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SEASONAL VARIATION IN THE ESSENTIAL OIL COMPOSITION OF *COMMIPHORA QUADRICINCTA* AND ITS EFFECT ON THE MATURATION OF IMMATURE ADULTS OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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Key Word Index—*Schistocerca gregaria*; Acrididae; *Commiphora quadricincta*; Burseraceae; essential oil; terpenoids; sexual maturation; male pheromone; kairomone; maturation–acceleration; oocytes; copulation; oviposition.

Abstract—Extracts of steam distillates of the desert shrub *Commiphora quadricincta* were tested for their effects on the maturation of gregarious immature males and females of the desert locust, *Schistocerca gregaria*. Maturation was significantly faster in immature adults exposed to the extract obtained before winter rains than in those exposed to the extract obtained after the rains, with respect to locust colour changes (yellowing of body), mating activity, aggregation–maturation pheromone titres (as measured by phenylacetonitrile levels in males), ovulation (as determined by the length of oocytes in females) and oviposition time. A large number of the compounds in the crude extracts were identified as terpenoids by GC-mass spectrometry. Twenty-two electrophysiologically active compounds were located by GC-EAD in the extract of plant samples taken before the rains, while extracts from plant samples taken after the rains contained 12 such active components. These results confirm a previous finding that at the onset of the rainy season, the essential oil of *Commiphora* shrubs accelerates the maturation of immature adults of the desert locust. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Sexual maturation of the adult desert locust has been reported to coincide with the bud burst of certain desert shrubs at the beginning of the rainy season and a few weeks before the appearance of the annual vegetation [1–3]. These shrubs include species of *Boswellia neglecta* S. Moore and *Commiphora myrrha* (Nees) Engler (Gaffal) which have resinous buds that are the source of the biblical frankincense and myrrh.

Desert locusts in the field apparently respond to the scent of the shrubs that first break bud [1]. Follow-up laboratory experiments have suggested that exposure to the aromatic emissions of commercial articles derived from *Commiphora* shrubs trigger maturation in the insect [2]. Accordingly, it was postulated that essential oils produced by floral parts of many desert shrubs might initiate maturation of locusts in desert areas [3]. On the other hand, introduction of the leafing twigs of *C. opababumum* (L.) Engl. into a diet on which locusts were reared had no apparent effect in inducing maturation, despite the consumption of the leaves by the insects [4]. It has also been observed that maturation may be delayed by certain seasonal changes, such as the onset of drought or infrequent rain when young adults have to feed on senescent plants. Under these conditions young adults remain sexually immature. However, maturation may be normalized when senescent diet is supplemented with gibberellin A_3 and eugenol [5].

These somewhat anecdotal findings suggest that the desert locust might rely on secondary metabolites associated with changing physiology of food and other desert plants to regulate its maturation. Accordingly, an in-depth elucidation of plant–insect relations and the mediating signals in the recession areas could help throw light on some of the mechanisms that underlie population and phase dynamics of the insect. As a first step in the process, we have analysed the composition of the oils of the desert shrub *C. quadricincta* before and after the winter rains around Port Sudan, and investigated their effects on the maturation of gregarious adults of the desert locust.

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Table 1. EAG-active compounds identified in the essential oil from *C. quadricincta* by GC and GC-MS analysis on methyl silicone and carbowax columns

Peak number	Compound	Before rains	After rains	Evidence
16	4-Carene	+	–	MS, RT
18	γ-Terpinene	+	–	MS
	Phenylacetaldehyde	–	+	MS, RT
	Unknown	+	–	
31	2-Nonen-4-one	+	+	MS
40	Cyclopropane, 1,2-dimethyl-1-pentyl	+	–	MS
41	Germacrene B	+	+	MS
	Cyclopropane, 1,1,2-trimethyl	+	–	MS
	Linalol	+	+	MS, RT
	Unknown	+	–	
	Unknown	+	–	
	4-Terpinenyl	–	+	MS
	Unknown	+	–	
	Linalyl-propanoate	+	+	MS
	7-Octen-1-ol,2-methyl-6-methylene	+	–	MS
	1(2H)-Naphthalenone, 3,4,4a,5,8,8a-hexahydro-8-methyl-,*trans*-	+	–	MS
	Unknown	+	–	
	4-Hydroxy-2-methylacetophenone	+	–	MS
	Cedr-8-ene	+	–	MS
50	Caryophyllene	+	+	MS, RT
54	Alloaramadendrene	+	+	MS, RT
55	δ-Cadinene	–	+	MS
	1*R*-(1*R*,2*R*,5*S*,6*E*,10*R*)-8-methylene-5-(1-methylethyl)-spiro-11-oxabicyclo-8.1.0 undec-6-ene-2,2′-ox	+	–	MS
	Unknown	–	+	
	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, *S*-(*Z*)-	+	–	MS
	(–)-Spathulenol	–	+	MS
75	α-Farnesene	–	+	MS, RT
81	α-Guainene	+	+	MS, RT
82	(*E*)-Isoeugenol	+	–	MS, RT

MS = sample spectrum compared to library spectrum.
RT = confirmed by retention time with authentic sample.
Peak number indicated identified using carbowax column.

RESULTS

Analysis of oil. GC-EAD analysis revealed 22 electrophysiologically active compounds from the steam-distilled oil of *C. quadricincta* collected before the winter rains, while the oil obtained after the winter rains showed 12 active compounds (Table 1). Compounds were identified by GC-mass spectrometry, by comparison of their spectral data with those in the library of the mass spectrometer and, in certain cases, by comparison of GC-mass spectrometry and retention times of authentic samples. A significant number of the compounds identified were terpenoids (Table 2 and Fig. 1).

Bioassays. The effects of crude steam distillate extracts of *C. quadricincta* obtained before and after winter rains on the maturation of gregarious immature males and females, as assessed by colour, copulation and oviposition, are summarized in Tables 3 and 4. Although both volatiles gave significant acceleration of maturation compared to untreated controls, the sample collected before the winter was clearly more potent than that collected after the rains (compare particularly 0.1 μl doses for effects on males in Table 3 and on females in Table 4). Moreover, at 0.1 μl doses, maturation times were not significantly different from treatments involving exposure of the insects to mature males (Tables 3 and 4).

Figure 2 shows the effects of the crude steam distillates of *C. quadricincta* on the titres of phenylacetonitrile, the major component of adult aggregation–maturation pheromone emitted by maturing males. The phenylacetonitrile titres in males exposed to the distillate collected before the rains were similar to those exposed to mature males, and those exposed to the distillate collected after the rains followed roughly the pattern of the control insects.

Table 5 gives the period of the first gonotrophic cycles of immature females under different treatments. Females exposed either to *C. quadricincta* volatiles collected before winter rains or to mature males had shorter gonotrophic periods compared either to controls or to those exposed to *Commiphora* volatiles collected after the winter rains.

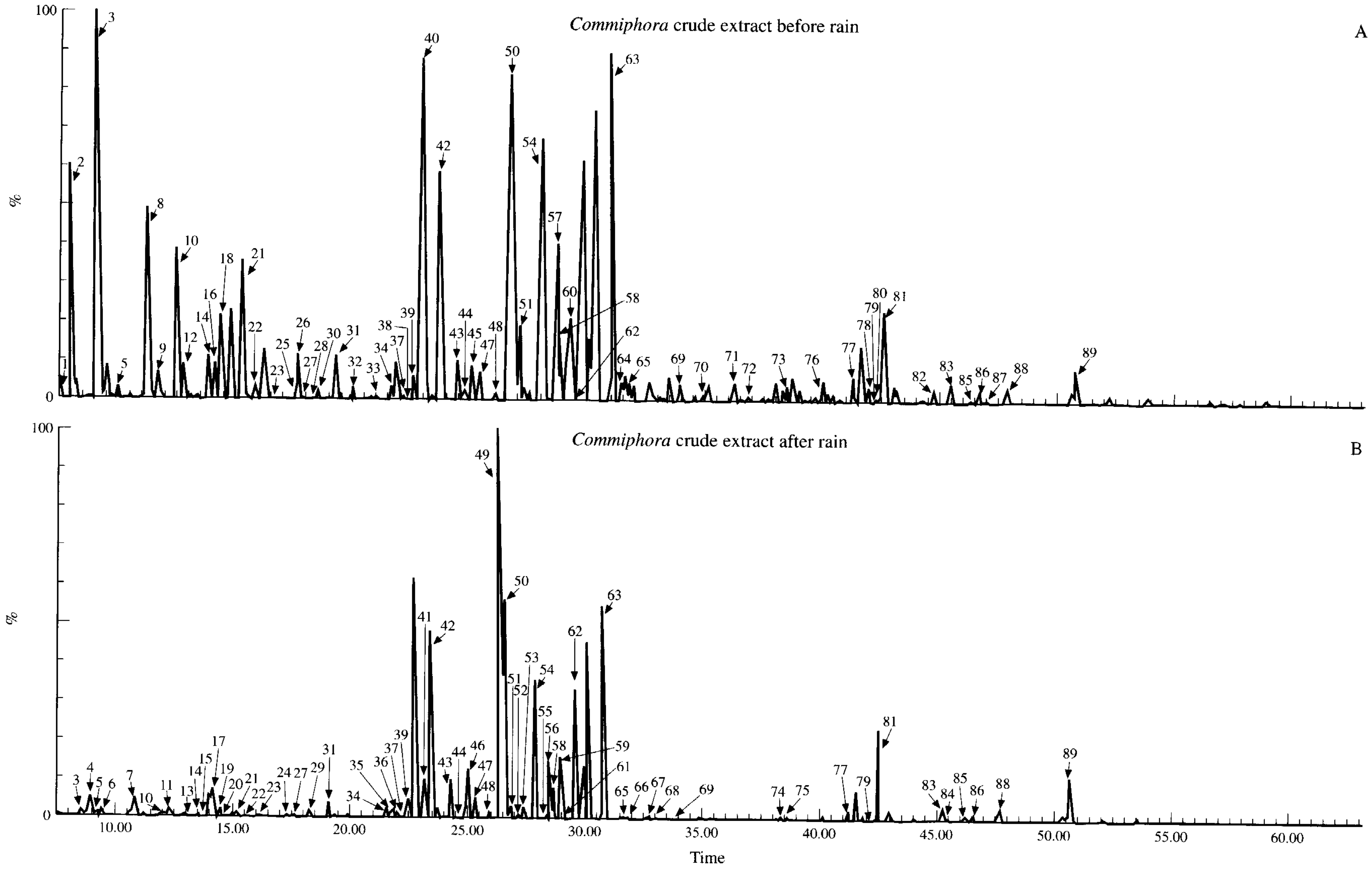

Fig. 1. Total ion chromatograms of crude steam distillate extracts of desert shrub *C. quadricincta* injected onto a 50-m carbowax 20 M capillary column: (A) collected in October 1994 2 weeks before winter rains; (B) collected in March 1995 after rains at winter breeding areas of the desert locust at Red Sea Coast, Sudan.

Table 2. Additional compounds identified in the essential oil from *C. quadricincta* (carbowax column)

Peak number	Compound	Before rains	After rains	Evidence
1	α-Pinene	+	−	MS, RT
2	Camphene	+	−	MS, RT
3	β-Pinene	+	+	MS, RT
4	*o*-Xylene	−	+	MS, RT
5	*m*-Xylene	+	+	MS, RT
6	*p*-Xylene	−	+	MS, RT
7	Isopropylbenzene	−	+	MS
8	β-Myrcene	+	−	MS, RT
9	4-Terpinenyl acetate	+	−	MS
10	Limonene	+	+	MS, RT
11	*n*-Propylbenzene	−	+	MS
12	α-Phellandrene	+	−	MS, RT
13	Undecane	−	+	MS
14	(*E*,*E*)2,4-Nonadienal	+	+	MS, RT
15	β-Ocimene	+	+	MS, RT
17	Styrene	−	+	MS, RT
19	α-Ocimene	−	+	MS, RT
20	4-Methylstyrene	−	+	MS
21	*p*-Cymene	+	+	MS, RT
22	α-Terpinolene	+	+	MS, RT
23	2-Octen-4-one	+	+	MS
24	3-Phenylpropyl acetate	−	+	MS
25	α-Methylstyrene	+	−	MS
26	4-Nonanone	+	−	MS, RT
27	6-Methyl-5-hepten-2-one	+	+	MS, RT
28	3,4-Dimethyl-octane	+	−	MS, RT
29	1-Hexanol	−	+	MS, RT
30	Citronellyl formate	+	−	MS
32	Nonanal	+	−	MS, RT
33	AR, α-Dimethylstyrene	+	−	MS
34	Linalool oxide	+	+	MS
35	1-Methyl-4-methylene-cycloheptane	−	+	MS
36	1-Heptanol	−	+	MS, RT
37	1-Heptyl-2-methyl-cyclopropane	+	+	MS
38	(*E*)-Solanone	+	−	MS
39	α-Cubebene	+	+	MS, RT
42	Copeane	+	+	MS, RT
43	β-Bourbonene	+	+	MS
44	(*Z*)-β-Farnesene	+	+	MS, RT
45	Ledol	+	−	MS
46	Linalyl acetate	−	+	MS, RT
47	(*E*)-2-Nonenal	+	+	MS
48	β-Cubebene	+	+	MS
49	4-Terpineol	−	+	MS
51	(+)-Aromadendrene	+	+	MS, RT
52	*cis*-Sabinenehydrate	−	+	MS
53	Embellulone	−	+	MS
56	α-Humulene	−	+	MS
57	α-Caryophyllene	+	−	MS
58	(*E*)-β-Farnesene	+	+	MS, RT
59	Linalyl propionate	−	+	MS
60	1-α-Terpineol	+	−	MS
61	Ledene	−	+	MS, RT
62	γ-Cadinene	+	+	MS, RT
63	Cadinene	+	+	MS
64	Myrtenol	+	−	MS
65	(−)-α-Muurolene	+	+	MS
66	*p*-Mentha-1(7),8(10)-dien-9-ol	−	+	MS
67	*p*-Cymen-8-ol	−	+	MS
68	*trans*-*trans*-Octa-2,4-dienyl acetate	−	+	MS

Table 2. (*Continued*)

Peak number	Compound	Before rains	After rains	Evidence
69	Germacrene *D*	+	+	MS
70	Palustrol	+	–	MS
71	Patchoulane	+	–	MS
72	(–)-Globulol	+	–	MS
73	β-Cedrene	+	–	MS, RT
74	1,2,3,4,4a,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene	–	+	MS
76	1H Cycloprop e azulene-7-ol, decahydro-1,1,7-trimethyl-4-methylenper-hydro. [1a*S*-(1aα,4aα,7α,7aβ,7bα)]	+	–	MS
77	δ-Cadinol	+	+	MS
78	Terreyol	+	–	MS
79	2,4-Diphenyl-4-methyl-2-(*Z*)-pentene	+	+	MS
80	Cadalin	+	–	MS
83	Nonadecane	+	+	MS
84	2-Methyl-2-phenyl pentadecane	–	+	MS
85	Calemenene	+	+	MS
86	2,4-Diphenyl-4-methyl-2-(*E*) pentene	+	+	MS
87	4 t-Butyl-2-(dimethyl benzyl) phenol	+	–	MS
88	9-*M*-Octylheptadecane	+	+	MS
89	2,6-Bis(t-butyl)-4-(dimethylbenzyl)phenol	+	+	MS

MS = sample spectrum compared to library spectrum.
RT = confirmed by retention time with authentic sample.

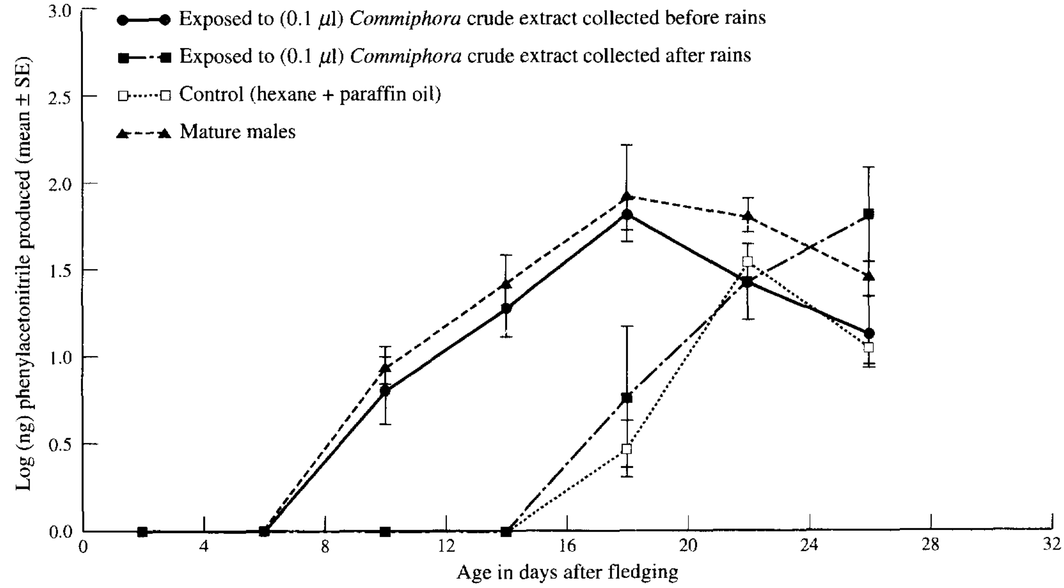

Fig. 2. Phenylacetonitrile production by males exposed to: (a) crude steam distillates of *C. quadricincta* collected before and after winter rains; (b) mature males; (c) immature males controls (hexane + paraffin oil).

Table 5 also gives the average number of eggs in pods oviposited by females under different treatments. Interestingly, females exposed to *Commiphora* volatiles collected prior to winter rains gave pods with significantly higher numbers of eggs.

DISCUSSION

Our results parallel those of Carlisle *et al.* [2], who had demonstrated that commercial articles derived from *Commiphora* spp., such as resins and essential oils, caused significant acceleration of maturation of laboratory reared desert locusts. In the present study, we confirmed this effect by monitoring body colour change and copulation time in both sexes, male-produced aggregation pheromone (monitored by phenylacetonitrile titres), and oocyte growth and oviposition time in females. We also compared the effects of oils directly collected from the same set of *C. quadricincta* shrubs before the winter rains and after the wet season. Although both oils caused significant

Table 3. Mean maturation times of the immature males exposed to different doses of crude extract of *C. quadricincta* before and after the winter rains, mature males, and paraffin oil + *n*-hexane (control)

Treatment (test insect/signal source)	Maturation time (days, X ± S.E.)* By colour	By copulation	*N*
Immature ♂/control (hexane + paraffin oil)	22.67 ± 0.71 a	23.07 ± 0.83 a	15
Immature ♂/0.1 μl crude extract (AWR)	18.85 ± 0.49 b	19.50 ± 0.55 b	20
Immature ♂/0.001 μl crude extract (BWR)	15.55 ± 0.84 c	16.55 ± 0.84 c	20
Immature ♂/10 μl crude extract (AWR)	14.50 ± 0.11 cd	15.05 ± 0.17 cd	20
Immature ♂/10 μl crude extract (BWR)	14.50 ± 0.80 cd	15.50 ± 0.77 cde	20
Immature ♂/0.1 μl crude extract (BWR)	13.50 ± 0.38 cd	14.25 ± 0.30 de	20
Immature ♂/mature ♂	13.00 ± 0.36 d	13.75 ± 0.30 e	20

Means with the same letter are not significantly different at $P = 0.05$ according to LSD.
N, number of surviving insects at the end of the experiment.
*No significant difference between maturation times by colour and copulation ($P > 0.05$, *t* test).
BWR, before winter rains; AWR, after winter rains.

Table 4. Mean maturation times of the immature females exposed to different doses of crude extract of *C. quadricincta* before and after the winter rains, mature males, and paraffin oil + *n*-hexane (control)

Treatment (test insect/signal source)	Maturation time (days (X ± S.E.)* By colour	By copulation	Oviposition time (days, X ± S.E.)	*N*
Immature ♀/control (hexane + paraffin oil)	22.31 ± 0.81 a	22.56 ± 0.82 a	24.94 ± 0.70 a	16
Immature ♀/0.1 μl crude extract (AWR)	18.50 ± 0.47 b	18.81 ± 0.44 b	22.75 ± 0.65 b	16
Immature ♀/10 μl crude extract (AWR)	16.76 ± 0.36 c	17.06 ± 0.35 c	19.18 ± 0.37 c	17
Immature ♀/0.001 μl crude extract (BWR)	14.69 ± 0.45 d	15.25 ± 0.49 d	17.19 ± 0.40 d	16
Immature ♀/10 μl crude extract (BWR)	14.59 ± 0.23 d	14.76 ± 0.25 d	17.35 ± 0.31 d	17
Immature ♀/0.1 μl crude extract (BWR)	12.75 ± 0.34 e	13.38 ± 0.26 e	15.13 ± 0.30 e	16
Immature ♀/mature ♂	12.00 ± 0.00 e	12.44 ± 0.13 e	14.94 ± 0.14 e	16

Means with the same letter are not significantly different at $P = 0.05$ according to LSD.
*No significant difference between maturation times by colour and copulation ($P > 0.05$, *t* test).
N, number of surviving insects at the end of the experiment.
BWR, before winter rains; AWR, after winter rains.

Table 5. Gonotrophic period and mean number of eggs per pod of females exposed to (a) *C. quadricincta* crude volatiles collected before and after winter rains, (b) mature males and (c) paraffin oil + hexane (control)

Treatment (test insect/signal source)	First gonotrophic period in days*	*N*#	Eggs per pod per female ± S.E.	*N*
Immature ♀/*Commiphora* extract (0.1 μl) (BWR)	14–16	16	58.80 ± 2.63 a	15
Immature ♀/*Commiphora* extract (0.1 μl) (AWR)	18–22	16	38.94 ± 1.70 b	17
Immature ♀/mature ♂	14–16	16	44.63 ± 2.62 b	16
Immature ♀/paraffin oil + hexane	22–24	16	44.44 ± 2.12 b	16

Means with the same letter in column are not significantly different at $P = 0.05$ according to LSD.
N#, Number of insects dissected for each treatment of the experiment.
N, Number of eggs pod counted for each treatment of the experiment.
BWR, before winter rains; AWR, after winter rains.
* Monitored by oocyte length.

acceleration of maturation, the former was significantly more potent and, in fact, comparable to the effect of mature males (Tables 3 and 4) [6, 7].

Our analysis of steam volatiles of *C. quadricincta* collected around Port Sudan before and after the winter rains showed the presence of a large number of compounds, predominantly terpenoid (Fig. 1 and Tables 1 and 2). Volatiles collected before the rains were comparatively richer, particularly in the more volatile fraction, and GC-EAD runs revealed that about 22 of these were electrophysiologically active, compared to about 12 in volatiles collected after the rainy season. Previously, Carlisle *et al.* [2] tested four major constituents of *C. myrrhae* oil comprising α-pinene, β-pinene, limonene and eugenol, for their maturation accelerating effects. Each was found to accelerate maturation independently. In our GC-EAD studies, the monoterpenes (α- and β-pinene and limonene) were identified in proportions which were not electrophysiologically active. Eugenol, on the other hand, elicited a strong EAG response. In our previous studies on the maturation accelerating effect of volatile emissions associated with maturing and fully mature male desert locusts, the active blend comprised mainly of a series of simple aromatic derivatives [7]. Thus, it appears probable that a range of volatile terpenoids and phenolic plant constituents in different blends may function as effective maturation accelerants for the desert locust.

A key question arising from these laboratory findings is whether plant secondary metabolites associated with physiological changes in desert plants in specific seasons provide useful signals to the desert locust relating to the timing of its reproductive activity. Carlisle *et al.* [2] have pointed out that maturation in the insect following a period of drought occurs more or less simultaneously at sites hundreds of km apart a few weeks before the arrival of rains. Moreover, laboratory experiments have failed to show any clear correlation between the onset of maturation in the insect and such factors as humidity, photoperiod or temperature changes [8]. Accordingly, it has been proposed that desert shrubs such as *Commiphora* in eastern Africa, and other plant species that may have the same ecological niche elsewhere in the locust recession areas, may provide the environmental cue by which the desert locust synchronizes its mating to the on-coming rains [2]. However, it may be that other desert perennials that function as food or shelter plants for the insect also play a similar role. Indeed, *Commiphora* plants are relatively rare in the Red Sea area of the Sudan, and are unlikely to be the sole source of such a cue there.

Another remarkable effect of the volatiles collected from *C. quadricincta* before the rains is on the number of eggs produced by each insect (Table 5). Significantly larger number of eggs relative to controls indicate that the volatiles provide an additional signal consistent with the insects' need to multiply rapidly during the short wet seasons. Interestingly, the oil collected after the winter rains showed a small, although not significant, drop in the number of eggs per pod, and suggests that more research is needed to explore its full implication.

In conclusion, we concur with speculations by Carlisle *et al.* [2] on the probable role of desert shrubs in providing semiochemical signals that elicit maturation in the desert locust prior to the onset of rains. Our results suggest that additional signals may also be involved in modifying the reproductive potential of the insect. An in-depth study of the chemistry of different desert plants in different seasons and their relation to locust physiology, behaviour and reproduction in the field is clearly warranted, to help elucidate the mechanisms that underlie the population and phase dynamics of the insect.

EXPERIMENTAL

Plant materials. Twigs and leaves from the same set of plants of *C. quadricincta* were collected at Klanieb in the southwest of Port-Sudan, the Red Sea coastal area in the Sudan, in October 1994 before the winter rains, and in March 1995 after the winter rains. The plant material was identified by M. O. Bashir (Applied Ecology Department, International Center of Insect Ecology and Physiology, ICIPE) and a voucher specimen (94/1) is deposited in the Chemical Ecology Department (CED) at ICIPE.

Insects. The desert locust *Schistocerca gregaria* was reared in a crowded colony at the Animal Breeding and Quarantine Unit at ICIPE. The colony originated from a stock kept by The Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Addis Ababa, Ethiopia. Insects (300–400) of both sexes were reared under crowded conditions in aluminum cages (50 × 50 × 50 cm). The rearing room (4.5 × 4.5 m) was well aerated through a ducting system that operated at a negative pressure and the room was maintained at a temp of 33 ± 1°C, a humidity of 60%, and a 12L:12D hr light–dark cycle. Insects used for the experiments were fledgling males and females from the same batch (1–2 days old) and mature males, to eliminate possible variability in their physiological status.

Extraction. Volatiles from the plants were collected by steam distillation using a Clevenger-type apparatus. Four samples of the plant material (1 kg each time) were each boiled in water and the generated blend of steam and plant volatiles condensed into the clevenger which contained hexane. The hexane extracts were pooled and concd using a short distillation apparatus (Kontes), and the concentrate of the crude extract was stored at −15° until use.

Bioassays. Previously described aluminum bichamber cages [7] were used to test the effect of the crude steam distillate extract of *C. quadricincta*, before and after the winter rains, on the maturation of gregarious immature adult males and females. Each cage consisted of two interlinked chambers, of identical size (15 × 15 × 15 cm), with sliding glass doors at the front, a wire gauze on end of the other three sides and the top, and a wire gauze between the two chambers to allow diffusion of volatiles [7]. Newly moulted immature adults (2–3 days old) were placed in the lower compartment of the cage and supplied with fresh wheat seedlings and wheat bran for food. Cages were kept under the same conditions as for rearing the insects throughout the experimental period. *C. quad-*

ricincta extracts, in vials (3.7 ml) with 2 mm vents, were placed in the upper compartment of the bichamber cages (referred to as the signal source) at the following doses: 10, 0.1 and 0.001 μl oil from plants collected before winter rains, and 10 and 0.1 μl oil from plants collected after the rains, each in 1000 μl light paraffin oil to moderate the release of the volatiles. In the control bichambers only, light paraffin oil (1000 μl) with hexane (100 μl) was used. For comparison purposes, mature males, known to accelerate maturation of young adults [7], were placed in the upper compartment of one set of control bichambers, and immature adults in another set of controls. Each set was replicated 4 ×. All signals sources were changed every 3 days to maintain the same degree of uniformity in release rates. For treatments involving females, an additional five replicates were set up to allow measurements of lengths of oocytes to be made.

Recipient insects were assessed daily for signs of maturation using the following parameters: (1) changes in colour (yellowing) in accordance with Norris [6] stage III classification (see also ref. [7]); (2) copulation time [carried out by pairing (a) test males which had begun to develop the yellow colouration on the abdomen with fully mature females, and (b) test females with mature males, for about 6 hr daily, and noting evidence of copulation]; (3) phenylacetonitrile titre [7, 9] of males at different ages (10–14, 14–18, 18–22, 22–26, and 26–30 days); (4) time of oviposition (i.e. the ages of the test females at first oviposition); (5) first gonotrophic period as measured by the maximum length of the oocytes in females from day 12 to day 30, every 2 days (the abdomens of adult females were cut open longitudinally, ovaries examined under a binocular microscope and the length of six developing terminal oocytes in the first gonotrophic cycle measured using a micrometer).

The egg pods laid by the insects were removed from the egg laying tube, and the eggs in each pod laid by each insect were counted.

Quantification of phenylacetonitrile. The major component of adult male pheromone, phenylacetonitrile, was monitored as previously described by entrainment of the pheromone on charcoal traps [9]. Briefly, air from a compressed air cylinder was passed through a charcoal filter, over locusts contained in a trapping chamber (10 cm long × 3.5 cm i.d.), and through the charcoal trap packed between two glass wool plugs in 6 cm long × 8 cm i.d. glass tubes at 106 ml min^{-1} for 16 hr at $30 \pm 1°$. Collections were made from sets of nine adult males in groups of three (aged 12–16, 16–20, 20–24, 24–28 and 28–32 days after final moult) exposed to: (a) crude extract of steam distillate of *Commiphora* collected before rains; (b) crude extract of steam distillate of the plant (collected after the rainy season); (c) mature males (control); (d) immature adults (control).

Dichloromethane extracts of insect volatiles trapped on charcoal were concd to 100 μl and 250 ng of *o*-methyl acetophenone were added as the int. standard. Aliquots (2.5 μl) of these extracts were used for analysis by capillary GC. Analyses were performed on a HP 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HP capillary column (Carbowax, 50 m × 0.32 mm i.d. × 0.3 μm film thickness) using nitrogen as the carrier gas at a flow rate of 0.35 ml min^{-1}. The oven temperature was initially isothermal at 60° for 10 min, then programmed at 5° min^{-1} to 180° for 5 min and at 10° min to 220° for 15 min. Chromatographic peaks including that of phenylacetonitrile, were integrated using a HP 3393 integrator, and the released amounts of this compound quantified.

Analysis of the oil of C. quadricincta. The crude steam distillate extracts (2 μl) were analysed by GC-EAD and GC-MS. For GC-EAD, GC conditions were as above. However, the GC was equipped with a 1:1 splitter to facilitate delivery of volatiles over an antennal preparation. The deactivated silica line delivering volatiles over the antenna via a steel tube (0.5 cm i.d.) was maintained at 150° by a temperature control unit (THC-3, Syntech). FID and EAD signals were synchronously monitored by a GC-EAD interface card (Syntech) installed in a PC (Harvard Professional Computers, American Megatrends Inc.). For GC-MS, a VG Masslab 12–250 mass spectrometer (EI, 70 eV) coupled to a 5790 gas chromatograph was used, the latter employing the same GC conditions as above.

Statistical analysis. All data collected were subjected to analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC 27512-8000, U.S.A., 1987). The means were compared by the least significant difference test.

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