobe, 1981a).

Phase diversification in locusts is elicited by the population density and influenced by certain environmental factors (Uvarov, 1921; Faure 1932; Gunn and Hunter-Jones, 1952). Production of volatile compounds was identified as an important component of stimuli by which locusts perceive the population density (reviewed by Loher, 1990). An array of such compounds was recently identified as

"gregarious pheromone" (Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994). One of the aims of our study was to examine relationship between the profiles of ecdysteroid titer and pheromone release.

Locusts were among the first insects in which presence of large ecdysteroid amounts in the adult stage was demonstrated (Hoffmann *et al.*, 1975). It was recognized that ecdysteroids are produced and largely confined to vitellogenic ovaries, with only minor representation in the hemolymph (Lagueux *et al.*, 1977). Ovarian ecdysteroids were traced from their synthesis in the follicle cells (Glass *et al.*, 1978; Goltzené *et al.*, 1978) to their deposition into eggs as polar conjugates (Gande *et al.*, 1979; Dinan and Rees, 1981). Both ovarian and egg ecdysteroids received much attention, whereas hemolymph ecdysteroids were specifically addressed in just three studies. Hoffmann *et al.* (1980) detected for *Locusta migratoria* considerable amounts (220 ng/ml) of hemolymph ecdysteroids in adult females, while Lagueux *et al.* (1977) claimed that hemolymph of the males is virtually devoid of ecdysteroids. Ismail and Gillott (1993), found in *Melanoplus sanguinipes* hemolymph ecdysteroids in the adults of both sexes, but in amounts nearly two orders of magnitude lower than in the larvae. We decided that time has come to detail investigation of hemolymph ecdysteroids, especially in relation to the phase dimorphism.

RESULTS

IDENTIFICATION OF ECDYSTEROIDS

Individual ecdysteroids were identified by HPLC/RIA in the hemolymph collected on day 16 after imaginal ecdysis, i.e. at the time of the major ecdysteroid peak. Elution profile of the RIA-positive material was the same as in the previous analysis of the larval hemolymph (Tawfik et al., 1995a): highly polar products (HPP), 20E, MaA, and E were identified (see Fig. 17 in chapter III). The highly polar products resisted hydrolysis with enzymes known to liberate ecdysteroids from their conjugates, similarly as in the case of HPP present in the larval blood (Tawfik et al., 1995a).

An apparent difference detected in the analysis of the four groups of adults concerned the sex-related ratio of E to 20E (Fig. 20). While in the females these ecdysteroids appear to be present in similar amounts (from 40 to 50% of all RIA-positive material), males are characterized by the domination of 20E that makes 73% of total ecdysteroids in the solitary, and 94% in the gregarious phase (Fig. 20). If combined, E and 20E make up at least 95% of hemolymph ecdysteroids and their content in both sexes is nearly identical.

Adults of the solitary phase clearly contain in the hemolymph much larger amounts of total ecdysteroids than the gregarious phase (Figs. 20 and 21 A&B). Ecdysteroid distribution in individual fractions does not seem to be related to the phase (Table 2). Highly polar products are most abundant (14%) in solitary females but absent in

solitary males; their content in the gregarious adults of both sexes is moderate (3-5%). The content of MaA is relatively high in gregarious females (7.5%) and solitary males (5%), and negligible or absent in solitary females and gregarious males. Significance of these variations is not clear.

ECDYSTEROID TITERS IN THE SOLITARY VERSUS GREGARIOUS ADULTS

Ecdysteroid titer established in freshly ecdysed adults with the H-22 antiserum varied randomly between 28 and 49 ng/ml. Sex and phase specific differences began to be manifested within the next two days and included shifts in the timing of some ecdysteroid peaks and generally higher peak values in the solitary than in the gregarious adults (Fig. 21 A&B). Ecdysteroid quantification with the aid of the 85-B/L2 antiserum yielded lower values due to its low sensitivity to 20E and HPP, and insensitivity to MaA. The curves established with H-22 and 85-B/L2 are mostly parallel indicating that the ratio of dominating 20E to the other ecdysteroids remains similar. The discrepance between values established with the two antisera is much larger in the males than in the females, confirming that males contain much more 20E than E.

Changes in total ecdysteroids measured with the H-22 antiserum and expressed in 20E equivalents obviously reflected hormone fluctuations that are of physiological importance. In the solitary

females, ecdysteroid titer rose to a small peak (101 ng/ml) around day 4, a high peak (409 ng/ml) between days 10 and 18, and another small peak (156 ng/ml) between days 28 and 40. The first peak and the second ecdysteroid rise were shifted in the gregarious females and the maximal concentration, which was reached in the second peak as in the solitary phase, amounted only to 142 ng/ml (Fig. 21A). Differences between the gregarious and solitary phases were obliterated on day 20 and ecdysteroid titres were thereafter practically identical.

Ecdysteroid fluctuations in the solitary and gregarious males followed identical patterns characterized by a sharp peak on day 4 and a broad peak with highest values on days 16-20 (Fig. 21B). The first peak of 338 ng/ml in the solitary and 136 ng/ml in the gregarious males was followed in either phase by a drop to about 60 ng/ml on days 6-8. Thereafter the titer grew rapidly to nearly 400 ng/ml in the solitary phase, and gradually to about 150 ng/ml in the gregarious phase. Ecdysteroid concentration declined in both phases between days 20 and 24, drooping in the gregarious phase to minimal values of 21-28 ng/ml that seemed to be then maintained permanently. By contrast, the solitary phase titer decreased as late as by day 40 after ecdysis only to a value (115 ng/ml) that is close to all-time gregarious maximum (136 ng/ml). Hence, ecdysteroid titer was continuously higher in the solitary than in the gregarious phase since day 12 until the end of our observations.

AGGREGATION PHEROMONE

Gas chromatographic analysis of trapped volatiles from the different groups of adults confirmed that aged gregarious males (over 10 days after emergence) liberate 6 aromatic compounds, viz., anisole, benzaldehyde, veratrole, guaiacol, phenylacetonitrile and phenol. Maximal production was detected on day 18, with phenylacetonitrile comprising 80—85 % of the volatiles (Fig. 21B). Listed compounds are liberated only in low and pheromonally inactive amounts by young males of either phase and females of either phase and any age (see Fig. 8 in chapter I).

TABLE 2.   Per cent ratios of ecdysteroid fractions in the solitary and gregarious adults.

Sex	Solitary				Gregarious			
	HPP	20E	MaA	E	HPP	20E	MaA	E
Male	n.d.	72.6	5.2	22.3	2.7	93.9	0.52	2.80
Female	13.7	43.8	n.d.	42.6	2.8	39.8	7.50	49.9

n.d. = not detected
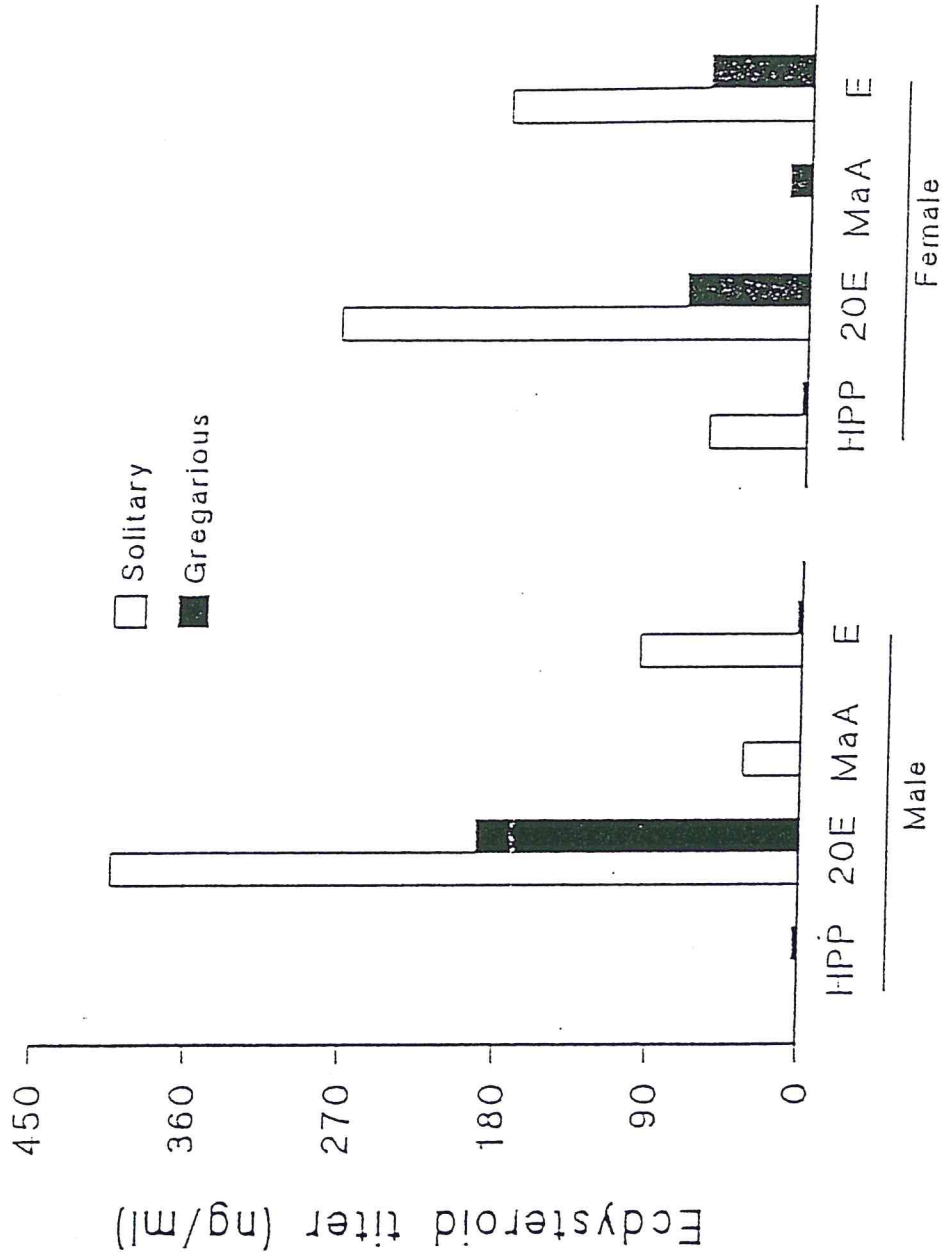
Fig. 20. Quatification of hemolymph ecdysteroids in the solitary (open columns) and gregarious (solid columns) adult male (left) and female (right). Identified fractions: HPP, highly polar products; 20E, 20-hydroxyecdysone; MaA, makisterone A; E, ecdysone (expressed in 20E equiv.).
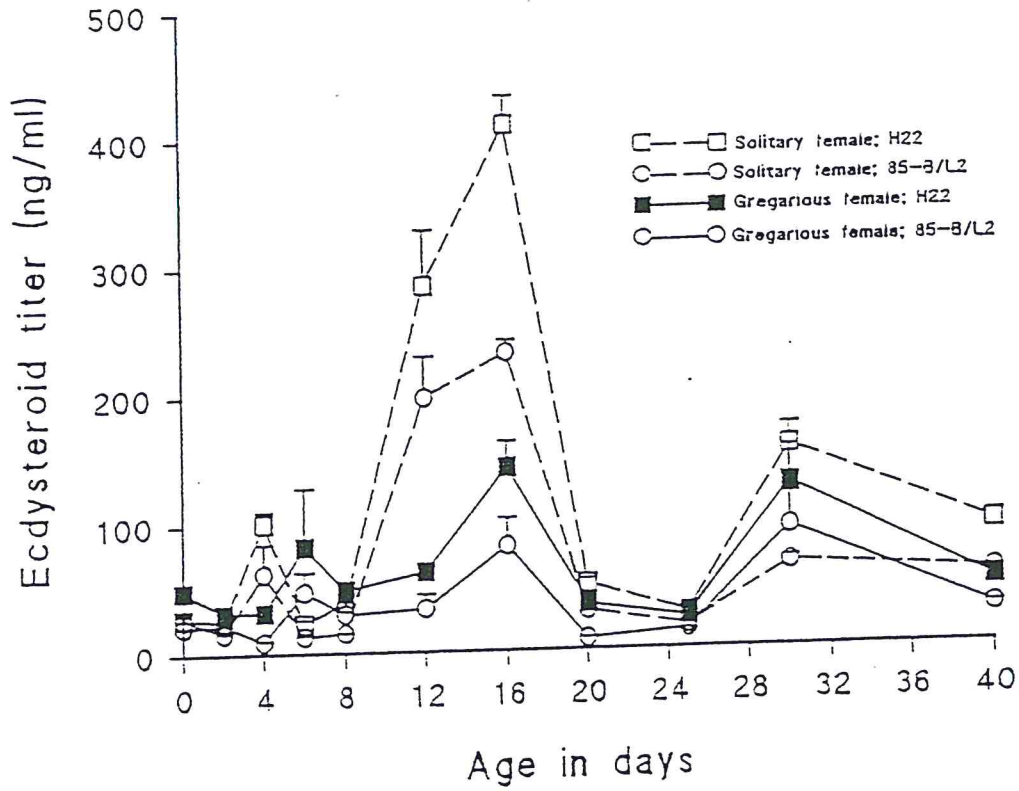
Fig. 21A. Profiles of ecdysteroid titer established in the solitary
(square data points) and gregarious (round data points)
adult female with H-22 antiserum and 20E as standard and
with 85-B/L2 antiserum and E as standard.
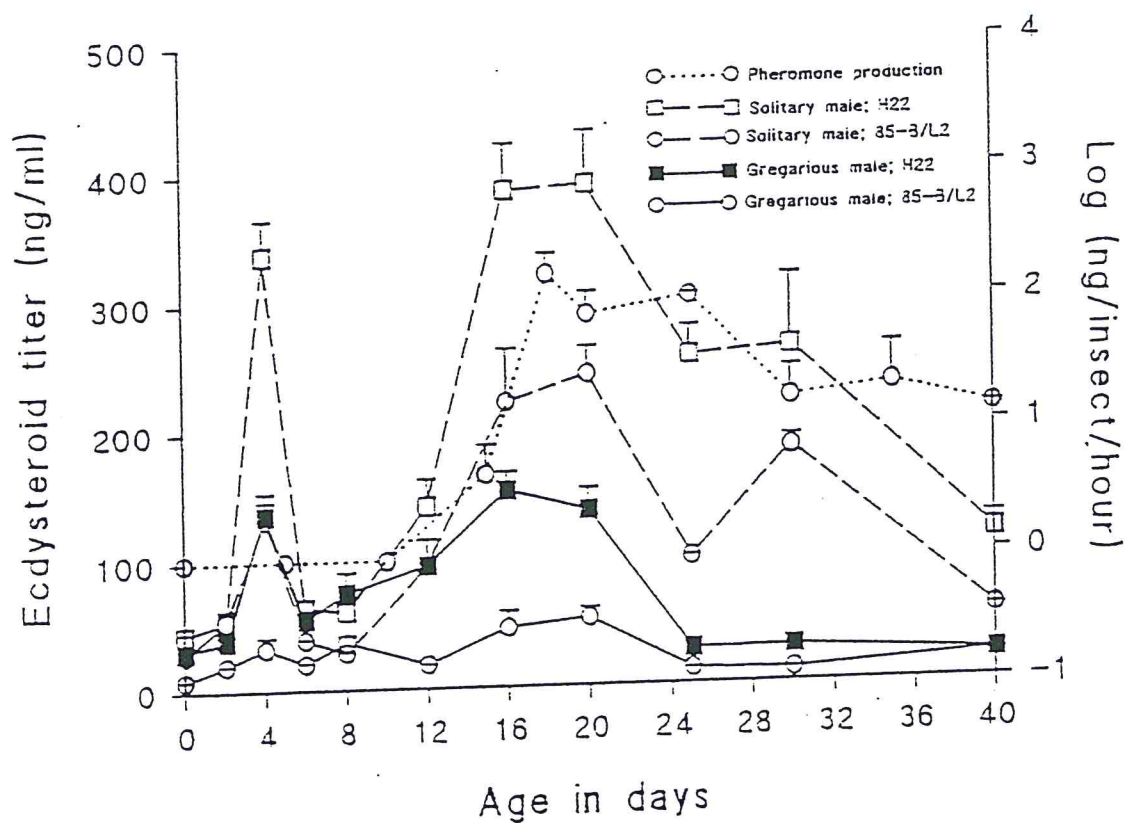
Fig. 21B. Profiles of ecdysteroid titer established in the solitary (square data points) and gregarious (round data points) adult male with H-22 antiserum and 20E as standard and with 85-B/L2 antiserum and E as standard; in relation to pheromone production (round data points and doted line).

DISCUSSION

POSSIBLE SOURCE OF HEMOLYPH ECDYSTEROIDS

It is now generally accepted that insect ovaries at a certain stage of the gonotrophic cycle possess the capacity to synthesize ecdysteroids. In a number of species with panoistic ovaries, including *L. migratoria*, follicle cells were indentified as the site of ecdysteroid biosynthesis (Laqueux *et al.*, 1977; Goltzene *et al.*, 1978). There is considerable evidence that some of the ecdysteroids produced by the ovary may end up in the hemolymph (Hagedorn, 1985; Ismail and Gillott, 1993; Romana *et al.*, 1995). For example, in ovariectomized or allatectomized adult females of *L. migratoria* Lagueux *et al.* (1977), found a significant decrease in hemolymph ecdysteroids, indicating that their production requires maturing ovaries. Our results show that ecdysteroid titers are higher in the solitary than in the gregarious *S. gregaria* adults, consistently with our previous finding for the larvae (Tawfik *et al.*, 1995a). The higher titer of solitary females (Fig. 21A) could be related to different fertility of the two phases which was documented by Norris (1952), Hunter-Jones (1958), Albrecht *et al.* (1959), Papillon (1960) and Injeyan and Tobe (1981a). High fecundity of solitary females is probably due to increased number of ovarioles allowing for higher number of eggs per a pod. Unknown physiological adaptations are responsible for increased number of pods and reduced proportion of sterile eggs.

A strict dependence of ecdysteroid hemolymph titer on the

ovarian production of ecdysteroids, however, is rendered unlikely by the fact that similar titer differences between the solitary and gregarious phases are also found in the males (Fig. 21B). We thus propose that prothoracic glands (PG) might be involved in ecdysteroid production in adult locusts, at least during the first 1-2 weeks after emergence. In contrast to most other insects, PG of locusts persist the imaginal moult and in the solitary phase they are retained at least for several weeks (Carlisle and Ellis, 1959). Carlisle and Ellis (1959), also reported for newly fledged adults of both sexes, that PG are considerably larger in animals that have been reared in isolation than in those bred under crowded conditions. Ultrastructural studies on solitary *L. migratoria* disclosed a link between structural changes in PG cells and sexual maturation (Cassier and Fain-Maurel, 1969).

POSSIBLE ROLE OF HEMOLYMPH ECDYSTEROIDS IN ADULT INSECTS

In gregarious female adult of *L. migratoria,* vitellogenin content in the fat body reaches two peaks coincidentally with terminal oocyte length of 2.5 and 5.5-6.5 mm, respectively (Bar-Zer *et al.*, 1975). Studies of Injeyan and Tobe (1981b) on *S. gregaria* revealed that vitellogenin appears in the hemolymph on day 4 of the 14-16 days-long gonotrophic cycle of the solitary females but on day 6 of the 12-14 days-long cycle of the gregarious females. The time coincidence with ecdysteroid titer changes (Fig. 21A) may indicate that the first peak of ecdysteroids after female emergence may play a role in *Schistocerca*

vitellogenesis. Stimulation of vitellogenesis by ecdysteroids was shown in many insect species (Hagedorn, 1985; 1989).

Second and third ecdysteroid peaks in female hemolymph occur in both solitary and gregarious females around days 16 and 30, i.e. around oviposition terminating the first and second gonotrophic periods. This is in agreement with whole-body ecdysteroid analysis in *S. gregaria* that also revealed maximal ecdysteroid levels just before oviposition (Gande *et al.*, 1979). By contrast, Ismail and Gillot (1993) reported for *Melanoples sanguinipes* that hemolymph ecdysteroids of adult females peak 48 hours after mating. They also found that high titer of ecdysteroids in the hemolymph coincides with high ecdysteroid content in the female reproductive system. Studies on various other insects with panoistic ovaries showed a parallel between the amounts of hemolymph and ovarian ecdysteroids that both reach their maxima at the end of vitellogenesis, i.e. when chorion is formed just before oviposition. This is the case of the cockroaches *Nauphoeta cinerea* (Zhu *et al.*, 1983) and *Blaberus craniifer* (Bulliere *et al.*, 1979), the cricket *Gryllus bimaculatus* (Hoffman *et al.*, 1981; Weidner and Hoffmann, 1990), and the earwig *Labidura riparia* (Vancassel *et al.*, 1991; Sayah *et al.*, 1993).

Although the profile of hemolymph ecdysteroids in adult males of *S. gregaria* resembles the situation in the females, the role of ecdysteroids in the males is probably different. The early ecdysteroid peak (on day 4 in *Schistocerca*), which was also detected in *Melanoplus sanguinipes* by Ismail and Gillott (1993), may play a role in

spermatogenesis. Using organ cultures of *L. migratoria* testes Dumser (1980), demonstrated that 20E in concentrations as low as 10^{-8} M accelerates the flow of spermatogonial cells from G1 into S phase (the period of DNA synthesis), and from G2 (chromosome condensation) into M (mitosis). The drops in hemolymph ecdysteroids may also release specific processes; for example, in adult gypsy moth, *Lymantria dispar*, such a drop seems to be essential for the initiation of the rhythmic release of sperm from the testis (Giebultowicz *et al.*, 1990). Regulations effected by ecdysteroids are certainly complex, as indicated by the occurrence of ecdysteroids in various tissues. Lagueux *et al.* (1977), demonstrated ecdysteroids in the hemolymph, fat body and testis of adult *L. migratoria* males. Possibly even more tissues can release ecdysteroids when incubated in vitro: a study with adult males of *Gryllus bimaculatus* revealed that testes secrete within 16 hr 20.2 ng 20E equiv., tergites 28.5 ng, oenocytes 18.9 ng, thoracic fat body 8.2 ng, and Malpighian tubules 6.4 ng (Bressel *et al.*, 1990).

ARE ECDYSTEROIDS INVOLVED IN THE REGULATION OF PHASE DIMORPHISM?

Our results demonstrate that hemolymph ecdysteroid titer in adult *Schistocerca* reaches significantly higher values in the solitary than in the gregarious phase, indicating that it may play a role in the phase dimorphism. A similar conclusion can be drawn from several previous studies. Ellis and Carlisle (1961), showed that partial ablation of the ventral (= prothoracic) gland in the larvae of *S. gregaria* causes

appearance of some gregarious features, while El-Ibrashy *et al.* (1976), found that injection of phytoecdysteroids from *Podocapus gracilior* into newly ecdysed gregarious fourth instar nymphs of the desert locust elicits changes in morphometric ratios of the resulting adults to an intermediate state between the gregarious and solitary phases. Girardie and Joly (1967), also concluded from their surgical investigations that the ventral gland modifies morphometric proportions, pigmentation and general activity of acridids in favour of the solitary or gregarious phase. Moreover, they reported that a gregarious sensorial flux seems to inhibit activity of this gland.

Differences in ecdysteroid titer between the solitary and gregarious phases of adult *Schistocerca* are particularly striking in the males (Fig. 21B). The titer rise beginning around day 10, i.e. just before mating, is small and transient in the gregarious but high and persistent in the solitary males. This increase in ecdysteroids is closely followed by elevated production of physiologically active volatiles that were identified as a mixture of anisole, benzaldehyde, veratrole, guaiaol, phenylacetonitrile (major product, comprising about 80—85% of the mixture) and phenol. These odour components of mature males of the gregarious desert locust are active in an electrophysiological test and elicit aggregation behavior in intact locusts (Torto *et al.*, 1994). It is significant that they are never emanated in effective amounts by the solitary males and by either solitary or gregarious females, and that in the gregarious males their production reaches a sufficient level only after mating. The coincidence between ecdysteroid rise and increased

production of the volatiles indicates that both these processes occur in response to a single cue (mating) but it cannot be excluded that ecdysteroids in low concentration stimulate and in high concentration inhibit the release of volatiles. Adams *et al.* (1984) found that ovariectomized houseflies do not produce their sex pheromone unless implanted with the ovaries or injected with ecdysteroids.

CONCLUSION
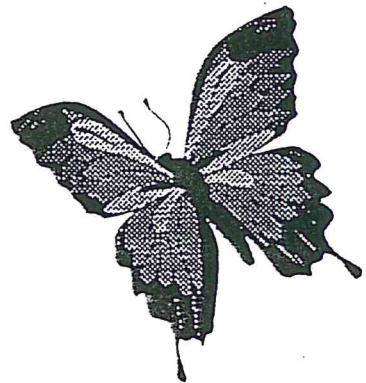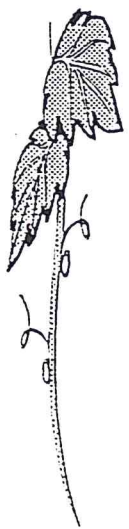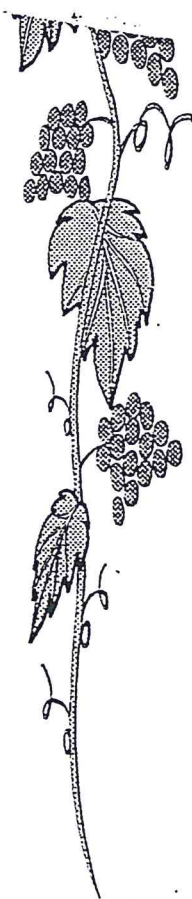
CONCLUSIONS

The present results clearly demonstrate that the corpora allata (CA) and their product, juvenile hormone (JH) play a crucial role if not the primary intrinsic factor on phase changes and pheromone production in the desert locust, *S. gregaria*.

On the other hand, available information suggests that hemolymph ecdysteroids belong to factors that influence phase dynamics and pheromone production in *S. gregaria*. Changes in phase characters (behavioral, morphometric, coloration, physiological and pheromone production) are likely to be brought about by interactions of juvenile hormone and ecdysteroids under the over-riding control by the central nervous system.

Schematic representation of the possible control of phase changes by endocrine [IGRs (JH and ecdysteroids)] and exocrine [environmental, density and food,...] factors are shown in Fig. 22.
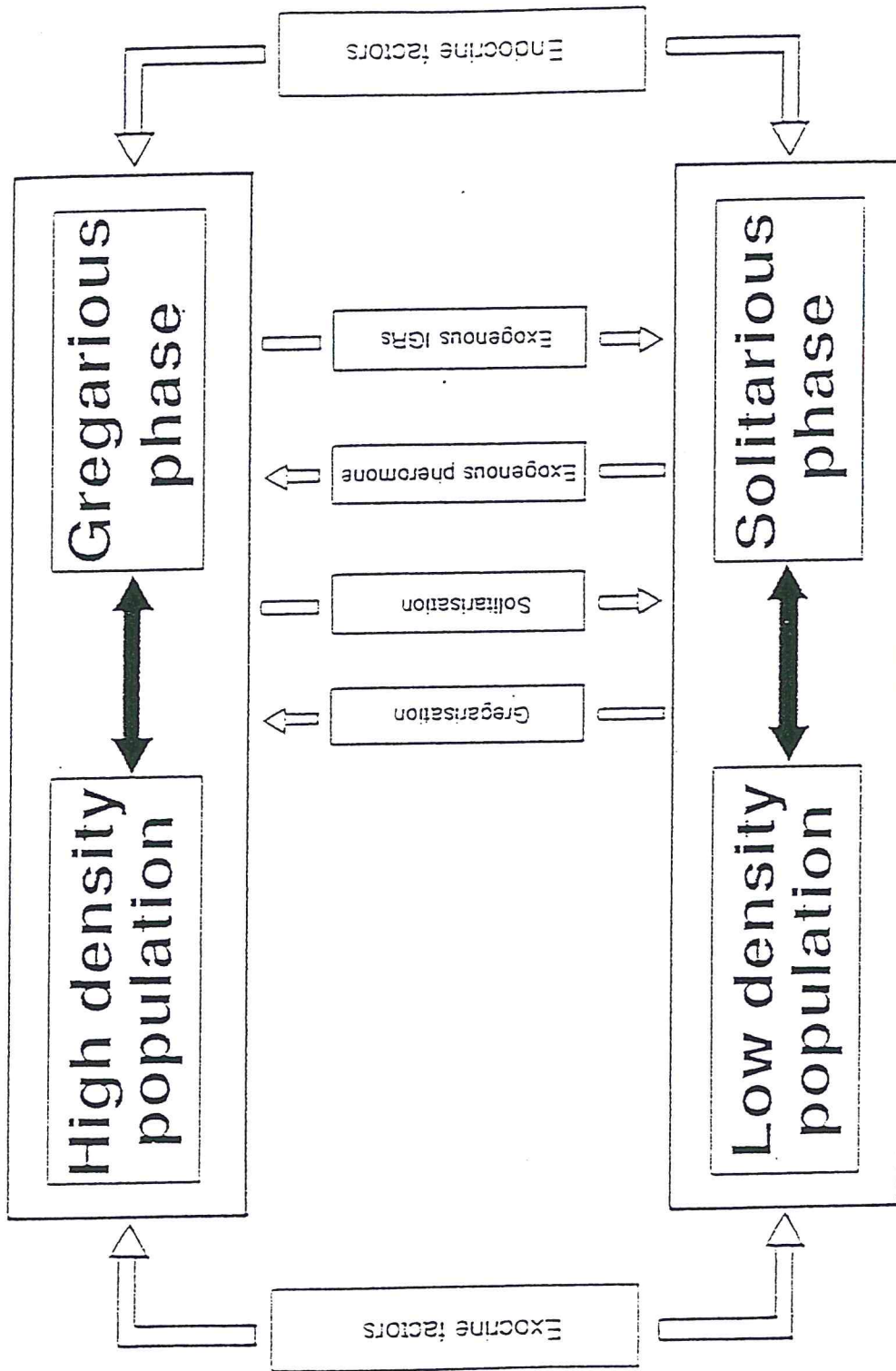
Fig. 22.   Schematic representation of the possible control of phase changes by endocrine and exocrine factors.
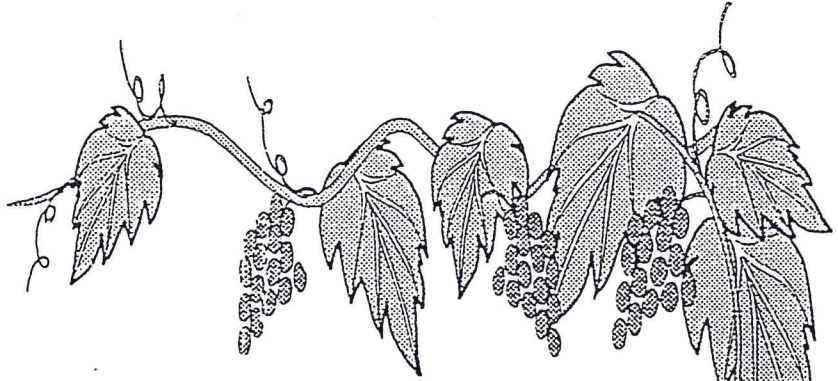
SUMMARY
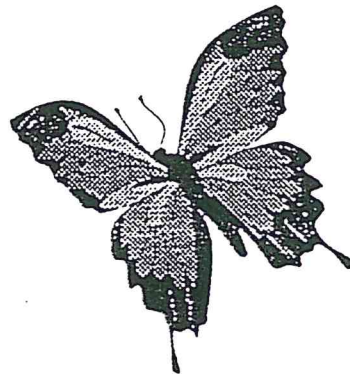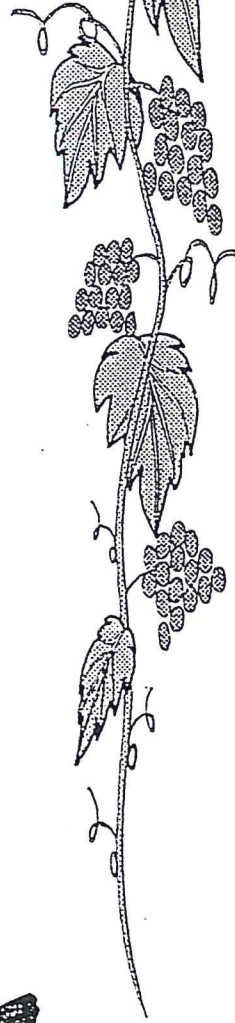
SUMMARY

CORPUS ALLATUM VOLUME AND JUVENILE HORMONE TITER

The volumes of the corpora allata (CA) were determined for the last two stadia and the adult stage of both sexes in the solitary and gregarious phases of *S. gregaria*. In addition, the oocyte length was also studied. The CA volumes of the solitary females were generally larger than those of gregarious females in all the three stages examined. On the other hand, there was no clear relationship in the CA volume between solitary and gregarious males in the last two stadia. Whereas, in adult males the CA volumes of gregarious were larger than those of their solitary counterparts between days 2 and 18 after fledging. However, there was a reduction in the CA volume after day 20 in gregarious phase and *vice versa* in solitary males. The CA volume of the solitary and gregarious females varied cyclically in relation to the growth of the oocytes during the first and second gonotrophic cycles.

The JH titers in the hemolymph of gregarious adult females were generally higher than those of solitary females at all ages examined. However, in gregarious adult males, the JH titer was high at day 10 (197 nmol/ml) and then decreased to 26 nmol/ml at day 20. Thereafter, the JH titer increased again to 101 nmol/ml at day 30 after fledging. On the other hand, in the solitary adult males, the JH titer was low (12 nmol/ml) at day 10 and then increased to 67 nmol/ml and 46 nmol/ml at day 20 and at day 30 after fledging, respectively. In gregarious adult

males, it was observed that the pheromone production coincides with JH threshold and a reduction in the CA volume.

THE EFFECTS OF EXOGENOUS JUVENILE HORMONE (JH III) AND ANTI-JH (PRECOCENE II)

Generally, last instar nymphs exhibited a higher sensitivity to applications in comparison to adult stage. The results of the present experiments showed that JH plays an important role in the regulation of pheromone production of the gregarious adult male, *S. gregaria*. In treated locusts both maturation, sexual behavior and mating were affected by JH and precocene. Application of JH also, resulted in the fading of the yellow color of gregarious adult males and shifting the hemolymph pigment ratio from the gregarious to the solitary phase ranges. In general, precocene exerted a somewhat weaker effects on pheromone production and coloration in comparison to the effects of JH. While, injection of JH or precocene had no or only minor influences on phase changes and pheromone production. Topical applications and JH in the vapor phase, on the other hand, exerted a high effects on both phase changes and pheromone production.

COMPOSITION AND TITER OF HEMOLYMPH ECDYSTERODS IN LARVAE

Four types of ecdysteroids were identified in the hemolymph of larvae as follow: 20-hydroxyecdysone (20E), ecdysone (E), makisterone A (MaA) and high polar metabolites (HPP). 20E makes up 74-84% of

detected ecdysteroids in the female, and 63-74% in the male locust larvae. The content of ecdysone and putative MaA were higher in the solitary, while that of polar metabolites was higher in the gregarious phase.

The phases also differed in that the molt-inducing ecdysteroid peaks last longer but seem less high in the gregarious than in the solitary. Thus, in the solitary penultimate (5th) instar, ecdysteroid titer peaked on day 2, drops on day 3 and then remains low until ecdysis, while in the gregarious penultimate (4th) instar the peak persisted through day 3. In the last larval instar, a small elevation of ecdysteroids occured in both phases on day 1 or 2, and another rise on day 4; in the gregarious phase this rise continued to a peak extending from days 5 to 7 and tapering on day 8. Whereas, in the solitary phase the titer droped on day 5 and the molt-inducing surge was limited to day 6.

COMPOSITION AND TITER OF HEMOLYMPH ECDYSTERODS IN ADULTS

Also, four types of ecdysteroids (20E, E, MaA and HPP) were identified in the hemolymph of adults as in the larval stages. In the adult females the ecdysteroid ratio of E to 20E appeared to be present in similar amounts (from 40-50% of all RIA-positive material). Whereas, adult males were characterized by the domination of 20E that makes 73% of total ecdysteroids in the solitary and 94% in the gregarious phase.

Adults of the solitary phase clearly contained in the hemolymph much larger amounts of total ecdysteroids than gregarious phase. In both the solitary and gregarious adult females, ecdysteroid titer rose to a small peak around day 4, a high peak between day 10 and 18, and another small peak between days 28-40. However, ecdysteroide fluctuations in the solitary and gregarious males followed identical patterns characterized by a sharp peak on day 4 and a broad peak on days 16-20.
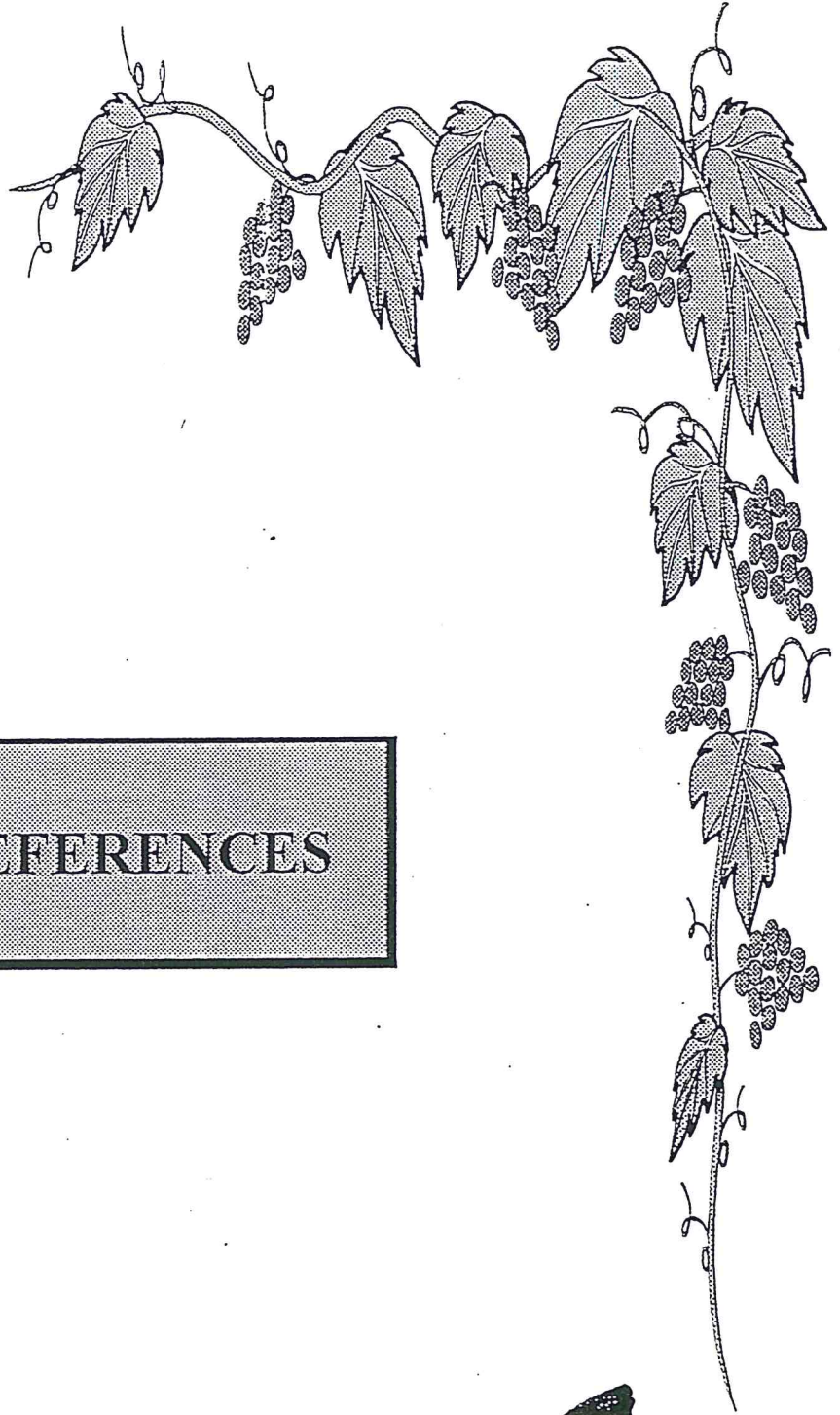
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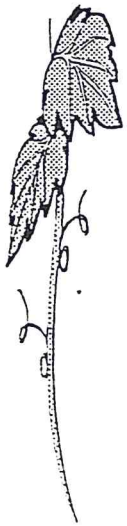
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SUMMARY