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Influence of wind temporal variation to the initiate of host searching behaviour of the blood sucking bug, *Linshcosteus karupus* Galvao et. al. (Insecta: Hemiptera: Triatominae)

M. Anto Claver* and Amit Yaqub

Department of Zoology, St. Andrew's College, Gorakhpur 273001, UP, India.

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ABSTRACT

Surveillance was carried out in the xerophytic climatic condition of Kalakkad forest area (8°41' N 77°19'E) to study the arousal and foraging behaviour of the *Linshcosteus karupus* (Galvao et. al.) during different dusk timing in two successive years. The photoperiod, temperature and humidity recorded at the time of investigation were 12 ± 2 hrs, 22°C – 26°C and 76% – 82% respectively. Triatomine bugs are most important vector of trypanosomiasis or Chaga's disease, a protozoan infection of man and other mammals caused by *Trypanosoma cruzi* (Chagas). These bugs are nocturnal and prefer to feed and mate after the sunset. Therefore our study was conducted precisely after dusk. In our study maximum number of *L. karupus* were noticed late after sunset i.e. between 8- 9 pm in two years, May 2013 and May 2014. The wind formation timing was a key factor in the arousal and foraging behaviour of this bug. Similarly when the wind velocity reached to the upper day's limit i.e. ≤ 6 but > 3 Km/hr again maximum individuals were collected. The total of thirteen bugs was collected when the wind was at high velocity, among them eight adults and five fifth nymphal instars. Among adults greater numbers was of males which provide a very clear idea about the attraction of adults males to females and to their hosts through the olfactory sense by wind. Observation was also made about the response of these bugs to the air temperature, as the temperature declined the number of bugs seemed aroused in an increased number. At the temperature below 22°C at kalakkad hills forest, the total collected individuals of *L. karupus* were eleven; this number was more than twice the bugs reported at temperature above 24°C. In the delayed condition of wind formation, the bugs suddenly started to appear when wind picked speed and the number of individuals reached to maximum limit all at once. It was presuming that insects are waiting for the wind to blow and it may be the conditional respond of its feeding behaviour. In our surveillance studies all attempted were done to understand the natural arousal and behaviour of these haematophagous bugs in its natural habitat

Introduction

Mostly temperature, precipitation, and humidity are considered important variables in the investigations of insect arousal and searching behaviour, and wind effects are rather considered a possible or likely additional side effect of the overall ecological process under investigations. Some insect species benefit from this effect by letting themselves drift away—termed ballooning in the scientific literature—to explore new habitats using a short thread that increases their updraft and thus drifting distance at higher wind speeds. Many populations of high-flying nocturnal insect migrants are plainly influenced by wind-related factors when taking up their flight headings. For example, in some radar studies, the mean heading closely followed the downwind direction despite veering of the wind with altitude (Schaefer 1976; Riley and Reynolds 1983).

Triatomine bugs are most important vector of trypanosomiasis or Chaga's disease, a protozoan infection of man and other mammals caused by *Trypanosoma cruzi* (Chagas). These haematophagous bugs inhabit palm trees, bird's nests and burrows of small mammals in the wild, and peridomestically the crevices and corners of human dwelling and animal habitats (Patterson et al. 2001). Biotic and abiotic factors that have been shown to increase the desire to feed these bugs include a temporal influence, whereby the bugs prefer to feed at post dusk as opposed to predawn (Lorenzo and Lazzari 1998, Barrozo and Lazzari 2004a) and a physiological response whereby an increase in starvation time promotes the search for food (Ryckman 1952, MacCord et al. 1986, Barrozo and Lazzari 2004b). Most studies of orientation and attraction of bugs to host cues have focused on short-distance attraction. The flight activity of triatomine bug is crepus cular and almost all our collection were made between sunset and the following 5 hrs under total darkness when the temperature and wind was fluctuating from high to low and vice - versa respectively. The genus *Linshcosteus* Distant, 1904, is restricted to the Indian subcontinent. At night, they are active, searching for food, mating opportunities and oviposition sites (Ampleford and Davey 1989; Lazzari 1991, 1992; Lorenzo and Lazzari 1998; Figueiras et al., 1994).

Tele: 9335896735
E-mail addresses: antoclaver@yahoo.co.in

Triatomines exhibit different degree of response to a blood source; most species prefer to bite at night in the dark (Marroquin et. al. 2003). Adults of triatomine species have a high potential for dispersal by noctumal flight during the hot and dry months (August-November) (Wisnivesky-Colli et. al. 1993, Noireau et al. 1998). Studies applied to various triatomine species have shown that starvation was apparently the main cause of flight initiation (Sjogren and Ryckman 1966, Ekkens 1981, Lehane and Schofield 1982, McEwen and Lehane 1993). Therefore the nutritional status may allow a prediction of the proportion of wild adults likely to fly (Lehane and Schofield 1982). Most species of Triatomine group of blood sucking insects leave their refuges at the beginning of the night and display the major part of their activities (e.g. host search, feeding, dispersion etc) in discrete temporal windows during this dark period (Amplefold and Davey 1989, Lazzari 1991, 1992, McEwen and Lehane 1993, Figueiras et. al. 1994, Roca and Lazzari 1994, Lorenzo and Lazzari 1998, Guarneri et al 2002, Minoli and Lazzari 2003, Barrozo et. al. 2004).

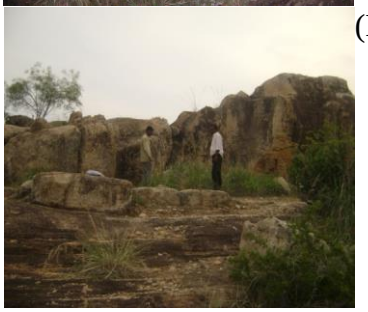
Influence of wind coolness on the initiation of flight activity of *Linshcosteus karupus* (Galvao et. al.) was examined at three adjacent air temperatures prevailed from the natural ecotype. In our study we were also focused on arousal of *L. karupus* at the same locality but at different wind temperature, initiation timing and its progressive wind speed. The data were collected for arousal of *L. karupus* at different abiotic condition in their natural habitat. This provided knowledge about the arousal pattern of these salvatic bugs in their natural habitats. This was the first time when these bugs in India were studied for such factors in their natural habitat.

Material and Method

Study Area

A team of three persons periodically visited the Kalakkad hills forest at Southern Tamil Nadu (8°41' N 77°19'E) to investigate the population dynamics and behaviour of the Triatomine bugs after 6 pm in two successive years 2013 - 2014. The total area of this forest region is about 895 km². The average temperature of this region in both summer and winter are 28±6°C. Annual rainfall recorded in this area is about 140 inches. The days chosen for collection were dry and mostly seen area was covered with patches of xerophytic plants. At the time of our investigation, temperature and humidity varied between the range 22°C – 26°C and 76% – 82% respectively as study was carried in natural habitat of the bugs (Fig1 A, B).

(A)



(B)

(C)

Fig 1. Micro-habitat of *L. karupus* at kalakkad hills forest at Tamil Nadu after Sunset (A, B) and laboratory reared 5th instar feeding on pigeon (C)

Observation pattern

The habitat of *L. karupus* was inspected with the help of torch light and long forceps once in every 30 minutes interval to each possible site of their habitat on the observation days. Observations were made by showing much care to avoid noise or vibration so that natural arousal of individuals may be undisturbed. Entrance of major cracks in the rocks was carefully inspected as nymph and adult of these bugs have a very high camouflage potential.

Reptiles and small rodents were also observed in this area which might be the key hosts for these bugs. It was also observed that whether the activity timing of both hosts and these bugs were very closely synchronized or not. No light traps or chemical attractants were use in our study to ensure nature arousal of these bugs from their habitats.

Influence of wind

This study was aimed to analyse the Triatomine bug appearance out of its hiding niche whether coincidence with the wind initiation timing or speed or its coolness after sun set. The gathered field data were re-arranged according to variation of wind initiation timing for the appearance of these bugs outside their niche. The wind initiation timing of the dusk were categorised as early dusk (before 6 pm), mid dusk (between 6 and 7 pm), late dusk (between 7 and 8 pm) and very late dusk (after 8 pm) so that to understand that is this wind initiation timing pattern has showed any influence on its arousal stimulus of searching behaviour.

The second investigation was made to analyse whether the speed of wind had any influence on Triatomine outside appearance i.e., noticed out of their hiding places for host searching and mating behaviour. The collected wind speed data were re-clustered into four categories like wind speed 0.01 km/hr, ≤1 but > 0.01 Km/hr, ≤3 but > 1 Km/hr and ≤ 6 but > 3 Km/hr.

The third analysis is to detect whether air coolness has caused any impact on the arousal response of these bugs for coming out of its hiding area in search of its host. The recorded temperature during dusk at the time of field observation was segregated as below 22^0 C, between 22^0C and 24^0 C and above 24^0 C to find out whether temperature had any influence on its searching behaviour while these bugs come out of their niches.

For recording the wind temperature, humidity and velocity, instruments like digital thermometer, hygrometer and anemometer were used in our study. All this investigations were made on a clear day with no rainfall.

Haematophagous bugs collected during the study were carefully stored in separate plastic containers with good aeration and were brought in laboratory for further studies to

establish the concept; experiment was being designed in the laboratory to study the effects of wind coolness on the foraging behaviour of these bugs with the help of olfactometer (Fig1 C).

Result and Discussion

The collected bugs in the Kalakkad hills forest was not in a very huge number as collection of these bugs in recent years have been a challenge and such observation has been made by many researchers and scientist in recent time in different parts of new world (Galvao et. al. 2002, Rajan 2011, Claver and Yaqub 2013). The Triatoma species were so rare (collected less than 10 times in total by any researcher in Texas since 2000) that they are presumed not to be important for establishing Chagas disease transmission cycles in the state (Sarkar et. al. 2010). More over they tend to behave like dead and show no movement unless and until any stimulus is very close to it. Nymphal camouflaging among triatominae was observed by Breddin (1904) and this phenomenon was described in more detail in the nymph of *T. dimidiate* (Latreille) in the laboratory by Hase (1940) and Zeledon et.al., (1969) as well as in the field by Hase (1940).

Host searching behaviour of these nocturnal insects is exhibited by a sequence of events which is normally initiated by odour and heat radiation of its vertebrate host. These insects not always wait for the host which come near by its vicinity but be ready to go out from their hiding places when climatic situation turns to their favour. In this study we were monitored that wind temperature and speed of breeze or wind, its initiation timing when the arousal response of these insects happened. In our study, we have found that the arrival of cool wind after dusk had greater influence for their arousal response in foraging the host. As the day temperature gradually drops soon after sun set and the wind velocity slowly rises in the rocky areas of their niches, while these bug come out of there hiding habitats in order to obtain blood meal of vertebrates. Similar observation was also made by Galvao et. al. (2002).

Flying is one of the main mechanisms involved in long-distance movements in insects, and thus, in their colonization capability (Minoli et. al. 2006). A maximum of individuals of *L. karupus* was collected between 8- 9 pm in two years i.e. May 2013 and May 2014 (Table 1). This collected data gives a clear picture that as the night progress these bugs emerge out of their niche for mating and feeding on their hosts in large number. The peak activity of the triatomine bugs are almost unimodal and covered the period 61- 180 min after dark, according to the species and sex (Noireau and Dujardin 2001). The search of new habitats, food and mates are some of the phenomena that instigate them to fly. Nutritional (Lehane et. al. 1992; McEwen and Lehane 1993) and reproductive (McEwen and Lehane, 1994) states, as well as population density (McEwen et. al. 1993) and environmental conditions (Lehane et. al. 1992; Schofield et. al. 1992) are factors known to modulate this activity in triatomines.

Table 1. Timing of the wind initiation for the appearance of *L. karupus* outside of its niche for searching its host after sunset.

Wind formation timing	Number of insects approached out of the hiding place	
	Immature	Adult
Before 6 pm (early dusk)	0	0
6 – 7 pm (mid dusk)	1	4 (3M + 1F)
7 – 8 pm (late dusk)	2	5 (4M + 1F)
8 – 9 pm (very late dusk)	6	10 (7M + 3F)

M= Male, F= Female

Although they are mainly walking insects, the active arrival to new habitats seems to occur mostly by flight. In this way, flight might be an important factor not only for the colonization of new houses by domestic bugs coming from nearby dwellings, but also for the arrival of sylvatic bugs to human houses to feed and eventually establish.

Wind Initiation Timing

All triatomine bugs are nocturnal and prefer to feed and mate after the sunset when the surrounding gets cooler (Rajan 2011, Claver and Yaqub 2013). During our investigation arousal of L. karupus noted between 6- 7 pm for both the years i.e. May 2013 and 2014, there were about four adults collected, out of which three males, one was female and one fifth instar. But as the time passed slowly more number of individual were seen aroused in both the year (Table 1). Most flights occur during the first 4 hrs after sunset (Ekkens 1981). Our study in the micro habitat of *L. karupus* clearly reflects that these individuals prefer a late arousal time for feeding and mating. No arousal was reported before 6 pm for these bugs.

Wind Speed

As the wind catches up the speed it carries more phenomenal information to wide area, this also stimulate arousal of the triatomine bugs. In our study, we were noticed that as the wind picked its speed and slowly reached its upper limit of the day's wind velocity, more individuals of *L. karupus* were aroused from their micro habitat (Table 2). Maximum number of bug's appearance was recorded when wind was blowing at its top speed i.e. ≤ 6 but > 3 Km/hr and this data suggested that the wind velocity had direct influence on arousal and searching behaviour of *L. karupus*, it's also clear that wind velocity is directly proportional to appearance of these bugs. Bugs presences were observed more on one side of rocks which is too in wind's direction. When wind velocity increased from ≤1 but > 0.01 Km/hr to ≤ 6 but > 3 Km/hr the arousal rate also increase more than six times. Many populations of high-flying nocturnal insect migrants are plainly influenced by wind-related factors when taking up their flight headings. For example, in some radar studies, the mean heading closely followed the downwind direction despite veering of the wind with altitude (Schaefer 1976; Riley and Reynolds 1983).

Table 2. Influence of wind velocity during dusk for the appearance of *L. karupus* in the exposed rocky area.

Wind velocity (Km/hr)	Number of insects appeared out of its niche	
	Immature	Adult
0.01	0	0
≤1 but > 0.01	0	2 (2M + 0F)
≤3 but > 1	2	4 (4M + 0F)
≤ 6 but > 3	5	8 (6M + 2F)

Wind Coolness

Drop in the air temperature stimulates the foraging behaviour of triatomine bug and these bugs initiate their search of hosts (Patterson et. al. 2001). In this section of our study we focused on the arousal of *L. karupus* at different temperatures. Temperatures of the collection days were divided into three categories i.e. below 22°C, between 22°C - 24°C and above 24°C at kalakkad hills forest during the studies was carried out. The total collected individuals of *L. karupus* reported were eleven, six and four for above mentioned respective temperatures (Table 3). The bugs collected at the temperature below 22°C at kalakkad hills forest were more than twice the bugs reported at temperature above 24°C. Both the nymphal instar and adult male were seemed aroused in higher number at lower temperature than at

higher temperature (Table 3). More males were active during lower temperature timing than the females (Table 3). Most triatomines aggregate in refuges during day and search for blood during night when the host is asleep and the air is cooler (http://en.wikipedia.org/wiki/Triatominae). Though very less data were available on *L. karupus* for its foraging behaviour regarding temperature of wind thus more study in needed in this line.

Table 3. Impact of the Coolness of wind (air temperature) for the foraging behaviour of *L. karupus* after sunset.

Temperature range (°C)	Number of insects observed in the rocky area	
	Immature	Adult
Below 22°C	5	(4M + 2F)
22°C - 24°C	2	4 (3M + 1F)
24°C & above	1	3 (2M + 1F)

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Conclusion

In our study, the collected data showed that the temperature of the surrounding as well as the wind coolness and wind velocity and formation of wind timing directly affect the arousal and foraging behaviour of *L. karupus*. These above data collected during our investigation reveals the direct influence of abiotic factors on the behaviour of *L. karupus*. The individuals collected from the micro habitat of kalakkad hills forest were brought to the laboratory to set up more experiments to with regard to different wind velocity and temperature for its searching behaviour.