*Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, Vol. *88* (4): 535-539, oct./dec. 1993

# AGGREGATION PHEROMONE IN FIVE SPECIES OF TRIATOMINAE (HEMIPTERA: REDUVIIDAE)

LEOPOLDO CRUZ-LOPEZ; EDI A. MALO & JULIO C. ROJAS

Laboratorio de Ecología Química, Centro de Investigaciones Ecológicas del Sureste, km 2.5 Carretera Antiguo Aeropuerto, Apdo Postal 36, Tapachula, 30700, Chiapas, México

*An aggregation pheromone found in the faeces of 5th instar nymphs and adults of* Triatoma mazzottii *Usinger,* Triatoma longipennis *Usinger,* Triatoma pallidipennis *(Stal),* Triatoma barberi *Usinger and* Rhodnius prolixus *(Stal) was studied under laboratory conditions. Bioassays were performed using a 30 cm-diameter arena and a wind tunnel.* T. longipennis *nymphs showed a stronger response than the other triatomine nymphs tested. There were no significant differences in faecal attractiveness to nymphs, but the faeces of* T. longipennis *and* T. pallidipennis *were most active. The responses of all species to male and female faeces of* T. mazzottii *was significantly different, but there was no significant difference in the responses of the development stages to male and female faeces of* T. mazzottii. *However, male faeces were more active than female faeces. The feeding status of nymphs did not affect the response.*

Key words: Hemiptera – Triatominae – faeces – pheromone

Semiochemical studies in blood-sucking bugs have focused mainly on pheromones. Evidence of sexual and aggregation pheromones has been reported in *Triatoma infestans* (Klug), *Rhodnius prolixus* (Stal), *Panstrongylus megistus* (Burmeister), and *Triatoma mazzottii* Usinger (Velazquez 1968; Baldwin et al., 1971; Schofield & Moreman, 1976; Schofield & Patterson, 1977; Neves & Paulini, 1981, 1982a; Ondarza et al., 1986; Rojas et al., 1991). The source of sex pheromone is unknown, but the aggregation pheromone has been found in the bug faeces. In addition, secretion from the metasternal glands of *Dipetalogaster maxima* (Uhler) (3-methyl-2-hexanone) has been suggested as a possible alarm pheromone (Rossiter & Staddon, 1983).

This study was undertaken to examine the response of two main vectors of Chagas disease in Mexico, *Triatoma barberi* Usinger and *R. prolixus* (Zarate & Zarate, 1985), and three species of the *Triatoma phyllosoma* complex (Lent & Wygodzinsky, 1979) to aggregation pheromones present in their faeces.

Supported by UNDP/World Bank/WHO Special Programe for Research and Training in Tropical Disease (Grant No. ID870212).

Received 16 March 1993.
Accepted 23 September 1993.

## MATERIALS AND METHODS

*Insects* – 5th instar nymphs and adults of *T. mazzottii, Triatoma longipennis, T. pallidipennis, T. barberi* and *R. prolixus* were obtained from stocks of the insectary of the Centro de Investigaciones Ecológicas del Sureste. The insects were mantained at 27 ± 1 °C, 70 ± 5% RH and a 8:16 (L:D) photoperiod. Nymphs and adults were fed on rabbit for 30 min each eight days (unless otherwise specified) using the technique of Ryckman (1952).

*Collection of faeces* – Immediately after feeding, the insects were placed in plastic containers (0,320 l) with a piece of filter paper (Whatman grade 3) on the bottom to collect the faeces, for a period of 15 days.

*Behavioural test* – The bioassays were performed using two systems: (1) the nymphal faeces were tested for aggregation properties in a circular arena (10 cm high and 30 cm in diameter), which was made of cardboard wrapped with aluminium foil. The tests were carried out using two pieces of 25 cm filter paper folded along their width three times and placed three cm away from the wall of the arena. One contaminated piece with faeces was used as sample and another clean filter paper as control. Then 25 insects were placed in the centre of the arena, and the insect response

was recorded after 1 hr by counting the number of insects on each piece of paper (sample or control); (2) the activity of adult faeces was evaluated in a wind tunnel as described by Ondarza et al., (1986). Experiments were done using either 15 adults or 5th instar nymphs, and after each test the apparatus was cleaned with acetone. The bioassays were conducted in a dark room at 25 °C and 50% RH. The response of the insects was calculated using an index Ai = Npc-Np1/Nt, where: Ai = aggregation index; Npc = number of aggregated insects on contaminated paper; Np1 = number of aggregated insects on clean paper; Nt = Number of released insects in each biossay. The index value ranged from +1 to –1, positive values indicate aggregation, negative values repulsion.

*Homospecific and interspecific responses of nymphs to nymphs faeces* – 25 fifth instar nymphs of *T. mazzottii, T. pallidipennis, T. longipennis, T. barberi* and *R. prolixus* were used, and the tests were done in the circular arena. The aggregation of the insects to the 5th instar nymph faeces was determined by testing each species with their own faeces and with the faeces of the other species. The aggregation indexes were analyzed by ANOVA multifactorial and Tukey's test. The first factor was nymphs-species and the second the nymphs-faeces.

*Response of adults and nymphs to faeces of males and females of T. mazzottii* – Males, females and 5th instar nymphs of *T. mazzottii, T. longipennis, T. pallidipennis, T. barberi* and *R. prolixus* were tested separately in the wind tunnel using male and female faeces of *T. mazzottii.* The aggregation indexes were analyzed by ANOVA multifactorial and Tukey's test. The first factor was the species tested, the second the development stage, and the third was the faeces source.

*Effect of feeding period on the aggregation response* – In order to determine the effect of feeding period in the insect response, 5th instar nymphs of *T. mazzottii* under different feeding patterns were tested with mixed faeces of nymphs of *T. mazzottii, T. longipennis,* and *T. pallidipennis* in the circular arena. The aggregation indexes were analyzed by ANOVA and Tukey's test.

## RESULTS

*Homospecific and interspecific responses of nymphs to nymphs faeces* – Table I shows the results of aggregation indexes of different species to the faeces of the nymphs. The response of the species differed significantly ($F = 8.12$; $df = 4, 100$; $P < 0.05$). *T. longipennis* showed a stronger response than *T. pallidipennis, R. prolixus, T. mazzottii* and *T. barberi.*

TABLE I

Aggregation response of Triatominae 5th instar nymphs to 5th instar nymphs faeces

| First factor (Nymphs-species)[1] | Insects on: Sample | Control | Aggregation index (mean ± SE)* |
|---|---|---|---|
| *Triatoma barberi* | 250 | 42 | $0.13 \pm 0.009^{c}$ |
| *Triatoma longipennis* | 776 | 132 | $0.42 \pm 0.06^{a}$ |
| *Triatoma mazzottii* | 323 | 53 | $0.18 \pm 0.03^{c}$ |
| *Triatoma pallidipennis* | 594 | 125 | $0.31 \pm 0.04^{b}$ |
| *Rhodnius prolixus* | 492 | 129 | $0.24 \pm 0.02^{b}$ |
| **Second factor (Nymphs-faeces)[1]** | **Insects on: Sample** | **Control** | **Aggregation index (Mean ± SE)*** |
| *Triatoma barberi* | 528 | 112 | $0.27 \pm 0.07^{a}$ |
| *Triatoma longipennis* | 550 | 83 | $0.31 \pm 0.08^{a}$ |
| *Triatoma mazzottii* | 412 | 108 | $0.20 \pm 0.01^{a}$ |
| *Triatoma pallidipennis* | 530 | 88 | $0.29 \pm 0.060^{a}$ |
| *Rhodnius prolixus* | 415 | 90 | $0.21 \pm 0.06^{a}$ |

*1*: total results of 60 replicates.
*: means followed by the same letter are not significantly different ($P < 0.05$, Tukey's test), and analyzed by ANOVA multifactorial.

TABLE II

Aggregation response of Triatominae adults and nymphs to faeces of males and females from *Triatoma mazzottii*

| First factor (Species)[1] | Insects on: Sample | Control | Aggregation index (Mean ± SE)* |
|---|---|---|---|
| *Triatoma barberi* | 183 | 68 | $0.20 \pm 0.04^c$ |
| *Triatoma longipennis* | 231 | 40 | $0.35 \pm 0.02^b$ |
| *Triatoma mazzottii* | 230 | 42 | $0.34 \pm 0.03^b$ |
| *Triatoma pallidipennis* | 255 | 44 | $0.38 \pm 0.02^a$ |
| *Rhodnius prolixus* | 170 | 49 | $0.22 \pm 0.02^c$ |
| **Second factor (Development stage)[2]** | **Insects on: Sample** | **Control** | **Aggregation index (Mean ± SE)*** |
| Females | 332 | 85 | $0.26 \pm 0.03^a$ |
| Males | 361 | 63 | $0.32 \pm 0.02^a$ |
| Nymphs | 376 | 90 | $0.31 \pm 0.04^a$ |
| **Third factor (Faeces source)[3]** | **Insects on: Sample** | **Control** | **Aggregation index (Mean ± SE)*** |
| Females | 472 | 129 | $0.25 \pm 0.03^b$ |
| Males | 597 | 114 | $0.35 \pm 0.02^a$ |

*1:* total results of 36 replicates.
*2:* total results of 60 replicates.
*3:* total results of 90 replicates.
*: means followed by the same letter are not significantly different ($P < 0.05$, Tukey's test) and analyzed by ANOVA multifactorial.

TABLE III

Effect of different regimens of feeding of nymphs of *Triatoma mazzottii* on the response to mixed faeces to *T. mazzottii, T. longipennis* and *T. pallidipennis*

| Condition | Insects on: Sample | Control | Aggregation index[1] (Mean ± SE)* |
|---|---|---|---|
| Recently feeding | 26 | 2 | $0.26 \pm 0.058^a$ |
| 8 days of starved | 30 | 2 | $0.31 \pm 0.060^a$ |
| 15 days of starved | 26 | 8 | $0.20 \pm 0.057^a$ |

*1:* total results of 6 replicates.
*: means followed by the same letter are not significantly different ($P < 0.05$, Tukey's test).

On the other hand, the faeces no exhibited a significative difference of attractiveness to the insects ($F = 1.93$; $df = 4, 100$; $P > 0.05$) (Table I). The interaction species-faeces was no significantly different ($F = 0.33$; $df = 16, 100$; $P > 0.05$). These results suggest that the aggregation pheromone in the studied species is interspecific, although the response is different between species.

*Response of adults and nymphs to males and females faeces of T. mazzottii* – The response of species to faeces of males and females of *T. mazzottii* was significantly different ($F = 4.70$; $df = 4, 116$; $P < 0.05$). *T. pallidipennis, T. longipennis* and *T. mazzottii* presented the greatest level of response (Table II). There was no significant difference ($F = 1.30$; $df = 2, 116$; $P > 0.05$) in the response of the females, males and nymphs to the faeces (Table II). However, in the faeces source (females or males) there was a significant difference ($F = 3.84$; $df = 1, 116$; $P < 0.05$), the male faeces were more active than female faeces (Table II). The results of this experiment suggest that the response to aggregation pheromone in faeces of *T. mazzottii* was not affected by the development stage, but was affected by the species and sex of the faeces-source.

*Effect of feeding period on the aggregation response* – There was no significant difference in the response of *T. mazzottii* nymphs under different regimens of feeding ($F = 1.45$; $df = 2, 15$; $P > 0.05$), although insects starved for

eight days presented a higher response (Table III).

## DISCUSSION

This work extends the knowledge of pheromones in triatomine bugs. An interspecific aggregation pheromone is present in the faeces of nymphs and adults of several species of the subfamily Triatominae. Our results coincide with those of Schofield & Patterson (1977), who found the presence of substances in the faeces of *T. infestans,* which elicited assembly behaviour in nymphs of *T. infestans* and *R. prolixus.* But we disagree with Neves & Paulini (1982a), who stated that the biological activity of the faeces of *T. infestans* and *Panstrongylus megistus* is species-specific, and that a repellent factor was found in the faeces of *T. infestans, P. megistus,* and *T. sordida* (Stal) when these were tested interspecifically (Neves & Paulini, 1982b). Ondarza et al. (1986) claimed the presence of two aggregation pheromones in *T. mazzottii,* one in the faeces of nymphs and females that attract only fed females and nymphs, and another present in males faeces that attracts fed and unfed males. Our results differ (Table II and III), since the aggregation pheromone in *T. mazzottii* and other triatomines was found to be independent of sex, and the feeding status of the insects did not affect the aggregation behaviour.

Schofield & Patterson (1977) reported that the assembly pheromone of *T. infestans* and *R. prolixus* could be composed of two active fractions, one that attracts unfed bugs, and another that inhibits the movement of fed insects, whereby the possible function of this pheromone is to indicate a suitable food source. However, another possible function of the aggregation pheromone could be to assemble males and females for mating. Rojas & Cruz-López 91992) point out that sexual behaviour of *T. mazzottii, T. phyllosoma* and *T. pallidipennis* seems to be determined mainly by the fact that these insects occur in groups influenced by an aggregation pheromone. So it is possible that the pheromone deposited near to the host acts as an arrestant to maintain the bugs feeding, mating, and breeding near to the host. Further studies will be performed to establish the function of the pheromone in the chemical ecology of these insects.

The fact that aggregation pheromone is interspecific suggest that the same or very similar chemicals are present in the faeces of species studied here. Also it could indicate that these compounds have a common ancestor. Schofield & Patterson (1977) suggested that the aggregation pheromone could be a product of the final breakdown of blood meal. Results of our study could support this hypothesis. Data of the last authors showed that blood itself is not responsible for the aggregation.

Finally it will be necessary to determine the chemical nature of the aggregation pheromone. Preliminary separation showed that the active compounds were extracted with polar solvents (water and ethanol) (Cruz-López, unpublished data). Also the behavioural and chemical evidence suggests that the pheromone is a mixture of several components (Rojas, 1991). The aggregation pheromone identified and synthesized could be used for monitoring the bugs in the field.

## ACKNOWLEDGEMENTS

This work is dedicated to Alfredo Ramírez-Rovelo, who was an excellent friend and coworker. We thank Octavio Jaramillo-Monroy and Salvador Meza (CIES, Tapachula, Chiapas) for statistical analysis. Early drafts of this manuscript were reviewed by Mario H. Rodriguez (Centro de Investigaciones de Paludismo, Tapachula, Chiapas) and Juan Cibrián-Tovar (Centro de Entomología y Acarología, Colegio de Postgraduados, Montecillo, Texcoco, Edo de México).

## REFERENCES

BALDWIN, W. F.; KNIGHT, A. G. & LYNN, K. R., 1971. A sex pheromone in the insect *Rhodnius prolixus* (Hemiptera: Reduviidae). *Can. Entomol., 103:* 19-22.

LENT, H. & WYGODZINSKY, P., 1979. Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas' disease. *Bull. Am. Mus. Nat. Hist., 163:* 123-520.

NEVES, D. P. & PAULINI, E., 1981. Atração sexual em *Panstrongylus megistus* e *Triatoma infestans* (Hemiptera: Reduviidae) por feromonio. *Rev. Bras. Entomol., 25:* 301-306.

NEVES, D. P. & PAULINI, E., 1982a. Atração social em *Panstrongylus megistus* e *Triatoma infestans* (Hemiptera: Reduviidae) por feromonio. *Rev. Bras. Entomol., 26:* 23-28.

NEVES, D. P. & PAULINI, E., 1982b. Repelencia entre *Panstrongylus megistus, Triatoma infestans* e *Triatoma sordida* (Hemiptera: Reduviidae), por ação de feromonio. *Rev. Bras. Entomol., 26:* 349-354.

ONDARZA, R. N.; GUTIERREZ-MARTINEZ, A. & MALO, E. A., 1986. Evidence for the presence of

sex and aggregation pheromones from *Triatoma mazzottii* (Hemiptera: Reduviidae). *J. Econ. Entomol., 79:* 688-692.

ROJAS, J. C., 1991. Feromona de agregación y conducta de aparecimento en algunas especies mexicanas de *Triatoma* (Hemiptera: Reduviidae). *Bol. Soc. Mex. Entomol., 9:* 28-33.

ROJAS, J. C.; RAMIREZ-ROVELO, A. & CRUZ-LOPEZ, L., 1991. Search for a sex pheromone in *Triatoma mazzottii* Usinger (Hemiptera: Reduviidae). *J. Med. Entomol., 28:* 469-470.

ROJAS, J. C. & CRUZ-LOPEZ, L., 1992. Sexual behavior in two species of *Triatoma phyllosoma* complex (Hemiptera: Reduviidae) under laboratory conditions. *J. Med. Entomol., 29:* 13-18.

ROSSITER, M. & STADDON, B. W., 1983. 3-methyl-2-hexanone from the triatomine bug *Dipetalogaster maximus* (Uhler) (Heteroptera: Reduviidae). *Experentia, 39:* 380-381.

RYCKMAN, R., 1952., Laboratory culture of Triatominae with observations on behavior and a new feeding device. *J. Parasitol., 38:* 210-214.

SCHOFIELD, C. J. & MOREMAN, K., 1976. Apparent absence of sex attractant in adult *Triatoma infestans* (Klug), vector of Chagas' disease. *Trans. R. Soc. Trop. Med. Hyg., 70:* 165-166.

SCHOFIELD, C. J. & PATTERSON, J. W., 1977. Assembly pheromone of *Triatoma infestans* and *Rhodnius prolixus* nymphs (Hemiptera: Reduviidae). *J. Med. Entomol., 13:* 727-734.

VELAZQUEZ, A. A., 1968. Atracción por olor en ninfas y adultos de *Rhodnius prolixus. Rev. Inst. Med. Trop. São Paulo, 10:* 242-246.

ZARATE, L. G. & ZARATE, R. J., 1985. A checklist of the Triatominae (Hemiptera: Reduviidae) of Mexico. *Int. J. Entomol., 27:* 102-127.