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Research

Sampling, Distribution, Dispersal

Development of an operational trap for collection, killing, and preservation of triatomines (Hemiptera: Reduviidae): the kissing bug kill trap

Gabriel L. Hamer[1,*,‡], Juan P. Fimbres-Macias[2,‡], Jose G. Juarez[1,3], Christopher H. Downs[1,4], Ester Carbajal[5], Melinda Melo[5], Danya Y. Garza[1], Keswick C. Killets[2], Gregory K. Wilkerson[6,7], Rogelio Carrera-Treviño[8], Enrique Corona-Barrera[9], Arturo Arabied Tello-Campa[8], Martha Rocío Rojas-Mesta[8], John H. Borden[10], Michael G. Banfield[4], Sarah A. Hamer[2]

[1]Department of Entomology, Texas A&M University, College Station, TX, USA, [2]Veterinary Integrative Biosciences, Texas A&M School of Veterinary Medicine & Biomedical Sciences, College Station, TX, USA, [3]Centro de Estudios en Salud, Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala, [4]BanfieldBio, Inc., Woodinville, WA, USA, [5]Texas A&M AgriLife Research, Weslaco, TX, USA, [6]MD Anderson Cancer Center, Michale E. Keeling Center for Comparative Medicine and Research, Bastrop, TX, USA, [7]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill NC, USA, [8]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nuevo León, General Escobedo, México, [9]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Ciudad Victoria, México, [10]JHB Consulting, Burnaby, BC, Canada [*]Corresponding author, email: gabe.hamer@ag.tamu.edu
[‡]Both authors contributed equally to this manuscript.

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Surveillance of triatomines or kissing bugs (Hemiptera: Reduviidae: Triatominae), the insect vectors of *Trypanosoma cruzi*, a Chagas disease agent, is hindered by the lack of an effective trap. To develop a kissing bug trap, we made iterative improvements over 3 years on a basic design resulting in 7 trap prototypes deployed across field sites in Texas, United States and Northern Mexico, yielding the capture of 325 triatomines of 4 species (*Triatoma gerstaeckeri* [Stål], *T. sanguisuga* [LeConte], *T. neotomae* [Neiva], and *T. rubida* [Uhler]). We began in 2019 with vertical transparent tarpaulin panel traps illuminated with artificial light powered by AC current, which were successful in autonomous trapping of flying triatomines, but were expensive, labor-intensive, and fragile. In 2020, we switched to white LED lights powered by a solar cell. We tested a scaled-down version of the vertical panel traps, a commercial cross-vane trap, and a multiple-funnel trap. The multiple-funnel traps captured 2.6× more kissing bugs per trap-day than cross-vane traps and approached the performance of the vertical panel traps in number of triatomines captured, number of triatomines per trap-day and triatomines per arthropod bycatch. Multiple-funnel traps required the least labor, were more durable, and had the highest triatomines per day per cost. Propylene glycol in the collection cups effectively preserved captured triatomines allowing for molecular detection of *T. cruzi*. The trapping experiments established dispersal patterns for the captured species. We conclude that multiple-funnel traps with solar-powered LED lights should be considered for adoption as surveillance and potentially mass-trapping management tools for triatomines.

Key words: trapping, surveillance, multiple-funnel trap, Texas, Mexico

Chagas disease, caused by the protozoan parasite, *Trypanosoma cruzi* Chagas (Trypanosomatida: Trypanosomatidae) is transmitted by triatomine insects known as kissing bugs (Hemiptera: Reduviidae: Triatominae). The global burden of Chagas disease is estimated at 6–7 million people mostly in Latin America (WHO 2024) and these estimates include 288,000 people in the United States (Irish et al. 2022). Autochthonous vector-borne transmission in humans in the United States is increasingly recognized due to blood donor screening and active surveillance, and Chagas disease is reportable in 6 states (Curtis-Robles et al. 2017, Gunter et al. 2017, Bennett et al. 2018). In the United States, much of the risk of human exposure to *T. cruzi* occurs as adult triatomines disperse by flight from the natural sylvatic habitat and enter the peridomestic and domestic habitat (Hodo and Hamer 2017) where they may be attracted by artificial light and can colonize homes (Beatty et al. 2018). This "spillover" is aided by infrequent but significant dispersal flights, which can extend over several kilometers (Schweigmann et al. 1988, Vazquez-Prokopec et al. 2004, Carbajal de la Fuente et al. 2007, Pacheco-Tucuch et al. 2012). The World Health Organization identifies Chagas disease as a neglected tropical disease that does not have a cost-effective control tool. Disease control relies heavily on reducing insect vector populations and the proposed goal is to interrupt vectorial transmission by 2030 (WHO 2024). However, with some 150 species widely distributed mainly in the Americas (Gorla and Noireau 2017), sustainable prevention of spill-over from sylvatic to domestic habitats has proven to be difficult (Bezerra et al. 2020). Indoor residual spraying has been effective in regions of Latin America where triatomines commonly colonize homes (Peterson et al. 2019), but insecticide resistance (Calderón et al. 2020) and lack of sustained abatement has led to the resurgence of triatomines in many regions (Dohna et al. 2007). No vaccines for humans exist; treatment is limited to 2 drugs not fully approved for adults in the United States (Bern et al. 2019). While these drugs are effective if given early after infection, their efficacy diminishes during chronic stages and adverse reactions have been reported (WHO 2024). In addition to human disease, canine Chagas disease is widespread in the United States in companion animals (Busselman et al. 2021) and government-owned working dogs (Meyers et al. 2020). Animals in zoological parks and research facilities are also at risk of *T. cruzi* infection (Hodo et al. 2018, Reis et al. 2020).

Surveillance for triatomines is challenging due to their unique biology and low population densities; attempts to improve collection methodology have had limited success (Curtis-Robles et al. 2018b). A productive method for triatomine surveillance of kissing bugs utilizes members of the public. An early example is the program of Dr. Sherwin Wood in Arizona in 1941 with public advertisements of "Nab that bug at one cent each for Dr. Wood at City College to keep" (Wood and Wood 1961). More recently, the Kissing Bug Community Science Program at Texas A&M University has received >8,000 triatomines from citizens of 28 US states, 2013–2021 (Curtis-Robles et al. 2015, Busselman and Hamer 2022). While surveillance of kissing bugs by public submissions has value (Hamer et al. 2018), it does not adequately determine spatio-temporal patterns of triatomines across the range of sylvatic, peridomestic, and domestic environments.

In the southern United States and Mexico, active sampling by searching around lights at night, excavating harborages in dead wood, and collecting triatomines landing on a white cloth under a blacklight has yielded 0–4.5 kissing bugs/h of searching (Rebollar-Téllez et al. 2009, McPhatter et al. 2012, Curtis-Robles et al. 2018b, Dye-Braumuller et al. 2019, Busselman and Hamer 2022). In Brazil, 75 triatomines were collected in the forest canopy using white cloth traps and different types of light suspended on a platform 45 m above the ground (Castro et al. 2010). Major limitations of most active collection techniques include the need for humans to be present during the night when safety is a concern, and habitat destruction such as cutting down palm trees or digging up nests (Angulo et al. 2013).

Traps have been developed and used to maintain continuous surveillance, enhance early detection of triatomines, minimize manual collection by personnel, and obtain live specimens for laboratory colonization. The simplest trap is a double-sided sticky tape affixed in strategic places in dwellings (Enriquez et al. 2020). Another non-baited device is the Gómez-Nuñez cardboard box, which provides refuge for the triatomines, and is used to indirectly detect the presence of triatomines *via* their feces and exuviae, but also to collect individuals (Feliciangeli et al. 2007). Noireau traps are containers covered with double-sided adhesive tape, baited with live mice, and placed on trees, rock piles and crevices; they have been used with success in South America (Noireau et al. 2002). A modified version of this trap collected 18 triatomines in 30 traps in western Mexico (Martínez-Ibarra et al. 2008). A similar mouse-baited design was used along the Peninsula of Baja California, Mexico, where 433 traps captured 17 triatomines of 2 species (Waleckx et al. 2024). Another design used a wire cage containing a bird placed above a plastic box containing live triatomines (Angulo and Esteban 2011). Both devices trapped diverse triatomine species and life stages in various ecotopes but required daily maintenance, making them unsuitable for large-scale and long-term deployment. Other traps have been baited with one or more attractants (e.g., CO_2, host odors, and heat) to simulate a live animal. In one example, traps that employed yeast to generate CO_2 were more effective than unbaited control traps (Lorenzo et al. 1998), but they would have required daily maintenance. An attempt at the development of a trap using semiochemicals, heat sources, CO_2, and blue LED light resulted in zero triatomine collections in Houston, Texas (TX), United States (Dye-Braumuller et al. 2019).

In contrast to traps baited with live animals or other attractants, traps using artificial light as the attractant would not require daily maintenance. In 1954, 3 light traps around a rural residence in Arizona, United States captured 398 *Triatoma protracta* (Uhler) over 5 mo (Sjogren and Ryckman 1966). This trap employed a fluorescent blacklight placed next to a vertical white panel surrounded by a Malathion-treated gutter that trapped and killed all attracted triatomines. That year the homeowners noticed fewer triatomines within the residence compared to typical years (Sjogren and Ryckman 1966), suggesting that triatomine populations could be reduced by mass-trapping (Day and Sjogren 1994). In 1966 and 1967, 4 small blacklight cross-vane traps designed for general insect surveys were deployed in San Antonio, TX, United States and captured 698 specimens of *T. gerstaeckeri* (Stål) and 13 *T. sanguisuga* (LeConte), with peaks occurring in June and July, respectively (Pippin 1970). In 2011, 2 light traps with a solar panel and a photo sensor in Southeast Brazil captured 4 specimens of 3 different triatomine species over 1 yr (Leite Dias et al. 2011). Similarly, in the Brazilian Pantanal region researchers used light traps with a flap that led into a container and captured 2 triatomines with 7,560 h of trap effort (Santos et al. 2015). In 2016, cross-vane traps baited with UV light, a human-mimic chemical lure or both were deployed in Panama and captured 45 *Rhodnius pallescens* (Barber; Updyke and Allan 2018). The light trap alone captured approximately 0.5 triatomines/trap night and the combination traps captured 0.4 specimens/trap night, but a white sheet and black light captured approximately 0.75 specimens/trap night. The trap designs mentioned above targeted flying triatomines; however, pitfall traps with artificial light have also been used to collect crawling triatomines. This design was more efficient than active sampling, yielding 7 triatomines, while none were collected by active sampling within the Caatinga biome in the state of Rio Grande do Norte, Brazil (Vasconcelos Queiroz et al. 2021).

Some commercially available traps designed for other hematophagous insects have been tested against triatomines. Between 2012 and 2016, Curtis-Robles et al. (2018b), captured no kissing bugs in Universal Black Light Traps (BioQuip Products Inc., Compton, California, USA, now defunct) or the MegaCatch ULTRA Mosquito Trap baited with 1-octen-3-ol (Megacatch, Memphis, Tennessee, USA) in TX, United States. Between 2016 and 2019, Rodriguez et al. (2021) collected only 17 triatomines using the Universal Black Light Traps in TX and New Mexico while 225 individuals were collected by manual searching and community submissions.

The objective of this study was to build on past triatomine traps to develop an operational, autonomous, low-maintenance trap with lights as the attractant that automatically turn on every night to attract, kill, and preserve adult triatomines.

Materials and Methods

The study occurred in 4 TX locations in years 1–3: (Mission-South [private residence], Mission-North [private residence], College Station [30° 36.601′ N, 96° 21.864′ W] and Bastrop [30° 12.372′ N, 97° 18.190′ W]), and 3 northern Mexico locations in year 3 (Jaboncillos, Coahuila [25° 58.278′ N, 102° 48.474 W], General Bravo, Nuevo Leon, [25° 47.630′ N, 99° 11.789′ W], and Ciudad Victoria, Tamaulipas [23° 41.502′ N, 99° 6.914′ W]; Fig. 1). Locations were selected based on past community science submissions (Curtis-Robles et al. 2015) or access to secure property where traps could be deployed and operated. The Bastrop location was a biomedical research facility with a history of Chagas disease in semi-outdoor-housed nonhuman primates (Hodo et al. 2018, Kiehl et al. 2023).

2019: Evaluation of Experimental Vertical Panel Traps

The initial prototype trap was intended to present a tall and wide vertical catchment surface (Sjogren and Ryckman 1966, Updyke and Allan 2018) around an attractive light source, acknowledging that we and Sjogren and Ryckman (1966) have observed that triatomine flight orientation is not precise and that triatomines often land short of a light source.

The traps (Fig. 2) employed a vertical transparent tarpaulin (2 × 3 m) suspended with nylon rope attached to 4 upright 3-m-high metal fencing T-posts over a horizontally held tarpaulin funnel (2.4 × 2.4 m) tethered tightly with nylon rope to 4 upright 2.4-m-high T-posts and sloping inward to a central funnel aperture. This design created a large catchment surface to intercept flying kissing bugs, after which they would fall into the funnel. The tarpaulin funnel led to an aluminum funnel, which led into an elbow-curve tunnel, with small drain holes that allowed rainwater to flow out but allowed insects to pass through and fall into a collection container containing propylene glycol (Bluewater Chemgroup, Fort Wayne, IN; Supplementary Fig. S1), a nontoxic preservative that kills captured insects and preserves nucleic acids for diagnostic analysis (Martoni et al. 2021).

Light units with extension cords connected to a source of AC power and a programmable timer (BN-LINK, Santa Fe Springs, California, USA) were suspended directly above the vertical transparent tarpaulin with nylon rope from the 4 3-m-high T-posts. One trap was equipped with a 20-W fluorescent black light and photo switch (BioQuip Products Inc., now defunct; Fig. 2A). The other trap was equipped with a 2-socket light fixture holding 2 120-W blue LED PAR38 flood lights (Epilux Commercial Lighting, Busan, Busan, Republic of Korea; Fig. 2B). These 2 lights were selected based on published information that kissing bugs are attracted to ultraviolet (UV) and blue wavelengths (Pacheco-Tucuch et al. 2012).

The fluorescent black light trap was deployed from 8 April–28 October 2019 at the Mission-South site, at a private residence near the Mexican border, and the blue LED flood light trap was deployed from 13 June—24 October at the Bastrop, TX site outside a research enclosure containing baboons, *Papio anubis*. Weekly visits were made to retrieve the contents of the collection cups, replenish the propylene glycol, remove cobwebs, and ensure that the light was working. Captured arthropods were stored at 4.0°C in propylene glycol or transferred to 70% ethanol until further processing. The contents of each collection were strained, and all arthropods were separated by order and counted. Honey bees were identified and

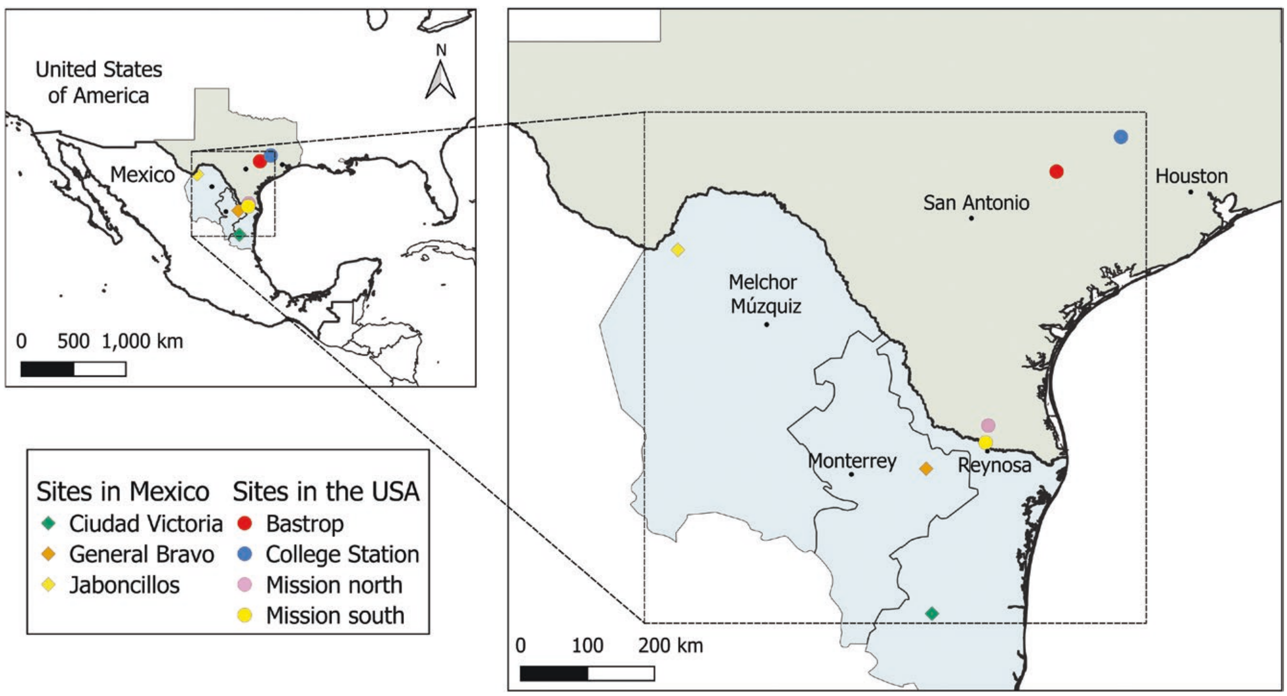

Fig. 1. Locations for kissing bug trap experiments, 2019–2021 in Texas, United States and Northern Mexico.

Fig. 2. Experimental vertical single panel traps with fluorescent blacklight A) and blue LED flood light B) tested in Texas, United States in 2019. Components include a transparent tarpaulin barrier panel, tarpaulin collecting funnel, lower aluminum funnel, white U-tube drainage tunnel, collection container and support T-posts with nylon ropes.

tabulated separately. Triatomines were then separated, identified into species (Lent and Wygodzinsky 1979) and counted.

2020: Evaluation of Modified Commercial Traps

Although the vertical panel traps developed and tested in 2019 caught flying adult triatomines, we judged them to be unsuitable for operational use because of the need for AC-powered lights, a large amount of labor required for construction and maintenance, and poor durability. Therefore, we designed a smaller version of the vertical panel trap and searched for commercial traps that could potentially be modified for catching kissing bugs. The only commercial traps that resembled the vertical panel embodied in the 2019 experimental traps were large cross-vane traps originally designed for catching wood-boring beetles and wood wasps (McIntosh et al. 2001, Morewood et al. 2002). Traps of a similar design had previously been used to catch kissing bugs (Pippin 1970, Updyke and Allan 2018). Another commercially available product was the multiple-funnel trap, designed to catch small ambrosia and bark beetles (Lindgren 1983), and which presents a narrow multifaceted silhouette very different from the tall and wide panels embodied in traps previously shown to be effective for kissing bugs (Sjogren and Ryckman 1966, Updyke and Allan 2018).

Three types of traps were experimentally tested in randomized complete blocks in the field in 2020. The first (Fig. 3A) was the scaled-down version of the 2019 vertical panel trap. A transparent tarpaulin (1.0 × 1.5 m) was suspended from 4 3-m-high T-posts embedded in the ground at an angle to create a teepee over a black polystyrene plastic funnel (243.8 × 121.9 × 0.09 cm) below which was a 22.7-L bucket with the bottom 2 cm filled with propylene glycol. The bucket was held in place with 3 cinder blocks. The second trap (Fig. 3B) was a commercial cross-vane trap (Synergy Semiochemicals Corp., Delta, BC, Canada). The trap had 4 20.3 × 61.0 cm upright vanes connected to a funnel leading to a collection cup with the bottom 4 cm filled with propylene glycol. The third trap was a 6-unit multiple-funnel trap (Synergy Semiochemicals Corp., Delta, BC, Canada; Fig. 3C), with a wet collection cup filled to 4 cm with propylene glycol. The cross-vane and multiple-funnel traps were suspended between 2 2.4-m-high upright metal T-posts.

Mounted at the top of each trap on a 45 × 25 cm rectangular 3.8 cm angle-iron frame attached to the support posts were 2 40-unit 25-W LED lights emitting white light and pointed in opposite directions, and a 35.0 × 24.0 cm 6V/8W solar panel connected to a 5V 8,000-mAh Li-ion battery with a built-in photo-activated on–off switch (Shenzhen Lovefindahome Lighting & Furnishing Company Ltd., Jiangmen, China). This apparatus eliminated the need for AC power and maintained continuous light intensity all night.

At a study site, trap locations were at least 15 m apart and placed to allow access to sunlight and visibility of flying insects. Trap sites and dates of deployment were: Mission-South (6 May–3 November 2020), Mission-North (7 May–3 November), and College Station (27 May–27 October). Captured arthropods were collected weekly and identified as above.

2021: Importance of the Light Stimulus

Based on the results of the 2020 trials, we adopted the multiple-funnel trap as the leading prototype and focused on evaluating lights with different wavelengths and intensities. Three treatments, all with multiple-funnel traps, were compared: 1) the same solar panel, battery, and large LED light assembly as tested in 2020 (Fig. 2C), 2) 2 40-LED lights (6,000 K temperature) connected to a 17.8 × 15.2 cm solar panel and a 3.2V 4,500-mAh battery (Shenzhen EMANER Lighting Co. Ltd., Shenzhen, China), and 3) no light, solar panel or battery. Four replicates were set up as randomized complete blocks as in 2020, with the 3 traps spaced ≥15 m apart. One replicate was at Mission-South (20 April–2 December), and the other 3 were at locations in Mexico, Jaboncillos, Coahuila (3 June–25 October), General Bravo, Nuevo Leon (31 May–5 November), and Ciudad Victoria, Tamaulipas (28 May–4 November). Traps were serviced weekly, and the arthropods were identified as above.

Fig. 3. Prototype traps deployed in Texas, United States comprising scaled-down vertical single panel trap A) tested in 2020, cross-vane trap B) tested in 2020, and multiple-funnel trap C) tested in 2020 and 2021. All traps have 2 identical large LED flood lights and solar panels with internal batteries mounted above.

Detection of *T. Cruzi* in Triatomines Preserved in Propylene Glycol

To evaluate the ability to detect *T. cruzi* DNA in triatomines captured and preserved in propylene glycol, we randomly tested approximately half of the specimens from the Mission-South location in 2020 using previously described PCR methods (Duffy et al. 2013). First, we soaked the triatomines in 50% bleach for 15 s to mitigate contamination, then rinsed them in sterile distilled water. Next, we dissected the triatomines and recorded bloodmeal scores from 1 to 5 (Curtis-Robles et al. 2018b) wherein: 1 = no blood, desiccated guts; 2 = no blood, guts visible; 3 = traces of blood in gut; 4 = blood present, but not much; 5 = large amount of blood. We then extracted DNA using the MagMax-96 DNA Multi-sample Extraction Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and amplified a 166-bp region of nuclear satellite DNA of *T. cruzi* (Piron et al. 2007, Duffy et al. 2013) using a Bio-Rad CFX96 thermocycler according to previously reported methods (Curtis-Robles et al. 2018a).

Statistical Analysis

For the 2020 and 2021, experiments, the total numbers of kissing bugs captured in each treatment were compared by Chi-square tests ($\alpha = 0.05$) against an equal catch for each treatment that would be expected if there were no difference in performance among the traps. Infection prevalence was calculated as the total number of infected triatomines divided by the total tested.

Results

2019: Evaluation of Experimental Upright Barrier Traps

The large vertical panel trap with a black light in Mission-South, Texas captured 122 *T. gerstaeckeri* and 3 *T. neotomae* from 15 April–7 October, with peaks in April, May–June, and July (Fig. 4). The identical trap with the blue LED floodlights near Bastrop, Texas captured 5 *Triatoma sanguisuga* from July to October (Fig. 4). These traps comprised relatively expensive materials, took over 2 h to install, and the plastic tarpaulin funnel degraded after several months in the sun and tore when exposed to strong wind. The container with glycol and insects resting on the ground was damaged by unknown wild animals at the Bastrop site.

2020: Evaluation of Modified Commercial Traps

Traps with solar-powered LED lights as the attractant in 2020 captured 91 triatomines, 67.0% ($n = 61$) of which were at Mission-South (Table 1). The large vertical panel traps captured the most triatomines followed by the multiple-funnel traps and the cross-vane traps, and the distribution of catches among all 3 traps differed significantly from equality ($\chi^2 = 8.00208$, df = 2, $P = 0.01829$). The multiple-funnel traps captured 2.6× more kissing bugs per trap-day than the cross-vane trap and approached the performance of the vertical panel traps in the total number of kissing bugs captured, number of kissing bugs per trap-day and number of kissing bugs per 1,000 other arthropods (Table 1).

When all supplementary materials were considered, the vertical panel trap was the most expensive ($211.30 USD), followed by the cross-vane trap ($138.32), and the multiple-funnel trap ($136.89; Table 2). Traps at Mission-North were exposed to strong winds from Hurricane Hanna on 25–27 July 2020, resulting in severe damage to the vertical panel traps, lesser damage to the cross-vane traps, and almost no damage to the multiple-funnel traps (Table 3). The labor required for set up and maintenance varied among the traps (Table 3). To assess the cost-effectiveness of each design we calculated the triatomines per day trap and divided per the trap cost * 1000. This resulted in the multiple-funnel as the highest (0.525), followed by

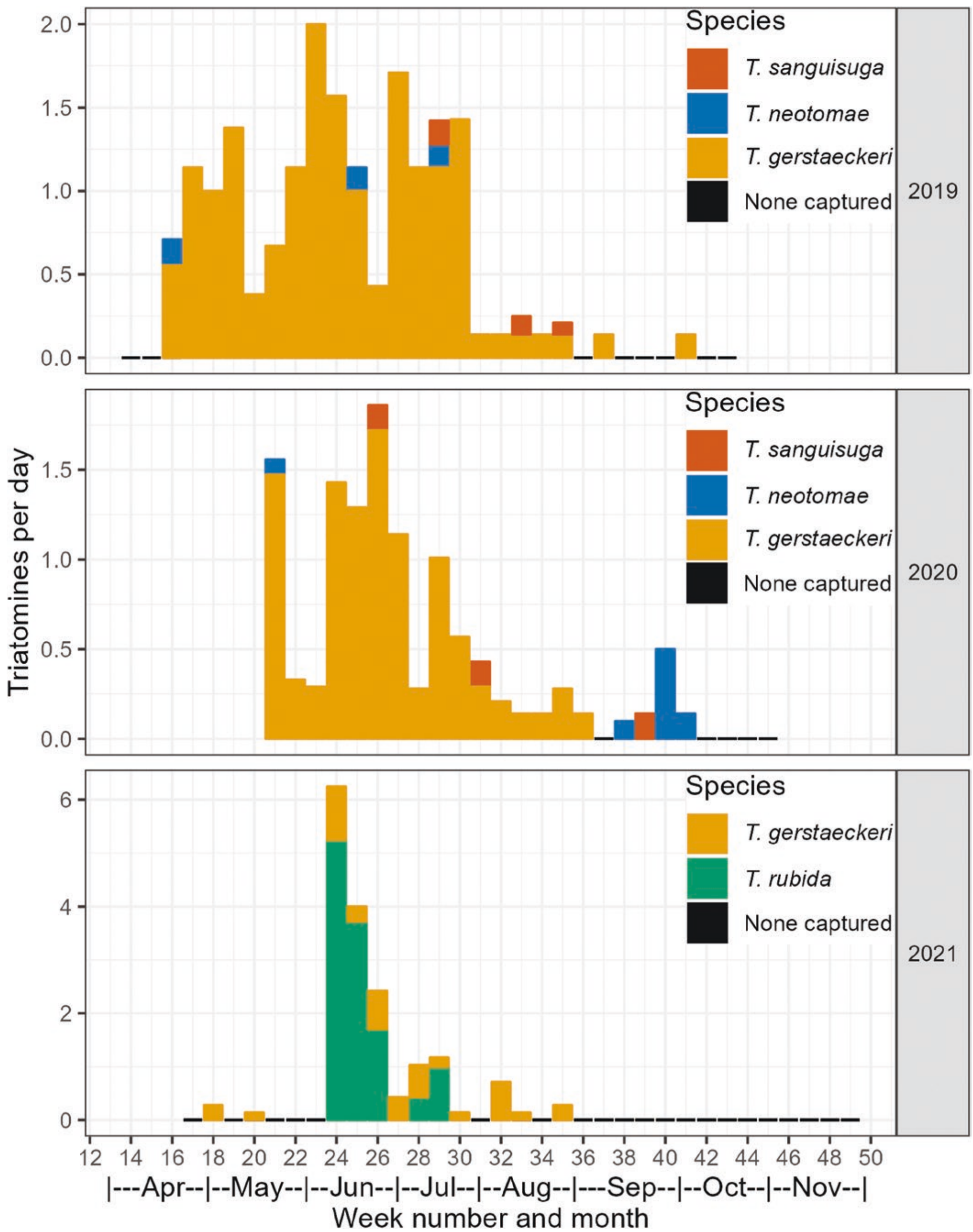

Fig. 4. Seasonal capture phenology (triatomines per trap per day) of 4 *Triatoma* spp. Pooled for all traps and locations in 2019 (South Texas and Bastrop, Texas), 2020 (South Texas and College Station, Texas), and 2021 (South Texas and Northern Mexico). None captured indicates that the traps were active but no triatomines were captured.

the vertical panel trap (0.459), and cross-vane trap (0.195; Table 3). While the large LEDs and solar panels had no technical difficulties, they required a mounting frame that took time to assemble and were heavy enough that it necessitated 2 supporting T-posts.

The traps in Mission-South captured 56 *T. gerstaeckeri*, and 5 *T. neotomae* from 18 May to 6 October (Fig. 4). Traps in Mission-North captured 27 *T. gerstaeckeri* from 26 May to 25 August (Fig. 4), while traps in College Station captured 3 *T. sanguisuga,* one each in June, July, and September (Fig. 4).

2021: Importance of the Light Stimulus

In 2021, multiple-funnel traps with large LED lights captured 6.3× more kissing bugs per trap day and 2.9× more kissing bugs per 1,000 other arthropods than traps with small LED lights; traps with no light captured 1 kissing bug (Table 4). The distribution of triatomines captured in the 3 types of traps was significantly different from equality ($\chi^2 = 64.4464$, df = 2, $P < 0.0001$). Although the total cost of traps with small LED lights was less than that of the traps with large LED lights, the reduction in cost (Table 2) was accompanied by an even larger reduction in trap catch (Table 4).

Traps in Jaboncillos, Coahuila, captured a fourth triatomine species, 69 *T. rubida* (Uhler), from 7 June to 12 July 2021, traps in General Bravo, Nuevo Leon captured 24 *T. gerstaeckeri* from 7 June to 23 August, and traps in Mission-South captured 11 *T. gerstaeckeri* from 27 April to 26 August (Fig. 3). While the onset of flight was as early as in 2019, most flights were over by September, and there was a single peak in July. No triatomines were captured in traps deployed in Ciudad Victoria, Tamaulipas.

Table 1. Comparative performance in 2020 in capturing triatomines by 3 types of experimental traps in Texas, United States

Criterion evaluated	Vertical single panel trap	Cross-vane trap	Multiple-funnel trap
Number of operational days			
Mission-South	180	180	180
Mission-North	79	180	180
College Station	153	153	153
Number of kissing bugs captured			
Mission-South	21	10	30
Mission-North	19	3	5
College Station	0	1	2
Total kissing bugs captured	40	14	37
Number of kissing bugs per trap day	0.097	0.027	0.072
Number of other arthropods captured	14,395	31,992	16,063
Number of kissing bugs per 1,000 other arthropods	2.78	0.44	2.30

Table 2. Compilation of experimental kissing bug traps tested in 2020 and 2021, with component and total costs in USD

	Component costs					
Year and trap description	Trap	Bucket and blocks	Light	Light bracket	Support posts	Total cost
2020: Vertical single-panel trap	$66.80	$14.50	$64	$10	$56	$211.30
2020: Cross-vane trap	$44.32		$64	$10	$20	$138.32
2020 and 2021: Multiple-funnel trap (large light)	$42.89		$64	$10	$20	$136.89
2021: Multiple-funnel trap (small light)	$42.89		$40	$5	$20	$107.89

Table 3. Summary of evaluation criteria for experimental vertical panel traps and commercial cross-vane and multiple-funnel traps as tools for capturing flying adult kissing bugs. Triatomines per day per cost based on 2020 data with collections in Texas, United States

Trap type	Durability	Set up and maintenance requirements	Triatomines per day trap per cost *1000
Vertical single-panel trap	Low	High, set up very labor-intensive, some traps destroyed in wind and not repairable	0.459
Cross-vane trap	Medium	Medium, setup requires some assembly, and some wind damage to panels, but repairable	0.195
Multiple-funnel trap	High	Low, set up easy, almost no maintenance required	0.525

Table 4. Comparative performance of multiple-funnel traps fitted with large or small LED lights or no light in Texas, United States, and Northern Mexico, 2021

Criterion evaluated	Traps with 2 large LED lights	Traps with 2 small LED lights	Traps with no lights
Number of operational days			
Mission-South, Texas	210	210	210
Jaboncillos, Mexico	144	144	144
General Bravo, Mexico	158	158	158
Ciudad Victoria, Mexico	160	160	160
Number of kissing bugs captured			
Mission-South, Texas	6	4	1
Jaboncillos, Mexico	62	7	0
General Bravo, Mexico	21	3	0
Ciudad Victoria, Mexico	0	0	0
Total kissing bugs captured	89	14	1
Number of kissing bugs per trap day	0.132	0.021	0.001
Number of other arthropods captured	23,595	10,855	1,897
Number of kissing bugs per 1,000 other arthropods	3.77	1.29	0.53

Other Arthropods (Bycatches)

Over the 3 yr, we collected 120,713 other arthropods (bycatch), with the highest bycatch per trap (>10,000) in large vertical panel traps in 2020 (Supplementary Table S1). Most abundant were Coleoptera (41.4% of the total), followed by Hymenoptera (non *Apis* spp.; 20.1%). We found a total of 111 honey bees, *Apis mellifera* (0.09%; Supplementary Table S1).

Detection of *T. Cruzi* in Triatomines Preserved in Propylene Glycol

From 19 female and 14 male *T. gerstaeckeri* triatomines from Mission-South in 2020, 13 females and 8 males tested positive for *T. cruzi*, for an overall infection rate of 63.6%. During the dissections, 19 specimens (58%) scored levels of 2 on the bloodmeal scale (no blood), 12 specimens (36%) scored bloodmeal levels of 3 (traces of blood), and 2 specimens (6%) scored bloodmeal levels of 4 (blood present). Furthermore, from the 19 females dissected: 5 (26.3%) were gravid, with 3 carrying 10+eggs, and the remaining 2 carrying 1 and 6 eggs.

Discussion

During 3 years of field trials, we evaluated 7 trap designs in TX and Mexico, captured 325 triatomines of 4 different species, and demonstrated the utility of a trap that autonomously captures, kills, and preserves insect vectors of *T. cruzi*. We demonstrated that a powerful light source is an important component of an effective trap used to capture dispersing adult triatomines (Table 4), and that using solar-powered LED lights can eliminate the need for an AC power source. Our results surprisingly indicated that a tall and wide vertical panel is not an essential trap component and that a narrow vertical column of funnels as in commercially available multiple-funnel traps can be similarly effective for triatomines as they have been for beetles (Table 1). When factors such as cost (Table 2), ease of set up, maintenance, and durability (Table 3) are added to trapping capability, we conclude that the multiple-funnel trap with a strong solar-powered LED light as the attractant is suitable for operational implementation as a surveillance tool for triatomine vectors of *T. cruzi*, and that it merits consideration for mass-trapping and reduction of kissing bug populations. A limitation of this design could be the price, particularly in low- and middle-income Latin American countries. However, the use of the Biogents Sentinel 2 trap (Biogents AG, Regensburg, Germany), which costs approximately $250 USD, for mosquito surveillance and mass trapping (Degener et al. 2014) suggests that trials of multiple-funnel traps for the control of triatomines are feasible. Moreover, as the multiple-funnel light trap improves and becomes a commercial product, the price could be reduced. Additionally, components of the trap, such as the supporting post, could be sourced locally or use existing support (e.g., a fence post).

The results of our 3-yr study reveal large differences in triatomine collections among trapping sites, e.g., 25×greater catches at Mission-South than Bastrop in 2019, 67% of all bugs captured in 2020 harvested at Mission-South, only 3 bugs captured at College Station in 2020 and zero in Ciudad Victoria in 2021. Spatio-temporal heterogeneity in dispersing adult triatomines is expected (Vazquez-Prokopec et al. 2012, Chico-Avelino et al. 2022), and is confirmed by this study using the same trap design deployed in multiple regions. The maximum number of triatomines captured per trap day was 5, which occurred in Jaboncillos, Coahuila, Mexico, between the last week of June and the first week of July 2021. In TX the number of triatomines per trap day ranged from zero to a maximum of 2, the latter of which occurred in Mission-South between the last week of May and the first of June 2019. For comparison, Updyke and Allan (2018) captured approximately 0.5 *R. pallescens* per trap night in cross-vane traps fitted with a fluorescent blacklight, and Leite Dias et al. (2011) captured approximately 0.005 per trap night with a similar design. Some research traps, e.g., the large panels with a fluorescent black light surrounded by a Malathion-treated gutter (Sjogren and Ryckman 1966), or the large vertical panel trap tested by us in 2019 (Fig. 2), may capture more kissing bugs but are not suitable for scaled-up operational use.

During this study, we collected 4 different triatomine species known to occur in the trap locations, but we did not collect other endemic species (*Paratriatoma lecticularia* [Stål] comb. nov. and *Triatoma protracta* [Uhler]; Curtis-Robles et al. 2018a). Furthermore, some species were found in high numbers while some were scarce (Fig. 4). This source of heterogeneity in capture success could be due to variation in population densities of the different triatomine species, which we know exists, but also to variation in attraction to lights while dispersing at night. Studies have identified blue (430 nanometers) as the most attractive wavelength of light for *T. dimidiata* (Latreille; Pacheco-Tucuch et al. 2012) and violet (470 nm) for *T. rubida* (Indacochea et al. 2017). The white lights used in this study emit a broad-spectrum range of wavelengths.

Some species considered important vectors of Chagas disease in Latin America, such as *Triatoma infestans* (Klug; Di Iorio and Gürtler 2017), *T. dimidiata* sensu lato (Pacheco-Tucuch et al. 2012), and *Rhodnius prolixus* (Stål; Erazo and Cordovez 2016), commonly infest houses and are known to be attracted to artificial lights. We captured triatomines that had apparently left sylvatic habitats and were approaching light traps placed next to buildings, suggesting that multiple-funnel traps could be used after residual insecticide treatment to intercept triatomines that could reinfest dwellings. Additionally, *T. infestans* dispersal away from a simulated domiciliary habitat following host removal was observed (Castillo-Neyra et al. 2015), suggesting that these traps could also capture dispersing adults leaving infested homes.

Our longitudinal data identified adult triatomine dispersal periods for 4 *Triatoma* spp. (Fig. 4), which are comparable to similar data obtained from community science submissions (Curtis-Robles et al. 2018b) and active surveillance accomplished by persons collecting bugs after they landed on structures such as illuminated walls of buildings (Fimbres-Macias et al. 2023). Little is known about variation in adult triatomine dispersal over the years and what factors regulate this process. Our results suggest that a standardized trap, such as the multiple-funnel trap and LED light, as explored in this study, could be deployed over large spatial scales and during multiple years to define spatio-temporal variation in the occurrence and abundance of dispersing triatomines.

There was little apparent difference caused by trap design or location within years, but there was pronounced temporal variation due to unknown causes among all species between years (Fig. 4). For example, the number of flying adult triatomines captured in multiple-funnel traps was 80% lower in 2021 than in 2020 at the same Mission-South location (Tables 1 and 4). We suggest that Winter Storm Uri, which brought unprecedented extended freezing temperatures to the southern United States from 13 to 17 February 2021, could have killed many overwintering triatomines, as it is hypothesized to have killed large numbers of bats (McSweeny and Brooks 2022) and fish (National Weather Service 2021) and some insect pests (Russell 2021). While we visited traps weekly, more frequent visitation could enable the investigation of fine-scale abiotic factors. Daily visitation would be needed to explore the influence of temperature, humidity, moon phase, wind speed, and wind direction on flight dispersal (Vazquez-Prokopec et al. 2004, Fimbres-Macias et al. 2023). If the collection cups were used dry, frequent visitation could allow for the collection of living triatomines for research or establishing laboratory colonies.

We propose that multiple-funnel traps could be used to provide a barrier and intercept triatomines as they approach residences (Waleckx et al. 2018) and could also be used to reduce kissing bug populations, as suggested by Sjogren and Ryckman (1966). Mass-trapping in limited peridomestic areas that are much smaller than vast sylvatic habitats is a management tactic that would specifically target non-domiciliated vectors of Chagas disease (Barbu et al. 2011). If implemented, a mass-trapping tactic for triatomines would be similar to the approach implemented in 1982 for ambrosia beetle pests that infest similarly limited areas where logs and lumber are stored on the west coast of Canada. This approach, which uses semiochemical-baited traps (Lindgren and Borden 1983), now constitutes the major component of the world's longest-running integrated pest management program (Borden and Stokkink 2021). If triatomine semiochemicals (Bohman et al. 2018, Lazzari 2021) could be developed as attractive trap lures, they could potentially be used to supplement attractive light stimuli to increase the efficacy of multiple-funnel traps. While previous light traps for triatomines baited with host odors did not yield improved captures based on one study in Panama (Updyke and Allan 2018), more research is warranted to confirm the utility of host odors given that many dispersing triatomines are starved.

In addition to employing traps in large-scale surveillance, each captured bug has the potential to offer information on vector biology and control. Because propylene glycol is a safe food-grade preservative (Stenn et al. 2019) there is little danger of traps harming humans and domestic animals. We confirmed that *T. cruzi* DNA could be detected in specimens captured and preserved in propylene glycol at Mission-South in 2020, and prevalence estimates were within the values of previous analyses of specimens collected at the same location from 2013 to 2020 (Curtis-Robles et al. 2015). None of the triatomines captured in our traps appeared fully engorged, which is consistent with reports suggesting triatomines metabolize most of the ingested blood prior to dispersal flight (Abrahan et al. 2011). We found 42% of the dissected triatomines to have a bloodmeal score ≥3, implying the presence of trace amounts of blood which should be sufficient for identifying the most recent vertebrate host species (Curtis-Robles et al. 2018c), either by conventional molecular techniques or by rapid DNA barcoding (Ondrejicka et al. 2017, Lumsden et al. 2021).

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Author contributions

Gabriel Hamer (Conceptualization [lead], Funding acquisition [lead], Investigation [lead], Methodology [lead], Writing—original draft [equal]), Juan Fimbres-Macias (Data curation [lead], Formal analysis [lead], Investigation [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Jose Juarez Valdez (Investigation [equal], Writing—review & editing [equal]), Christopher Downs (Methodology [equal], Writing—review & editing [equal]), Ester Carbajal (Investigation [equal], Writing—review & editing [equal]), Melinda Melo (Investigation [equal], Writing—review & editing [equal]), Danya Garza (Investigation [equal], Writing—review & editing [equal]), Keswick Killets (Investigation [equal], Writing—review & editing [equal]), Gregory Wilkerson (Investigation [equal], Writing—review & editing [equal]), Rogelio Carrera-Treviño (Investigation [equal], Writing—review & editing [equal]), Enrique Corona-Barrera (Investigation [equal], Writing—review & editing [equal]), Arturo Arabied Tello-Campa (Investigation [equal], Writing—review & editing [equal]), Martha Rocío Rojas-Mesta (Investigation [equal], Writing—review & editing [equal]), John Borden (Conceptualization [equal], Funding acquisition [equal], Methodology [equal], Writing—review & editing [equal]), Michael Banfield (Conceptualization [equal], Funding acquisition [equal], Methodology [equal], Writing—review & editing [equal]), and Sarah Hamer (Conceptualization [equal], Funding acquisition [equal], Methodology [equal], Writing—review & editing [equal])

Supplementary data

Supplementary data are available at *Journal of Medical Entomology* online.

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Data Availability

Data are on file with the corresponding author and are available on request.

Conflict of Interest

The researchers declare that the prototype trap developed during this study is associated with a provisional patent application filed on behalf of GLH, MGB, and JHB and assigned to their respective institutions with an assignment to the US government.

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