ROLE OF SOME FACTORS IN PHASE CHANGE IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSKAL) (ORTHOPTERA: ACRIDIDAE)

BY

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DECLARATION

I hereby declared that the work presented in this thesis is the result of my own investigation during the three years research undertaken under the supervision at the ICIPE, Nairobi, Kenya and that it has not been submitted elsewhere for any degree.

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DEDICATION

To my father Garang Malual,

mother Ayot Piok,

and my wife Achol Atem

whose love, care

and encouragement

have help me strive to this level.

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ABSTRACT

The effect of food distribution on the degree of gregarization of solitarious desert locust, *Schistocerca gregaria* (Forskal), has been studied using the solitarious nymphal densities of 4, 6, 8, 10 and 12 per (100 x 100 x 50cm) cage. Nymph were reared on localized and delocalized wheat and kale plants. The change was monitored through parameters, comprising of adult aggregation pheromone titres measured as phenylacetonitrile, haemolymph pigment composition measured as absorbance ratio at 460 and 680nm, integumental colour and morphometrics. The results showed that locusts at low densities of 4 and 6 in both treatments, and 8 in delocalized treatment, failed to gregarize and, were similar to solitary-reared adults. Locusts from solitarious nymphs at density of 8 in localized treatment, and at densities of 10 and 12 locusts / cage in both localized and delocalized food, promoted gregarization by becoming yellow and produced pheromone levels which were intermediate between the controls (gregarious and solitarious). However, for the same locust density, gregarization proceeded much further for locusts on the localized than delocalized treatments. The distribution of host-plants is an important factor initiating locust gregarization by concentrating the solitarious locusts. On the other hand, absorbance and morphometric ratios were not different from solitarious control, but differed significantly from the gregarious control. These characters change were slowly in response to density.

Comparison of the effect of plant type on the rate of gregarization was

studied by rearing solitarious locusts on two different plants, wheat and kale separately. The results showed that for the same locusts density and same food distribution treatment, gregarization was higher in wheat than in kale. Possibly reason for this difference is that the nature of food affects level of contact between the solitarious insects. The experimental insects were initially reared on wheat for many generations and those on kale only one generation, therefore no definite conclusion can be drawn. The two sets of insects would need to be reared on the respective plants for the same number of generations, then compared.

The rate of solitarization of isolated gregarious nymphs and adults, with and without exposure to pheromone emissions of conspecifics, was investigated in the absence of visual and contact cues. The rate of solitarization was monitored using pheromone titres (the levels of nonanal plus nonanoic acid in nymphs and phenyacetonitrile in adults volatile emissions), behaviour, body colour changes and weights. The presence of pheromone slowed down the process of solitarization in isolated recipients (nymphs and adults), and was dependent on the quality and dose of the stimulus. Adults exposed to their own volatile emissions solitarized more slowly than when exposed to nymphal volatiles. In contrast, solitarization in nymphs appeared to be independent of the quality of the volatile signal. In both stages, increasing pheromone emission dose, provided by a higher locust source density, decreased the solitarization process in recipients, although the effect was stronger in adults than in nymphs. Continued physiologically gregarious status (because of pheromone emissions) was reflected

in the retention of gregarious behaviour, colour and higher body weights of recipients, compared to non-recipients of pheromones. Those pheromone emissions alone are only effective in slowing down the process of solitarization, but not effective in sustaining gregarization in isolated gregarious locusts, and the volatile pheromones are not responsible for inducing gregarization of solitarious locusts. This accounts for previous observations that isolated gregarious insects kept in the same room with crowded insects retained somewhat gregarious character.

The effect of egg and sand-associated oviposition pheromones, secreted by gregarious females of desert locust, on the phase shift of hatchlings that emerged from solitarious eggs were compared with controls in grouping bioassays. Hatchlings from different treatments were randomly released into a ring-like bioassay arena and their distribution per equal segments was noted. The grouping behaviour of gregarious control nymphs and those nymphs that emerged from exposed solitarious eggs was highly significant, whereas solitarious control nymphs were distributed randomly across the segments of the bioassay arena. The behaviour was density-dependent. The greater the number of gregarious egg-pods used as the source of pheromone, the more behaviourally gregarious the hatchlings. On the contrary, hatchlings that emerged from solitarious eggs exposed to solvent extracted or nitrogen flushed sand, previously oviposited by gregarious females, failed to change behaviourally. Their behaviour being similar to that of solitary control. This indicated that primer signals had been removed by solvent extraction or flushed-off by nitrogen, showing the mediation of non-

polar, volatile compounds as primer signals. Thus, oviposition aggregation pheromone components, secreted into the sand by gregarious females, are responsible for the primer effect, which induces gregarization of the hatchlings developing from solitary or gregarious eggs.

ملخّص الأطروحة

دور بعض العوامل في تغير الطور في الجراد الصحراوي (*S. gregaria Forskal*)

تمت دراسة توزيع الغذاء و أثرها على درجة التجمّع لدي الجراد الصحراوي (*Schistocerca gregaria Forsk.*) ، مستخدمين الطور الانفرادي من الحوريات (solitarious phase) في مجموعات مكونة من ٤ ، ٦ ، ٨ ، ١٠ و١٢ فرداً داخل أقفاص أبعادها ١٠٠×١٠٠×٥٠ سم . هذه الحوريات مغذّاة على نبات القمح أو نبات الكيل (Kale) . تحول الحوريات تم تتبعه طبقاً لعدة معايير من بينها :-

١- مستوى إنتاج فيرومون التجمع (Aggregation Pheromone) ويقاس بمستوى مركب الفنيل إيستوناترايل (Phenylacetonitrile) .

٢- التركيب الصبغي للدّم(Haemolymph pigment composition) ويقاس بنسبة امتصاص الأشعة (absorbance pigment ratio) على الموجتين ٤٦٠ و٦٨٠ (نم) نانومتر بالإضافة الي معياري تغيير اللون (Colour change)، وحاصل نسب أبعاد اجزاء جسم الحشرة (Morphometrics) .

النتائج أثبتت أن الجراد ذو الكثافة المنخفضة (بين ٤ و٦، أفراد) في كلا المعاملتين ، و٨ أفراد من المعاملة غير المتوزّعة (delocalized)، فشل في التجمّع وكان يشبه الجراد الانفرادي البالغ. كما أن الجراد الانفرادي الناتج عن حوريّات إنفرادية مكثّفة في (٨ أفراد) في معاملة غير موزّعة (localized) والذي بين ١٠ إلى ١٢ فرداً في القفص الواحد والمغذىّ على كلا الغذائين الموزع وغير الموزّع يؤدّي إلى التجمّع بعد أن يصبحوا صفراً وينتجوا مستويات من الفيرومون كان وسطيه بين الجراد غير المعامل (التجمّعي والإنفرادي) رغماً عن حالة التجمع سبقت بكثير في حالة الجراد المعامل معاملة غير موزعة عن الجراد المعامل معاملة موزّعة .

هذه النتائج أكدت النظريات المتوقعة أن توزع النباتات العائلة يعتبر عـــاملا مـــهما فـــي إحداث التجمع لدى الجراد عندما تزداد كثافة الجراد الانفرادي. من جهـــة أخـــرى لامتصـــاص ودراسة نسب اجزاء جسم الحشرة لم يختلفا مع المعايير الخاصة بالجراد الإنفرادي غير المعـــامل ولكن هنالك اختلاف معنوي بينه وبين الجراد التجمعي غير المعامل مؤكدا النظريات التي تـــرى أن هذه التغيرات تسير ببطيء طبقا للكثافة .

لقد تمت أيضا دراسة مقارنة تأثير نوع النبات على نسبة التجمع بعـــد تربيـــة الجـــراد الانفرادي وتغذيته على نوعين من النباتات ، القمح والكيل كل على حده .

أثبتت النتائج أنه تحت نفس كثافة الجراد أو نفس توزيع الغذاء لنفس كثافة الجراد، التجمع كان كبيرا في حالة الجراد الذي كان يغذى على القمح هنالك أسباب ممكنة اقـــترح ان طبيعـــة الغذاء أثرت علي معدلات الاحتكاك بين الجراد الانفرادي.

الجدير بالذكر ان العينات قد أخذت من جراد تمت تربيته علي قمح لأجيال عديدة لكـــن اثناء التجربة تمت تغذية الحوريات علي نبات الكيل لمدة جيل واحد فقط. لا يمكـــن إذا الجـــزم بخلاصة واضحة في هذا الشأن ، حيث أن الجراد المستخدم في التجربتين يجب أن يربى علـــى كلا النباتات التي ستجري عليها التجارب ولمدة أجيال متساوية ثم تجرى مقارنة بينهما.

معدل الانفرادية للحوريات التجمعية المعزولة والجراد التجمعي البالغ والمعـــزول قـــد أجريت بعد تعريضه أو عدم تعريضه لإفرازات الفيرومون من طرف فرقائهم في غياب إشارات بصرية أو لمسية منهم . إن معدل تم تقييمه مستخدمين مقاييس الفيرومون والذي يقاس بمستويات النونال و حمـــض النونـــاويك (Nonanal Plus nonanoic acid) فـــي الحوريـــات و الفينـــايل أسيتونايترايل (phenylacetonitrile) في الجراد البالغ عـــن طريـــق الإفـــرازات المتطـــايرة و السلوكيات و تغيير لون الجلد والوزن .

أثبتت النتائج أن وجود الفيرومون يبطئ عملية الانفراديـــة فـــي الحشـــرات المســـتقبلة والمعزولة (حوريات وجراد بالغ) ويعتمد ذلك علي نوعية وجرعة المادة المنبهة. تعريض الجراد

البالغ لإفرازاته المتطايرة يؤدي إلى التفرد أكثر من الذي عرض للمواد المتطايرة للحوريات . التفرد في الحوريات يبدو انه مستقلا عن نوعية الإشارة الطيارة ، وفي كلا الطوريين وجد انه عند ازدياد جرعة الفيرومون الناتجة عن الكثافة العالية للجراد تسببت في خفض عملية التفرد لدي الجراد المستقبل، مع أن التأثير كان أقوى لدى الجراد البالغ منه في الحوريات. الحالة التجمعية الفسيولوجية المستمرة (بسبب إفرازات الفيرومون) قد انعكست في الحفاظ بالسلوك التحمعي مقارنة مع المستقبلين للفيرومونات.

أثبتت النتائج أن إفراز الفيرومون وحده يؤثر فقط في تأخير عملية التفرد ولكن لا يؤثر في الاحتفاظ على التجمع لدى الجراد التجمعي المعزول وهذا يتطابق مع الاكتشافات التي ترى أن الفيرومونات الطيارة ليست مسؤولة عن إحداث التجمع لدى الجراد الانفرادي. هذه ميزة للنظريات المتوقعة بأن الحشرات التجميعية المتواجدة في نفس المكان مع الحشرات المكدسة تحتفظ لدرجة ما بالملامح التجميعية .

لقد قورن تأثير فيرومونات وضع البيض الموجودة بالبيض والرمل المحاذي لمكان وضع البيض والتي افرزن بواسطة إناث الجراد الصحراوي التجمعي، تأثيرها على مرحلة التغيير لدى الحوريات الحديثة النشأة، والتي نتجت من البيض الانفرادي، قورن مع فيرومون بيض الجراد غير المعامل التجريبي المزدحم . الحوريات الحديثة النشأة والمنحدرة من الجراد المعامل تم إطلاقها عشوائيا في قفص تجريبي دائري وروعي توزيعها المتجانس في أقسام القفص المختلفة.

السلوك التحمعي للحوريات الجماعية غير المعاملة كانت موزعة عشوائيا عبر أجزاء القفص التجريبي، هذا السلوك كان مقيدا بالكثافة كلما إزداد عدد البيض المستخدم كمصدر للفيرومون، كلما ازداد السلوك التجمعي لدى الحوريات حديثة النشأة والعكس حدث في الحوريات حديثة النشأة والتي نتجت عن البيض الانفرادي المعرض للمادة المستخلصة أو تيار رمل تم امراره سبق وضع بيض فيه بواسطة إناث تجمعيه، فشلت في تغيير سلوكياتها. ماثلت سلوكياتها سلوكيات الشاهد الانفرادي وهذا قد يشير الي الإشارة البادئة (المخضرة) قد أزيلت من

طريق المذيب أو امرار تيار النايتروجين مظهراً توسط مركبات غير قطبية متطايرة كإشـــارات بادئة أو مخضرة.

هذه النتائج أثبتت أن مكونات فيرومون وضع البيض الذي أفرز فـــي مكـــان التبويـــض بواسطة إناث الجراد كانت مسؤولة عن التأثير الأوّلي الذي أدّى إلى نمو ظاهرة التجمّـــع لـــدى الحوريّات الحديثة النشأة من البيض الإنفرادي أو التجمّعي.

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ABBREVIATIONS

ARPPIS	The African Regional Postgraduate in Insect Science
A	Absorbance
ANOVA	Analysis of Variance
AZA	Azadirchtin
AKH	Adipokinetic hormone
BCED	Behavioural and Chemical Ecology
C	Greater width of the Head Capsule
^{O}C	Degree(s) Celsius or Centigrade
CA	Corpora Allatum
CC	Corpora Cardiaca
Cm	Centimetre
DAAD	German Academic Exchange Programme
DLCO-EA	Desert Locust Control Organization for Eastern Africa
E	Length of the fore-wing or Elytron
E/F	Length of Elytron to Hind-femur Ratio
F	Length of the Hind-femur
F/C	Length of the Hind-femur to Head Capsule Ratio
FID	Flame Ionization
FIG	Figure
GC	Gas Chromatography
G/C	Gregarious Control
G/I	Gregarious Isolated (Uncrowded)

g	gram
h	Hour
HP	Hewlett Packard
HPLC	High Performance Liquid Chromatography
ICIPE	The International Centre of Insect Physiology and Ecology
IFAD	International Fund for Agricultural Development
ID	Internal Diameter
IABU	Insect and Animal breeding Unit
JH	Juvenile Hormone
JHAs	Juvenile Hormone Analogues
kd	kilo dalton
LD	Light and Dark
Ltd.	Limited
LSD	Least Significant Different
mg	milligram
Min.	minute
ml	milititre
mm	milimetre
µl	microlitre
µm	micrometre
ng	nanogram
nm	nanometre

PG	Prothoracic Gland
VG	Ventral Gland
SAS	Statistical Analysis System
S/C	Solitarious Control
SE	Standard Error
UV	Ultra-Voliet
UK	United Kingdom
$\lambda_{max.}$	Maximium wave length

CHAPTER ONE

1. INTRODUCTION

1.1. General Introduction

The desert locust, *Shistocerca gregaria* (Forskal), is a short-horned grasshopper, belonging to the family Acrididae, order Orthoptera. It differs from ordinary grasshoppers in that it is polymorphic, that is, it is able to interconvert between two distinct forms or phases: *solitaria* and *gregaria*, which differ in morphology, physiology, pigmentation and behaviour (Gillett, 1988; Steedman, 1988). Transformation between the two extreme phases encompasses a series of intermediate forms known as transiens (Gunn and Hunter- Jones, 1952; Pener, 1991).

At some irregular periods, when weather conditions, especially rainfall become suitable, individual locusts concentrate, multiply, and gregarize. They form large numbers of strongly aggregating hoppers, which march in bands. These hoppers develop into winged adults, which swarm over long distances (Steedman, 1988; Meinzingen, 1993). Gregarious *S. gregaria* can migrate up to 3000 or 4000 km (Rainey, 1963; Pedgley, 1981).

As a result of this tendency to gregarize, the desert locust is an economically important pest in North and West Africa, the Middle East and Southwest Asia. Four other economically important species of locusts have been recorded in Africa and

include the African migratory locust, *Locusta migratoria migratorioides* (Reiche and Fairmaire); the red locust, *Nomadacris septemfasciata* Serville; the tree locust, *Anacridium melanorhodon melanorhodon*Walker; and the brown locust, *Locusta pardalina* Walker. All these locust species are polyphagous (they can consume different types of vegetation), although they prefer plants belonging to the family graminae (grasses), which include all the major staple food crops in locust-affected countries.

The damage caused by the desert locust stems from the fact that it can consume food equivalent to its own weight, 1.5-3g of vegetation daily (Meinzingen, 1993). However, damage varies with the locust stages as Meinzingen (1993) indicated; 8% of the total damage by locusts is due to hoppers, 69% to immature and 23% to mature adults. Whereas the gregarious phase of *S. gregaria* is a destructive agricultural insect pest (Walsh, 1988), the non-migrating solitary phase is of little economic importance. It is most of the time restricted to a recession area in the arid and semi-arid locust breeding belt, an area of about 16 million km^2, which extends from the Atlantic ocean to North-west India (Fig 1) (Uvarov, 1977; Steedman, 1988). However, during plagues, gregarious swarms of the desert locust can invade an area of about 29 million square kilometres, about 20% of the total land surface of the world (Fig 1) and potentially threaten food of about 10% of the world population (Steedman, 1988).

Crop protection against swarming locusts is a major concern of national, regional and international organizations, especially at the time of outbreaks. In the

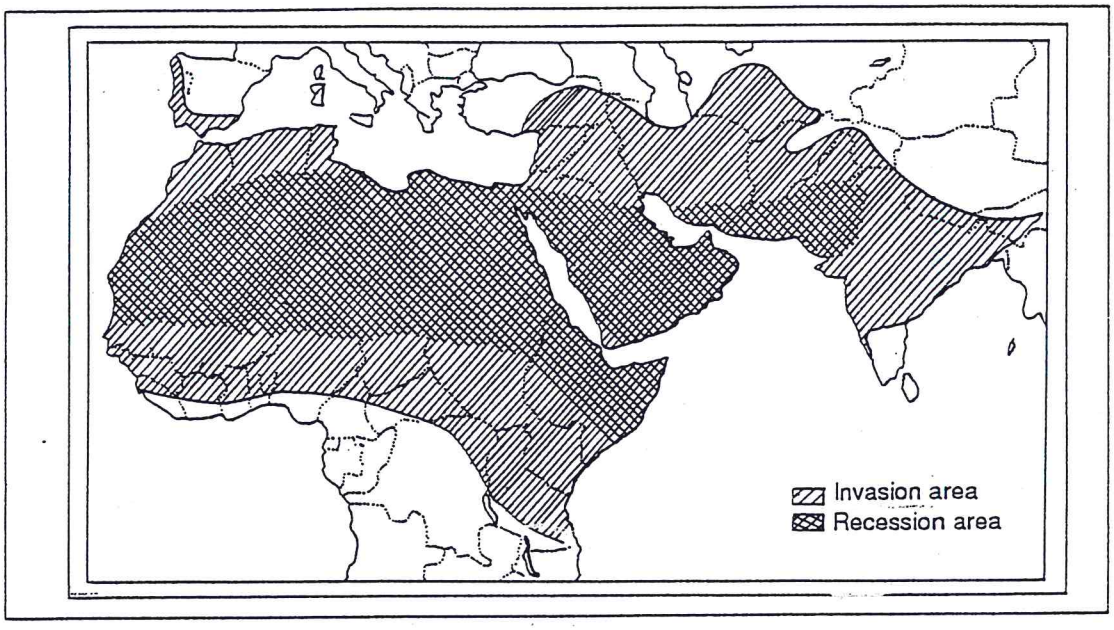


Fig. 1. Desert locust (*S. gregaria*) recession and invasion areas (from Steedman, 1988).

past, traditional methods such as burning, beating and digging of trenches to bury hoppers were employed in the locust control. Current locust control campaign is heavily dependent on the use of insecticides, which affect a wide range of non-target beneficiary organisms including insects, birds, livestock and wildlife. They also constitute a risk to humans and the environment (Everts, 1990). Alternative control agents, such as entomopathogenic fungi *Metarhizium anisopliae var.acridum* (Merschnikoff) and *Beauveria bassiana* (Balsamo) and protozoans, such as *Nosema locustae* Canning (Lomer and Prior, 1992; Thomas *et al.*, 1992), are currently being tested for locust control. They are effective against locusts in some areas, but not in others because special weather conditions are needed for the diseases these organisms inflict in the locusts to develop. Botanical insecticides from the neem tree (*Azadirchata indica* A. Juss) and *Melia volkensii* (Gurke) act best as insect growth-disruptants but expensive to use on large scale (Diop and Wilps, 1997; Mwangi *et al.*, 1997). Recently, tests with behaviour-modifying chemicals, particularly pheromones, which have a solitarizing effect on gregarious locusts have been carried out on field populations of hopper bands at the Red Sea area of Port Sudan with promising results (Anon., 1999).

Apart from control agents, in principle, three strategies or approaches for locust control are possible:

(a) Reactive: delay of control until upsurge develops into outbreaks and then control with insecticides or other agents. This strategy was practised in 1930s,

1940s, and focused on swarms control in order to maximize limited crop protection resources and minimize the sprayed area.

(b) Proactive: earlier intervention by destroying initial gregarizing or gregarious populations to stop upsurge development and prevent plague formation. This depends on continuous and extensive surveys of large locust aggregations in breeding areas, using aerial and ground survey, satellite imagery, weather reports and information from local locust scouts. This strategy was put into practice after the mid-1960s and considered achievable during the long recession period after 1963 (Joffe, 1995). The onset of both 1986-89 and the 1992-94 outbreaks showed its shortcomings. The main weakness has been lack of understanding of the process of gregarization.

(c) Preventive: much earlier intervention at the onset of gregarization when locusts have gathered in small patches to prevent development of significant gregarious groups. The approach is dependent on a thorough understanding of factors associated with gregarization of solitarious locusts, including habitat attributes as well as the rate and pattern of spatial spread of gregraization. ICIPE`s long-term goal is to develop such an approach for the desert locust as well as for other locusts.

1.2 Objectives of the Study

Gregarization of *S. gregaria* is predicated on population density which is promoted by a number of factors including convergent winds, environmental heterogeneity, habitat topologies, distribution of food plants, favourable oviposition

sites, improved rain and vegetation, etc. Recent studies on the desert locust, have confirmed that forced crowding results in rapid gregarization. Deng (1995) demonstrated that forced crowding of solitarious locusts in cages led to rapid production of the gregarious phase aggregation pheromone. On the other hand, uncrowding led to rapid loss of the pheromone. Bouaichi *et al.* (1996) showed that habitat microstructures, such as patchy distribution of food plants and perching sites, do indeed facilitate the concentration of and encounter between solitarious desert locusts. In time, this led to active cohesive behaviour. No studies on the effect of such food distribution on physiological changes, such as pheromone production, have been undertaken to complement this work.

Earlier work on semiochemicals implicated pheromonal effects in gregarization of locusts. Nolte (1963) and Gillett (1968) observed that gregarious isolated hoppers in the same room with crowded hoppers retain their gregarious characteristics (pigmentation, morphometrics, grouping traits). Nolte *et al.* (1973) proposed that the active volatile pheromone was 5-ethylguaiacol (locustol). However, subsequent studies in different laboratories failed to locate this compound in locusts of either phase or any stage (Fuzeau-braesch *et al.*, 1988; Obeng-Ofori *et al.*, 1994b; Francke and Schmidt, 1994; Torto *et al.*, 1994 and 1996). Heifetz *et al.* (1996) demonstrated that short-range olfaction of air borne volatiles was only slightly effective in gregarizing locusts, whereas chemotactile cues derived from cuticular lipid extracts of gregarious nymphs induced a marked behavioural gregarization in isolated nymphs. However, these studies as well as those of Deng

(1995) showed that exposure of solitarious nymphs to volatiles or faecal extracts from crowded nymphs, without visual and physical contact, had no effect on phase change. Roessingh *et al.* (1998) confirmed Deng`s work that olfactory stimuli alone was ineffective in gregarizing locusts, but its combination with visual cues caused some behavioural change in isolated nymphs. These authors undertook detailed studies of the effect of tactile, visual and olfactory cues and found those tactile stimuli alone or in combination induced a behavioural gregarization, whereas olfactory or visual alone was ineffective. However, visual stimuli play a role in the attraction of gregarious locusts to each other over short distances and in the repulsion of solitarious locusts. The authors concluded that a complete phase transformation is induced by a combination of stimuli (tactile, visual, and olfactory) together. Each stimulus may elicit only a partial response when acting alone. However, in view of the earlier work in 1960s, it is possible that once gregarious, the volatile aggregation pheromones may provide sufficient primer stimuli to delay solitarization of isolated gregarious insects. This possibility has not been investigated.

Several studies have focused on the mechanisms by which gregarization might spread across a population and across generations. Studies on the electrophysiological and behavioural assays have shown that solitarious *S. gregaria* respond to the aggregation pheromone equally as their gregarious counterparts (Njagi *et al.*, 1996). The implication of this finding is that solitarious individuals that encounter foci of pheromone-emitting groups of gregarizing or gregarious insects

would be arrested by pheromone fog and recruited into the group. The new recruits will rapidly acquire gregarious characteristics through either contact or mating with gregarious individuals (Ellis, 1963; Dale and Tobe, 1990; Roessingh *et al.*, 1993; Islam *et al.*, 1994a, b). Thus, clusters of pheromone-emitting gregarious locusts in preferred microenvironments may constitute effective nuclei for the horizontal spread of the gregarious characters across the population (Njagi *et al.*, 1996).

Two sets of pheromonal effects have been shown to operate in the transmission of gregarious characteristics across generations: releaser signals mediate group oviposition behaviour, which ensures cohesiveness of the succeeding hopper generation. This behaviour is mediated by a signal (nonanal) from ovipositing females (Njagi *et al.*, submitted), benzene compounds (acetophenone, and veratrole) associated with froth of egg-pods (Saini *et al.*, 1995; Rai *et al.*, 1997), and unsaturated ketones ((Z)-6-octen-2-one, (E, E)-3,5-octadien-2-one and its isomer (E, Z)-3,5-octadien-2-one) secreted into the sand during oviposition and which are also found in the eggs (Torto *et al.*, 1999). Similar behaviour is found in the migratory locust, *L. migratoria* (Lauga and Hatte, 1977, 1978). In addition to these releaser signals, a primer signal in the aqueous extracts of egg froths of gregarious females of the desert locust promoted gregarious characters in solitarious hatchlings (McCaffery *et al.*, 1998), thus effecting transgenerational transfer of phase characters. These authors proposed that a polar compound of less than 3k daltons was responsible for this effect. It is possible that these workers had in fact extracted from froths unsaturated ketones rather another polar compound(s). The

ketones referred to above (Torto *et al.*, 1999) and secreted into the sand may have both releaser as well as primer effects. Their volatility could ensure diffusion around the soil particles to effect neighbouring egg pods. This possibility was examined in the present study.

1.3 Specific objectives

Accordingly, the present study was undertaken to answer the questions raised above; specifically, (a) the effect of food distribution on gregarization of solitarious insects as measured by physiological manifestations, particularly pheromone release; (b) the possible primer role of aggregation pheromones in delaying solitarization of isolated gregarious locusts; and (c) the possible primer effects of unsaturated ketones secreted into the sand by ovipositing females in transgenerational transmission of gregarious characters.

CHAPTER TWO

2. LITERATURE REVIEW

Phase transformation in the desert locust refers to its ability to exist reversibly in two extremes phases: *solitaria* and *gregaria*. The two phases differ by many traits, collectively termed phase characteristics. In the present chapter, phase-dependent characteristics are first reviewed and then factors (causative, mediating and physiological) associated with phase change are discussed.

2. 1. Phase characteristics in locusts

Recent research has revealed some new phase characteristics in addition to old ones. Some of these traits respond very rapidly to changes of density. Important characteristics are outlined below.

2. 1. 1. Behaviour

Behaviourally, solitary phase locusts live as sedentary, harmless individuals far apart from another (Roessingh *et al.*, 1993) and show strong repulsive reactions to confrontations with other hoppers (Wiesel *et al.*, 1996). The adult stages fly individually by night. In contrast, in the gregarious phase, locust hoppers actively aggregate, forming large crowded groups, termed bands. Hoppers in a band have a higher locomator activity in the same direction (Uvarov, 1977). Likewise, adults fly together in swarms during the day with a higher flight activity compared to solitarious ones (Michel, 1980b). Male sexual behaviour is more intense in crowded than in isolated adults (Pener, 1967a).

2. 1. 2. Morphometrics

Morphologically, solitary or isolated locusts are somewhat larger than conspecific gregarious or crowded ones, but they can be differentiated using morphometric ratios such as the ratio of adult forewing length or elytron (E) to hind femur length (F), (E/F), and hind femur length to the greatest width of the head capsule (C), F/C. The F/C ratio is higher, whereas the E/F ratio is lower in solitary than in gregarious locusts (Dirsh, 1951, 1953). Different authors have proposed different E/F ratios ranges for the phases; *e.g* 1.92-2.08 for *solitaria* and 2.11-2.27 for *gregaria* (Rao, 1942; Dirsh, 1951, 1953; Meinzingen, 1993; Ochieng Odero *et al.*, 1994). F/C ratios are higher in both locust phases; *e.g* 3.77-4.10 in *solitaria* and 3.24-3.59 in *gregaria* (Jackson *et al.*, 1978). These ratios vary with locust strain (Gunn and Hunter-Jones, 1952). Deng *et al* (1996) suggested that morphometrics ratios are not good phase indicators since they change slowly over several generations.

2. 1. 3. Body colour

Body colour pattern in locusts can be used to differentiate solitary from gregarious ones. Solitary hatchlings are uniform light grey (Hunter and Hunter-Jones, 1952, Hunter-Jones, 1958). Solitary hoppers exhibit uniform green colour at high humidity, whereas at lower humidity colour is uniformly whitish-creamy, straw yellow, beige, buff, brown, grey or intermediate shades to match the colour of the background in the field or colour of the cage in the laboratory (Faure, 1932; Staal, 1961; Albencht, 1964, 1965). Solitary adults are characterized by greyish brown

colour (Nickerson, 1956; Stower, 1959; Pener, 1991). In contrast, gregarious first-instar nymphs are dark, dirty orange with black pattern in the second and third instar, and bright yellow with black pattern in the later ones (Nickerson, 1956; Stower, 1959; Pener, 1991), while gregarious fledglings are pink and bright yellow after the onset of full sexual maturation (Chauvin, 1941; Norris, 1954; Pener, 1967a; Steedman, 1988; Ferenz, 1990; Loher, 1990).

2. 1. 4. Flight activity

A major behavioural difference between solitary and gregarious adult locusts is the migratory group flight of the latter (Michel, 1972a, b, 1973a, b). Solitarious adult stages fly individually by night, whereas crowded adults fly together in swarms during the day with a higher flight activity compared to solitarious ones (Michel, 1980b). The flight related phase dependent difference is exhibited by *S. gregaria* (Michel, 1970a, b, 1980a, b) and by *L. migratoria* (Ayali and Penner, 1992, 1995; Ayali *et al.*, 1994). *Corpora cardiaca* (CC) and its secretion adipokinetic hormone (AKH) ensure provision of fuel and energy for sustained flight. Possible difference in AKH production, AKH content of CC and the rate of AKH release response contribute to the difference in flight activity between crowded and isolated locusts. Flight activity results in release of AKH from the CC of the locusts (Cheeseman *et al.*, 1976; Cheeseman and Goldsworth, 1979). AKHs induced mobilization of lipids, which serve as the major fuel for migratory flight. Also flight activity induced increase of haemolymph lipid level and hyperlipaemic response both which were higher in crowded than in isolated adult males of *L.*

migratoria (Ayali *et al.*, 1996a, b) and in adult females of *S. gregaria* (Schneider and Dorn, 1994).

In crowded locusts, adipokinetic response, lipid-content of the fat body and hyperlipaemic response were higher than in isolated adults of *L. migratoria migratorioides* (Ayali and Pener, 1992, 1995; Ayali *et al.*, 1994), but they quickly decline when crowded fledglings are isolated. Conversely, Chino *et al.* (1992) found poor fat body with a low triacylgcerol-content in a solitary phase.

2. 1. 5. Fecundity

The reproductive potential (or fecundity), as expressed by number of ovarioles, egg pods, eggs per pod, of isolated females is higher than that of crowded *S.gregaria* (Papillon, 1960, 1970). For example, Injeyan and Tobe (1981a) reported that average number of the ovarioles was 110 in crowded females of *Schistocerca* and range of 130-154 ovarioles in isolated locusts. However, despite the difference in the number of ovarioles, the average weight of an egg pod and vitellin-content per ovary, are about equal in crowded and isolated females, because eggs of isolated are small and lighter. Thus the number of eggs per pod laid by isolated females is higher at the expense of egg size and vitellin-content per egg. The number of egg pods produced per female during life span is lower in crowded than in isolated. Gregarious females lays 2-3 egg pods, each with about 60 to 80 eggs, whereas solitarious females 3-4 egg pods, each pod containing 95 to 160 eggs (Norris, 1950; Albencht *et al.*, 1958; Injeyan and Tobe, 1981a; Anon., 1982; Steedman, 1988).

2. 1. 6. Nymphal development

Nymphal developmental time depends on temperature, humidity and locust phase status. It varies from 3-8 weeks. Under optimium conditions, gregarious nymphs develop faster than solitarious ones. In crowded locusts, the nymphal stage develops through five instars (Fig 2) and through six instars in the solitarious phase. The developmental duration in days of each instar is as follows: 4, 4, 3, 3 and 8 for first, second, third, fourth and fifth, respectively, and the sixth instar required 7-8 days in the laboratory, making a total of 22 and 29-30 days for gregarious and solitarious phase, respectively. In the field, it takes 5, 5, 6, 7 and 11 days, which makes a total of 34 days for gregarious in Sahel conditions. The extra instar depends on parental or hopper density experience, which affects the weight and size of hatchlings and determined the number of instars in progeny (Albrencht, 1955; Injeyan and Tobe, 1981; Anon., 1982; Steedman, 1988).

2. 1. 7. Eye stripes

The number of eye stripes can be used as a phase marker in locusts. The number of stripes in an adult locust is less in the gregarious phase by one, compared to solitary phase. For instance, in *S. gregaria*, solitarious adults have seven stripes corresponding to the number of instars, whereas gregarious insects have six (Burnett, 1951; Albrencht, 19955).

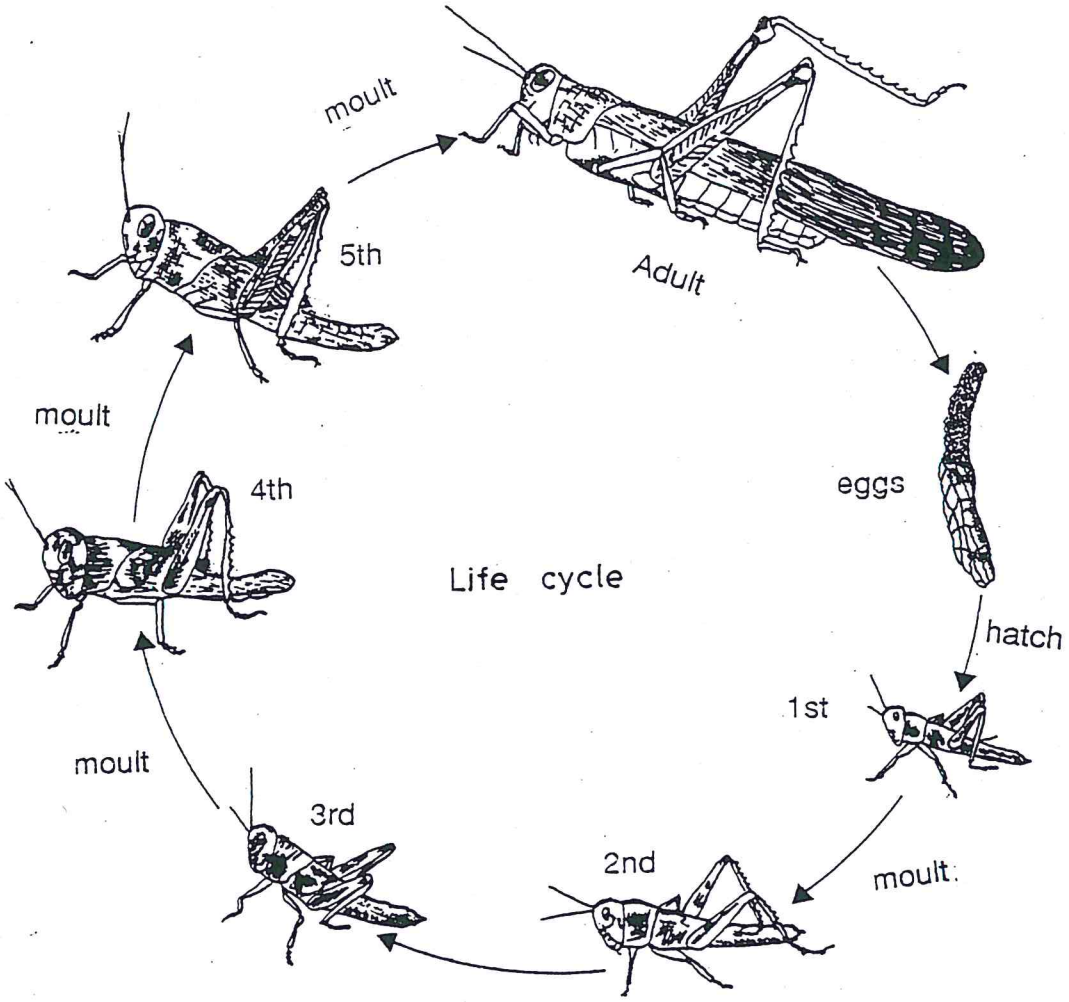


Fig. 2. Life cycle of the gregarious desert locust *S. gregaria*.

2.1. 8. Pheromone emission

The use of aggregation pheromone as a phase marker was first demonstrated by Torto *et al* (1994) and confirmed by Deng *et al* (1996). Phenyalcetonitrile a major component of the aggregation and maturation accelerating pheromone, is only produced by crowded adult males of *S. gregaria*, but it is not produced by isolated ones. Crowding of isolated mature adult males induces phenylacetonitrile production within a few days, whereas isolation of crowded mature males lead to a quick decrease in its production (Deng *et al.*, 1996).

2. 1. 9. Haemolymph pigment composition

The use of absorbance ratio of haemolymph pigment composition to differentiate solitary from gregarious locusts was recently demonstrated by Mahamat *et al* (1993). It was found that bivilerdin pigments in the haemolymph of *S. gregaria* are present in solitary insects, irrespective of their diets, but absent in gregarious ones. The absorbance ratio at 460nm (λ max. for the carotenoids) and 680nm (λ max. for the biliverdin) in an UV spectrophotometer was higher in crowded locusts with a range of 3.99-4.78 and lower in solitarious with a range of 0.64 -1.67.

2.2. Causative Factors Associated with Gregarization

Gunn and Hunter-Jones (1952) studied the effect of density on phase status of the desert locust and found that hatchlings isolated in cages gave nymphs (hoppers) which differed in colour from the natural hoppers. In contrast, adults were biometrically like the natural individuals of phase *solitaria*. When crowded in cages,

nymphs were chromatically like natural gregarious hoppers, while adults were biometrically unlike natural gregarious specimens. The authors also found that rearing locusts for a single-generation at low densities did not produce chromatic properties of solitarious hoppers. In a single-generation, the phase with an extra instar was morphologically associated with the solitary phase (Albrencht and Blackith, 1956).

Social aggregation behaviour of locusts is affected by rearing-density (Gillett, 1973), and manifests more significantly in adults and nymphs reared crowded than those reared singly. In another study, Gillett (1988) investigated the rate of solitarization in *S. gregaria* after different periods of isolation and found that gregarious nymphs lost their tendency to group more rapidly even after isolation for 24 hours. The fall in the level of grouping behaviour correlated with the number of days or period of isolation suggesting that the locusts were very responsive to their social environment.

Injeyan and Tobe (1981) studied the transition among successive generations of crowd-reared solitarious locusts and found that 50% of these locusts gregarised in the second generation. This proportion increased gradually in the late generations, but did not reach 100% by the sixth generation. The relationship between locust density and flight behaviour of *S. gregaria* reared isolated and, then grouped, was studied by Michel (1980). He found that as locust density increased, flight activity increased. This migratory behaviour was more pronounced in locusts

reared isolated for one to several generations before being regrouped, than in individuals permanently raised at high densities over several successive generations.

Recently, Duranton and Lecoq (1990) suggested that locust densities, which are less than 250 per hectare in the field, are to be considered as solitarious, or transiens dissociant, and for densities ranging between 250 and 100,000 as transiens congregants. Locust densities greater than 100,000 per hectare are referred to as a gregarious population. On the other hand, Popov and Duranton (1991) observed that the least number of locusts capable of inducing gregarious characteristics in the field was between 5 and 50 insects per hectare, for both nymphs and adults, respectively. Pener (1991) reported that the shift in phase of typical locust species followed changes in population density and that full-scale phase differences seemed to be limited to the field. Showler (1995) reported that the cause of the 1992 desert locust outbreak in Africa and Asia, was due to an increase in the populations of the solitary phase over 2 or 3 generations in the summer of 1992. This build up induced gregarious behaviour in concentrating locusts, particularly after postdrought flushes of vegetation.

Crowding and re-isolation of adults of the desert locust changes their behavioural status in relation to phase (Bouaichi *et al.*, 1995). In laboratory experiments, crowding previously isolated adults for a period of 4 hours caused them to behave like gregarious insects (crowd-reared insects). Furthermore, young adults, which were 2-days old, were more responsive to crowding than older ones, but gregarious behaviour was lost rapidly within one day of re-isolation. When

parents previously reared in isolation were subjected to crowding for 48 hours at different times, even at the late stage in their reproductive cycle at the time of oviposition, they produced hatchlings that exhibited gregarious characteristics.

Deng (1995) studied the effect of crowding and uncrowding on phase characters of the desert locust and found that adult males of the Fo generation (from crowding solitary-reared) were sensitive to crowding by becoming yellow and produced phenylacetonitrile (gregarious older adult aggregation pheromone), which increased in the F1 generation, but decreased and leveled off in the F2 and F3 generations compared to control. On the other hand, adult males of the Fo generation, when solitarised (from crowd-reared insects), were dull yellow and did not produce phenylacetonitrile similar to solitary-reared adults (control). This study showed that crowding and uncrowding at different rates affected phase characters. In adults, pheromone production and, integumental colour were sensitive to the onset of phase change. Locust density may be promoted by specific microhabitat attributes or by interaction between different locust species.

2. 2. 1. Effect of microhabitats in facilitating concentration of locusts

In the field, dense populations evolve as a consequence of a variety of environmental (a biotic) and biotic factors (Rainey, 1963). Among these, convergent winds lead to concentration of scattered adult locusts (Rainey, 1989), while habitat topologies such as warm spots provided by stones and small heaps of soil at appropriate angles to the sun, or tall clumps of grass rising above uniform environment, limited in number and area, may serve as foci for attracting and

concentrating the locusts, nymphs and adults (Chapman, 1976). Localized precipitation, increased quality and quantity of vegetation when fed on by immature solitary adults hasten their sexual maturation (Carlisle and Ellis, 1965), followed by copulation and gravid females concentrate at suitable oviposition sites (Roffey and Popov, 1968). These promote population build up, initially maintained as cohesion hopper bands and later as adult swarms (Uvarov, 1966, 1977).

Evidence from a variety of observations indicates that the concentration or dispersion of their hosts can directly influence insect population. Habitat patchiness created by progressive drying out of vegetation during the dry season causes suitable habitats to gradually shrink in size, producing the islands of vegetation throughout the habitat. The individual locusts are attracted and converge on available green patches of vegetation. Maxwell-Darling (1937) observed that microhabitats forced the individual locusts together, such that those congregated under a single bush, were likely to emerge with a group moving from another plant to form a larger one. In field observations, Kennedy (1939) reported that the distribution of food plants affected the distribution of locusts. In sparse vegetation, encounters between individual locusts were frequent, leading to concentration and the onset of behavioural gregarization. Consequently active aggregation of locusts is observed. On the other hand, a more abundant resource increases the chances for solitarized locusts to avoid each other leading to further solitarization. These ideas have been investigated recently by Bouachi *et al* (1996) in the laboratory. The effects of distribution of food plants, perches and microclimatic sites on the behavioural phase

status of the desert locust were examined. Results showed that the provision of multiple resource sites tended to disperse locusts, which depicted a solitarizing effect while provision of only a single resource site promoted congregation and led to behavioural gregarization. These authors concluded that the effect of environmental microstructure on behaviour might yield insights into plague formation in the desert locust. Further observations have confirmed the effect of plant distribution on insect congregation (Culmsee, 1997). For instance, first-instar nymphs of the desert locust were observed to congregate on island-like patches of vegetation on a single *Boerhavia repens* plant (Nyctaginaceae) and were more evenly spread out when found on regularly spaced plants.

2. 2. 2. Effect of interaction between locust species in enhancing density effects

Interaction among locusts within and between trophic levels may have important implications on the population of locust and their life histories. Johnston and Buxton (1949) reported their observation on the interaction between two locust species, *L. migratoria migratoroides* and *S. gregaria* in northern Sudan. The two species laid their eggs simultaneously and upon hatching, the hoppers intermingled freely in bands with varying ratio. However, vegetation played an important role in segregating the species in a band. For instance, when a mixed band of the two species passed through a patch of *Sesbania tetraptera*, the *Shistocerca* hoppers remained to feed on it, while the *Locusta* hoppers, which fed on grasses, moved on.

Laboratory experiments have demonstrated that locusts affect phase polymorphism and development of one another when they are crowded in cages,

while grasshoppers do not (Gillett, 1968; Ba-Angood, 1976). Gillett (1968) studied the grouping behaviour of the desert locust, *S. gregaria,* reared singly and individuals reared with a non-swarming grasshopper species, *Humbe tenuicornis* (Schaum). Isolated locusts and those reared crowded with grasshopper were unable to form groups, compared to gregarious populations. Similarly, Ba-Angood (1976) compared crowd-reared *S. gregaria* and individuals of *S. gregaria* crowded with the grasshoppers, *Oedaleus spp., Aiolopus spp.,* and *Kraussaria spp.* on phase development of *S. gregaria,* and found that *S. gregaria* hoppers tended to aggregate together away from other immature grasshoppers. He concluded that crowding with grasshoppers had no effect on phase status of the desert locust with regard to biometric ratios, E/F, F/C and the number of eye-stripes. The author added that these insects depended on certain self expressing factors (presumably a pheromone) that contributed to the change of phase.

In a survey carried out in the desert locust recession and winter breeding habitats along the Red sea coast of Sudan, El Bashir and Abdel Rahaman (1991) observed a mixture of hoppers of the solitarious desert locust and those of the African migratory locust hoppers at different stages in a band at a ratio of 2:200 and that of adults at a ratio of 1:10. They suggested that these interactions and the dense population of gregarious stages of the African migratory locust hoppers induced morphological changes typical of gregarious phase in hoppers of the desert locust. Based on the field observations of El Bashir and Abdel Rahaman (1991), intra- and inter-specific aggregation responses of *L. migratoria migratorioides* and *S. gregaria*

and, a composition of the pheromone emissions of the two locusts species were compared in laboratory assays (Niassy, 1997). Results showed that significant changes occurred towards the gregarious phase in the characteristics (body colour, number of instars, pheromone titres, haemolymph composition and morphometrics) of solitarious nymphs and immature adults of *S. gregaria* reared with gregarious *L.migratoria*. Similarly, solitarious immature adults of *L. migratoria* changed their phase characters to gregarious phase. In another study, Niassy (1997) reared solitarious desert locust with pheromone-producing grasshopper, *Phymateus viridipes* and non-pheromone-producing grasshopper, *Eyprepocnemis plorans*. He found that solitarious *S. gregaria* reared with *P. viridipes* shifted to a transient phase with respect to colour, morphometrics and pheromone production, whereas no such phase shift was observed in the desert locust reared with the grasshopper, *E. plorans.*

2. 3. Mediating Factors

Gregarization is mediated by pheromonal signals. Two principal pheromonal effects have been demonstrated: primer pheromones provide the physiological stimuli for transformation; releaser pheromones mediate behavioural aggregation.

2. 3. 1. Primer pheromones

Two primer pheromones have been implicated in gregarization of locusts: a primer signal that contributes to phase shift from solitary to gregarious, and another one responsible for transfer of gregarious characteristics from parents to offsprings.

Recent work on factors underlying locust gregarization revealed that sensory stimuli are effective in inducing phase change. Heifetz *et al.* (1996) demonstrated that chemotactile cues derived from the cuticle of gregarious nymphs elicit significant behavioural gregarization in isolated nymphs. On the other hand, Roessigh *et al.* (1998) showed that tactile stimulation, natural locusts' odour combined with prolonged visual contact caused behavioural gregarization in isolated nymphs. These authors proposed that self-stimulation by cuticular components, facilitated by self-grooming behaviour, a character of gregarizing insects (Roessigh *et al.*, 1993), distribute the cuticle components, which play a role in behavioural transformation, from the solitary to the gregarious phase. Thus, the precise role of gregarization pheromone is yet to be established.

McCaffery *et al.* (1998) showed that exposure of solitarious eggs to the chemicals from eggs, as well as egg froth of gregarious females, predisposed solitary offsprings to emerge with the characteristics of the gregarious phase. On the other hand, early separation of gregarious eggs from the egg pods laid by crowd-reared females led to solitarization of the hatchlings, confirming that a primer factor, either in or around the egg, that promotes gregarization had been removed. These authors indicated that a gregarizing factor is a small hydrophilic substance (< 3kD) which diffuses around and into the eggs and influence their embryonic development towards gregarious phase.

2. 3. 2. Releaser pheromones

Fuzeau-Braesch *et al.* (1988) studied the composition and role of volatile substances emanating from two gregarious locusts, *L. migratoria* and *S. gregaria.* Three aromatic derivatives, phenol, guaiacol and veratrole, were identified, but no locustol was found. Behavioural tests showed that phenol or guaiacol or mixture of the three compounds elicited significant aggregation behavior in both species and acted as a cohesion pheromone.

More recent studies revealed differences between phases, sexes and the different stages of *S. gregaria* in the production and chemistry of the pheromones as well as in their aggregation responses to different pheromone emissions (Obeng-Ofori *et al.*, 1993, 1994a, b; Torto *et al.,* 1994, 1996). Obeng-Ofori *et al.* (1993 and1994) demonstrated in olfactometric bioassays that crowded nymphs and adults were capable of choosing and aggregating in air stream permeated by odours from conspecific groups. Moreover, nymphs were attracted only to nymphal-produced odours and, mature adult odours specific to adults. The pheromone systems mediating aggregation behaviour in nymphal and adult stages of *S. gregaria* were characterized by Torto *et al* (1994, 1996). The adult pheromone produced by sexually mature males comprised anisole, veratrole, benzaldehyde, guaiacol, phenylacetonitrile and phenol (Torto *et al.*, 1994). Phenylacetonitrile, benzaldehyde, guaiacol, and phenol were found to elicit aggregation responses. Furthermore, it was shown that only gregarious mature adult male locusts produced the pheromone, and that solitary locusts responded to it (Torto *et al.,* 1994; Njagi *et al.,* 1996). The

nymphal (2nd to 5th instar) pheromone system was characterized as consisting of three sets of compounds, aliphatic C6, C8-C10 aldehydes, their corresponding acids and the facecal volatiles phenol and guaiacol (Torto *et al.*, 1996). These compounds elicited aggregation responses from nymphs (Obeng-Ofori *et al.*, 1994). Since electrophysiological studies showed that solitarious locusts responded equally to the aggregation pheromone as their gregarious counterparts (Njagi *et al.*, 1996), it was suggested that these signals may play a role in the arrestment and subsequent recruitment of solitarious individuals that encounter foci of pheromone-emitting groups of gregarious insects into the groups and, thus help in the build-up of locust outbreaks (Njagi *et al.*, 1996).

2. 4. Internal Physiological Factors

A number of other factors have been shown to cause varying degree of phase shifts, including growth regulators (both synthetic analogues of endocrine factors and natural products), dietary factors, meteorological factors and phase history of the insects. These are likely to be mediated by internal physiological processes.

2. 4. 1. Effect of endocrine factors on phase transformation

Involvement of endocrine factors (as an internal factor) in locust phase transformation has been reviewed (Dale and Tobe, 1990; Pener, 1991; Pener and Yerushalmi, 1998). These factors have been found to affect some phase characteristics and may play a role in locust phase change. Implantation of active *corpora allata* (CA) or administration of its product, the juvenile hormone (JH) or

JH analogues (JHA) into crowded gregarious hoppers induced green colouration and caused a decrease in E/F (the length of elytron/ length of hind femur) ratio associated with solitary phase (Jolly and Jolly, 1954; Couillaud *et al.*, 1987). This effect had first been demonstrated in *L. migratoria* (Jolly and Jolly, 1954), *S. gregaria* (Carlise and Ellis, 1959; 1962) and other grasshoppers (Rowell, 1967).

Injeyan and Tobe (1981) reported that JH biosynthesis activity of the CA, assessed by radiochemical assay *in vitro*, was higher in isolated than in crowded penultimate (4th instar) and last stadia as well as during the first gonotropic period of adults. Nijhout and Wheeler (1982) found excess JH induces the solitary phase. Dale and Tobe (1986) found a larger CA volume in isolated than in crowded females of *L. migratoria* during the first 8 days after fledging. The rates of JH biosynthesis of C.A. in isolated females were significantly greater than those of C.A. in crowded females. Pener (1990) found that CA and its products, the JH promoted many solitary characteristics, including the induction of green colour. Injection of neuropeptide, an extract of CC from gregarious 5th instar nymph of *L. migratoria* into solitary 4th instar nymphs, induced a darker colouration after ecdysis in 5th instar nymphs (Tanaka and Pener, 1994). On the hand, implantation of C.A. from crowded nymphs induced darkening in the integument, and the compound eyes of isolated green nymphs. The effect was dose-dependent. As the number of implanted C.A. increased, the more the black pattern appeared (Tanaka and Yagi, 1997). Application of methoprene (a JHA) on normal and attatectomized crowded adult males of *S.gregaria* induced yellow colour, indicating that corpora allata (CC)

promote yellowing, a characteristic of the gregarious phase (Hasegawa and Tanaka, 1994). Schneider *et al.* (1995) found that application of JHIII and JH Analogues to gregarious *S. gregaria* females; increased fecundity, suppressed fat body development and lipid mobilization, all these are solitarization effects. Wiesel *et al.* (1996) found in both locusts, *S. gregaria* and *L. migratoria* that higher JH levels and its analogues reduced the characteristics aggregation behaviour of crowded locusts, that is promoted solitary characteristics. However, JHAs intensified marching activity of crowded hoppers, gregarious phase characteristics, to levels surpassing that of crowded controls. Therefore, JH does affect certain phase characteristics, but not primary physiological factor, which induces the solitary phase. Application of JH III on gregarious 5th instar and newly fledglings of *S.gregaria* delayed pheromone production (phenylacetonitrile) and, also affected the integumental colouration and absorbance ratio of the haemolymph pigments, making a shift towards the solitary phase (Tawfik *et al.*, 1997). On the other hand, antennal allatectomy of gregarious nymphs, as well as adult males, resulted in complete loss of their gregarization behaviour and colour, associated with solitarious insects (Mordue, 1977), an effect that was reversed by re-implantation of CA or administration of JH (Pener and Lazarovici, 1979).

According to Ellis and Carlisle (1961), locusts in the solitary phase often possessed a larger prothoracic gland (PTG; often-termed ventral glands 'VG' in acridids) than their gregarious counterparts. The larger size of the PTG correlate to the higher F/C ratio in *Schistocerca*. Surgical removal of a part of the PTG from *S.*

gregaria resulted in a colour shift in the last instars within a generation towards the pattern of the gregarious phase (Carlisle and Ellis, 1959, 1962, 1963). In a subsequent study, these authors showed that injection of fresh extracts of PTG homogenate into gregarious hoppers reduced their marching behaviour. All these findings indicated a solitarizing effect of the PTG.

Recent studies showed that PTG were the same in isolated and crowded hoppers; they only differed in peak intensity in the rate of ecdysteroid biothynesis. In crowded peak, titres were lower with longer duration, whereas in isolated hoppers was higher, but of shorter duration during in last-instar hoppers of *L. migratoria* (Roussel, 1993) or in penultimate and last-instar hopper of *S. gregaria* (Tawfik *et al.*, 1996). Rowell (1967) demonstrated that implantation of a single CA from a young donor into the same species of certain African grasshoppers, *Acanthacris ruficornis*, *Humble tenuicornis* and *Gastrimargus africanus* caused them to have significantly more green colour in all post-operative instars than the controls. Ellis and Novak (1971) found that the balance between the two metamorphosis hormones played a role in determining the final colour of the hoppers in the desert locust. Comparison of the ecdysteriod level in fifth-instar *solitaria* and *gregaria* of *Schistocerca* revealed no significant differences (Wilson and Morgan, 1978).

Studies on neurochemicals in the nervous system and endocrine glands revealed that the amount of octapamine extract is higher in solitary than in gregarious adults of *L. migratoria* (Fuzeau-Braesch and David, 1978; Fuzeau-Braesch *et al.*, 1979). Conversely, dopamine is more abundant in gregarious than in

solitary (Fureau-Baesch, 1977a, b). Neuroparsin of the CC was higher in crowded than in isolated adults of *L. migratoria.* Amount of the ovarian parsin was similar in maturing isolated and immature crowded ones, but higher in crowded locusts after maturation (Ayali *et al.*, 1996b). Adipokinetic hormone (AKH) I and II content of the CC was higher in isolated than in crowded males at age of 12-19 days after fledging, but there was no phase difference in 25-30 days-old adult males (Ayali *et al.*, 1996c).

2. 4. 2. Effect of natural products on phase change

The effect of plant products in locust phase transformation has been studied (Schmutterer, and Freres, 1990; Langewald and Schmutterer, 1992 and 1995). These products have been found to affect phase shift towards solitary phase. Injection of azadirchtin (AZA) from neem into *L. migratoria* inhibits vitellegenin synthesis and activation of ovaries (Rembold and Sieber, 1981) both of which are controlled by JH. AZA also blocked corpus allatum activity, prevented or delayed the secretion of JH (Rembold *et al.*, 1987; Shalom *et al.*, 1993). Treatment of crowded hoppers with neem oil containing natural AZA induced pale yellow colour, reduced activity (Schmutterer and Freres, 1990) and, less intense aggregation behaviour on the resultant adults (Langewald and Schmutterer, 1992). Langewald and Schmutterer (1995) found that topically applying neem oil or extracts of AZA to nymphs and adults of *S. gregaria* tended to reverse their phase colour from gregarious to solitarious after 33 days (from black and yellow to pale yellow transciens then, to green solitary type). The authors found the effect on phase shift was much greater at

higher locust density (500 individuals/cage), than at a lower density (150 individuals/cage). Treatment of *S. gregaria* with *Melia volkensii* seed-extracts induced solitary colour in the hoppers and an extra eye-stripe in the resultant adults, a solitary characteristic reflecting the number of moults (Nasseh *et al.*, 1993).

2. 4. 3. Effect of diet on phase transformation

The desert locust is a polyphagous insect and uses its host plants for food and /or shelter (Hussain *et al.*, 1946; Evans and Bell, 1979). The moisture content of the food may be crucial in relation to the phase status of locust as reported by Gunn and Hunter-Jones (1952). Evaluation of the effect of moist air with fresh food, and dry air with dry food on phase characteristics in both *Locusta sp.* and *S.gregaria* showed that higher morphometric ratios (E/F and F/C) associated with gregarious characteristics correlated with drier conditions. However, in addition to moisture, other dietary constituents may also affect the physiology of the insects and transform phase.

Berneys (1955) compared the effect of different diets on longevity and egg-production of the lesser migratory grasshopper, *Melanoplus mexicanus* using alfalfa (*Medico sp.*), johngrass, nettle-leaf goosefoot (*Chenopodium murale*), hedge mustard (*Sisymbrium irio*) and a mixed diet of all these plants in her experiments. Her results showed that those reared on hedge mustard or the mixed diet favoured the gregariform, while those reared on Johnson grass were intermediate between the two phases. Those while were reared on alfalfa showed typical solitarious characteristics.

Comparing the effect of five food plants, which included two grasses (*Agropyron repens* and *Poa annua*), lime (*Tilia ceropoea*), spinacia (*Spinacia oleracea*) and privet (*Lingustrum vulgare*) on the development of the *S. gregaria* showed that *A. repens* was most suitable for locust development, while privet was the worst (Toye, 1973). Jackson *et al.* (1978) studied the effect of seven plants on the development, fecundity and phase of *S. gregaria.* They observed changes in colour, morphometrics and fecundity. Two of these plants (*Pennisetum typhoideum* and *Sorghum sp.*) were cultivated, while the remaining five (*Dipterygium glaucum, Zygophllum simplex, Chrozophora oblingifolia, Tribulus longipetulis*, and *Panicum turgidum*) were uncultivated. The cultivated plants were found to enhance gregarious characteristics in the desert locust, while *Dipterygium* promoted solitarious features.

2. 4. 4. Effect of temperature and humidity on phase transformation

At high temperatures (about 30 ^{o}C) crowded locusts exhibit E/F ratios closer to those characteristic of extreme phase *gregaria* (Hussain and Muther, 1943), while high humidity favours solitarious conditions (Albrecht and Lauuga, 1979). The prothoracic patterns of fifth-instar hoppers are more markedly affected by temperature changes in the laboratory (Dudley, 1964). For instance, at 38^{o}C, nymphs showed a marked reduction in pigmentation (or black pattern), even when reared under crowded conditions, showing a prothoracic pattern typical of the solitary phase. At 28^{o}C, prothoracic pattern typical of gregaria phase is obtained in

nymphs in the field (Stower, 1959; Meinzingen, 1993). Humidity changes on the other hand affect adults more than hoppers. High humidity is associated with a higher E/F ratio as observed in phase *gregaria.*

2. 4. 5. Effect of previous phase history on phase

As outlined earlier, the degree of crowding experienced by a locust generation affects not only the phase status of the individuals of that generation, but also subsequent generations (Roessingh *et al.,* 1993). However, in addition to the cumulative effects of crowding, the number of previous solitarious or gregarious generations of a given population of locusts has also been found to affect the degree of expression of phase characters of the insects. Crowding at high density of the first generation of isolated locusts, which had been solitary for one to three generations, showed significant increased flight activity, compared to those that were solitarious for longer cycles (Michel, 1980b). On the other hand, maintenance under-crowded conditions for several successive generations led to the reduction of flight performance.

CHAPTER THREE

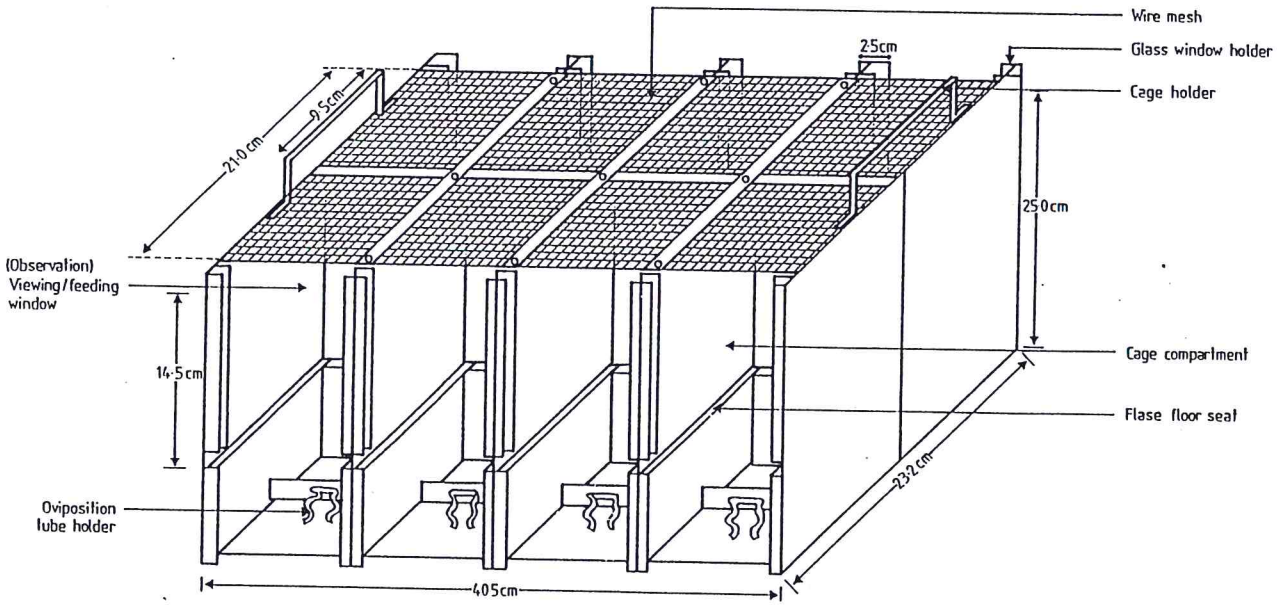
3. MATERIALS AND METHODS

3. 1 Insects

The experimental insects, both gregarious and solitarious, *S. gregaria* used, were obtained from the ICIPE's Insect and Animal Breeding Unit (IABU). The colony stock originated from the Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Addis Ababa, Ethiopia in 1989. They had been reared for 53 generations as separate solitary and gregarious cultures on a diet of wheat seedlings and bran.The insects used in conducting the studies reported in this thesis were first, third and fourth-instar nymphs, mature adults of the same generation, to avoid possible variation in physiological status.

Gregarious and solitarious locusts were reared in separate rooms, located in different parts of the building, each measuring 1.5 x 4.5 m. Standard aluminum cages (10 x10 x 24 cm) and (50 x 50 x 50 cm) described by Ochieng-Odero *et al.* (1994) were used for rearing solitarious and gregarious insects, respectively (Fig 3 and 4).

A photoperiod of 12:12 LD was maintained in the experimental cages using an electric timer connected to the source of power. Temperature was kept at 30±2 ^{o} C for both dark and light phases. The R.H was maintained at 40-50%. The rooms



Solitary locust cage

Fig. 3 Cage for rearing solitarious desert locust.

CAGE FOR REARING GREGARIOUS DESERT LOCUST

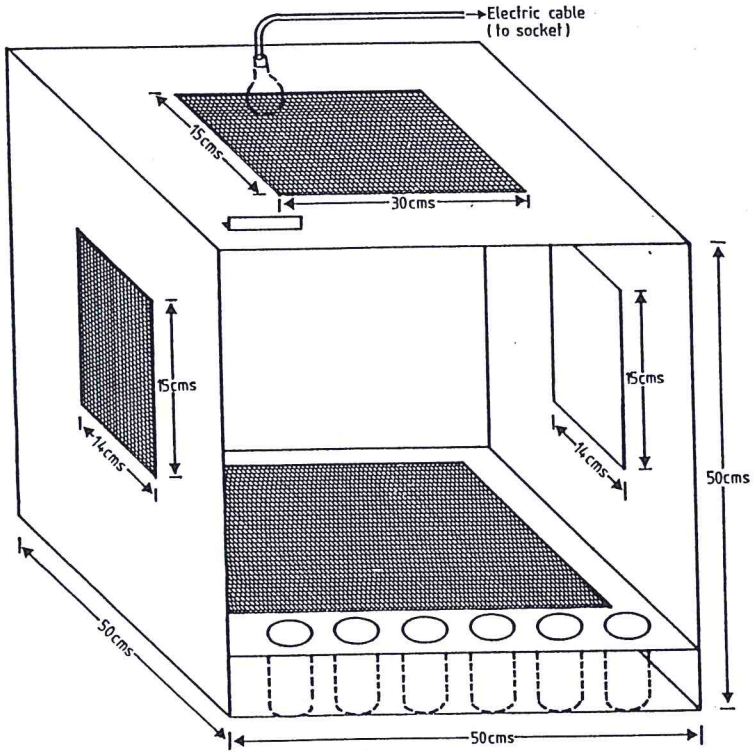


Fig. 4 cage for rearing gregarious desert locust.

were aerated by a duct system which maintained negative pressure that allowed about 10-15 air changes per hour throughout the experimental period.

3. 2 Experimental Cages

Three different cages were used in the present study:

(1) Galvanized aluminium wire mesh cages (100 x 100 x 50 cm) were used for studies on the effect of localized (L) and delocalized (D) food on the gregarization of solitary locusts (Fig 5).

(2) A bichamber cage (15 x 15 x 30 cm), with three sides made of aluminium and a glass front was used for studying primer effects (Fig 6).The upper portion 15 x 15 x15 cm, was similar in dimensions to the lower portion. Each cage had a sliding glass front for visibility, feeding and handling of insects, and wire gauze windows on the five sides, one in the middle and four at the sides for ventilation.

(3a) A uniform ring-like aluminium arena subdivided equally into 18-segments (Ellis, 1953b), each corresponding to 20^{o} from the centre, was used for behavioural assays to measure behavioural effects of gregarization in first instars. The diameter of the inner wall of the arena was 14.5 cm, the outer 29.0 cm, with the height being 10 cm. The top of the circular bioassay compartment was covered with a transparent glass sheet to allow for observation and insect count (Fig 7).

(3b) An arena similar to the one described in 3a, with 30-segments, each corresponding to 12^{0} from the centre, inner wall diameter 34.5cm, the outer 45.5cm,

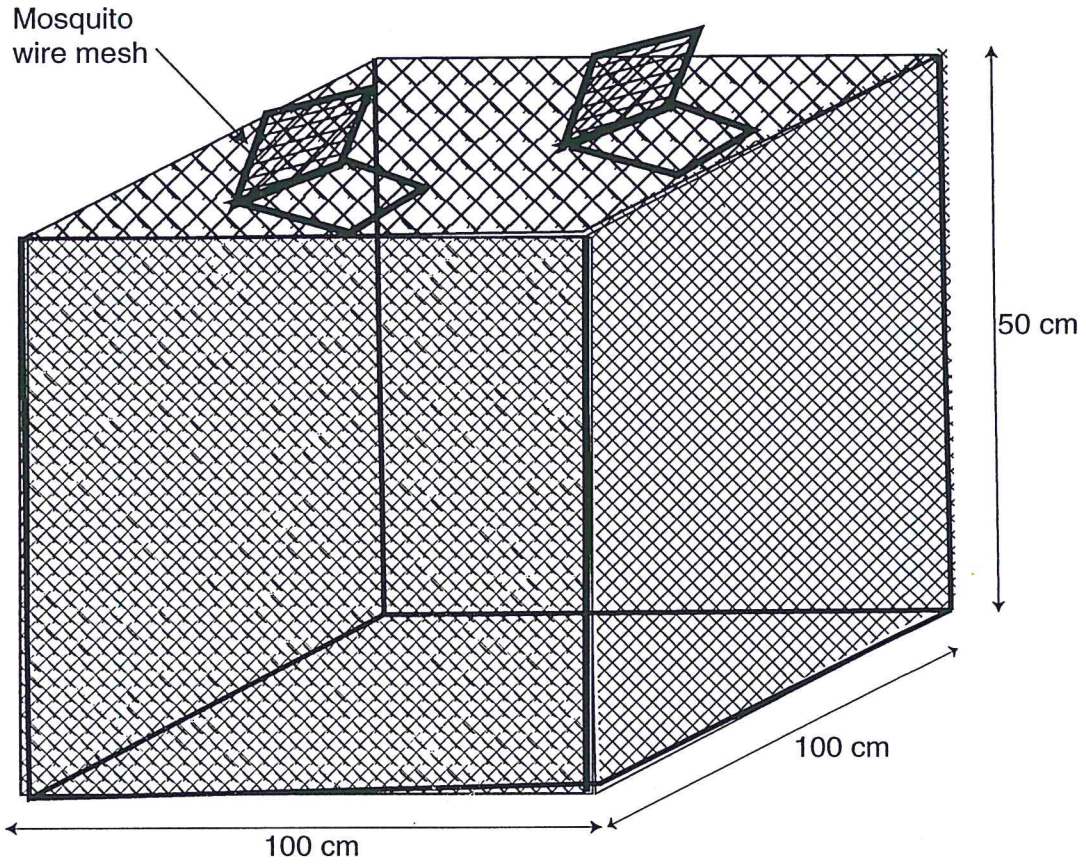


Fig. 5. Galvanized aluminium mesh wire cage for localized and delocalized food effects studies.

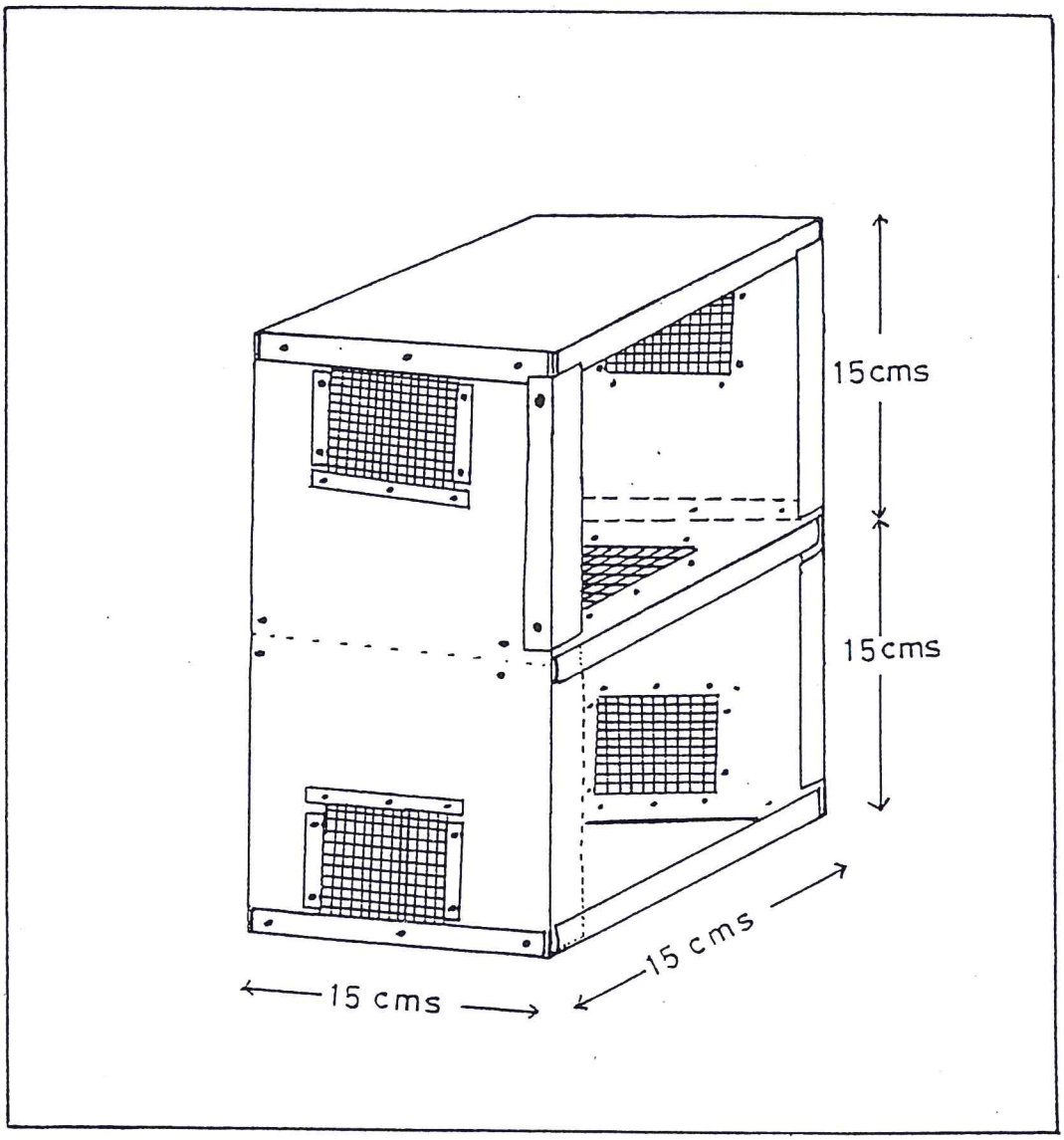
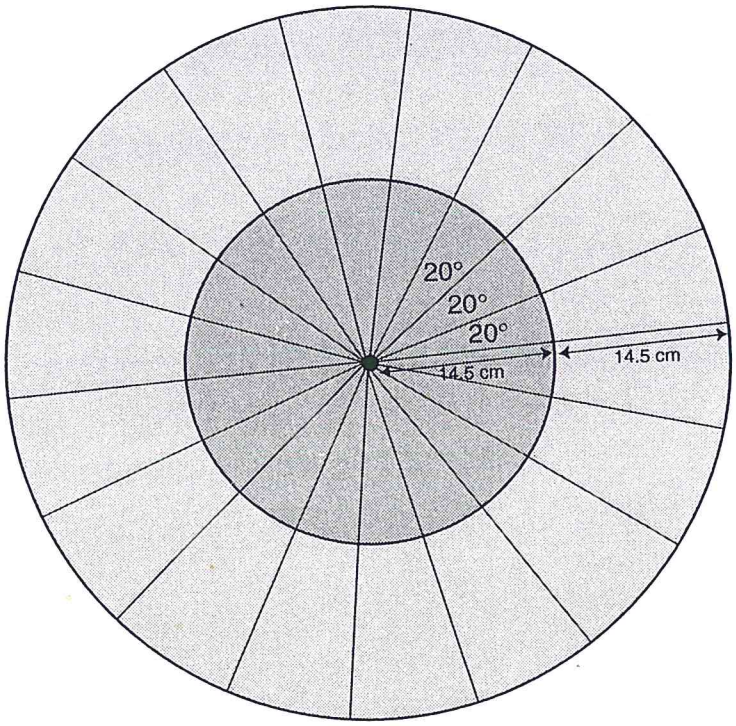


Fig. 6 Aluminium bichamber bioassay cage.



Aluminium circular arena

Fig. 7. Aluminium ring-like arena for behavioural assays.

with same height as in (3a), was used for behavioural assays in hgher nymphs (4^{th} and 5^{th} instars) and mature adult locusts.

3. 3. Food Plants

Fresh leaves of wheat (*Triticum sp*, variety nyangumi), bran and kale (*Brassica oleracea acephela* L.), were provided as food, at daily basis to the insects.

3.4. Soil Preparation and Exposure to Oviposition-Associated Signals

Sand sieved through a wire mesh (2 mm^2 mesh size) was washed sequentially and thoroughly with hot tap water, methanol, acetone and dichloromethane to remove all the inorganic and organic compounds that may be present in the sand. The sand was heat-sterilized in an oven at 205 ^{o}C for 24 hr. After cooling at room temperature, the sand was moistened with distilled water to give a moisture-content of 15 % (15 ml of water added to 100g of sand); (Norris, 1968). Standard aluminum oviposition cups (10 x 4 cm -ID) were filled with moist sand and placed in the holes at the front of the false floor of standard aluminum locust rearing cages (50 x 50 x 50 cm) containing gregarious laying females. After 48 hr, egg-pods deposited by gregarious females were removed from the sand and the content of the cups sieved to remove remnants or debris of eggs and froth. The moisture content of the sieved sand was restored to its original level of 15 % by adding an appropriate amount of distilled water before placing solitary egg-pods in its (as treated sand).

3.5 Chemicals

Phenylacetonitrile (PAN), methanol, acetone and dichloromethane (HPLC grade) were purchased from Aldrich Chemicals Ltd, Gillingham, Uk. The purity of each chemical was, ca. 99 % .

3.6 Parameters Monitored

Physiological markers monitored included phenylacetonitrile titres (Torto *et al.*, 1994) determined by gas chromatography and, haemolymph pigment composition (Mahamat *et al.*, 1993) determined by spectrophotometry. Physical indicators monitored were body colour change and the biometric ratios adult fore wing (E) to hind femur (F), E/F and, the hind femur to greatest width of head capsule (C), F/C.

3. 6. 1. Collection of volatiles

Volatiles were collected from test insects as described by Torto *et al.* (1994). Activated charcoal (30mg, 80-100 mesh, Chrompack International, Middelburg, Netherlands) was packed between two glass wool plugs in 6 cm long x 8 mm-ID glass tubes. Before use, the charcoal was cleaned thoroughly by Soxhlet extraction with dichloromethane (Merck, Germany) for 72 hours and, then followed by activation at 250^{o}C under a stream of nitrogen (20 ml / min.) for 30 minutes. Air from a compressed air cylinder was filtered through activated charcoal and then over locusts, either nymphs or adults, contained in a quick-fit glass tube (14 cm long x 2.5 cm-ID) and, then through the activated charcoal which adsorbed the locust

volatiles (Fig 8). All the quick-fit joints were sealed with Teflon tape to avoid air leakage during trappings. Aerations were performed at a flow rate of 106 ml / min., maintained for 15 hr at 31±2°C. Volatiles were collected from sets of nine adult male locusts selected randomly in groups of three, at seven different ages: 10-12, 15-17, 20-22, 25-27, 30-32, 35-37 and 40-42 days-old after fledging. The charcoal traps were eluted with 4ml HPLC grade (Aldrich Ltd, UK) dichloromethane. The volatile extracts were stored at -15°C and concentrated to 100µl under a stream of nitrogen at 0°C prior to analysis by gas chromatograpy (GC).

3.6.2 Analyses of volatiles

Volatiles analysis was carried out as described by Torto *et al.*(1994). An aliquot (5 µl) of the concentrated volatile samples were analyzed by capillary gas chromatograpy on a Hewlett-Packard (HP) 5890 Series II gas chromatograph, equipped with a flame ionization detector (FID) and a HP capillary column (Carbowax 20 M x 50 m x 0.2 mm ID x 0.2 µm), using nitrogen as a carrier gas at a flow rate of 0.35 ml / min. The oven temperature was initially isothermal at 60°C for 10 min., then programmed at a rate of 5 °C/min. to 180 °C and, then to 220°C at a rate of 10°C / min. Chromatographic peaks were integrated using a HP 3396 Series II integrator. Triplicate injections from three different collections for each age group were analyzed. The mean level of phenylacetonitrile released was estimated by GC using an authentic sample of the compound. estimate the mean level of phenylacetonenitrile released.

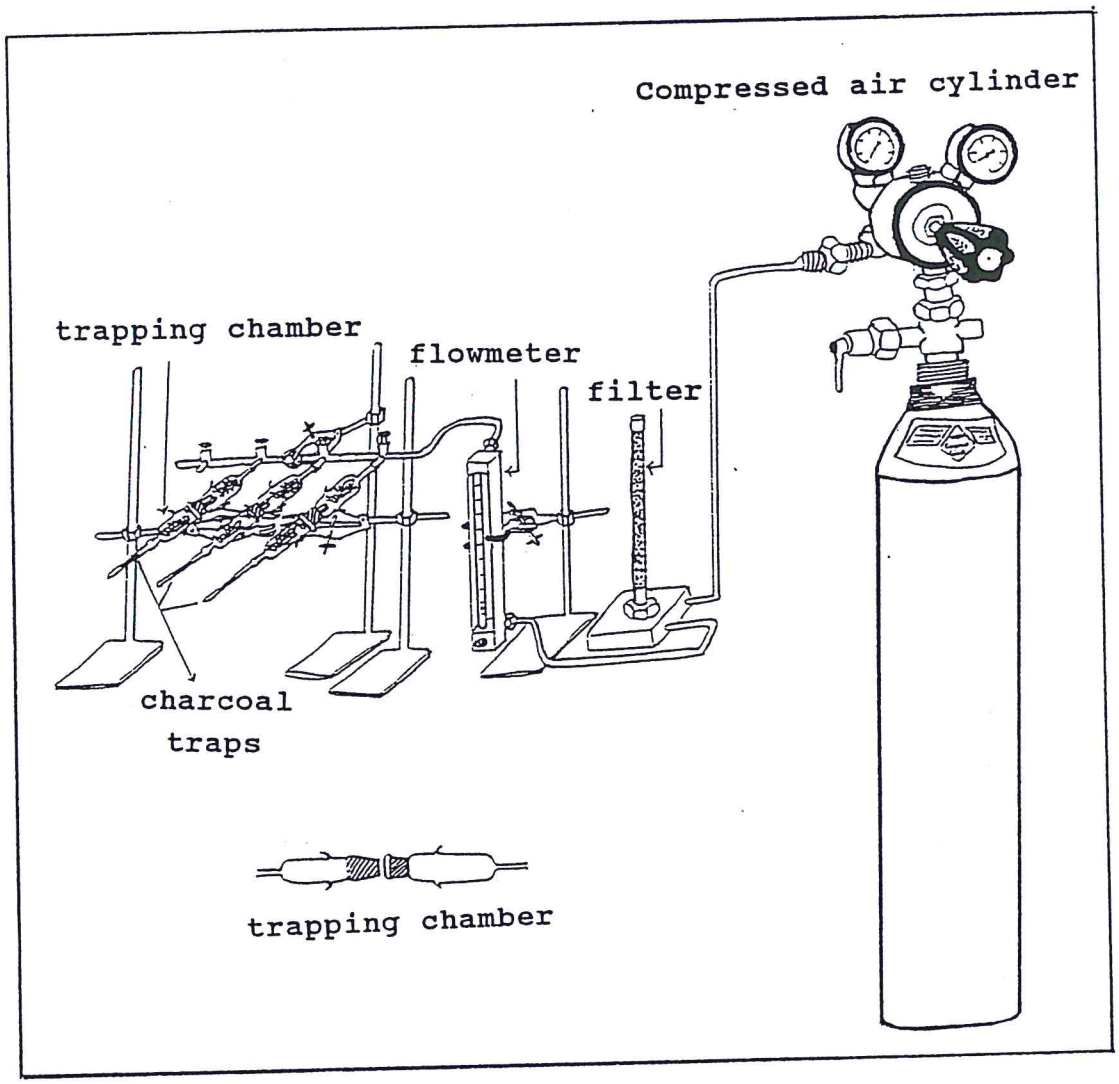


Fig. 8 Volatiles collection assembly for both nymphs and adult *S. gregaria*.

3. 6. 3 Collection of haemolymph samples

Haemolymph samples (10µl) were collected as described by Mahamat *et al.*(1995) from adults (25-27 days old after final moult). Samples were collected by first puncturing the pre-coxal cavity of the hind femur of 26 day-old adults with a microlane needle (0.5 mm ID). The haemolymph was sucked with a micro-capillary tube and, then drained into a Eppendorf tube containing a bleeding buffer (490µl) or mordue. Samples were preserved at -15 ^{o}C prior to analysis.

3.6.4 Analysis of haemolymph

Spectral analysis of the haemolymph samples were carried out on a Beckman DU-50 spectrophotometer between the wavelength range of 300-700nm. The ratio of absorbance at 460 and 680 nm (Fig. 9) were computed for each sample and compared for the control and treated insects. Ten samples were analyzed per group (Mahamat *et al.*, 1995).

3.6.5 Morphometrics

Morphometrics measurements were taken as described by Ochieng-Odero *et al.* (1994) for both sexes from 15-17- day -old adult locusts of both sexes. This was carried out by measuring the lengths of fore-wing or elytron, E, hind femur,F and the greatest width of head capsule, C (Fig.10), using an electric caliper (Trimos Syvac Meteorology Ltd, London, UK), with a range of 0-150mm and an accuracy of 0 ± 0.03mm. The E/F and F/C ratios from these measurements were calculated using the

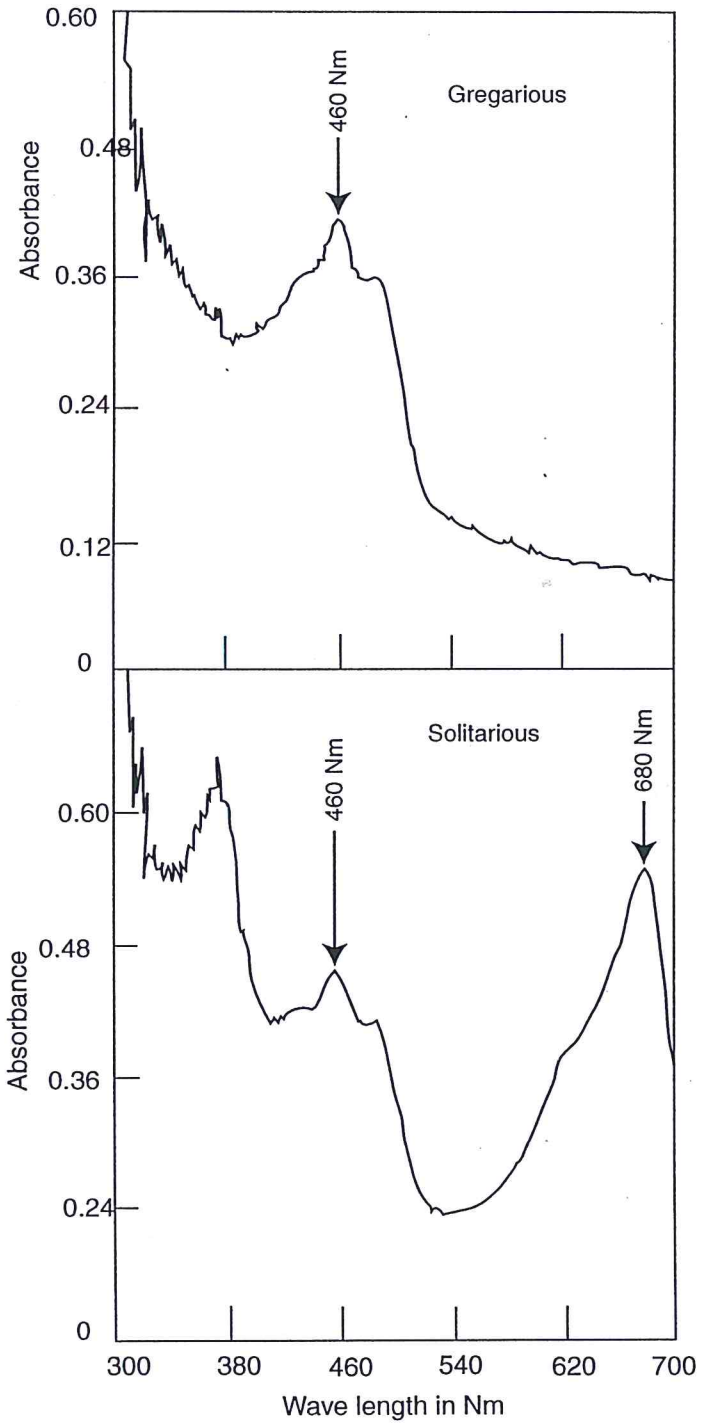


Fig. 9. UV visible absorption of S. gregaria (Forskal) haemonymph.

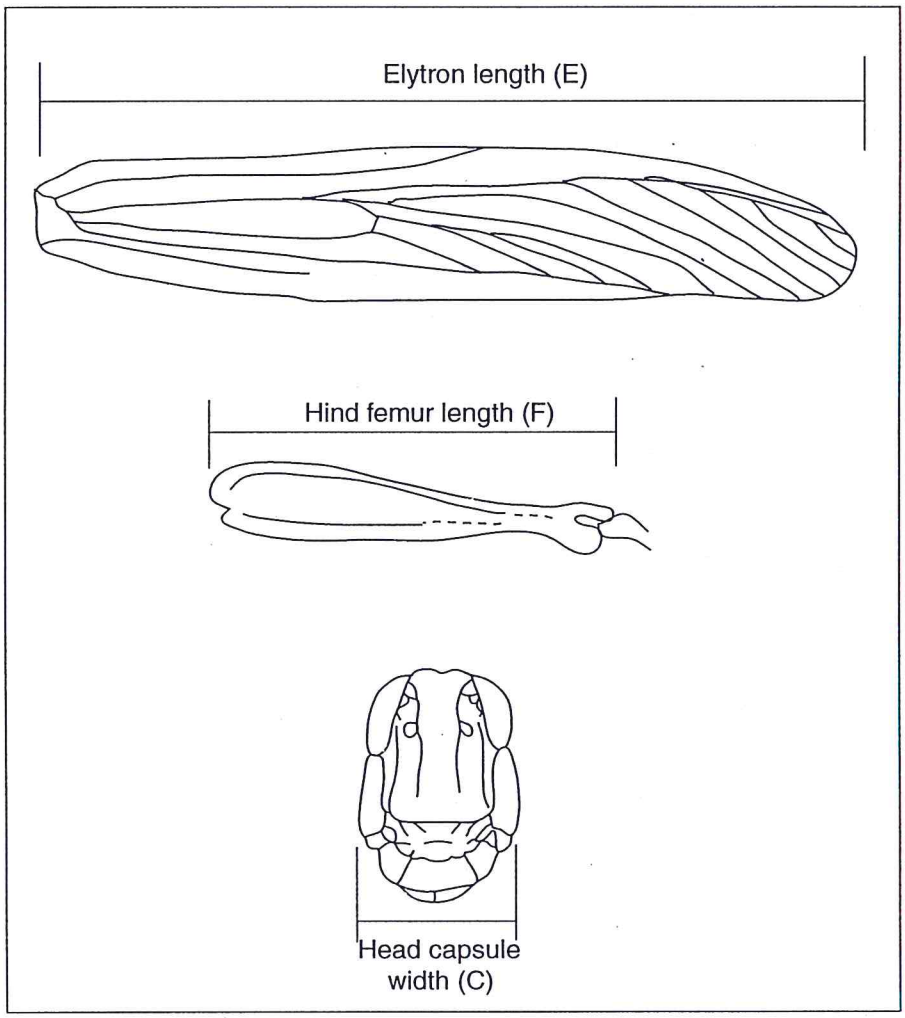


Fig. 10. Desert locust (S. gregaria) body parts measured.

method described by Dirsh (1953) and compared for phase differentiation. Ten locusts from each sex were used per treatment.

3.6.6 Body Colour Changes

Visual observation on body colour changes were carried out on nymphs and adults of the test insects and compared to control. The appearance of black colour (melanin) on the yellow background in nymphs, while pink and bright yellow colour in fledglings and in the mature adults males, respectively, were checked in tested solitarious locusts which were raised at densities of 4, 6, 8, 10 and 12 in both localized (L) and delocalized (D) food situations. For exposure experiments, appearance of green or decreased in gregarious colour was monitored in nymphs and adults. These observations were compared in both test and control insects.

3. 7. Data Analysis

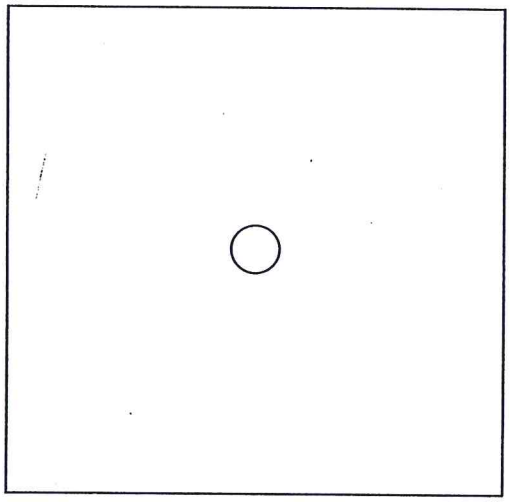
Mean titres of pheromone and those of E/F and F/C ratios were transformed to Log (x + 1) for normality before being subjected to analysis of variance (ANOVA). Differences between transformed means were compared using the least significant difference test (LSD) at p (= 0.05) with a SAS statistical package 1988 (Version 6.04). Logarithmic means of absorbance ratios of haemolymph pigment composition, body weights were first square root transformed and before being subjected to ANOVA followed by LSD at $P < 0.05$ using SAS (1988).

3. 8. Experimentations

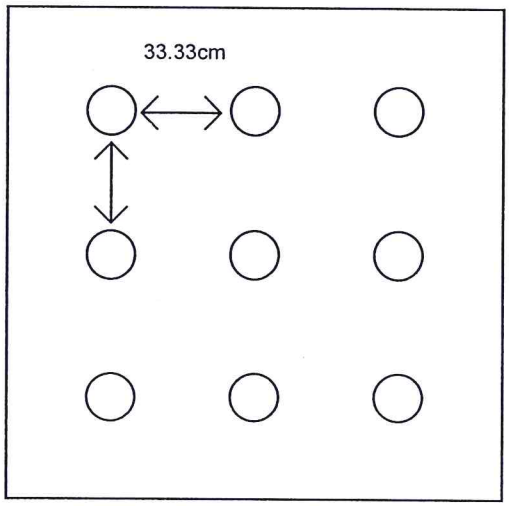
3. 8. 1. Effect of localized and delocalized food on gregarization of solitarious hoppers (Objective I).

In order to investigate the effect of food distribution on gregarization of solitarious locusts, newly moulted third-instar (1-day-old) nymphs of a solitary population were reared on two plants, via wheat and kale, separately as localized (L) and delocalized (D) food until adult stage at densities of 4, 6, 8, 10 and12 locusts per cage (100 x 100 x 50 cm). Each situation was replicated three times. The control consisted of a single nymph from the same solitarious nymphal population and age, placed in a standard solitary rearing cage (10 x 10 x 24 cm). Twenty such nymphs of the stock were kept isolated. A second control consisted of gregarious insects provided with food located randomly. It was assumed that because of the strong cohesion naturally maintained between individual gregarious insects, the degree of gregarization in these insects reared on localized and delocalized food would be insignificant.

The same food biomass (30g each) was presented to a group of nymphs in a treatment arena, either as a bunch placed centrally in a plastic cup (localized food) or clumps equally distributed in nine positions in a film holder (delocalized food), separated by an equal distance of 33.33cm throughout in the cage (Fig11). Fresh wheat leaves and bran were provided daily for all treatments. A temperature regime of 30-32^{o}C and a photoperiod of 12 : 12 L:D maintained by three diffused



Localized food



Delocalized food

Fig. 11. Treatments, localized and delocalized food distributions

fluorescent light tubes (2ft each, 20 watts) were placed above the cage to avoid insects gathering around possible hot points (see preliminary study, appendix 1).

Two separate rooms were used for crowding and isolation experiments. Gregarization was monitored using pheromone titres, haemolymph pigment ratio, body colour changes and biometric ratios as described in section 3.6

3. 8. 2. The effect of gregarious locust volatiles on the rate of solitarization of isolated conspecifics (Objective II).

In order to investigate Objective II, individual locusts (referred to as recipients) drawn from the gregarious stock of fourth-instar nymphs or mature adults, were exposed to gregarious nymphs or mature adults of the same population in a double storey aluminum cage, described in section (3. 2). The double cage was separated in the middle with a wire gauze which allowed the recipients in the lower portion to perceive the volatile emitted by the pheromone source from the upper portion. To avoid visual and tactile contact a piece of black cloth was placed on the wire gauze between the two portions. Conspecific nymphs (1-day-old 4th instar) or mature adults (3-weeks-old) of mixed sexes at densities of 5, 10 or 20 were placed in the upper compartment of the double storey cage. These constituted the source of the pheromone signal. Individual recipients were placed in the lower compartment without visual or tactile contact with the insects in the upper compartment. Treatments were as follows:

RECIPIENT	PHEROMONE SOURCE	NO. OF INSECTS RECIPIENT: SOURCE
a) mature adult male	mature adults of mixed sexes	1: 5, 10 or 20
b) fourth-instar male or female	mature adults of mixed sexes	1: 5, 10 or20
c) fourth-instar male or female	fourth-instar of mixed sexes	1: 5, 10 or 20
d) mature adult male	fourth-instar of mixed sexes	1: 5, 10 or 20

Each treatment was replicated three times. The experimental insects were daily fed with fresh wheat seedlings and bran. The rearing conditions were as described under Materials and Methods. Recipients were checked after 5 days for signs of gregarization as assessed by: (1) pheromone titre, by measuring phenylacetonitrile levels in mature adult males and total acids and aldehydes in nymphs (since the volatiles of nymphs exposed to the pheromone emissions of conspecifics or mature adults contained very low amounts of pheromone constituents, aldehydes and acids; the levels of these constituents were summed up for each treatment); (2) behavioural change measured by distribution of nymphs per equal segments of arena; (3) body colour change; and (4) weight.

3. 8. 3. Primer effect of sand-associated oviposition pheromone on nymphs that emerged from solitarious eggs (Objective III).

In order to investigate Objective III, outlined above, egg-pods from solitarious females were placed in sand treated as follows:

(i) Clean sand with 15% moisture in which gregarious females had previously laid 3, 5 or 10 egg-pods.

(ii) Sand as above which was sequentially washed with methanol, acetone and dichloromethane and, then dried in an aluminum foil tray under room temperature maintained at 30 ± 2 ° C for 24h and moistened to 15%. as in (i).

(iii) Sand as in (i) which was flushed with nitrogen for 24h and its moisture-content restored to 15 % by adding appropriate amount of distilled water.

All solitarious egg-pods in the treated sand were transferred into an incubator prior to hatching. After 7 days, they were checked daily for hatching and the dried samples were moistened by adding distilled water to maintain their moisture-content until hatching, which took 12-15 days.

After hatching from each oviposition cup, 12 newly hatchlings (1-24 h-old) which emerged from solitarious egg-pods from different treatments were released randomly into the bioassay arena, previously described under section (3. 2). Their grouping behaviour was recorded as number of nymphs per segment after 20 min when they had settled down. The number of cage segments that contained 0, 1, 2, 3 and more nymphs was noted. Preliminary assays showed that groups of 4 and 5 were rare. So these were included into the group with 3 nymphs. After each reading, the test insects were disturbed by tapping the top of the cage several times after which they were allowed to settle before the next recording after 20 minutes. The procedure was carried out repeatedly for 2 hrs which resulted into 6 readings /

replicate. The mean score per replicate was computed. Each treatment was replicated five times. The aggregation behaviour of solitary hatchlings from the treatments was compared to those of solitary and gregarious controls. Between experiments, the bioassay arena was thoroughly cleaned with a detergent and, then rinsed with acetone to remove odour stains before drying in an oven at of 100^{o}C for 15 min.

CHAPTER FOUR

4. RESULTS

4. 1. Effect of Localized and Delocalized Wheat Seedlings on the Degree of Gregarization of Desert Locust.

4. 1. 1. Phenylacetonitrile titre

The means of phenylacetonitrile (PAN) titres of adult males reared on both localized and delocalized wheat seedlings increased with increasing locust density. However, the titre levels were intermediate between the levels for controls, gregarious and solitarious (Fig 12, 13, 14). For the same locust density, gregarization proceeded significantly faster for locusts reared on localized than delocalized food (Fig 15 and 16). In the localized food situation, at 10 and 12 locusts / cage, pheromone production reached a maximum in males at 26 days after fledging (DAF) and then dropped gradually. At 8 locusts / cage, maximum pheromone production occurred 10 days later (36 DAF).

In the delocalized food situation, at 10 locusts / cage, PAN titre reached an optimum at 21 DAF and, did not change significantly with locust age. However, these maxima were significantly lower than the maximum achieved by 12 locusts/ cage, which occurred at 31 DAF. Unlike the localized food situation for 8 locusts / cage, where PAN was detected in the volatile emissions of males, no pheromone was detected for the same locust density exposed to delocalized food. Similarly,

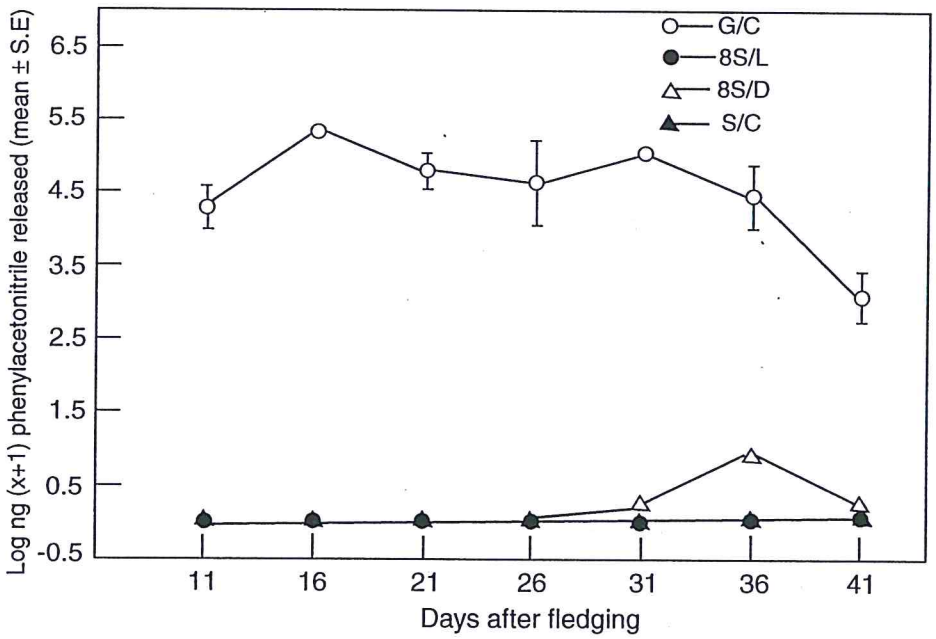


Fig. 12. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) and delocalized (D) wheat (W) at a density of 8 locusts/cage. G/C=gragarious control, 8S/L=8 solitary locusts on localized, 8S/D=8 solitary on delocalized and S/C= solitarious control

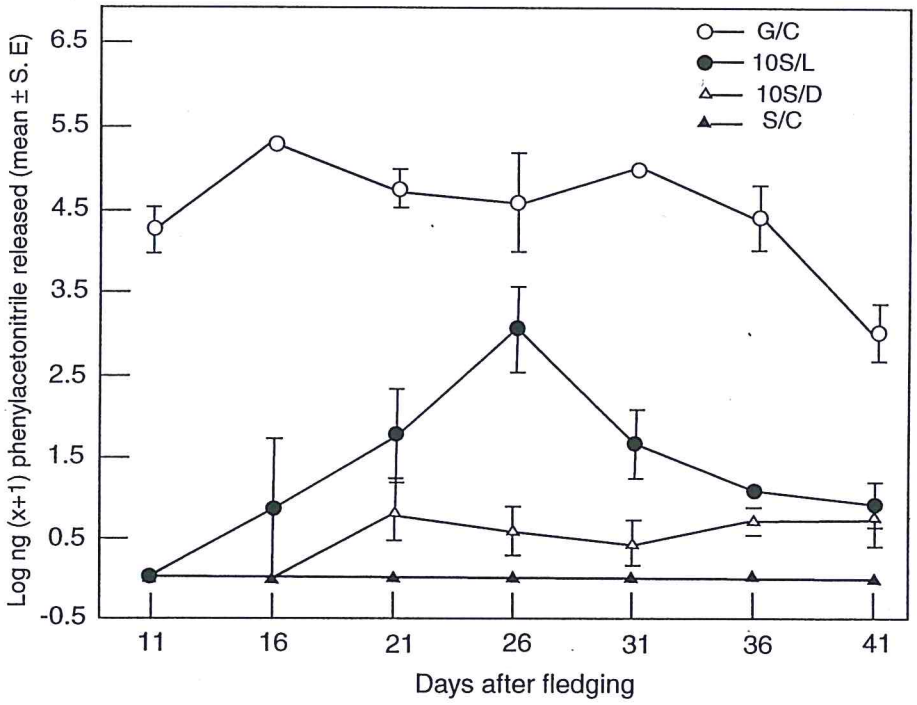


Fig. 13. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) and delocalized (D) wheat (W) at a density of 10 locusts/cage. G/C= gragarious control, 10S/L=10 solitary locusts on localized, 10S/D=10 solitary ondelocalized and S/C=solitarious control.

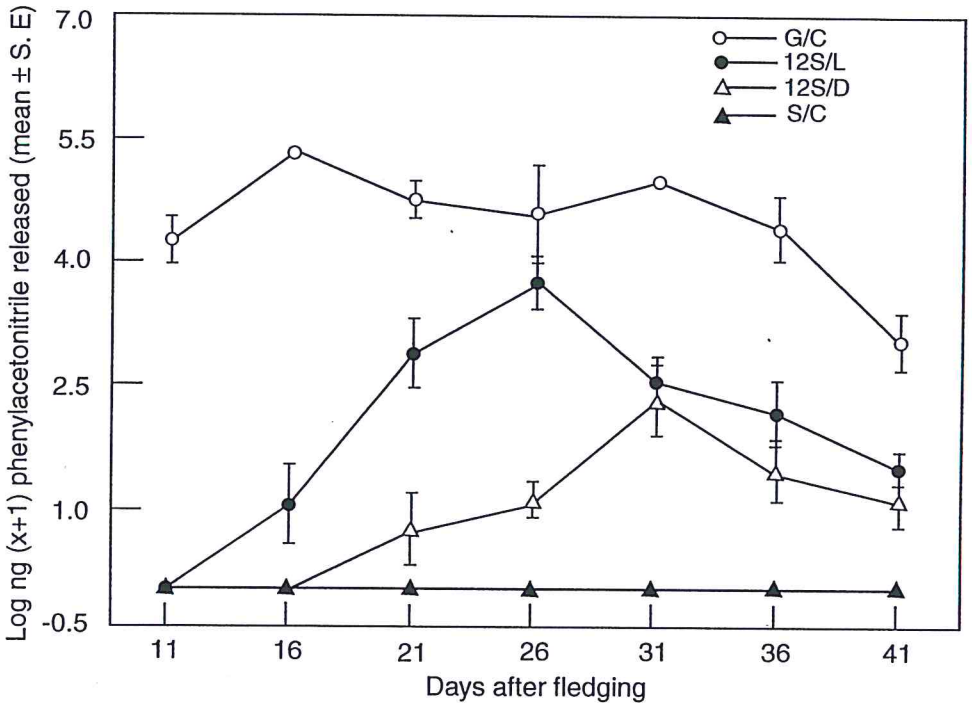


Fig. 14. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) and delocalized(D) wheat (W) at a density of 12 locusts/cage. G/C=gregarious control, 12S/L=12 solitary locusts on localized, 12S/D=12 solitary on delocalized and S/C=solitarious control.

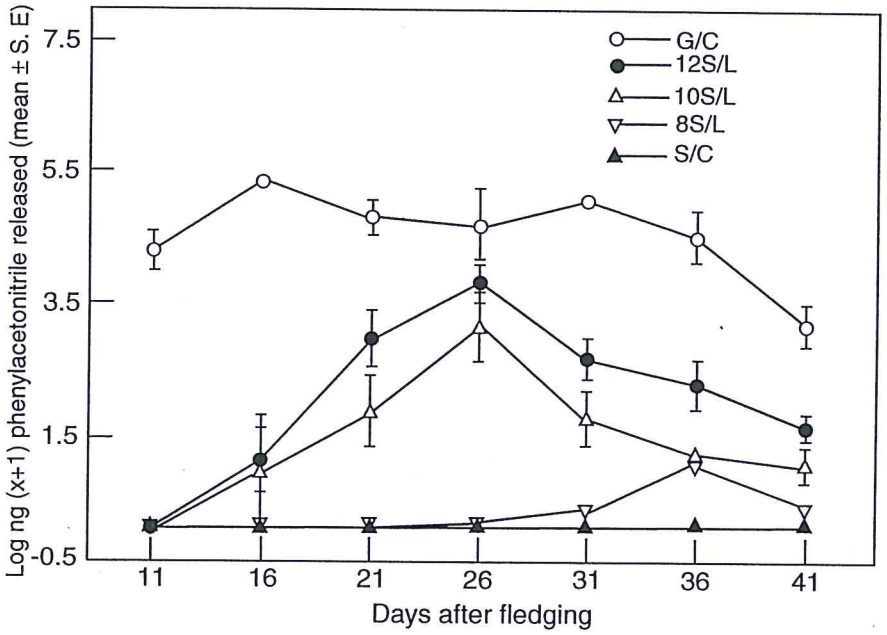


Fig. 15. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) wheat (W) at 8, 10 and 12 locusts/cage. G/C= gregarious control,12S/L=solitary locusts on localized, 10S/L=10 solitary on localized, 8S/L=8 solitary on localized and S/C= solitarious control.

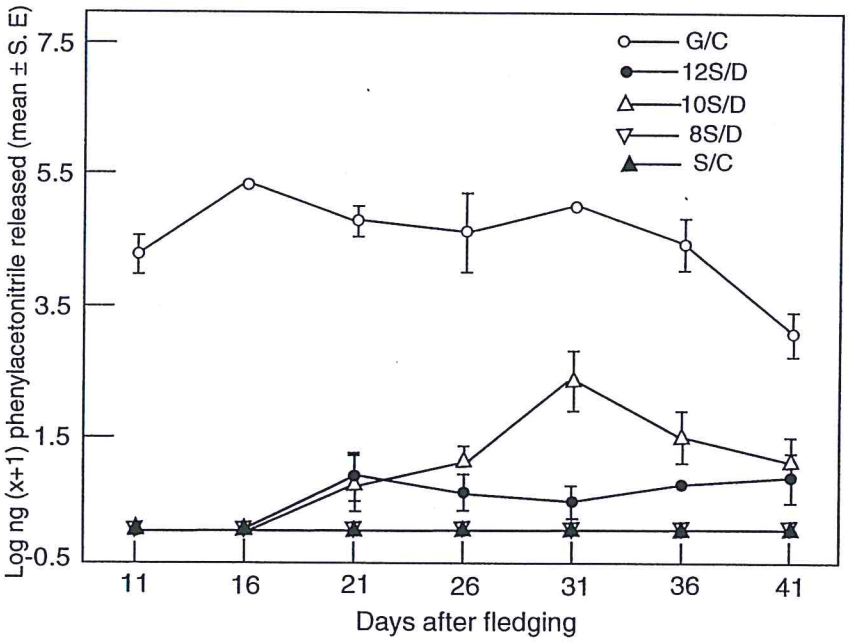


Fig.16. Phenylacetonitrile titres in the pheromone emissions of adult males reared on delocalized (D) wheat at 8, 10 12 locusts/cage. G/C=gregarious control,12S/D10 solitary on delocalized and 8S/D=8 solitary on delocalized.

no pheromone was detected for locust densities of 4, 6 and 1/ cage for both localized and delocalized food situations.

4. 1. 2. Absorbance ratio (Haemolymph Pigment Ratio)

The mean absorbance ratios of the haemolymph pigments of adults emerged from solitarious nymphs reared on localized and delocalized wheat seedlings at densities of 4, 6 and 8 per cage were not measured. The mean UV absorbance ratios of locusts raised at 10 and 12 per cage for the two situations (localized and delocalized) were not significantly different from solitarious control, but differed significantly from gregarious control (Fig 17).

4. 1. 3. Morphometrics

The F/C ratios calculated for both adult males and females that emerged from solitarious third-instar nymphs reared on wheat seedlings, as localized and delocalized food, were significantly different from gregarious controls. On the other hand, the F/C ratios of the treated insects were closer to solitarious control (Fig 18).

The mean E/F ratios computed for adults in the experiments with 8, 10 and 12 locusts per cage were more like solitarious control than gregarious locusts (Fig 19).

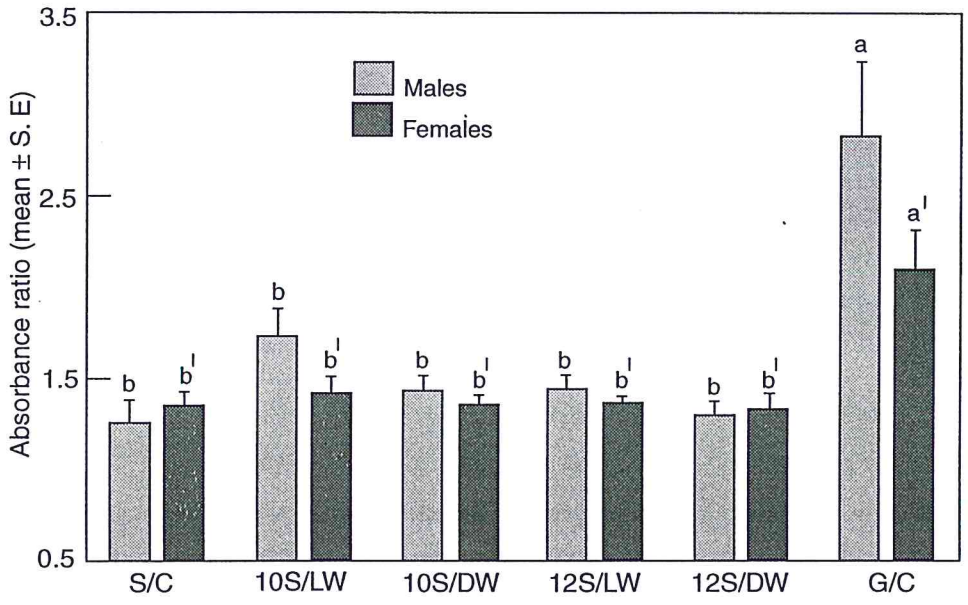


Fig. 17. UV Absorbance ratios at 460 and 680 nm of haemolymph of locusts reared on wheat(W) seedlings. S/C=solitarious control, 10S/LW=10 solitary locusts on localized wheat,10S/DW=10 solitary on delocalized wheat, 12S/LW=12 solitary on localized wheat, 12S/DW=10 solitay on delocalized wheat, G/C=gregarious control.

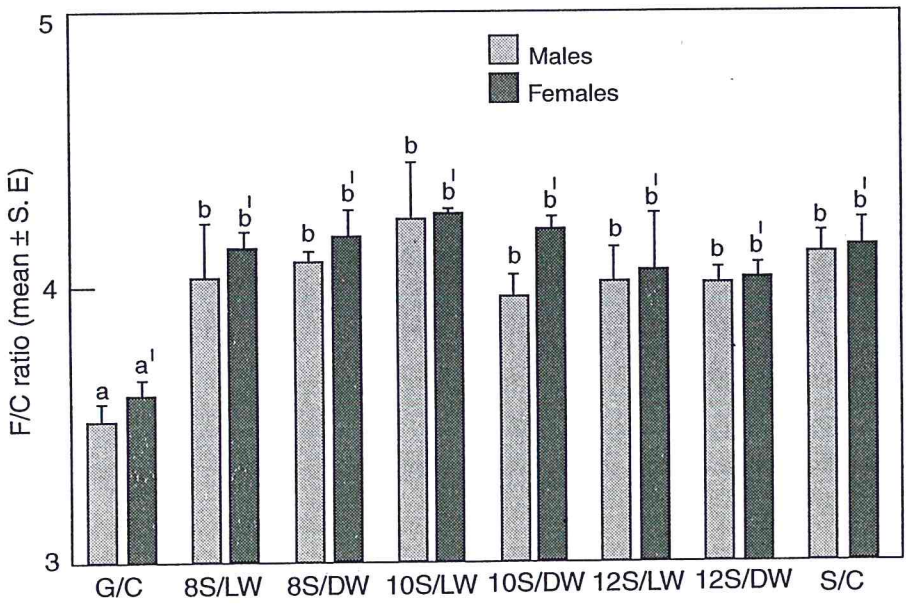


Fig. 18. F/C ratios of solitarious locusts reared on localized (L) and delocalized (D) wheat (W) seedlings. G/C=gregarious control, 8S/LW=8 solitary on localized, 8S/DW=8 solitary on delocalized, 10S/LW=10 solitary on localized, 10S/DW=10 solitary on delocalized 12S/LW=12 solitary on localized, 12S/DW=12 solitarious on delocalized and S/C= solitarious control

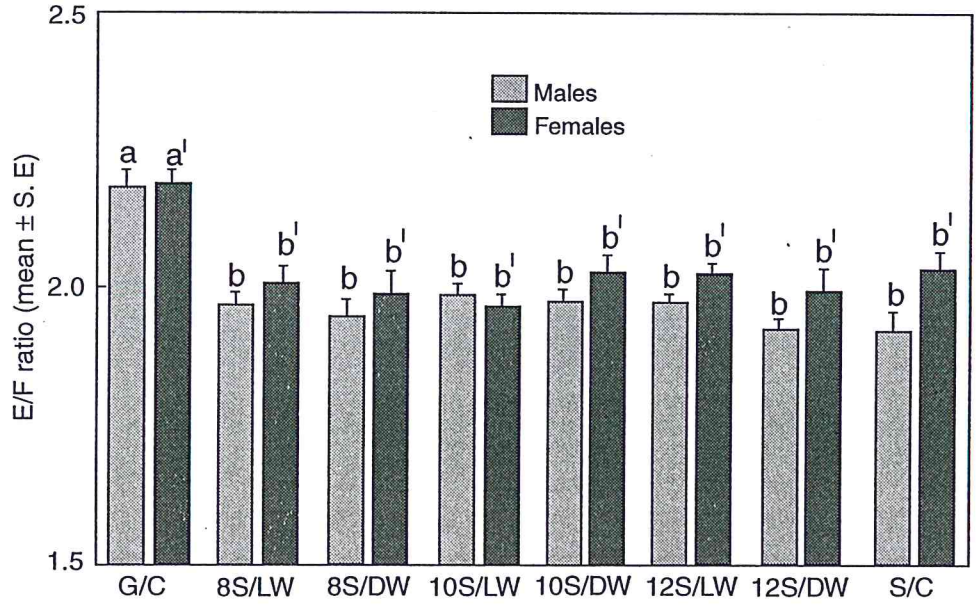


Fig.19. E/F ratios of solitarious locusts reared on localized (L) and delocalized (D) wheat (W) seedlings. 8S/LW=8 solitary on localized, 8S/DW=8 solitary on delocalized, 10S/LW=10 solitary on localized, 10S/DW=10 solitary on localized, 12S/LW=12 solitary on localized, 12S/DW=12 solitary on delocalized, G/C=gregarious control, S/C=solitarious.

4. 1. 4. Colour

The body colour of fifth-instar, fledglings and mature adult males emerged from third-instar reared at densities of 4, 6 and 8 / cage in both localized and delocalized situations were similar to solitarious insects, *i.e.* fifth-instars green or brown, fledglings-greyish and mature adults-beige or greyish. For 8 locusts on localized food and for 10 and 12 locusts in both localized and delocalized food situations, the body colour of nymphs appeared greyish, while fledglings appeared beige or brown similar to their solitarious counterparts. Mature adult males were somewhat different, appearing dull yellow from their solitarious counterparts at a more mature stage of development 30 days after final moult.

4. 2. Effect of Localized and Delocalized Kale Plant on the Degree Gregarization of Desert Locust.

4. 2. 1. Phenylacetonitrile (PAN) titre

Again, the means of PAN titres of adult males reared on localized and delocalized food with kale seedlings increased with increasing locust density. However, the titre levels were intermediate between the levels for controls, gregarious and solitarious (Fig 20, 21, 22). For the same locust density, gregarization proceeded much faster for locusts reared on localized than delocalized food (Fig 23 and 24). In the localized food situation, at 10 and 12 locusts / cage, pheromone production reached a maximum in males at 31DAF and, then dropped gradually. At 8 locusts / cage, maximum pheromone production occurred 5 days later (36 DAF).

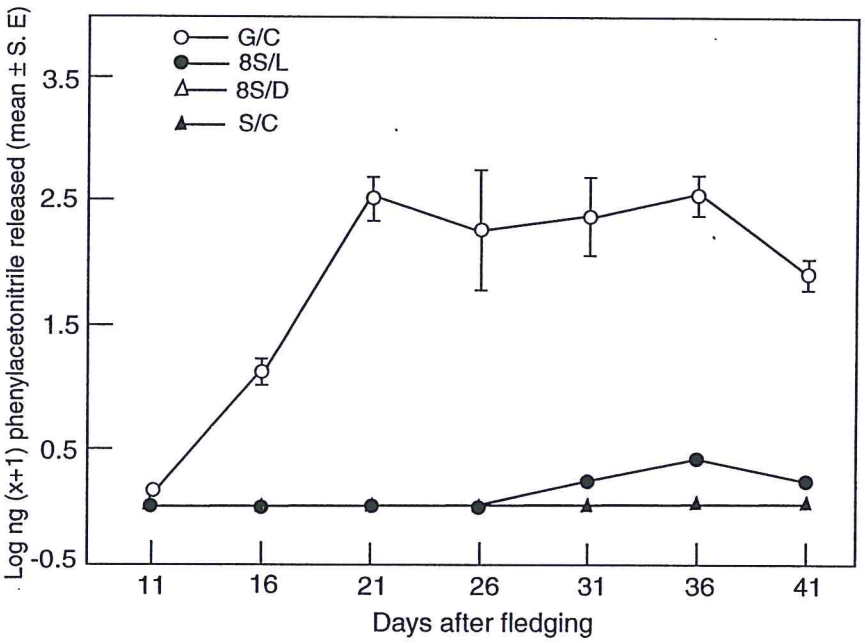


Fig. 20. Phenylacetonitrile titres in the pheromone emissions of adult males reared onlocalized(L) and delocalized (D) kale (K) at a density of 8 locusts/cage. G/C=gregarious control, 8S/L=8 solitary locusts on localized, 8S/D=8 solitary on delocalized and S/C=solitarious control.

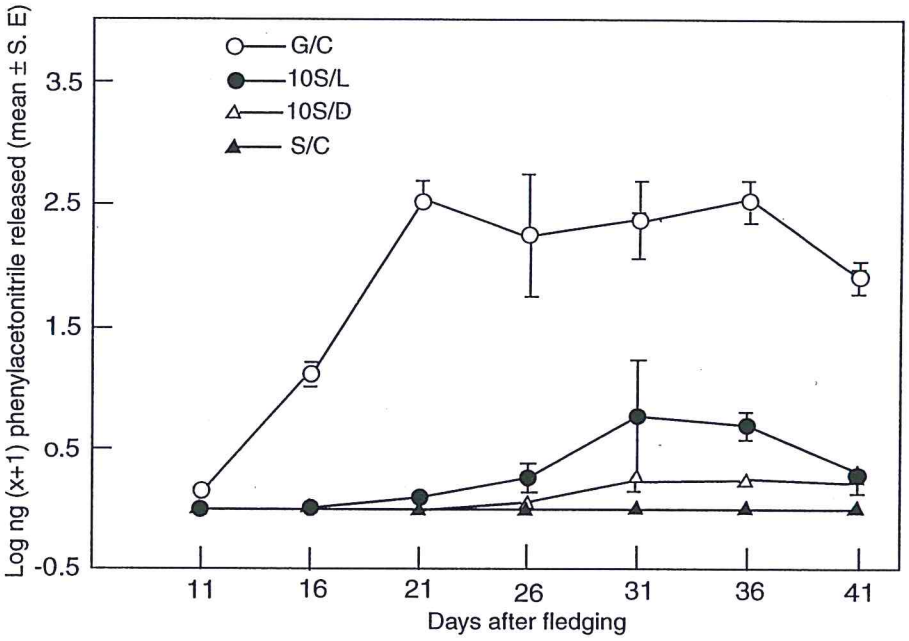


Fig. 21. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) and delocalized (D) at a density of 10 locusts/cage. G/C=gregariouscontrol, 10S/L= 10 solitary locusts on localized, 10S/D=solitary on delocalized and S/C=solitarious control.

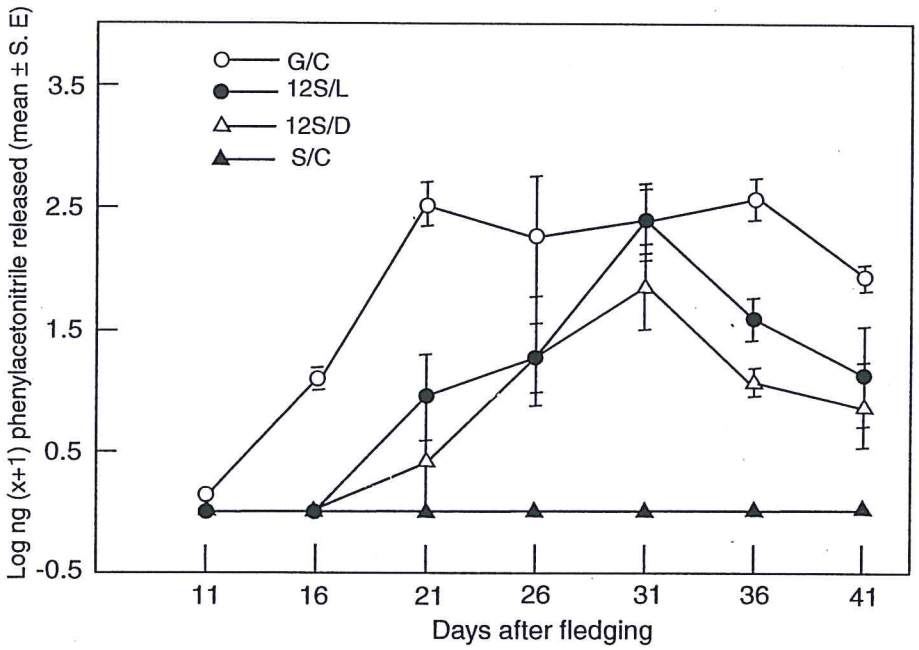


Fig. 22. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) and delocalized (D) kale (K) at a density of 12 locusts/cage. G/C=gregarious control, 12S/L=12 solitary locusts on localized, 12S/D=12 solitary on delocalized and S/C=solitarious control.

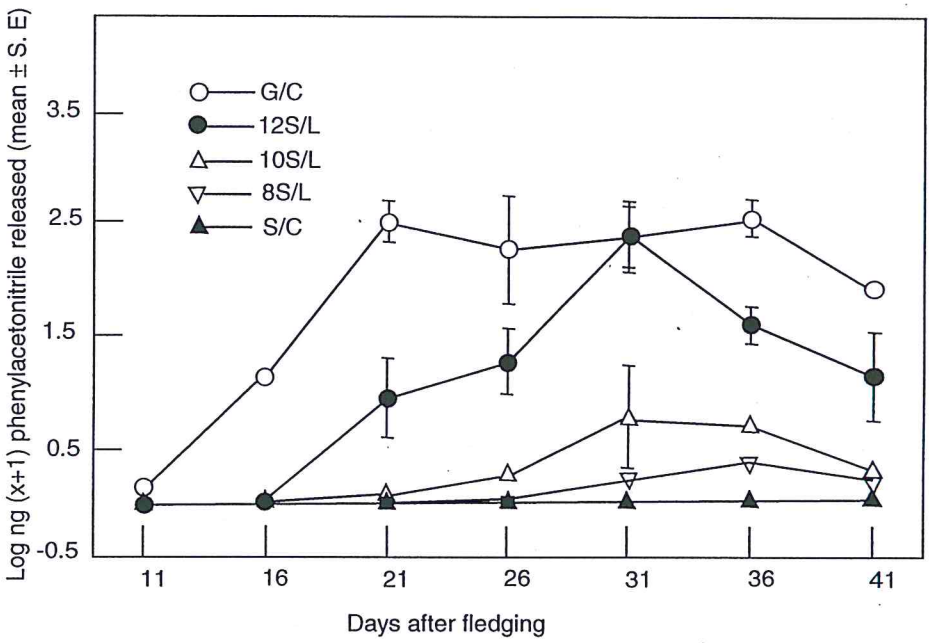


Fig. 23. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) kale (K) at 8, 10 and 12 locusts/cage. G/C=gregarious control, 12S/L-12 solitary locusts on localized, 10S/L=10 solitary on localized, 8S/L= solitary on localized and S/C=solitarious control.

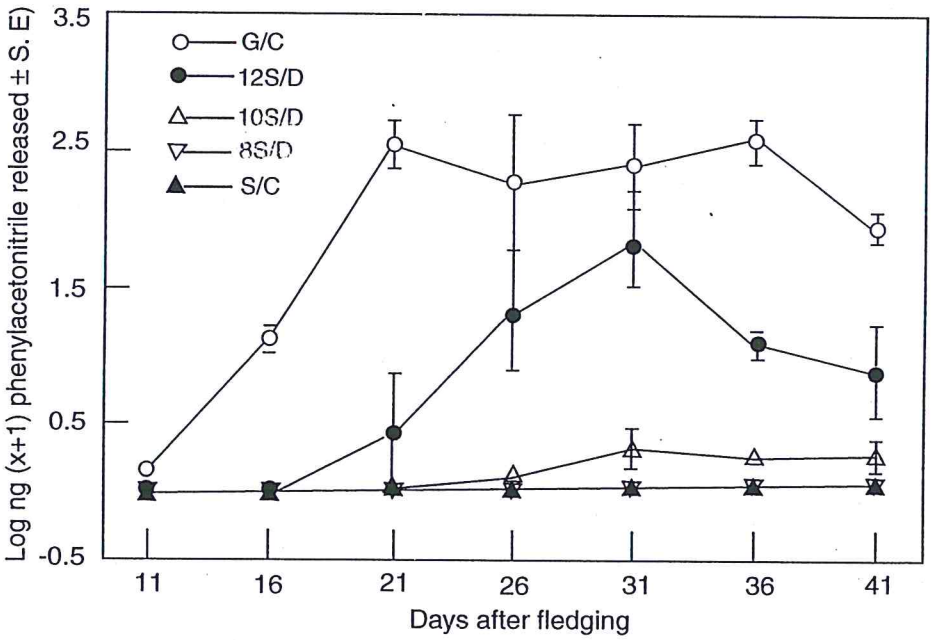


Fig. 24. Phenyacetonitrile titres in the pheromone emissions of adult males reared on delocalized (D) kale (K) at 8, 10 and 12 locusts/cage. G/C=gregarious control,12S/D=12 solitary locusts on delocalized, 10S/D=10 solitary on delocalized, 8S/D=solitary on delocalized and S/C=solitarious control.

In the delocalized food situation, at 10 locusts / cage, PAN titre reached an optimum at 31 days after fledging. However, this maximum was significantly lower than the maximum achieved by 12 locusts / cage which occurred at the same time (31 DAF). Unlike the localized food situation for 8 locusts / cage, where PAN was detected in the volatile emissions of males, no pheromone was detected for the same locust density exposed to delocalized food. Similarly, no pheromone was detected for locust densities of 1, 4 and 6/ cage for both localized and delocalized food situations.

4. 2. 2. Absorbance ratio (Haemolymph Pigment Ratio)

The mean UV absorbance ratios of locusts reared on localized and delocalized food at densities of 10 and 12 per cage, were not significantly different from solitarious control. It differed significantly from gregarious control (Fig 25).

4. 2. 3. Morphometrics

The F/C ratios calculated for both adult males and females that emerged from solitarious third-instar reared on localized and delocalized kale seedlings were significantly different from gregarious control. On the other hand, the F/C ratios of treated insects were closer to solitarious control (Fig 26).

The mean E/F ratios computed for adults which emerged from rearing 8, 10 and 12 locusts per cage on localized and delocalized food were more like solitarious control than their gregarious counterparts (Fig 27).

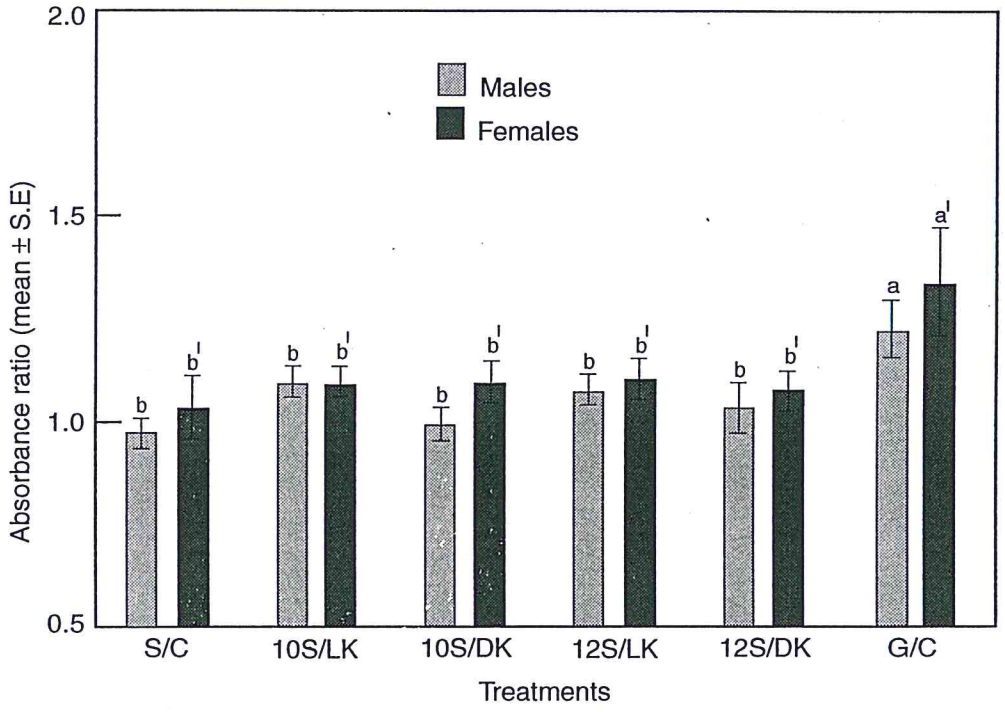


Fig. 25. UV absorbance ratio at 460 and 680 nm of haemolymph of locusts reared on kale (k) seedlings. S/C = solitarious control, 10S/LK = 10 solitary locusts on localized kale, 10S/DK = 10 solitary locusts on delocalized, 12S/LK = 12 solitary locusts on localized, 12S/DK = 12 solitary locusts on delocalized, G/C = gregarious control.

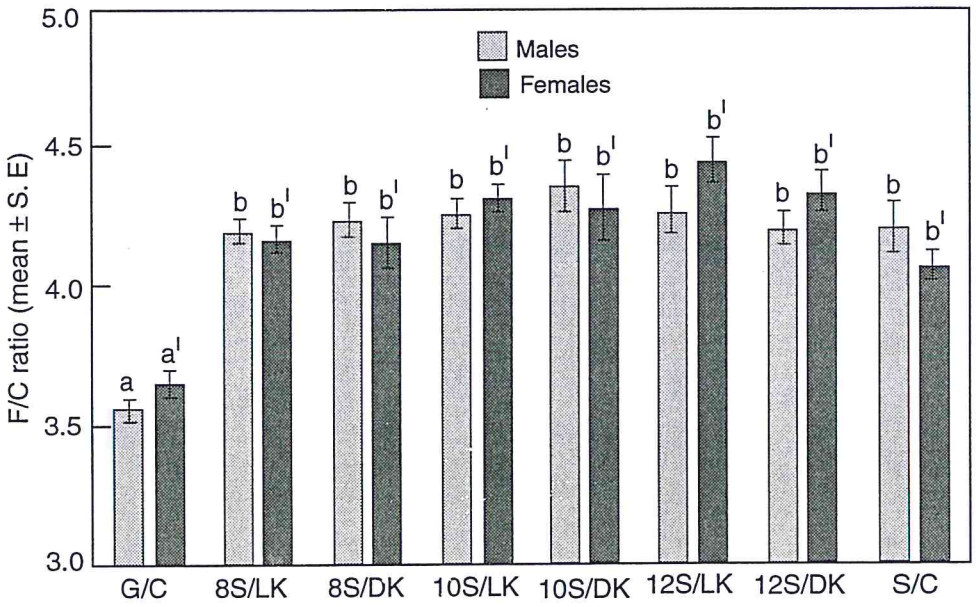


Fig. 26. F/C ratios of solitarious locusts reared on localized (L) and delocalized (D) kale (K) seedlings. G/C=gregarious control, 8S/LK=8 solitary on localized kale, 8S/DK=8 on delocalized. 10S/LK=10 on localized, 10S/DK=10 on delocalized, 12S/LK=12 on localized 12S/DK=12 on delocalized, S/C=solitarious control.

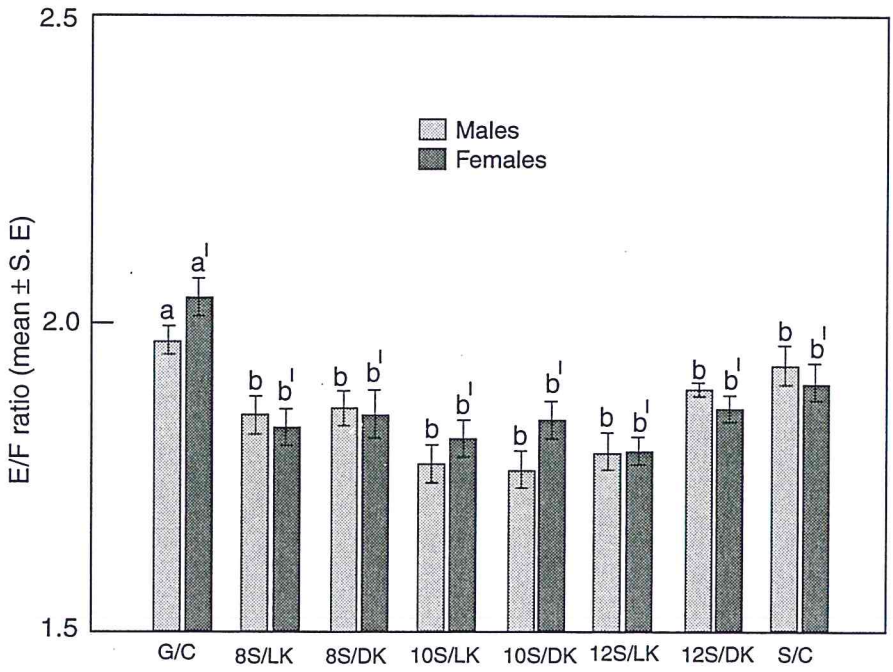


Fig. 27. E/F ratios of solitary locusts reared on localized (L) and delocalized (D) kale (K) seedlings. G/C=gregarious control. 8S/LK=8 solitary on localized kale, 8S/DK=8 solitary on delocalized, 10S/L=10 on localized, 10S/DK=10 on delocalized, 12S/LK=12 on localized, 12S/DK=12 on delocalized, S/C=solitarious control.

4. 2. 4. Colour

The body colour of fifth-instar, fledglings and mature adult males emerged from third-instar reared at densities of 8 per cage on localized food and 10 and 12 locusts / cage in both localized and delocalized food situations was similar to their solitarious counterparts (Plate 1). However, males seemed dull yellow after 30 days as mature adults.

4. 3. The Effect Of Plant Type on the Rate of Gregarization Of Solitarious Locusts

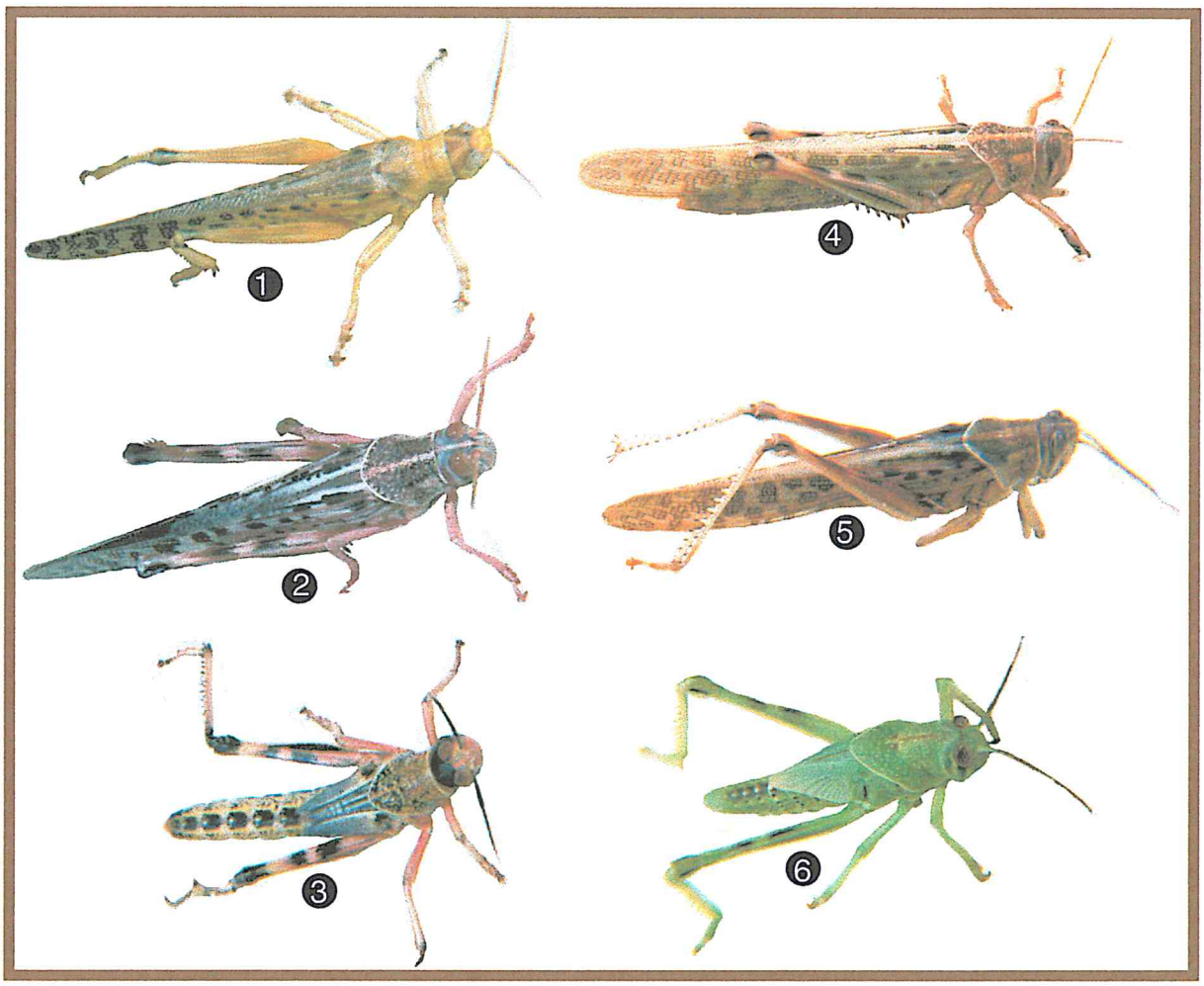
Comparison of the pheromone titres of locusts reared on kale and wheat seedlings separately at densities of 8, 10 and 12 per cage, showed that for the same locust density and for the same food distribution situation, PAN titres were higher in wheat than in kale (Fig 28, 29,30, 31 and 32). For the same locust density, gregarization rate proceeds much further for locusts reared on wheat than on kale.

4. 4. The Effect of Gregarious Locust Volatiles on Solitarization of Isolated Conspecifics

4. 4. 1. Pheromone titres

Pheromone-producing gregarious mature adult males and fourth-instar nymphs which were isolated (and which were not exposed to volatile emissions

Plate 1: S. gregaria mature (1-1), immature adult (1-2), fifth-instar (1-3) from gregarious, mature (1-4), immature adult (1-5), fifth-instar (1-6) from solitarious control.



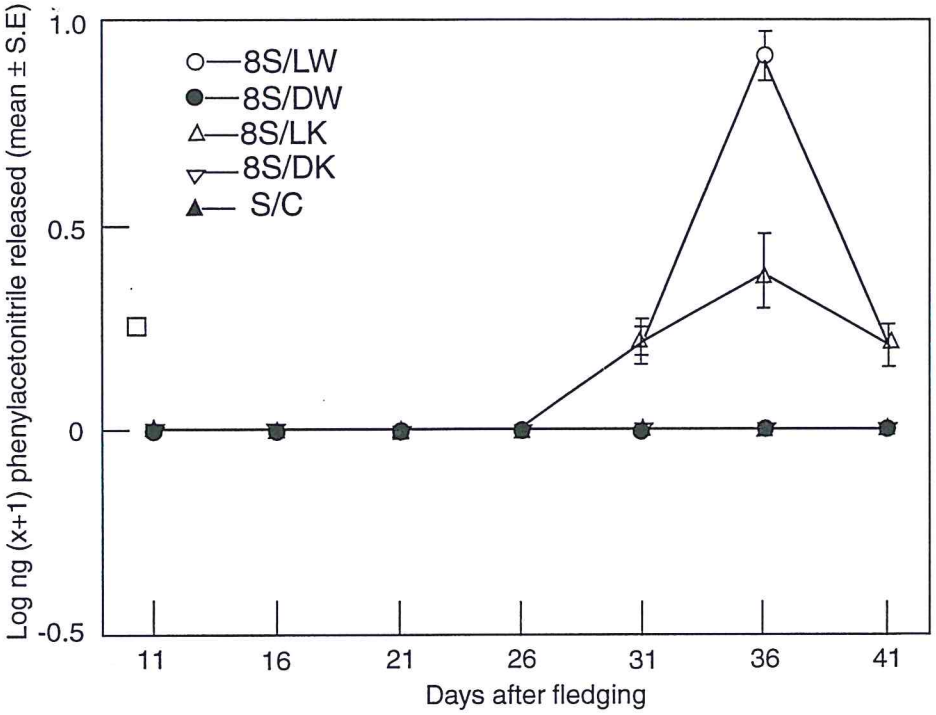


Fig. 28. Phenylacetonitrile titres in the pheromone emissions ofadult males reared on localized (L) and delocalized (D)kale (K) and wheat (W) at a density of 8 locusts/cage.8S/LW = 8 solitary locusts on locolized wheat, 8S/DW = 8 solitaryon delocalized wheat, 8S/LK = 8 solitary on localized kale,8S/DK = 8 solitary on delocalized kale and S/C = solitarious control.

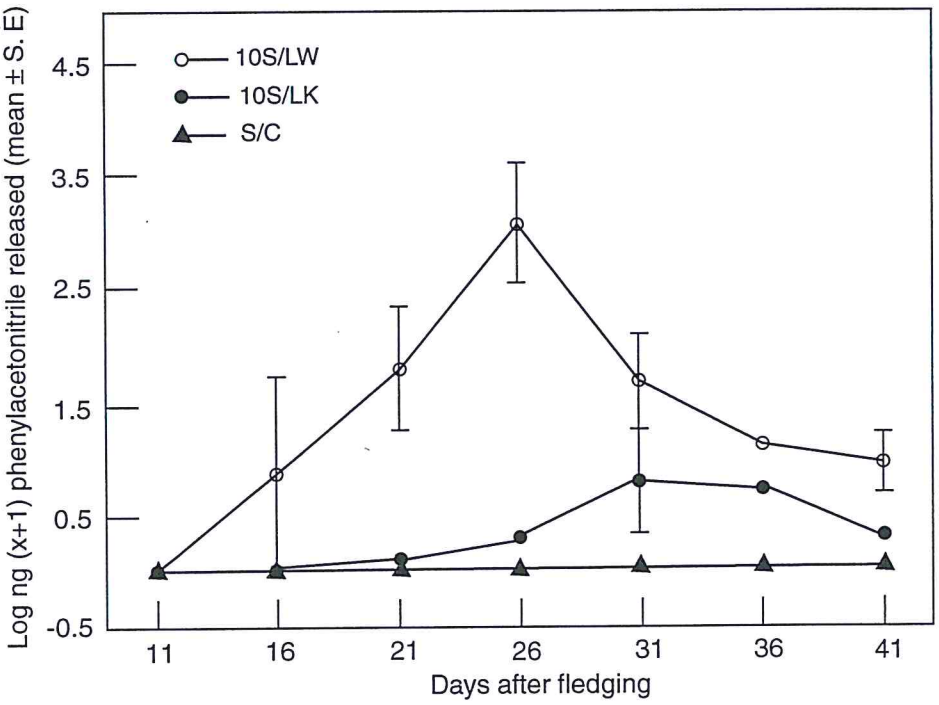


Fig. 29. Phenylacetonitrile tritres in the pheromone emissions of adult males reared on localized (L) wheat (W) and kale (K) at a density of 10 locusts/cage. 10S/LW=10 solitary locusts on localized wheat, 10S/LK=10 solitary on localized kale S/C=solitarious control.

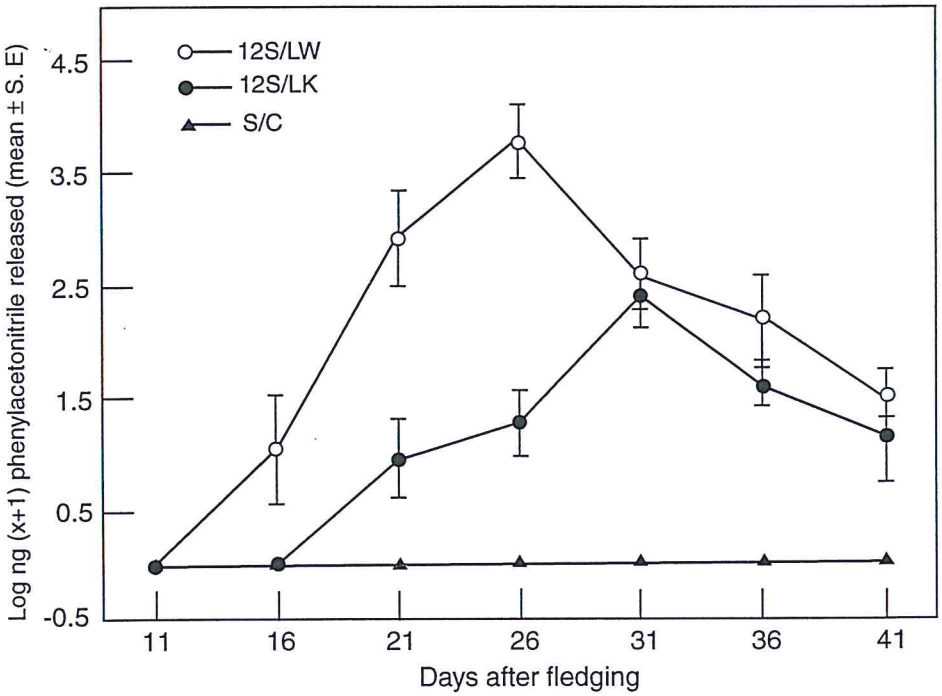


Fig. 30. Phenylacetonitrile titres in the pheromone emissions of adult males reared on localized (L) wheat (W) and kale (K) at a density of 12 locusts/cage. 12S/LW=12 solitary locusts on localized wheat, 12S/LK=12 solitary on localized kale S/C=solitarious control.

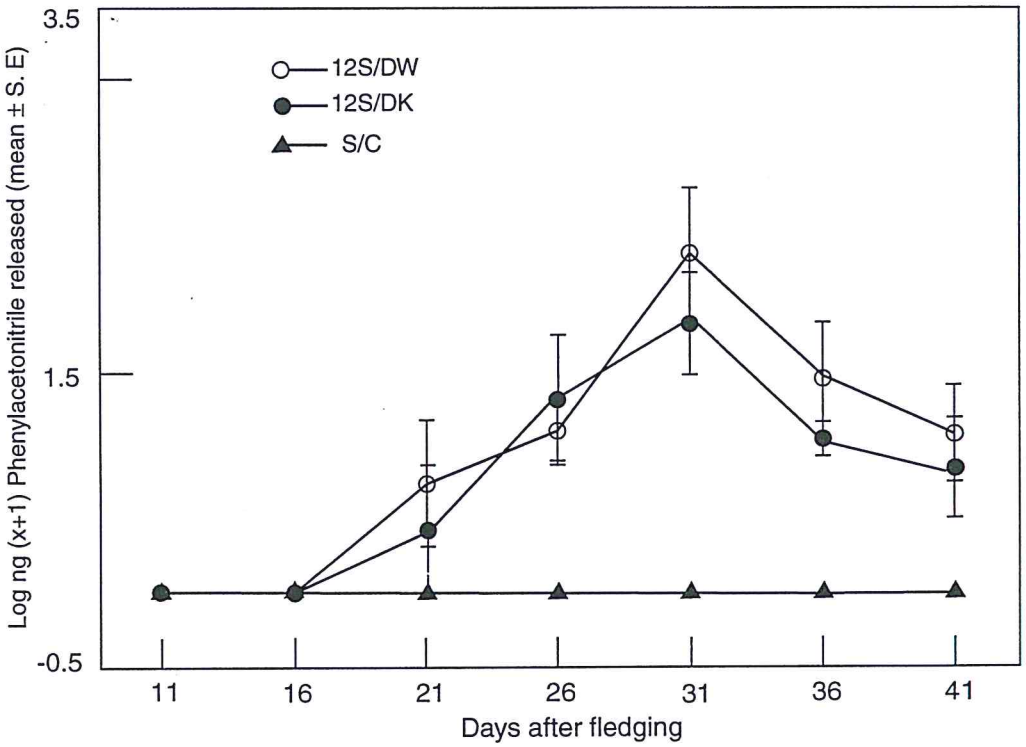


Fig. 31. Phenylacetonitrile titres in the pheromone emissions of adult males reared on delocalized (D) wheat (W) and kale (K) at a density of 12 locusts/cage. 12S/DW=12 solitary locusts on delocalized wheat, 12S/DK=12 solitary on delocalized kale and S/C=solitarious control.

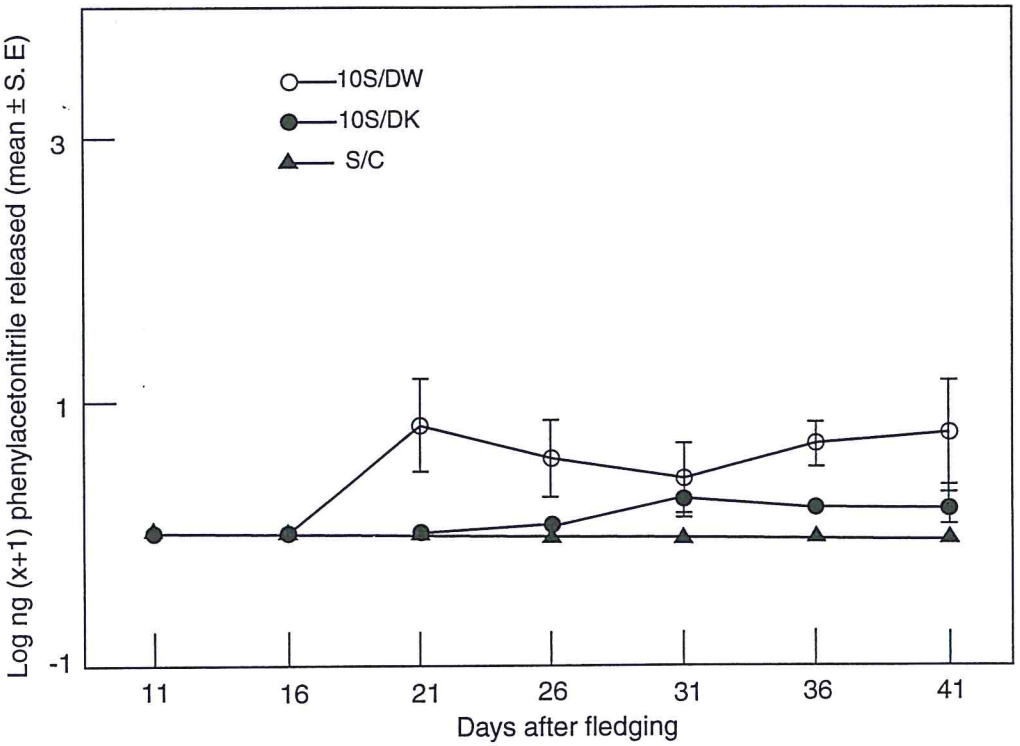


Fig. 32. Phenylacetonitrile titres in the pheromone emissions of adult males reared on delocalized (D) wheat (W) and kale (K) at a density of 10 locusts/cage. 10S/DW=10 solitary locusts on delocalized wheat, 10S/DK=10 solitary on delocalized kale and S/C=solitarious control.

from conspecifics) both stopped producing their respective pheromones by the fifth-day of isolation [Fig 33, 34, 35(a), 35(b) and 36]. On the other hand, pheromone-producing nymphs and adults exposed to the volatile emissions of 5, 10 and 20 gregarious conspecifics continued to produce their respective pheromones until after 10 and 15 days, respectively, when the level of pheromone production decreased. Pheromone production in gregarious controls continued beyond 15 days, although at lower levels. The pheromone emission of insects exposed to 20 locusts was not significantly different from gregarious control, whereas the emissions of those exposed to 5 and 10 locusts differed significantly from the control by the fifth-day. Following these periods, pheromone production decreased until after 10 days, when pheromone emissions in the test locusts was not detectable.

Overall, pheromone production was density-dependent, *i.e.* increasing with increasing the density in both adults and nymphs. Mature adults and nymphs exposed to their cross-volatiles continued to produce their respective pheromones up to 10 days after exposure when test locusts shifted to solitary state. However, they produced significantly lower levels of pheromone than those exposed to their respective stage volatile emissions.

4. 4. 2. Behaviour

The aggregation behaviour change of isolated gregarious nymphs exposed to their own volatiles at density of 5 locusts/exposure and, isolated (exposed to no

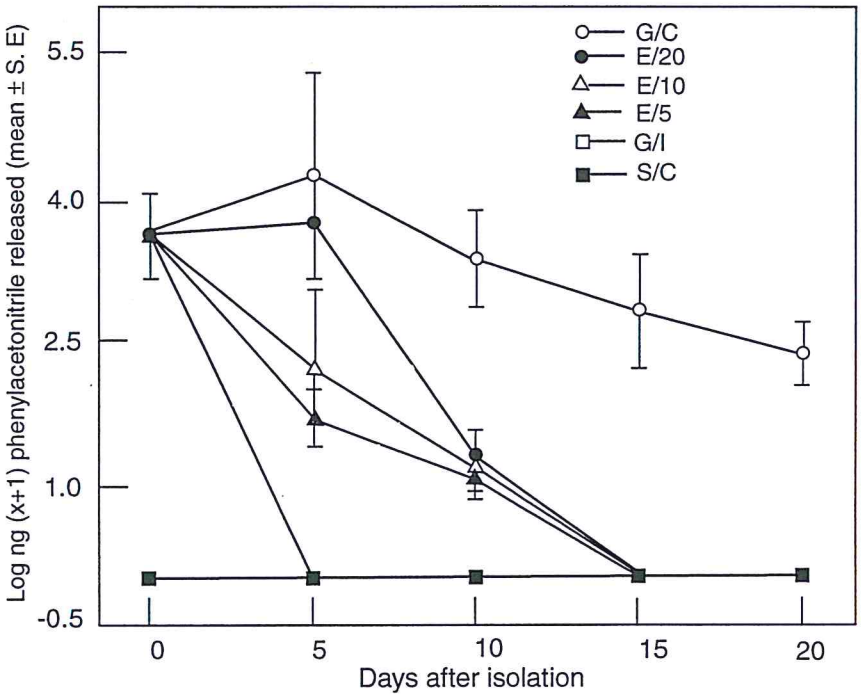


Fig. 33. Phenylacetonitrile titres in the pheromone emissions of gregarious mature adult males exposed to the volatile emissions of gregarious conspecifics. G/C=gregarious control, E/20=exposed to 20 conspecifics, E/10=exposed to 10 E/5= exposed to 5, 1/G= isolated gregarious, S/C=solitarious control.

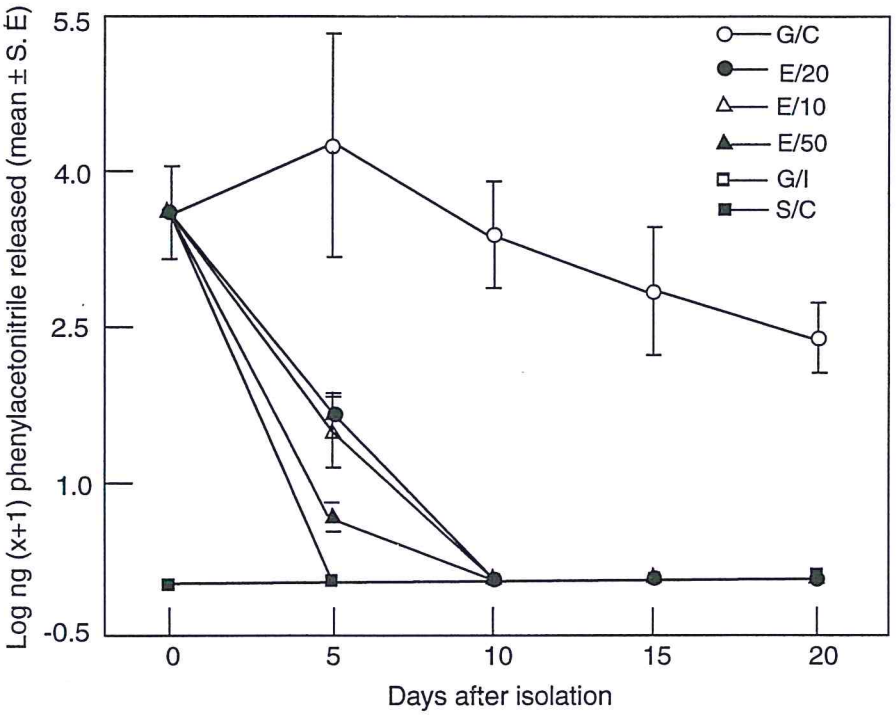


Fig. 34. Phenylacetonitrile titres in the pheromone emissions of gregarious mature adultmales exposed to the volatile emissions of gregarious nymphs. G/C=gregarious control E/20=exposed to 20 nymphs, E/10=exposed to 10 nymphs E/5=exposed to5 nymphs, 1/G=isolated gregarious, S/C= solitarious control.

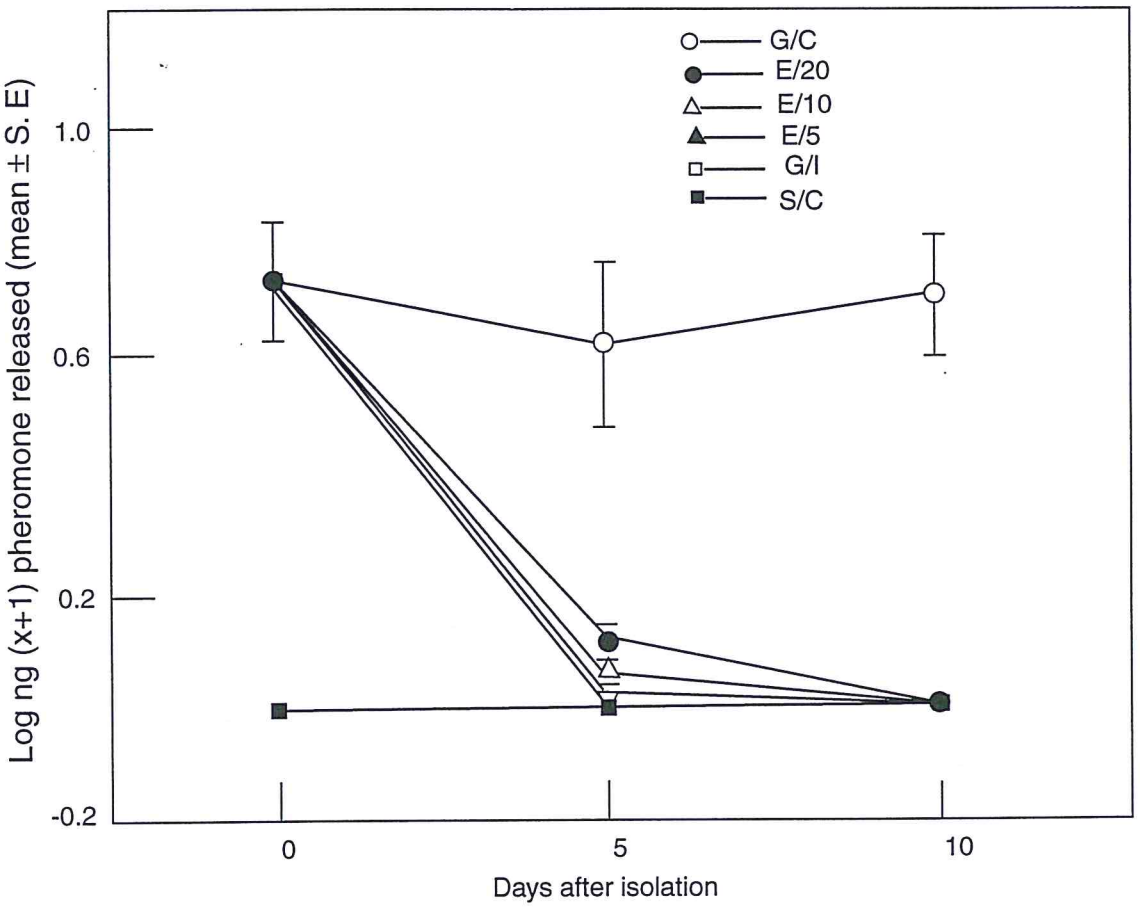


Fig. 35a. Nymphal pheromone titres after isolation in the presence or absence of the volatiles of conspecifics. G/C=gregarious control, E/20=exposed to 20 gregarious nymphs, E/10=exposed to10 nymphs, E/5=exposed to 5 nymphs, 1/G=isolated gregarious and S/C= solitarious control.

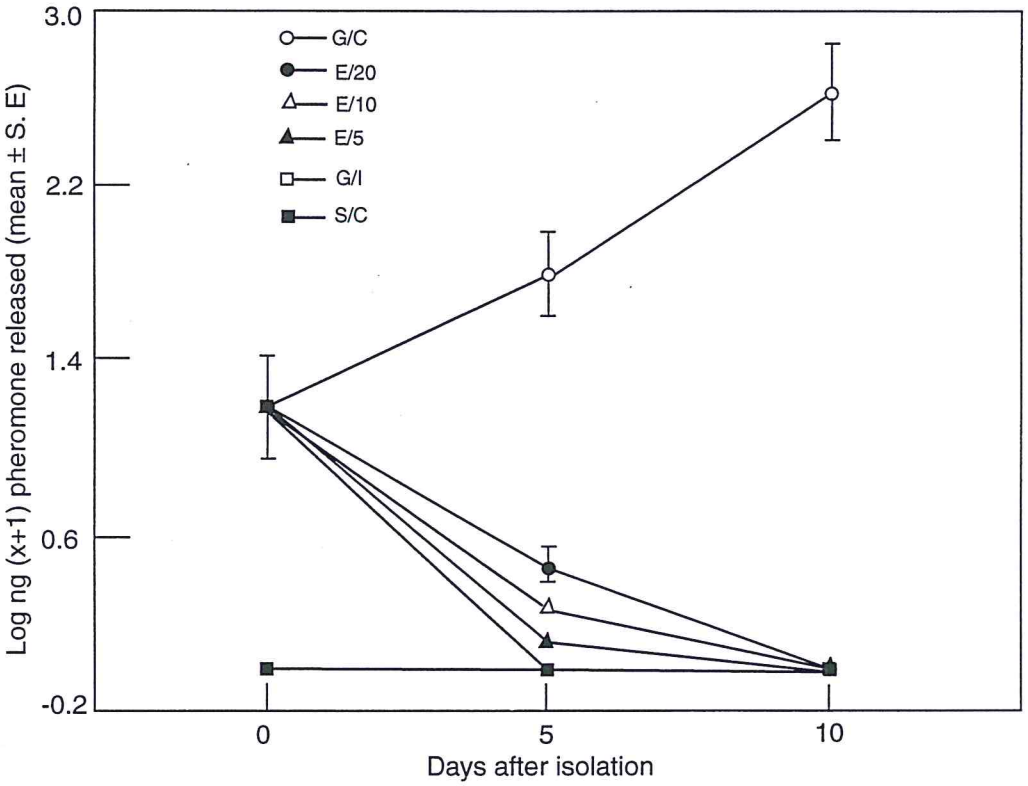


Fig. 35b. Nymphal pheromone titres after isolation in the presence or absence of the volatiles ofconspecifics. G/C = gregarious control, E/20 = exposed to 20 gregarious nymphs, E/10 = exposedto 10 nymphs. E/5 = exposed to 5 nymphs, G/I = gregarious isolated and S/C = solitarious control.

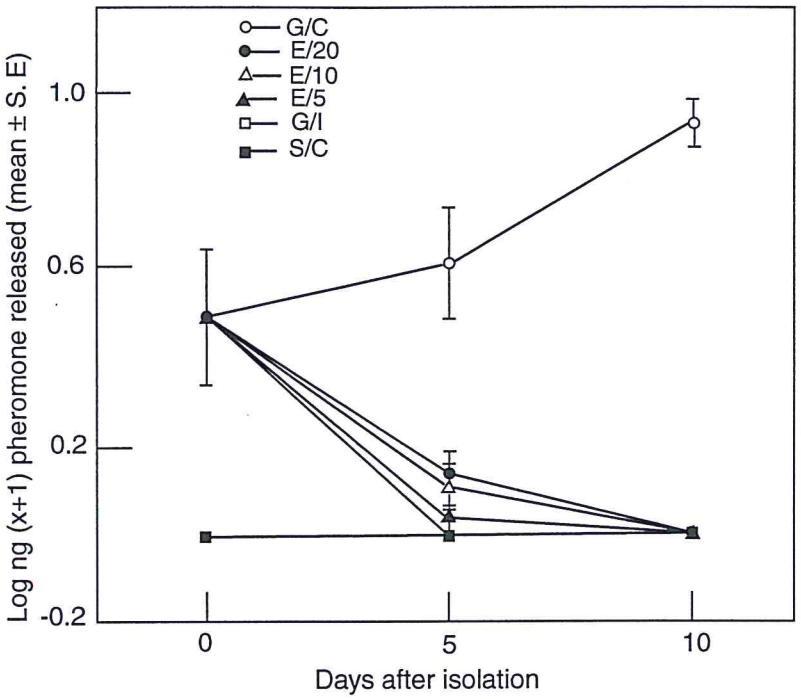


Fig. 36. Nymphal pheromone titres after isolation in the presence or absence of the volatiles of mature conspecifics. G/C=gregarious control, E/20=exposed to 20 conspecifics, E/10=exposed to 10, E/5=exposed to 5, 1/G=isolated gregarious and S/C=solitarious control.

volatiles), were not significant from the control solitarious (S/C)nymphs. However, the test insects showed a gradual decrease in the rate of behavioural solitarization with time at all exposure densities (5, 10 and 20). The results followed more or less the same trend as pheromone release in exposed nymphs and, decreased with time and the quantity of pheromone source (Fig. 37). The behavioural change in mature male adults was not monitored because it was interrupted by copulation behaviour, which interfered with the distribution of insects across the segments.

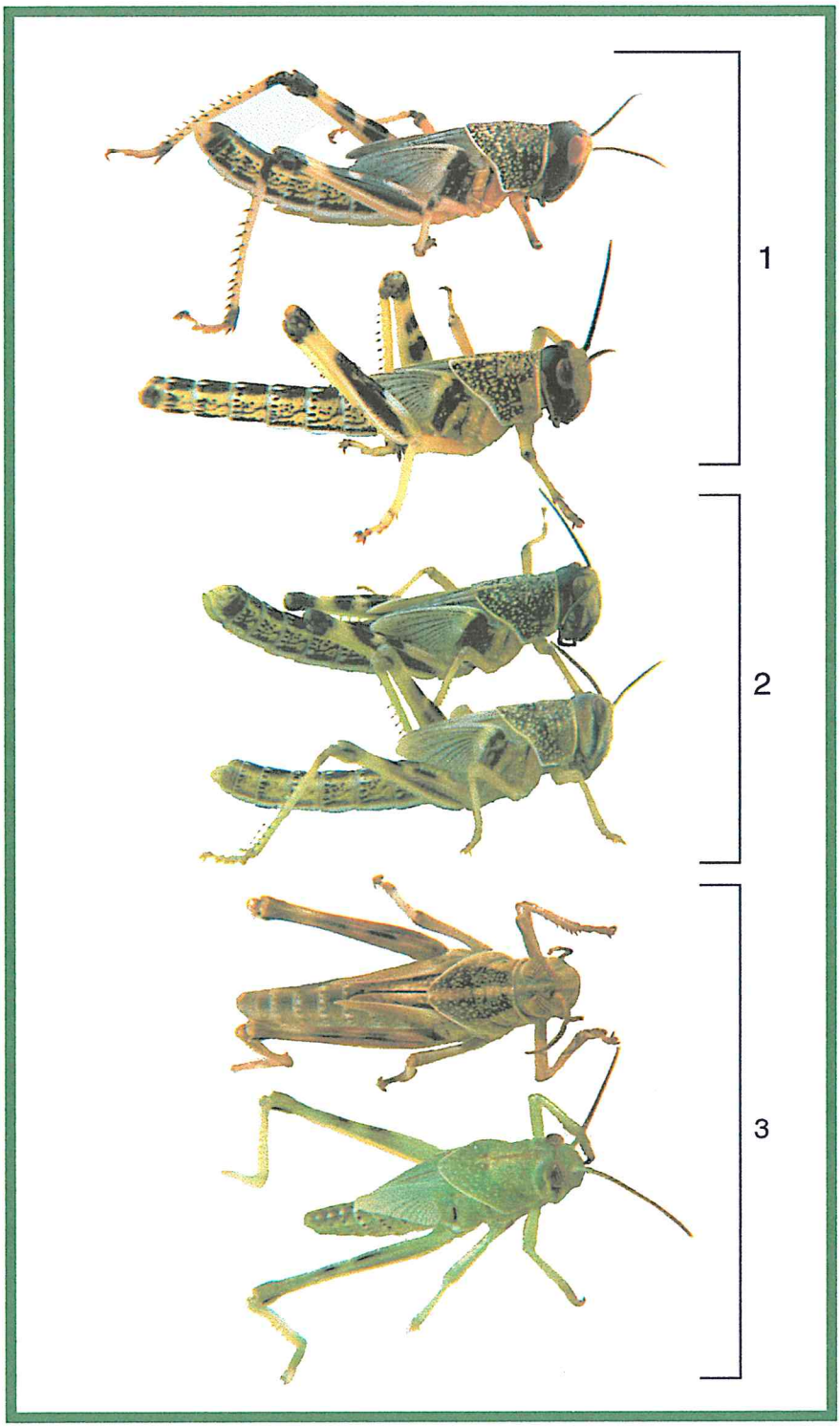
4. 4. 3. Colour

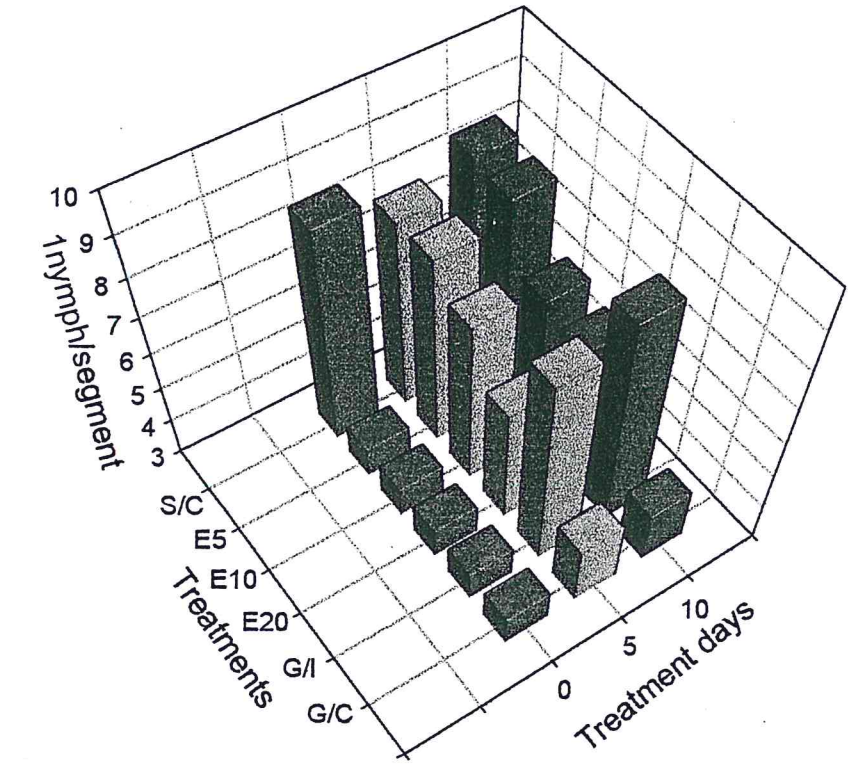
At all densities, mature adults exposed separately to the volatiles of conspecifics and 4th-instars turned faded yellow, corresponding to the grade III colour classification of Norris (1954). Gregarious controls were bright yellow, corresponding to grade IV. Fifth-instar nymphs, exposed separately to the volatiles of gregarious conspecifics and mature adults, turned pale-yellow and showed reduced melanization on their bodies (plate 2). There were no apparent density effects on locust body colour.

4. 4. 4. Weight

The mean weights of nymphs exposed to their own volatiles at densities of 10 and 20 nymphs were not significantly different at ($p = 0.05$). The mean weight of the tests insects was however closer to the control. Nymphs exposed to volatiles of conspecifics (5 per cage) tended to lose weight during the first five-days before showing some measurable weight-gain (Fig 38). Similarly, nymphs

Plate 2: Gregarious fifth-instars (2-1), exposed isolated gregarious fifth-instars (2-2) and isolated gregarious fifth-instars (2-3).





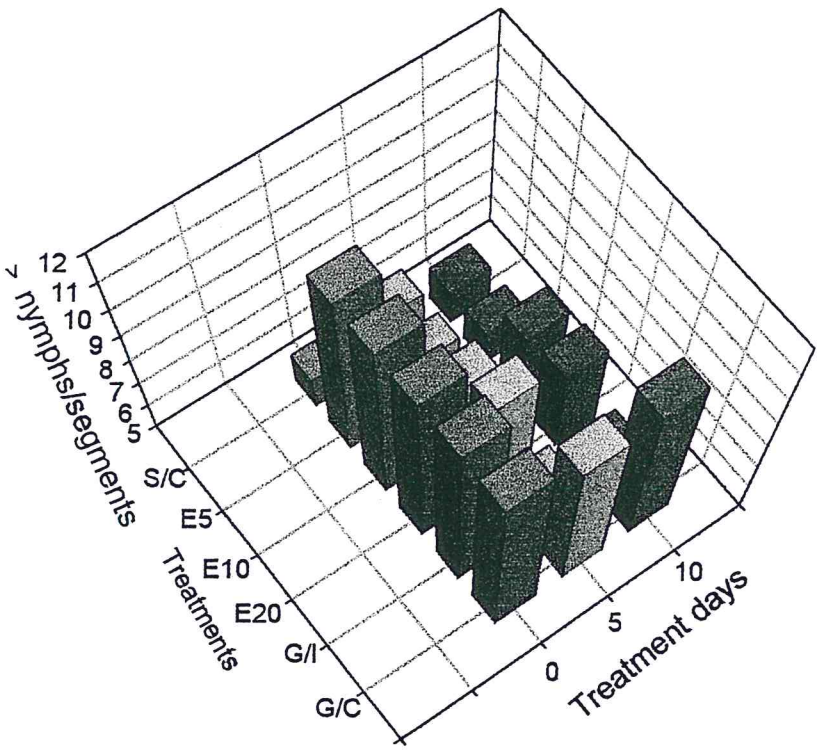


Fig.37. The pheromonal effect on the aggregation behaviour of isolated gregarious nymphs exposed to their own volatiles. S/C=solitarious control, E5=exposed to 5, E10=exposed to 10, E20=exposed to 20 nymphs, G/I=isolated gregarious and G/C=gregarious control nymphs.

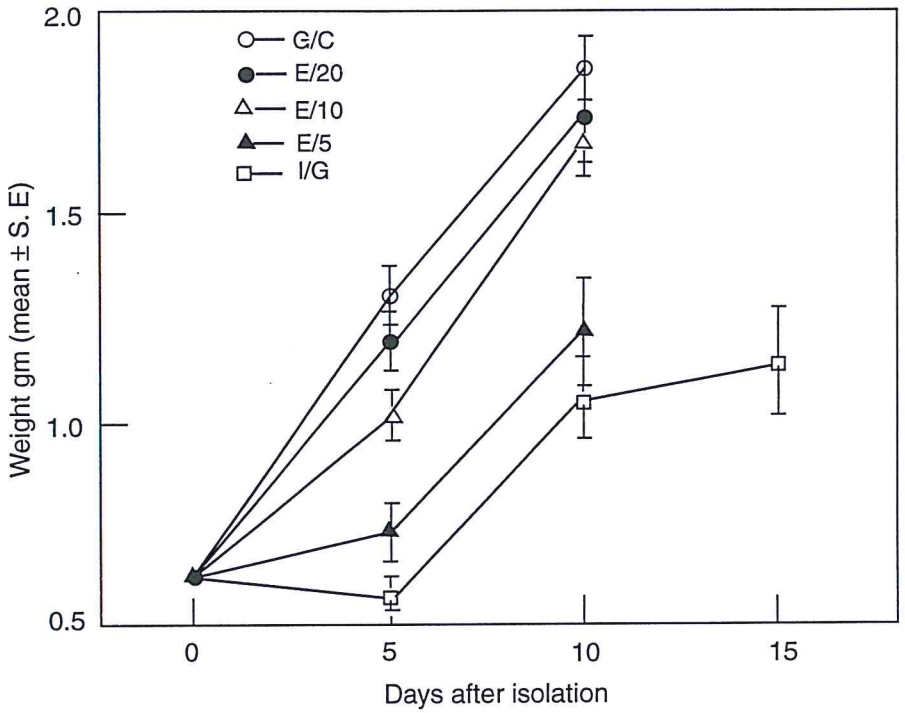


Fig. 38. Mean weights of isolated gregarious nymphs exposed to the volatiles of conspecifics at densities of 6, 10 and 20. G/C=gregarious control, E/20=exposed to 20 gregarious nymphs, E/10=exposed to 10 nymphs, E/5=exposed to 5 nymphs and 1/G=isolated gregarious.

exposed to the volatiles of mature adults showed a decrease in weight with time at all densities (5, 10 and 20) during the first five days before showing signs of weight-gain (Fig.39).

The mean weights of adults exposed to their own volatiles at densities of 5 and 10 were not significantly different (Fig. 40). The mean weight of adults exposed to nymphal volatiles was not significantly different from control (Fig. 41).

4. 5. Primer Effect of Sand-Associated Oviposition Pheromone on Nymphs that Emerged From Solitarious Eggs

The grouping behaviour of gregarious and solitary first-instars was compared with that of solitary first-instars that emerged from sand laid in by gregarious females. To test for aggregation, a theoretical random distribution was calculated using the Poisson Series Prediction (Sokal and Rohlf, 1981), and compared with those of test results. A deviation of the observed results from the Poisson prediction indicated aggregation.

In behavioural assays, using the aluminium circular-arena described in section 3.2, solitary nymphs were found to follow the Poisson Series, while their gregarious counterparts did not. Fewer gregarious nymphs occupied the segments individually, with more of the nymphs occupying the segments in groups of 2 or more. Thus, aggregation behaviour was highly significant for nymphs under gregariform.

Using sand laid in by gregarious females from which three different egg-pod densities (3, 5 and 10 egg-pods) had been removed and replaced with solitary

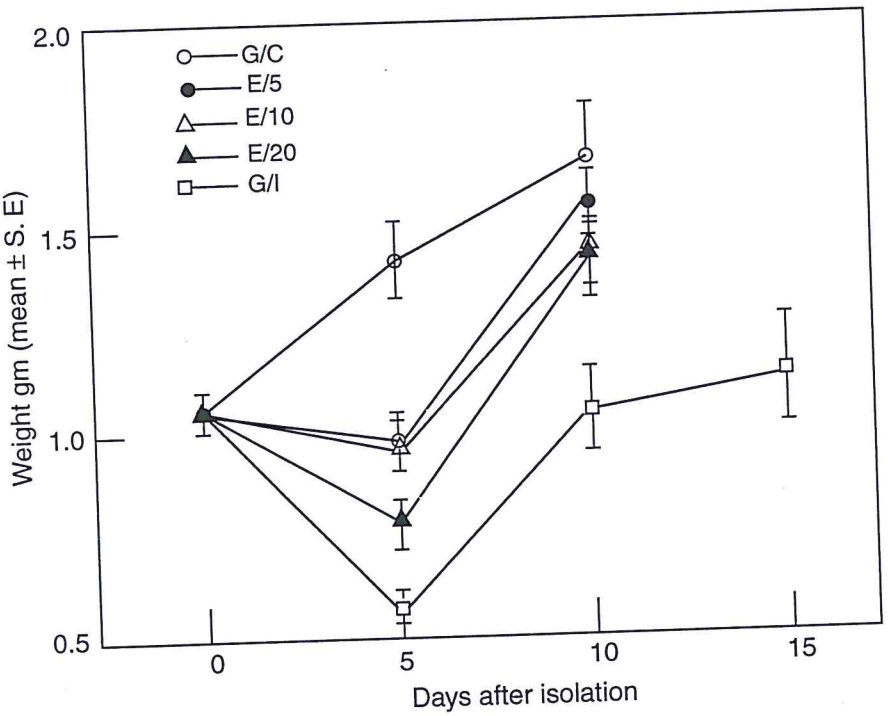


Fig. 39. Mean weights of isolated gregarious nymphs exposed to mature gregarious adult volatiles at densities of 5, 10 and 20. G/C=gregarious control, E/20=exposed to 20 gregarious adults, E/10=exposed to 10 adults, E/5=exposed to 5 adults and 1/G=isolated gregarious.

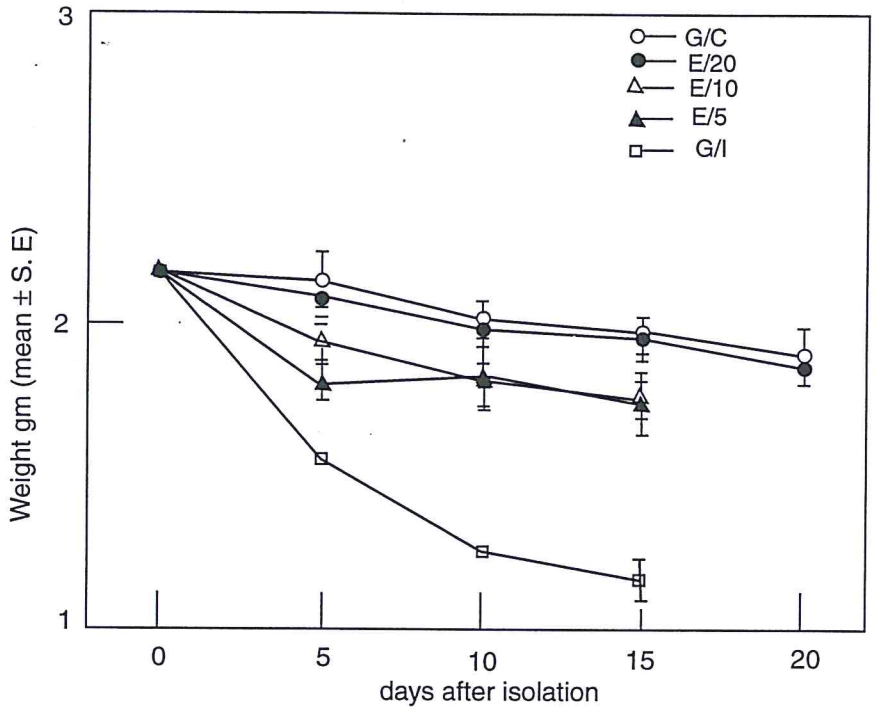


Fig.40. Mean weights of isolated gregarious mature adults males exposed to gregarious mature adult volatiles at densities of 5, 10 and 20. G/C=gregarious control, E/20=exposed to 20 gregarious mature adults, E/10=exposed to 10 adults, E/5=exposed to 5 adults and 1/G=isolated gregarious.

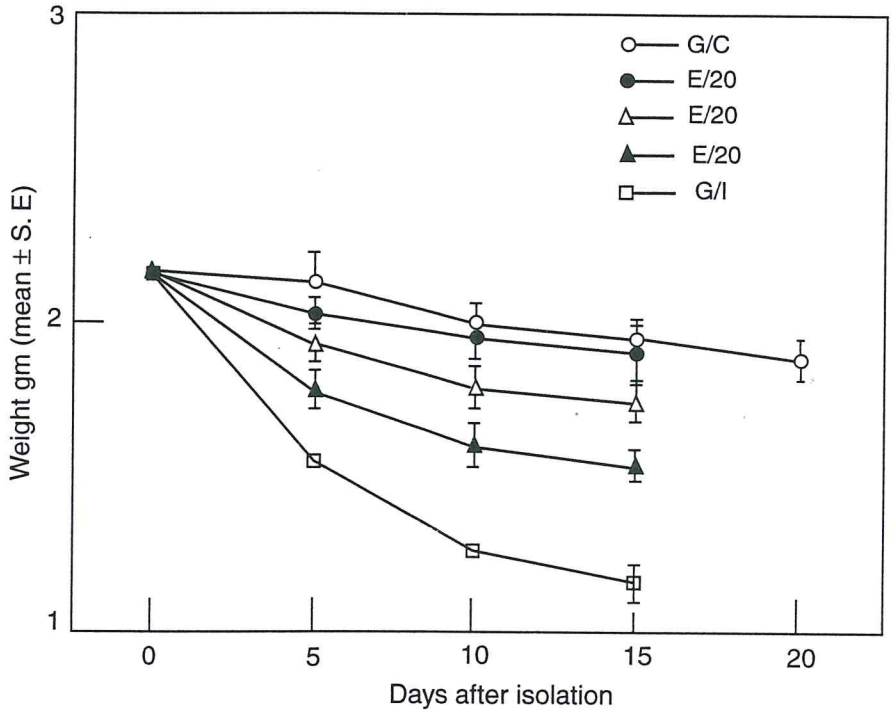


Fig. 41. Mean weights of isolated gregarious mature adults males exposed to gregarious nymphal volatiles at densities of 5, 10 and 20. G/C=gregarious control, E/20=exposed to gregarious adults,E/10=exposed to 10 adults, E/5=exposed to 5 adults and 1/G=isolated gregarious.

egg-pods, the grouping behaviour of solitary first-instars was observed. (Fig. 42). The change in behaviour from solitary to gregarious-like was highly significant in all treatments. Behavioural gregarization was density-dependent, increasing with increasing egg-pod densities.

On the other hand, solvent extracted sand in which gregarious females had oviposited, did not induce significant grouping behaviour in solitary hatchlings (Fig. 43), Likewise, nitrogen-flushed sand in which gregarious females had previously laid, did not induce significant grouping in solitary hatchlings similar to solitary control (Fig. 44).

Plotting percent of 1 nymph per segment against that of > 2 or ≥ 2 nymphs per segment from sand previously laid 3, 5 or 10 egg-pods, showed that nymphs occupying segments singly (% of 1 nymph per segment) decreased with increasing egg-pod densities used as source of primer signals. The percent 0f 1 nymphs from either solvent extracted sand or nitrogen-flushed sand occupied segments similar to solitarious control [Fig 45 (a) and Fig. 45(b)].

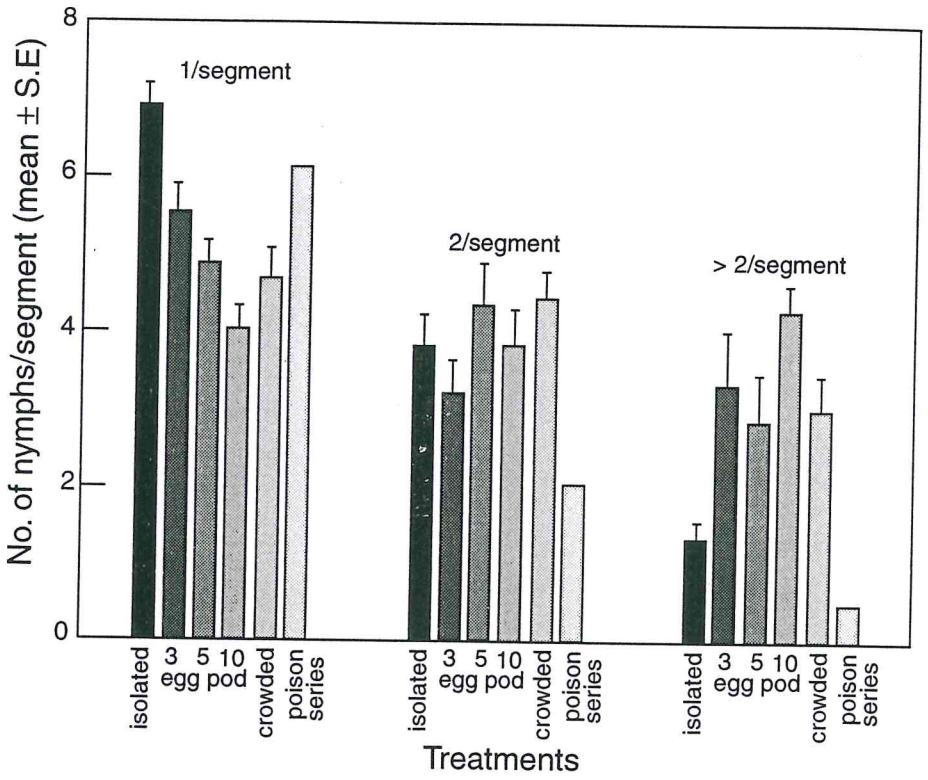


Fig. 42. Effect of exposed solitarious eggs to sand previously exposed to gregarious eggs(3, 5 & 10 egg-pods).

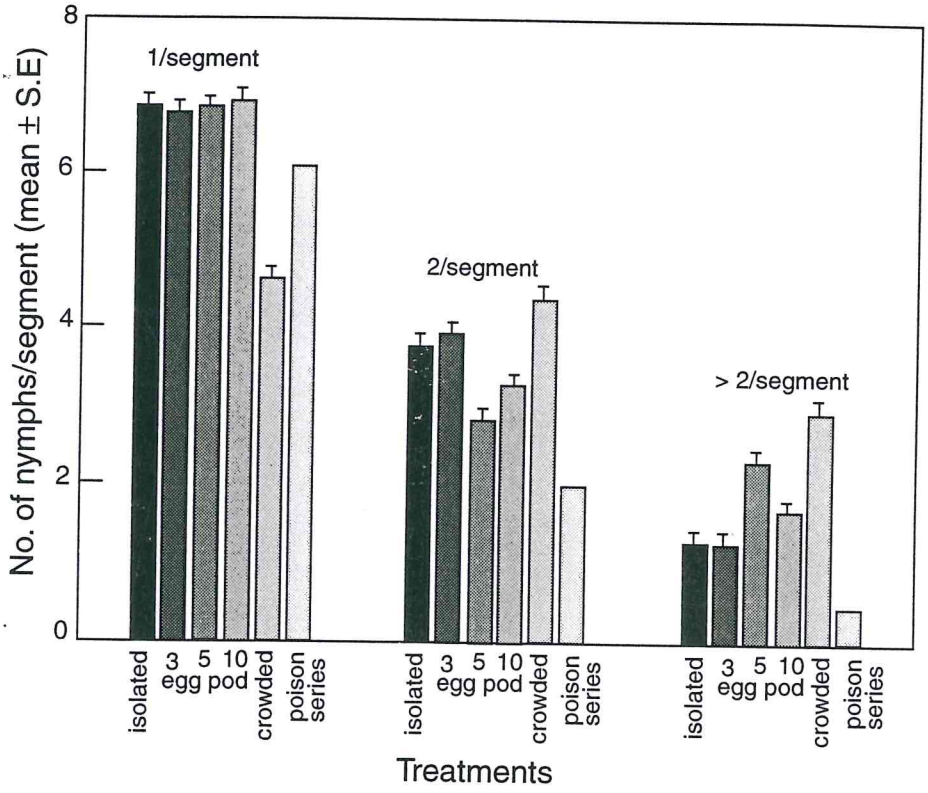


Fig. 43. Effect of exposed solitarious eggs to solvent washed sand previously exposed to gregarious eggs (3, 5 & 10 egg-pods).

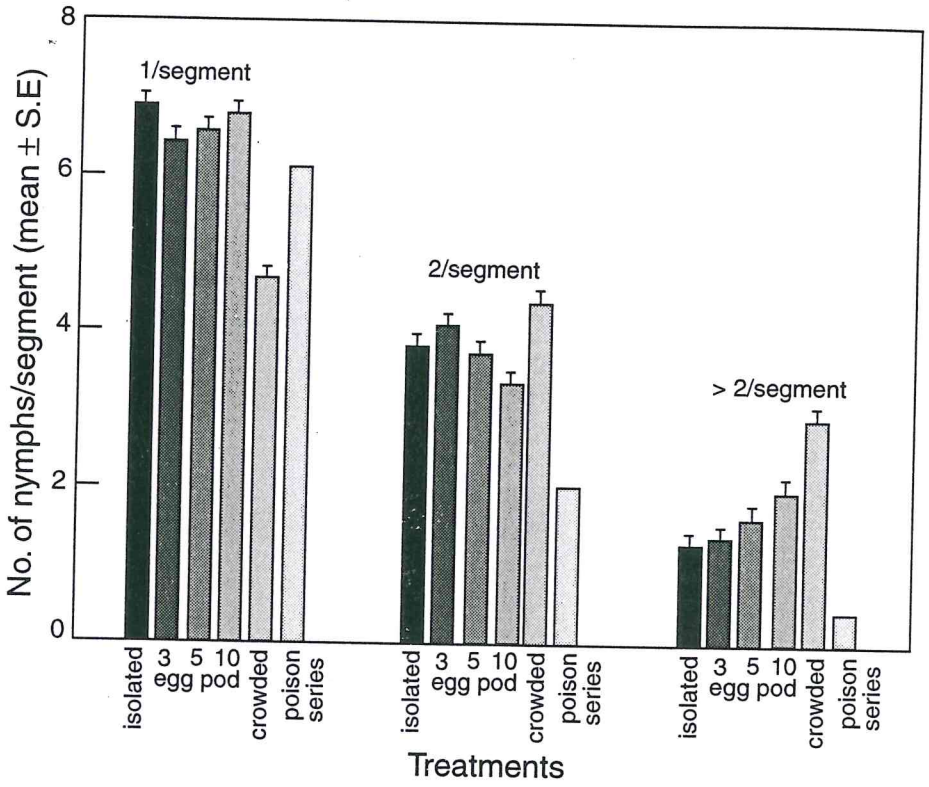


Fig. 44. Effect of exposed solitarious eggs to nitrogen-flushed sand previously exposed to gregarious eggs (3, 5 & 10 egg-pods).

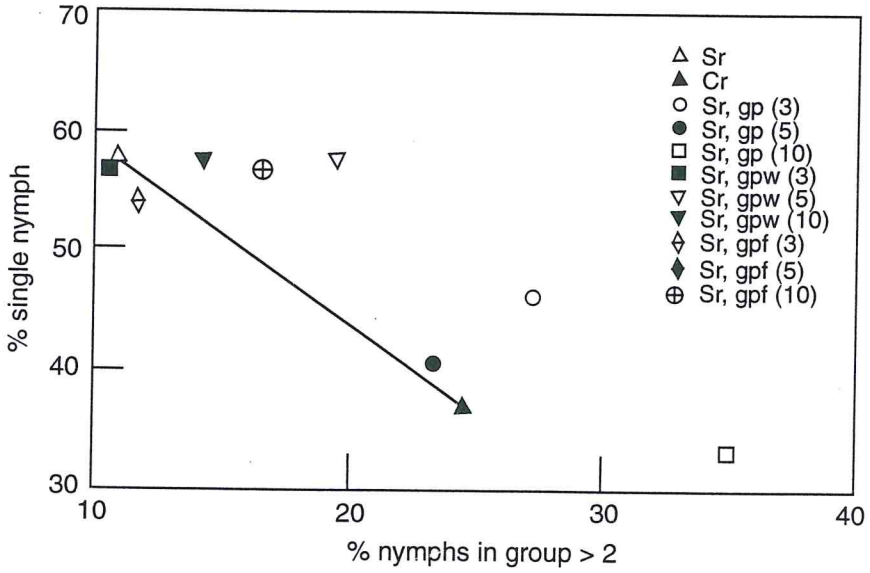


Fig. 45a. Effect of exposed solitarious eggs to (a) sand previously exposed to gregarious egg-pods (3, 5 & 10); (b) as in (a) but solvents washed; (c) as in (a) but nitrogen-flushed. sr=solitary-reared, cr=crowd-reared, sr,gp(3)=solitary eggs in three gregarious egg-pods sand, sr,gp(5)=solitary eggs in five gregarious egg-pods sand, sr,gp(10)=solitary eggs in ten gregarious egg-pods sand, sr,gpw((3)=solitary eggs in three gregarious egg-pods sand washed with solvents, sr,gpw(5)= solitary eggs in five gregarious egg-pods sand washed with solvents, sr,gpw(10)= solitary eggs in ten gregarious egg-pods sand washed with solvents, sr,gpw(3)=solitary eggs in three gregarious egg-pods sand flushed with nitrogen, sr,gpw(5)=solitary eggs in five gregarious egg-pods sand flushed with nitrogen, sr,gpw(10)= solitay eggs in ten gregarious egg-pods sand flushed with nitrogen

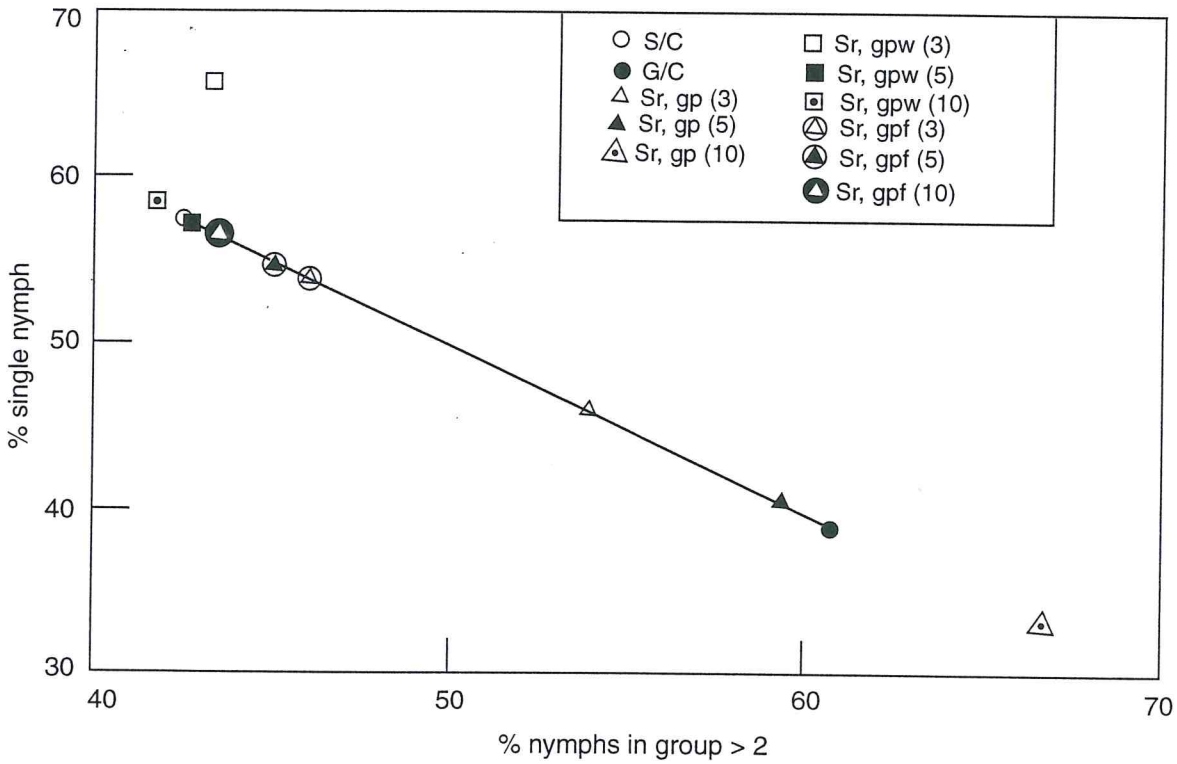


Fig. 45b Effect of exposed solitarious eggs to (a) sand previously exposed to gregarious egg-pods (3, 5 & 10); (b) as in (a) but solvents washed; (c) as in (a) but nitrogen-flushed. sr=solitary-reared, cr=crowd-reared, sr,gp(3)=solitary eggs in three gregarious egg-pods sand, sr,gp(5)-solitary eggs in five gregarious egg-pods sand, sr,gp(10)= solitary eggs in ten gregarious egg-pods sand, sr,gpw(3)= solitary eggs in three gregarious egg-pods sand washed with solvents, sr,gpw(5)= solitary eggs in five gregarious egg-pods sand washed with solvents, sr,gpw(10)= solitaryeggs in ten gregarious egg-pods sand washed with solvents, sr,gpw(3)= solitary eggs in three gregarious egg-pods sand flushed with nitrogen, sr,gpw(5)= solitary eggs in five gregarious egg-pods sand flushed with nitrogen, sr,gpw(10)= solitary eggs in ten gregarious egg-pods sand flushed with nitrogen

CHAPTER FIVE

5. DISCUSSION

5.1 Effect of Food Distribution and Plant Type

The present study confirms the effect of food distribution on the rate of gregarization of solitarious desert locust previously observed in the field (Kennedy, 1937; Volkensky, 1942; Ellis and Ashall, 1957) and recently demonstrated experimentally by Bouiachi *et al.* 1996. In their study on behavioural phase status of locusts, Bouiachi *et al.* (1996) showed that provision of a multiple resource site tended to disperse locusts, while a single resource site promoted congregation. In the present study, the results showed that the provision of a single resource site had a significant effect in causing a change in locust body colour pattern, aggregation pheromone production and overall physiological gregarization in treated locusts, compared to the provision of a multiple resource site. Locust body colour pattern changed more slowly in all treatments, compared to pheromone production. The yellow body colour of mature adult males appeared between ages 25-27 and 30-32 days, compared 10-15 days in gregarious control. These confirm that locust body colour pattern is not a sensitive measure for monitoring initial phase shifts resulting from crowding or uncrowding, in agreement with results obtained by Deng (1995).

Locust density per unit area appeared to be an important factor promoting gregarization. A single resource site appeared to increase locust mutual interaction through various stimuli (olfaction, visual and tactile). Locusts forced to congregate

during feeding at densities of 4 and 6 in experimental cages (100 x 100 x 50cm) failed to gregarize in the present study as revealed by the parameters monitored. Deng (1995) showed that one solitarious locust paired with another in a 10 x 10 x 24 cm cage could provide each other with the necessary stimuli to gregarize. The smaller cages used by Deng, about 200 times smaller than those used in the present study, may explain the differences in the results. Confining locusts to a small space increases their chances of stimulating each other to gregarize, with greater vision, touch and exchange of chemical signals. In the natural situation, small pockets of locust populations may congregate on different parts of the same plant to initiate the process of gregarization. If individuals in a pocket are in contact with each other sufficiently long, gregarization of these individuals will take place. Various gregarizing locust pockets will move to merge with others to cause the spread of the gregarization across the population.

Microhabitats, such as warm spots provided by stones, tall vegetation or any localized resource may also serve as foci for concentrating initial low-density population (Maxwell, 1937; Chapman, 1976). Warm spots were eliminated in the experimental design by using diffused light provided by 3 fluorescent light tubes. Therefore, the results of this study were entirely due to the distribution of food resource.

Comparison of the effect of wheat and kale on the rate of gregarization showed that at the same locust density and food distribution, gregarization was

higher in wheat than in kale. Learning behaviour and adaptation to wheat may have caused this difference. Locusts in this study had been reared on wheat for 53 generations and may have become adapted to this food plant, while other set of test locusts were switched from wheat and reared for a single generation on kale. This needs to be substantiated with more detailed studied by comparing the effects of the two plants species on gregarization after rearing locusts on them for the same length of time.

In the present study, there were no significant differences in haemolymph pigment absorbance ratios between the treatments and controls at all densities for both wheat and kale. Gunn and Hunter-jones (1952) and Deng (1995) reported that forced crowding of solitarious locusts for a single generation at low densities, such as densities used in the present study, was unlikely to alter all the phase characters of test locusts. Deng (1995) studied the haemolymph pigment ratio of shifting crowd-reared locusts to solitary conditions and found that pigment ratio required several generations to change from one phase to the other. Thus, the current results are in agreement with earlier results that the pigment ratio is not a sensitive measure for monitoring rapid phase shifts in adults.

The pattern of morphometric (E/F and F/C) data observed appeared to parallel the data for haemolymph pigment composition. Solitarious locusts subjected to a single or multiple food resource did not respond to treatment effects. These results confirm those previously obtained by Deng (1995) on morphometric

changes and the insensitivity of this parameter as a method for monitoring initial phase change resulting from crowding/uncrowding.

5.2. The Effect of Gregarious Locust Volatiles on Solitarization of Isolated Conspecifics

The present study on the effect of olfactory stimulus from nymphal and mature adult stages of the gregarious locusts on conspecifics showed that the quality and dose of the stimulus determined the degree of solitarization in isolated gregarious counterparts. The pheromone emissions from mature adult males appeared to slow down considerably, by an extra five days, the solitarization process in conspecifics compared to nymphs treated similarly. Furthermore, a higher residual pheromone titre was found in isolated adults exposed to a higher adults density compared to nymphs treated similarly. These results suggest stage-dependent differences to solitarization in the presence of respective aggregation pheromones.

In *S. gregaria*, olfactory stimulus alone does not cause phase-change in solitarious locusts as measured by behavioural changes and pheromone production (Deng, 1995; Heifetz *et al.*, 1996; Roessingh *et al.*, 1998). The results of the present study show that solitarization can be significantly retarded in isolated gregarious individuals in the presence of their own olfactory pheromones, and as measured by pheromone titres and aggregation behaviour, showing that of olfactory pheromonal signal percieved by recipients has some primer effect. The effect is dose-dependent.

The present results are in agreements with those of Heifetz *et al.* (1996) and Roessingh *et al.* (1998) who found that short-range olfactory or locusts odour did cause some behavioural change in the recpients.

The change in locust body colour, visually observed, in pheromone exposed and unexposed gregarious hoppers, showed that the exposed ones maintained much of their colour, compared to the isolated ones. In unexposed hoppers, much of their body colour was lost between moults, while in adults the colour pattern faded with time. It appeared that pheromone loss is correlated with locust body colour, which is consistent with the results obtained by Deng *et al.* 1996 on the effect of density on locust body colour.

Isolated and exposed gregarious conspecifics to pheromonal emissions recorded a slight increase in their overall body weight compared to their isolated and unexposed counterparts, a characteristic feature associated with gregarious insects (Hunter-Jones, 1958; and Injeyan and Tobe, 1981). Delayed solitarization *i.e* continued gregarious physiological state accounts for the increase in body weight, which is consistent with the Deng's (1995) findings on the effect of pheromonal emissions from gregarious insects on the body weight of conspecifics.

Earlier studies had implicated pheromonal effects in gregarization of locusts, because isolated gregarious nymphs kept in the same room with crowded nymphs retained their gregarious characteristics. It was assumed that the olfactory signal was the gregarization pheromone (Nolte, 1963; Gillett, 1968). However,

recent studies have shown that exposure of nymphs or fledglings to pheromone emissions or faecal extracts from crowded locusts without visual and physical contact, did not transform *solitaria* to *gregaria* (Deng, 1995; Heifetz *et al.*, 1996; Roessingh *et al.*, 1998). On the other hand, the present results have shown that once gregarious, the volatile pheromones provide sufficient primer stimuli to delay solitarization, thus account for Nolte`s (1963) and Gillett`s (1968) observations.

5.3. Phase Status of Hatchlings from Solitarious Egg Pods Exposed to Sand Laid in by Gregarious Females.

The present study confirmed the hypothesis that oviposition aggregation pheromone components, secreted into the sand by gregarious females, also primes the gregarization of hatchlings of developing embryos in solitarious eggs exposed to the pheromones. The secretions from sand previously laid in promoted phase shift in hatchlings from solitarious eggs exposed to it resulting in more gregarious insects as measured by grouping assays. Furthermore, the greater the number of gregarious egg-pods used as source of the pheromone, the more behaviourally gregarious the hatchlings. The amount of secretions in sand was shown to increase with the number of egg pods used per oviposition cup. This is in agreement with Torto *et al.* (1999) findings that the amount of active ketones in the secretions of laid in sand by gregarious females increased with consecutive use of sand for oviposition.

Passing of the gregarious characters from the mother to offsprings has been repeatedly observed in the past, but the mechanism remained obscure (Gun and

Hunter-Jones, 1952; Hunter-Jones, 1958; Injeyan and Tobe, 1981). However, a recent study, implicated the froth of egg-pods from gregarious females as the source of the primer signal, induced gregarious characteristics in hatchlings from solitarious eggs. In the present study, sand (without the froth and the eggs) previously laid in by gregarious females also primed gregarization of hatchlings from solitarious eggs. The gregarious females secreted oviposition aggregation pheromone components into the sand which are adsorbed to the soil particles. These components are desorbed upon moistening, and their volatility ensure difussion around the soil particles to influence the development of any solitary eggs nearby and, to modify their embryonic development towards the gregarious phase. In this regard, the present work results are consistent with the findings of McCaffery *et al.* (1998). However, These authors traced the primer signal to the egg froth of gregarious females extractable by aqueous methanol. Moreover, the same authors found that sand previously laid in by gregarious females had no effect on phase characteristics from hatchlings from *solitaria.* Torto *et al.* (1999) showed that the active components associated with sand strongly adhered to the sand particles and could be released only when the sand was moistened. On the other hand, McCaffery *et al.* (1998) evaluated sand samples previously laid in by gregarious females without moistening. This could account for the differences between the present work and their results. Moreover, Torto *et al.*, (1999) showed that the active compounds associated with sand were present in relatively higher amounts in the egg, but lesser relative amounts in the froth, which is consistent with the primer function of these

signals, although the amounts present in the froth were clearly enough to cause phase shifts in hatchlings in McCaffery *et al.*, (1998) experiments. Failure by these workers to extract the primer signal from eggs is attributed to a choice of inappropriate solvent and extraction method (McCaffery *et al.*, 1998).

Hatchlings that emerged from exposed solitarious eggs to solvent-extracted or nitrogen-flushed sand previously used by gregarious females failed to gregarize in the present study as shown by lack of aggregation behaviour which was similar to that of solitary control. These findings confirm that the gregarization factor deposited into the sand during oviposition, is soluble in organic-solvents and volatile enough to be flushed off by nitrogen. These results then show strongly that the unsaturated polar ketones secreted into the sand by ovipositing gregarious females during oviposition, identified previously by Torto *et al.* (1999), are responsible for the primer effect, which gregarizes the hatchlings from solitary eggs. The present results differ from those of McCaffery *et al.* (1998), which implicated polar compounds of less than 3 kd.

Group oviposition is characteristic of the desert locust, *S. gregaria*, and has been shown to be mediated by signals from ovipositing females, egg froth and compounds secreted into the sand during oviposition (Norris, 1950; Njagi *et al.*, submitted; Saini *et al.*, 1995; Rai *et al.*, 1997; Torto *et al.*, 1999). Such behaviour ensures that resultant hatchling locusts are in close proximity to each other, thus promoting and maintaining shifts towards the extreme gregarious phase (Uvarov,

1977). In the desert locust habitat, resource localization, such as water source and greenery is restricted to oasis or wadis in the desert (Uvarov, 1966). Field observations have shown that pioneering gravid females prefer to lay at such sites in the open, perhaps dictated by moisture level. Once laid, these egg-pods then attract other females to oviposit in the vicinity. When solitarious females are offered a choice of gregarious egg-pods and solitarious egg-pods, they prefer to lay near the former (Bashir *et al.*, in press). This preference may be related to the need of the species to facilitate phase shift and associated behavioural changes early in the population build up.

5. 4. Suggestions for future study

1. The distribution of host-plants has now been shown in the laboratory to affect gregarization of locusts at appropriate densities. This now needs to be undertaken under the field conditions, where the rate of gregarization needs to be studied at different host-plants and solitarious locust densities, different plant constituents comprising preferred and non preferred desert plants and varying host-plant physiology (seedling, growing, senescent, etc.). Such studies will give a quantitative understanding of the relation between vegetational attributes and locust numbers on one hand, probability and extent of gregarization on the other hand. This would provide a basis for development of (Geographical Information System (GIS) and remote-sensing techniques and the development of an early warning system.

2. Although unsaturated ketones are clearly implicated as primer signals for maternal transfer of crowding experience (gregarization) to the progeny, the compounds need to be synthesize and evaluated in the laboratory and in the field. The availability of the compounds would make it possible to evaluate the effect/role of such components and in blends as oviposition attractant(s) and as primer pheromone(s). An intriguing future follow up is to determine the biosynthetic pathways of the ketones and possibility of the enzymes involved in key steps in their synthesis. This may open up the possibility of inhibiting their synthesis and a novel approach to forestalling locust gregarization.

5.5. Conclusions

1. Adults males which emerged from solitarious nymphs at densities of 4 and 6 reared on both localized and delocalized food failed to gregarize as measured by pheromone titres, indicating that the population was too low to provide critical level of stimuli for phase change.
2. Adult males which emerged from a density of 8 on localized food situation gregarized, as measured by pheromone emission, while in delocalized food situation, no such gregarization took place, indicating localized resources of food promoted congregation and mutual stimulation.
3. Adult males emerged from densities of 10 and 12 locusts per cage in both situations gregarized, indicating that higher densities in cages are effected in enough mutual stimulation, irrespective of the pattern of food distribution.

4. The ratios (haemolymph absorbance ratio, F/C and E/F), as well as colour, did not change during the experimental period, indicating that these characters change very slowly in response to density.
5. Comparison of locusts gregarization reared on wheat and kale plants separately, showed that gregarization was higher in wheat than kale, suggesting that plant species may play a role in gregarization; however, since the insects reared on kale were originally reared on wheat, further work needs to be done to verify this.
6. Pheromone emissions from live gregarious insects (nymphs and adults) delayed solitarization of isolated recipient locusts, which retained their gregarious colour; behaviour and body weights, showed that the aggregation pheromones also have a primer effect (physiological effect) in gregarious insects.
7. Unsaturated ketones secreted into the sand by gregarious females and, which are also present in the eggs, constitute the primer gregarization pheromone responsible for transgenerational transfer of gregarious characters.

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* Seen as abstract.

7. APPENDICES

Appendix 1. Preliminary investigation on the effect of localized and delocalized food on solitarious locust gregarization.

7. 1. Preliminary experiments

Preliminary experiments were conducted with newly moulted third-instar (1-day-old) nymphs which were reared on wheat. The following factors were considered in the preliminary experiments: type and size of cages, illumination conditions and locust density. Two types and sizes of cages, standard aluminium (50 x 50 x 50cm) and galvanized aluminium wire mesh (100 x 100 x 50cm), two light bulbs (40 watts) were suspended in each cage and diffused fluorescent light tube (2ft each, 20 watts) placed above the cages. Locust densities of 4, 6, 8, 10 and 12 were used to determine which would be suitable for present studies. The 50 x 50-x 50cm cage was found to be small to provide the difference between treatments (localized and delocalized). The 100 x 100-x 50cm cage was suitable for the study, because it had wider space for delocalized food distribution. Suspended light was not suitable because the insects were observed to gather around the hot bulbs, which interfered with the effect of treatments. The diffused fluorescent light tube was found to be suitable as it avoids insects aggrgation around possible hot points.

7. 2. Preliminary Results

7. 2. 1. Phenylacetonitrile (PAN) titre

Comparisons of the means of PAN titres in both localized and delocalized situations for male locusts, which emerged from solitarious third instar nymphs reared at density of 4 locusts per cage of (50 x 50 x 50cm), showed no significant difference between the treatments (Fig.47.). Similarly, for locust densities of 10 and

12 per cage (100 x 100 x 50cm), gregarization in terms of PAN was not significant between the two treatments localized and delocalized food; (Fig .48, 49). No pheromone was detected for low locust densities of 4 and 6 per cage (100 x 100 x 50cm) for both localized and delocalized food treatments.

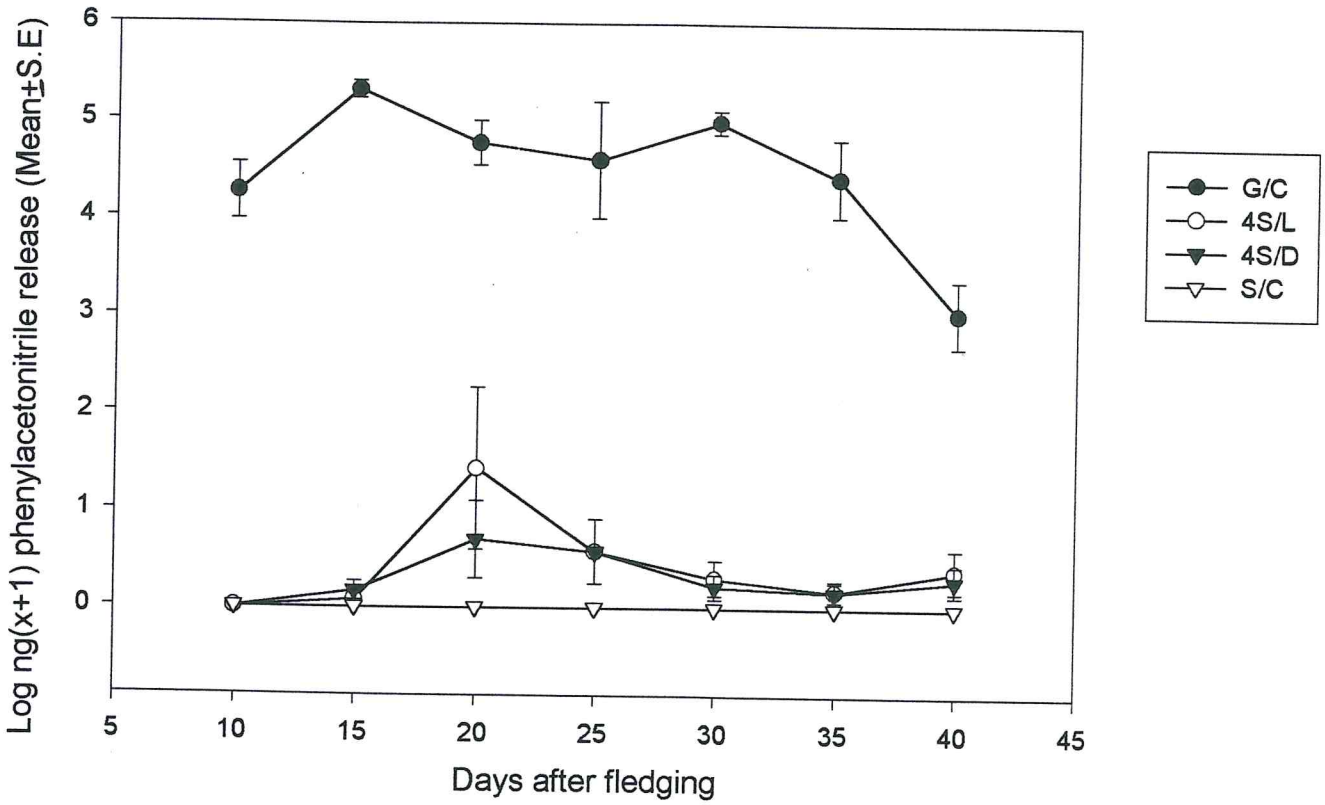

Fig 47 phenylacetonitrile titre in the pheromone emissions of adult males reared on localized(L) and delocalized (D) wheat (W) at a density of 4 locusts/cage (50 x 50 x 50cm).G/C=gregarious control, 4S/L=solitary on localized, 4S/d=solitary on delocalized and S/C=solitarious control.

12 per cage (100 x 100 x 50cm), gregarization in terms of PAN was not significant between the two treatments localized and delocalized food; (Fig .48, 49). No pheromone was detected for low locust densities of 4 and 6 per cage (100 x 100 x 50cm) for both localized and delocalized food treatments.

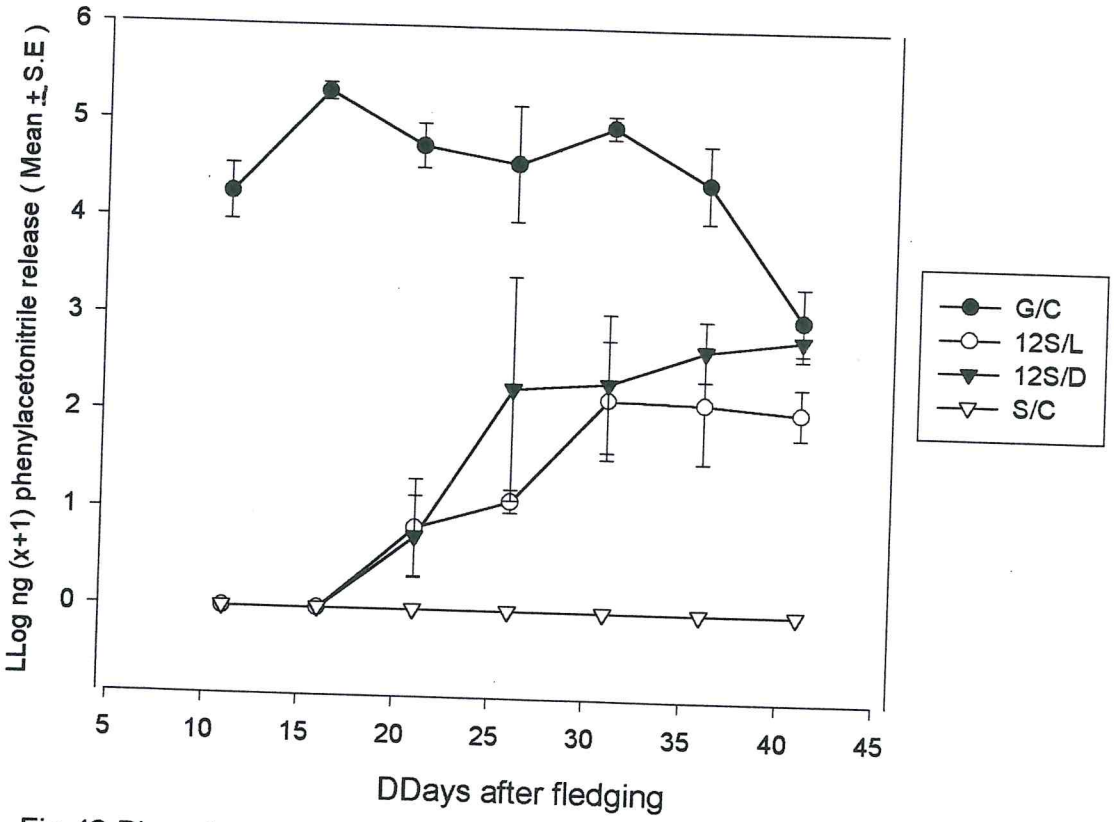

Fig 48 Phenylacetonitrile titre in the pheromone emissions of adult males reared on localized (L) and delocalized (D) wheat at a density of12 locusts/cage. G/C=gregarious control, 12S/L=solitary locusts on localized, 12S/D=12 solitary on delocalized and S/C=solitarious control.

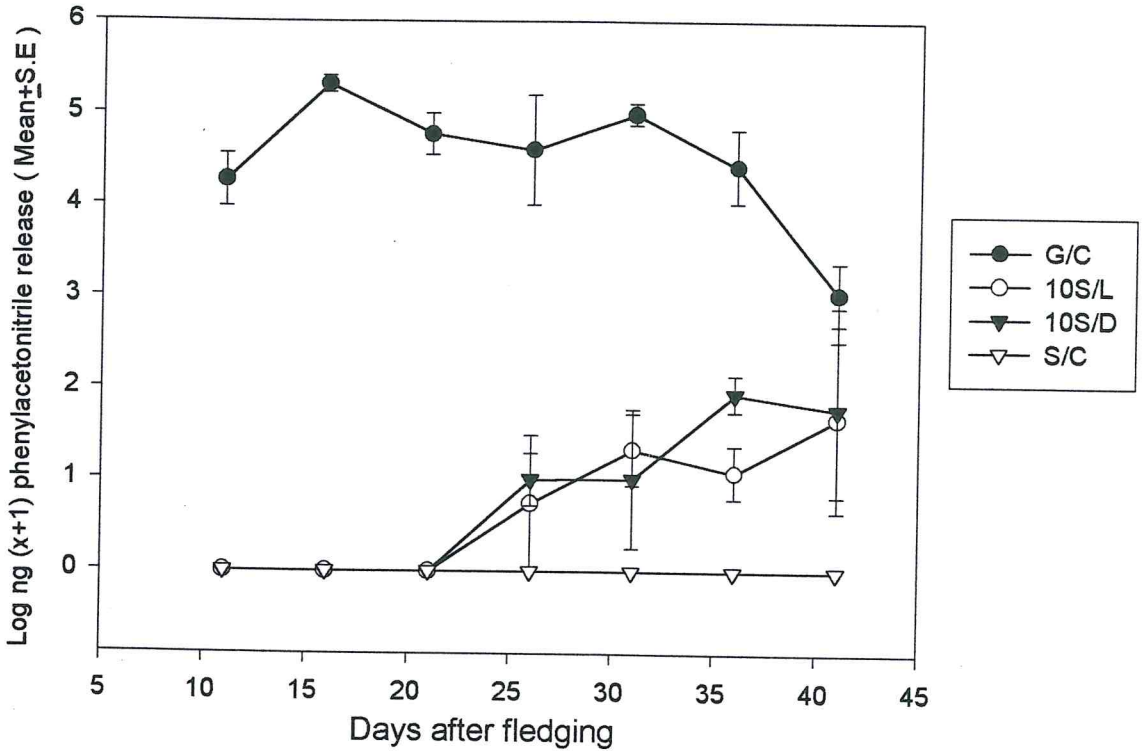

Fig 48 Phenylacetonitrile in the pheromone emissions of adult males rearedon localized and delocalized wheat at a density of 10 locusts/cage (100x100x50cm).G/C=gregariouscontrol, 10S/L=10solitary locusts on localized, 10S/D=10 solitary on delocalized and S/C=solitarious control

Appendix 2. Mean absorbance ratios of solitarious locusts reared on localized (L) and delocalized(D) wheat (W) seedlings. S/C = solitarious control, 10S/LW = 10 solitary locusts on localized, 10S/DW = 10 solitary on delocalized, 12S/LW = 12 solitary on localized, 12S/DW = 12 solitary on delocalized and S/C = solitarious control.

Treatments	Absorbance Ratio (Mean ± S.E)	
	Males	Females
S/C	1.26 ± 0.13b	1.35 ± 0.08b
10S/LW	1.74 ± 0.14b	1.42 ± 0.09b
10S/DW	1.43 ± 0.08b	1.35 ± 0.05b
12S/LW	1.44 ± 0.07b	1.36 ± 0.03b
12S/DW	1.29 ± 0.07b	1.32 ± 0.08b
G/C	2.80 ± 0.41a	2.08 ± 0.21a

Means with the same letter are not significantly different (p=0.05)

Appendix 3. Mean F/C and E/F ratios of solitarious locusts reared on localized (L) and delocalized (D) wheat (W) seedlings at densities of 8,10 and 12 locusts/cage. S/C=solitarious control, 8S/LW=8 solitary locusts on localized, 8S/DW=8 solitary on delocalized, 10S/LW=10 solitary on localized, 10S/DW=10 solitary on delocalized, 12S/LW=12 solitary on localized, 12S/DW=12 solitary on delocalized and G/C=gregarious control

Treatments	F/C ratio (Mean ± S.E)		E/F ratio (Mean ± S.E)	
	Males	Females	Males	Females
S/C	4.14±0.04b	4.03±0.05b	1.93±0.03b	2.04±0.03b
8S/LW	4.07±0.03b	4.11±0.05b	1.97±0.02b	2.01±0.03b
8S/DW	4.05±0.05b	4.08±0.07b	1.95±0.03b	1.99±0.04b
10S/LW	4.00±0.04b	4.20±0.04b	1.99±0.02b	1.97±0.02b
10S/DW	3.96±0.07b	3.96±0.06b	1.98±0.02b	2.03±0.03b
12S/LW	4.00±0.06b	3.99±0.06b	1.98±0.01b	2.03±0.02b
12S/DW	4.13±0.07b	4.15±0.06b	1.93±0.02b	2.00±0.04b
G/C	3.52±0.06a	3.61±0.05a	2.18 ± 03a	2.19±0.02a

Means followed in a column by the same letter are not significantly different (p=0.05).

Appendix 4. Absorbance ratios of solitarious locusts reared on localized(L) and delocalized(D) kale(K) seedlings at densities of10 and 12 locusts /cage. S/C=solitarious control, 10S/LK=10 solitarious locusts on localized, 10S/DK=10 solitary on delocalized, 12S/LK=12 solitary on localized, 12S/DK=12 solitary on delocalized and G/C=gregarious control.

Treatments	Absorbance Ratio (Mean ± S.E)	
	Males	Females
S/C	0.97 ± 0.04a	1.03 ± 0.08b
10S/LK	1.09 ± 0.04ab	1.09 ± 0.05b
10S/DK	0.99 ± 0.04b	1.09 ± 0.04b
12S/LK	1.07 ± 0.06b	1.10 ± 0.05b
12S/Dk	1.03 ± 0.06b	1.07 ± 0.05b
G/C	1.22 ± 0.07a	1.33 ± 0.13a

Mean followed by the same letter are not significantly different (p=0.05)

Appendix 5. Mean F/C and E/F ratios of solitarious locusts reared on localized(L) and delocalized(D) kale (K) seedlings at densities of 8, 10 and 12 locusts/cage. S/C=solitarious control, 8S/LK=8 solitary locusts on localized, 8S/DK=8 solitary on delocalized, 10S/Lk=10 solitary on localized, 10S/DK=10 solitary on delocalized, 12S/LK=12 solitary on localized,12S/Dk=12 solitary on delocalized and G/C=gregarious control

Treatments	F/C ratio (Mean ± S.E)		E/F ratio (Mean±S.E)	
	Males	Females	Males	Females
S/C	4.14±0.04b	4.03±0.05b'	1.93±0.03b	2.04±0.03b'
8S/LK	4.07±0.03b	4.11±0.05b'	1.97±0.02b	2.01±0.03b'
8S/DK	4.05±0.05b	4.08±0.07b'	1.95±0.03b	1.99±0.04b'
10S/LK	4.00±0.04b	4.20±0.04b'	1.99±0.02b	1.97±0.02b'
10S/DK	3.96±0.07b	3.96±0.06b'	1.98±0.02b	2.03±0.03b'
12S/LK	4.00±0.06b	3.99±0.06b'	1.98±0.01b	2.03±0.02b'
12S/DK	4.13±0.07b	4.15±0.06b'	1.93 0.02b	2.00±0.04b'
G/C	3.52±0.06a	3.61±0.05a'	2.18 ± 03a	2.19±0.02a'

Means followed in a column by the same letter are not significantly different (p=0.05).

Appendix 6. Mean distribution of insects exposed to their own volatiles at different ages and locust densities of 5, 10 and 20/exposure.

Treatment/Days	No. insects (nymphs) per segment (Mean±S.E)		
	1	2	3
S/C_0	8.78±0.50a	4.55±0.40	1.67±0.10
S/C_5	8.06±0.24a	5.11±0.11ced	1.83±0.17
S/C_{10}	8.72±0.24a	4.78±0.22ced	1.50±0.29g
$E5_0$	3.83±0.58e	6.78±0.87b	4.44±0.44ced
$E5_5$	8.11±0.24a	4.33±0.69ced	1.56±0.47g
$E5_{10}$	8.72±0.40a	4.00±0.19ced	1.50±0.00g
$E10_0$	3.83±0.58e	6.78±0.87b	4.44±0.44ced
$E10_5$	7.44±0.56ab	5.89±0.49c	2.00±0.58f
$E10_{10}$	7.89±0.61ab	5.22±0.29ced	1.89±0.49g
$E20_0$	3.83±0.58e	6.78±0.87b	4.44±0.44ced
$E20_5$	5.17±0.19ced	5.61±0.34cd	3.89±0.39e
$E20_{10}$	6.83±0.82cd	4.22±0.78ced	3.94±0.49e
G/I_0	3.83±0.58e	6.78±0.87b	4.44±0.44ced
G/I_5	8.16±0.48ab	4.45±0.56ced	3.83±0.98e
G/I_{10}	8.61±0.57ab	5.11+0.44ced	1.28+0.11g
G/C_0	3.83±0.58e	6.78±0.87b	4.44±0.44ced
G/C_5	4.67±0.03ced	5.67±0.03cd	4.67±0.10ced
G/C_{10}	5,56±0.11cd	5.55±0.11cd	5.00±0.59ef

Means followed in a column by the same letter are not significantly different (P=0.05)

Appendix 7. Mean distribution of nymphs that emerged from solitarious eggs exposed to sand laid in by gregarious females.

Treatments	Nymphs per segment (Mean ± S.E)		
	1	2	3
S/C	6.90 ± 0.29a	3.80 ± 0.40ced	1.30 ± 0.20f
3egg pods	5.53 ± 0.37b	3.20 ± 0.43ed	3.27 ± .69ed
5egg pods	4.87 ± 0.30bc	4.33 ± 0.53bcd	2.80 ± 0.60e
10egg pods	4.00 ± 0.30ced	3.80 ± 0.46ced	4.20 ± .33cd
G/C	4.67 ± 0.40bc	4.40 ± 0.37bcd	2.94 ± 0.44e

Means followed in a column by the same letter are not significantly different (P=0.05).

Appendix 8.Mean distribution of nymphs from solitarious eggs exposed to sand laid in by gregarious females but solvents washed (methonal, cetone, dichloromethane).

Treatments	Nymphs per segment (Mean ± S.E)		
	1	2	3
S/C	6.90 ± 0.29a	3.80 ± 0.40bcd	1.30 ± 0.20h
3egg pods	6.80 ± 0.26a	3.93 ± 0.19bc	1.27 ± 0.17h
5egg pods	6.87 ± 0.27a	2.80 ± 0.44ef	2.33 ± 0.24fg
10egg pods	6.97 ± 0.33a	3.33 ± 0.53cde	1.70 ± 0.36hg
G/C	4.67 ± 0.40b	4.40 ± 0.37b	2.94 ± 0.44def

Means followed in a column by the same letter are not significantly different (P=0.05).

Appendix 9. Mean distribution of nymphs from solitarious eggs exposed to sand laid in by gregarious females but nitrogen-flushed.

Treatments	Nymphs per segment (Mean ± S.E)		
	1	2	3
S/C	6.90±0.29a	3.80±0.40bcd	1.30±0.20f
3egg pods	6.47±0.17a	4.13±0.36bc	1.40±0.33f
5egg pods	6.60±0.38a	3.73±0.33bcd	1.66±0.28f
10egg pods	6.80±0.24a	3.33±0.49cd	1.98±0.40ef
G/C	4.67±0.40b	4.40±0.37b	2.94±0.44ed

Means followed in a column by the same letter are not significantly different (P=0.05).