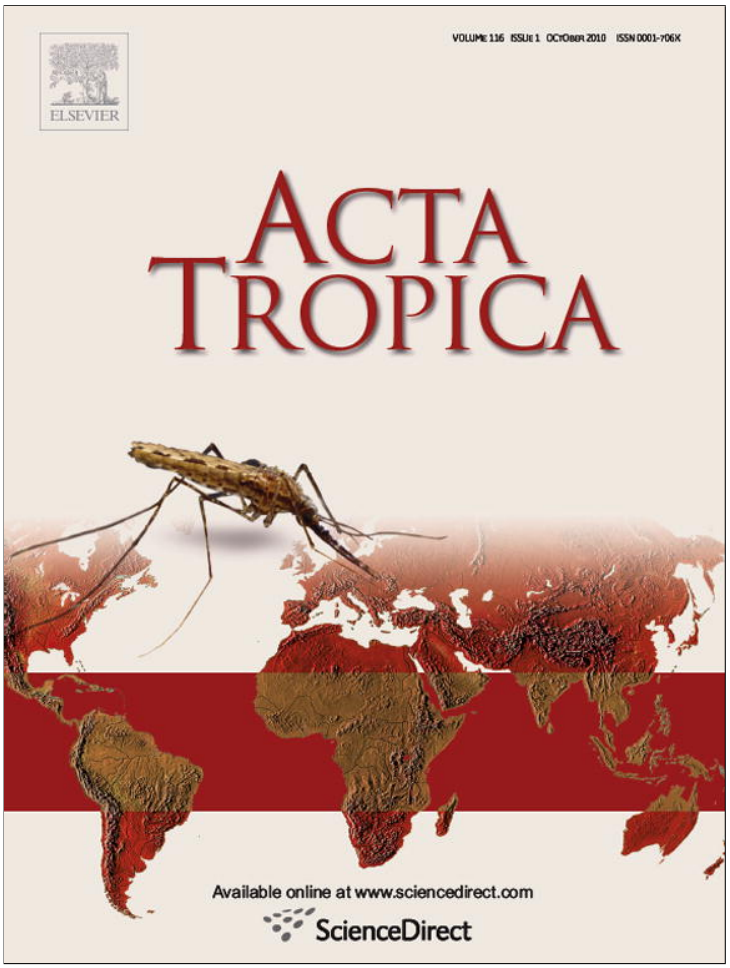

Acta Tropica 116 (2010) 111–114

Contents lists available at ScienceDirect

Acta Tropica

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

Short communication

Searching for triatomines. A new method for field search using UV light

Silvia Catalá

Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR/CONICET), Anillaco, 5301, La Rioja, Argentina

ARTICLE INFO

Article history:
Received 8 January 2010
Received in revised form 20 March 2010
Accepted 3 April 2010
Available online 10 May 2010

Keywords:
Chagas surveillance
Triatominae
Ultraviolet light

ABSTRACT

Detection of triatomine bugs within a house is essential for the estimation of Chagas disease transmission risk and for evaluating the success of insecticidal control attempts. Small residual populations could represent an important risk but are difficult to detect by time manual sampling. Faecal marks from triatomines are clearly detectable with an ultraviolet (UV) light on most of the materials frequently used in rural buildings. A new method for finding triatomines is proposed here, based on the unexplored property of faeces to fluoresce when exposed to UV light.

Searching for Triatominae bugs in rural houses of Latin America is an important activity carried out by Health Services in every country affected by Chagas disease. This endemic illness, produced by the *Trypanosoma cruzi* flagellate and transmitted by triatomines, causes fifty thousand deaths a year within an infected population estimated on 5–6 million people. Achieving eradication of the vectors from houses is the main goal for the World Health Organization (WHO) as more than 85% of the cases are produced by vectorial transmission (WHO, 2002).

Chagas disease is closely related to social and economic development and to poverty in rural populations of Latin America. Vector control by means of insecticides is effective and has been shown to interrupt transmission; however, it requires continuous entomological surveillance before and after periodical chemical control. The entomological research is necessary for the acquisition of information on the number of species present in houses and peridomestic structures, as well as their infestation and dispersion rates in order to estimate the transmission risk of *T. cruzi*. In areas where native species colonize the domiciles, the goal should be to keep housing free of intra-domiciliary colonies. Regarding introduced species – for which the goal is total elimination – the finding of a single bug, regardless of its stage of development and whether it is infected or not, should be sufficient reason for control to start (WHO, 2002).

In general, infestation by triatomines in the domestic and peridomestic structures is recorded by active search by means of timed manual collection using a dislodgant spray. The activity requires trained people, usually from Vector Control and Health Care Programs, who go to each rural house and carefully revise intra and peri-domiciliary buildings. The houses and their annexes, like store rooms, chicken coops, corrals, etc, are very complex structures which make difficult to find triatomines. Most of poor rural houses lack electricity, so darkness is a characteristic inside the rooms where windows are small and scarce, or absent. The house's walls are made of adobe bricks, wood logs or stones with many crevices where the insects rest during the day. Searching for triatomines in those conditions of reduced visibility is especially hard at low vector densities, as well as in the case of new colonisations.

A good evidence of a house infestation is the presence of triatomine faeces. One method used for detecting low density populations of domestic triatomine bugs is the Gomez-Nuñez box (and its derivatives) which acts as an artificial refuge for the bugs. It is a sensitive research tool but very expensive and very time consuming for wide use in large scale control campaigns as it requires to be examined by trained people at different times after being placed within the house (Schofield et al., 1986).

The objective of the present work is to propose a new method that would help entomological surveillance of Chagas disease vectors, through a more efficient way of detecting triatomines faeces and refuges. The method uses the unexplored property of faeces to fluoresce when exposed to UV light.

Laboratory experiments to determine the fluorescence of faeces emitted at different times after a meal were conducted during 1 week. Ten fifth-instar nymphs and 10 adults of *Triatoma infestans* – laboratory reared under constant temperature ($28 \pm 2\,^{\circ}C$), humidity (80%), and light, were placed in two plastic bottles with a white absorbent non-fluorescent paper. The insects were fed on a restrained chicken for 30 min, as usual. After the meal, the paper was removed and replaced by a new sheet every day, and it was analysed for fluorescence with a commercial UV Black Light Blue

E-mail address: scatala@crilar-conicet.com.ar.

0001-706X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2010.04.017

tube (UV BLB, 365 nm), connected to a 12 V battery. This probe was carried out under an illumination of 10–50 lux, determined by a Tenmars TM-201 luxometer. Pictures were obtained with a Panasonic Lumix digital camera.

The second step was to determine whether faecal fluorescence could be detected on building materials and common objects found in rural houses. Bricks (adobe, cement and commercial red), wood, cardboard, paper, plastics and fabrics, were spotted with faeces obtained by pressing the abdomen of bugs. After 24 h all those materials were exposed to UV BLB and photographed. Additionally, some bricks from the walls of an infested chicken coop (no longer in use) were checked for faecal drops using the UV BLB lamp.

The colourless urine, emitted by *Triatoma infestans* during the first 24 h after the blood meal was clearly detectable under UV BLB (Fig. 1) when deposited on absorbent paper. The pale excreta (white, yellow or beige drops) showed marked fluorescence under UV BLB meanwhile the dark excreta (brown and black) were non-fluorescent or showed a fluorescent green halo when deposited on absorbent paper (Fig. 1).

Faecal material mixed with water lost the fluorescence in a few hours. When this solution was observed under light microscopy the typical spherical crystals of uric acid were replaced by needle-like crystals.

The colourless and pale faecal drops deposited on adobe or cooked bricks, plastics, journal's paper, and wood, showed green fluorescence. On the other hand, excreta deposited on fabrics and cardboard were barely or no fluorescent at all. The phenomenon was less evident when the excreta were covered with dust, as on the bricks obtained from the chicken coop dismantled several months before (Fig. 2).

It was observed that the colourless and pale excreta deposited on a wood stick spread very fast on the surface producing a large fluorescent mark. This effect was not too evident on adobe bricks, probably as consequence of the different absorption property of this surface.

During the experiments detailed above, another substance was unexpectedly fluorescent: the spermatophores secreted by males during mating showed an intense green-blue colour (Fig. 3). Moreover, as a consequence of contamination with faecal material, the insects themselves showed green fluorescent spots above their body. It was also noted that, when they walked on fresh excreta, small footprints appeared on the walked surface (Fig. 1).

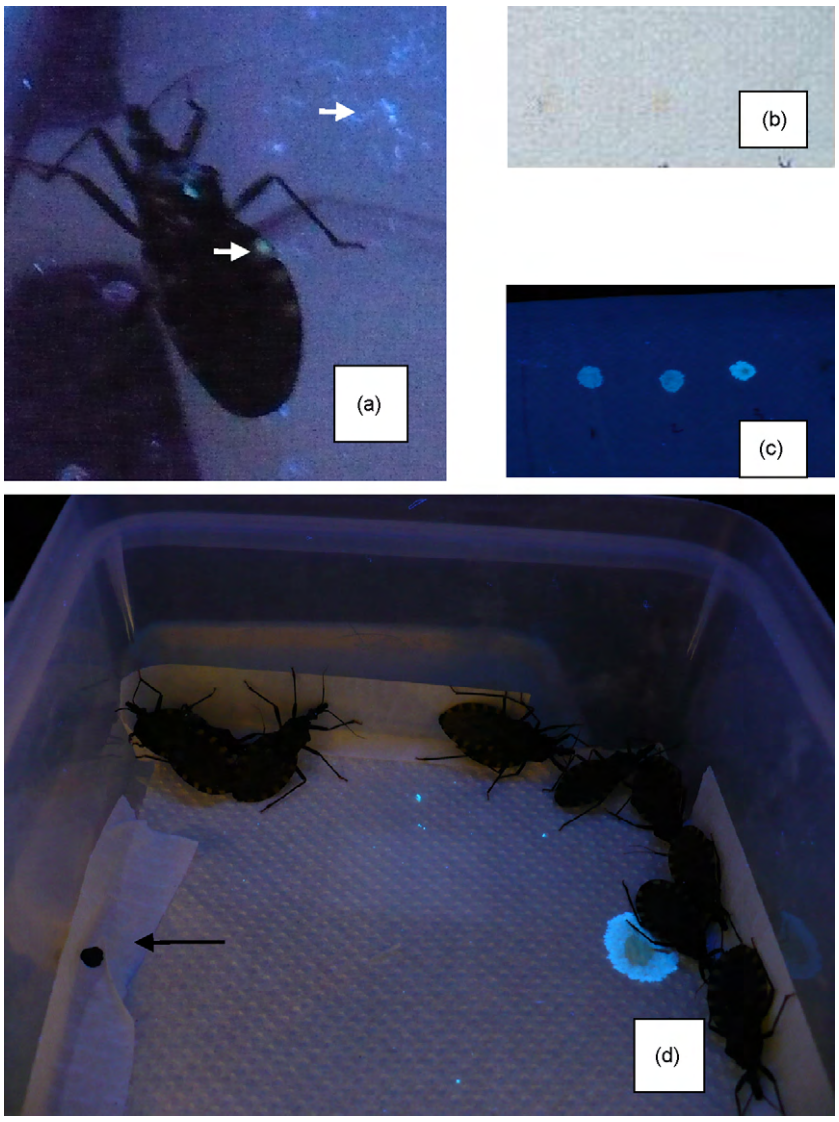

Fig. 1. (a) An adult of *Triatoma infestans* shows two fluorescent spots of faecal material on its body. Other fluorescent marks on the ground are foot prints. (b and c) Colorless urine collected on paper 4 h after feeding and observed under white (b) and UVBLB (c). (d) A box with several *Triatoma infestans* after feeding. The arrow points at a black dejection (non-fluorescent) emitted immediately after feeding. Some foot prints and colorless urine fluoresce intensely on the right.

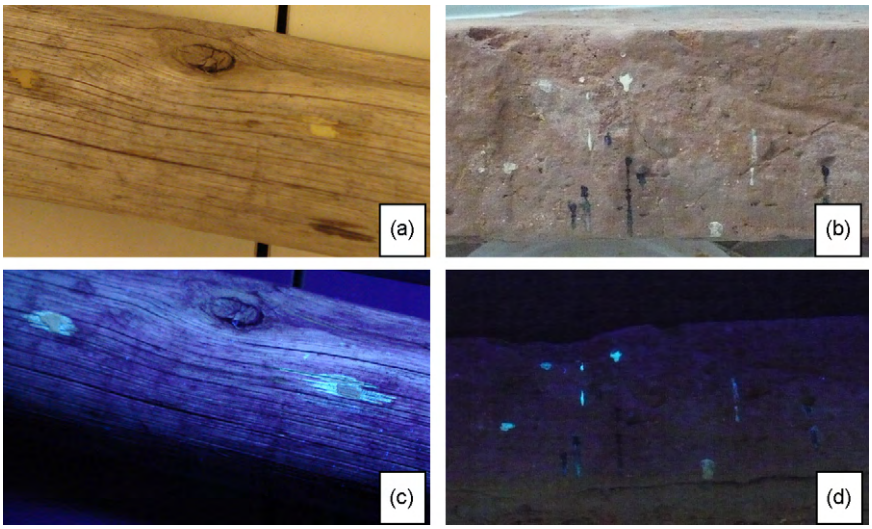

Fig. 2. A piece of wood (a) and a red brick (b) with faecal spots of *Triatoma infestans* observed under common light or UVBLB (c and d). Colorless and pale dejections fluoresce with UVBLB.

The fluorescence of insect excrement when exposed to UV light was first exploited by Lamkim et al. (1991) to evaluate insect contamination in stored grain. The same principle could be applied for detecting the presence of Triatominae excrement on different substrates, as showed here.

UV light could detect more triatomine dejections than a normal visual search within the domestic environment. Even the clear fluid excreted during the first few hours after a meal (Catalá, 1991), invisible to human eyes, is clearly detectable using an UV BLB device. Triatomines defecate every time they feed – this behavior is influenced by seasonal climatic changes. During the hot season, when the infection risk by *T. cruzi* is the highest (Giojalas et al., 1990), *Triatoma infestans* feed every 2–7 days, producing a large amount of faeces and colourless urine in particular (Catalá, 1991). The faeces are frequently deposited at the entrance of their refuges and act as chemical marks that help the insects finding them (Guerenstein and Lazzari, 2009). Using UV light for detecting faecal spots would also help to recognize new infestations, as the old excreta, covered by dust, are poorly fluorescent or non-fluorescent at all.

The general course of excretion in triatomines (Wigglesworth, 1931) is characterized by changes in the chemical composition and colour of faeces. Almost immediately after feeding, the insect voids the black residue of its previous meal; then, a few minutes later, a drop of cloudy watery fluid. For the next 3–4 h it passes, at intervals of a few minutes, a perfectly clear colourless fluid; and then the passage of urine ceases. The longer the appearance of the next drop is delayed, the greater is the proportion of sediment it contains, which dries as a yellow powder. In the later stages of digestion the urine is contaminated with haematin (a dark pigment) from the intestine; but it frequently contains no faecal material for a week or 10 days. In this paper it is shown that the intense fluorescence of colourless, yellow or white excreta is produced by the abundant rounded crystals of uric acid, derived from the protein metabolism (Wigglesworth, 1931). On the other hand, the brown and black faeces show a variable quantity of dark particles of haematin that diminish the faecal fluorescence produced by the uric acid.

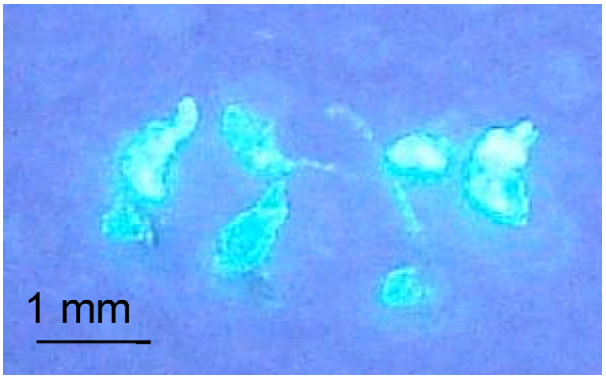

Fig. 3. *Triatoma infestans* spermatophores observed under UVBLB.

The excreta of other insects, birds and reptiles also contain uric acid but they are not likely to be confused with triatomines faeces because of their particular size and shape ("dripping candle wax") (Schofield et al., 1986).

The fluorescence of faecal marks from triatomines is clearly detectable with an UVBLB lamp on most of the materials frequently used in rural buildings: adobe, cooked bricks, cement, and wood.

Visual inspection for evidence of bug faeces is commonly used to determine whether a house is infested. There is generally no simple way of knowing objectively how long ago those faecal streaks were deposited, and much of the evaluation decision is based on personal field experience. Finding of faecal streaks alone indicates that a house has been infested at some time in the past, but it might not indicate a current infestation. By contrast, UV fluorescence will be more apparent in relatively recent faeces, prior to degradation of the uratic spheres, or the effect of dust that eventually will cover them. A disadvantage of this method could be that UV light requires a more careful use than the normal light.

The fluorescence of urate in faeces of triatomine bugs has been known since 1934 (Wigglesworth, 1931), but this is the first time it has been proposed for use in entomological surveillance. Normally, a torch light is used to search for triatomines within the houses. Replacing the regular light bulb by an UVBLB one could substantially improve the early detection of residual or emergent populations of domestic triatomine bugs and contribute to a successful evaluation and vigilance of Chagas disease vectors.

Acknowledgements

To Dr. C. Schofield who revised the manuscript and to Dr. Nahuel Romero and Tec. Natalia Folguera who helped with laboratory work. The work was partially founded by PICT 2006-878 ANCyT (Argentina). SC is a member of CONICET.

References

Catalá, S., 1991. The biting rate of *Triatoma infestans* under natural climatic conditions in Argentina. Med. Vet. Entomol. 5, 325–333.

Giojalas, L., Catalá, S., Asin, S., Gorla, D., 1990. Seasonal changes in infectivity of domestic populations of *Triatoma infestans*. Trans. R. Soc. Trop. Med. Hyg. 84, 39–442.

Guerenstein, P., Lazzari, C., 2009. Host seeking: how triatomines acquire and make use of information to find blood. Acta Trop. 110 (2–3), 148–158.

Lamkim, W.M., Unruh, N.C., Pomeranz, Y., 1991. Use of fluorometry for the determination of uric acid in grain. Elimination of interfering fluorescence. Cereal Chem. 68 (1), 81–86.

Schofield, C., Williams, N., Kirk, M., Garcia Zapata, M., Marsden, P., 1986. Key for identifying faecal smears to detect domestic infestations of triatomine bugs. Rev. Soc. Bras. Med. Trop. 19 (1), 5–8.

Wigglesworth, V., 1931. The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). III. The mechanism of uric acid excretion. J. Exp. Biol. 8, 443–454.

World Health Organization, 2002. Second Report of the WHO Expert Committee on the Control of Chagas Disease. World Health Organization WHO Tech Rep Series, 905.