Journal of Insect Physiology 56 (2010) 640–645

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

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

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ARTICLE INFO

Article history:
Received 24 July 2009
Received in revised form 17 January 2010
Accepted 26 January 2010

Keywords:
Desert locust
Schistocerca gregaria
Adult pheromone
Phenylacetonitrile
Nymphs
Solitarisation

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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0022-1910/$ – see front matter
doi:10.1016/j.jinsphys.2010.01.012

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