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Assessing methods for the density of *Triatoma dimidiata*, the principal vector of Chagas' disease in Guatemala

Carlota MONROY¹⁾, Mildred MEJÍA¹⁾, Antonieta RODAS¹⁾,
Tomoyuki HASHIMOTO^{2), 3)} and Yuichiro TABARU^{2), 4)}

¹⁾School of Biology, Faculty of Chemistry and Pharmacy University of
San Carlos of Guatemala Zone 12, Guatemala City, Guatemala

²⁾Japan International Cooperation Agency -JICA-, 5a Ave. 11-40, Zona 11, Guatemala

³⁾Japan Environment Sanitation Center 10-6, Yotsuya-kamicho, Kawasaki,
Kanagawa, 210-0828 Japan

⁴⁾100-24, Makinohara 2, Matsudo, Chiba, 270-2267 Japan

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Abstract: We found a close relation between the number of *Triatoma dimidiata* collected by the man-hour method and the number of excrement spots of the vector on white paper placed on the wall for a week or more. The sticky trap, hiding box or animal trap was not effective for detecting *T. dimidiata* because of their dull and slow behavior. The flushing-out method is not effective either in estimating the density in the case of *T. dimidiata*, because *T. dimidiata* were not easily flushed-out from their hiding-places by spraying pyrethroid insecticides. We recommend use of the man-hour collection or the white paper method for assessing the density of *T. dimidiata* in Guatemala.

INTRODUCTION

To detect the population density of triatomine vectors of Chagas' disease in houses is an important issue for entomological studies and control strategies. The traditional man-hour collection for these vectors of the Chagas' disease is frequently used in research of population density or evaluation of insecticide efficacy in the field. However, this method depends basically on the collector's ability or experience. The principal object of this study was to acquire techniques and methods to detect or collect *Triatoma dimidiata* in houses and then use them as indicators of insecticide evaluation. Schofield (1978, 1980), Pinchin *et al.* (1981) and Gürtler *et al.* (1993, 1995) compared detecting techniques for domestic populations of tri-

atomines, mainly of *T. infestans* in South America, and Schofield *et al.* (1987) commented on the advantages and disadvantages of several detection methods. There are a few data in this field on *T. dimidiata*, the principal vector of Chagas' disease in Guatemala. The most widely used way of assessing the domiciliary density of this species is the man-hour collection. Monroy *et al.* (1995) and Tabaru *et al.* (1998) reported the results on *T. dimidiata* using man-hour collection. Besides this method, we have tried other methods using sticky papers or animal traps, counting excrement spots on white paper, flushing-out after pyrethroid spraying and mark-release-recapture in Guatemala.

MATERIALS AND METHODS

The experiments were performed at the

Laboratory of Malaria, the Ministry of Public Health and in rural houses at villages of San Juan de Arana, Santa Maria Ixuhatan and Agua Zarca in Department of Santa Rosa, Villa Canales in Department of Guatemala and San Miguel Huite in Department of Zacapa. *Triatoma dimidiata* and partially *Rhodnius prolixus* were targetted in these studies.

Traditional man-hour collection

Experienced technicians collected the bugs inside a house for a certain time period (one person for one hour, two persons for 30 min. or three persons for 20 min.) with a flashlight and a pair of tweezers. The tests were conducted in mud wall houses in four localities. The results of this method were compared with those of other methods.

Counting excrement spots on walls

A calibrated plastic frame of one square foot was put on the wall where there were spots of triatomine excrement and the number of spots inside the frame including new and old ones was counted. We chose 188 mud wall houses in Santa Maria Ixhuatan. The relation between the number of excrement spots and the number of triatomines collected by the man-hour method was examined.

Adhesive traps in the laboratory and in the field

Two commercially provided Victor Roach Glue traps were placed at the bottom inside of a small fish tank (50 cm × 35 cm × 40 cm h) with open or closed covers. This study was carried out in the laboratory under natural temperature. Then we set free 48 bugs in the first and the second instar in the tank. For comparison, we set free the same number of male adults of German cockroach, *Blattella germanica*, in the same type of tank under the same conditions. Beside the glue trap, two hiding boxes made with corrugated cardboard were placed in the bottom of each tank for their resting places. The accumu-

lative numbers adhered on the traps were recorded daily. In the field the same type of trap was put for a week on the wall inside the house where there were many excrement spots. Then we checked the presence of bugs adhered on the traps. We compared the numbers of bugs with those captured by man-hour collection in the same house.

Animal trap collection

A wooden box (38 cm × 38 cm × 38 cm) fitted with metal mesh on one side and wood panels on the other three sides, keeping a chicken inside, was set under a bed in a rural mud wall house for a week. Three folded carton shelters (20 cm × 5 cm) were set each along the three sides of the box for bug hiding. Each box had passages for triatomines of 30 cm width and 3 cm height at each side at the bottom. Food and water for the chicken were prepared every day. One week after, the carton shelters were checked to detect bugs.

White paper method

We placed a letter-size sheet of white paper (21 cm × 29 cm) on walls close to beds or chicken nests inside a house for a week; then, we counted the number of excrement spots on the paper. The numbers of spots on the paper were compared with the numbers by the man-hour collection. We tried to use this method for the evaluation of insecticide efficacy before and after the intradomiciliar insecticide treatment.

Flushing-out activity with pyrethroides spraying

We sprayed 0.075% water suspension of the commercial formulation of 10% lambda-cyhalothrine wettable powder at a rate of 50 ml/m² to mud walls against *T. dimidiata* and to palm-thatched roofs against *R. prolixus*. Ten to 30 minutes after spraying we captured flushed-out bugs and the number was compared with that by the man-hour collection in the same house.

Mark, release and recapture

At first we collected the bugs in a house by the man-hour collection; then we marked them with white paint and released them at the entrance of the house. After two weeks, we made another collection in the same way.

RESULTS AND DISCUSSION

The mean number of *T. dimidiata* collected by the traditional man-hour method in the pilot area of Santa Maria Ixhutan, Department of Santa Rosa was 6.24 in 79 positive houses. We obtained similar numbers of bugs in three other localities: 5.63 in 9 positive houses in San Juan de Arana, 4.72 in 6 positive houses in Agua Zarca, Department of Santa Rosa and 6.90 in 30 positive houses in Villa Canales, Department of Guatemala. The advantage of this method is to be able to obtain live samples. However, it is indispensable for an experienced person to collect bugs. Most of the investigation, surveillance and detecting for triatomines was based on this method (Schofield, 1978, 1980; Pinchin *et al.*, 1981; Gürtler *et al.*, 1993, 1995). This is also appropriate for *T. dimidiata* (Tabaru *et al.*, 1998). Schofield (1978) reported that experienced collectors could collect 23.3 *T. infestans* in 15 minutes in one house, whereas inexperienced collectors collected 12.7 in Brasil. This relatively large number of collected *T. infestans* may be due to the difference in population level of the vector.

We compared the numbers of bugs in the same house by the man-hour collec-

tion and by counting after demolition (Monroy *et al.*, 1998). The rate of the number by the man-hour collection to the total number counted after the demolition varied from 0.7 to 23.5. The number by the man-hour collection method did not very accurately reflect the total number obtained after demolition of the house.

In the pilot area of Santa Maria Ixhutan we counted the number of excrement spots of bugs on walls, using a calibrated plastic frame of one foot square in 170 houses. The mean number of excrement spots was 28 in 74 houses positive for bugs by the man-hour collection, whereas it was only 1.7 in 110 negative houses. A large number of excrement spots on walls suggested the possibility of bug presence in the house. However, we did not use it for evaluation of insecticide efficacy, because the excrement spots were mixed, new and old together.

Fig. 1. Bug excrement spots inside a calibrated plastic frame (one foot square) on a wall. The frame was put on the wall where spots were most abundantly found.

Table 1. Results of the traditional man-hour collections for *T. dimidiata* in various locations in Guatemala.

Location	Number of houses investigated	Mean number of bugs	Maximum number	Minimum number
Santa Maria Ixhutan	74	6.24	37	1
San Juan de Arana	9	5.63	17	1
Villa Canales	30	6.90	37	1
Agua Zarca	6	4.72	15	1

Houses without bugs were excluded from the data.

Table 2. Comparison of attractiveness to adhesive traps between *T. dimidiata* and *B. germanica* in laboratory.

Insect	Condition	Day 1 (%)	Day 2 (%)	Day 3 (%)
<i>T. dimidiata</i>	Cover open	1	1	2
	Covered	1	2	2
	Total	2 (4.2)	3 (6.3)	4 (8.3)
<i>B. germanica</i>	Cover open	12	14	17
	Covered	14	19	31
	Total	26 (58.3)	33 (68.8)	48 (100)

Each 48 number of insects was set free into the same type of fish tank. One trap was without a cover and the other was closed.

Table 3. Comparison of the numbers of *T. dimidiata* by three different methods in 9 rural houses and one chicken house in San Juan de Arana.

House no.	Man-hour collection	Animal traps	Shelter traps
1	15.0	1.0	0
2	6.7	0	0
3	8.6	0	0
4	10.0	0	0
5	3.3	0	0
6	0.7	0	0
7	4.7	0	0
8	2.7	0	0
9	2.3	0	0
10 (Chicken house)	2.3	0	0

The man-hour collection was triplicated. Traps were set for a week.

A commercially provided Victor Roach Glue trap was evaluated as detecting material for bugs in the laboratory. Table 2 shows the results. All German cockroaches were captured in three days, whereas the number of *T. dimidiata* captured was only 2 (4.2%) in day 1 and 4 (8.3%) in day 3. The bugs seemed to repel the glue traps. In the laboratory, the rest of the bugs hid themselves in the shelter. The glue traps are not effective for bug detection even in the laboratory.

Table 3 shows the results comparing the numbers of bugs obtained by three different methods, in nine rural houses in San Juan de Arana. The mean number collected by the man-hour collection method was 5.63, whereas only one bug was captured

by animal traps in house No. 1, and no bugs were captured by glue traps at all. These poor results with the latter two passive methods are due to the dull and slow activity of *T. dimidiata*, and they seem to hesitate due to the inexperienced substance. We abandoned the idea of using the glue trap and the animal trap for detecting bugs and evaluating insecticide efficacy in further studies. Schofield *et al.* (1987) reported the efficacy of the commercialized, artificial refuge box for *T. infestans* with good results even when the bug population was not high. Gürtler *et al.* (1995) also demonstrated good efficacy with this type of box for the same species in the field.

Schofield (1978), Pinchin *et al.* (1981), Chuit *et al.* (1992) and Gürtler *et al.* (1995) used a sensor box (Biosenor, Biocientifica de Avanzada, Buenos Aires, Argentina) for detecting *T. infestans* in the field. However, Pinchin *et al.* (1981) and Schofield (1978) emphasized that the sensor box was not suitable for insecticide evaluation, because a long time-period, more than a month, would be required to capture a sufficient number of bugs. Chuit *et al.* (1992) reported that this sensor box was promising for community-based surveillance in a vector control programme in Argentina, where *T. infestans* was abundant. Gürtler *et al.* (1995) reported the results using six methods including the sensor box, and he reported that the sensor box was applicable for detecting *T. infestans*. However, *T. dimidiata* did not

Table 4. Comparison of changes in the number of bugs man-hour collection and in the number of excrement spots on white paper in the same house, before and after spraying of diazinon wettable powder (600 mg/m²).

House number	Bugs collected by man-hour		Excrement spots in white papers	
	Before	After 1 month	Before	After 1 month
1	5.7	0	3.5	0
2	6.3	1	2.6	0
3	2.0	0	0.6	0.6
4	6.0	0	0.7	0
5	3.0	0	0.8	0
6	4.3	0	3.2	0
7	21.5	1	6.6	0.7
Mean	6.97	0.29	2.57	0.19
Reduc. %		95.6		93.0
Control	4.7	5	6.0	5.7

The man-hour collection was done two or three times before insecticide treatment and once after treatment. Five white papers were placed on walls for one week and the number of excrement spots was counted.

Reduction % was calculated after the adjustment by the numbers of bugs in the control.

enter the carton shelters, which were comparable to the sensor box, placed for a week with the animal trap in the present experiment. The behavior seems to be quite different between *T. dimidiata* and *T. infestans*; the former is slower and duller than the latter.

When sheets of white paper are placed on walls for a week, new spots of bug excrement certainly appear on them (Table 4). We used this phenomenon for insecticide evaluation (Tabaru *et al.*, 1998). The number of spots of excrement after insecticide treatment decreased notably, and the change paralleled that by the man-hour collection. The important issue is to select the proper place for the paper, which should be placed where a large number of excrement spots is found.

The disadvantage of this indirect method in the evaluation of insecticides is that twice placement of the paper, before and after treatment, is required and we can not collect live bugs for natural infection studies.

After spraying pyrethroid insecticide some bugs are flushed-out from their resting places. Schofield (1978) suggested that calculating the number of flushed-out bugs should be useful to evaluate insecti-

cide efficacy in fields. Table 5 shows the numbers of flushed-out *T. dimidiata* and *R. prolixus* after lambda-cyhalothrin spraying. The number of bugs by the man-hour collection and the number flushed-out in *T. dimidia* were not so different (5.8 vs 7.6), whereas in *R. prolixus* they were quite different (6.7 vs 24.7).

T. dimidiata seems more difficult to be flushed-out than such other species as *T. infestans* and *R. prolixus*. This method is acceptable not for assessing the density but for detecting the presence of *T. dimidiata*. For evaluating insecticide efficacy it does not seem appropriate because it is necessary to confirm bug density prior to insecticide spraying. In addition, all the bugs and parasites are killed immediately after the treatment of pyrethroid for flushing-out and, accordingly, this method is not acceptable for natural infection studies.

We collected bugs by the man-hour collection, and released them at the entrance of a house after marking them with white paint on the back of the thorax. We tried to recapture them after two weeks in the same house. Table 6 shows the result of this study. Only a small number of the released bugs were recaptured at 2–6 m

Table 5. Comparison of the numbers of bugs by the man-hour collection and the flushing-out method in *T. dimidiata* and *R. prolixus*.

House number	<i>T. dimidiata</i>		House number	<i>R. prolixus</i>	
	Man-hour collection	Flushed-out		Man-hour collection	Flushed-out
1	5.5	8	1	13	26
2	11.5	9	2	6	34
3	3.0	9	3	2	7
4	1.0	4	4	8	30
5	8.0	8	5	3	7
			6	8	44
Mean	5.8	7.6		6.7	24.7

Test with *T. dimidiata* was done in Agua Zarca, Department of Santa Rosa and with *R. prolixus* in San Miguel Huite, Department of Chiquimula.

Table 6. Movement of *T. dimidiata* in rural houses using the mark, release and recapture method in Santa Maria Ixhuatan, Department of Santa Rosa.

House number	No. of released bugs with mark	No. of captured bugs	No. of marked bugs recaptured	Distance from released place (m)
1	7 (2♂♂, 2♀♀, 3 nf)	4 (2♂♂, 2 nf)	2 (1♂, 1 nf)	2.3
2	4 (2♂♂, 1♀, 1 nf)	2 (1♂, 1♀)	0	—
3	5 (1♂, 1♀, 3 nf)	4 (2♂♂, 1♀, 1 nf)	0	—
4	3 (2♂♂, 1♀)	4 (1♂, 2♀♀, 1 nf)	1 (1♀)	6.0
5	6 (2♂♂, 2♀♀, 2 nf)	5 (2♂♂, 1♀, 2 nf)	1 (1 nf)	2.0
6	7 (1♂, 2♀♀, 4 nf)	4 (2♀♀, 2 nf)	0	—
7	3 (1♂, 2 nf)	1 (1♂)	0	—

from the releasing spot. Schofield (1978) reported on the estimation of population density using this method with *T. infestans* in Brazil. But we failed to estimate the density of *T. dimidiata* due to the very small number of bugs recaptured.

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摘 要

家屋内での *Triatoma dimidiata* の生息密度推測について

Carlota MONROY¹⁾, Mildred MEJÍA¹⁾, Antonieta RODAS¹⁾, Tomoyuki HASHIMOTO^{2), 3)} and Yuichiro TABARU^{2), 4)}

¹⁾School of Biology, Faculty of Chemistry and Pharmacy, University of San Carlos of Guatemala, Zone 12, Guatemala City, Guatemala

²⁾国際協力事業団, グアテマラ共和国, 熱帯病研究プロジェクト 5a Ave. 11-40, Zona 11, Guatemala

³⁾日本環境衛生センター

(〒210-0828 川崎市川崎区四谷上町 10-6)

⁴⁾〒270-2267 千葉県松戸市牧ノ原 2-100-24

サシガメが生息する民家の壁に白紙 (21 cm×29 cm) を一定期間設置してその上に新しく付着する糞の数をサシガメの生息数の指標及び効果判定の評価にできる事を明らかにした。ゴキブリ捕獲用粘着トラップ, シェルター, 動物囿等を「限定時間採集」と比較したが, 何れも *T. dimidiata* には有効でなかった。また, ビレスロイド系殺虫剤散布後の Flush-out する数も少なかった。これは, 本種の行動に起因すると判断した。