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Novel cross-stage solitarising effect of gregarious-phase adult desert locust (*Schistocerca gregaria* (Forskål)) pheromone on hoppers

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ABSTRACT

Previous studies had demonstrated stage differentiation in the cohesion (aggregation) pheromone systems of the desert locust, *Schistocerca gregaria*. In laboratory arena, the nymphal and adult stages responded aggregatively to their own pheromone, but dispersed evenly within the arena in the presence of the other. In the present study, we explored the effects of longer-term contact of field gregarious hopper bands and laboratory crowd-reared nymphs with the major constituent of the adult pheromone. During the first few days, hoppers in treated bands became relatively hyperactive. Over the next few days, their movements became random and they stopped marching as coherent groups, they started to roost for longer periods on vegetations, and they fragmented into smaller and smaller groupings and individuals. When attacked by birds, they demonstrated subdued levels of collective defensive behaviour compared to normal hoppers, and there were clear signs of increased predation and cannibalism at the roosting sites. In cage experiments, crowd-reared nymphs treated with the pheromone component became hyperactive, showed abnormal diel patterns and reduced feeding on plants but increased cannibalism. Our observations show that the major adult pheromone constituent has a solitarising effect on gregarious hoppers. The mechanism underlying this effect and the potential of the agent in desert locust control are discussed.

1. Introduction

Locusts are characterized by tendency to transform reversibly between solitarious and gregarious phases that differ in behaviour, physiology, biochemistry, pigmentation, and morphology (Uvarov, 1966; Applebaum et al., 1997; Pener and Yerushalmi, 1998; Ainstey et al., 2009; Verlinden et al., 2009). Phase dynamics associated with this phenotypic plasticity is the key to the biology and pest status of locusts. It is predicated on the insect density in the primary breeding areas associated with certain environmental and biotic factors that determine frequency of encounters between the insects (Roffey and Popov, 1968; Bouaïchi et al., 1996; Hassanali et al., 2005a). Forced crowding of solitarious desert locusts leads to rapid shifts in some phase-related traits like aggregation behaviour (Roessingh et al., 1998); others, such as morphometrics, take several generations (Uvarov, 1966). Studies in different laboratories have identified two different contact stimuli as responsible for priming solitarious insects to shift toward the gregarious phase: a chemotactile signal associated with the cuticular hydrocarbon fraction of the insect (Heifetz et al., 1997) and a site-specific mechanosensory stimulus associated with repeated tactile stimulation (Simpson et al., 2001). A combination of visual and olfactory cues also primes some behavioural gregarisation (Roessingh et al., 1998), which may be particularly important in recruiting solitarious individuals into gregarious groups. Individual locusts that experience tactile stimulation rapidly produce the neurochemical serotonin (Ainstey et al., 2009), which may be responsible for switching on traits typical of the gregarious phase. These include emission of a series of pheromones that mediate key behavioural or physiological traits of gregarising or gregarious locusts, such as social cohesion, synchronous maturation, communal oviposition, and trans-generational transfer of phase traits (McCaffery et al., 1998; Hassanali and Torto, 1999; Hassanali et al., 2005b). On the other hand, isolation of previously crowded locusts leads to rapid inhibition of the production of these pheromones (Deng et al., 1996).

An interesting finding relates to the mediation of distinct releaser pheromones in the cohesive (aggregation) behaviours of adult and nymphal desert locusts (Obeng-Ofori et al., 1993). In a

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laboratory arena with two columns of air, one permeated with volatiles from nymphs or adults and the other untreated, groups of the nymphs or adults of the insect responded strongly by clumping together in the presence of their own pheromone but were indifferent to that of the other. The adult pheromone, which is made up of a blend of benzene derivatives, including phenylacetonitrile (the major component present in about 80%), benzaldehyde, guaiacol, and phenol, is emitted by older and mature males (Torto et al., 1994). These constituents contribute differentially to the cohesion effect of the pheromone with the major component (phenylacetonitrile, PAN) being the most potent. The nymphal pheromone, emitted by both sexes, consists of a mixture of aliphatic (C_6, C_8–C_{10}) aldehydes and acids and the two phenolic constituents (guaiacol and phenol) present in the adult pheromone (Torto et al., 1996). Synthetic blend of the eight aliphatic compounds and two phenols promoted nymphal aggregation to a level comparable to that induced by the natural nymphal volatile blend. This stage differentiation in the cohesion pheromone systems of adult and nymphal desert locusts may have evolved in response to the need to separate the adults from hoppers and thus to minimize competition in environments with patchy food resources where the insect primarily breeds and multiplies (Uvarov, 1966; Obeng-Ofori et al., 1993). In natural field populations, cross-stage pheromonal contact is relatively transient since both stages are mobile, particularly the adults that can fly off in swarms to search for its food elsewhere. We hypothesised that longer-term exposure of gregarious nymphal groups to the adult pheromone could gradually lead to significant dispersal and perhaps effective solitarisation of the treated insects. During the last 10 years we undertook a series of studies in cages, enclosed field arenas, and, whenever opportunity availed, field populations of the desert locust in northern Sudan to study the effects of exposing crowd-reared nymphs or field hopper bands to the adult pheromone. In this paper, we outline behavioural and physiological evidence of the solitarising and associated effects of the major constituent of the adult pheromone on crowd-reared groups in cages and on natural hopper bands in the field.

2. Materials and methods

2.1. Reared locusts

For cage trials, locusts were crowd-reared at ICIPE field station in Port Sudan in aluminium cages (50 cm × 50 cm × 50 cm) similar to those described by Ochieng-Odero et al. (1994) in a room with open windows to allow temperature and humidity conditions to equilibrate with the outside. The colony was developed initially from egg pods collected from the field and the laboratory culture was regularly refreshed with new field collections. Insects were fed on natural desert food plants collected from the field, mainly millet and *Heliotropium* spp., with some *Dipterygium glaucum* Dene and *Crotolaria microphylla* Vahl. Other desert plant twigs such as those of *Aerva javanica* (Burm. f.) Shult. and *Panicum turgidum* (Forsk) were provided for roosting. During shortage of field plants, a diet consisting of these plants stored in the freezer (−20 °C), supplemented with fresh alfalfa, millet and wheat bran, was provided.

2.2. Field bands

Field tests were conducted on natural bands in an area of Adarat (18°0634′N/38°1501′E) 215 km south of Port Sudan during the months of December/February in 1998–1999. Distinct small bands of marching hoppers had originated from oviposition sites of small sand beds at the base of the mountains on the western side that were kept separate by the rugged terrain and small khors with water courses (following rainfall in the area). The bands were found marching eastwards toward silt-enriched plateau with millet cultivations.

These multiple relatively small bands provided a rare opportunity for detailed replicated observations on the effect of the major component of the adult pheromone on their behaviour. Ten bands spatially separated from one another were selected. The age structure and approximate number of nymphs per band were estimated during their roosting stage on shrubs of *Acacia tortilis* (Forssk.) Hayne and *Lycium persicum* Miers. Most of the bands were in late 3rd to 4th instars (ratio ranging from 4:1 to 2.3:1) with the rest in 4th to 5th instars (ratio ranging from 6:1 to 1:2.7). The number of hopper individuals in the bands ranged from ∼9150 to 34,800 (average of ∼20,806) initially occupying areas ranging from 28 to 87 m^2 (average: ∼58 m^2).

2.3. Phenylacetonitrile (PAN) treatments

For marching bands in the field, three concentrations of PAN (∼98%, Sigma) in 50% aqueous acetone (0.1, 0.5 and 1%) were used, each on two replicates. The treatments were made early in the morning while roosting nymphs were concentrated on bushes. In each case, 1 ha of land around the target bushes was delineated and the whole area, including the bushes with roosting hoppers, sprayed uniformly. For control, one pair was treated with aqueous acetone and the other was left untreated. In all treatments, knapsack mist blower was used for spraying PAN-containing formulations (or aqueous acetone control). The mist blower was calibrated and deployed as follows: flow rate, 80 ml/min; height of sprayer nozzle, 1 m; track space, 8 m; operator speed, 3.6 km/h; and volume application rate (VAR), 2 l/ha. In cage tests, 4th instar nymphs were initially placed in a 100 m^2 enclosed area (10 m × 10 m bomas) on the ground for treatment. A battery operated Micro Ulva, spinning disc type hand-held sprayer was used to deliver uniformly the required dose of PAN (equivalent to 10 ml/ha) in diesel over the whole area in about ∼36 s. The sprayer was calibrated and deployed as follows: flow rate, 35 ml/min; height of sprayer nozzle, 0.5 m; disc speed, 7500–8000 rpm; and volume application rate (VAR), 2 l/ha. After treatments, the nymphs were transferred to cages where appropriate observations were made.

2.4. Observations on field hopper bands

These were carried out with the help of a group of 10 well-trained technical personnel. The following attributes of each band were monitored (facilitated by the relatively small sizes of the bands) and recorded every 2 h daily from 6 am to 6 pm by the team of observers: displacement direction and distance marched; number of leading edges (representing relatively higher concentrations of hoppers in the front compared to the rest of the band behind) of the marching band; and number of roosting groups of each band in different bushes at the start of daily observations. At the start of day 3 after treatments, number of nymphs affected by predation (presence of two hind legs, four forelegs, a gut or a head capsule, parts typically unconsumed by bird species in the area, was taken as representing one individual), at the roosting sites were recorded. This involved careful inspection of all the sites occupied by roosting groups (a total of 45 PAN-treated and 7 untreated). Concurrently, assessment of levels of cannibalism (based on number of dead individual hoppers located under the bushes that were not completely consumed and that showed clear signs of having been mauled by conspecifics, e.g. crawling nymphs without abdominal parts) was made. The number of hoppers affected by predation and cannibalism, respectively, in each band relative to the total number of individuals in all observed bands (presented as predation and cannibalism indices) were computed. In addition, defensive behaviours of treated and untreated

gregarious hoppers when attacked by birds during marching (concerted hopping typically demonstrated by most individuals in a normal hopper band or lack of this) and while roosting on bushes (hoppers dropping together on the ground and running in a concerted fashion toward adjacent bushes) were recorded whenever such attacks were encountered from day 3 of the observation period for up to 20 cases for each replicate treatment.

2.5. Cage experiments

2.5.1. Effect on diel patterns

Since cage experiments restricted the nymphs to crowded conditions (unlike in the field where they were free to disperse), the effect of varying replicate doses of PAN (equivalents of 20, 10, 5, 1, or 0.5 ml/ha in diesel, with untreated insects as control) on selected daily behavioural patterns (feeding, mobility and roosting) typical of the gregarious-phase hoppers was initially observed to establish the minimum dose that showed optimum effects. Groups of 100 4th instar crowd-reared nymphs, that had moulted 2 days earlier, were treated with the different doses of PAN. Nymphs from each treatment were transferred in three replicates to 1 m × 1 m × 1 m cages with open bottoms erected on outdoor plots cultivated with millet (beds 15 cm apart, 20 plats per hole). Some *A. javanica* and *P. turgidum* twigs were also placed inside each cage to provide roosting sites to the insects. Observations were made for 2 days after treatment at 8 am (normal period of feeding and mobility by gregarious hoppers) and 8 pm (normal period of roosting) for 1 h. The number and percentage of nymphs engaged in feeding, moving, or roosting were noted.

The above test was repeated (under slightly modified conditions) to see if some of the observed effects of PAN extended beyond the two periods. The experiment was carried out with groups of 50 4th instar nymphs treated with either PAN equivalent of 10 ml/ha (found to be the optimum dose in the above test) or pure diesel, or left untreated, each in four replicates. The nymphs were transferred to 50 cm × 50 cm × 50 cm wire mesh cages with wooden frames. Fresh *Heliotropium ovalifolium* Forssk was provided as food on each of the 2 days of observations. *A. javanica* (Burm. f.) Shult. twigs inside each cage provided roosting sites for the insects. The cages were kept indoors and observed every hour from 6 to 9 am, 12 to 3 pm, 3 to 5 pm and 6 to 9 pm for 2 days. The number and percentage of nymphs engaged in feeding, moving, and roosting during these periods were recorded.

2.5.2. Effect on feeding rates

Five replicates of 20 4th instar nymphs that had molted 2 days earlier were treated with PAN as above (at 10 ml/ha) and transferred into 50 cm × 50 cm × 50 cm cages as above. For control, a similar number of replicates treated with diesel or left untreated were deployed. Fresh food consisting of *H. ovalifolium* (100 g) was provided early in the morning (6 am). After 24 h, the remaining food and spill were carefully collected, dried for 24 h in an oven (80 °C) and weighed twice on an infra red balance. 100 samples (each 1.000 g) of fresh *H. ovalifolium* were similarly dried and weighed to obtain the average dry weight of the plant (0.2138 ± 0.0745 g).

2.5.3. Effect on cannibalistic behaviour

150 4th instar nymphs (that also had moulted 2 days earlier) were treated with PAN as above and divided into three sets, each kept in a wire mesh 50 cm × 50 cm × 50 cm cage. As above, the cages were provided with fresh *H. ovalifolium* as food and *A. javanica* twigs for roosting. Controls comprised of cages with nymphs sprayed with PAN carrier (diesel) and untreated nymphs. The cages were kept indoors and observed on hourly basis every day. Dead nymphs were immediately removed and inspected. Number of nymphs that were cannibalised (evidence of having been mauled and partially fed) was recorded. The percentage of cannibalised nymphs was computed.

2.5.4. Comparison of the effects of PAN and antennectomy on diel patterns

Replicate groups (three each) of 50 4th instar nymphs were either treated with PAN (at 10 ml/ha in diesel), antennectomised, or left untreated (control) and the number of nymphs engaged in different behaviours (feeding, roosting, moving) at 8 am and 8 pm compared for 3 successive days after treatment as for previous experiment on diel patterns.

2.6. Data analyses

For treatments on field bands, there were no discernible differences between effects of the three doses of PAN on distance marched, number of leading edges of the marching band, and number of roosting groups in different bushes, so the time-course data for the three treatments were pooled. Likewise, the corresponding data for the two control treatments were also pooled together. The data were subjected to regression analyses using Mathematica 6.0 (2007); non-linear regression package was used to obtain the best fit parameters and ANOVA to determine the correlation coefficients. Data from cage experiments were analysed by Excel statistics package (Microsoft Office 2007 and Statistica 7.0) and means compared by Student–Newman–Keul (SNK) test at 5% level of significance.

3. Results

3.1. Effect of PAN on field bands

Data on marching behaviour (distance covered, number of leading edges) indicated that although there were no measurable differences between PAN-treated and untreated bands during the first 2 days, these became apparent by the third and fourth day. Whereas control bands continued to march toward the millet cultivations at more or less the same rate, there was a significant (t tests, $P < 0.05$) slowing down of the treated bands (Fig. 1a) by the third day. Thereafter, these bands appeared to move to and fro with no clear direction, and with even some negative overall displacement. Concurrently, the treated bands began to fragment and disperse as reflected in increasing number of leading edges of the bands during day time movements (Fig. 1b) and a gradual, exponential increase in the number of roosting groupings (Fig. 1c). By day 7, no cohesiveness was evident in the treated groups and nymphs behaved largely like solitarious individuals.

The collective defensive responses of hopper bands when attacked by birds during marching (mainly by individual wagtails, *Motacilla* spp., and Sudan golden sparrow, *Passer luteus* (Lichtenstein)) and roosting (mainly by African bulbul, *Pycnonotus barbatus* Desfontaines, and *Falcon* spp.) were significantly affected by PAN, both with respect to their relative frequency (Table 1; based on observations on 20 different occasions) and the vigour with which these occurred (not ranked). Comparison of remains of dead hoppers at the roosting sites on day 3 after treatments (Table 1; cannibalism and predation indices are averages of the number of affected hoppers in each band relative to the total number of hoppers in all observed bands) indicated that significantly higher levels were affected by cannibalism and predation in treated bands than in the control groups on that day.

3.2. Effect of PAN diel patterns in cages

Fig. 2a shows that the different dosages of PAN used in the experiment affected the behaviour of crowd-reared nymphs in a

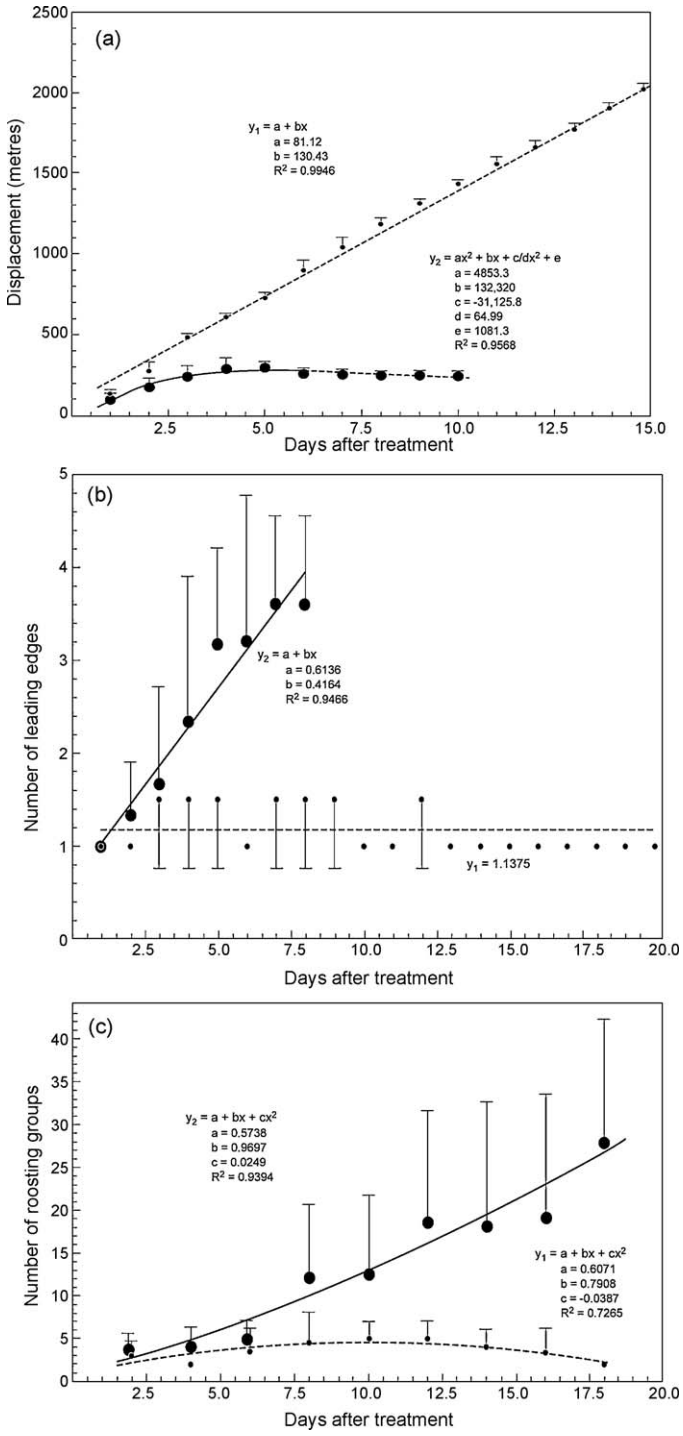

Fig. 1. Time–course regression relationships of the status of field hopper bands treated (•) and untreated (•) with PAN. (a) Distance covered during marching; (b) number of leading edges of the mobile bands; (c) number of roosting blotches on the vegetation early in the morning.

dose-dependent manner. Greater proportions of treated individual nymphs were active at both times, unlike their untreated counterparts, the majority of which showed a clear pattern of feeding in the morning and roosting in the evening. Observations on groups of nymphs at four different times (including closer to mid-day and afternoon) showed that this pattern continued across the whole day and that PAN-treated nymphs spent significantly less time feeding or roosting compared with the untreated or diesel-treated counterparts (Fig. 2b).

Table 1
Frequency of collective defensive responses of *S. gregaria* hopper bands to attacks by birds during marching and roosting and estimates of levels of predation and cannibalism at the roosting sites.

Trait observed/estimated	Treated bands	Control bands
Concerted hopping[a]	40.00 ± 6.67^{a}	73.33 ± 5.76^{b}
Dropping and running[b]	38.33 ± 7.14^{a}	73.77 ± 7.70^{b}
Predation index[c]	0.13 ± 0.04^{a}	0.04 ± 0.01^{b}
Cannibalism index[c]	0.17 ± 0.02^{a}	0.03 ± 0.01^{b}

Means with different letters in each row are significantly different at $P = 0.05$.
[a] During marching.
[b] During roosting at the start of observations in the morning.
[c] Numbers affected relative to the number of hoppers in all observed bands (see text for details).

3.3. Effect of PAN on feeding rates and cannibalistic behaviour in cages

Daily food consumption (Table 2) by crowd-reared (gregarious) 4th instar nymphs exposed to PAN was found significantly reduced (0.26 ± 0.06) g d^{-1} compared to diesel-treated and untreated controls (0.50 ± 0.02 and 0.48 ± 0.03 g d^{-1}, respectively). On the other hand, cannibalistic behaviour was significantly higher in PAN-treated nymphs compared with diesel-treated or untreated controls (Table 2).

3.4. Comparison of the effects of PAN and antennectomy

Table 3 summarises the effects of PAN and antennectomy on the diel pattern of nymphs as reflected in the percentages engaged in

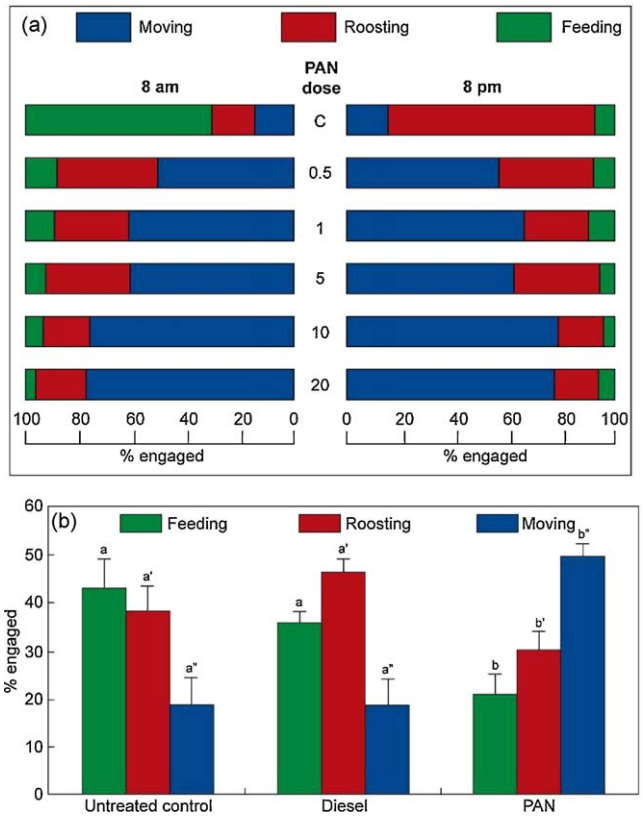

Fig. 2. Percentages of crowd-reared 4th instar nymphs treated or untreated with PAN engaged in feeding, moving or roosting. (a) Different groups of nymphs treated with 0 (untreated control), 0.5, 1.0, 5.0, 10.0 and 20.0 g ha^{-1} PAN in diesel observed in the mornings and evenings; (b) cumulative percentages of nymphs treated with PAN in diesel at 10.0 g ha^{-1}, diesel without PAN, and untreated, observed at four different periods (morning, late morning, afternoon and evening). ANOVA indicated significant differences between nymphal behaviours due to PAN dose. Bars (in (b)) representing the different behaviours with different letters are significantly different ($P < 0.05$, SNK test).

Table 2
Feeding rates and cannibalism in the 4th instar desert locust in cages under different treatments.

Treatments	PAN-treated	Diesel-treated	Untreated
Feeding rates (g day^{-1})	0.26 ± 0.06^{b}	0.50 ± 0.02^{a}	0.48 ± 0.03^{a}
Total mortality	70.00 ± 2.00^{a}	45.53 ± 14.19^{b}	26.66 ± 3.06^{c}
% Cannibalism	31.33 ± 4.16^{a}	8.00 ± 4.00^{b}	7.33 ± 3.06^{b}

Means in the same row sharing a common letter are not significantly different at $P = 0.05$ (SNK test).

Table 3
Percentages of nymphs (PAN-treated, antennectomised or untreated control) engaged in different behaviours for 3 days following treatments in the mornings and evenings.

	Control	PAN-treated	Antennectomised
8 am			
Feeding	71.60 ± 4.86^{a}	17.97 ± 1.80^{b}	17.10 ± 2.53^{b}
Roosting	11.53 ± 5.08^{a}	28.83 ± 3.65^{b}	29.30 ± 3.70^{b}
Moving	16.87 ± 0.75^{a}	53.07 ± 2.16^{b}	53.53 ± 4.50^{b}
8pm			
Feeding	6.40 ± 1.01^{a}	$10.40 \pm 5.41^{a,b}$	13.30 ± 3.46^{b}
Roosting	77.07 ± 3.13^{a}	32.83 ± 3.87^{b}	28.43 ± 6.49^{b}
Moving	16.20 ± 3.70^{a}	56.63 ± 9.23^{b}	57.97 ± 9.35^{b}

Means with same letter in a row are not significantly different at $P = 0.05$ (SNK test).

feeding, roosting or moving, in the mornings and evenings. PAN-treated nymphs demonstrated the same behavioural pattern as those that were subjected to antennectomy. This was significantly different from that of the untreated control group, which showed normal circadian pattern of behaviour.

4. Discussion

The possibility of solitarising (anti-gregarisation) effect of a chemical signal associated with crowd-reared adults on crowd-reared nymphal stages was first suggested by Gillett and Phillips (1977). These workers observed that nymphs treated with adult faeces appeared to be less gregarious in behaviour and colour than their untreated counterparts. However, the differences between treated and untreated controls were not statistically significant, and follow up laboratory studies failed to confirm this effect (Gillett, 1983).

The present series of study was inspired by the discovery that distinct releaser pheromones mediate the cohesive (aggregation) behaviours of adult and nymphal desert locusts (Obeng-Ofori et al., 1993; Torto et al., 1994, 1996) and, specifically, by loss of cohesiveness and apparent restlessness of crowd-reared nymphs in the presence of the adult pheromone in a laboratory arena (Hassanali and Bashir, 1999). The results of the present study show that field hopper bands were significantly affected by the major component of the adult pheromone (PAN). During the first few days, they became relatively hyperactive, and by the third day they virtually stopped marching as coherent groups. Over the next few days, their movements became random; they started to roost for longer periods on vegetations, and the bands fragmented into smaller and smaller groupings and individuals. Interestingly, there were no discernible differences between the effects of the three doses of PAN (representing a tenfold dose range) on distance marched, number of leading edges of the marching band, and number of roosting groups in different bushes. This indicates that relatively small concentrations of PAN (~2 ml ha^{-1}, equivalent to 0.2 μl m^{-2}) are sufficient to trigger the sequence of effects observed on field hopper groups.

Loss of cohesiveness was also apparent when attacked by birds. Hoppers in PAN-treated bands demonstrated more individualized or subdued levels of collective defensive behaviour compared to those typical of normal marching hoppers. Likewise, at the roosting sites, the collective defensive responses of treated hoppers also appeared impaired, and there was a clear indication of increase in the levels of predation and cannibalism. In cage experiments, during the 2 days following treatment with PAN, crowd-reared nymphs were found to be hyperactive, and showed abnormal diel patterns, reduced feeding on plants but increased cannibalism. Some of these behavioural traits (e.g. hyperactivity and reduced food intake) may be direct outcomes of exposure to PAN; others (such as cannibalism) may be secondary consequences of the reduced food intake. A recent study by Bazazi et al. (2008) under controlled laboratory conditions suggests that cannibalistic interactions among individuals, and specifically, the threat of attack by those approaching from behind, may be a principal factor in the onset of collective movement among healthy bands. A similar detailed behavioural study of hoppers exposed to PAN in the field and in cages is needed to shed some light on possible interactive causal links between the observed traits. Interestingly, our findings on cannibalistic behaviour in the present study appear to parallel previously observed enhanced cannibalism (estimated at over 50%, in some cases) in solitarising gregarious hopper populations that encounter either poor supplies of young food plants, because of insufficient rain, or senescing plants at the start of a dry season following a rainy period (e.g. Ashall and Ellis, 1962). This may have direct survival value since it may enable part of the population to undergo phase change and survive as scattered, low-density solitarious individuals, or to continue in the gregarious phase to the adult stage that can migrate to more favourable environments.

What then underlies the observed solitarising and associated effects of PAN on gregarious hoppers? In a recent study, the effect of PAN on adult desert locusts was found to be concentration dependent, eliciting cohesion effects at low doses but repelling them at higher concentrations (Rono et al., 2008). Are the observed effects of PAN in the present study due to a repellent action on hoppers? At concentrations used in laboratory arena (Obeng-Ofori et al., 1993; Torto et al., 1994; Rono et al., 2008) and in the present field study, we found no evidence of repellency of PAN on nymphal stages. In the laboratory arena, nymphs did not avoid the column of air enriched with PAN. They simply lost their cohesiveness and were evenly distributed between the two sides of the arena. In the field, a significant repellent effect would be expected to lead to almost immediate dispersal of hoppers in all directions. However, treated bands stayed together for the first 2 days before the effects of PAN became clearly discernible.

At certain doses, oral intake of PAN is known to be toxic to mammals (e.g. 210–304 mg/kg bw of rats; Jackson et al., 1982) and fish (e.g. 66–134 mg/kg bw of *Cyprinus carpio*; Loeb and Kelly, 1963). No similar data are available for insects and the possibility that the observed effects of PAN on hoppers may be due to its toxicity needs to be considered. In a recent eco-toxicological study with PAN (and PAN with Green Muscle) on desert locust and non-target test organisms that occur in the field, we found that PAN topically applied at doses above 10 μl/individual was toxic to desert locust nymphs and some other insects (Bashir et al., in preparation). However, application rate of 10 ml/ha we have used in most of our studies in the field is equivalent to 1 μl m^{-2}, and therefore, much less per individual hopper. As pointed above, in the present study, the lowest dose in the field (2 ml/ha) was as effective in eliciting the observed effects on hopper bands as the other two. It corresponds to application rate of 0.2 μl m^{-2}, which represents many-fold less than the threshold of 10 μl/individual required for observable toxic action of PAN on locust nymphs. It thus seems unlikely that toxicity is the primary mode of action of PAN on hoppers at doses deployed in our study.

Previous electrophysiological studies undertaken at Lund, Sweden, have shown that the adult pheromone inhibits perception

by nymphs of their own aggregation pheromone (Ochieng, 1997). This has been confirmed at ICIPE by Njagi et al. (in preparation) who showed that antennal electrophysiological response of nymphs to their pheromone blend is substantially depressed (by >90%) if they are exposed to PAN vapour for 6 h. Moreover, when exposure of nymphs to PAN is terminated, recovery of antennal sensitivity to the nymphal pheromone blend occurs only gradually. Significantly, in our cage experiments, the disruption of normal pattern of circadian rhythm observed among PAN-treated nymphal groups was indistinguishable from that in groups that were subjected to antennectomy. The resulting loss of olfactory communication between the gregarious individuals accounts for the observed shift to the solitarious phase, and is also consistent with behavioural phase change of crowd-reared nymphs resulting from disruption of antennal chemoreception by antennectomy reported by Heifetz et al. (1996). Other behavioural traits demonstrated by PAN-treated hoppers in the early stages of phase change may arise from a confusing set of signals individual hoppers encounter. Close proximity between hoppers in the cages and before dispersal in the field may continue to provide the tactile and chemotactile signals associated with shift to the gregarious phase (Heifetz et al., 1997; Simpson et al., 2001). This may explain the relatively slow pace of phase change in hoppers exposed to PAN. However, these stimulations are not accompanied by olfactory contact between hoppers, which may account for the abnormal and stressful behaviour of affected hoppers during the early stages of phase transition. Further studies that augment detailed behavioural observations with measurements of titers of key physiological and neuro-physiological markers may help to shed further light on the chain of events triggered by PAN in gregarious groups of hoppers.

An intriguing question that arises from the observed effects of PAN is whether it has a potential in the control of hopper bands. Phase change by itself without concurrent mortality of large proportions of the insect would not be expected to be very effective, since the nymphs could develop into solitarious adults, which could give rise to gregarious populations in the following generations under favourable conditions (Hassanali et al., 2005a). In the present study, no detailed counts of hopper mortality were carried out on field bands treated with the pheromone constituent. However, our overall observations suggested that the cumulative mortality of hoppers resulting from hyperactivity, cannibalism and predation during the 3 weeks could be relatively high. In follow up studies, to be reported elsewhere, we pursued this question further and confirmed that treatment of hoppers in the field with PAN does indeed lead to very high levels of mortality and that the pheromone shows promise as a subtle control agent for this stage of the desert locust.

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