STUDIES ON SOME FACTORS THAT INFLUENCE PHASE DYNAMICS OF THE DESERT LOCUST, *Schistocerca gregaria* (FORSKÅL) (ORTHOPTERA: ACRIDIDAE)

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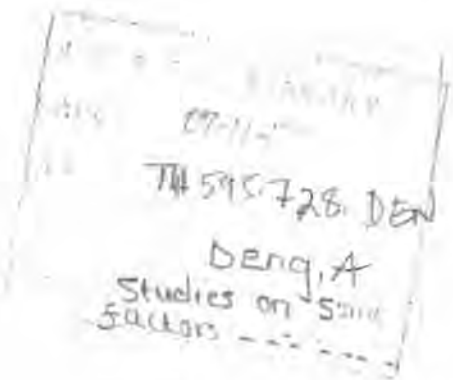

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DECLARATION

I hereby declare that the work embodied in this thesis is a result of my own investigations during the three years research undertaken under supervision at the (ICIPE), Nairobi, Kenya, has not been submitted before for any degree in any other University.

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DEDICATION

To the memory of my parents late Leek (Agerkuei) and Akur, brother Kon, and wife Achol.

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ABSTRACT

The rate and the degree of reversible change in the phase characteristics of the desert locust, *Schistocerca gregaria* (Forskal), on crowding and uncrowding have been compared by two new parameters, aggregation pheromone titre (as measured by released phenylacetonitrile) and haemolymph pigment composition (as measured by absorbance ratio at 460 and 680nm) with colour and morphometrics. Changes in the four parameters followed different patterns. Adults of the F_0 generation resulting from uncrowding crowd-reared (gregarious) nymphs, fledglings or mature adults were grey or dull yellow and did not produce phenylacetonitrile similar to solitary-reared adults. Conversely, adults of the F_0 generation which emerged from crowding solitary-reared (solitarious) nymphs, fledglings or mature adults were yellow in colour and produced pheromone levels which were not significantly different from those of the control adults from crowd-reared colony. The levels of pheromone increased in the F_1 generation relative to those of the control but decreased and levelled off in the F_2 and F_3 generations. Extreme sensitivity to crowding effects was demonstrated by the fact that adult males of the F_0 generation resulting from crowding solitarious hoppers in groups of only two per cage produced phenylacetonitrile, although in significantly lesser amounts compared to

those from normal experimental crowding condition (four/cage) or from crowd-reared control insects. Haemolymph pigment composition, on the other hand, gave a contrasting trend. It changed rapidly in nymphs, but slowly and erratically in adults both resulting from uncrowding or crowding at hopper stage. Significant shifts were recorded in both situations in nymphs within the F_0 generation and in the adults by the end of the F_2 and F_3 generations of solitarisation and gregarisation, respectively. In contrast to pheromone emission and haemolymph pigment composition, morphometrics changes were slowest, significant shifts taking several generations. The F/C (hind-femur length to head capsule width) ratio was more sensitive to treatment effects than E/F (fore-wing to hind-femur length) ratio in agreement with previous findings.

The primer effects of the volatiles emitted by live gregarious nymphs (first, third to fifth and fifth instars and their faeces), on solitarious nymphs and fledglings did not follow the same pattern. The volatiles from live gregarious first instar nymphs and faeces had no significant effect on haemolymph pigment composition but was associated with slight increase in the weight of test solitarious first instar nymphs and their development compared to control. In contrast, the volatiles from live gregarious third to fifth instar and their faeces induced formation of black patterns (melanization) on the bodies of first instar solitarious

nymphs, changed their background colour from green to pinkish or pale green and arrested their development which was followed by death within three to four weeks as second or early third instar nymphs. On the other hand, this pheromone system had no notable effects on haemolymph pigment composition, weight and developmental time of solitarious second instar nymphs but had a slight effect on their colouration. Likewise, the colour, pheromone emission and haemolymph pigment composition of adults resulting from exposure of solitarious fledglings to the pheromone were not affected.

In summation, this study has shown that: (a) the different phase characters change at different rates during uncrowding and crowding of the desert locust. Of the four parameters monitored, the emission of adult aggregation pheromone and integumental colour are more sensitive measures of the onset of phase change in adult desert locust. At the nymphal stages, integumental colour and haemolymph pigment composition are more suitable for monitoring phase change; (b) chemical communication (pheromones) in the desert locust appears to play an important primer role in phase change. The releaser/primer pheromone produced by nymphal gregarious locusts is stage- dependent and that of the late instars significantly affect the development of first instars of solitarious locusts.

ملخص الإطروحه

تمت مقارنة درجة التحول العكسي في صفات مظهر الطور الشكلي في الجراد الصحراوي Schistocerca gregaria (FORSKAL) بمعيارين مستحدثين هما (١) مستوي انتاج فيرمون التجمع (Aggregation Pheromone) ويقاس بمستوي افراز مركب فينيل اسيتو نيتريل (Phenylacetonitrile) و (٢) الصبغات الموجودة بالدم (Haemolymph digment composition) ويقاس بنسبة امتصاص الضوء علي الموجتين ٤٦٠ و ٦٨٠ نم . هذا بالاضافة الي معياري تغير اللون (Colour) وحاصل قسمة مقاييس ابعاد اجزاء من جسم الحشره (Morphometrics) (اثنين من المعايير المتبعه قديما) . هذا وقد ثبت ان درجة التحول او التغير في هذه المعايير الاربعه لا يسير علي وتيره واحده . وقد اثبتت التجارب ان ذكور الجيل الاول من سلالة المظهر الجماعي المرباه علي انفراد (Uncrowding) من طور الحوريه او حتي في مرحلة الحشرة الكاملة غير البالغة او البالغة اضحت رماديه اللون او فاتره الاصفرار ولم تنتج مركب الفنيل اسيتو نيتريل ، مشابهه في ذلك مع الطور البالغ من المظهر الانفرادي المعروف (Solitarious Phase) علي نقيض ذلك فان ذكور الحشرة الكاملة في الجيل الاول من سلالة المظهر الانفرادي المرباه في مجموعات (Crowding) سواء من طور الحوريه او الحشرة الكاملة غير البالغة او البالغه اضحت صفراء اللون وانتجت المركب الفيرموني بمستويات لم تكن مختلفة عن الذي تنتجه الاطوار البالغه من المظهر الجماعي (Gregarious Phase) .يجب الاشاره الي ان معدل افراز الفيرمون في هذه الحالة كان عاليا في الجيل الثاني مقارنة بالجيل الاول والمظهر الجماعي غير المعامل (Control) ثم تقلص هذا المعدل الي مستوي ادني في الجيلين الثالث والرابع . كذلك اثبتت الدراسه حساسية الافراد من المظهر الانفرادي للتربية في مجموعات، حيث ان الذكور البالغة من الجيل الاول الناتجه عن تربية حوريات المظهر الانفرادي في مجموعات تحتوي علي حوريتين فقط قامتا بانتاج مركب الفينيل اسيتو نيتريل وان كان بمعدل اقل من الذي تفرزه الذكور البالغه من المظهر الجماعي غير المعامل او الذكور البالغه والناتجه من تجربة التربية في مجموعة مكونة من اربع حوريات في حيز التجربة الواحدة . اما بالنسبة لتركيب الصبغات الموجودة بالدم فقد اتخذ طابعاً مخالفاً في التغير حيث تغير بمعدل سريع في الحوريات وببطيء وبنمط غير منتظم في الطور الكامل الناتج عن التربية الجماعية او الانفراديه للحوريات . التحول الكامل في هذه الصفه حدث في الجيل الاول في حالة الحوريات وفي الجيلين الثالث والرابع من الحشره الكاملة في حالتي التربية المنعزله والجماعيه علي التوالي .

علي سبيل المقارنه فان التغير الشكلي (Morphometric Changes) كان بطيئا مقارنة بالتغير في اللون ، معدل افراز الفيرمون وتركيب الصبغات الموجودة بالدم، حيث ان التحول الكامل استغرق عدة اجيال . وقد لوحظ ان نسبة طول الفخذ الخلفي الي عرض الراس (F/C)كانت اكثر حساسية للتغير من نسبة طول الجناح الامامي الي طول الفخذ الخلفي (E/F) . هذه الملاحظة تتفق مع الملاحظات السابقة في هذا المجال .كذلك تمت دراسة تاثير المواد الكمياوية الطياره المفرزه من حوريات المظهر الجماعي (الاول، الثالث الي الخامس والخامس) وبرازها علي حوريات المظهر الانفرادي . اتضح ان المواد الطياره الناتجه من الطور الاول الجماعي لم تؤثر علي تركيب الصبغات الموجودة بالدم في حوريات الطور الاول من المظهر الانفرادي ، وانما تسببت في ازدياد طفيف في وزن هذه الحوريات ومعدل نموها مقارنة بالحوريات غير المعامله . بالمقارنة فقد تسببت المواد الطياره المفرزه من حوريات الطور الجماعي الثالث الي الخامس والخامس في تكوين بقع سوداء علي جسم حوريات الطور الاول الانفرادي المعامله وغيرت لون عامة الجسم من اخضر الي بنفسجي او اخضر باهت، ثم ادت الي تاخير معدل نموها وموتها خلال فترة تراوحت بين ٣ الي ٤ اسابيع وهي في مرحلة الطور الثاني او الثالث . من ناحية اخري فان هذه المواد الطياره لم تؤثر علي تركيب الصبغات الموجودة بالدم او الوزن او معدل النمو في طور الحوريه الثاني من المظهر الانفرادي المعامل وانما اثرت قليلا عل لونها . كذلك لم يتاثر اللون الافراز الفيرموني او تركيب الصبغات الموجودة بالدم في الحشره الكامله من المظهر الانفرادي يتعرضها لفيرمون حوريات الطور الخامس من المظهر الجماعي .

في الخلاصه فقد اثبتت هذه الدراسه الاتي:-

١- ان جميع صفات التحول المظهري في الجراد الصحراوي تتغير بدرجات ومعدلات متفاوته اثناء الانفراد والتجمع .

٢- ان افراز فيرومون التجمع ولون الجلد كانتا اكثر المعايير ملاءمة لمعرفة حدوث التحول المظهري للجراد الصحراوي البالغ، وان تركيب الصبغات الموجودة بالدم ولون الجلد هما انسب المعايير لمعرفة التحول المظهري في الحوريات .

٣-ان للفيرمونات علاقة وثيقة بالتحول المظهري للجراد الصحراوي حيث ان المواد الطياره او الفرمونات الناتجه عن الطور الاول الجماعي تختلف نوعا ووظيفة عن فيرمون الحوريات المتقدمة في العمر حيث ان فيرمون الطور الاول الجماعي يؤدي الي ازدياد في وزن ومعدل نمو حوريات الطور الاول من المظهر الانفرادي بينما يؤدي فيرمون الاطوار المتقدمة الي تاخير نمو هذا الطور وموته لاحقا .

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ABBREVIATIONS

A	Absorbance
ANOVA	Analysis of Variance
C	Greatest Width of the Head Capsule
^{o}C	Degree(s) Celsius or Centigrade
CA	Corpora Allatum
cAMP	Cyclic Adenosine 3, 5 monophosphate
CED	Chemical Ecology Department
cm	Centimetre
E	Length of the Fore-wing or Elytron
E/F	Length of Elytron to Hind-femur Ratio
F	Length of the Hind-femur
F_0	Parental Generation
F_1	First Generation
F_2	Second Generation
F_3	Third Generation
F/C	Length of the Hind-femur to Head Capsule Ratio
FID	Flame Ionization Detector
FIG.	Figure
GC	Gas Chromatography
G/C	Gregarious Control
G/I	Gregarious Isolated (Uncrowded)
h	Hour
HP	Hewlett Packard
HPLC	High Performance Liquid Chromatography

(ABBREVIATIONS CONTD.)

ICIPE	The International Centre of Insect Physiology and Ecology
ID	Internal Diameter
INT.	International
JH	Juvenile Hormone
LD	Light and Dark
Ltd	Limited
LSD	Least Significant Difference
M	Metre
mg	Milligram
Min	Minute
ml	Millilitre
µl	Microlitre
mm	Millimetre
µm	Micrometre
ng	Nanogram
nm	Nanometre
ns	Not significantly different
PG	Prothoracic Gland
SAS	Statistical Analysis System
S/C	Solitarious Control
SE	Standard Error
S/G	Solitarious Grouped (Crowded)
TR	Total Transformation Range

1. INTRODUCTION

1.1. General Introduction

Locusts are polymorphic species which belong to the family Acrididae and composed of a large group of short-horned grasshoppers. They are capable of changing phase reversibly both in terms of behaviour and physiology in response to the prevailing biotic and a biotic factors (Gillett, 1988). Locusts become highly gregarious when crowded, and a social when in isolation. The two extreme forms, *gregaria* and *solitaria* encompass several intermediate forms known as transients (Gunn and Hunter-jones, 1952; Pener, 1991).

The economically important species of locust in Africa are: the red locust, *Nomadacris septemfasciata* Serville; the brown locust, *Locusta pardalina* Walker; the African migratory locust, *Locusta migratoria migratorioides* (Reiche and Farmaire); the tree locust, *Anacridium melanorhodon* (Walker); and the desert locust, *Schistocerca gregaria* (Forskal) (Schmidt, 1988; Steedman, 1988). Among these species, the desert locust is the most pestiferous due to its ability to form giant swarms across widely distributed breeding and invasion zones in Africa and parts of Asia (Fig. 1) (Uvarov, 1977; Steedman, 1988). All these different types of locusts show similar characteristics, significant

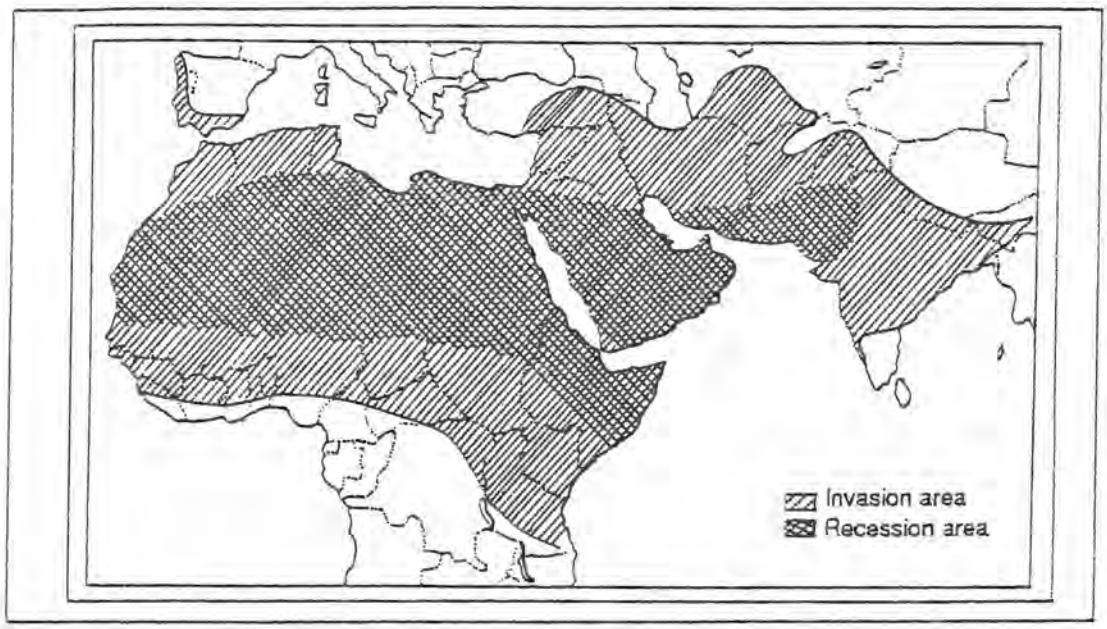

Fig. 1 Desert locust, *Schistocerca gregaria* recession and invasion areas within Africa, Middle East and Asia. (Source: Steedman, 1988).

among these include their efficient migratory behaviour, swarm formation, and polyphagous nature of feeding on both wild and cultivated plants.

The desert locust, *S. gregaria* is the most widely studied species among the five different types of locusts. Individuals in the two phases differ in morphology, behaviour, and physiology (Steedman, 1988; Loher, 1990; and Pener, 1991). An adult in the solitary phase is often pale-grey or beige 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; Steedman, 1988; Loher, 1990; and Pener, 1991).

Behaviourally, solitary locusts live as harmless scattered individuals. The adults usually fly at night and occasionally during the day when disturbed (Steedman, 1988). Gregarious adults, on the other hand, fly together in swarms diurnally, exhibiting a relatively higher flight activity compared to solitarious individuals (Michel, 1980). The immature swarming locust is capable of flying continuously for a period of 6-20 hrs with an average speed of 16-19 Km/hr (Steedman, 1988). The gregarious hopper stages march in bands (Roffey, 1963; Steedman, 1988), a feature which is not associated with solitary hoppers.

Solitary locusts generally appear larger than gregarious forms, but can be distinguished using more rigorous morphometric measurements. Typical among these include ratios of adult forewing or elytron(E) to hind femur length (F) (E/F) ratio and hind femur length (F) to the greatest width of head capsule (C) (F/C) ratio. Various authors have reported different values for these ratios. For example, E/F values are less than 2.05 in *solitaria*, greater than 2.15 in the *gregaria* and range between 2.06 to 2.15 in *transient* (Rao, 1937); 1.92 to 2.20 in *solitaria* and 2.11 to 2.46 in *gregaria* (Dirsh, 1951); 1.98 to 2.18 in *solitaria* and 1.94 to 2.28 in *gregaria* (Gunn and Hunter-jones, 1952) and greater than 2.23 and 2.27 in *gregaria*, and less than 2.03 and 2.08 in *solitaria* for males and females, respectivley (Meinzingen, 1993; Ochieng Odero *et al.*, 1994). The different F/C values reported include for example a range of 3.77 to 4.10 in *solitaria* and 3.24 to 3.59 in *gregaria* (Jackson *et al.*, 1978); greater than 3.75 in males and 3.85 in females of *solitaria* and less than 3.15 in both males and females of *gregaria* (Meinzingen, 1993; Ochieng Odero *et al.*, 1994). These ratios (E/F and F/C) have been found to vary with locust strain (Nolte, 1977). More egg-pods and eggs per pod are produced by solitary females than by their gregarious counterparts (Jackson *et al.*, 1978; Injeyan and Tobe, 1981; Steedman, 1988).

Locust control involves the use of insecticides which are harmful to both human and animal health and pose serious environmental risks. Alternative control strategies require a detailed knowledge of the behaviour and ecology of locusts. At the International Centre of Insect Physiology and Ecology (ICIPE), research is geared towards this goal, and involves the investigation of the factors associated with different behavioural patterns which are related to locust phase transformation, with special emphasis on semiochemical communication. Since 1990, a comprehensive reinvestigation of the factors mediating gregarisation, solitarisation, synchronized maturation, oviposition and host-plant selection has been carried out, the details of which are reviewed in Chapter 2.

1.2. Objectives of The Study

In the past, colour and morphometrics, were used to characterize the phase status of the desert locust, *S. gregaria*. These measurements sometimes did not correlate with other features associated with changes in the transformation of the desert locust. A good example is behaviour which does not always match with the physical characteristics of the insect, as in the case of a gregarious-like swarm of locusts studied by Kennedy (1939) which showed typical *solitaria* morphometrics. In a different

study, Jackson and co-workers (1978) found that colour patterns of the test locusts fed on the same food plants differed even within individuals. These observations were confirmed by Steedman (1988) who reported that the changes in locust behaviour and appearance often occurred at different rates. These examples show that a detailed systematic study involving a comparison of the sensitivities of several phase traits is required to demonstrate the changes that occur in the desert locust during phase transformation. In this regard, the present study was initiated to systematically investigate some physiological and morphological changes associated with phase transformation in the desert locust with the following specific objectives:

1. To investigate the effect of uncrowding and crowding of different locust stages (nymphs, fledglings and mature adults) on the rate and degree of phase-related changes as reflected in pheromone titres, haemolymph pigment composition, integumental colour and morphometrics.

2. To investigate the primer effects of the releaser pheromones produced by gregarious nymphs and adults on the rate and degree of phase-change and development in solitarious nymphs and adults with respect to the four indicators described in 1.

2. LITERATURE REVIEW

Phase transformation in the desert locust, *Schistocerca gregaria* (Forskal) from gregarious to solitarious form or vice versa has been shown to be influenced by various factors including density (Gunn and Hunter-jones, 1952; Gillett, 1972, 1973; Michel, 1980; Injeyan and Tobe, 1981; Gillett, 1988; Duranton and Lecoq, 1990; Popov *et al.*, 1991), temperature and humidity (Hussain and Mathur, 1943; Goodwin, 1952; Stower, 1959; Hunter-jones, 1962; Dudley, 1964), diet (Gunn and Hunter-jones, 1952; Barnes, 1955; Jackson *et al.*, 1978), hormone (Joly, 1954; Staal, 1961; Ellis and Carlisle, 1961; Rowell, 1967; Ellis and Novak, 1971; Willson and Morgan, 1978; Dale and Tobe, 1986; Pener, 1991), previous phase history (Hunter-jones, 1958; Michel, 1980; Roessingh *et al.*, 1993 and Islam *et al.*, 1994), interaction with other locust species (Johnston and Buxton, 1949; Loher, 1990; El Bashir and Abdel Rahaman, 1991) and chemical stimuli (Nolte, 1963, Gillett, 1968; Nolte *et al.*, 1973; Gillett, 1975a and b; Nolte, 1977; Gillett and Phillips, 1977; Gillett, 1983; Fuzeau-Braesch *et al.*, 1987; Obeng-Ofori *et al.*, 1993, 1994a and b; Torto *et al.*, 1994). Tactile and visual stimuli have also been implicated in phase change by Ellis (1959) and Ellis and Pearce (1962), respectively, but no detailed studies have been carried out

to investigate the role played by these stimuli in the transformation process.

2.1. Density

The first record of the effect of density on phase status of the desert locust was reported by Gunn and Hunter-jones (1952). They found that when locusts are isolated in cages their hatchlings usually gave hoppers which differed in colour from naturally occurring hoppers, while adults appeared to be biometrically similar to individuals of the naturally occurring *solitaria*. However, when these insects are crowded in cages, hoppers appeared to be chromatically like naturally occurring gregarious nymphs, while adults appeared to be biometrically unlike specimens of naturally occurring *gregaria*. They further observed that chromatic properties of solitary *S. gregaria* hoppers were not readily produced by merely rearing locusts for a single generation at low densities (two or more per cage). On the other hand, rearing hoppers singly did not produce brown ones typical of phase *solitaria*. In their experiments only one out of the 16 hoppers studied turned out as truly *solitaria*.

Kennedy (1956) and Steedman (1988) reported that individuals of phase *solitaria* of the desert locust, in the field lived as scattered individuals and transformed into

highly gregarious and mobile forms as population density rose. Pener (1991) stated that phase transformation in the desert locust followed changes in population density. He reported that full-scale phase change seemed to be limited to field conditions. The author concluded that locusts raised in groups or isolated in the laboratory only approached the characteristics of the gregarious or solitarious, if reared for several generations. Laboratory experiments were, however, not carried out to confirm these observations. Injeyan and Tobe (1981) investigated the degree of transformation among successive generations of the solitarious desert locust and found that although at the second generation about 50% of the insects had transformed into gregarious ones, full transformation was not attained even by the sixth generation.

In a study to investigate whether locusts became temporarily less gregarious after final moult, Gillett (1972) found out that there was no reduction in grouping after fledging, but rather a sharp reduction occurred in the formation of large groups by mature *S. gregaria*. Gillett (1973) also studied the effect of different locust densities on subsequent grouping behaviour and found that significantly more nymphs and adults reared under high density conditions formed groups than did those reared in isolation. The nymphs more readily showed touching behaviour typical of the extreme phase of *gregaria* than adults.

Michel (1980) studied the relationship between the density and flight behaviour of the desert locust, raised in isolation then in a group and found that increasing density favoured flight activity. However, his results suggested that migratory behaviour of locusts, regrouped after one to several generations of isolation, was greater than that of individuals permanently raised at high densities over numerous successive generations.

Gillett (1988) measured the rate at which gregarious nymphs of *S. gregaria* lost their tendency to form groups following different periods of isolation and observed a marked loss of social grouping after isolation for 24 hrs. The fall in the level of social grouping was found to be highly correlated with the number of days the nymphs had spent in isolation.

Recently, it was suggested that the phase status of locusts can be described in accordance with their density in the field. Locust densities of less than 250 per hectare in the field may be considered as solitarious or transients dissociants, and between 250 to 10000 as transients congregants, while more than 10000 may be referred to as gregarious population (Duranton and Lecoq, 1990). On the other hand, Popov *et. al.* (1991) have observed that the least number of locust that can induce gregarious characteristics in the field was 5 and 50 insects per hectare for both nymphs and adults, respectively.

2.2. Temperature and Humidity

Hussain and Mathur (1943) and Dudley (1964) showed that rearing temperature affected the E/F ratios of *S. gregaria*. At high temperatures, crowded locusts exhibited ratios closer to those regarded as characteristic of extreme phase *gregaria*. In their investigations, F/C ratios were not studied. While temperature also affected the distribution of melanin pigment in locust (Goodwin, 1952), humidity affected the background colour of solitary nymphs (Stower, 1959; and Hunter-jones, 1962).

2.3. Dietary Factors

Different-food plants, under different conditions, affect phase transformation in locusts and grasshoppers in different ways. In a study to evaluate the effect of moist-air with fresh food, and dry-air with dry food on phase characteristics in both *Locusta* spp. and *S. gregaria*, Gunn and Hunter-jones (1952) found slight differences in E/F ratios of the extreme forms of both species. The higher ratios were correlated with drier conditions, which appeared to favour gregarious characteristics. In *S. gregaria* the E/F ratios of laboratory specimens of the solitary phase were similar to those of field collected insects. In a different study, Barnes (1955) tested the effects of different diets

comprising alfalfa (*Medicago* sp.), Johnson grass (*Chenopodium murale*), Hedge mustard (*Sisymbrium irio*) and a mixture of the three on morphometrics of adult lesser migratory grasshopper, *Melanoplus mexicanus mexican.* He found that adults which were reared on hedge mustard or a mixed diet showed a greater degree of gregariousness than those reared on Johnson grass. Those raised on alfalfa showed little or no change towards the gregarious phase, maintaining typical solitarious characteristics.

The plant community in a typical habitat of solitarious desert locust is diverse and includes *Heliotropium* spp, *Dipterygium glaucum*, *Tribulus* sp., *Schouwia* spp, *Zygophylum* sp., *Hyoscyamus muticus*, *Aerva persica* and *Pennisetum* sp. and some cultivated crops, such as sorghum and millet which serve both as shelter and food plants (Jackson *et al.*, 1978; Steedman, 1988).

Jackson *et al.* (1978) studied the effect of seven of these natural food-plants on the phase status of the desert locust, and monitored changes in colour, morphometrics, number of eye stripes and fecundity. Their results revealed that *Pennisetum typhoides* (Burm.f.) and *Sorghum* bicolor enhanced gregarious characteristics, while *Dipterygium glaucum* Oecn. accentuated solitary traits.

2.4. Hormones

Density is known to be the primary external factor influencing phase change in the desert locust, but the intrinsic factors affecting the physiological and molecular basis of phase transformation are insufficiently known. While *Corpora allata* (CA) and its product, juvenile hormones (JHs) had been implicated as the primary causal factor (Joly, 1954; Rowell, 1967) the prothoracic gland (PG) and its products, ecdysteroids, apparently played no major role in the process (Ellis and Carlisle, 1961; Willson and Morgan, 1978).

Joly (1954) and Staal (1961) demonstrated that implantation of an extra CA, or administration of JH or JH analogues to crowded hoppers of *L. migratoria migratorioids* induced the green colour of solitary locusts. This finding also has been demonstrated in *Schistocerca* by Novak and Ellis (1967) and more recently by Pener (1991). On the other hand, removal of 75% of the PG in green hoppers of solitary *S. gregaria* induced black pigmentation and a yellow or creamy background colour in operated locusts (Ellis and Carlisle, 1961). Rowell (1967) found that CA controlled the green/brown polymorphism in Acridoidea in experiments involving implantation of a single CA from a young adult donor of the same species in the larvae of the African grasshoppers *Acanthacris ruficornis*, *Humbe tenuicornis* and

Gastrimorgus africans. In all three species, the post-operated larvae included significantly more green forms than controls. Ellis and Novak (1971) observed that a balance between the two metamorphosis hormones, JH and ecdysone determined the final colour of hoppers in *S. gregaria*.

An estimate of the JH titre biosynthesis in isolated and crowded adults of *L. migratoria migratorioids* showed that there were no significant differences between isolated and crowded one-day-old fledglings, although by day seven the titre showed a two-fold increase in isolated adults. However, no differences were observed between the females of the two phases in JH biosynthesis by CA from day zero through day six (Dale and Tobe, 1986). Similarly, the ecdysone levels in the bodies of fifth instar nymphs of crowded and isolated locusts were found not to be significantly different (Wilson and Morgan, 1978).

2.5. Previous Phase History

The effects of crowding and isolating parent locusts as nymphs or adults and progeny of crowded and isolated parents on the changes in progeny colour has been studied in *S. gregaria* and *L. migratoria migratorioides* (Hunter-jones, 1958). In both species, crowded parents were found to produce dark and relatively heavy hatchlings, while isolated

parents gave pale and less heavy ones. It was concluded that the relationship between parental density and hatchling colour was associated with crowding, but not with the presence of male. This was confirmed by crowding virgin females which produced parthenogenetically dark hatchlings.

Roessingh *et al.* (1993) demonstrated that previous rearing history had a marked effect on subsequent behaviour of nymphal desert locust. For instance, nymphs reared in isolation avoided a group of locusts whereas, crowd-reared insects were more often attracted to the group. These results corroborated those of previous workers (Ellis and Pearce, 1962; Gillett, 1973).

In studies designed to investigate the effect of maternal and paternal phase status and the density under which mating and/or oviposition occurred on the behaviour of the offspring in *S. gregaria*, Islam *et al.* (1994) found that both colour and behaviour of the offspring were significantly affected by the phase status of the father, but the influence of the mother is more pronounced with respect to colour. Likewise, isolated parents either mated in a crowded situation, followed by crowded oviposition or reared at birth in a crowded situation induced gregarious behavioural phase characteristics in their offspring.

2.6. Interaction Between Locust Species

Evidence of the interactions between locust species has been reported for *L. migratoria migratorioides* and *S. gregaria*, but the effect these interactions have on phase transformation on either species is not well known. A mixed band of hoppers composed of both species in a habitat in northern Sudan was reported by (Johnston and Buxton, 1949). Both species were found to lay their eggs simultaneously, with emerged hoppers mingling freely in bands in varying proportions. These authors further observed that vegetation played an important role in segregating the two species in a band. For example, whereas *Schistocerca* hoppers in a mixed band remained to feed on encountering a patch of *Sesbania tetraptera*, *Locusta* hoppers which fed on grasses moved on. These observations were corroborated by reports by Loher (1990) of interactions between *S. gregaria*, on the one hand, and *L. migratoria migratoroides* and *L. pardalina*, on the other.

In a survey carried out in the winter breeding habitats of the desert locust along the western coast of the Red Sea, Sudan, El Bashir and Abdel Rahaman (1991) observed mixed bands of different stages of solitarious desert locust and other species of locusts, particularly the migratory locust. They suggested that these interactions may have an effect on the behaviour and phase change of the desert locust. Typical

solitarious hoppers were observed to have acquired gregarious colour patterns. Furthermore, they noted that the hopper density in the field was approximately two desert locusts to every two hundred African migratory locusts while it was 1:10 for adults. The desert locust hoppers were either solitary or transient segregating, while hoppers of the migratory locust were either gregarious or transient congregating. A mixed population of fifth instar nymphs and young adults of *Schistocerca* with young adults of *Locusta* was recently encountered in the Red Sea Coastal area of Sufia, Sudan by Torto (1995, personal communication).

2.7. Chemical Stimuli

The first experiments to demonstrate the influence of chemical stimuli on phase transformation of locusts were carried out by Nolte (1963). He found that an external airborne factor, emanating from locust faeces, influenced the loss of black pigmentation in hoppers reared crowded and then isolated. Nolte also observed that this factor influenced adult morphometrics. Gillett (1968) confirmed Nolte's previous findings by demonstrating that visual and tactile isolation was insufficient to prohibit development of gregarious characteristics in *S. gregaria*. Her behavioural and morphometrical observations showed the existence of an airborne factor, believed to be a pheromone,

which increased the level of grouping of locusts reared in visual and tactile isolation.

Nolte *et al.* (1973) isolated from the faeces of gregarious hoppers a pheromone which they called gregarisation pheromone or locustol. Bioassays of this pheromone on solitarious desert locust hoppers showed quantitative changes in phase traits, such as: chiasma frequencies, adult morphometrics, colour of the integument and the behaviour of the hoppers. Their morphometric measurements revealed that different strains of locust possessed different strengths of phase traits. During transformation from solitarious to gregarious form, other changes may occur, such as an increase in the production of adenosine-3,5-monophosphate (cAMP) (Nolte, 1977).

Gillett (1975a) examined the action of this proposed pheromone on five non-behavioural characteristics by exposing solitary-reared nymphs to the source of this pheromone. Her results showed that while the pheromone decreased the number of nymphs showing the green background colour associated with phase *solitaria*, increased the amount of melanin on them and accelerated their developmental time, it had no effect on the number of eyestripes and morphology. Pheromonal attraction also appeared to be of much less importance than vision in desert locust grouping in test with live insects and cuticular extracts (Gillett *et al.*, 1976).

Gillett and Phillips (1977) also demonstrated the existence of this chemical factor in the faeces of crowded nymphs and found that it promoted gregarious characteristics, such as colour and grouping behaviour in isolated nymphs. Crowded adult faeces, on the other hand, promoted more solitarious characteristics in the isolated nymphs, while immature adult faeces had little effect on locust behaviour (Gillett, 1983).

A more detailed study of the components of air-borne volatiles surrounding gregarious locusts of *S. gregaria* and *L. migratoria* was carried out by Fuzeau-Braesch *et al.* (1988). Three aromatic compounds, viz, phenol, guaiacol and veratrole were identified. Behavioural tests showed that essentially phenol, guaiacol and mixture of the three products tended to increase the aggregation behaviour in both species. In a recent study, Obeng-Ofori *et al.* (1993) showed that aggregation in *S. gregaria* is mediated by two sets of releaser pheromone systems, a juvenile aggregation pheromone produced by nymphs and an adult aggregation pheromone produced by older adults. While aggregation pheromone production in the nymphs was sex independent, young adults of either sex did not produce a stimulus with significant activity. In older adults, males produced the aggregation stimulus to which both sexes were equally responsive (Obeng-Ofori *et al.*, 1994a). The involvement of gregarious nymphs faecal volatiles in the aggregation of

different stages of the desert locust was recently reconfirmed by Obeng-Ofori *et al.*(1994b). They found that faecal volatiles of gregarious nymphs were part of the aggregation pheromone complex of the desert locust and that they aid aggregation in both nymphs and adults in addition to maintaining of cohesion in young adults which do not produce any aggregation stimuli. The stimulus mediating aggregation in gregarious older adults of the desert locust described by Obeng-Ofori *et al.*(1993 and 1994a) was characterized as a blend of phenylacetonitrile, benzaldehyde, anisole, veratrole, guaiacol and phenol (Torto *et al.*, 1994). The blend produced by mature males has been demonstrated to play a parsimonious role as an adult aggregation signal and as a maturation accelerant in young adults (Mahamat *et al.*, 1993). Phenylacetonitrile has been found as a phase specific component present only in the volatiles of gregarious adult males (Torto, personal communication).

3. MATERIALS AND METHODS

3.1. Insects

First instar nymphs, fledglings and mature adults of both solitarious and gregarious desert locust, *S. gregaria*, from ICIPE colonies derived from a culture obtained from the Desert Locust Control Organization for East Africa (DLCO-EA), Addis Ababa, in 1989, were used for crowding and uncrowding experiments. Insects were reared in rooms (1.5 x 4.5m). Standard aluminum cages (50 x 50 x 50cm) and (10 x 10 x 24cm) were used for rearing crowded (gregarious) and isolated (solitarious) locusts, respectively (Ochieng Odero *et. al.*, 1994). A photoperiod of 12:12 LD was maintained using an electric timer connected to the source of power. Temperature was kept at 36±1°C in the light phase and 30±1°C in the dark phase. The relative humidity was maintained at 40-50%. Air flow through the rooms was also maintained at a negative pressure of about 10-15 air changes in an hour. For studies on the primer effects of releaser pheromones, first, second instar nymphs and fledglings of solitarious locusts of the same strain as their gregarious counterparts were used as test insects while first, third to fifth, fifth instar nymphs of gregarious individuals were used as the source of chemical stimuli (plate 1 and 2).

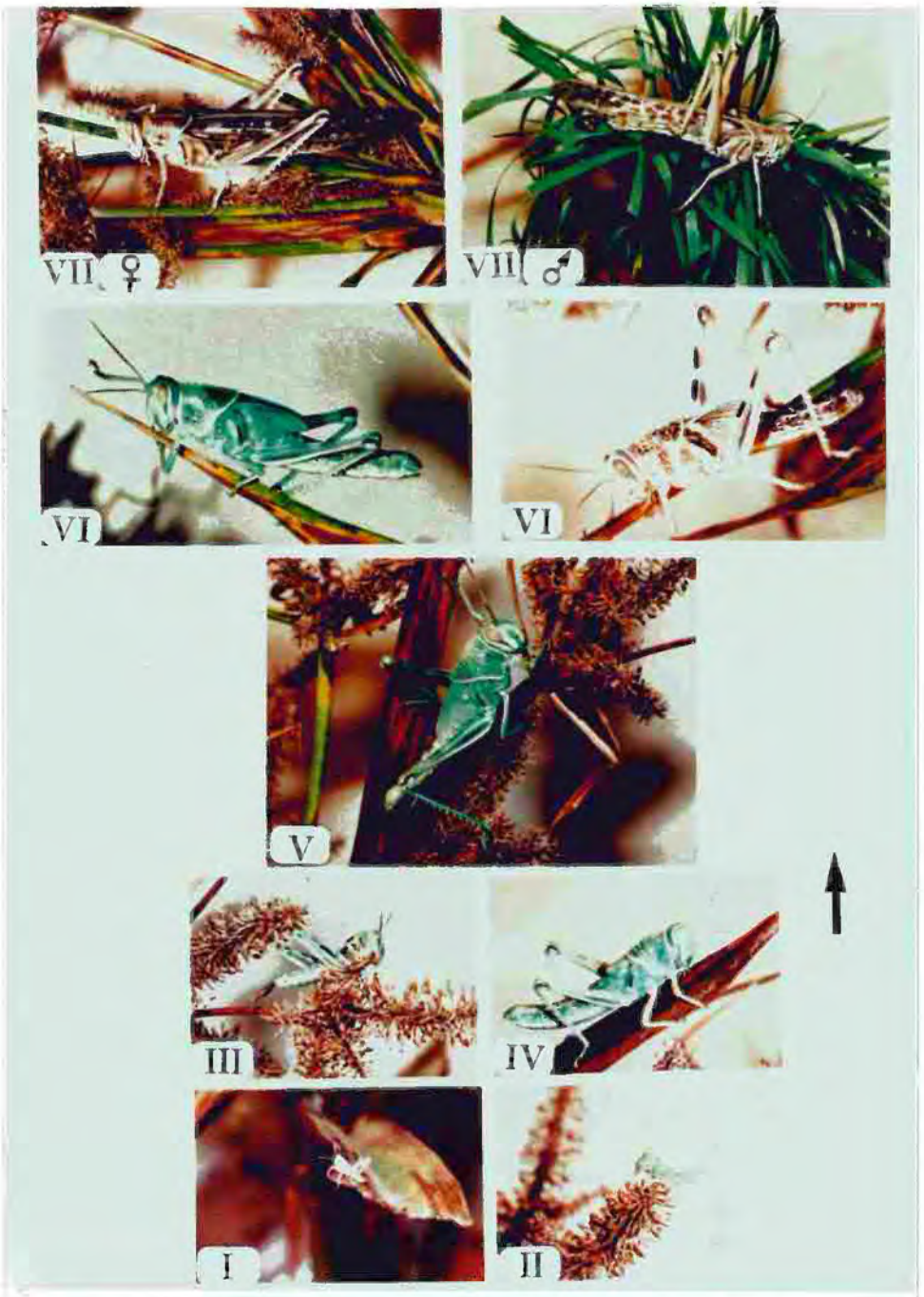
VII ♀
VII ♂
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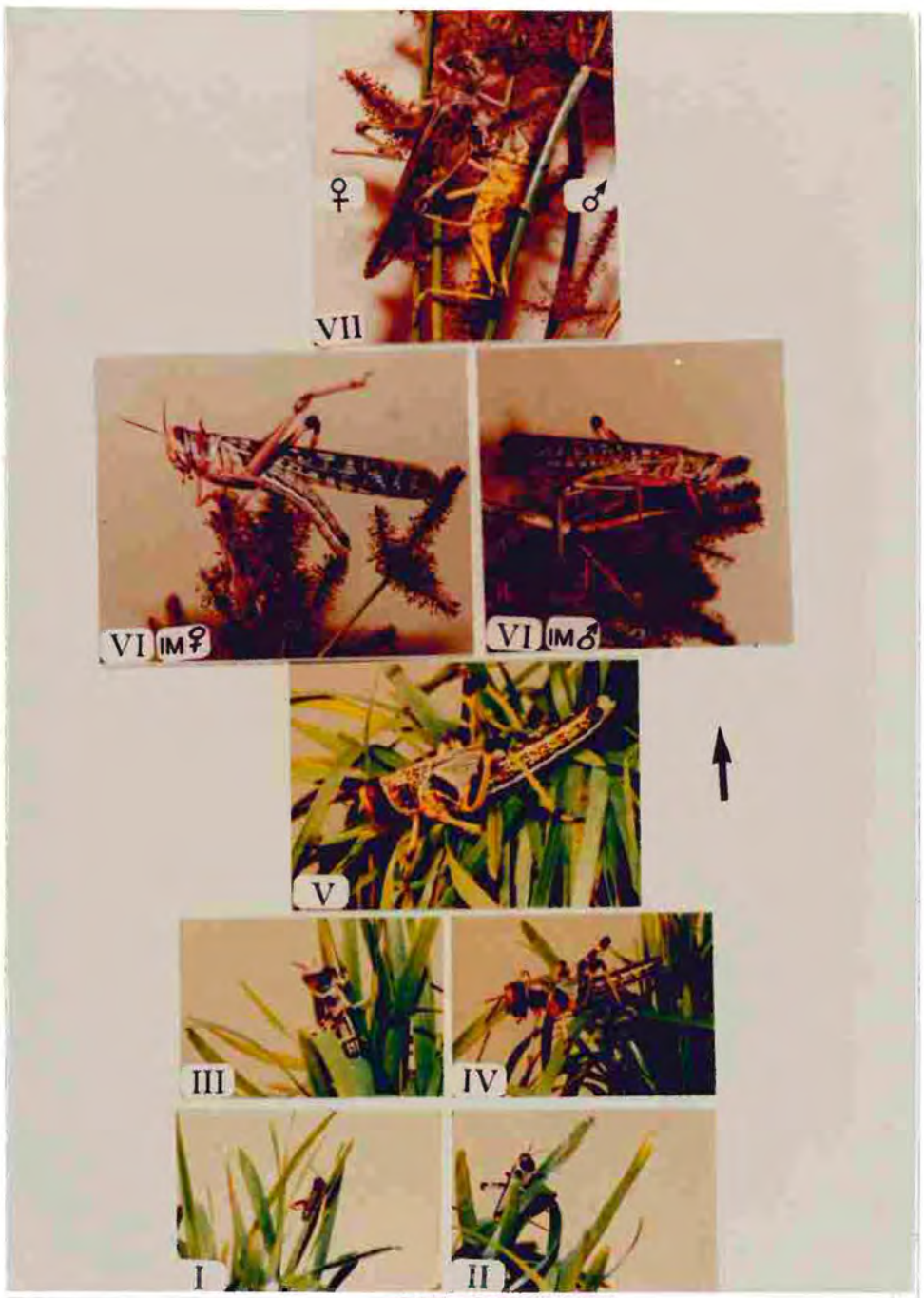
Plate 2. Different stages of solitarious desert locust, *S. gregaria*. i-vi= first to sixth nymphal instars, vi= brown and green colour bimorphism in nymphs, and vii= immature or mature adults.


♀
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VI IM♀
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Plate 1. Different stages of gregarious desert locust, *S. gregaria*. i-v= first to fifth nymphal instars, vi= male and female immature adults, and vii= male and female mature adults.

3.2. Food Plants

Fresh leaves of wheat (*Triticum sp*, variety 'Nyangumi') seedlings and wheat bran were provided daily to the test insects.

3.3. Experimental Rearing Conditions

Gregarious and solitarious locusts were reared separately in rooms located in different parts of the building, each measuring 1.5 x 4.5m. Standard aluminum cages (50 x 50 x 50cm) and (10 x 10 x 24cm) described by Ochieng-Odero *et al.* (1994) were used for crowding and uncrowding experiments, respectively (Fig. 2 and 3). A bichamber or double storey cage (15 x 15 x 30cm) was used for studies on primer effects (Plate 3).

A photoperiod of 12:12h LD was maintained in the arena using an electric timer. Temperature was kept at 30±2°C both in the light and dark phases. The R.H. was maintained at 40-50%. Air flow through the rooms was maintained at a negative pressure of about 10-15 air changes per hour throughout the experimental period.

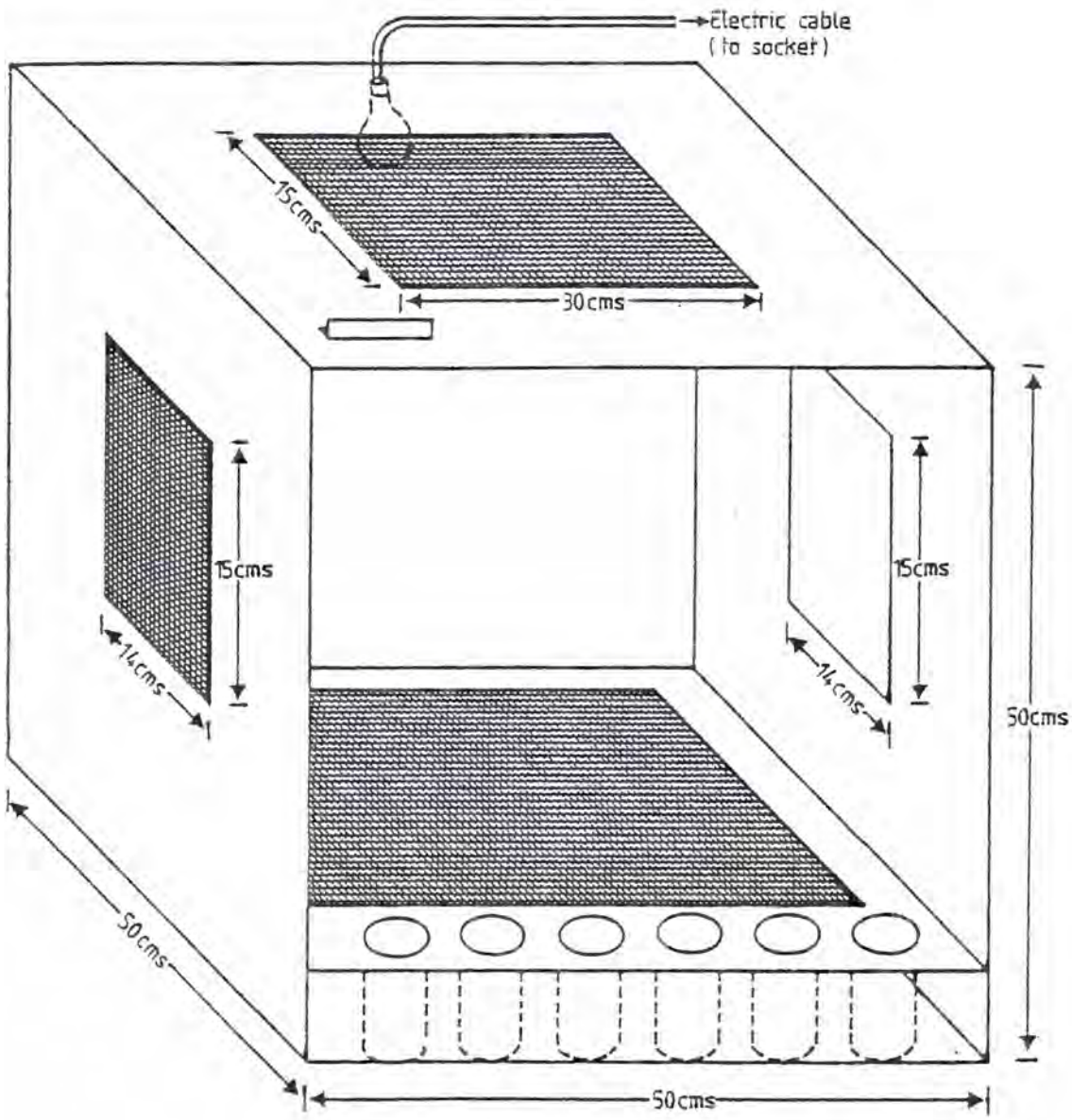


Fig. 2 Cage for rearing gregarious desert locust.

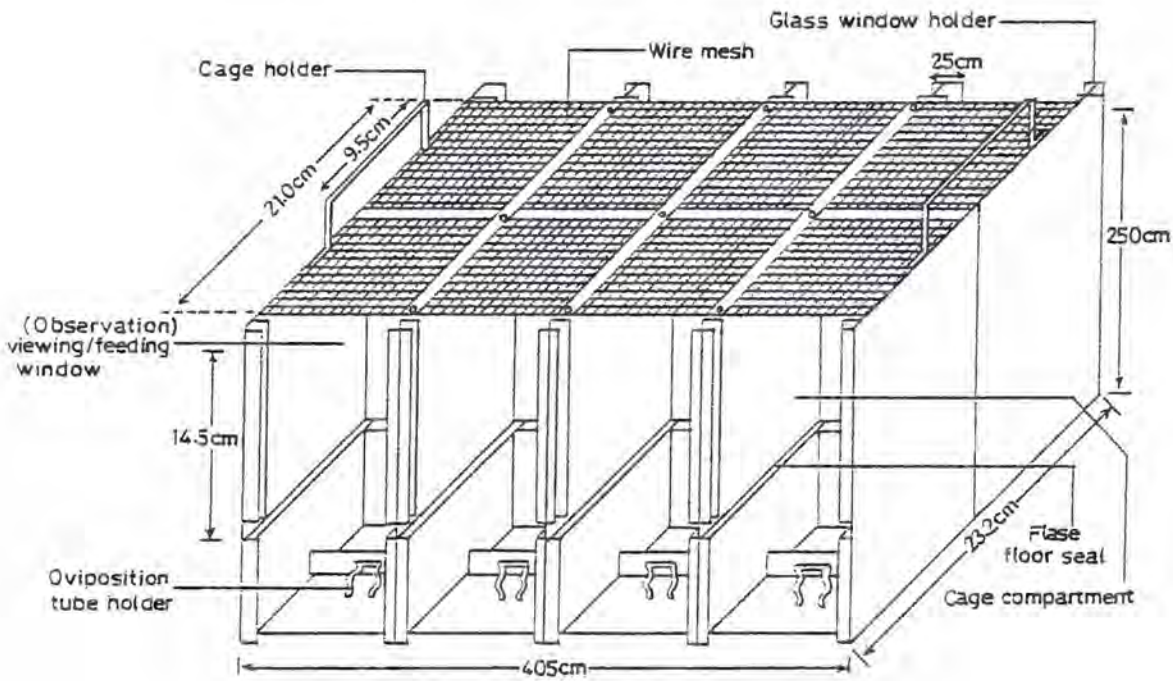


Fig. 3 Cage for rearing solitarious desert locust.

Plate 3. Double storey (bichamber) cage for primer effects studies.

3.4. Chemicals

Phenylacetonitrile and o-methylacetophenone were purchased from Aldrich Chemicals Ltd, Gillingham, UK. The purity of each sample was >98% by GC.

3.5. Parameters Monitored

Physiological and physical changes were both monitored during experimentation. The physiological markers studied included the volatile pheromone component (phenylacetonitrile) measured by its titre (Torto *et al.*, 1994) and haemolymph pigment composition described by Mahamat *et al.* (1995). The physical indicators which were monitored included integumental colour (qualitatively), and ratios of adult forewing (E) to hind femur length (F) i.e. E/F and hind femur length to the greatest width of head capsule (C) i.e. F/C.

3.5.1. Collection of volatiles

Volatiles were collected from groups of solitarious and gregarious adult males of different age (5-7, 10-12, 15-17, 20-22, 25-27, 30-32, 35-37, and 40-42 days-old after final moult) (Torto *et al.*, 1994). Aerations were carried out at 106 ml/min and volatiles adsorbed on thermally conditioned

charcoal (80-100 mesh, Chrompak International Middleburg, The Netherlands) packed between two glass wool plugs in 6-cm long x 8-mm-ID glass traps for 15h at 30±1°C (Fig. 4 and Plate 4A). The charcoal traps were cleaned by Soxhlet extraction with dichloromethane for 72 hrs followed by activation under a stream of nitrogen (20 ml/min) at 250°C for 1hr. Three adult locusts were randomly selected from each of the rearing conditions placed in a quickfit glass tubes (14-cm-long x 2.5-cm-ID) connected to a manifold (plate 4B) and aerated. For each age group aerations were replicated three times. All the collections were replicated three times. The charcoal was eluted with 4ml HPLC grade dichloromethane (Aldrich Ltd, UK) and preserved at -15°C (Plate 5A). Prior to analysis, each sample was concentrated under a stream of nitrogen at 0°C to 100μl and 250ng of o-methylacetophenone were added as the internal standard (Plate 5B).

3.5.2. Analysis of volatiles

Volatile extracts were analyzed by gas chromatography (GC), Hewlett-Packard (HP) 5890 Series II, equipped with a flame ionization detector (FID) and a HP capillary column (Carbowax 20M x 50m x 0.2 mm id x 0.2 μm) using nitrogen as the carrier gas at a flow rate of 0.35 ml/min. The flow

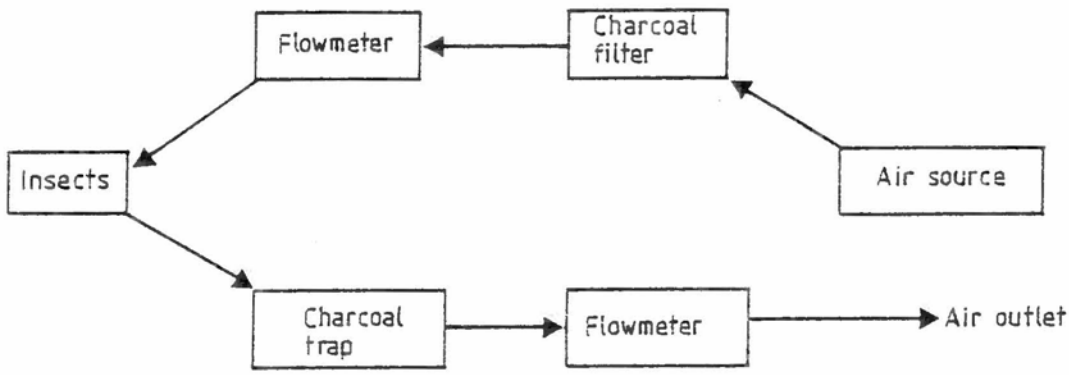


Fig. 4 Diagramatic representation of volatiles collection assembly.

Plate 4 (A). A setup for volatiles collection.

a= Compressed air cylinder

b= Copper connections

c= Charcoal filter

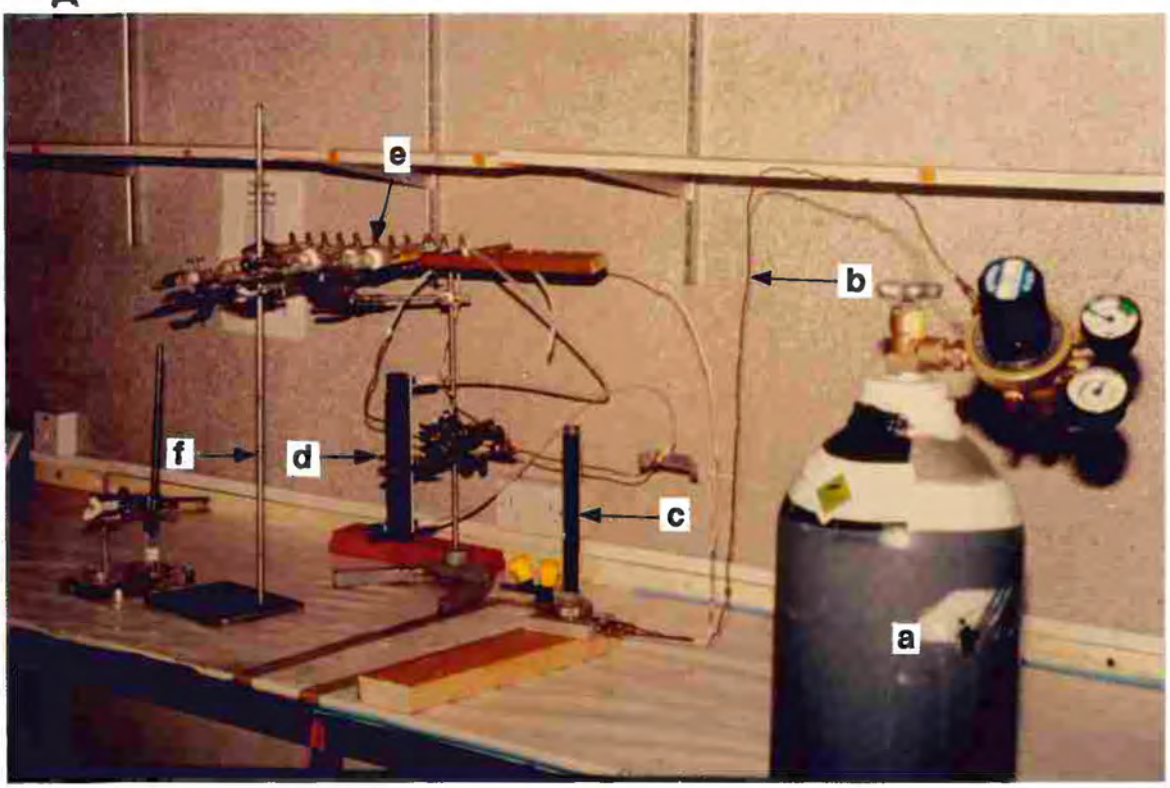
d= Flowmeter

e= Quickfit trapping chambers

f= Stand

Plate 4 (B). Quickfit trapping chambers containing locusts and connected to charcoal traps

A



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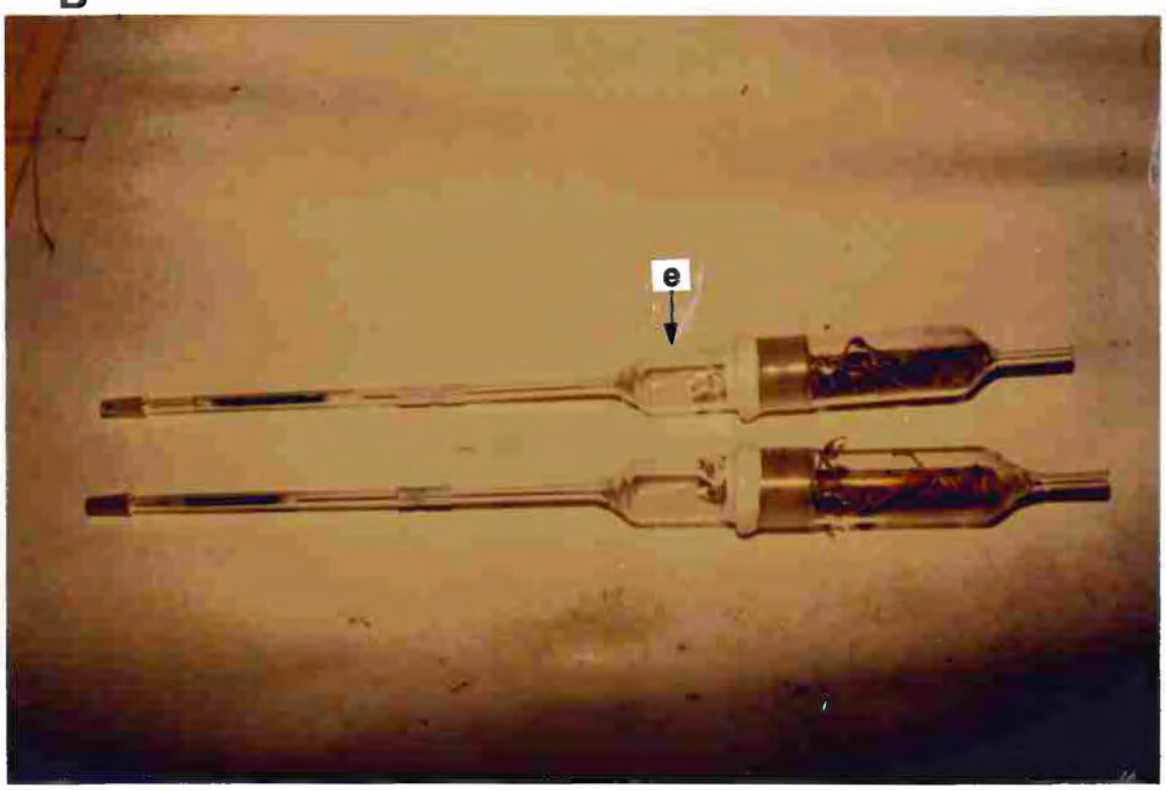


Plate 5 (A). Elution of trapped volatiles.

a= Charcoal trap

b= Sample vial

c= Petri-dish containing ice

Plate 5 (B). Concentration of trapped volatiles.

a= Compressed nitrogen cylinder

b= Copper connections

c= Charcoal filter

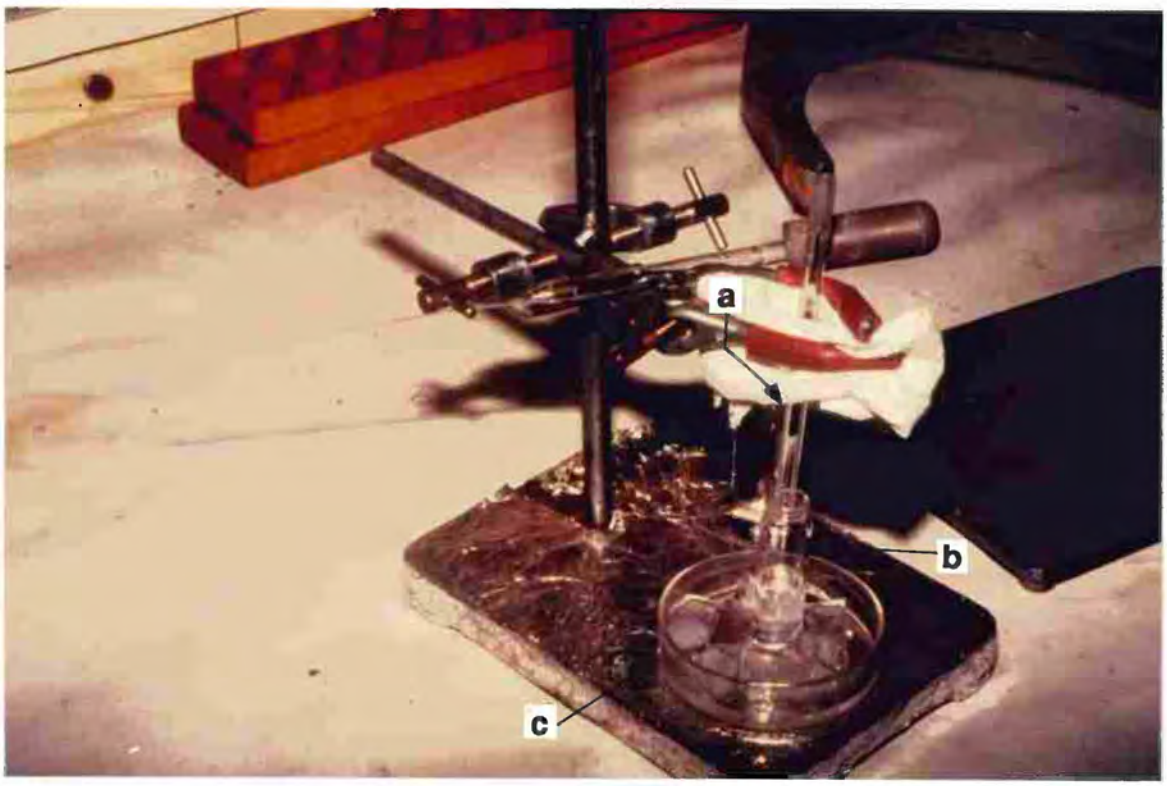
d= Stand

e= Sample

f= Petri-dish containing ice

g= Laboratory jack

A



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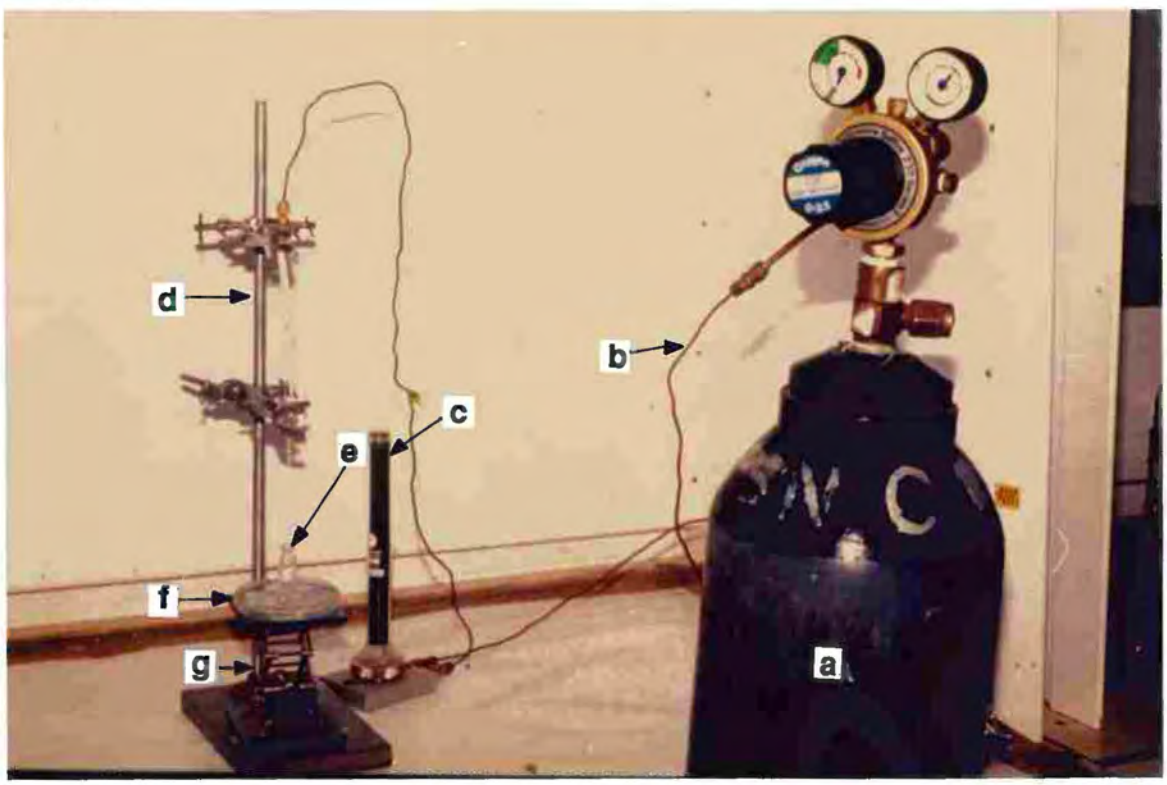


rates of hydrogen and oxygen were 86 ml/min and 400 ml/min respectively. The oven temperature was initially isothermal at 60°C for 10 min, then programmed at 5°C/min to 180°C, maintained for 5min and then to 220°C at 10°C/min. Nitrogen was the makeup gas and was set at flow rate of 50ml/min. The injector and detector temperatures were set at 220°C and 230°C, respectively. Chromatographic peaks were integrated using a HP 3396 Series II integrator. 2μl aliquots of the volatile extracts were analyzed. Triplicate injections from three different collections were examined for each age group. The mean release rate of phenylacetonitrile from these collections was estimated by GC using an authentic sample of the compound.

3.5.3. Collection of haemolymph

Haemolymph samples (10μl), were collected as described by Mahamat *et. al.* (1995) from both 3-5 day-old fifth instar nymphs and adults (10-12, 20-22, and 30-32 days-old after final moult). This was carried out by puncturing the pre-coxal cavity of the posterior leg with a microlance needle (0.5mm ID). The haemolymph sample was drawn into a bleeding buffer (490μl) by a micro-capillary tube. For each age, 10 samples were prepared and were preserved at -15°C prior to analysis.

3.5.4. Analysis of haemolymph

Spectral analysis of the haemolymph samples were carried out on a Beckman DU-50 spectrophotometer at a wavelength range of 300-700nm. The ratios of absorbances at 460 and 680nm were calculated for each sample and compared for phase differences. Ten samples were analyzed for each age group.

3.5.5. Morphometrics

Morphometric measurements were recorded from 15-17 day-old adult males and females as described by (Ochieng-Odero *et al.*, 1994). An electronic caliper (Trimos Sylvac Meteorology Ltd, London, UK) with a range of 0-150mm and an accuracy of ±0.03mm was used. The body parts of adults measured were lengths of fore-wing or elytron (E), posterior femur (F) and the greatest width of the head capsule (C) (Fig. 5). E/F and F/C ratios were calculated and compared for phase differences. Except in treatments where mortality was high, 20 male and 20 female locust per each treatment were used for the measurements.

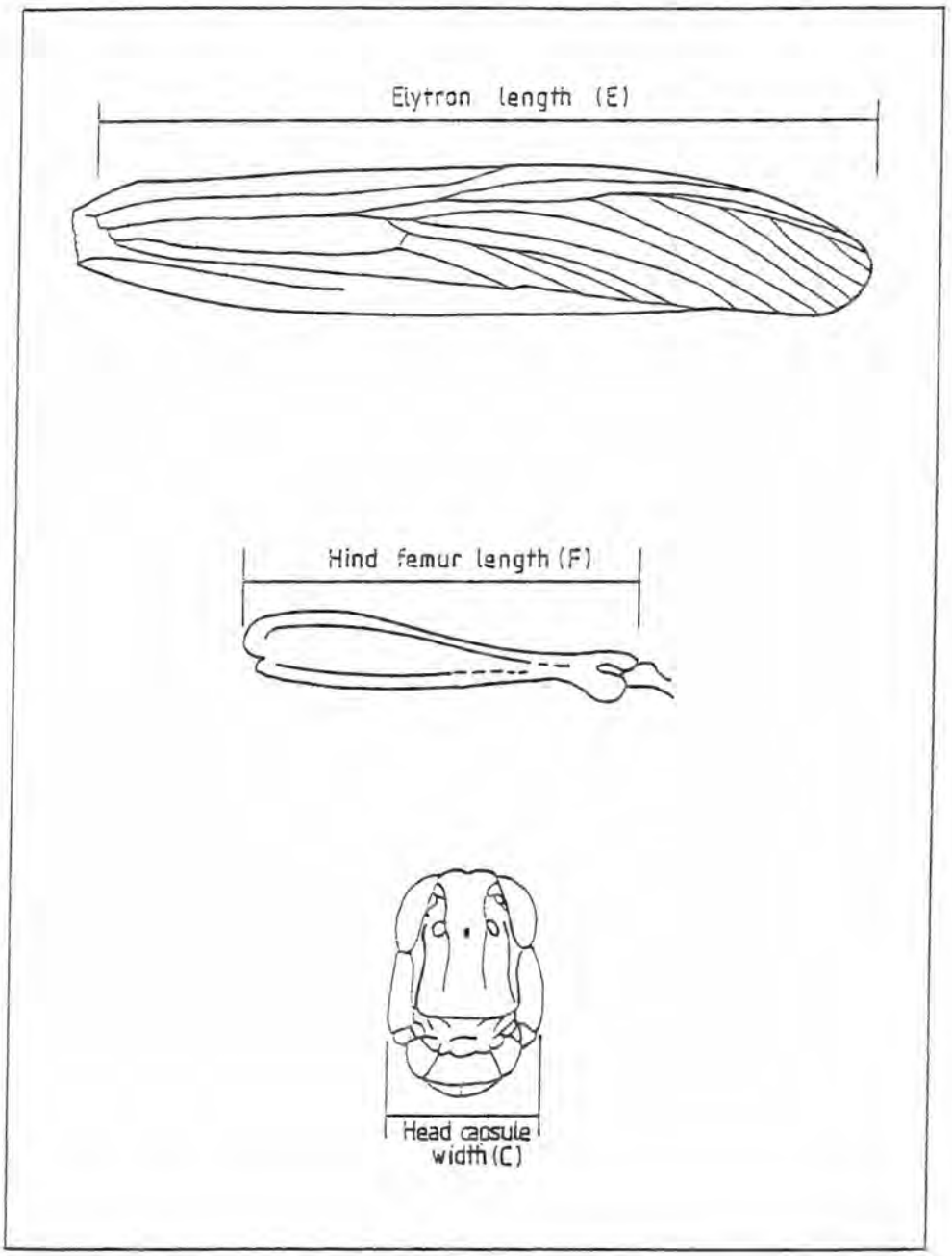


Fig. 5 Desert locust (*Schistocerca gregaria*) body parts measured.

3.5.6. Colour

Visual observations on colour changes were carried out on nymphs and adults of the test insects and compared with controls. The appearance of green or straw colour in nymphs and grayish-brown colour in adults were visually monitored in gregarious locusts, which were uncrowded. For crowding, black patterns (melanization) on a yellow background was checked for in the nymphal stages, pinkish colour in immature individuals and a bright yellow colour in the mature adult males.

3.6. Data Analysis

Mean values of pheromone titres and those of F/C and E/F ratios were tested for normality, transformed to $\log_{10}(x+1)$, and analyzed by analysis of variance (ANOVA). Separation of the transformed means was carried out using Least Significant Difference test (LSD) at $P<0.05$ with a SAS statistical package (Version 6.04). Logarithmic means of absorbance ratios of haemolymph pigment composition, body weights and developmental times were square root transformed and were subjected to ANOVA followed by LSD test at $P<0.05$ using SAS. On the other hand, independency of the primer responses (melanization and mortality) of solitarious nymphs to gregarious nymphal volatiles was examined by

Chi-square test at $P< 0.05$ using SAS.

The degree of transformation was calculated from the means of each of the measurements made; viz, pheromone titre, haemolymph pigment composition and morphometrics by computing data from treated and control locusts. A treatment/control ratio was computed as a measure of transformation from the difference between treated and control locusts, and the difference in the means between the two controls in the two sets of experiments (uncrowding and crowding of hoppers). The percentages with negative values were considered zero, while those over hundred as 100%. Percent change based on morphometrics (m) was calculated from both experiments from the formulae:

Percent shift toward gregarious values

= ((SCm - SGm)/ TRm) X 100 or ((SGm - SCm)/ TRm) X 100

Percent shift toward solitarious values

= ((GCm - GIm)/ TRm) X 100 or ((GIm - GCm)/ TRm) X 100

where total range of change (TR)= absolute value of the difference in the means between the two controls (GC - SC), SC= solitarious control, SG= solitarious grouped, GC= gregarious control, GI= gregarious isolated and m= morphometrics.

Where the rate of shift was with respect to pheromone and haemolymph pigment composition, the same equations were used, with (m) alternated with (p) and (h) for pheromone and haemolymph pigment composition, respectively.

3.7. Experimentation

3.7.1. Uncrowding of gregarious stages

In order to investigate Objective I, described in section 1.2, different stages of the desert locust were subjected to uncrowding and crowding conditions and the parameters described in section (3.5) were monitored in each situation.

(i) Uncrowding at nymphal stage

One-day-old first instar nymphs (64) from gregarious colony (generation 28) were isolated (1 nymph/cage) in standard aluminum cages (10 x 10 x 24cm). The control consisted of one-day-old first instar nymphs (100) of the same stock maintained crowded in a standard cage (50 x 50 x 50cm) as described by Ochieng Odero *et al*. (1994). Food type, arena and conditions were as described in sections 3.2 and 3.3.

The process of solitarisation was monitored in the isolated locusts for three generations by measurements of pheromone titres, haemolymph pigment composition, morphometrics and colour changes described in section 3.5.

(ii) Uncrowding at fledgling stage

As in 3.7.1 (i), but with 1-2 days old fledglings (48) of the gregarious locust (generation 26). These locusts were isolated and maintained for one generation in cages for solitary rearing (Fig. 3). The control consisted of 100 insects of the same age maintained grouped in cages for rearing crowded locusts (Fig. 2). The process of solitarisation was monitored as described previously in section 3.5.

(iii) Uncrowding at mature adult stage

As in section 3.7.1 (i) but with 20-22 days old mature adults (24) of the gregarious stock (generation 31). These locusts were isolated for a period of two weeks in cages shown in (Fig. 3). The control consisted of 100 locusts kept crowded in a cage (Fig. 2). Solitarisation in these insects was monitored using pheromone titre and colour as indicators.

3.7.2. Crowding of solitarious stages

(i) Crowding at nymphal stage

In order to investigate the effect of grouping on solitarious stock, one-day-old first instar nymphs (100) of the same stock (generation 19) were crowded in standard cages (50 x 50 x 50cm; Fig. 2). The control set up consisted of nymphs (64) of the same stock kept isolated (1 nymph/ cage) in the standard cage (10 x 10 x 24cm), previously described (Fig. 3). Food type, arena, conditions and phase shift from solitarious to gregarious were monitored as described in Sections 3.2, 3.3 and 3.5.

(ii) Crowding at fledgling stage

As in section 3.7.2 (i), but with 1-2 day-old fledglings (40) of solitarious stock (generation 21). These locusts were crowded for one generation in the standard crowd rearing cage (Fig. 2). The control consisted of 48 individuals of the same age kept isolated in cages for rearing solitarious locusts (Fig. 3). Gregarisation was monitored as in section 3.5.

(iii) Crowding at mature adult stage

As in section 3.7.2 (i) but with 20-22 days-old mature adults (24) of solitarious individuals (generation 21). These locusts were crowded for two weeks in a standard cage for rearing crowded locusts (Fig. 2). The control treatment consisted of 24 individuals of the same age maintained singly in cages (Fig. 3). Gregarisation was monitored for a period of two weeks only using pheromone titre and colour as measures.

3.7.3. Crowding of solitarious nymphs at different densities

As in section 3.7.2 (i); but with solitarious nymphs of (generation 21) raised as 1, 2 and 4 locusts per cage (Fig. 3).

3.8. Primer Effects of Releaser Pheromones

In order to investigate Objective II, described in section 1.2, experiments which involved the exposure of first and second instar nymphs, and fledglings from solitarious stock to the volatiles emanating from different nymphal instars of gregarious locusts were carried out. Pheromone titre, haemolymph pigment composition and body colour described in section 3.5 were monitored in addition to weight where appropriate.

3.8.1. Effect of gregarious first instar volatiles on solitarious first instar.

Three-days-old solitarious first instar nymphs (15) were placed in the lower compartments of double storey (bichamber) cages (Plate 3) as test insects. Each of them was exposed to volatiles emanating from one-day-old gregarious first instar nymphs (4) and their faeces placed in the upper compartment of the bichamber cage. Both the source and test insects were allowed to develop simultaneously. The controls consisted of three-day-old solitarious first instar nymphs (15) kept individually and not exposed to any source of chemical stimuli. The rearing conditions were as described in section (3.3). Phase shift was monitored qualitatively by colour (black patterns or

melanization and background colour), pheromone release and haemolymph pigment composition described in section 3.5. Insects were also assessed by comparing their developmental time, in addition weights were recorded in each instar and after every 10 days in adults.

3.8.2. Effect of gregarious third to fifth and fifth instar volatiles on solitarious first instar.

As in 3.8.1, but with individual three-days-old solitarious first instars (16) exposed to volatiles emanating from gregarious third instars (4 per cage, with faeces), which were allowed to develop upto the fifth instar. The control consisted of solitarious nymphs (14) of the same age, but were not exposed to any source of chemical stimuli. Phase shifts were monitored by changes in colour (melanization or black patterns on body and background colour) and haemolymph pigment composition as described previously.

3.8.3. Effect of gregarious fifth instar volatiles on solitarious second instar.

As in 3.8.1, but with individual three-days-old solitarious second instars (15) exposed to volatiles from gregarious fifth instars (4) and their faeces. The control

treatment consisted of 15 solitarious second instars kept individually and not exposed to any source of chemical stimuli. Changes in colour, haemolymph pigment composition and weight were monitored.

3.8.4. Effect of gregarious fifth instar volatiles on solitarious fledglings.

As in 3.8.1, but with individual 1-2 days-old solitarious fledglings (8) exposed to volatiles from gregarious fifth instar (4) and their faeces. The control consisted of fledglings (7) of the same age maintained unexposed to any source of chemical stimuli. Phase shift was monitored by colour, haemolymph pigment composition and pheromone release as described in section 3.5.

4. RESULTS

4.1. Uncrowding of Different Stages of Gregarious Locust

4.1.1. Phenylacetonitrile titre

(i) Uncrowding at nymphal stage

The mean phenylacetonitrile titre of adult males which emerged from uncrowding (isolation) gregarious nymphs and that of solitarious control adult males were not significantly different in each of the three successive generations F_0, F_1 and F_2 (Figs. 6 and 7). The degree of shift estimated was 100% at the F_0 generation similar to F_1 and F_2 (Fig. 8).

(ii) Uncrowding at fledgling stage

Adult males resulting from uncrowding gregarious fledglings failed to produce phenylacetonitrile similar to their solitarious control counterparts within the F_0 generation (Fig. 9). Consequently, no further experimentation was carried out beyond this generation.

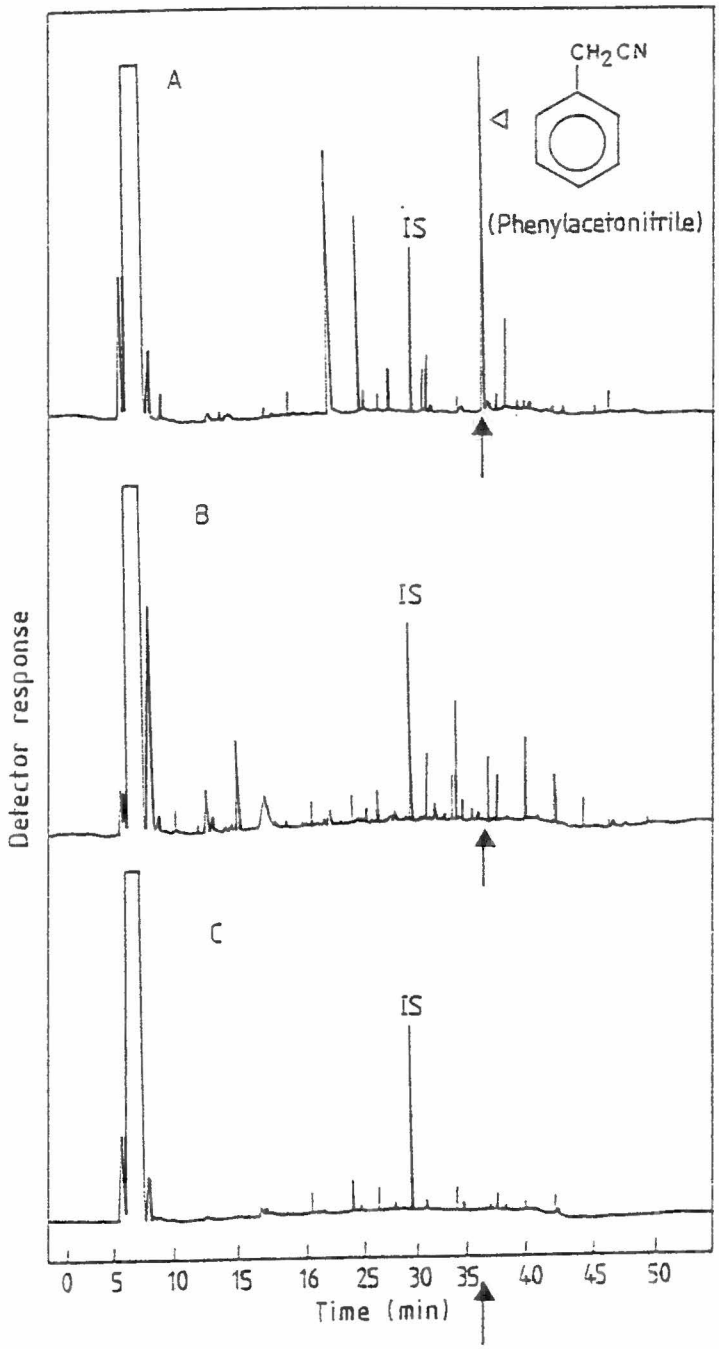


Fig. 6 Chromatograms of the air-borne volatiles collected from 20-22 day old adult males of (A) gregarious, (B) gregarious isolated and (C) solitarious *S. gregaria* injected into a 50m carbowax 20m capillary column. IS= Internal standard.

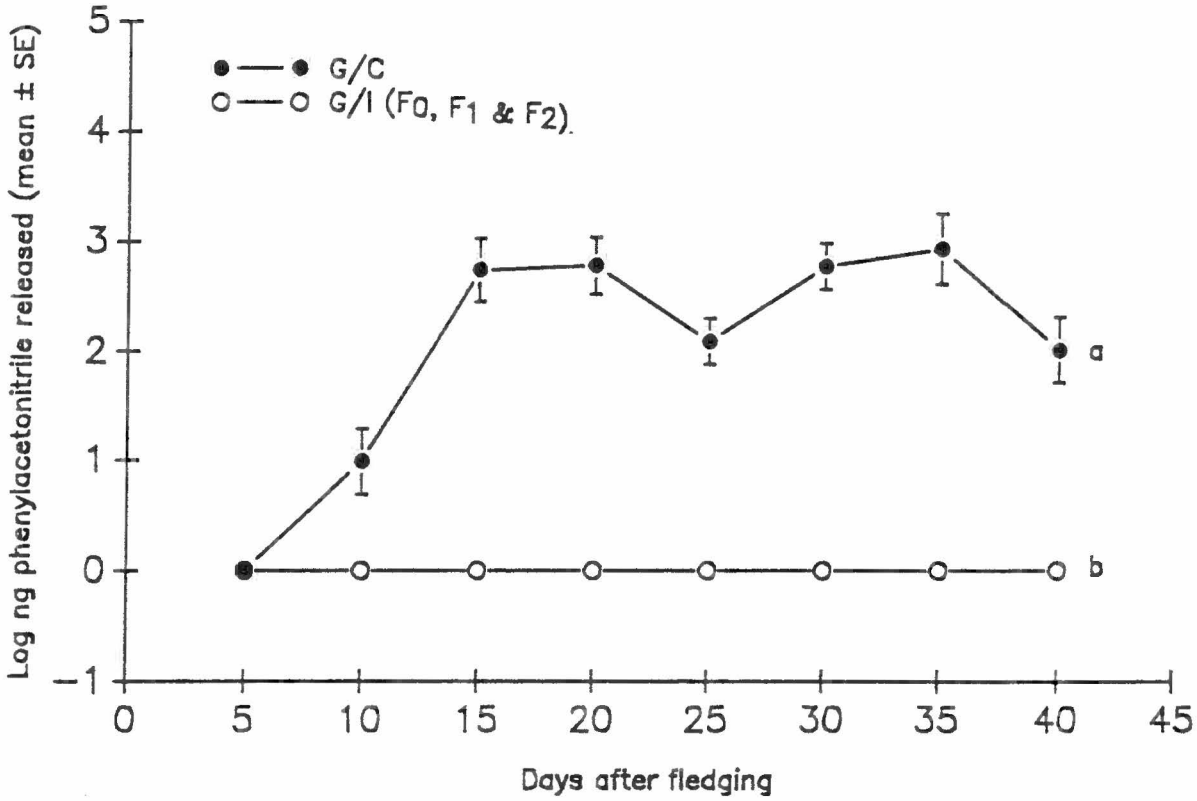


Fig. 7 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* which emerged from uncrowding gregarious nymphs for three generations (F_0-F_2). G/C= gregarious control and G/I= gregarious isolated.

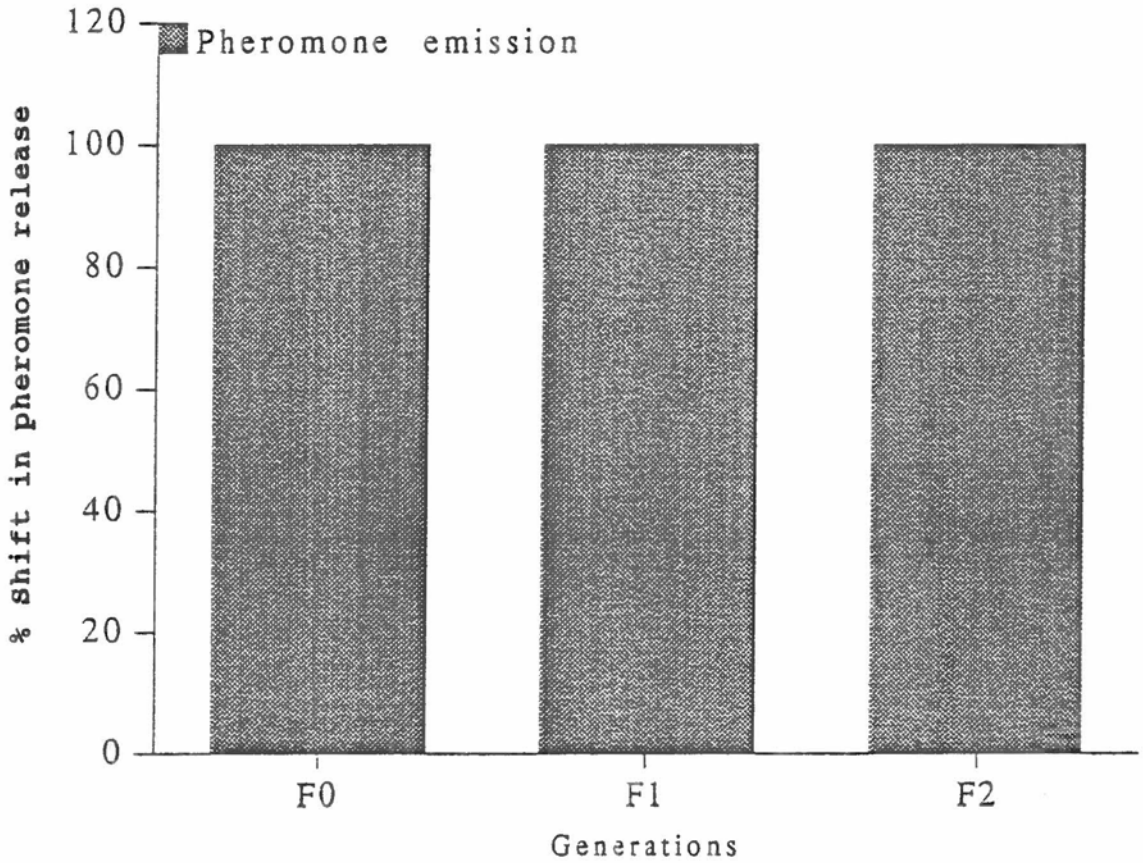


Fig. 8 Percentage shift in adult pheromone emission for three generations (F_0-F_2) of uncrowding of gregarious *S. gregaria* nymphs.

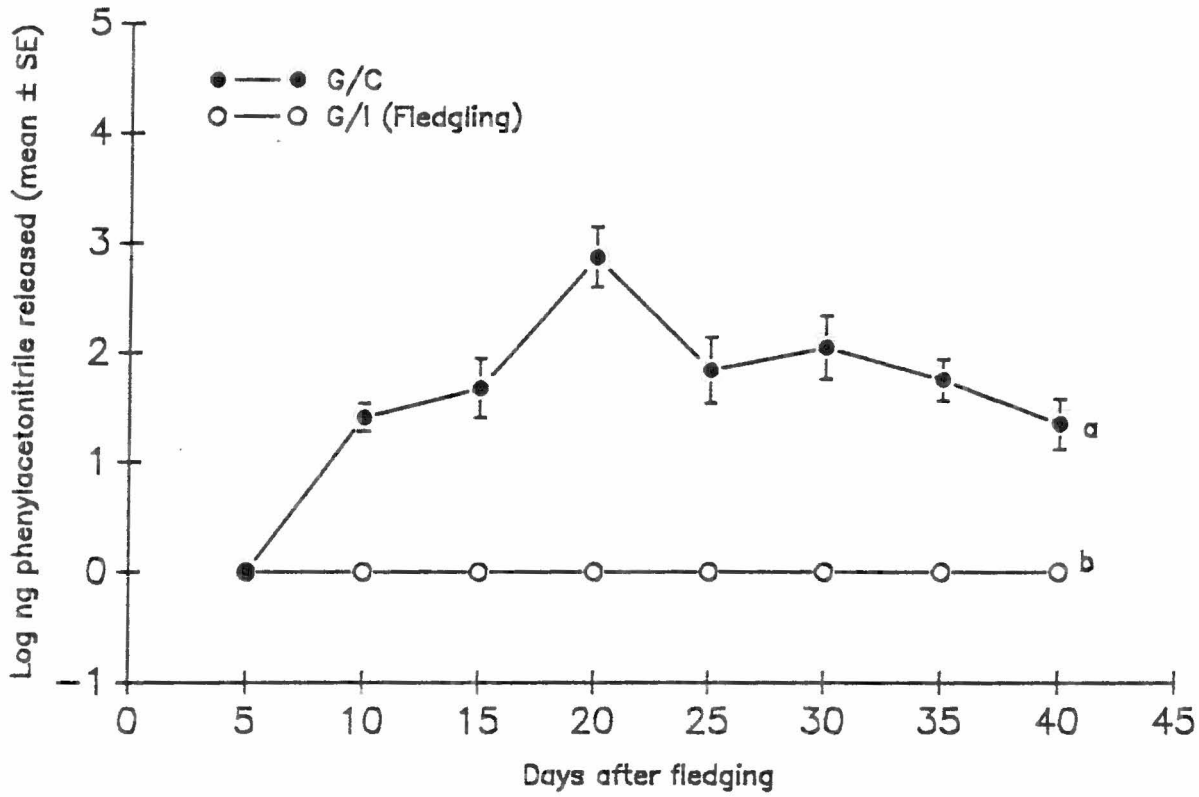


Fig. 9 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* which emerged from uncrowding gregarious fledglings for one generation (F_0). G/C= gregarious control and G/I= gregarious isolated.

(iii) Uncrowding at mature adult stage

Some phenylacetonitrile was detected in three-week-old gregarious mature adults which were uncrowded for 14 days, but the level was not significantly different from the titre in control solitarious locusts ($P > 0.05$). After 12 days of uncrowding, the phenylacetonitrile titre dropped to undetectable levels similar to solitarious control (Fig. 10).

4.1.2. Haemolymph pigment composition

(i) Uncrowding at nymphal stage

There were significant differences between the means of absorbance ratios of the haemolymph pigments at 460 and 680nm of solitarious and gregarious locusts (Fig. 11). The results showed that there was no sex differentiation within a phase (Table 1a & 1b). However, there was a significant stage and age differentiation within and among generations in each phase (Figs. 12, 13 and 14).

The mean absorbance ratio of the haemolymph pigments of gregarious locusts which were uncrowded (isolated) showed a significant shift in nymphs toward the solitarious ratio within F_0 generation (Fig. 15). The estimated degree of change in nymphs was 99%, 79%, and 74% in the F_0, F_1 and F_2

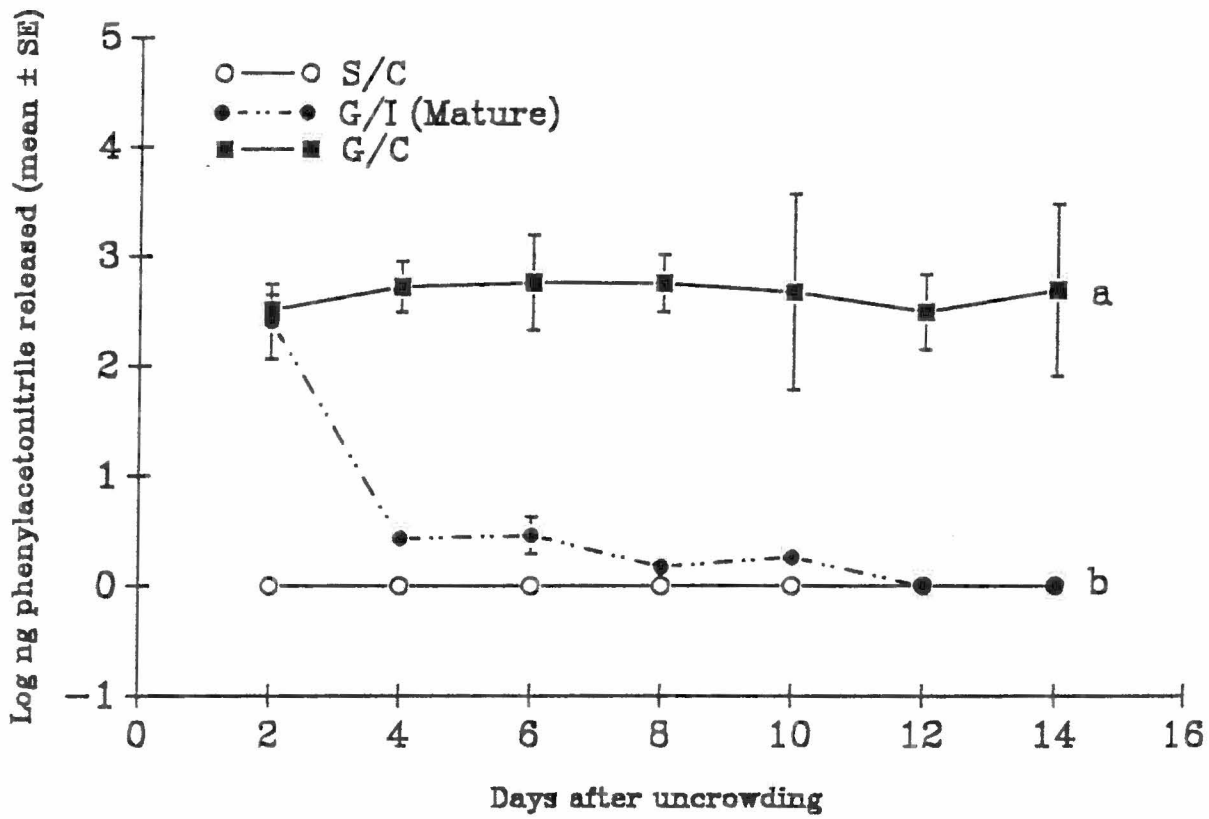


Fig. 10 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* . which emerged from uncrowding gregarious mature adults for two weeks. G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

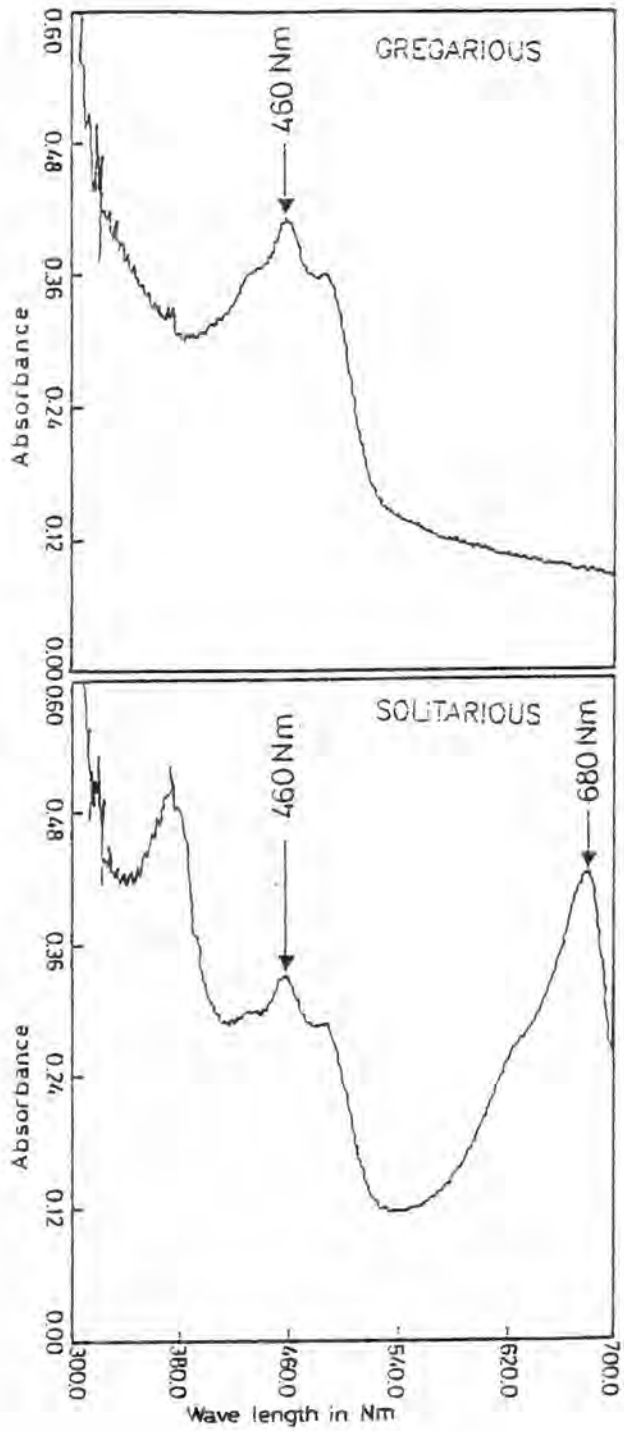


Fig. 11 UV and visible spectra of haemolymph of 20-22 days-old solitarious and gregarious *S. gregaria* adults.

Table 1a. Haemolymph absorbance ratio in the two sexes in different stages of gregarious *S. gregaria*

Stage	Male Mean(A) ± SE	Female Mean(A) ± SE	Ttest (P= 0.05)
Nymph			
(3rd)	2.23±0.20	1.75±0.05	ns
(4th)	1.83±0.11	2.02±0.13	"
(5th)	1.75±0.12	1.72±0.12	"
Adult			
(5 days old)	1.96±0.21	1.60±0.11	"
(10 " ")	2.24±0.21	1.70±0.17	"
(20 " ")	2.04±0.15	2.03±0.16	"
(30 " ")	1.82±0.17	1.54±0.05	"

Table 1b. Haemolymph absorbance ratio in the two sexes in different stages of solitarious *S. gregaria*

Stage	Male Mean(A) ± SE	Female Mean(A) ± SE	Ttest (P= 0.05)
Nymph			
(3rd)	1.35±0.17	1.17±0.08	ns
(4th)	1.64±0.22	1.51±0.07	"
(5th)	1.09±0.10	1.21±0.15	"
Adult			
(5 days old)	1.10±0.14	1.49±0.21	"
(10 " ")	1.42±0.13	1.31±0.03	"
(20 " ")	1.19±0.02	1.21±0.14	"
(30 " ")	1.86±0.08	1.49±0.14	"

ns= Not significantly different.
A= Absorbance ratio

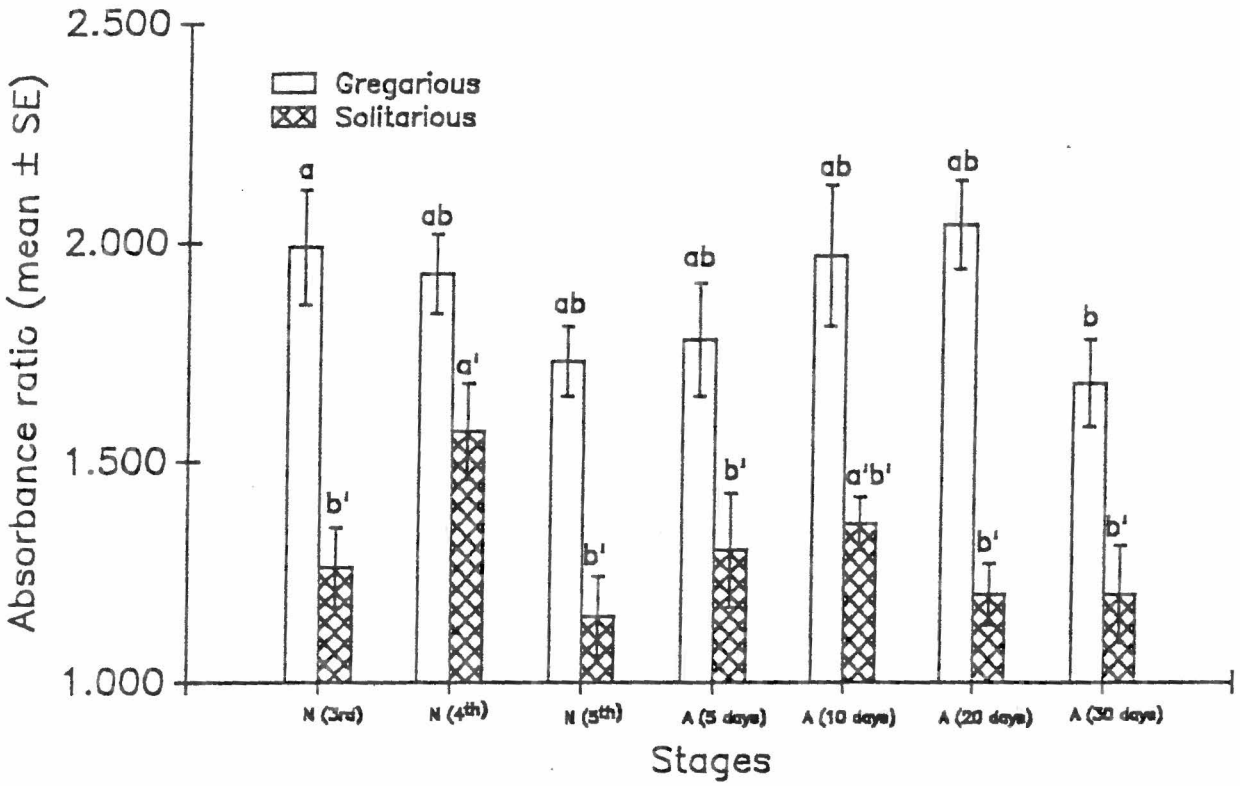


Fig. 12 Stage and age differentiation within a generation of solitarious and gregarious *S. gregaria* based on haemolymph absorbance ratio. N= nymph, A= adult.

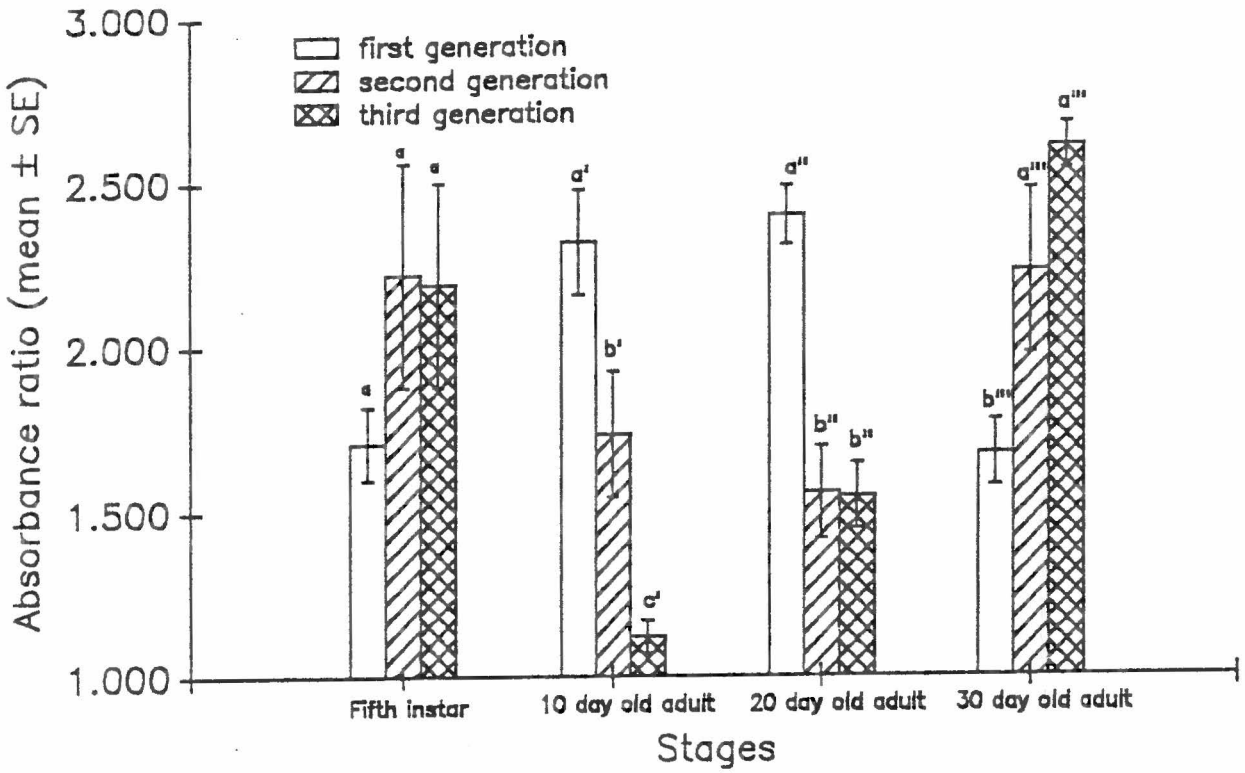


Fig. 13 Stage and age differentiation between generations of gregarious *S. gregaria* based on haemolymph absorbance ratio.

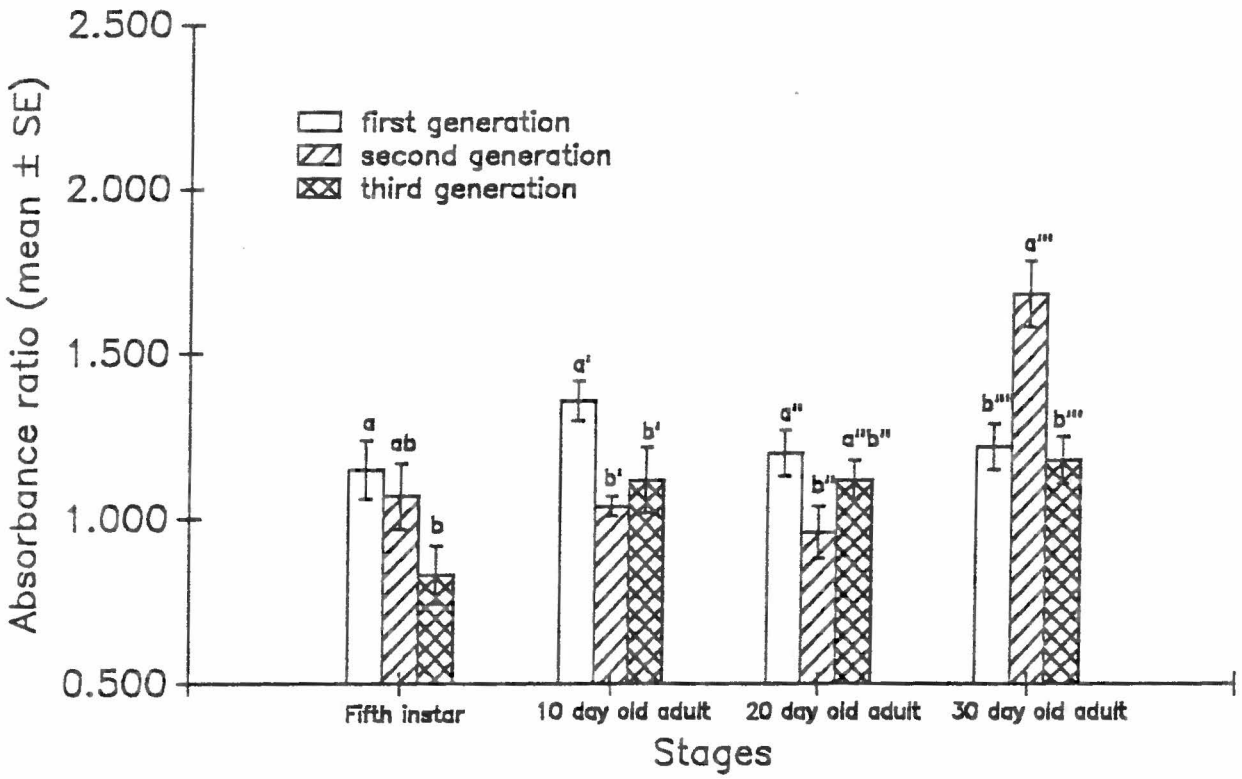


Fig. 14 Stage and age differentiation between generations of solitarious *S. gregaria* based on haemolymph absorbance ratio.

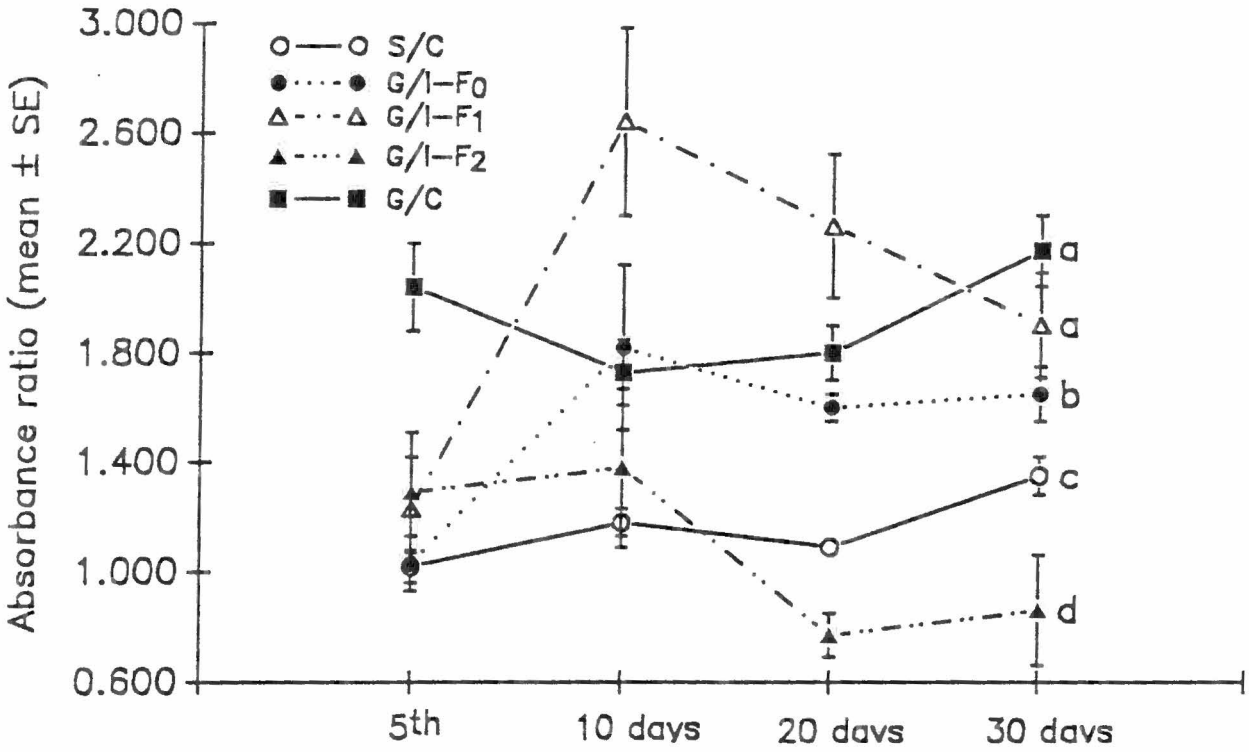


Fig. 15 Haemolymph pigment changes in gregarious *S. gregaria* uncrowded for three generations (F_0-F_2). G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

generations, respectively (Fig. 16a and b). In the adults, a significant shift toward solitarious ratios was recorded by the end of the F_2 generation (Fig. 15).

(ii) Uncrowding at fledgling stage

The mean absorbance ratio of the haemolymph pigments of gregarious fledglings which were isolated was not significantly different from that obtained for gregarious counterparts within the F_0 generation ($P< 0.05$) (Fig. 17). Consequently, no further experimentation was carried out beyond this generation.

(iii) Uncrowding at mature adult stage

Haemolymph collection and analysis were not carried out since similar treatment of fledglings effected no significant change in haemolymph pigment composition.

4.1.3 Morphometrics

(i) Uncrowding at nymphal stage

The mean F/C ratios calculated for isolated gregarious females by the end of the F_0 and F_1 generations were intermediate (31% and 66% transformation, respectively) and

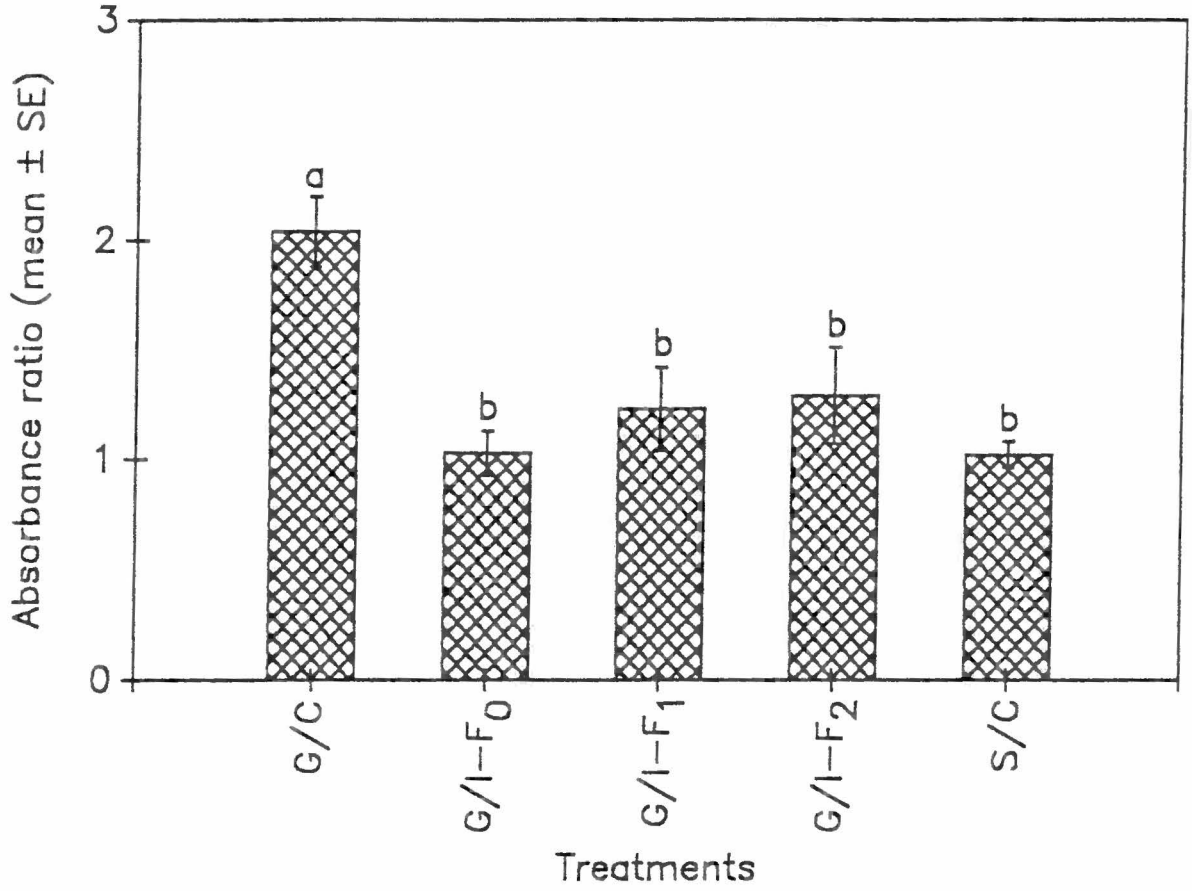


Fig. 16 (a) Haemolymph pigment ratio for three generations (F_0- F_2) of uncrowding of gregarious *S. gregaria* nymphs.

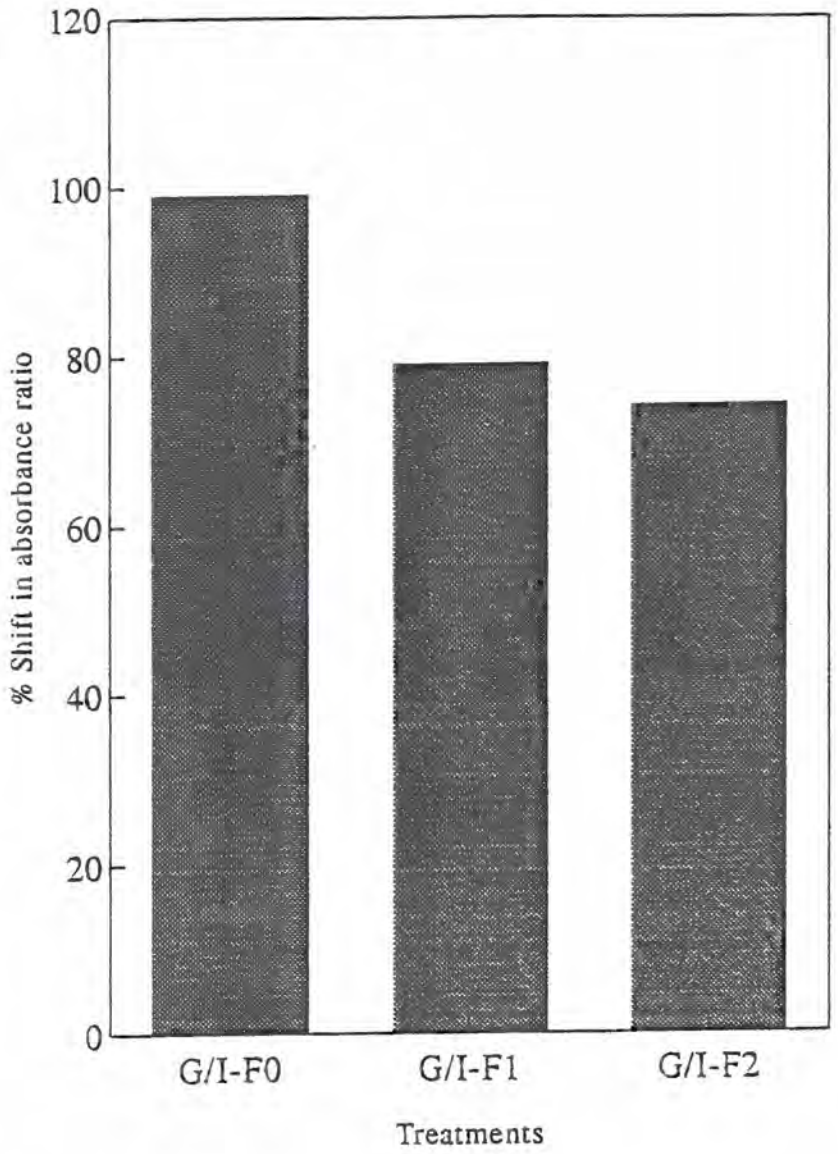


Fig. 16 (b) Percentage shift in haemolymph pigment ratio for three generations (F_0-F_2) of uncrowding of gregarious *S. gregaria* nymphs.

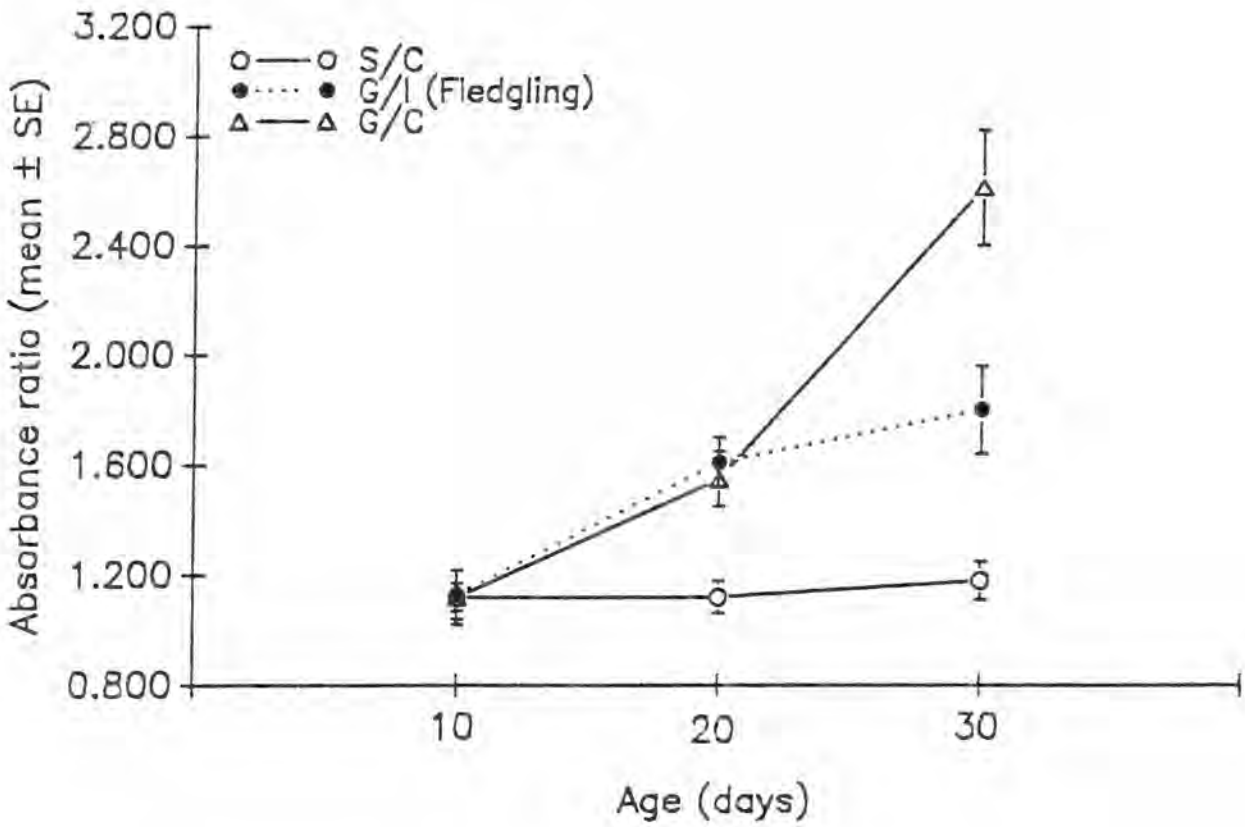


Fig. 17 Haemolymph pigment changes in gregarious *S. gregaria* uncrowded for one generation (F_0) at fledgling stage. G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

were significantly different from those of the two controls ($P< 0.05$). The ratio at the F_2 generation was not significantly different from that of the control solitarious females (86% transformation) (Figs. 18 and 19). Males showed no significant change in the F_0 generation (13% transformation) but responded significantly to uncrowding by the end of the F_2 generation (69% transformation (Figs. 18 and 19).

The mean E/F ratio calculated for adult females of the same group of locust showed no significant change by the end of F_0 generation (25% transformation) ($P< 0.05$). The ratios at the F_1 and F_2 were not significantly different from that of the control solitarious females (each 100% transformation) (Figs. 20 and 21). The mean E/F ratio for males on the other hand, showed no significant change within the F_0 generation (33% transformation) and became intermediate at F_1 (83% transformation). In F_2 generation, this mean ratio of the isolated males was not significantly different from that of the control solitarious males (100% shift toward solitarious F/C) ($P< 0.05$) (Figs. 20 and 21).

(ii) Uncrowding at fledgling stage

The mean F/C ratios for both males and females and the E/F ratios for males resulting from uncrowding gregarious fledglings remained unchanged within the F_0 generation

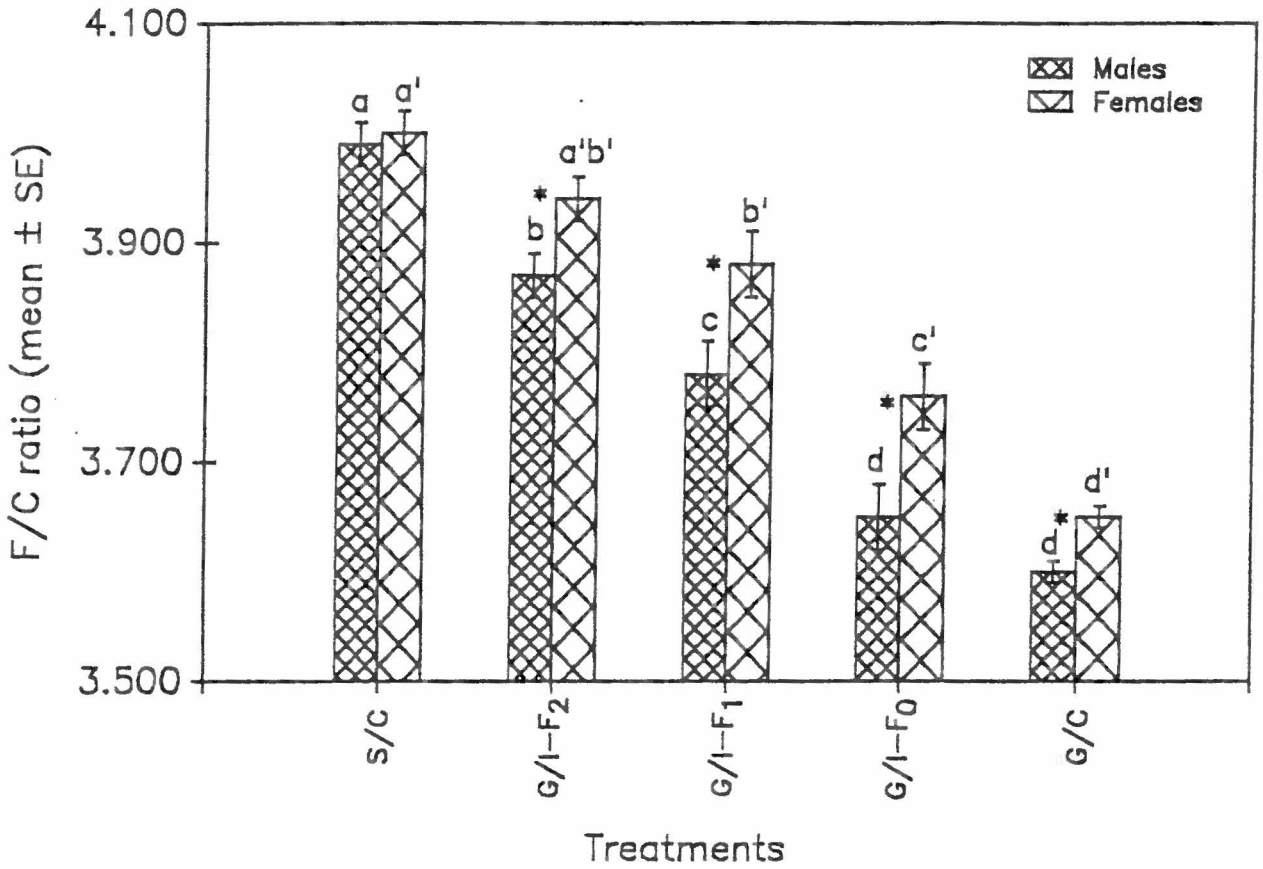


Fig. 18 Changes in F/C ratios (mean ± se) of adult *S. gregaria* which emerged from uncrowding gregarious nymphs for three generations (F_0-F_2). G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

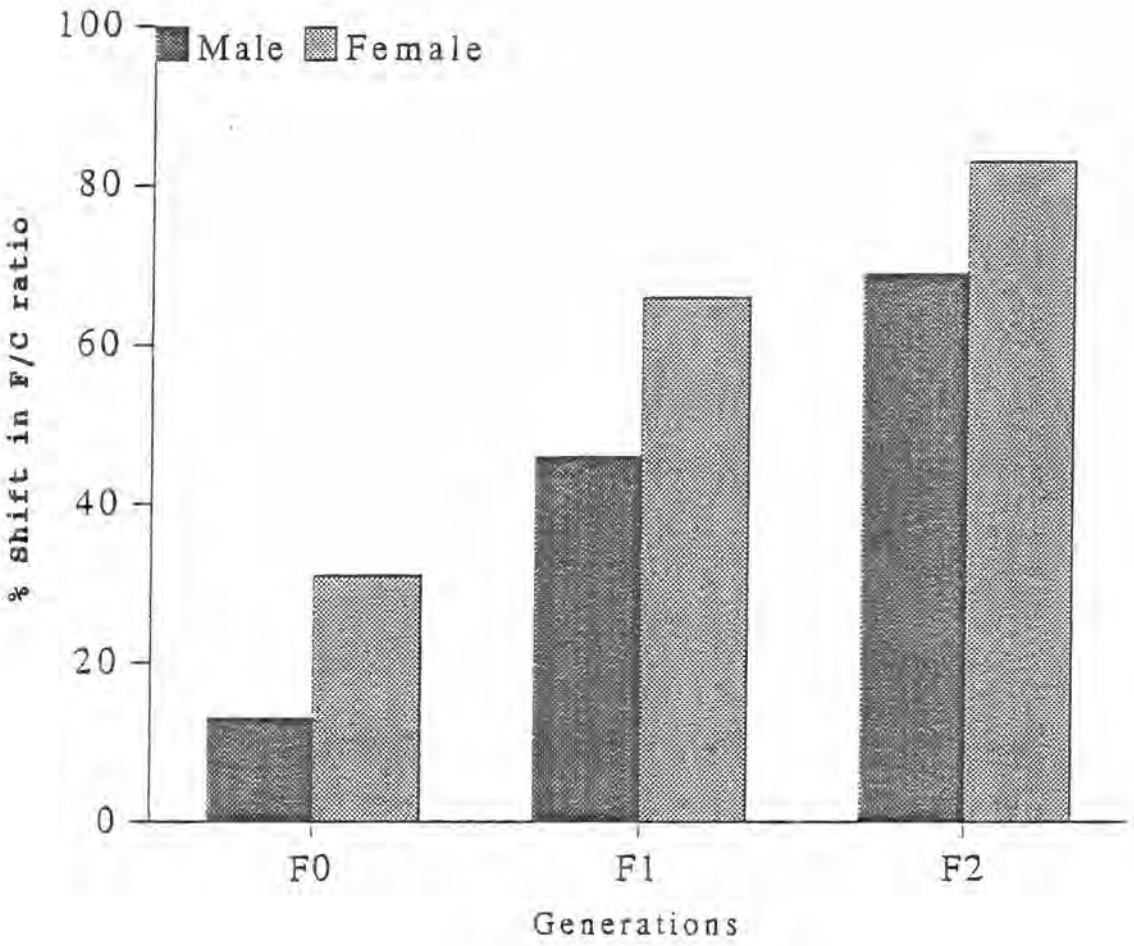


Fig. 19 Percentage shift toward solitary F/C ratio for three generations (F_0-F_2) of uncrowding of *S. gregaria* starting from gregarious nymphs.

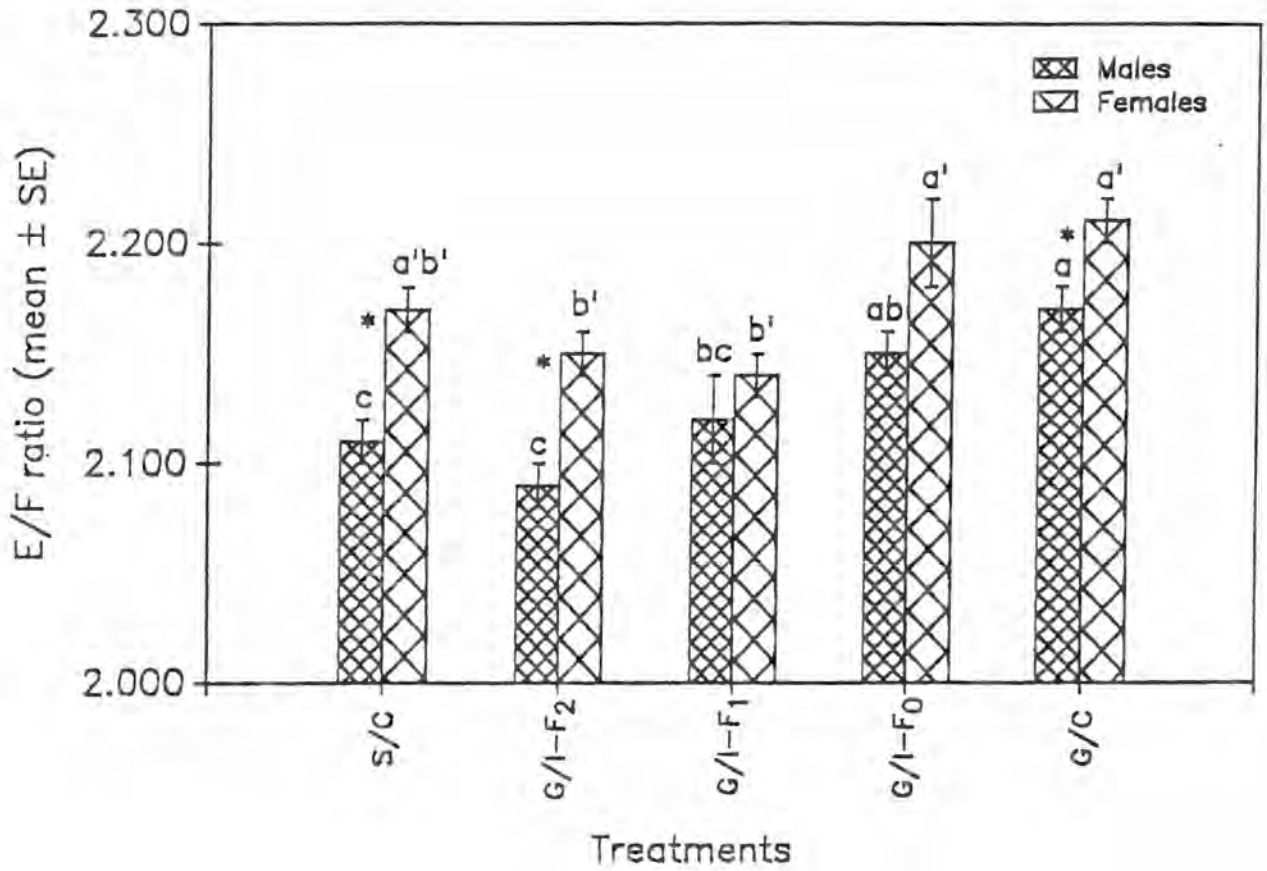


Fig. 20 Changes in E/F ratio (mean ± se) of adult *S. gregaria* which emerged from uncrowding gregarious nymphs for three generations (F_0-F_2). G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

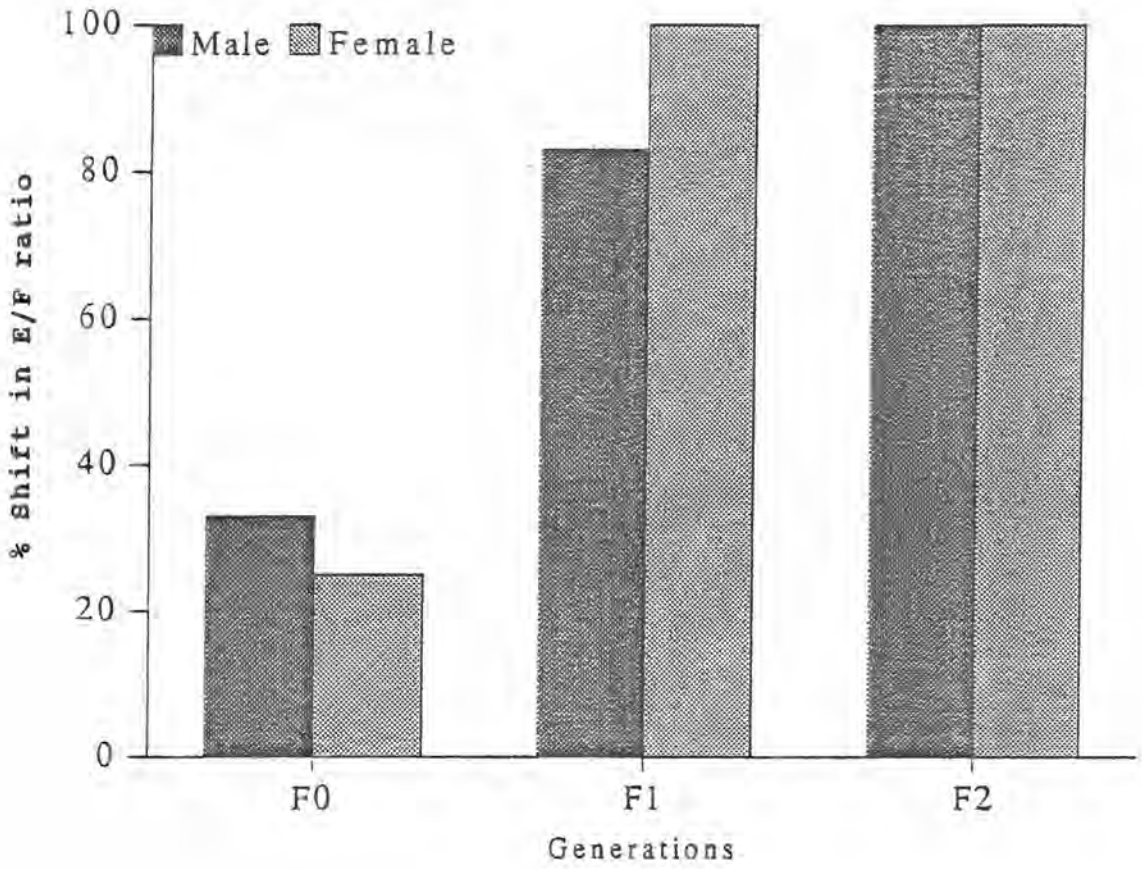


Fig. 21 Percentage shift toward solitary E/F ratio for three generations (F_0-F_2) of uncrowding of *S. gregaria* starting from gregarious nymphs.

(Figs. 22 and 23). The mean E/F ratio for females showed a significant shift toward solitarious ratios and was significantly different from that of the control gregarious females within the F_0 generation ($P< 0.05$) (Fig. 23).

(iii) Uncrowding at mature adult stage

Morphometric measurements were not carried out since similar treatment of fledglings effected no marked differences in F/C and E/F ratios between the control and isolated locusts.

4.1.4. Colour

(i) Uncrowding at nymphal stage

Gregarious nymphs which were uncrowded from the first instar turned green or straw brown by the fifth instar stage in the same generation and matured into either grey or grayish brown adults similar to solitarious controls (Table 2).

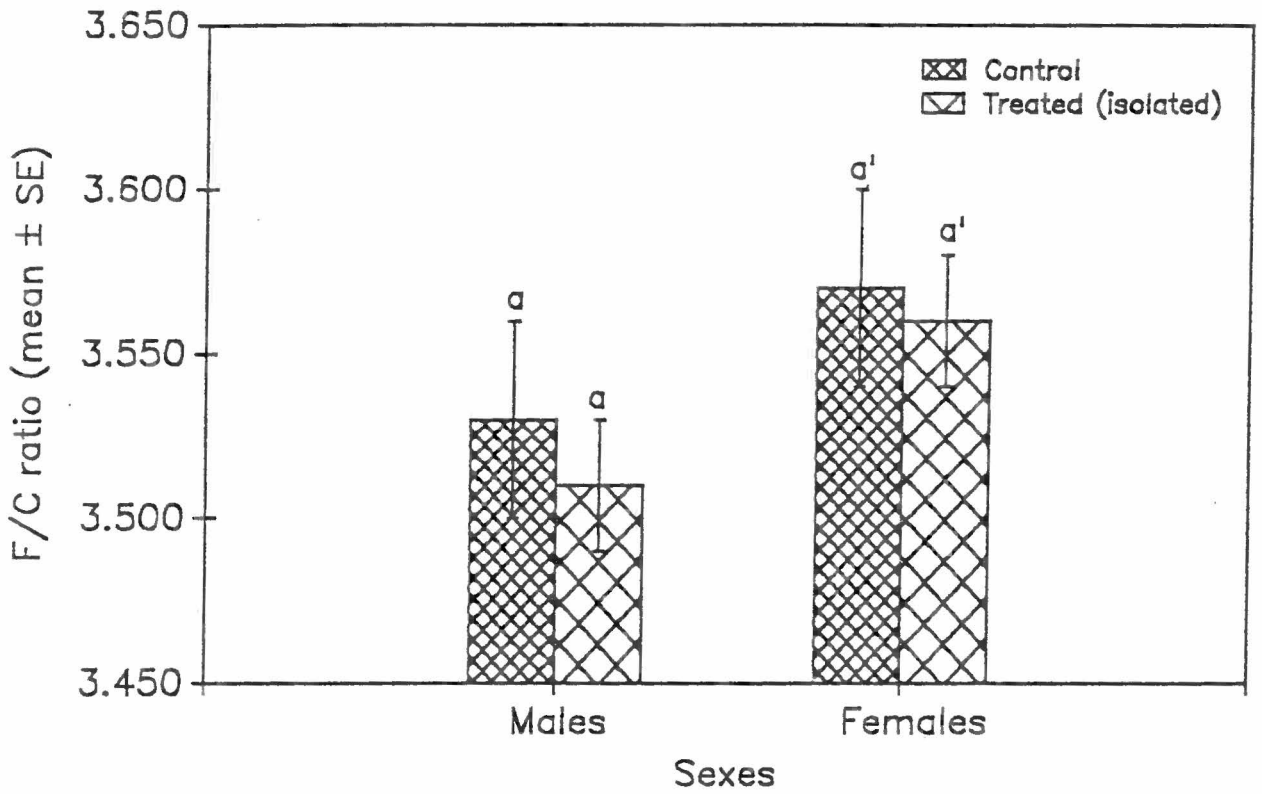


Fig. 22 Changes in F/C ratio (mean ± se) of adult *S. gregaria* from uncrowding gregarious fledglings in the same generation (F_0).

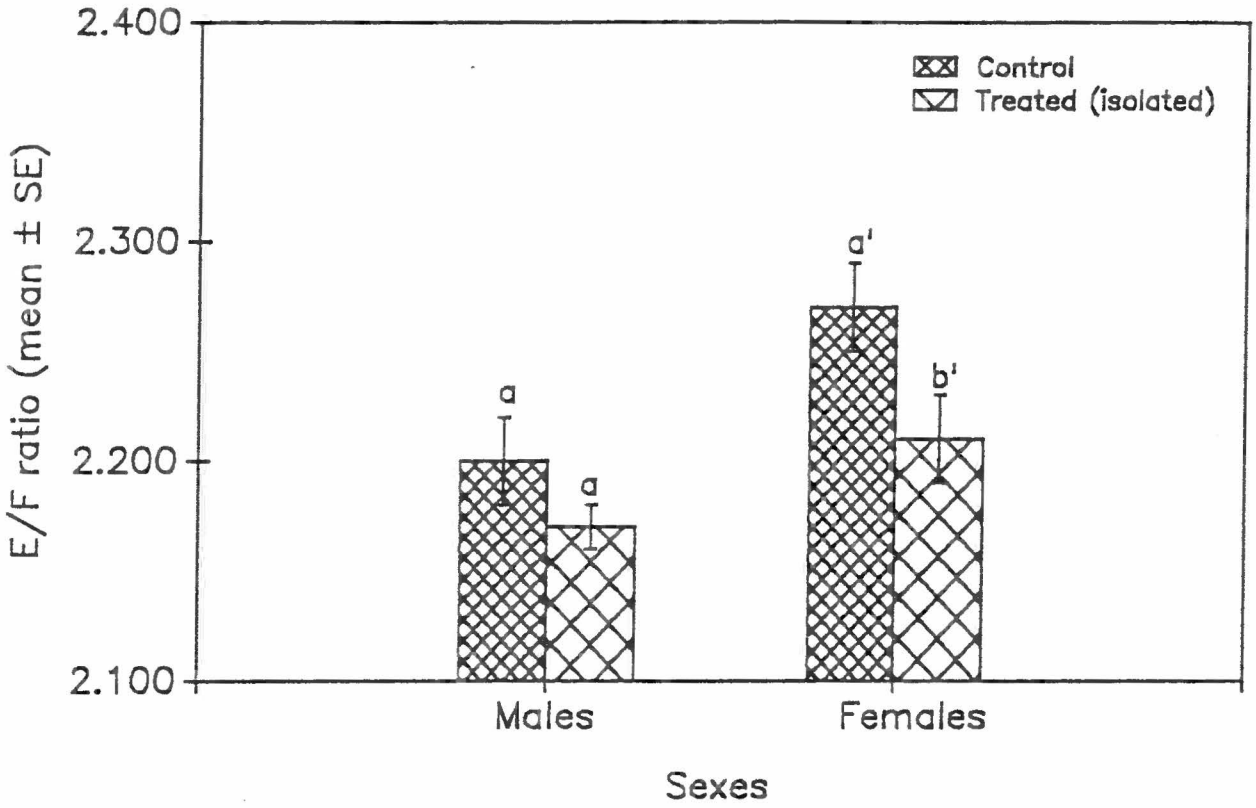


Fig. 23 Changes in E/F ratio (mean ± se) of adult *S. gregaria* from uncrowding gregarious fledglings in the same generation (F_0).

Table 2. Colour changes in nymphs, young (Y) adults and mature (M) adults of gregarious nymphs uncrowded for three generations (F_0-F_2). G/C= gregarious control, G/I= gregarious isolated and S/C= solitarious control.

Treatments	Colour		
	Nymph (5th)	Y. Adult	M. Adult
G/C	black pattern on yellow	pinkish	bright yellow
G/I-F_0	green or straw brown	grey or grayish brown	grey or grayish brown
G/I-F_1	green or straw brown	grey or grayish brown	grey or grayish brown
G/I-F_2	green or straw brown	grey or grayish brown	grey or grayish brown
S/C	green or straw brown	grey or grayish brown	grey or grayish brown

(ii) Uncrowding at fledgling stage

Gregarious fledglings which were isolated turned grey as they matured similar to solitarious mature adults (Table 3).

(iii) Uncrowding at mature adult stage

Bright yellow gregarious mature adults which were isolated turned dull yellow (Table 4).

4.2. Crowding of Different Stages of Solitarious Locusts

4.2.1. Phenylacetonitrile titre

(i) Crowding at nymphal stage

The mean phenylacetonitrile titre of adult males which emerged from crowding (grouping) solitarious nymphs and that of gregarious control adult males were not significantly different within the F_0 and F_1 generations ($P > 0.05$) (Figs. 24 and 25). In the F_2 and F_3 generations, phenylacetonitrile emission decreased and levelled off (Fig. 25). Percentages of phenylacetonitrile produced relative to gregarious

Table 3. Colour changes in adult males of gregarious *S. gregaria* fledglings uncrowded during the F_0 generation.

Treatments	Colour	
	Fledglings	Mature Adults
Control	pinkish	bright yellow
Treated (isolated)	pinkish	grey grayish brown

Table 4. Colour changes in adult males of gregarious *S. gregaria* uncrowded at mature adult stage for two weeks.

Treatments	Colour	
	Adults (20 days)	Adults (> 30 days)
Control	bright yellow	bright yellow
Treated (isolated)	bright yellow	dull yellow

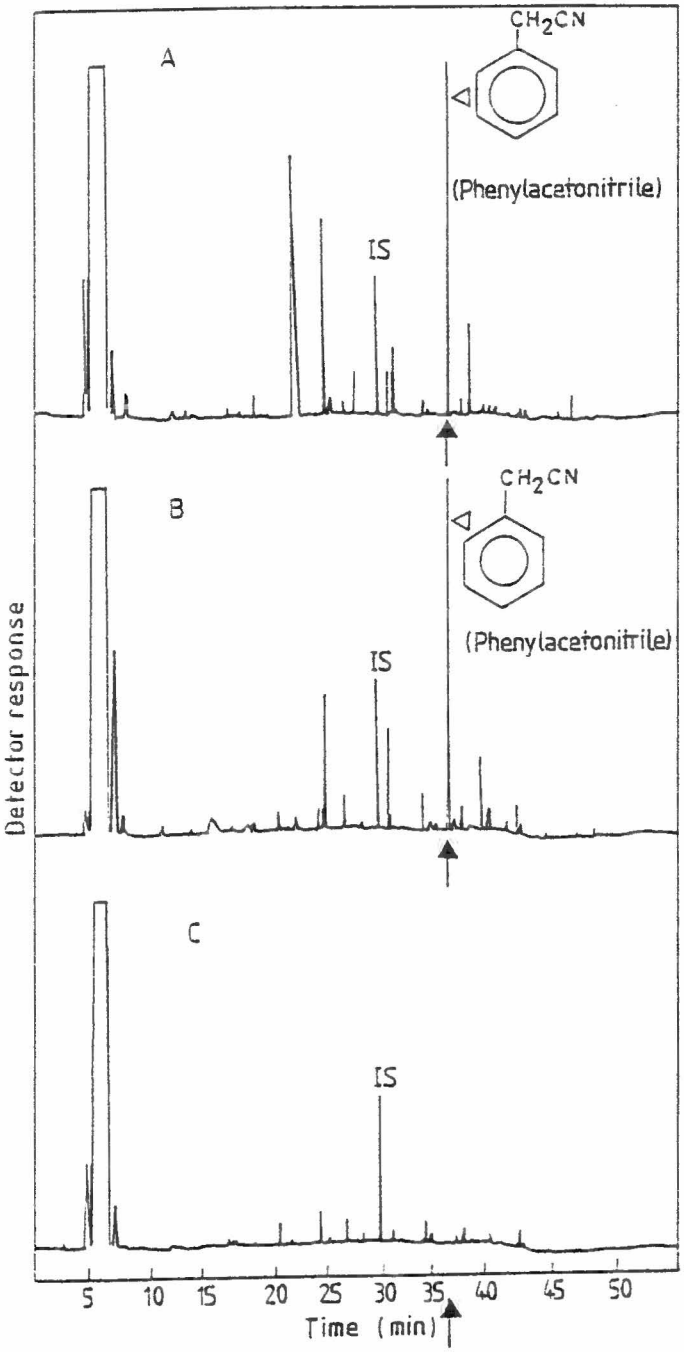


Fig. 24 Chromatograms of the air-borne volatiles collected from 20-22 day old adult males of (A) gregarious, (B) solitarious grouped and (C) solitarious *S. gregaria* injected into a 50m carbowax 20m capillary column. IS= Internal standard.

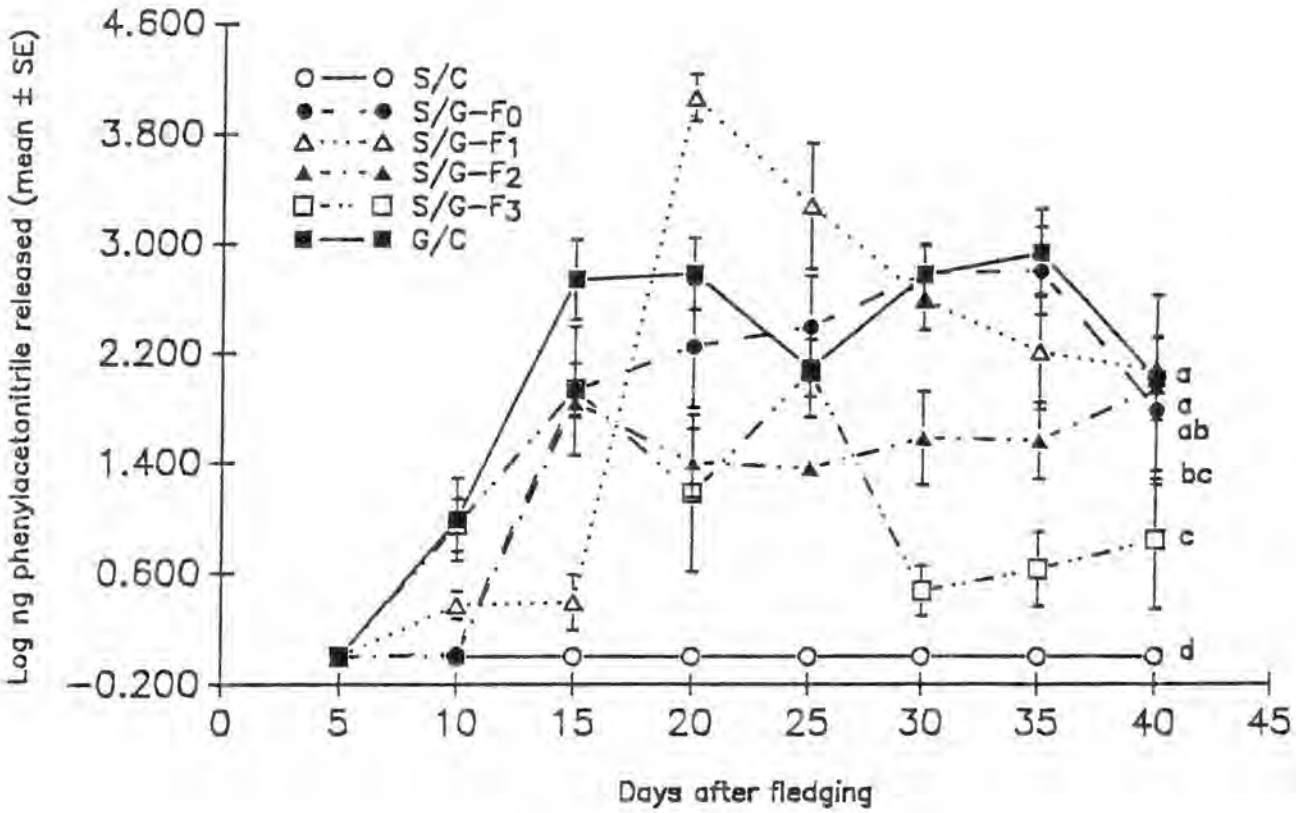


Fig. 25 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* which emerged from crowding solitarious nymphs for four generations (F_0-F_3). S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

control were 87%, 93%, 60%, and 50% in F_0, F_1, F_2 and F_3 generations, respectively (Fig. 26).

(ii) Crowding at fledgling stage

Adult males resulting from crowding solitarious fledglings produced phenylacetonitrile levels which were not significantly different from those of their control gregarious counterparts within the F_0 generation ($P< 0.05$) (Fig. 27).

(iii) Crowding at mature adult stage

The mean phenylacetonitrile titre computed for adult males resulting from crowding three-week-old solitarious mature adults was not significantly different from that of gregarious locusts 6 days after crowding (Fig. 28). This component was detected in the air-borne volatiles of the males two days after treatment but the levels were not significantly different from those of their solitarious control counterparts ($P> 0.05$) (Fig. 28).

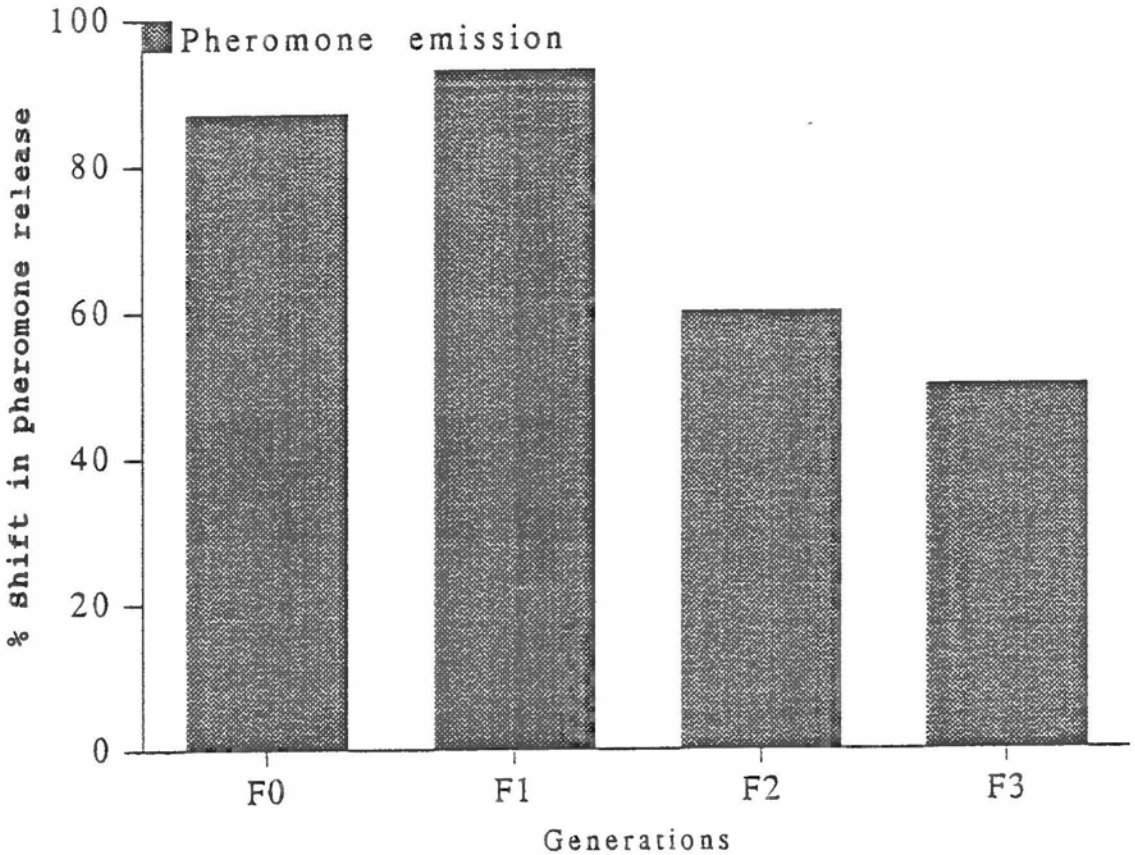


Fig. 26 Percentage phenylacetonitrile relative to gregarious control for four generations (F_0-F_3) of crowding of solitarious *S. gregaria* nymphs.

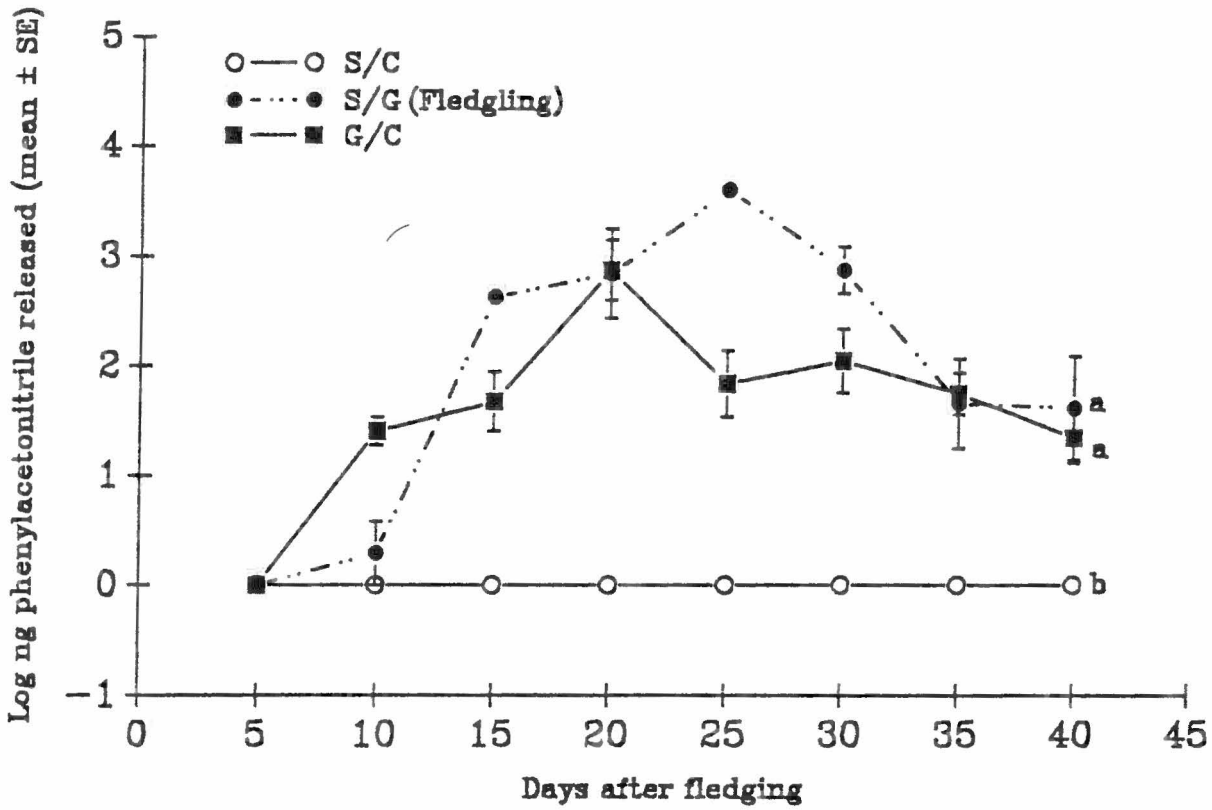


Fig. 27 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* . which emerged from crowding solitarious fledglings during the F_0 generation. S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

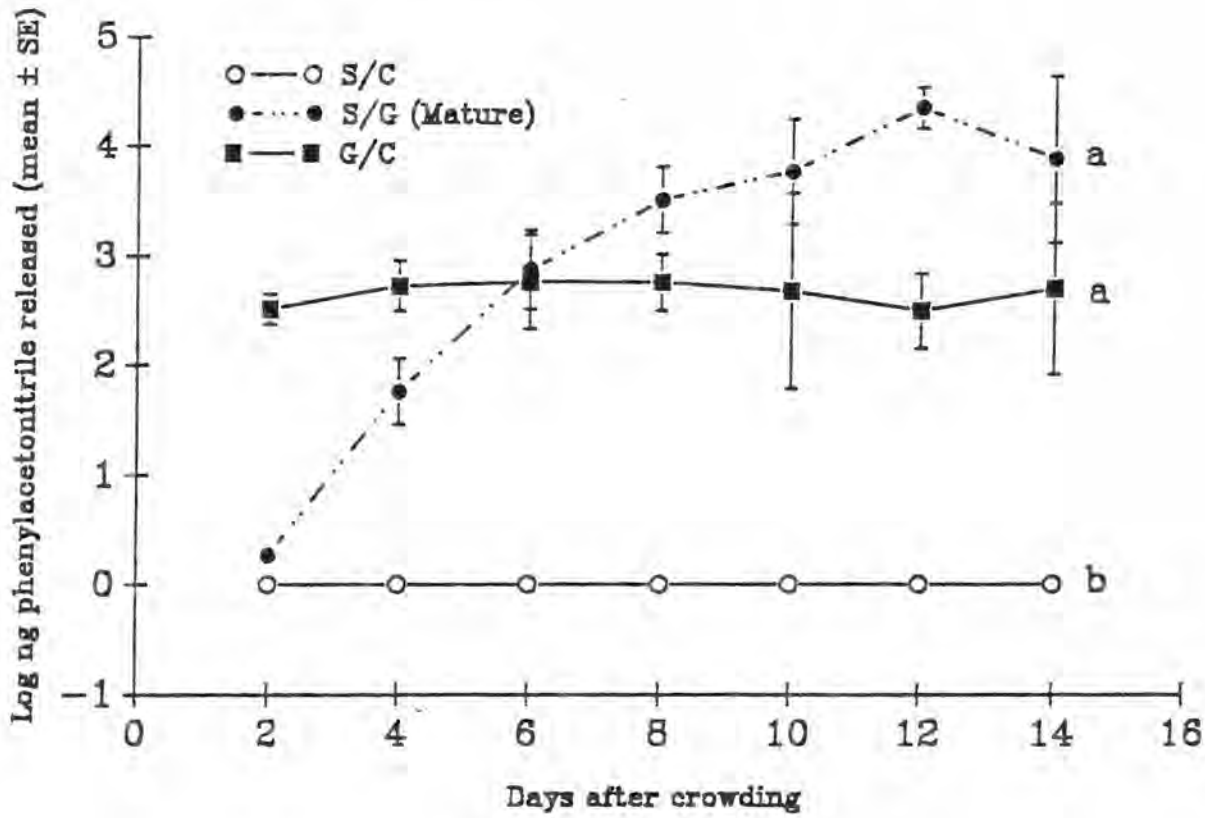


Fig. 28 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S. gregaria* which emerged from crowding solitarious mature adults for two weeks. S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

4.2.2. Haemolymph pigment composition

(i) Crowding at nymphal stage

The mean absorbance ratio of the haemolymph pigments of solitarious locusts which were crowded (grouped) showed a significant shift in nymphs toward gregarious ratio within F_0 generation(Fig 29). The estimated degree of change at this stage was 93%, 79%, 45% and 100% in the F_0, F_1, F_2 and F_3 respectively (Fig. 30a and b). The change in haemolymph pigment composition in the adults was slow and a significant shift toward gregarious ratio was registered in the F_3 generation (Fig. 29).

(ii) Crowding at fledgling stage

The mean absorbance ratio of the haemolymph pigments of the solitarious fledglings which were grouped was not significantly different from that of the control of solitarious locusts ($P< 0.05$) (Fig. 31).

(iii) Crowding at mature adult stage

Assessment of haemolymph pigment composition was not carried out since similar treatment on fledglings yielded no

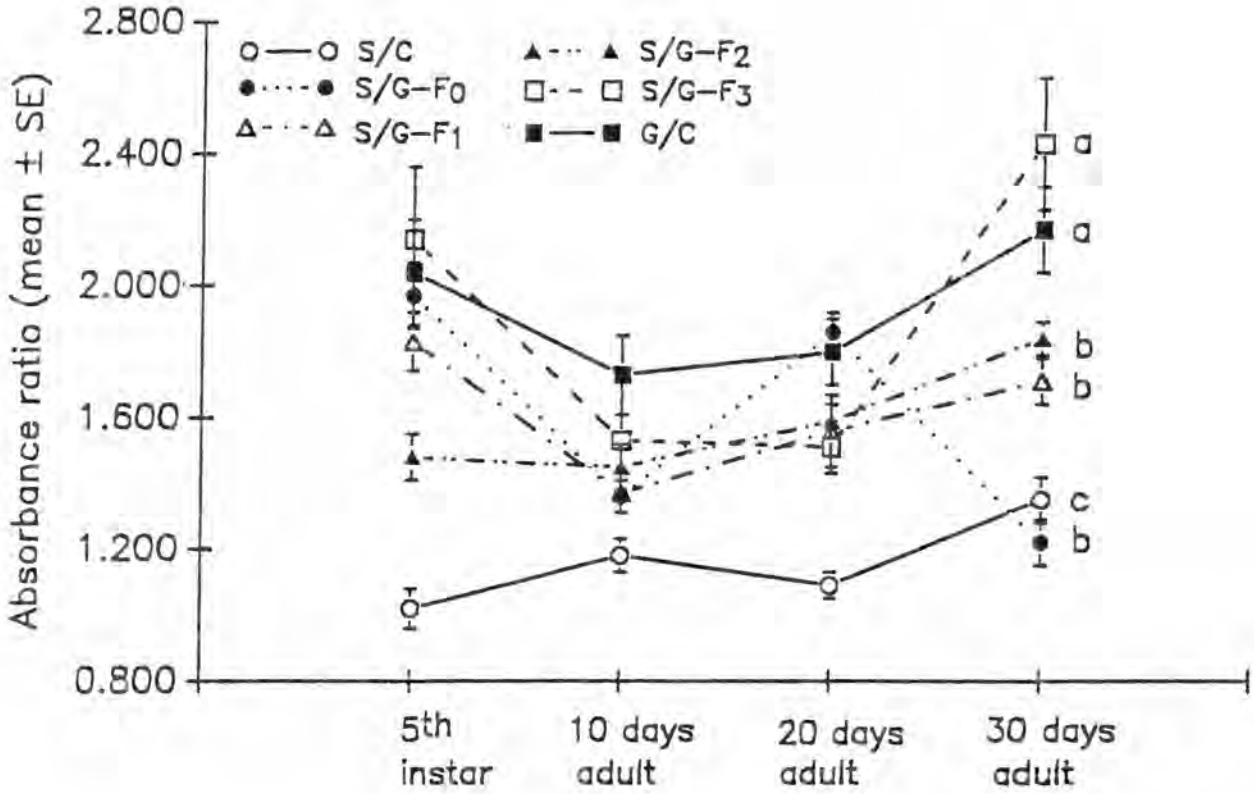


Fig. 29 Haemolymph pigment changes in solitarious *S. gregaria* crowded at nymphal stage for four generations (F_0-F_3). S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

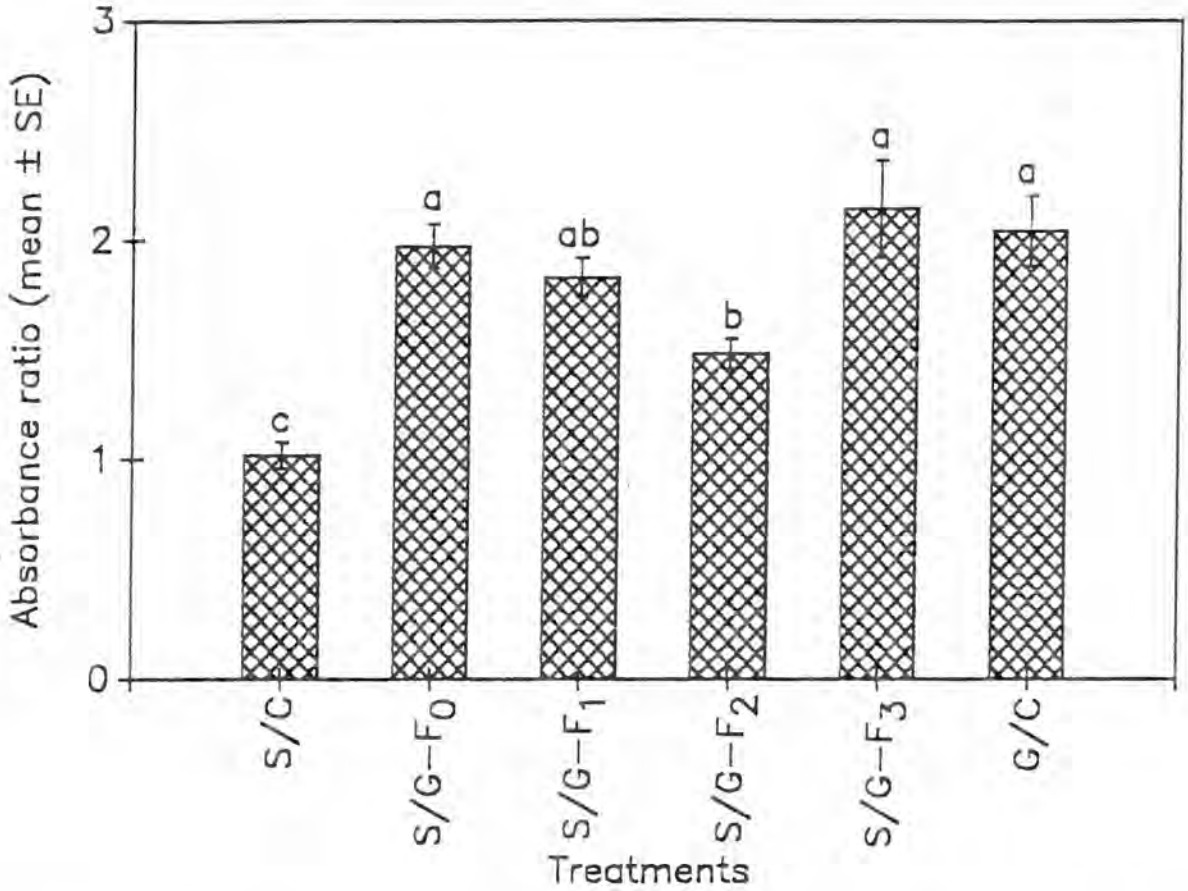


Fig. 30 (a) Haemolymph pigment ratio for four generations (F_0- F_3) of uncrowding of gregarious *S. gregaria* nymphs.

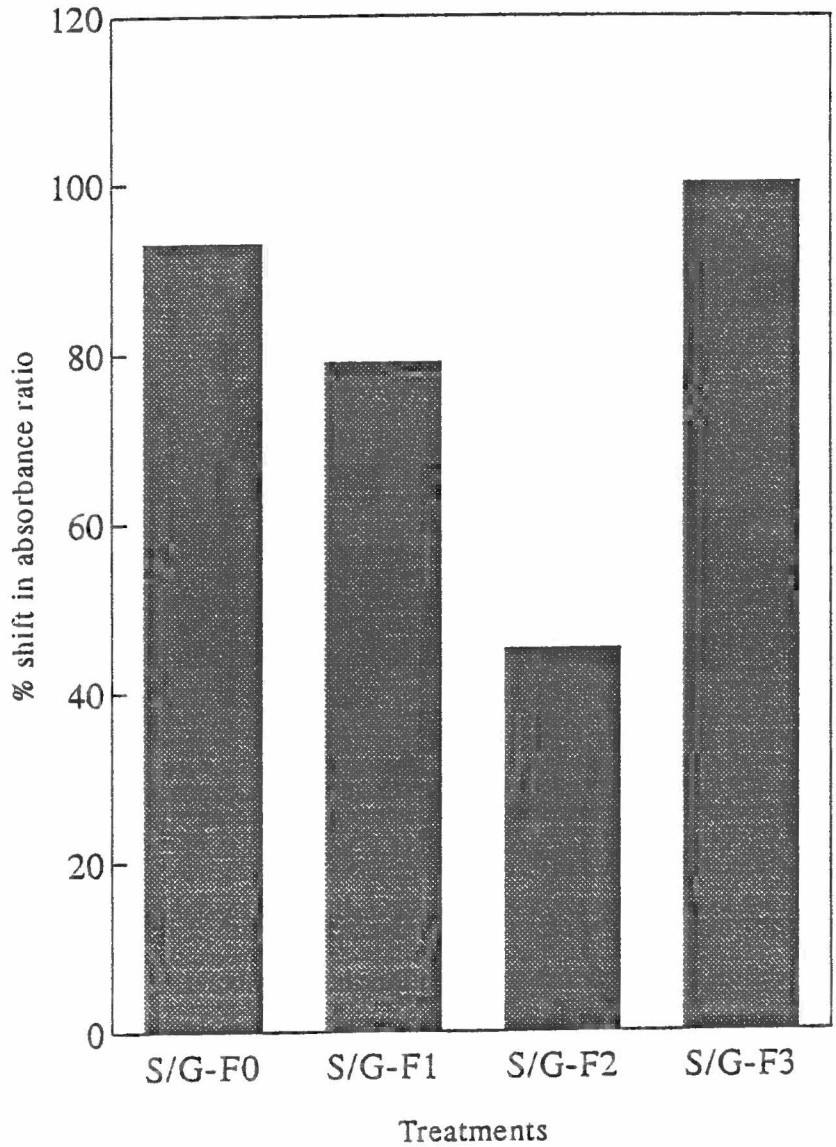


Fig. 30 (b) Percentage shift in haemolymph absorbance ratio for four generations (F_0-F_3) of crowding of solitarious *S. gregaria* nymphs.

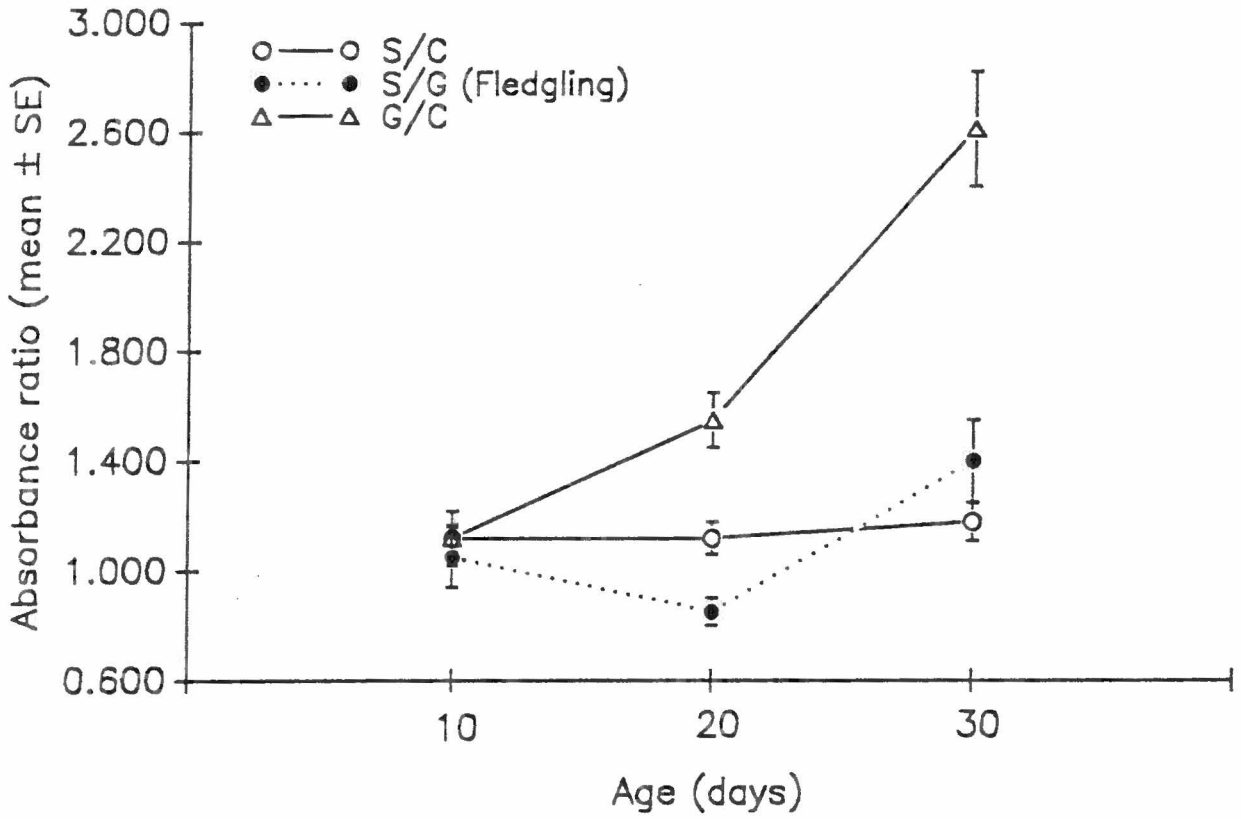


Fig. 31 Haemolymph pigment changes in solitarious *S. gregaria* crowded for one generation (F_0) at fledgling stage. S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

significant differences between the control and grouped locusts.

4.2.3. Morphometrics

(i) Crowding at nymphal stage

The mean F/C ratio computed for both males and females emerged from crowding solitarious nymphs was significantly different from that of the control gregarious locusts by the end of F_3 generation ($P< 0.05$) (Fig. 32). No significant change was recorded in treated females compared to the solitarious control counterparts within the F_0 generation. The estimated degrees of shifts toward gregarious F/C ratio at F_0, F_1, F_2 and F_3 generations were 21%, 26%, 64%, and 46% for males and 20%, 49%, 57%, and 57% for females (Fig. 33). The mean E/F ratios were not significantly different between treated females in all the generations studied and the two controls (Fig. 34). The mean E/F ratios for males differed significantly by the end of the F_0 generation. The shift estimates based on E/F ratios were 33%, 0%, 0%, and 0% for males and 0%, 0%, 25%, and 0% for females in F_0, F_1, F_2 and F_3 generations, respectively (Fig. 35).

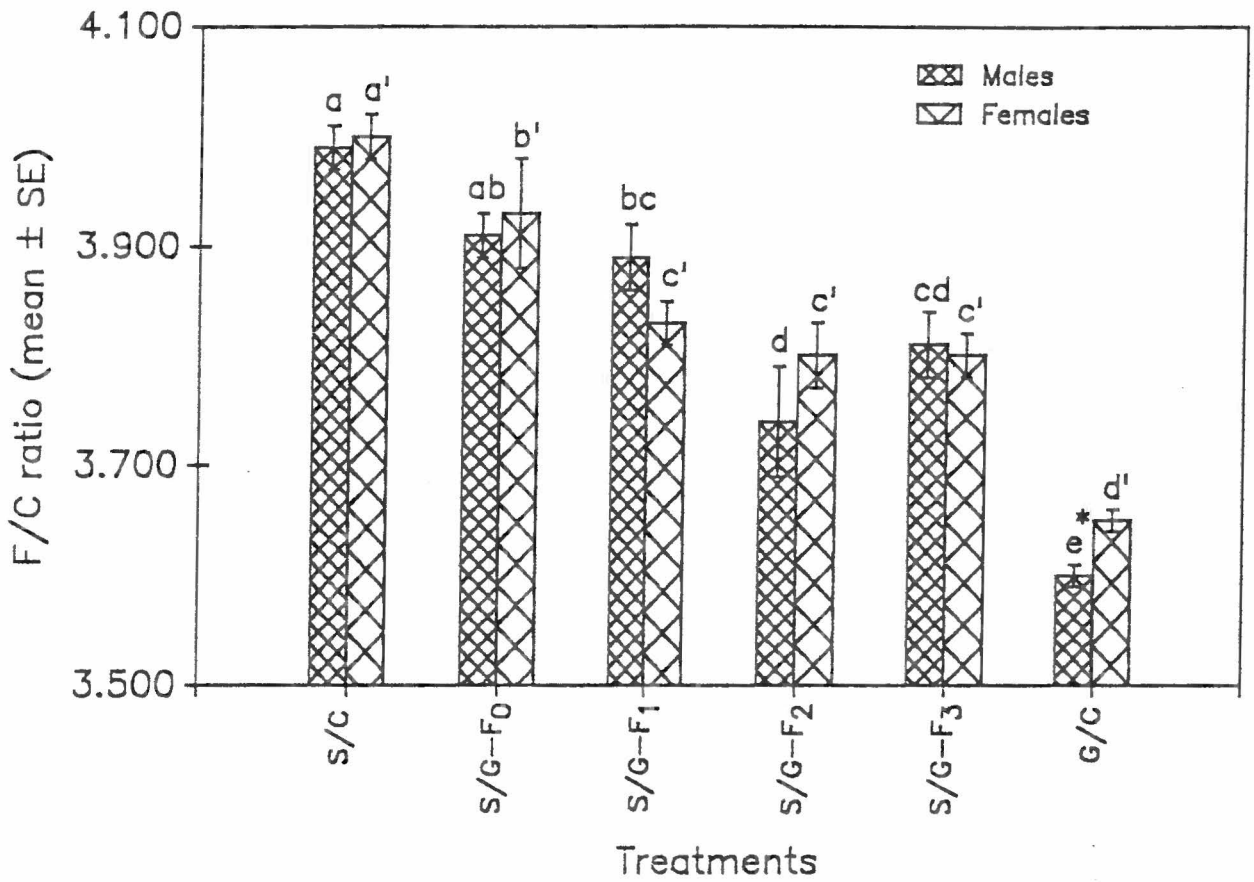


Fig. 32 Changes in F/C ratio (mean ± se) of adult *S. gregaria* which emerged from crowding solitarious nymphs for four generations (F_0-F_3). S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

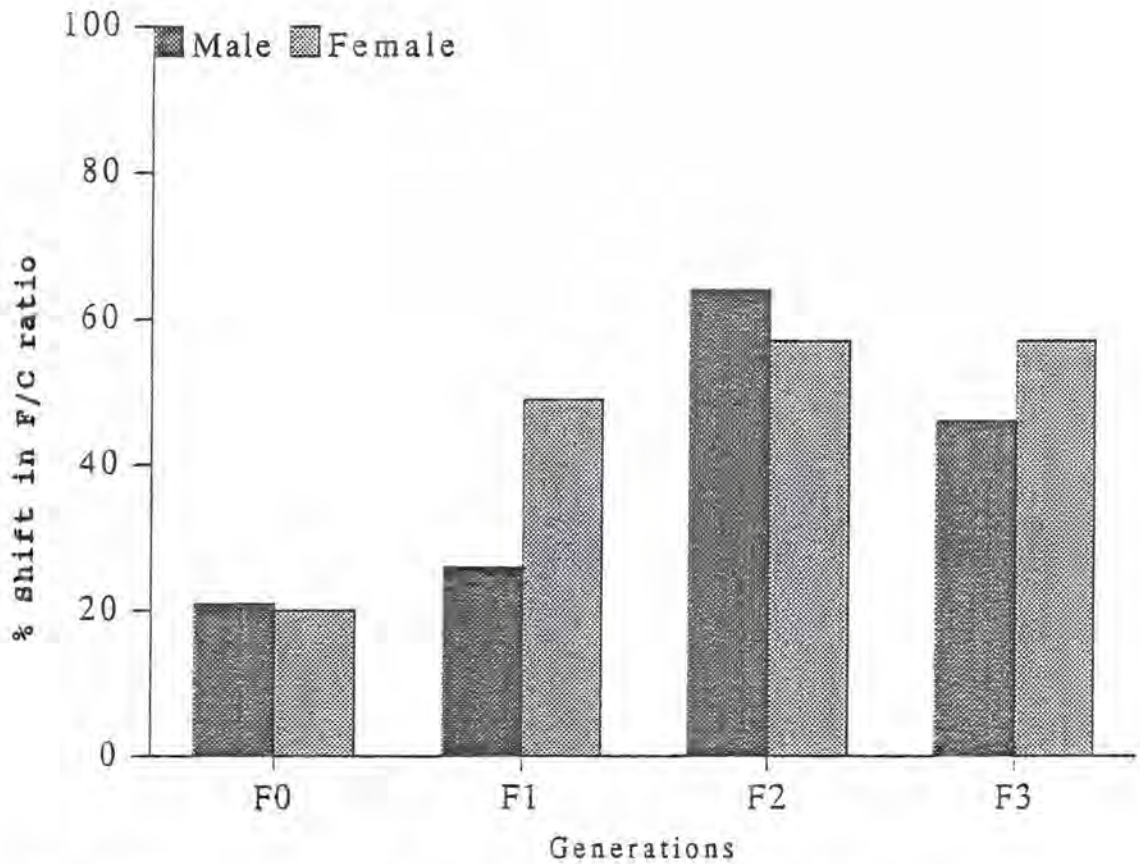


Fig. 33 Percentage shift in F/C ratio for four generations (F_0-F_3) of crowding *S. gregaria* starting from solitarious nymphs.

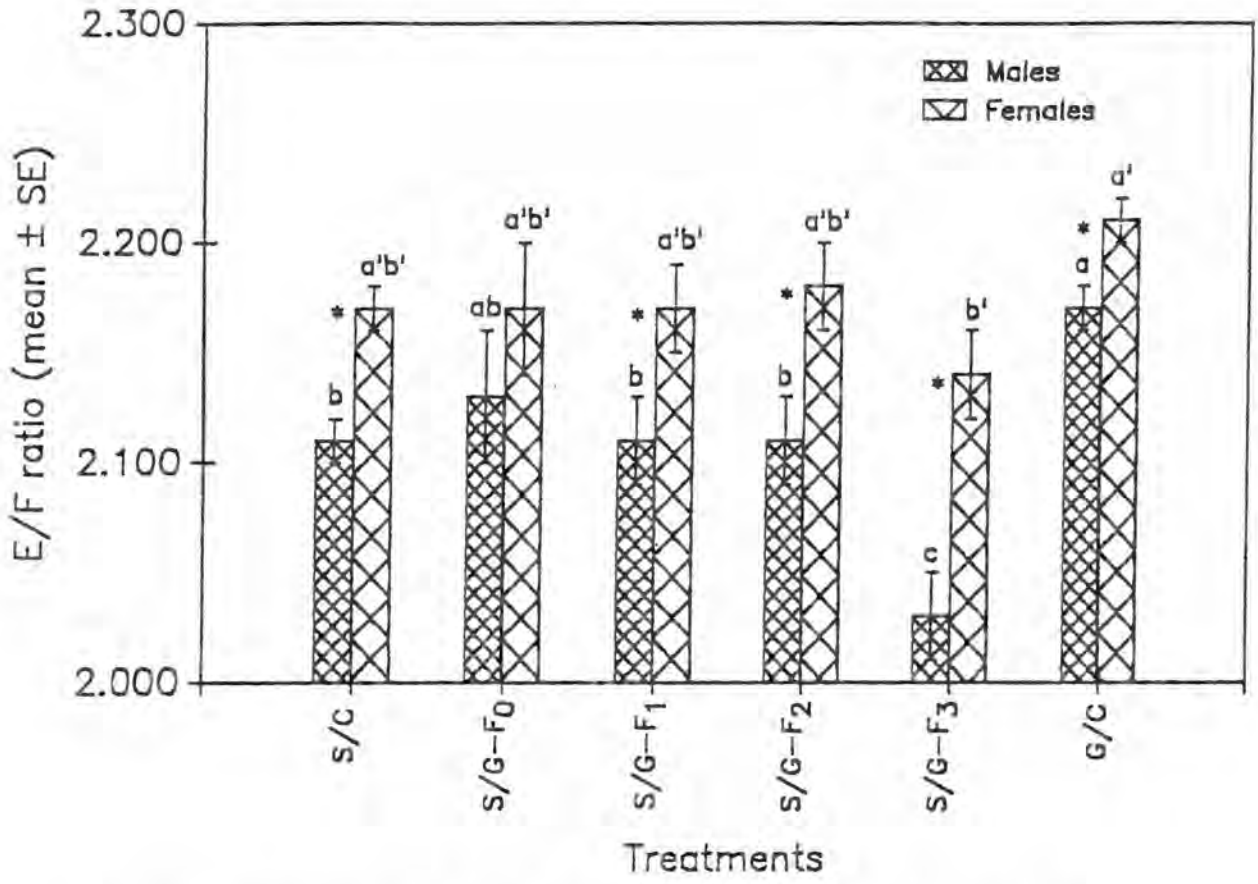


Fig. 34 Changes in E/F ratio (mean ± se) of adult *S. gregaria* which emerged from crowding solitarious nymphs for four generations (F_0-F_3). S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

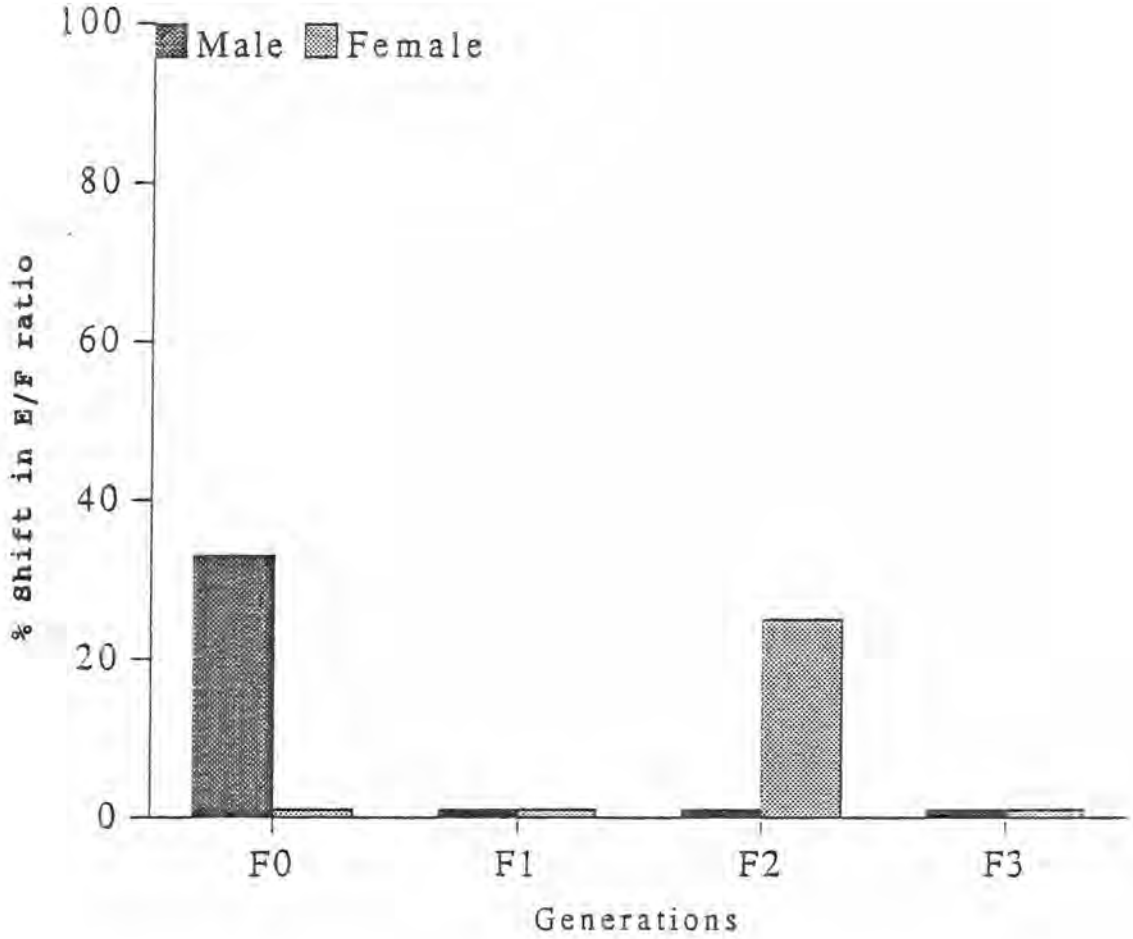


Fig. 35 Percentage shift in E/F ratio for four generations (F_0-F_3) of crowding *S. gregaria* starting from solitarious nymphs.

(ii) Crowding at fledgling stage

The mean F/C and E/F ratios for both males and females emerged from crowding solitarious fledglings were not significantly different from those of the controls ($P< 0.05$) (Figs. 36 and 37).

(iii) Crowding at mature adult stage

No assessment of this parameter was carried out in mature adults since similar treatment of fledglings did not yield significant differences between the control and grouped locusts.

4.2.4. Colour

(i) Crowding at nymphal stage

Fifth instar nymphs, immature and mature adults emerged from crowding solitarious nymphs were yellow with black patterns, pink and bright yellow, respectively similar to gregarious nymphs, immature and mature adults (Table 5).

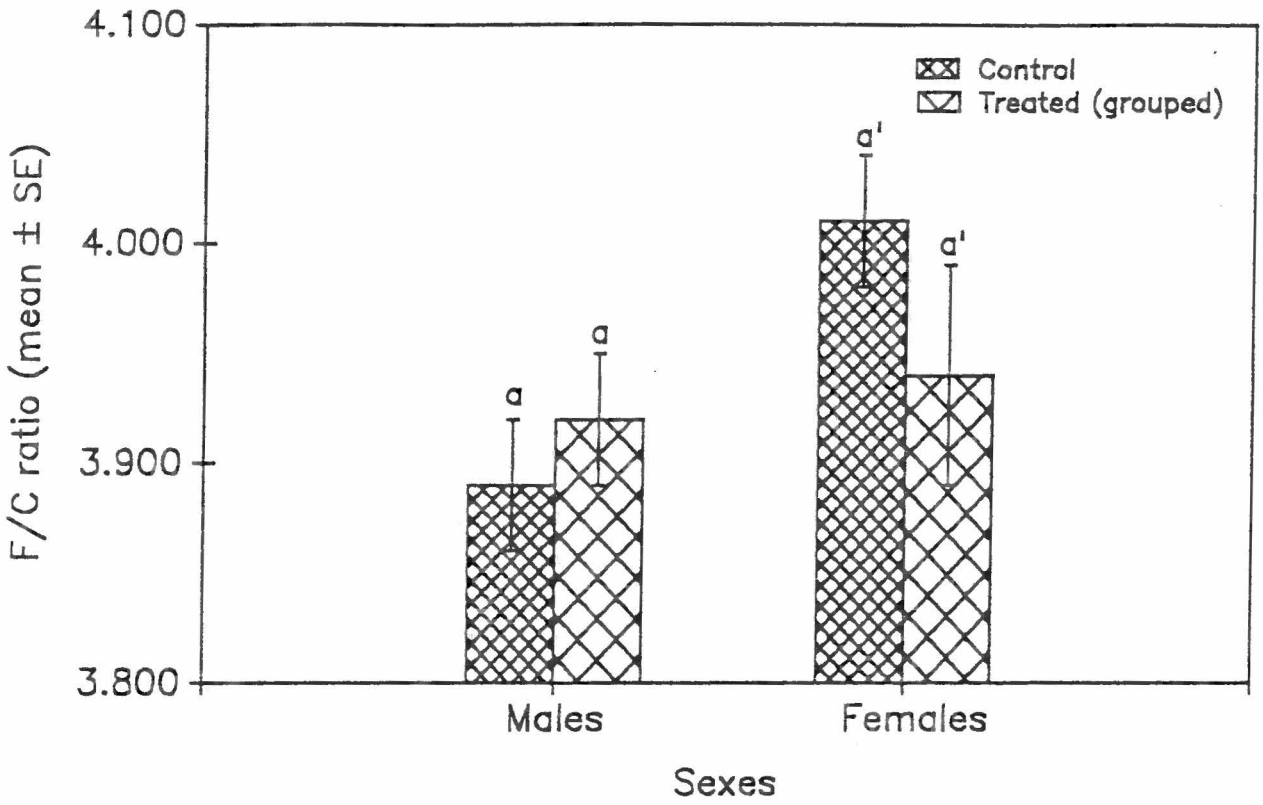


Fig. 36 Changes in F/C ratio (mean ± se) of adult *S. gregaria* from crowding solitarious fledglings in the same generation (F_0).

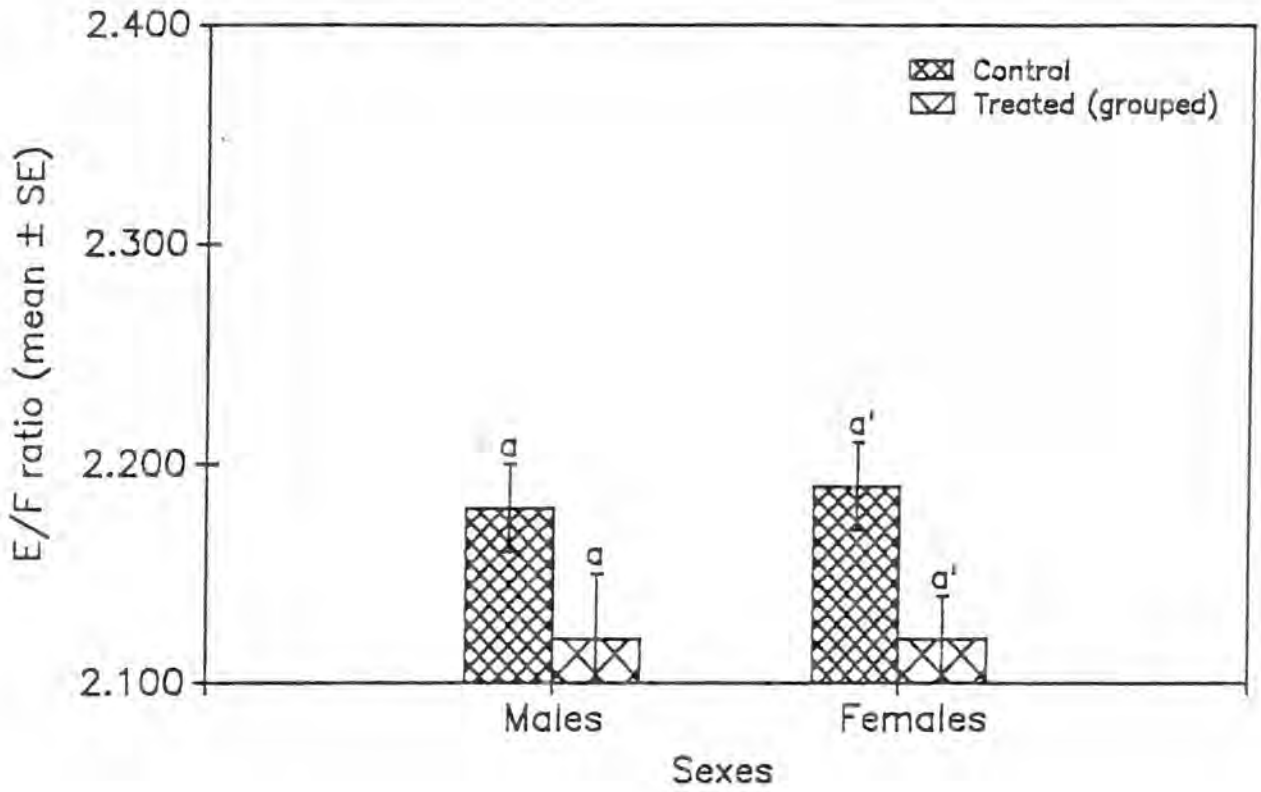


Fig. 37 Changes in E/F ratio (mean ± se) of adult *S. gregaria* from crowding solitarious fledglings in the same generation (F_0).

Table 5. Colour changes in nymphs, young (Y) adults and mature (M) adults of solitarious nymphs crowded for four generations (F_0-F_3). S/C= solitarious control, S/G= solitarious grouped and G/C= gregarious control.

Treatments	Colour		
	Nymph (5th)	Y. Adult	M. Adult
S/C	green or straw brown	grey grayish brown	grey grayish brown
S/G-F_0	black patterns on yellow	pinkish	bright yellow
S/G-F_1	black patterns on yellow	pinkish	bright yellow
S/G-F_2	black patterns on yellow	pinkish	bright yellow
S/G-F_3	black patterns on yellow	pinkish	bright yellow
G/C	black patterns on yellow	pinkish	bright yellow

(ii) Crowding at fledgling stage

Adult males emerged from crowding solitarious fledglings were bright yellow similar to their gregarious counterparts (Table 6).

(iii) Crowding at mature adult stage

Grayish solitarious mature adult males turned bright yellow after crowding for two weeks (Table 7).

4.3. Crowding Solitarious Nymphs at Different Densities.

4.3.1. Phenylacetonitrile titre

The mean phenylacetonitrile titre of adults which emerged from solitarious nymphs kept in groups of four, two, and one (control solitarious) per cage (used for rearing solitarious locust) followed different patterns. The total phenylacetonitrile titre computed for four locusts per cage was intermediate between that of control *gregaria* and one locust per cage within the F_0 generation. Locusts kept in two per cage also demonstrated measurable amounts of phenylacetonitrile but the amount was small (Fig. 38). Adult males from both four and two locusts per cage emitted

Table 6. Colour changes in adult males of solitarious *S. gregaria* fledglings crowded for one generation.

Treatments	Colour	
	Fledglings	Mature Adult
Control	grey or grayish brown	grey or grayish brown
Treated (grouped)	grey or grayish brown	bright yellow

Table 7. Colour changes in adult males of solitarious *S. gregaria* mature adults crowded for two weeks.

Treatments	Colour	
	Mature adults	Older Adults
Control	grey or grayish brown	grey or grayish brown
Treated (grouped)	grey or grayish brown	bright yellow

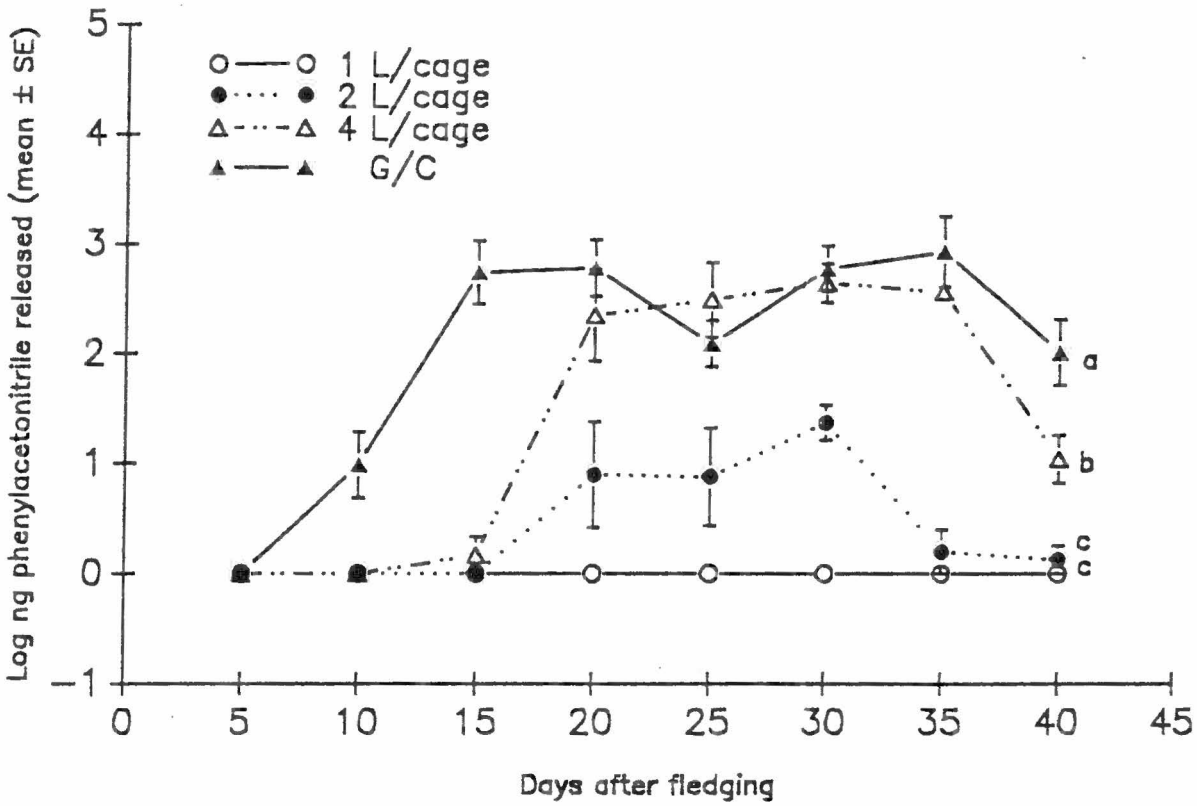


Fig. 38 Log ng phenylacetonitrile (mean ± se) released at different ages by adult males of *S.gregaria* which emerged from crowding solitarious nymphs in the same generation (F_0) at different densities. 1 L/cage= one locust per cage, 2 L/cage= two locusts per cage, 4 L/cage= four locusts per cage and G/C= gregarious control.

phenylacetonitrile at 15 and 20 days after final moult, respectively, compared to 10 days after final moult in gregarious control adult males (Fig. 38).

4.3.2. Haemolymph pigment composition

The mean absorbance ratios of the haemolymph pigments of locusts raised in groups of four and two per cage were significantly different from those raised as one locust per cage (solitarious) and control of gregarious locust ($P< 0.05$) (Fig. 39).

4.3.3. Morphometrics

The mean F/C value obtained for locusts reared in a group of four locusts per cage was intermediate between the two controls (*gregaria* and *solitaria*) and was significantly different from either of them. On the other hand, for two locusts per cage, the F/C ratio was not significantly different from that of one locust per cage (Fig. 40). There was no significant change in E/F ratios in adults which emerged from four, two and one locust per cage (Fig. 41).

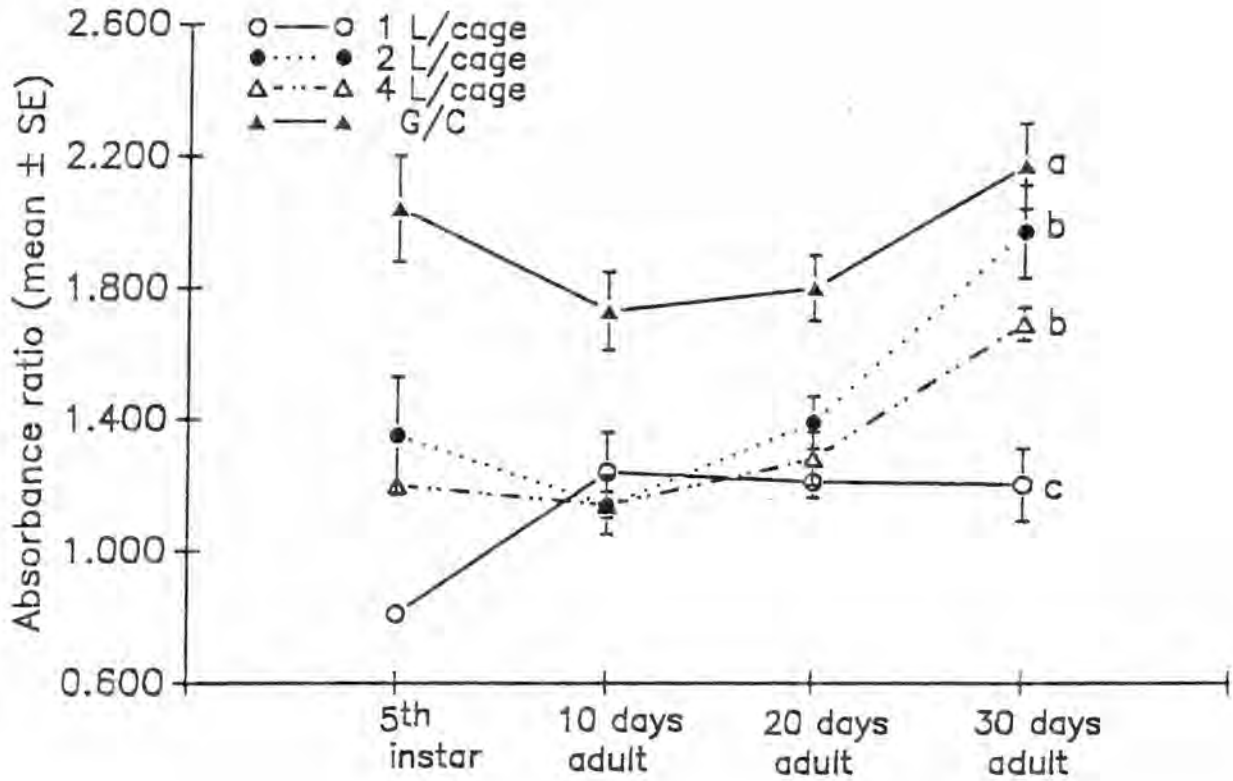


Fig. 39 Haemolymph pigment changes in solitarious *S. gregaria* nymphs crowded in the same generation (F_0) at different densities. 1 L/cage= one locust per cage, 2 L/cage= two locusts per cage, 4 L/cage= four locusts per cage and G/C= gregarious control.

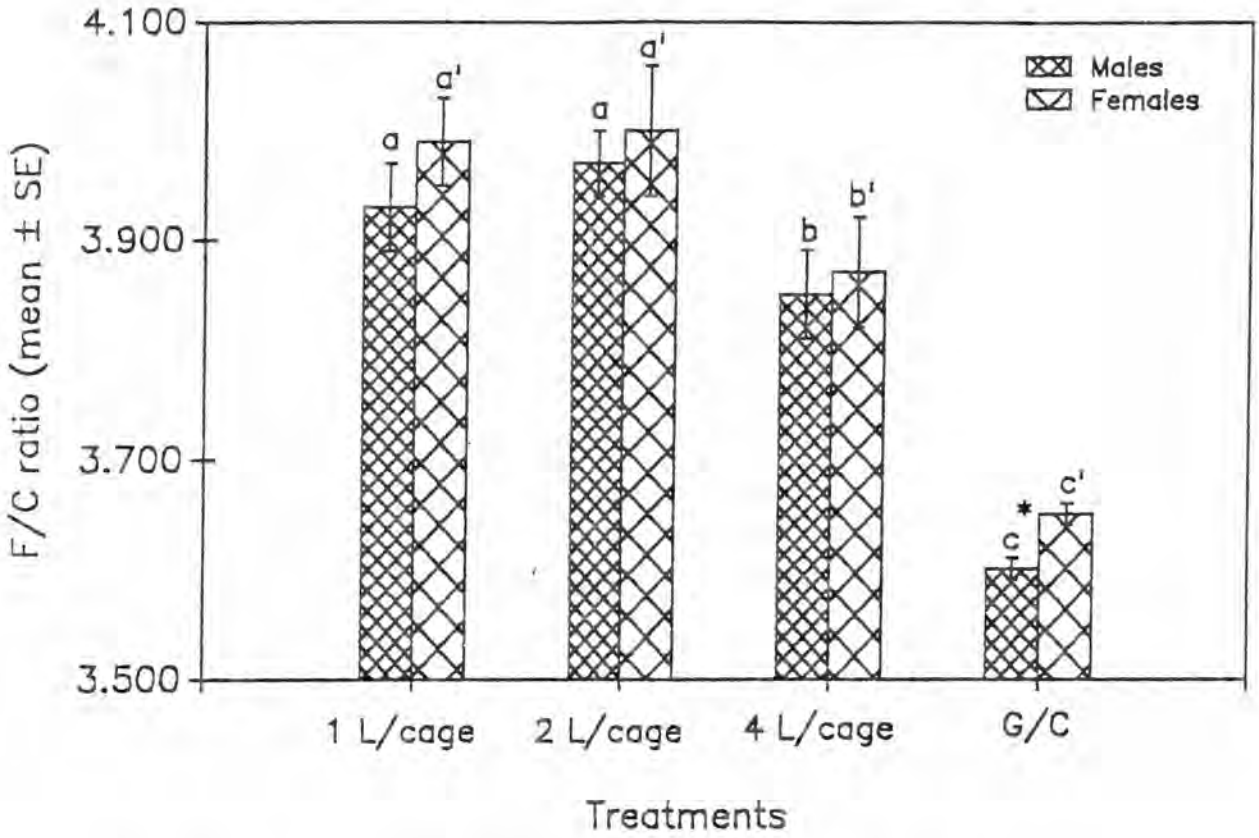


Fig. 40 Changes in F/C ratio (mean ± se) of adult *S. gregaria* which emerged from crowding solitarious nymphs in the same generation (F_0) at different densities. L= locust.

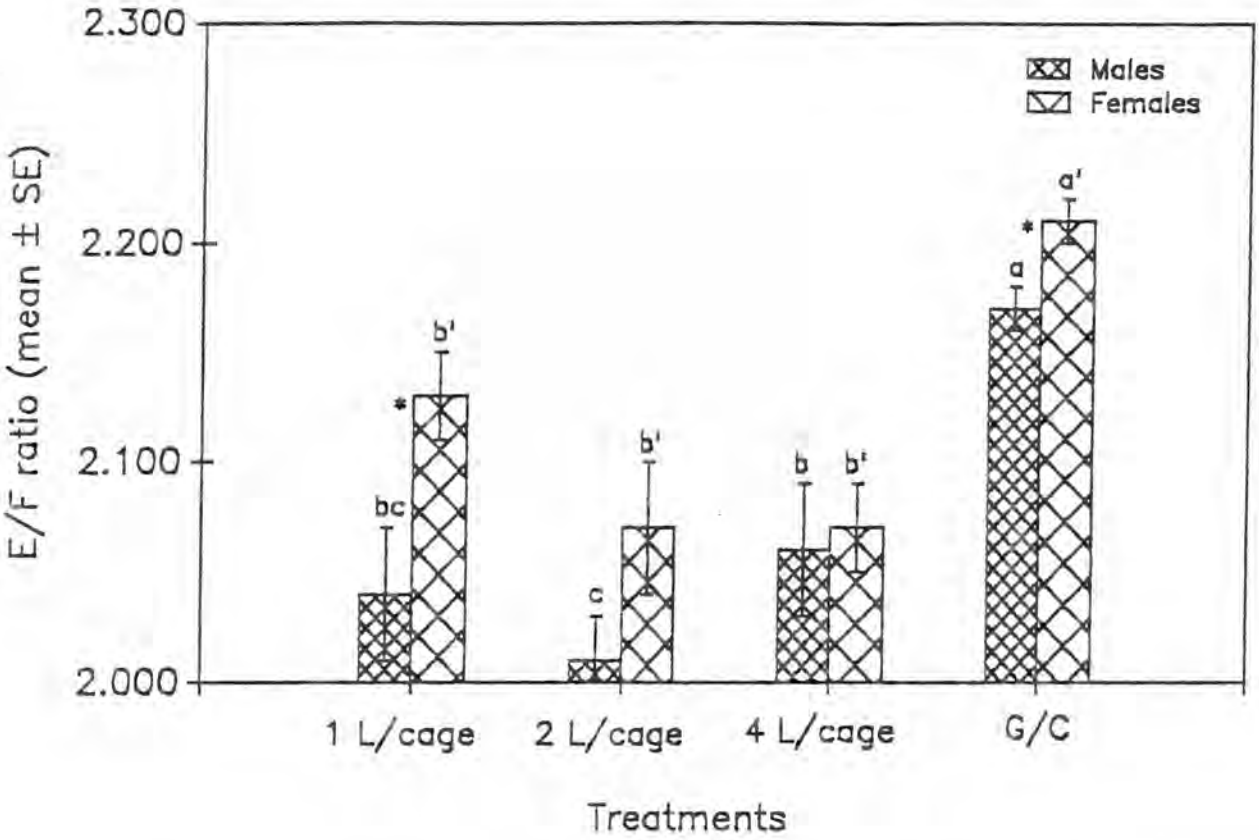


Fig. 41 Changes in E/F ratio (mean ± se) of adult *S. gregaria* which emerged from crowding solitarious nymphs in the same generation (F_0) at different densities. L= locust.

4.3.4. Colour

Fifth instar nymphs, fledglings and mature adult males from nymphs which were crowded as four and two locusts per cage were yellow with black patterns, pink, and yellow, respectively within the F_0 generation. One locust per cage maintained the solitarious grayish colour (Table 8).

4.4. Primer Effects of Releaser Pheromones

4.4.1. Effect of gregarious first instar volatiles on solitarious first instars

(i) Phenylacetonitrile titre

Both the control (unexposed) and treated (exposed) solitarious first instar nymphs failed to produce phenylacetonitrile at the mature adult stage (Fig. 42).

(ii) Haemolymph pigment composition

Both nymphs and adults obtained from solitarious first instar nymphs which were exposed to volatiles emanating from gregarious first instar nymphs and their faeces were not significantly different from their control (unexposed) counterparts ($P< 0.05$) (Fig. 43).

Table 8. Colour changes in solitarious nymphs crowded in groups of two or four per cage for one generation. 1 L/C= one locust per cage, 2 L/C= two locusts per cage and 4 L/C= four locusts per cage.

Treatments	Colour		
	Nymph (5th)	Y. Adult	M. Adult
1 L/C	green or straw brown	grey or grayish brown	grey or grayish brown
2 L/C	black patterns on yellow	pinkish	yellowish
4 L/C	black patterns on yellow	pinkish	bright yellow

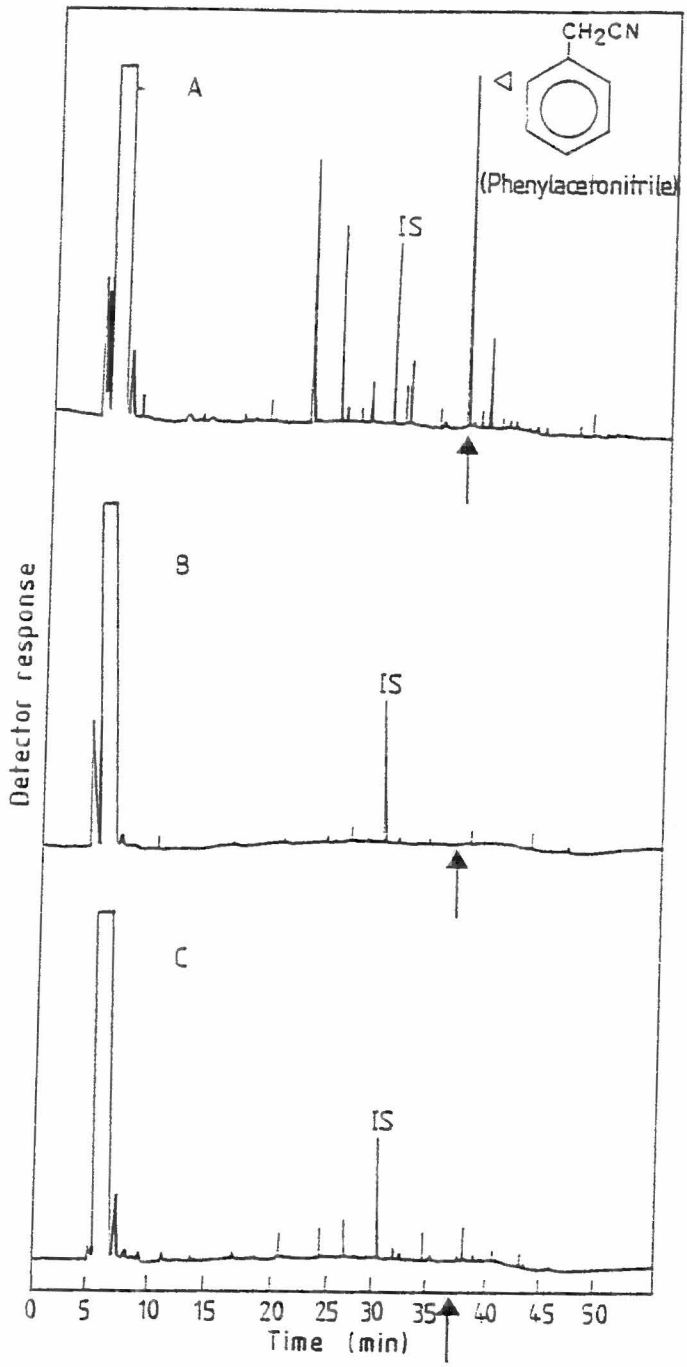


Fig. 42 Chromatograms of the air-borne volatiles collected from 20-22 day old adult males of *S. gregaria* (A) gregarious control (B) solitarious exposed to first instar gregarious nymphs and (C) solitarious unexposed injected into a 50m Carbowax 20M capillary column. IS= Internal standard.

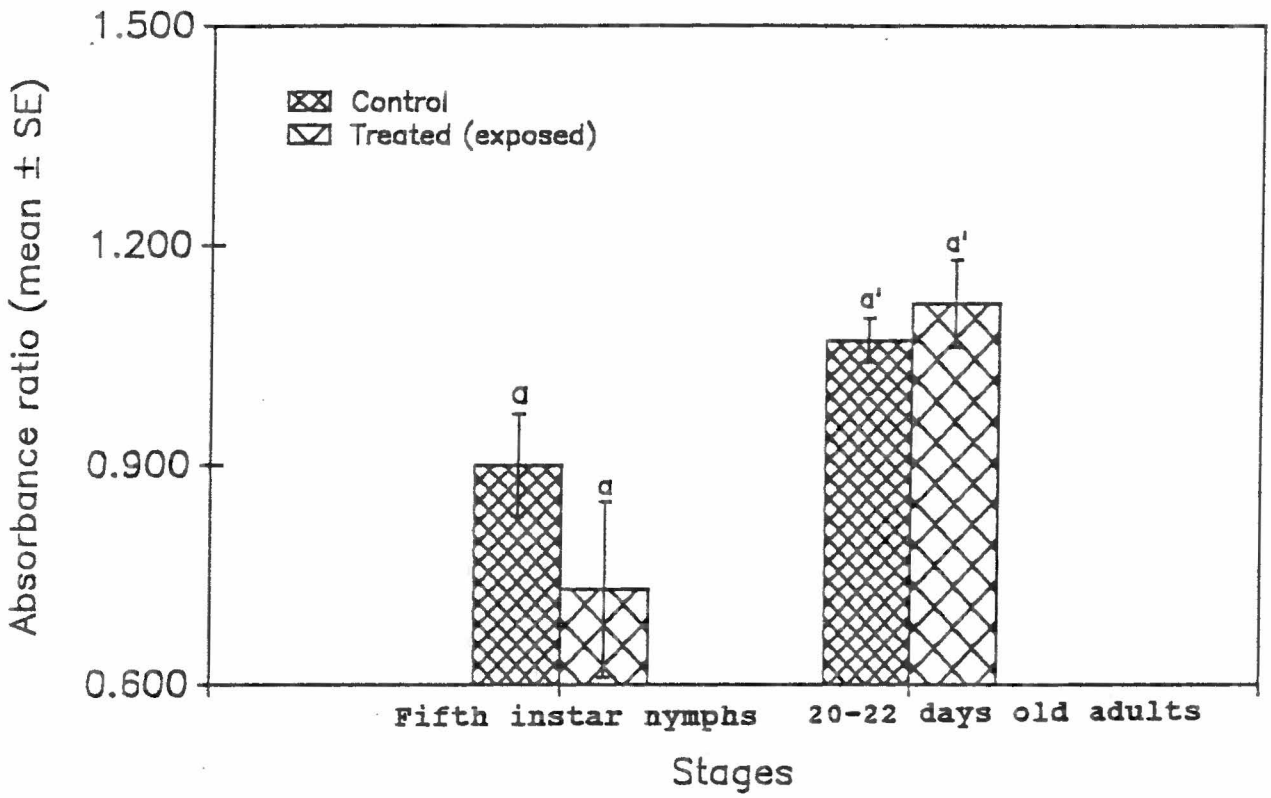


Fig. 43 Haemolymph pigment changes in *S. gregaria* solitaria exposed to gregarious first instar nymphal volatiles at first instar nymphal stage.

(iii) Developmental time

The treated (exposed) nymphal instars developed relatively faster compared to control, although the two mean developmental times were not significantly different ($P > 0.05$) (Fig. 44).

(iv) Weight

Exposed nymphs and adults were, respectively 1.3 and 1.2 times heavier than their unexposed counterparts, although there were no significant differences between their mean weights ($P > 0.05$) (Fig. 45).

(v) Colour

Both nymphs and adults which emerged from solitarious first instar nymphs which were exposed to gregarious first instar nymphal and faecal volatiles were green and grey similar to the unexposed control nymphs and adults, respectively.

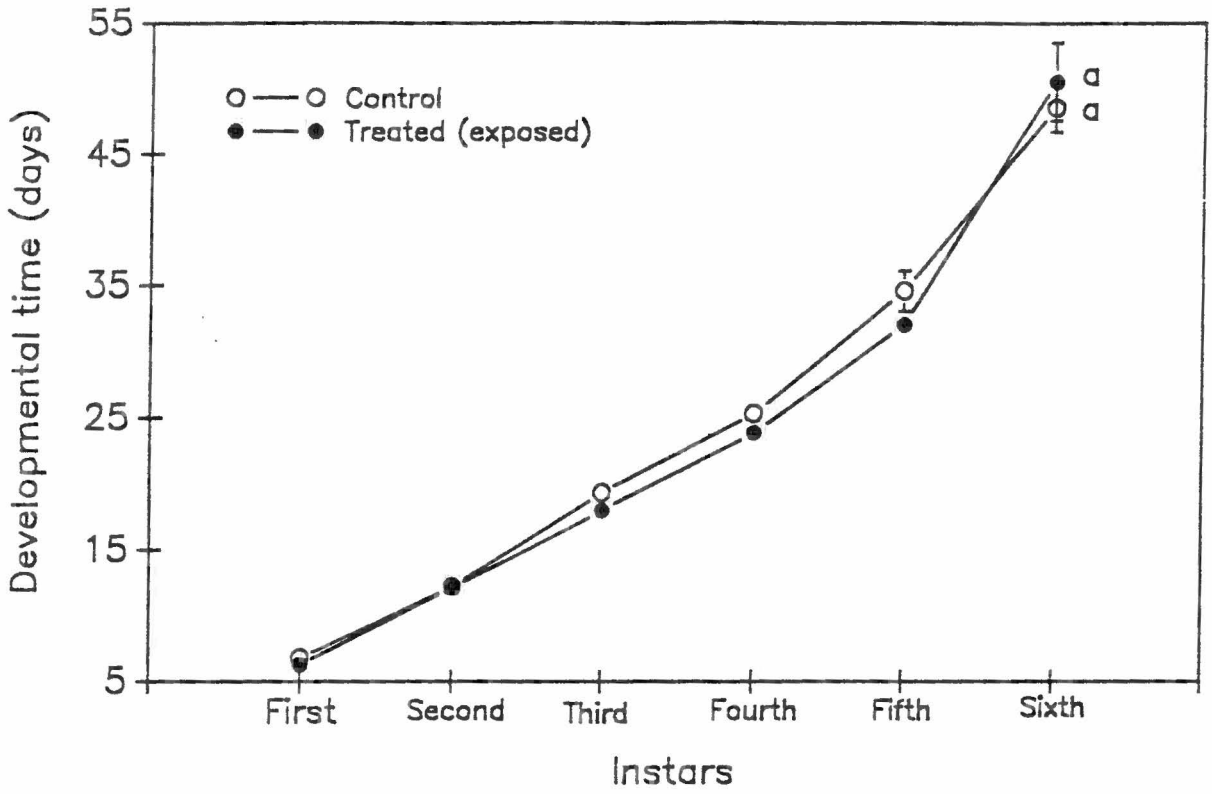


Fig. 44 Developmental time in *S. gregaria* solitarious first instar nymphs exposed to gregarious first instar nymphal volatiles.

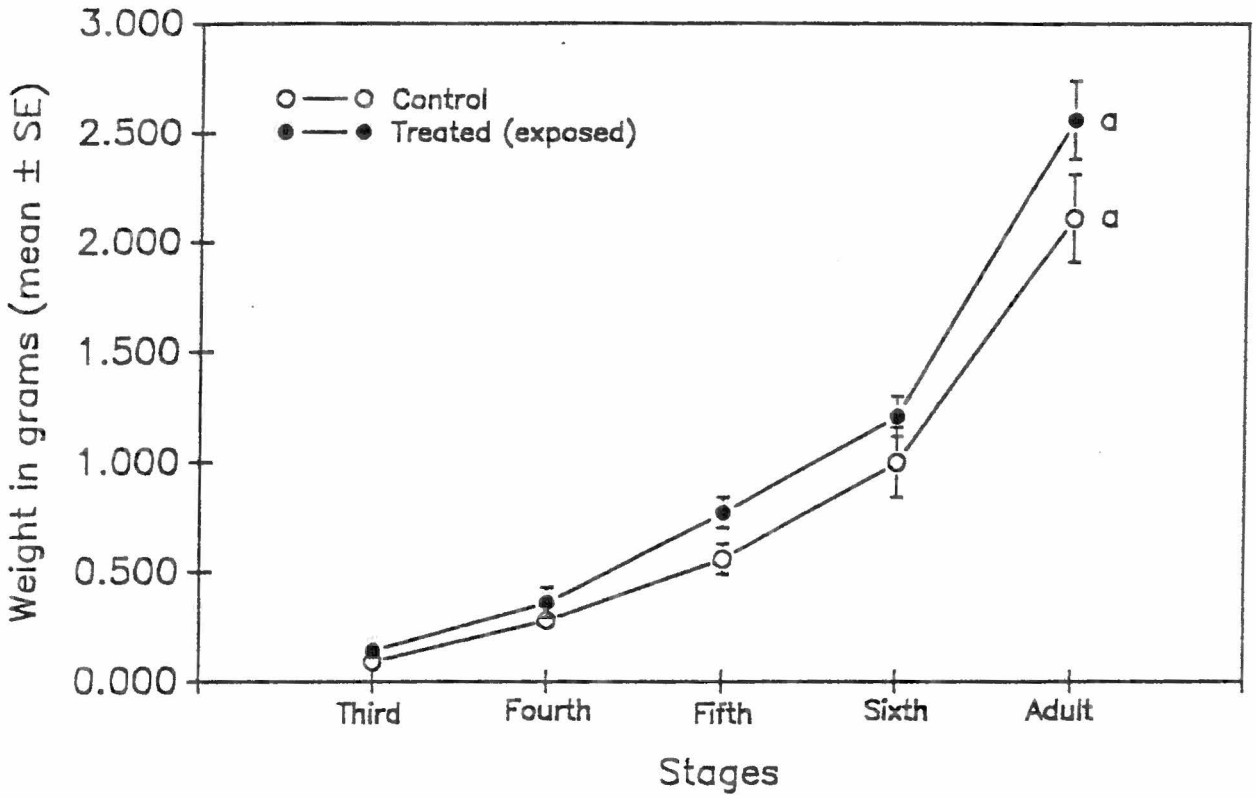


Fig. 45 Weight of nymphal instars and adults of *S. gregaria* which emerged from solitarious first instar nymphs exposed to gregarious first instar nymphal volatiles.

4.4.2. Effect of gregarious third to fifth and fifth instar volatiles on solitarious first instars

(i) Haemolymph pigment composition

Fourteen out of the sixteen solitarious nymphs exposed died 3-4 weeks after treatment and had developed to second nymphal or early third instar stage. Haemolymph collection and analysis were not carried out due to paucity of material (Table 9). On the other hand, only 4 out of the 14 control nymphs died during the same period, The surviving nymphs at that age had developed to third, fourth and fifth instar stages. Mortality in the exposed nymphs was significantly dependent on the volatile pheromones released by gregarious nymphs
($P<0.05$).

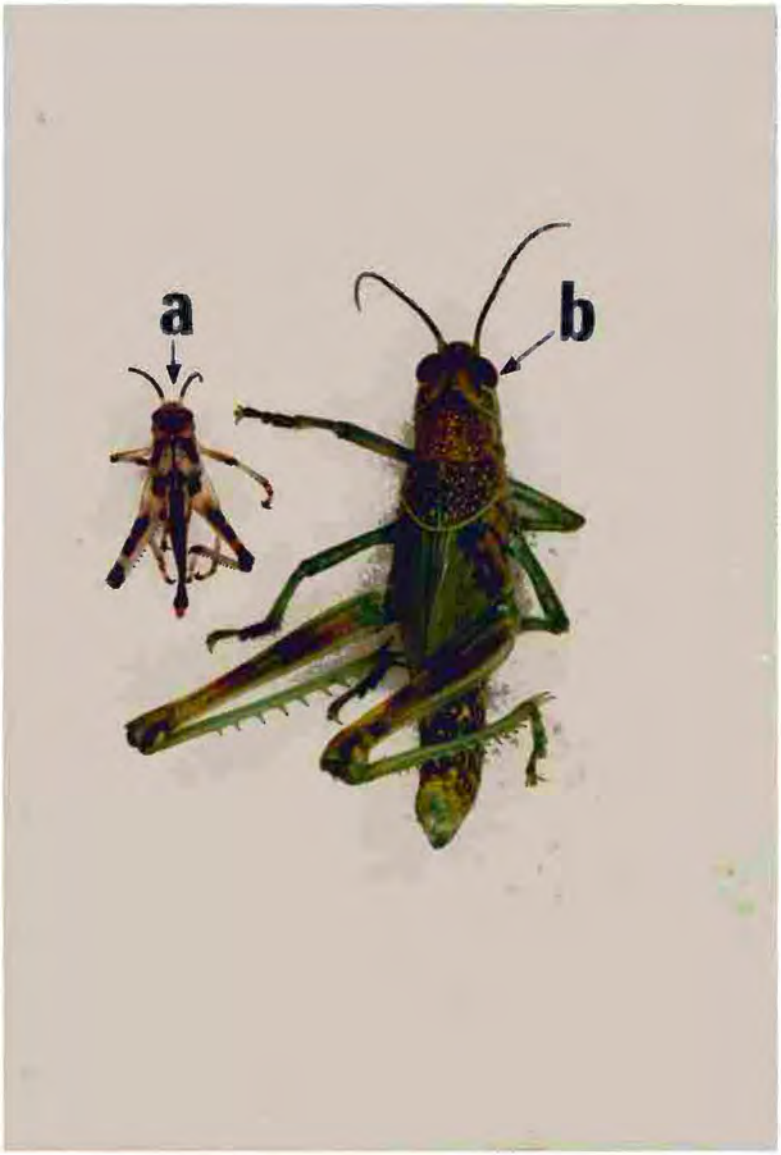
(ii) Colour

All the treated nymphs had a pinkish background with black patterns on their bodies (Plate 6b). four nymphs from the control treatment only showed some degree of colour change (melanization) and the rest were green (Table 9). Melanization in the exposed nymphs was significantly

Table 9. Effect of gregarious third to fifth and fifth instar nymphal and faecal volatiles on solitarious first instar nymphs

Trts	No of insects tested	Insect stage after 4 weeks of exposure	% Melanized after 4 weeks of exposure	% Mortality after 4 weeks of exposure
Cntl	14	3^{rd}, 4^{th}, 5^{th}	29%	36%
Test	16	2^{nd}, 3^{rd}	100%	88%

Plate 6. Effect of gregarious third to fifth and fifth instar nymphal volatiles on solitarious first instar nymphs four weeks after treatment.
a= Exposed and b= Unexposed control.


a
b

dependent on the volatile pheromones released by gregarious nymphs ($P<0.05$).

4.4.3. Effect of gregarious fifth instar volatiles on solitarious second instars

(i) Haemolymph pigment composition

Fifth instar nymphs which emerged from solitarious second instar nymphs exposed to volatiles emanating from gregarious fifth instar nymphs and their faeces were not significantly different from their control (unexposed) counterparts ($P> 0.05$) (Fig. 46).

(ii) Developmental time

The mean developmental times at any given instar stage of the exposed and those of the unexposed solitarious second instar nymphs were not significantly different ($P> 0.05$) (Fig. 47).

(iii) Weight

The mean weights of nymphs resulting from exposure of solitarious second instar nymphs at any given instar stage

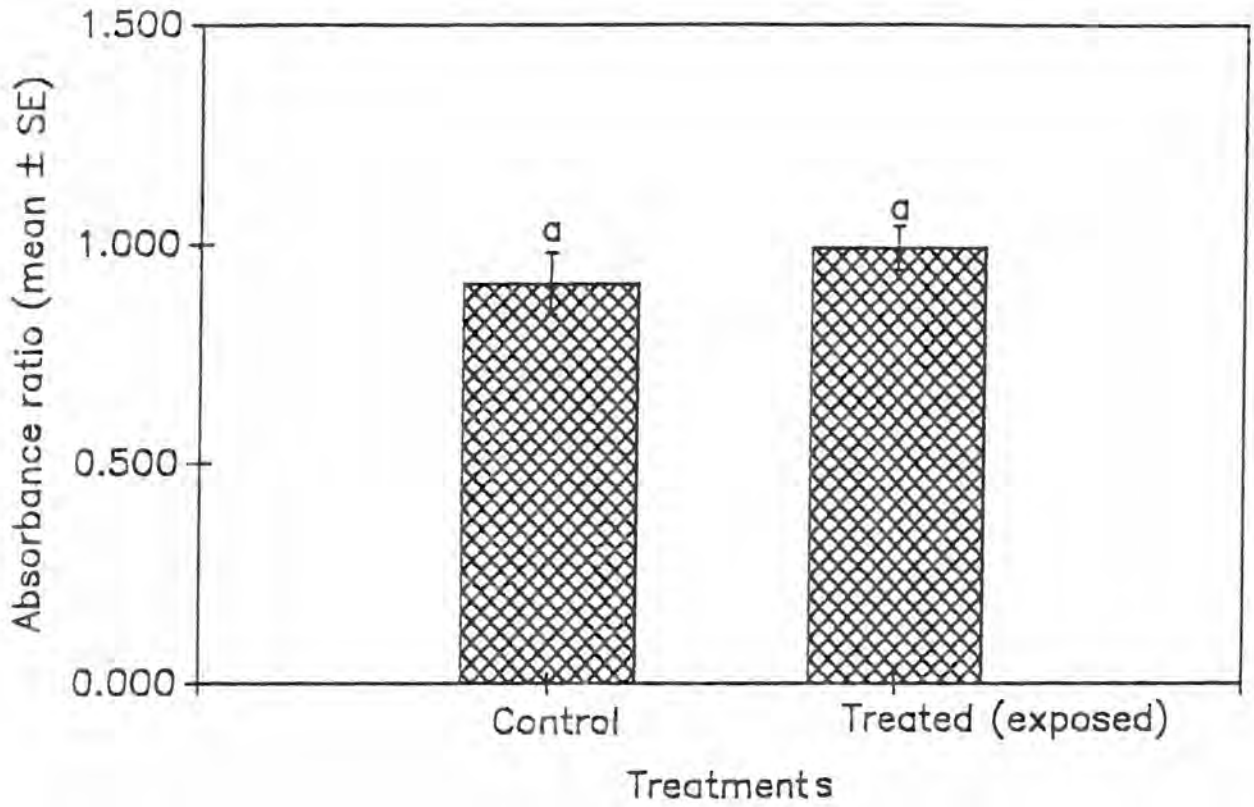


Fig. 46 Haemolymph pigment changes in *S. gregaria* solitarious second instar nymphs exposed to gregarious fifth instar nymphal volatiles.

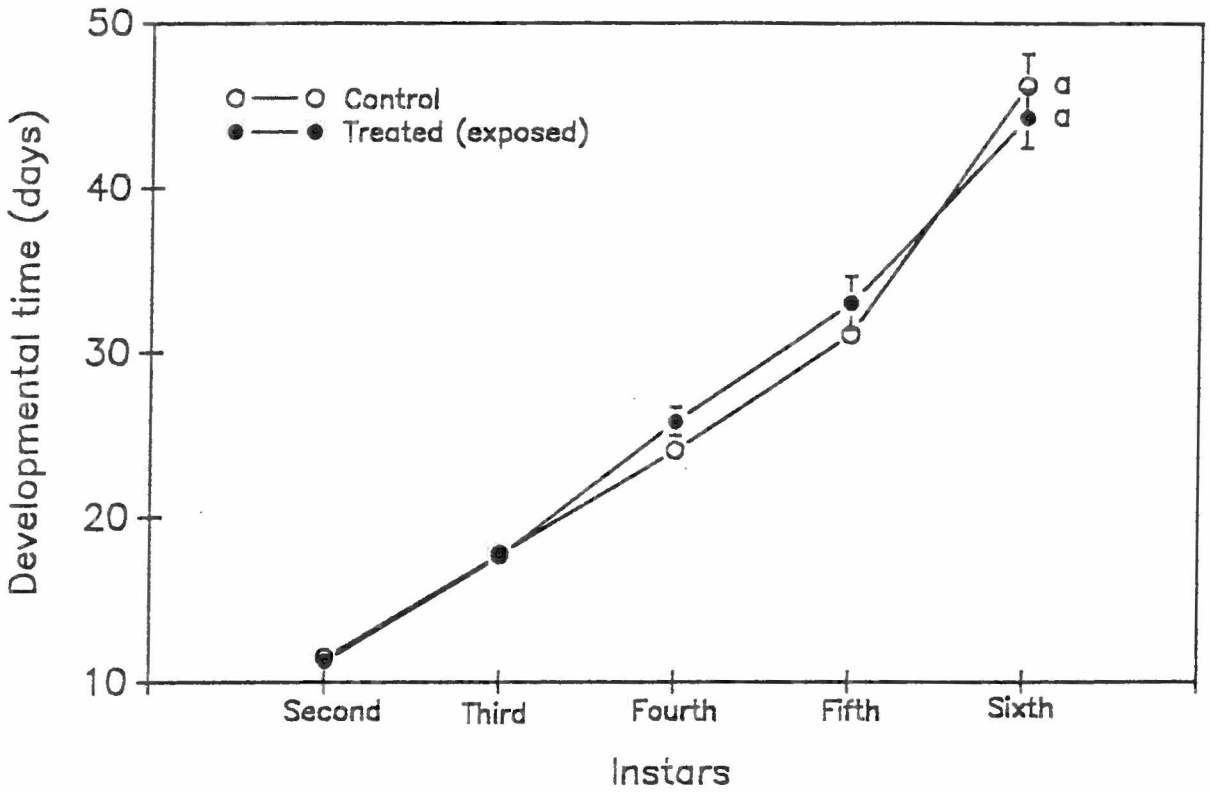


Fig. 47 Developmental time in *S. gregaria* solitarious second instar nymphs exposed to gregarious fifth instar nymphal volatiles.

were not significantly different from those of their control counterparts ($P< 0.05$) (Fig. 48).

(iv) Colour

Exposed last instar nymphs had a green background colour similar to their unexposed counterparts. In addition, about 20% of them developed an extensive black patterns on their bodies compared to controls (Plate 7).

4.4.4. Effect of gregarious fifth instar volatiles on solitarious fledglings

(i) Phenylacetonitrile titre

Both the control (unexposed) and treated (exposed) fledglings failed to produce phenylacetonitrile at the mature adult stage (Fig. 49).

(ii) Haemolymph pigment composition

Mature adult locusts resulting from fledglings which were exposed to gregarious fifth instar nymphal and faecal volatiles recorded higher absorbance ratios for their haemolymph pigments compared to controls, although the overall means of the ratios were not significantly different

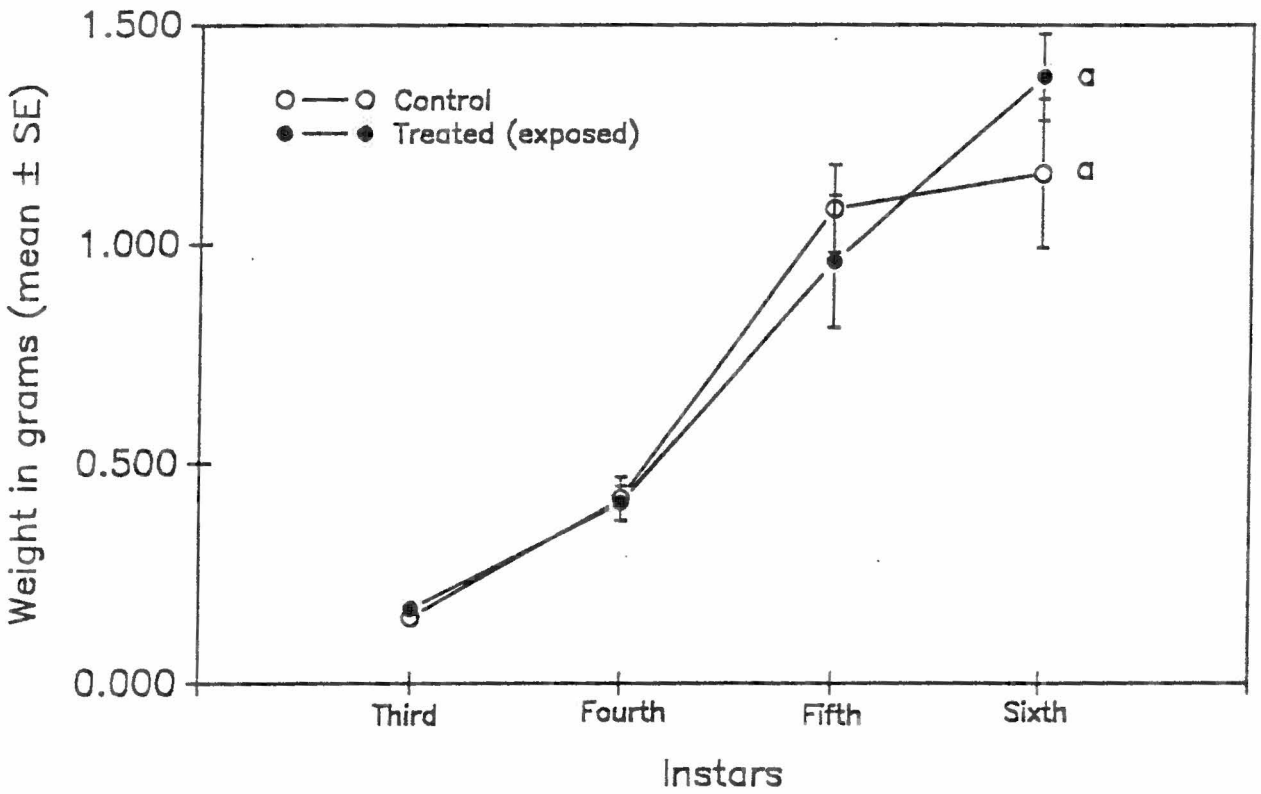
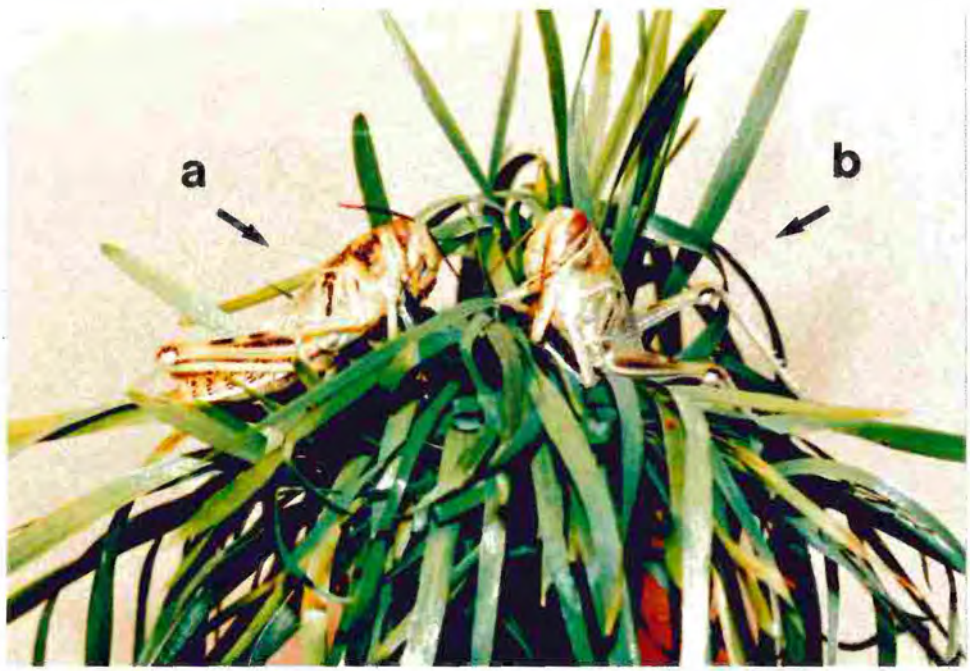


Fig. 48 Weight of nymphal instars of *S. gregaria* emerged from solitarious second instar nymphs exposed to gregarious fifth instar nymphal volatiles.

Plate 7. Effect of gregarious third to fifth instar nymphal volatiles on solitarious second instar nymphs four weeks after treatment.
a= Exposed and b= Unexposed control.


a
b

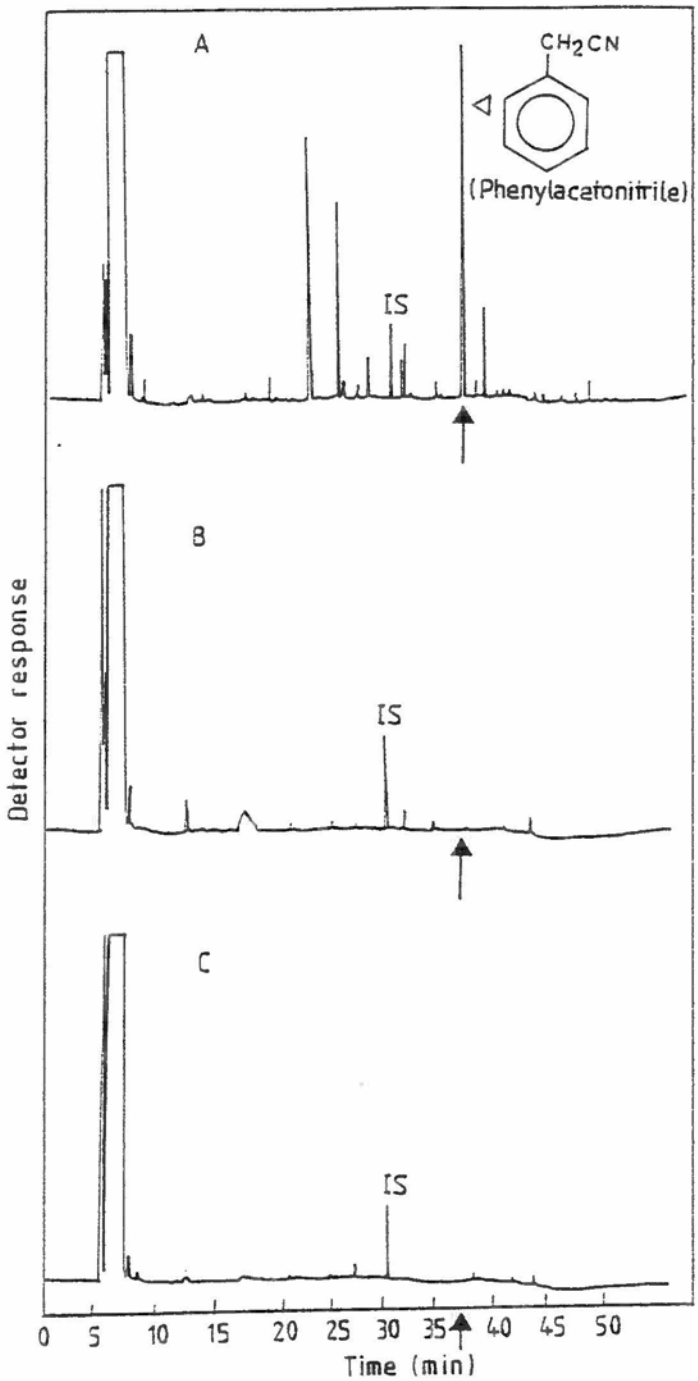


Fig. 49 Chromatograms of the air-borne volatiles collected from 20-22 day old adult males of *S. gregaria* (A) gregarious control (B) solitarious exposed and (C) solitarious unexposed injected into a 50m Carbowax 20M capillary column. IS= Internal standard.

($P > 0.05$). Significant differences emerged between the treated and controls after 40 days of exposure (Fig. 50).

(iii) Colour

Solitarious fledglings which were exposed to gregarious fifth instar nymphal and faecal volatiles were grey similar to the unexposed control individuals.

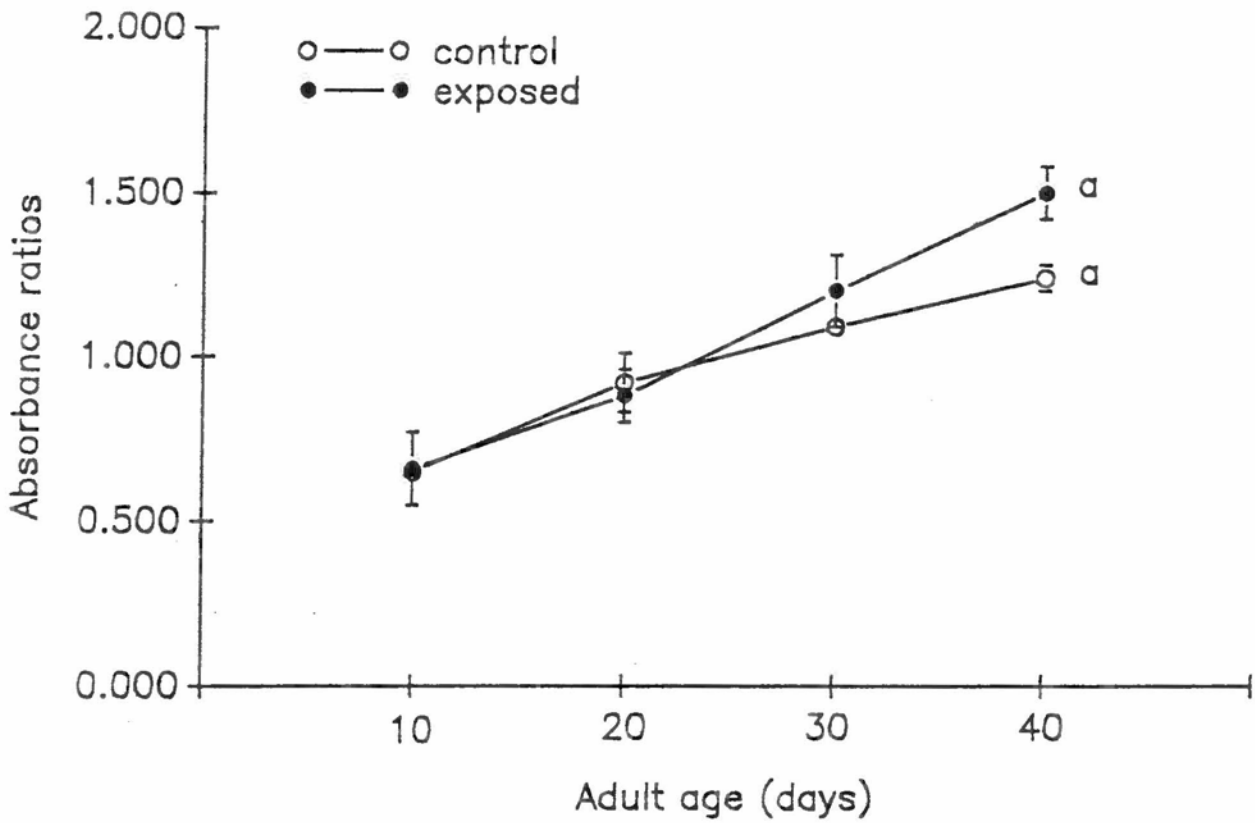


Fig. 50 Haemolymph pigment changes in *S. gregaria* solitarious fledglings exposed to fifth instar nymphal volatiles.

5. DISCUSSION

5.1. Uncrowding and Crowding Effects

The present study has shown that changes in adult pheromone emission, colour and nymphal haemolymph pigment composition in the desert locust occur rapidly in response to crowding and uncrowding. Thus, whereas on the basis of colour and pheromone emission, locusts appeared to have fully responded to density changes within the F_0 generation irrespective of the stage at which crowding or uncrowding took place (Figs. 6, 7, 9, 10, 24, 25, 27 and 28; Tables 2, 3, 4, 5, 6, 7 and 8). Similar to colour and pheromone emission, haemolymph pigment composition in nymphs changed rapidly within F_0 generation (Figs. 15 and 29). In the adults changes in haemolymph pigment composition were slow (Figs. 15, 17, 29, 31 and 39). On the other hand, morphometrics (F/C and E/F) revealed that no significant change occurred at this stage (Figs. 18, 20, 22, 23, 32, 34, 36, 37, 40 and 41). These findings may explain Kennedy's (1939) encounter of swarming locust individuals having typical *solitaria* morphometrics.

Adults which emerged in F_0 and F_1 generations of crowding solitary-reared hoppers registered a relatively high phenylacetonitrile titre compared to subsequent F_2 and F_3 generations of insects of the same origin and to gregarious controls (Fig. 25). It is possible that the

prolonged isolation of locusts for several generations (about 20 generations at the onset of this study), followed suddenly by crowding may have enhanced the propensity of these locusts to gregarise as compared to those that were reared crowded continuously for several generations in the control. These results parallel those of Michel (1980) who found relatively higher flight activity and migratory aptitude for a colony of crowded locusts originating from solitarious individuals which had been subjected to prolonged isolation for several generations. On the other hand, prolonged grouping over successive generations diminished the flight activity of crowded locusts. Whether these and results of the present study represent a general pattern of expression of phase characteristics remains to be established from more detailed studies on the relation between phase history and specific phase characters.

Extreme sensitivity to density changes is illustrated by two experiments in the study. First, uncrowding mature adults resulted in a rapid and significant drop in phenylacetonitrile titre within four days, and was not detected by the 12th day (Fig. 10). On the other hand, when solitarious adults were crowded they started to produce phenylacetonitrile within 2 days which reached levels comparable to those of gregarious controls four days later (Fig. 28). Second, analysis of volatiles from adult males resulting from rearing solitarious insects at two or four per cage (used for rearing

solitarious insects) showed that pheromone production is induced in both sets of insects albeit at a lower level in the former and follows some delay in both cases relative to that in gregarious controls (Fig. 38). Thus, it appears that even two insects confined together provide each other with the essential stimuli for inducing the onset of phase change. The nature of these stimuli now need to be elucidated through careful series of experiments which eliminate each of the possible candidates (tactile, visual and odour of solitarious insects) one at a time. Pheromone titres could provide a very sensitive means of monitoring the phase change in these experiments.

The rapid appearance of the adult pheromone on crowding is consistent with its releaser and primer role in that stage of the insect in its gregarious phase. Previous studies have shown that the pheromone plays a dominant role in the cohesive behaviour of gregarious adults (Obeng-Ofori *et al.*, 1993; Torto *et al.*, 1994) and is also involved in accelerating and synchronizing maturation in young adults of both sexes (Mahamat *et al.*, 1993). In view of the importance of these attributes to the gregarious phase locusts, the gregarisation process would be expected to be associated with early production of the signal. Conversely, uncrowding and resultant solitarisation of the insect is accompanied by rapid loss of the signal which now has no role.

These results also parallel those of Roessingh and

Simpson (1994) who found rapid behavioural gregarisation or solitarisation of locust nymphs on crowding and uncrowding respectively. The nymphal pheromone blend which has been characterized recently (Torto unpublished data), would allow studies on density effects on the emission of this pheromone. Furthermore, these studies could allow direct comparison with behavioural data, which may provide further insight on the sequence of manifestation of gregarious characters and their relationship at early stages of crowding of nymphal stages.

The pattern of the changes in locust body colour observed when locusts at different developmental stages were either crowded or uncrowded appeared to parallel the emission of pheromone (Tables 2, 3, 4, 5, 6, 7 and 8). Crowd-reared locusts responded to treatment effects giving different shades of green, brown, grey or dull yellow in the nymphal and adult stages depending upon the stage of the insect solitarised (Tables 2, 3 and 4). Interestingly, brightly yellow coloured adults emerged in F_0 generation of crowding where pheromone emission was relatively high (Tables 5, 6 and 7). The intensity of the yellow colour decreased in subsequent generations and appeared to parallel pheromone emission previously described above. Qualitative analysis of yellow colour of adult males resulting from rearing solitarious insects at two or four per cage showed that there was a delay in the appearance of the yellow colour and that the intensity

increased as the density increased particularly at lower densities (Table 8). However, the results of the present study show that in all situations of crowding, pheromone emission in adult males occurs earlier than the appearance of yellow colour. These results further explain the observations of Kennedy (1939) of swarming locust with typical solitarious characteristics.

Analysis of the data on haemolymph pigment composition show that nymphal stages respond more rapidly to solitarisation or gregarisation than the same group of insects that developed to adults (Figs. 15 and 29). A complete phase shift in this regard was recorded in nymphs within the F_0 generation but, in the adult, solitarisation was evident by the end of the F_2 generation unlike crowded locusts which transformed by the end of F_3 generation (Figs. 15, 16, 29 and 30). The results clearly show that the changes in the haemolymph pigment composition of adults were erratic (Figs. 15, 17, 29 and 31). It is possible that these changes may have been influenced by the transitional status of the young adult stage, particularly in the gregarious phase, where nymphal aggregation pheromone production shuts down to give way to the biosynthesis of the adult aggregation pheromone (Obeng-Ofori *et al.*, 1993 and Torto *et al.*, 1994).

Locusts emerging from crowding solitarious nymphs in groups of two or four per solitary rearing cage changed their haemolymph composition within the F0 generation

reflecting a relatively high sensitivity of this parameter to the treatment effects. The haemolymph pigment composition of individuals in the two groups was intermediate between solitarious and gregarious controls, reflecting, in this regard a transient status of these individual locusts (Fig. 39).

In this study, morphometric measurements changed rather slowly across generations compared to the other phase characters investigated (Figs. 18, 19, 32 and 33). F/C ratios were more sensitive to treatment effects than E/F ratios in both sexes, consistent with a previous study (Dirsh 1951, 1953). However, other authors have reported that the F/C ratio may be influenced by factors such as diet (Jackson *et al.*, 1978) and temperature (Stower *et al.*, 1960). Although, overall F/C ratios showed that solitarisation proceeds faster than gregarisation, it is clear that morphometrics constitutes a rather insensitive method of monitoring phase change associated with locust density and other factors, particularly in the early stages of phase transformation. Reliance on this method in the past severely limited the kind of information one could obtain in phase dynamics studies.

5.2. Primer Effects of Releaser Pheromones

The present study on the long term effects of releaser pheromones of nymphal stages of gregarious

locusts has shown that different nymphal stages of solitarious locusts respond differently to volatiles emanating from these nymphs and their faeces. Volatiles from live gregarious first instar nymphs appear to effect no significant change on integumental colour, pheromone emission and haemolymph pigment composition of solitarious locusts during development upto adult stage (Figs. 42 and 43), suggesting that a combination of pheromone with other signals such as vision and tactile might form a complimentary set for significant gregarisation to occur in solitarious nymphs. The present results on the confinement of two solitary locusts per cage where other signals are present supports this suggestion. However, subtractive bioassays involving the three types of signals will shed more light on the role played by each in the transformation process. Notably, the exposed solitarious nymphs registered a relatively high mean body weight compared to their unexposed control counterparts (Figs. 44 and 45) which is consistent with one of the features associated with gregarising/gregarious nymphs reported previously (Hunter-jones, 1958 and Injeyan and Tobe, 1981). The occurrence of a sixth instar stage is an important criteria in characterizing solitarious locusts (Injeyan and Tobe, 1981). In this experiment, both the exposed and control (unexposed) moulted to the sixth instar stage. Although the exposed nymphs moulted on the average a day or two earlier than their unexposed counterparts similar

to previous results (Gillett, 1975), there were no significant differences in their developmental times (Fig. 44). The relatively higher rate, quantity and utilization of ingested food material in gregarising/gregarious nymphs is for the purpose of muscle build up and accumulation of fuel in the form of lipids for subsequent marching or migratory flight when swarming as adults. Thus, it appears that in this experiment, the volatiles emanating from gregarious nymphs stimulated greater feeding in the exposed solitarious nymphs.

Unlike volatiles from first instar nymphs, the volatiles from third, fourth and fifth instar nymphs effected two noticeable changes in exposed solitarious nymphs. First, the typical solitarious green background colour faded to pale green or turned pink with striking black patterns on the body (Plate 6; Table 9), consistent with the observations made in similar experiments (Gillett, 1975). Second, exposed nymphs clearly showed severe delayed development followed by death in contrast to the results of (Gillett, 1975) who found accelerated development in exposed nymphs. The differences in current results and those of Gillett's (1975) merits a special comments in view of the fact that the stages of nymphs and the source of volatiles used in Gillett's experiments were not specified and also the fact that the mode of exposure of nymphs in the two sets of experiments were different.

Interestingly, solitarious second instar nymphs which were exposed to the total volatiles from third, fourth and fifth instar nymphs developed normally like their control counterparts (Figs. 46, 47 and 48; Plate 7).

A number of inferences could be made from these results. First, the two stages of the test solitarious nymphs (first and second instars) are physiologically different. Second, there are two primer pheromone systems in gregarious nymphal stages; a pheromone system associated with first instars and the other associated with later instars, in agreement with releaser pheromone results of behavioural assays conducted at the ICIPE (Obeng-Ofori, personal communication).

Extracts of the faeces of gregarious nymphs have been reported to induce certain phase traits in solitarious individuals (Nolte, *et al.*, 1973). Chiasma frequencies, adult morphometrics, colour of the integument and behaviour of the hoppers were among the characters notably altered by the extracts. Similarly, behaviour and colour of solitarious nymphs of the desert locust were found to be influenced by live gregarious nymphal and faecal volatiles (Gillett and Philip, 1977; and Gillett, 1983). Behaviourally, faecal volatiles have been shown to promote aggregation in nymphs and adults of gregarious phase Obeng-Ofori, *et al.* (1994), which is inconsistent with Gillett's, *et al.* (1976) results. Since both faecal and live insect volatiles were equally

important in altering some phase traits, it would be interesting to determine the relative importance of each in the changes associated with the exposure. Conducting experiments on possible effects of faecal volatiles alone on some phase traits of solitarious nymphs may assist in interpretation of the current results.

Solitarious fledglings exposed to volatiles emanating from gregarious fifth instar nymphs developed normally similar to solitarious controls. They did not produce phenylacetonitrile, neither was there any detectable change in haemolymph pigment composition (Figs. 49 and 50). These results further confirm the findings in the current study with regard to stage differentiation in response to effects of releaser pheromones. It appears that there could be a critical stage of exposure in the life cycle of solitarious individuals, where primer effects of the chemical factor could be sharply manifested in the behaviour, physiology and morphology of these insects.

In summation, the present study has shown that in addition to colour and morphometrics, phase change can be more precisely and quantitatively monitored by changes in pheromone emission and haemolymph pigment composition. A combination of the adult pheromone and nymphal haemolymph pigment composition constitute an early and sensitive measure of phase change in those stages of the insect and could provide an incisive probe for investigating the factors responsible for inducing phase

change and in understanding and modelling phase dynamics in natural habitats. The study also suggests that caution has to be exercised in interpreting data on phase transformation using only one phase character since full transformation of all phase characters take several generations (Pener, 1983 and 1991). The present study has also suggested that there is stage differentiation with regard to the primer effects of volatiles emanating from gregarious nymphs and their faeces on solitarious locusts.

5.3. Recommendations

From the present laboratory investigations, the following recommendations could be forwarded to acridiologists and crop protection specialists involved in the field surveys and locust control campaigns. This could assist them in determining the extent of transformation in any encountered foci of locust populations in the recession and invasion areas.

1. Phenylacetonitrile titre can be used in conjunction with locust colouration in determining the extent of transformation in gregarising groups of the field population of locusts. This is dependent on the presence of GC equipped laboratory in the vicinity.

2. Absorbance ratio of the haemolymph pigments together with colour can also be used to determine the extent of transformation in the field population of nymphs. This is also dependent on the presence of spectrophotometry equipped laboratory in the vicinity.

3. These recommendations are subject to use after validation of the current results in the field populations of locust.

4. After characterization and identification of the active components involved, pheromones from gregarious colonies of later nymphal instars should be explored in suppressing the development of the field population of solitarious first instar nymphs.

5.4. Suggestions for Future Study

The following lines for future research could be suggested based on results of the present study.

1. Investigation of the sensitivity potentials of the new phase markers (phenylacetonitrile titre and absorbance ratio of the haemolymph pigments) used in this study in the field populations of the desert locust and other related locust species.

2. To study the changes in haemolymph pigment ratio in

the early nymphal instars (second, third, and fourth) during uncrowding and crowding experiments.

3. To study the role of host plants (diet) on the transformation of these new phase parameters in the laboratory, semi-field and field conditions using both laboratory and field populations of the desert locust.

4. To screen the relative importance of each of the factors (vision, tactile, auditory, chemical and combination of them) in phase transformation using these new chemometric markers.

5. To study the biological significance of the green and brown solitarious hoppers in relation to phase change.

6. To test the effect of gregarious third, fourth and fifth instar nymphal volatiles on solitarious first and second instar nymphs in semi-field and field condition using both laboratory and field populations.

7. To test the effect of gregarious third, fourth and fifth instar nymphal volatiles on gregarious first and second instar nymphs in semi-field and field condition using both laboratory and field populations.

8. To study primer effects of live gregarious adult volatiles on solitarious nymphs and adults.

9. To investigate the primer effects of gregarious nymphal and adult faecal volatiles on solitarious nymphs and adults.

10. To study the primer effects of synthetic aggregation pheromone complex of gregarious desert locust on solitarious locusts.

6. SUMMARY AND CONCLUSIONS

6.1. Summary

1. Adult males which emerged from uncrowding gregarious nymphs, fledglings and mature adults failed to produce phenylacetonitrile indicating that these locusts have shifted into solitarious phase with regard to this measure within the F_0 generation.

2. Absorbance ratio of the haemolymph pigments of locusts emerged from uncrowding gregarious nymphs showed a significant shift toward solitarious ratios by the end of the F_2 generation in the adult, but a complete shift was recorded at nymphal stage in the F_0 generation.

3. Absorbance ratio of the haemolymph pigments has also shown that there was no sex differentiation within generations of both gregarious and solitarious locusts. However, there was stage and age differentiation within and between generations of both gregarious and solitarious locusts.

4. The mean F/C ratios of adult females which emerged from uncrowding gregarious nymphs changed toward solitarious

ratios by the end of the F_2 generation, male ratios remained intermediate by the same generation. However, the mean F/C ratios for adults which emerged from uncrowding gregarious fledglings remained unchanged within the F_0 generation.

5. The mean E/F ratios of the same group of locusts which emerged from uncrowding gregarious nymphs and fledglings generally did not show a definite pattern in most cases.

6. Nymphs and adults emerged from uncrowding gregarious nymphs were either green or brown at last nymphal stage and grey in adult stage similar to solitarious nymphs and adults, respectively. Similarly, adult males emerged from uncrowding gregarious fledglings and mature adults turned either grey or dull yellow, respectively, indicating a significant shift with regard to colour within the F_0 generation.

7. Adult males emerged from crowding solitarious nymphs, fledglings and mature adults emitted phenylacetonitrile indicating that these locusts had shifted into gregarious phase with regard pheromone emission within the F_0 generation.

8. Absorbance ratio of the haemolymph pigments of locusts which emerged from crowding solitarious nymphs showed a significant shift toward the gregarious ratio by the end of the F_3 generation, but a complete change was recorded at nymphal stage within the F_0 generation.

9. The mean F/C ratios of adult males and females which emerged from crowding solitarious nymphs remained intermediate even by the end of the F_3 generation. However, the mean F/C ratios for adults which emerged from crowding solitarious fledglings remained unchanged in the F_0 generation.

10. The mean E/F ratios of the same group of locusts showed an inconsistent pattern.

11. Nymphs and adults which emerged from crowding solitarious nymphs were yellow with black pattern at last nymphal stage, pink at fledgling stage and bright yellow at mature adult stage similar to gregarious nymphs, fledgling and adults, respectively. Similarly, adult males which emerged from crowding solitarious fledglings and mature adults turned bright yellow indicating a complete shift with regard to colour within the F_0 generation.

12. Adult males which emerged from crowding solitarious nymphs in groups of two or four per cage produced phenylacetonitrile within the F_0 generation. Both treatments registered a delayed release of the compound compared to that of their gregarious counterparts. The level of the titres were comparable to those of gregarious control in the latter and much lower in the former.

13. Absorbance ratio of the haemolymph pigment composition of locusts of all stages which emerged from crowding solitarious nymphs in groups of two or four per cage were intermediate between those of gregarious and solitarious within the F_0 generation.

14. The mean F/C ratios of adult females which emerged from crowding solitarious nymphs in groups of four per cage were intermediate within the F_0 generation, the mean ratio for two per cage remained unchanged similar to that of solitarious within the same generation.

15. The mean E/F ratios for adults which emerged from crowding solitarious nymphs in groups of two or four per cage remained unchanged within the F_0 generation similar to that of solitarious.

16. Nymphs and adults which emerged from crowding solitarious nymphs in groups of two or four per cage were yellow with black pattern at the last nymphal stage, pinkish when fledglings and bright yellow at mature adult stage within the F_0 generation, particularly in the latter treatment similar to gregarious nymphs, fledglings and mature adults, respectively.

17. Volatiles emanating from live gregarious nymphs from first instar onwards and their faeces have no effect on colour, phenylacetonitrile release, haemolymph pigment composition, but slightly influenced the weight and developmental time of the exposed solitarious nymphs.

18. Volatiles emanating from live gregarious third, fourth and fifth instar nymphs and their faeces changed the background colour from green to pinkish, promoted melanization, delayed the development and caused death of the exposed solitarious first instar nymphs.

19. Volatiles emanating from live gregarious fifth instar nymphs and their faeces have no effect on weight and development, but slightly affected the colouration of the exposed solitarious second instar nymphs.

20. Volatiles emanating from live gregarious fifth instar nymphs and their faeces have no effect on colour, phenylacetonitrile release and haemolymph pigment composition of the exposed solitarious fledglings.

6.2. Conclusions

The following conclusions can be drawn from the above synopsis of the current research results.

1. Different phase characters change at different rates when in uncrowded and crowded situations and these are cumulative over several generations.

2. Pheromone (phenylacetonitrile) titres and colour changed concurrently and more rapidly than haemolymph pigment composition (absorbance ratio) and morphometrics and provide early indicators of phase change..

3. Phenylacetonitrile level is useful in indicating the phase status and assessing the degree of shift in adult males of the desert locust while absorbance ratio of the haemolymph pigment composition is more sensitive at nymphal stages.

4. Based on absorbance ratio of the haemolymph pigments and morphometrics, solitarisation proceed more rapidly than

gregarisation. However, the two processes seem to proceed more or less at a similar rate with regard to pheromone emission and colour.

5. Solitarisation and gregarisation are largely density driven processes.

6. Intraspecific chemical communication in the desert locust among others, appeared to play a considerable role in the process of locust phase polymorphism.

7. Gregarious nymphal stages possess two different sets of primer pheromones, one for first instars and the other for the later instars, which confirms previous behavioural assays.

8. Solitarious nymphs (first and second instars) are physiologically different in response to primer effects of the releaser pheromones of the gregarious phase.

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*Seen in abstract only.

8. APPENDICES

Appendix 1. Analysis of variance table for pheromone release by adult males of *S. gregaria* emerged from uncrowding gregarious mature adults for two weeks.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	79	39	90	0.0001
Error	54	24	0.44		
C. Total	56	103			

R. Square	C.V.	Root MSE	Transformed Mean
0.77	58	0.66	1.15

Appendix 2. Analysis of variance table of stage and age differentiation within a generation of gregarious *S. gregaria*.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	6	0.14	0.02	1.54	0.18
Error	63	0.95	0.02		
C. Total	69	1.09			

R. Square	C.V.	Root MSE	Transformed Mean
0.13	11.69	0.12	1.05

Appendix 3. Analysis of variance table of stage and age differentiation within a generation of solitarious *S. gregaria*

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	6	0.22	0.04	2.06	0.07
Error	63	1.13	0.02		
C. Total	69	1.35			

R. Square	C.V.	Root MSE	Transformed Mean
0.16	16.35	0.13	0.82

Appendix 4. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* emerged from uncrowding gregarious nymphs for three generations (F_0-F_2).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	7	1.52	0.22	26	0.0001
Treatment	4	1.40	0.35	43	0.0001
Age (Stage)	3	0.11	0.04	4.78	0.0028
Error	352	2.90	0.01		
C. Total	359	4.42			

R. Square	C.V.	Root MSE	Transformed Mean
0.34	7.71	0.09	1.18

Appendix 5. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* emerged from uncrowding gregarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	0.36	0.18	23	0.0001
Error	117	0.95	0.01		
C. Total	119	1.31			

R. Square	C.V.	Root MSE	Transformed Mean
0.28	7.65	0.09	1.18

Appendix 6. Analysis of variance table for F/C ratios of adult females of *S. gregaria* emerged from uncrowding gregarious nymphs for three generations (F_0-F_2).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	4	0.196	0.049	71.65	0.0001
Error	187	0.128	0.001		
C. Total	191	0.324			

R. Square	C.V.	Root MSE	Transformed Mean
0.61	1.66	0.03	1.58

Appendix 7. Analysis of variance table for E/F ratios of adult females of *S. gregaria* emerged from uncrowding gregarious nymphs for three generations (F_0-F_2).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	4	0.011	0.003	3.93	0.0043
Error	187	0.134	0.001		
C. Total	191	0.145			

R. Square	C.V.	Root MSE	Transformed Mean
0.08	2.31	0.03	1.16

Appendix 8. Analysis of variance table for F/C ratios of adult males of *S. gregaria* emerged from uncrowding gregarious nymphs for three generations (F_0-F_2).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	4	0.24	0.06	86.86	0.0001
Error	191	0.13	0.001		
C. Total	195	0.38			

R. Square	C.V.	Root MSE	Transformed Mean
0.65	1.68	0.03	1.57

Appendix 9. Analysis of variance table for E/F ratios of adult males of *S. gregaria* emerged from uncrowding gregarious nymphs for three generations (F_0-F_2).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	4	0.014	0.004	6.64	0.0001
Error	191	0.102	0.001		
C. Total	195	0.116			

R. Square	C.V.	Root MSE	Transformed Mean
0.12	2.03	0.02	1.14

Appendix 10. Analysis of variance table for sex differentiation with regard to F/C ratio in adults of gregarious *S. gregaria*.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Sex	1	0.0029	0.0029	7.62	0.0067
Error	118	0.0448	0.0004		
C. Total	119	0.0477			

R. Square	C.V.	Root MSE	Transformed Mean
0.06	1.27	0.02	1.53

Appendix 11. Analysis of variance table for sex differentiation with regard to E/F ratio in adults of gregarious *S. gregaria*.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Sex	1	0.0066	0.0066	26.75	0.0001
Error	118	0.0291	0.0002		
C. Total	119	0.0357			

R. Square	C.V.	Root MSE	Transformed Mean
0.18	1.35	0.02	1.16

Appendix 12. Analysis of variance table for sex differentiation with regard to F/C ratio in adults of solitarious *S. gregaria*.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Sex	1	0.0006	0.0006	0.57	0.4507
Error	158	0.1561	0.001		
C. Total	159	0.1566			

R. Square	C.V.	Root MSE	Transformed Mean
0.004	1.95	0.03	1.14

Appendix 13. Analysis of variance table for sex differentiation with regard to E/F ratio in adults of solitarious *S. gregaria*.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Sex	1	0.0164	0.0164	15.70	0.0001
Error	158	0.1652	0.001		
C. Total	159	0.1816			

R. Square	C.V.	Root MSE	Transformed Mean
0.09	2.83	0.03	1.14

Appendix 14. Analysis of variance table for F/C ratios of adult females of *S. gregaria* emerged from uncrowding gregarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.00001	0.00001	0.03	0.8710
Error	34	0.01891	0.00056		
C. Total	35	0.01892			

R. Square	C.V.	Root MSE	Transformed Mean
0.0008	1.55	0.02	1.52

Appendix 15. Analysis of variance table for E/F ratios of adult females of *S. gregaria* emerged from uncrowding gregarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0027	0.0027	5.10	0.0305
Error	34	0.0179	0.0005		
C. Total	35	0.0205			

R. Square	C.V.	Root MSE	Transformed Mean
0.13	1.95	0.02	1.18

Appendix 16. Analysis of variance table for F/C ratios of adult males of *S. gregaria* emerged from uncrowding gregarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0001	0.0001	0.23	0.6320
Error	34	0.0150	0.0004		
C. Total	35	0.0151			

R. Square	C.V.	Root MSE	Transformed Mean
0.01	1.39	0.02	1.51

Appendix 17. Analysis of variance table for E/F ratios of adult males of *S. gregaria* emerged from uncrowding gregarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0007	0.0007	1.24	0.2728
Error	34	0.0180	0.0005		
C. Total	35	0.0186			

R. Square	C.V.	Root MSE	Transformed Mean
0.04	1.99	0.02	1.16

Appendix 18. Analysis of variance table for pheromone release by adult males of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	5	84	17	14	0.0001
Error	180	211	1		
C. Total	185	295			

R. Square	C.V.	Root MSE	Transformed Mean
0.28	73.20	1.08	1.48

Appendix 19. Analysis of variance table for pheromone release by adult males of *S. gregaria* emerged from crowding solitarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	52	26	32	0.0001
Error	67	54	0.8		
C. Total	69	106			

R. Square	C.V.	Root MSE	Transformed Mean
0.49	76	0.90	1.18

Appendix 20. Analysis of variance table for pheromone release by adult males of *S. gregaria* emerged from crowding solitarious mature adults for two weeks.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	109	55	59	0.0001
Error	60	55	0.92		
C. Total	62	164			

R. Square	C.V.	Root MSE	Transformed Mean
0.66	52	0.96	1.86

Appendix 21. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	8	1.18	0.15	29	0.0001
Treatment	5	1.04	0.21	41	0.0001
Age (Stage)	3	0.14	0.05	8.96	0.0001
Error	393	1.98	0.01		
C. Total	401	3.16			

R. Square	C.V.	Root MSE	Transformed Mean
0.37	5.96	0.07	1.19

Appendix 22. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* emerged from crowding solitarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	0.47	0.23	31	0.0001
Error	117	0.88	0.01		
C. Total	119	1.35			

R. Square	C.V.	Root MSE	Transformed Mean
0.35	7.62	0.09	1.14

Appendix 23. Analysis of variance table for F/C ratios of adult females of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	5	0.193	0.039	50.18	0.0001
Error	214	0.164	0.001		
C. Total	219	0.357			

R. Square	C.V.	Root MSE	Transformed Mean
0.54	1.76	0.03	1.58

Appendix 24. Analysis of variance table for E/F ratios of adult females of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	5	0.011	0.002	2.44	0.0357
Error	214	0.187	0.001		
C. Total	219	0.198			

R. Square	C.V.	Root MSE	Transformed Mean
0.05	2.56	0.03	1.16

Appendix 25. Analysis of variance table for F/C ratios of adult males of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	5	0.231	0.046	53.11	0.0001
Error	214	0.187	0.001		
C. Total	219	0.418			

R. Square	C.V.	Root MSE	Transformed Mean
0.55	1.88	0.03	1.57

Appendix 26. Analysis of variance table for E/F ratios of adult males of *S. gregaria* emerged from crowding solitarious nymphs for four generations (F_0-F_3).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	5	0.031	0.006	9.15	0.0001
Error	214	0.146	0.001		
C. Total	219	0.178			

R. Square	C.V.	Root MSE	Transformed Mean
0.18	2.30	0.03	1.14

Appendix 27. Analysis of variance table for F/C ratios of adult females of *S. gregaria* emerged from crowding solitarious fledglings for generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0006	0.0006	1.07	0.3113
Error	23	0.0131	0.0006		
C. Total	24	0.0137			

R. Square	C.V.	Root MSE	Transformed Mean
0.04	1.48	0.02	1.61

Appendix 28. Analysis of variance table for E/F ratios of adult females of *S. gregaria* emerged from crowding solitarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.002	0.002	2.96	0.0985
Error	23	0.016	0.001		
C. Total	24	0.018			

R. Square	C.V.	Root MSE	Transformed Mean
0.11	2.25	0.03	1.16

Appendix 29. Analysis of variance table for F/C ratios of adult males of *S. gregaria* emerged from crowding solitarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0002	0.0002	0.27	0.6099
Error	30	0.0229	0.0007		
C. Total	31	0.0231			

R. Square	C.V.	Root MSE	Transformed Mean
0.01	1.74	0.03	1.59

Appendix 30. Analysis of variance table for E/F ratios of adult males of *S. gregaria* emerged from crowding solitarious fledglings for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0026	0.0026	3.38	0.0758
Error	30	0.0232	0.0008		
C. Total	31	0.0258			

R. Square	C.V.	Root MSE	Transformed Mean
0.10	2.42	0.03	1.15

Appendix 31. Analysis of variance table for pheromone release by adult males of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	94	31	30	0.0001
Error	137	144	1.05		
C. Total	140	238			

R. Square	C.V.	Root MSE	Transformed Mean
0.40	79	1.02	1.30

Appendix 32. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	0.63	0.21	33	0.0001
Error	236	1.49	0.01		
C. Total	239	2.12			

R. Square	C.V.	Root MSE	Transformed Mean
0.30	6.66	0.08	1.19

Appendix 33. Analysis of variance table for F/C ratios of adult females of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	0.0851	0.0284	49.33	0.0001
Error	83	0.0477	0.0006		
C. Total	86	0.1328			

R. Square	C.V.	Root MSE	Transformed Mean
0.64	1.54	0.02	1.56

Appendix 34. Analysis of variance table for E/F ratios of adult females of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	0.0275	0.0092	22.29	0.0001
Error	83	0.0341	0.0004		
C. Total	86	0.0616			

R. Square	C.V.	Root MSE	Transformed Mean
0.45	1.75	0.02	1.16

Appendix 35. Analysis of variance table for F/C ratios of adult males of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	0.1009	0.0336	78.65	0.0001
Error	90	0.0385	0.0004		
C. Total	93	0.1394			

R. Square	C.V.	Root MSE	Transformed Mean
0.72	1.33	0.02	1.55

Appendix 36. Analysis of variance table for E/F ratios of adult males of *S. gregaria* emerged from crowding solitarious nymphs in groups of two or four per cage for one generation (F_0).

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	3	0.0443	0.0148	34.07	0.0001
Error	90	0.0391	0.0004		
C. Total	93	0.0834			

R. Square	C.V.	Root MSE	Transformed Mean
0.53	1.83	0.02	1.14

Appendix 37. Analysis of variance table for adult absorbance ratio of the haemolymph pigments of *S. gregaria* solitarious first instar nymphs exposed and unexposed to gregarious first instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.006	0.006	0.42	0.5325
Error	9	0.132	0.015		
C. Total	10	0.14			

R. Square	C.V.	Root MSE	Mean
0.04	11.03	0.12	1.10

Appendix 38. Analysis of variance table for nymphal absorbance ratio of the haemolymph pigments of *S. gregaria* solitarious first instar nymphs exposed and unexposed to gregarious first instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.07	0.07	1.59	0.2425
Error	8	0.36	0.05		
C. Total	9	0.44			

R. Square	C.V.	Root MSE	Mean
0.17	26.06	0.21	0.82

Appendix 39. Analysis of variance table for developmental times of *S. gregaria* solitarious first instar nymphs exposed and unexposed to gregarious first instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.006	0.006	0.22	0.6361
Error	125	3.37	0.027		
C. Total	126	3.37			

R. Square	C.V.	Root MSE	Transformed Mean
0.002	8.99	0.16	1.83

Appendix 40. Analysis of variance table for adult weights of *S. gregaria* solitarious first instar nymphs exposed and unexposed to gregarious first instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.02	0.02	3.17	0.0901
Error	20	0.10	0.01		
C. Total	21	0.12			

R. Square	C.V.	Root MSE	Transformed Mean
0.14	5.46	0.07	1.30

Appendix 41. Analysis of variance table for nymphal weights of *S. gregaria* solitarious first instar nymphs exposed and unexposed to gregarious first instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.02	0.02	1.06	0.3108
Error	38	0.71	0.02		
C. Total	39	0.72			

R. Square	C.V.	Root MSE	Mean
0.03	14.48	0.14	0.94

Appendix 42. Analysis of variance table for nymphal absorbance ratio of the haemolymph pigments of *S. gregaria* solitarious second instar nymphs exposed and unexposed to gregarious fifth instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.014	0.014	0.74	0.4158
Error	8	0.15	0.02		
C. Total	9	0.17			

R. Square	C.V.	Root MSE	Mean
0.08	14.66	0.02	0.95

Appendix 43. Analysis of variance table for developmental times of *S. gregaria* solitarious second instar nymphs exposed and unexposed to gregarious fifth instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.00002	0.00002	0.00	0.9716
Error	104	1.34285	0.01291		
C. Total	105	1.34287			

R. Square	C.V.	Root MSE	Transformed Mean
0.00001	6.03	0.11	1.88

Appendix 44. Analysis of variance table for nymphal weights of *S. gregaria* solitarious second instar nymphs exposed and unexposed to gregarious fifth instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	1	0.0005	0.0005	0.02	0.8855
Error	38	0.8206	0.0216		
C. Total	39	0.82			

R. Square	C.V.	Root MSE	Mean
0.0005	14.85	0.15	0.99

Appendix 45. Analysis of variance table for absorbance ratio of the haemolymph pigments of *S. gregaria* solitarious fledglings exposed and unexposed to gregarious fifth instar nymphal volatiles.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Treatment	2	0.06	0.06	0.57	0.4565
Error	38	4.35	0.11		
C. Total	39	4.42			

R. Square	C.V.	Root MSE	Mean
0.01	33.3	0.34	1.02

Appendix 46. Chi-square statistics table on the effect of gregarious third to fifth and fifth instar nymphal volatiles on solitarious first instar nymphs.

Frequency Percent Row Pct Col Pct	Melanization	Mortality	Total
Control	43.00	57.00	100.00
	09.05	12.00	021.05
	43.00	57.00	
	17.70	24.57	
Treated	200.00	175.00	375.00
	42.11	36.84	78.95
	53.33	46.67	
	82.30	75.43	
Total	243.00	232.00	475.00
	51.16	48.84	100.00

Chi-square Statistics

Statistic	Df	Value	Prob.
Chi-square	1	3.374	0.066

9. GLOSSARY OF SPECIAL TERMS

Absorption (of light): Complete retention, without reflection or transmission.

Absorption Spectra or Absorbance: A graph of light absorption versus wavelength of light which shows how much light is absorbed at each wavelength.

Hopper Band: A group of nymphs joined together to form a larger group which moves about.

Crowded (=gregarious or crowd-reared or phase *gregaria*): Existence of locusts in high density groups and the term is usually used for laboratory colonies in the context of the present study.

Elution: A process of washing out the adsorbed volatiles from the charcoal traps using a solvent.

Fledging: Final moult from last instar nymph to young adult.

Fledgling: A young adult locust emerging from final moult.

Gregaria **(gregarious or crowded or crowd-reared or):** Existence of locusts as numerous groups throughout invasion area and the term is usually used for field populations.

Gregarisation: A process involving change in certain characters (phase characters) of individual locusts from solitarious to gregarious phase.

Hatchlings: First instar nymphs emerging from eggs.

Immature Adult: Sexually immature fledgling which became gradually hard and able to fly strongly.

Isolated (= solitarious or solitary or solitary-reared or *solitaria*): Existence of locust as scattered individuals and the term is usually used for laboratory colonies in the context of the present study.

Mature Adult: Sexually mature adult which are able to mate and oviposit.

Morphometrics: Pertaining to measurement of parts of an insect.

Phase -dynamics or -transformation or -polymorphism: A process of change in certain characters (phase characters) of a locust from one form to another and vice versa.

Phase Characters: Traits which are used to distinguish the scattered individuals (solitarious) from those in crowded (gregarious) situation and these includes colour, behaviour, morphometics, physiology, phenylacetonitrile titres and absorbance ratio of the haemolymph pigment composition.

Phenylacetonitrile (= benzyl cyanide): A major component of adult desert locust aggregation pheromone system which constitutes 70-80% of its air-borne volatiles.

Pheromone: Chemical involved in intraspecific communication between living organisms.

Polyphagous: An organism which feeds on a wide range of host plants.

Primer Effects: Long term physiological changes in the recipient organism mediated by a chemical factor (primer pheromone) released by other individuals of its own kind.

Releaser Effects: Behavioural changes in the recipient organism mediated by a chemical factor (releaser pheromone) released by other individuals of its own kind.

Semiochemicals: Chemicals involved in the interaction between living organisms.

***Solitaria* (solitarious or isolated or solitary or solitary-reared):** Existence of a locust as scattered individuals in a recession area and the term is usually used for field populations.

Solitarisation: A process involving change in certain characters (phase characters) of individual locusts from gregarious to solitarious phase.

Swarms: A mobile large group of adult locusts flying from one region to another.

***Transient*:** Locust individuals showing phase characters which are intermediate between those of *gregaria* and *solitaria*.