A Simple Non-Powered Passive Trap for the Collection of Mosquitoes for Arbovirus Surveillance

Author(s): Scott A. Ritchie , Giles Cortis , Christopher Paton , Michael Townsend , Donald Shroyer , Paul Zborowski , Sonja Hall-Mendelin , and Andrew F. Van Den Hurk

Source: Journal of Medical Entomology, 50(1):185-194. 2013.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/ME12112

A Simple Non-Powered Passive Trap for the Collection of Mosquitoes for Arbovirus Surveillance

SCOTT A. RITCHIE,[1,2,3] GILES CORTIS,[4] CHRISTOPHER PATON,[1] MICHAEL TOWNSEND,[1] DONALD SHROYER,[5] PAUL ZBOROWSKI,[1] SONJA HALL-MENDELIN,[6] AND ANDREW F. VAN DEN HURK[6]

J. Med. Entomol. 50(1): 185–194 (2013); DOI: http://dx.doi.org/10.1603/ME12112

ABSTRACT Mosquitoes often are collected as part of an arbovirus surveillance program. However, trapping and processing of mosquitoes for arbovirus detection is often costly and difficult in remote areas. Most traps, such as the gold standard Center for Disease control light trap, require batteries that must be charged and changed overnight. To overcome this issue we have developed several passive traps for collection of mosquitoes that have no power requirements. The passive traps capture mosquitoes as they follow a CO_2 plume up a polyvinyl chloride pipe leading to a clear chamber consisting of a plastic crate. We believe the translucent, clear windows created by the crate inhibits escape. Once inside the crate mosquitoes readily feed on honey-treated Flinders Technology Associates cards that then can be processed by polymerase chain reaction for viral ribonucleic acid. Of the two designs tested, the box or crate-based passive trap (passive box trap, PBT) generally caught more mosquitoes than the cylinder trap. In Latin square field trials in Cairns and Florida, PBTs collected mosquitoes at rates of 50 to 200% of Center for Disease Control model 512 light traps. Mosquito collections by PBTs can be increased by splitting the CO_2 gas line so it services two traps, or by placing an octenol lure to the outside of the box. Very large collections can lead to crowding at honey-treated cards, reducing feeding rates. Addition of fipronil to the honey killed mosquitoes and did not impact feeding rates nor the ability to detect Kunjin viral ribonucleic acid by polymerase chain reaction; this could be used to minimize crowding affects on feeding caused by large collections. The passive traps we developed are made from inexpensive, commonly available materials. Passive traps may thus be suitable for collection of mosquitoes and potentially other hematophagous dipterans for pathogen surveillance.

KEY WORDS mosquito, surveillance, arbovirus, light trap, encephalitis

The emergence of arboviruses, such as Schmallenberg virus purportedly carried by *Culicoides* midges (Śmietanka et al. 2012), and the range expansion of viruses such as West Nile virus (Kramer et al. 2008), Japanese encephalitis virus (JEV) (van den Hurk et al. 2009), and blue tongue virus (Wilson and Mellor 2009) emphasize the need for effective and efficient arbovirus surveillance systems. Hematophagous flies frequently are collected in CO_2-baited traps, sorted by species, pooled, and sent to an appropriately equipped laboratory for virus detection. However, this can be a laborious and logistically difficult process. For instance, a cold chain requiring specimens be kept at −70°C is essential to maintain viral integrity during transport (Ritchie et al. 2003). To facilitate routine detection of virus in remote areas where maintaining a cold chain is difficult, reverse transcriptase polymerase chain reaction (PCR) assays (RT-PCR) can be used to detect viruses in mosquitoes that have been stored and transported at ambient temperature (Ritchie et al. 2003). During a field trial in remote northern Australia, mosquitoes were collected in stand-alone propane or solar-powered traps baited with CO_2 and forwarded via surface mail to a diagnostic laboratory for analysis by using real-time RT-PCR (Ritchie et al. 2007).

Because only a small percentage of mosquitoes are infected, large numbers must be collected and processed to detect virus. For instance, during the trial in northern Australia, it was estimated that over 47,000 mosquitoes would have to be processed to obtain one JEV positive pool (Ritchie et al. 2007). To avoid the need to process thousands of mosquitoes, we developed and validated a battery-powered updraft trap that houses honey-baited nucleic acid preservation

[1] School of Public Health, Tropical Medicine and Rehabilitative Sciences, James Cook University, Cairns, Queensland 4870, Australia.
[2] Tropical Regional Services, Cairns Population Health Unit, Queensland Health, Cairns, Queensland 4870, Australia.
[3] Corresponding author, e-mail: scott.ritchie@icu.edu.au.
[4] Private Contracting Engineer.
[5] Indian River Mosquito Control District, 5655 41st St., Vero Beach, FL 32967.
[6] Public Health Virology, Queensland Health Forensic and Scientific Services, Coopers Plains, Queensland 4108, Australia.

cards (Hall-Mendelin et al. 2010). Mosquitoes attracted by CO_2 are drawn into the trap by a motorized fan, where they feed and expectorate virus on the honey soaked Flinders Technology Associates (FTA) filter paper cards (Whatman International, Maidstone, United Kingdom) used for preservation of nucleic acid. The cards then are submitted for virus detection by using real-time RT-PCR. This system subsequently yielded numerous detections of Ross River (RRV) and Barmah Forest viruses during field surveillance of wild mosquito populations in Cairns and Bunbury, Australia (Hall-Mendelin et al. 2010).

The updraft trap outlined above, like many other CO_2-baited traps (reviewed in Silver [2007]), relies on electricity to power a fan motor that draws attracted mosquitoes into a collection or holding container. In these traps, electricity can be provided by mains power, batteries, solar power, or by heat generated by the combustion of propane. Unfortunately, access to any of these forms of electricity in the field can be difficult. Furthermore, long-term deployment under field conditions can damage trap components, preventing them from operating.

In the current paper we describe the development and field trial of a nonmotorized, CO_2-baited passive trap, the passive box trap (PBT), which collects mosquitoes and provides them access to the honey-baited nucleic acid preservation cards. The trap design is simple, does not contain a fan (or any other suction device), and thus does not require electricity to function. The design is based upon the strategy used in the development of the glass McPhail fly trap and derivative plastic versions (Multilure trap, and others) that use kairomone baits to lure fruit flies into an opening in the bottom of a container (Thomas et al. 2001, Díaz-Fleischer et al. 2009). The top section of the container is clear, and the trapped insects unsuccessfully attempt to escape by flying through the clear glass or plastic trap top. Although a clear passive trap previously had been developed for collection of mosquitoes, such as the Plexiglas trap of Schreck et al. (1970), it consists of a complex arrangement of wire mesh cones housed in a Plexiglas box that do not rely on the escape behavior exploited by the McPhail trap.

To further optimize the deployment of the PBT in the field, we examined various combinations of attractants and methods of CO_2 delivery. For instance, 1-octen-3-ol (octenol) when added to carbon dioxide (CO_2), significantly increases collections of many mosquitoes (Kline 2006, 2007), including Australian vectors of RRV (Kemme et al. 1993) and Japanese encephalitis (van den Hurk et al. 2006), as well as other biting flies such as *Culicoides* (Ritchie et al. 1994) and *Phlebotomus* (Beavers et al. 2004). The trapping system we describe herein has the potential to transform the way that arboviruses within mosquito populations are monitored, especially in areas where access to a source of electricity can be difficult.

Materials and Methods

Optimizing the Passive Trap

Study Sites. All Australian trials were conducted at the Smithfield Waste Disposal Facility (Smithfield tip) near Cairns, Australia. The site contains a fence line adjacent to a mixed melaleuca and mangrove swamp that has been the site of several trapping trials (van den Hurk et al. 1997, Johansen et al. 2003, Ritchie et al. 2008). The Florida site, Graves Swamp, is a cypress–oak–cabbage palm depression swamp located in Indian River County. This isolated site has dense herbaceous ground cover and is surrounded by citrus groves.

Trap Design and Field Assessment

Screening for Successful Passive Trap Designs. Four different passive traps were developed and tested at Smithfield: 1) short cylinder, 2) tall cylinder, 3) small box, and 4) large box. All traps were constructed from materials that could be sourced from most hardware stores. The descriptions of the traps, including their design and components are provided in Fig. 1. The Centers for Disease Control model 512 light trap (John W. Hock, Gainesville, FL) was used as the "control" trap in all field trials.

Carbon dioxide from 1 kg of dry ice was released from an insulated cooler via a tube extending from the top of the cooler into the passive trap interior. With the exception of the tall cylinder, the gas was released just inside the top of the bottom PVC entry pipe. The CO_2 release point for CDC traps was just above the center of the trap lid as per Ritchie et al. (2008).

The honey-baited cards used for the trials were either FTA cards, which contain proprietary chemicals that assist in binding nucleic acids and inactivate viruses, or, for optimization trials, filter paper (Bio-Rad, Hercules, CA). Previous laboratory experiments had demonstrated that there is no significant difference between the FTA cards and filter paper for the detection of arboviruses (Hall-Mendelin et al. 2010). A mixture of honey and blue food dye was added to FTA cards or filter paper as described by (Hall-Mendelin et al. 2010). Six honey-soaked 2.5- by 2.5-cm FTA cards or filter paper squares were attached to the inner walls of the passive traps.

Traps were set ≈30 m apart and deployed between 1600 and 0800 hours. Upon retrieval, mosquitoes were identified using a stereo microscope, and counted by species or taxonomic group. The presence of blue food dye within the mosquito body was indicative of feeding on the honey-soaked cards. For analysis, the mean number of mosquitoes was compared using an analysis of variance (ANOVA) on log $(x + 1)$ data by using ezANOVA software. A Tukey's HSD test was used to compare means.

Two 3 by 3 Latin square trials were used to compare the large and small passive box traps to the model 512 CDC light trap in August 2011 near Vero Beach, FL. Traps were baited with 1 kg of dry ice and set using a

Trap components	 Short cylinder	 Tall cylinder	 Small box	 Large box
Body	Cylinder of rolled clear polycarbonate plastic sheeting 250 mm long, 230 mm diameter, held by bungy cord attached to opposing ends of 165 mm diameter PVC spigot with 230 mm flange	Cylinder of rolled clear polycarbonate plastic sheeting, 500 cm long, 150 mm diameter, pop riveted along seem and fitted into open ends of ends of 150 mm PVC spigot. Tension from a stretched bungy cord attached to opposing ends of the PVC spigot held the sheeting in place.	8.5 L clear plastic storage container, 260 x 260 x 260 mm with pop off lid.	20 L storage box 290 x 370 mm, 270 mm deep, with clip on lid
Entry bowl and neck	Bowl: 200 mm diameter drain, waste and vent PVC pipe fitting; Neck is 150 mm PVC pipe extending 70 mm into trap body	Bowl: 200 mm diameter drain, waste and vent PVC pipe fitting; Neck: 110 mm PVC pipe extending 70 mm into trap body	Bowl: Clear plastic storage container, 20 x 20 x 10 cm, attached to 100 mm PVC pipe. Neck: 100 mm PVC pipe extending 70 mm into trap body.	Bowl: Clear plastic storage container, 20 x 20 x 10 cm, attached to 100 mm PVC pipe. Neck: 100 mm PVC pipe extending 70 mm into trap body.
CO_2 feed	Plastic tubing with elbow joint that directs tube into PVC entry pipe	Plastic tubing that feeds directly into top of cylinder	Plastic tubing with elbow joint that directs tube into PVC entry pipe	Plastic tubing with elbow joint that directs tube into PVC entry pipe
Ventilation	Top of PVC pipe open but screened	Top of PVC pipe open but screened	Screened 90 cm hole on top lid.	90 mm PVC elbow with screen set on box side
Lid	510 mm diameter black plastic potplant base	510 mm diameter black plastic potplant base	510 mm diameter black plastic potplant base	510 mm diameter black plastic potplant base

Fig. 1. Components of cylinder and box passive traps used in screening trial trials conducted from February to March 2011 at Smithfield, northern Australia.

similar protocol as in Australia. Honey-treated No. 3 Whatman filter paper (GE Healthcare, Waukesha, WI) was used to examine feeding rates in the passive traps. Statistical analysis of mean mosquito collections was conducted as before.

Optimization of the Large Passive Box Trap. Initial trials indicated that the large PBT was the most successful passive trap, collecting significantly more mosquitoes than both cylinder traps, and the CDC trap. Nonetheless, several modifications were made to the design of this trap with aim of improving the design: 1) the clear plastic inlet bowl was fragile and was replaced by a hard PVC spigot with neck diameter of 100 mm and bowl diameter of 170 mm; 2) the PVC entry neck into the box bottom was too long and shortened from 75 mm to 45 mm; and 3) the right angle ventilation pipe at the side of the box prevented stacking of boxes, so it was replaced with a 90-cm-diameter screen on top of the box. Two 5 by 5 Latin square trials were conducted at the Smithfield tip to compare these three modifications to the original large box trap and the CDC light trap. The initial trial took place along the exposed fence line at the tip, the second within a heavily wooded section of an adjacent swamp.

Use of Octenol to Increase Mosquito Collections. To examine whether octenol increases mosquito collections in the large PBT, 7-ml plastic vials were filled with octenol, and fitted with a lid and wick to produce a release rate of ≈5 mg/h (Van Essen et al. 1994). The vial was fixed just above the end of the CO_2 hose inside the entry pipe into the box (internal release) or attached to the clip connecting the trap to the pot-plant base lid (external release). Mean ($\log X + 1$) mosquito collections from traps with internal and external octenol release and an untreated control were compared by ANOVA.

Splitting the CO_2 Line to Maximize Collections. Split line CO_2 delivery from a single CO_2 gas cylinder previously has been shown to increase collections of mosquitoes by 50% in updraft CDC traps set in Cairns (Ritchie et al. 2008). Therefore, we assessed whether a split CO_2 line, a single CO_2 gas cylinder that feeds gas into two separate lines, could be used to bait two separate large box passive traps, thus increasing trap collections. A t-splitter was used to divide the CO_2 hose into two lines that fed two large box passive traps set 20 m apart. We compared trap collections from two traps that received 250 ml/min CO_2 from a split line to collections from a single trap baited with CO_2 gas at 500 ml/min that was set ≈40 m away. The position of the traps was changed daily in a 2 by 2 Latin square design. The trial was replicated three times for six trap nights in total. The mean log ($x + 1$) of total number of mosquitoes collected by both traps was compared with the single trap receiving 500 ml/min CO_2 by using a paired *t*-test.

Use of a Toxic Honey Bait to Kill Mosquitoes in the Trap. The Florida results indicated that large numbers of mosquitoes crowded on and around the honey-soaked filter paper inhibiting access and feeding by other mosquitoes. If mosquitoes that fed on the honey-soaked cards were exposed to an insecticide and subsequently died, then this could allow more space for other mosquitoes to feed. Sugar baits treated with the phenylpyrazol fipronil previously have been shown to have high efficacy against mosquitoes (Allan 2011). Therefore, to examine the effects on mosquitoes collected in the large PBT, liquid fipronil (Termidor, 10% active ingredient) was mixed with honey and blue food dye to create a solution containing 0.06% active ingredient. FTA cards were treated with the fipronil honey mix as described earlier. Large box passive traps

Table 1. Mean and SD of mosquitoes collected by passive traps and CDC model 512 light trap during initial screening trial ($n = 10$) in Smithfield, Queensland 16 March–1 April 2011

Species	CDC light trap		Short cylinder		Large box		Small box		Tall cylinder	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aedes alboannulatus	0.1	0.3	0.3	0.7	0.2	0.4	0.6	1.0	0.4	0.7
Ae. alternans	0.3	0.9	0	0	0.1	0.3	0	0	0	0
Ae. kochi	29.8A	15.2	9.7B	5.2	51.3C	24.3A	21.9A	12.6	22.5A	13.9
Ae. notoscriptus	0.6	0.6	0.3	0.5	0.7	0.9	0.9	1.0	0.8	0.8
Anopheles farauti sensu lato	5.1	5.1	3.7	2.9	3.9	2.5	1.7	1.4	11.0	4.5
Coquillettidia spp. nr *crassipes*	3.7	3.3	3.9	3.3	5.9	5.5	2.1	2.2	1.6	1.7
Culex annulirostris	37.1A	26.4	16.1B	7.9	107.9C	53	28.6AB	21.4	23.9AB	19.0
Cx. gelidus	12.4A	10.1	0.7B	1.1	1.6B	2.3	0.6B	1.0	17.0A	20.8
Cx. hilli	0.4	1.0	0.1	0.3	0.2	0.4	0.3	0.9	0	0
Cx. pullus	1.0	1.1	1.5	1.8	5.4	5.1	1.2	1.5	2.9	3
Mansonia septempunctata	0.6	1.3	0.2	0.4	0.4	0.5	0.2	0.4	0.4	0.7
Man. uniformis	1.1	1.6	1.2	1.1	1.6	1.9	0.3	0.5	0.6	0.5
Verrallina carmenti	3.4	6.8	0.3	0.5	3.3	4.0	3.2	5.5	0.8	1.2
Ver. lineata	3.2	7.6	0.7	1.3	1.2	2.1	1.5	3.1	0.2	0.4
Total mosquitoes	98.8A	55.3	38.7B	14.7	183.7C	81.5	63.1A	41.6	82.1A	50.8
Total species	14		13		14		13		12	

Traps were baited with 1-kg dry ice and set overnight.
Means followed by a different letter are significantly different ($P < 0.05$) by Tukey's HSD.

had either six fipronil treated or six untreated FTA cards, and data on the total number of mosquitoes captured and the percentage killed in each trap (arcsine transformed) was compared by a paired *t*-test.

Impact of Fipronil-Laced Honey on Detection of RRV and KUNV From FTA Cards. Two viruses were examined to determine if the addition of fipronil affected the detectability of flaviviruses and alphaviruses on the honey-baited FTA cards. Kunjin virus (KUNV; strain MRM16), isolated from Kowanyama, northern Australia in 1960, previously had been passaged in suckling mouse brain before a final passage C6/36 [*Aedes albopictus* (Skuse)] cells. Ross River virus was isolated in 2008 from Cairns and had been passaged twice in C6/36 cells. FTA cards were prepared by soaking them with honey or honey plus fipronil and cutting them in half to produce a 15- by 15-mm square. Untreated FTA cards were used as additional controls. Twenty-five microliters of 10^4 Vero cell tissue culture infectious $dose_{50}$ ($TCID_{50}$) of KUNV or RRV was inoculated onto the center of the card. The FTA cards were air dried for 2 hr before being stored overnight at 23°C.

To elute viral RNA, FTA cards were cut into strips, placed in 5-ml vials and 1 ml of sterile H_2O added. The vials were placed on ice and vortexed for 10 s every 5 min for 20 min in total. Strips then were removed from each vial and placed into a 3-ml syringe. The syringe plunger was used to squeeze the liquid back into the original vial. Viral RNA was extracted from 140 μl of the eluate by using the Bio Robot Universal System (Qiagen, Hilden, Germany) and the QIAamp Virus BioRobot MDx Kit (Qiagen, Clifton Hill, Australia) according to the manufacturer's instructions. Viral RNA was detected using KUNV and RRV specific real-time TaqMan reverse transcriptase PCR assays (Pyke et al. 2004, Hall et al. 2011). The mean cycle threshold (Ct) score for either KUNV or RRV and the untreated controls was compared by ANOVA.

Results

Australian Screening Trial. The large PBT consistently collected more mosquitoes than the other passive trap designs (Table 1). It caught significantly more mosquitoes than both cylinder traps, the small PBT, and even the CDC light trap. Both cylinder traps caught similar amounts of mosquitoes, suggesting that diameter of the entry bowl and trap cylinder did not impact collection of mosquitoes. The majority of collected mosquitoes had fed on the honey-treated FTA card. Of the total of 2,495 mosquitoes collected in the first trial, 2,058 (82.5%) had fed on the cards. In the second trial, 95.6% of 1,131 mosquitoes collected had blue dye and had fed on the honey-soaked cards.

Results of the Screening Trial—Florida. Although both small and large PBTs collected large numbers of mosquitoes, especially *Culex nigripalpus* Theobald, total collections generally were lower than that for CDC light traps (Table 2). Percent of mosquitoes with blue food dye was 42 and 50% for the small and large box passive traps, respectively. Feeding was negatively correlated with catch size, with a correlation of total mosquito collection with percent honey feeding of −0.71 and −0.78 for small and large box passive traps, respectively. This indicates that large collections create crowding at the honey-treated filter paper, impeding feeding (Fig. 2).

Optimizing the Passive Trap Design

Assessment of Modifications to the Large Box Passive Trap. Modifications to the large PBT did not separately increase mosquito collections significantly. However, collectively the new version of the large PBT incorporating a top ventilation port and a PVC spigot entry bowl (Fig. 3) consistently collected the highest numbers of mosquitoes (Tables 3 and 4). Interestingly, CDC collections were relatively higher in

Table 2. Mean (SD) number of mosquitoes captured using CDC light traps, small PBTs, and large PBTs set near Vero Beach, Florida ($n = 6$)

Species	CDC		Small passive		Large passive	
	Mean	SD	Mean	SD	Mean	SD
Aedes albopictus	0	0	0.2	0.4	1.2	2.4
Ae. infirmatus	339.8A	532.1	105.8A	218.0	144.3A	257.9
Ae. taeniorhynchus	8.3	6.4	0.2	0.4	0.2	0.4
Ae. triseriatus	1.5	3.7	0	0	0	0
Ae. vexans	16.3A	17.6	0B	0	0B	0
Anopheles crucians	21.2A	16.6	0B	0	0B	0
Culex coronator	22.0A	26.5	9.4A	3.0	12.4A	10.5
Cx. erraticus	11.8A	10.9	4.3A	5.4	17.7A	30.8
Cx. nigripalpus	5,809.0A	2,436.7	2,734.2A	2,417.2	3,003.3A	2,520.3
Mansonia dyari	1.5	3.7	0	0	0	0
Ma. titilans	4.7	11.4	1.0	2.4	0	0
Psorophora ciliata	0.7	1.6	0	0	0.2	0.4
Ps. columbiae	32.3A	45.0	6.3A	8.2	9.5A	15.2
Ps. ferox	37.5A	54.7	4.8A	5.3	16.0A	22.3
Ps. howardii	1.2	1.8	0	0	0	0
Uranotaenia lowii	6.0	14.7	0	0	0	0
Ur. sapphirina	54.5A	60.1	0B	0	0B	0
Wyeomyia mitchellii	1.5	3.7	0	0	0	0
Total mosquitoes	6,369.8A	2,843.4	2,864.7B	2,622.9	3,202.7AB	2,816.6

Means followed by a different letter are significantly different ($P < 0.05$) by Tukey's HSD.

sheltered wooded sites but considerably lower (≈0.50) along the exposed fence line. This is also reflected in the results from Florida where the CDC Trap Index (ratio of total mosquitoes caught by the PBT/CDC light trap) (Ritchie et al. 2008) was ≈0.50 for trials conducted in a protected, heavily wooded site (Fig. 4). Conversely, the CDC Trap Index for the large box trap in all trials conducted at the exposed fence line were >1.8. Feeding rates on the honey-treated filter paper cards were high (generally >90%) for all passive trap combinations.

Use of Octenol to Increase Mosquito Collections. Octenol significantly increased collections of mosquitoes in the large PBT (Fig. 5). Placement of the octenol lure outside the box also significantly increased mosquito collections over traps baited with the lure inside the box ($P < 0.05$, Tukey's HSD).

Splitting CO_2 Line to Maximize Collections. Two large PBTs each fed by 250 ml/min CO_2 gas collectively collected a significantly greater number of mosquitoes than a single PBT baited with 500 ml/min of CO_2. The mean (± SD) number of mosquitoes col-

Fig. 2. Large numbers of mosquitoes, primarily *C. nigripalpus*, feeding on honey-treated filter paper within a passive trap set near Vero Beach, FL. Crowding of large numbers of mosquitoes can reduce feeding rates on treated paper cards. (Online figure in color.)

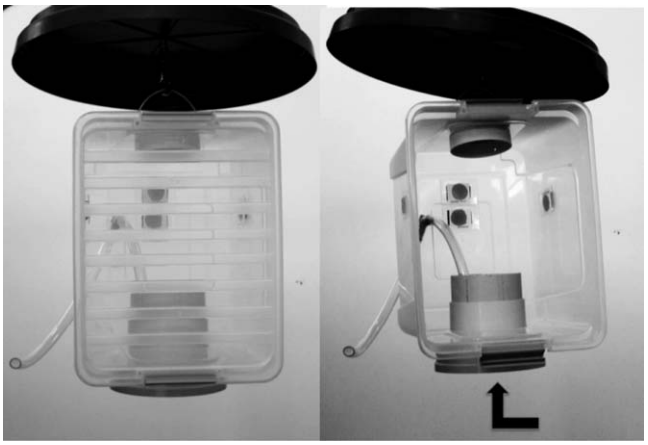

Fig. 3. Standard large PBT currently used, with crate lid removed to show trap elements (right): pot-plant base lid, PVC ventilation pipe, CO_2 hose, PVC entry pipe, and spigot. The entry point of mosquitoes into the PBT is depicted by the arrow; honey-treated FTA cards can be seen on walls of box.

lected by each trap baited with the split CO_2 line was 275 ± 88 and 334 ± 89, for a collective total of 609 ± 168. The single PBT baited with 500 ml/min of CO_2 caught a mean of 315 ± 124 mosquitoes, a significantly lower total than the collective paired traps (*t*-test, $n = 6$; $P = 0.006$).

Addition of Fipronil to Honey-Soaked FTA Cards to Kill Mosquitoes in Passive Box Traps. The addition of fipronil-laced honey to FTA cards had no significant impact on mosquito collections in the large PBT (*t*-test, $P = 0.82$) but did kill significantly more mosquitoes than traps with untreated cards (*t*-test, $n = 10$, $P = 0.0004$) (Fig. 6). The mean percent of mosquitoes containing blue dye as evidence of feeding on the FTA card was comparable (*t*-test, $P = 0.06$) in the untreated control (89.5%) than the fipronil laced honey (80.9%). The mean percent mortality of mosquitoes in traps with fipronil-laced honey versus untreated honey FTA cards was 94 and 5%, respectively.

Impact of Fipronil-Laced Honey on Detection of RRV and KUNV From FTA Cards. Fipronil-laced honey had no deleterious impact on detection of RRV and KUNV from FTA cards. The mean ± SD Ct score for FTA cards left untreated and treated with honey

Table 3. Mean (SD) number of mosquitoes captured using modified versions of the large PBT at exposed fence line at Smithfield rubbish tip ($n = 5$)

Species	CDC light trap		Original large box with side vent		Top vent, long neck		Top vent, short neck		Top vent, short neck PVC bowl	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aedes kochi	5.4A	3.4	27.6B	18.8	41.8BC	49.5	48.8BC	30.4	63.8C	43.5
Ae. lineatopennis	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4
Ae. notoscriptus	1.2A	1.1	10.8B	7.5	14.2B	11.8	13.6B	6.7	13B	8.2
Ae. tremulus	0.2	0.4	1.4	1.7	4.6	3.0	1.8	2.0	4.0	2.7
Ae. vigilax	14.4A	18.4	9.2A	7.9	25AB	24.4	19.6AB	13.4	41.4B	24.1
Anopheles farauti	20.2AB	34.5	6.4B	5.3	15.6AB	7.4	15.2AB	7.5	30.4A	17.7
Coquillettidia spp. nr *crassipes*	0	0	0.8	0.4	0	0	0.6	0.9	0.6	0.9
Culex annulirostris	5.4A	4.0	24.4B	15.2	16.2B	6.9	26.4B	18.8	23.2B	16.2
Cx. hilli	0.4	0.5	0.4	0.5	0.4	0.9	0.4	0.9	0.2	0.4
Cx. pullus	0	0	0	0	0	0	0	0	0	0
Cx. quinquefasciatus	0.2	0.4	1.2	1.3	0.6	0.9	0	0	0.4	0.5
Cx. sitiens	0	0	0	0	0.4	0.9	0	0	0	0
Mansonia septempunctata	1.2	2.7	1.2	0.4	1.8	2.5	1.0	1.0	2.2	1.3
Man. uniformis	0.4	0.5	0.4	0.5	0.4	0.5	1.2	1.1	0.6	1.3
Tripteroides magnesianus	0	0	0	0	0	0	0	0	0	0
Verrallina carmenti[a]	1.0A	1.2	5.4B	6.1	10.0BC	18.5	9.0BC	8.4	15.2C	8.3
Ve. funerea	0.6	1.3	4.0	4.1	10.2	11.7	5.2	2.4	6.8	5.4
Ve. lineata	0	0	1.6	3.0	2.6	4.2	1.4	1.1	3.6	2.7
Total mosquitoes	50.8A	64.6	95A	54.6	144AB	125.0	144.4AB	61.3	205.6B	98.5
Total species	13		15		15		14		15	
% feeding	NA		92%		84%		92%		94%	

Feeding is based upon presence of blue food dye in the mosquito indicative of feeding on honey-treated FTA card.
Means followed by a different letter are significantly different ($P < 0.05$) by Tukey's HSD.
[a] Because of their comparable morphology and ecology, multiple comparison test was conducted on pooled *Ve. funerea* and *Ve. carmenti*.

Table 4. Mean (SD) number of mosquitoes captured using modified versions of the large PBT at sheltered swamp at Smithfield rubbish tip ($n = 5$)

Species	CDC light trap		Original passive side vent		Top vent, long neck		Top vent, short neck		Top vent, short neck PVC bowl	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aedes alternans	0	0.0	0	0.0	0	0.0	1.8	4.0	0	0.0
Ae. kochi	31.0A	25.0	5.2B	1.6	15.4A	3.9	10.6AB	5.1	21.2A	9.1
Ae. notoscriptus	28.8A	12.2	30.4A	16.7	42.4A	23.6	43.6A	25.1	44.6A	22.6
Ae. tremulus	17.4A	22.4	2.2B	2.7	5.4B	6.5	3.4B	1.7	8.8B	9.4
Ae. vigilax	68.0A	60.3	8.2B	6.7	24.6A	11.6	37.0A	22.6	41.2A	24.1
Anopheles farauti s.l.	87.0A	48.6	6.0B	3.5	30.8C	20.1	27.4C	9.2	30.8C	15.3
Coquillettidia spp. nr *crassipes*	3.8	3.6	1.6	1.1	5.2	5.2	3.2	2.4	3.0	3.1
Culex annulirostris	50.2A	27.3	21.2B	14.5	28.6AB	6.8	37.8AB	10.6	38.4AB	23.9
Cx. biteaniorhynchus	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Cx. hilli	8.6	2.2	1.6	0.9	1.6	1.8	1.4	1.1	5.2	2.6
Cx. pullus	1.0	1.2	1.2	1.8	0.4	0.5	1.0	1.4	0.8	0.4
Cx. sitiens	10.0A	10.0	8.0A	4.0	14.6A	11.7	10.8A	7.7	19.6A	14.9
Mansonia septempunctata	4.8	5.6	0.4	0.5	1	1.0	2.4	3.8	2.2	0.8
Man. uniformis	0.4	0.9	0	0.0	0.2	0.4	0.2	0.4	0.4	0.5
Tripteroides magnesianus	0	0.0	0	0.0	0	0.0	0.2	0.4	0	0.0
Verrallina carmenti[a]	2.4	4.3	0	0.0	1.8	2.2	2.6	3.7	4.2	3.0
Ver. funerea[a]	12.8A	8.5	3.8B	4.7	10.6A	6.9	7.4AB	5.3	13.6A	10.2
Ver. lineata	0.8	1.3	0	0.0	1.6	1.5	1.0	0.7	2.4	1.8
Total	327A	188.1	89.8B	30.0	184.2A	50.6	191.8A	50.9	236.4A	69.9
Total species	15		12		15		17		15	
% feeding	NA		95%		96%		97%		96%	

For details on trap components see text.
Means followed by a different letter are significantly different ($P < 0.05$) by Tukey's HSD.
[a] Due to their comparable morphology and ecology, multiple comparison test was conducted on pooled *Ve. funerea* and *Ve. carmenti*.

and fipronil-laced honey was 29.9 ± 0.6, 29.7 ± 0.5, and 30.2 ± 0.3, respectively, for KUNV spiked cards, and 25.7 ± 0.2, 25.4 ± 0.7, and 22.8 ± 0.5, respectively, for RRV spiked cards. There was a significant difference by ANOVA ($P < 0.05$) for mean Ct score with fipronil-laced honey versus untreated- and honey-treated FTA cards for RRV.

Discussion

We have developed a passive trap that does not require any form of electric power, yet collects comparable, often higher, numbers of mosquitoes as a CDC light trap that requires batteries to power a light and fan. Of the two general passive trap designs tested, the clear plastic box passive trap (the PBT) appeared to be more successful, collecting more mosquitoes than the cylinder design. We believe the translucent, clear windows created by the crate inhibit escape, although further studies are needed to quantify this. Collections of some species could be enhanced by adding octenol, with placement of the reaction vial outside the box most effective. The simplicity of the PBT, comprised of materials sourced from common hardware and plumbing suppliers, ensures that traps can be constructed readily without need for purchasing complex or expensive components. Furthermore, the design concept involving a bottom entry port and a translucent holding chamber could be adapted de-

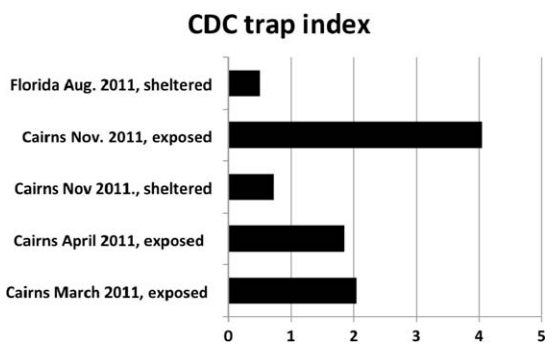

Fig. 4. CDC light trap index (total mosquitoes collected by large PBT/total mosquitoes collected by CDC light trap) for large box passive traps from Latin square field trials conducted in sheltered woodland and exposed, open field.

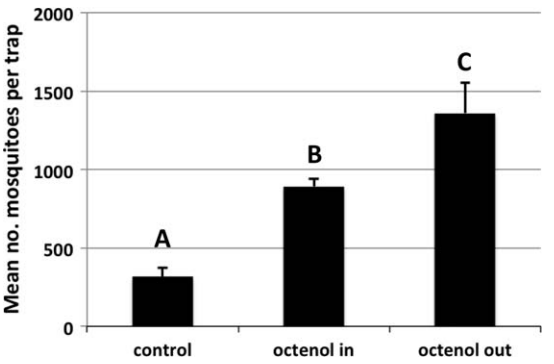

Fig. 5. Impact of octenol lure placed inside versus outside large PBT on mean (+ SEM) mosquito collection. Means were significantly different ($P < 0.05$ by Tukey's HSD) between all treatments.

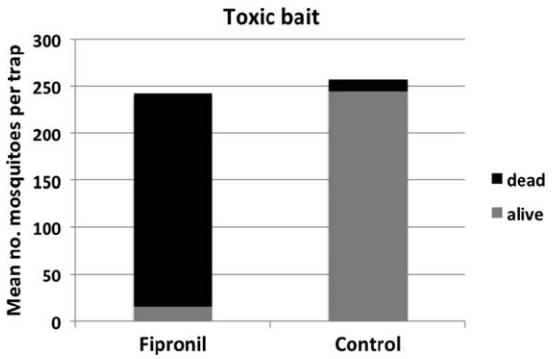

Fig. 6. Impact of honey soaked FTA cards treated with fipronil on mosquito catch and kill in large PBT ($n = 6$).

pending on the availability of components from local suppliers. Indeed, a version of the trap using PVC pipe inserted into the bottom of a 10-liter translucent bucket has collected large numbers of *Cx. nigripalpus* (D. S., unpublished data). Mosquito retention could be enhanced by placing a wire mesh cone at the top of the trap inlet similar to the method employed by Schreck et al. (1970), although this may impede entry by mosquitoes, and adds to the complexity of the trap.

Although the passive trap often collected more mosquitoes than the CDC light trap, there may be some species that the CDC trap is more effective at collecting. Comparative field trials should be conducted to ensure that target vectors are effectively collected before adopting passive traps for arbovirus surveillance. Furthermore, the trend for the passive trap to outtrap the CDC trap in exposed, windy areas (Fig. 3) is intriguing. The entry into the passive trap consists of a bowl that could minimize disturbance of the CO_2 plume. This would provide a direct olfactant trail for mosquitoes to follow into the trap. The release point for CO_2 with CDC trap (above the trap lid) is fully exposed to the wind, and turbulent destruction of the plume by the wind may make it difficult for mosquitoes to track the plume to the trap entry. Cooperband and Cardé (2006a) documented such turbulence in CO_2 plumes for traps where CO_2 release was exposed directly to wind. This, in turn, was reflected in poor trapping efficiency (Cooperband and Cardé 2006b). The entry bowl may also provide shelter for attracted mosquitoes, helping to herd them toward and into the trap entry pipe (Ritchie et al. 2008).

Honey-soaked FTA cards that harvest mosquito saliva are a promising system for arbovirus surveillance, especially in remote areas (van den Hurk et al. 2012). Currently, we have used the large PBT with fipronil- and honey-treated FTA cards to survey for arboviruses at four rural locations in Australia. To date we have obtained numerous detections of RRV, Barmah Forest Virus, and KUNV, and one trap set at Emerald, Queensland collected an estimated 29,000 mosquitoes within a week. Results of parallel trapping and sentinel chicken arbovirus detection trial will be provided in another paper.

The development of nonmechanical passive traps to house the FTA cards has the ability to further enhance this system, especially in areas where access to any form of electrical power, whether it be mains power, solar power, or long-life batteries, is logistically difficult and expensive. The nonpowered passive traps alleviate the need for large, heavy batteries to power trap fans, such as those that powered the updraft box trap that originally was used to house honey-soaked FTA cards (Hall-Mendelin et al. 2010). Batteries often are subject to vandalism and theft. The components within motorized traps often are damaged during transport or can malfunction after deployment for any period of time in hot, humid conditions, which are most often encountered while undertaking arbovirus surveillance. These factors are not an issue with the motorless traps we have developed.

Irrespective of the trap design, captured mosquitoes readily fed upon honey-soaked FTA cards. However, as evidenced during the Florida trial, large mosquito collections can cause crowding at honey-treated cards, reducing feeding rates, which, in turn, could affect arbovirus detection rates. Lacing the honey-soaked cards with insecticide, in this case fipronil, can be used to kill mosquitoes and potentially reduce crowding and feeding interference. In the current study, we did not test the fipronil-treated cards with large mosquito collections, and thus feeding rates in PBTs with treated and untreated cards were high. Thus, additional trials are needed when mosquito populations are high to confirm the benefit of the fipronil-treated cards on mosquito feeding. Our laboratory tests confirmed that both honey and fipronil-laced honey had no deleterious impact on detection of RRV and KUNV. This result likely would apply to other flaviviruses and alphaviruses targeted by surveillance programs.

Although the PBT originally was designed to collect and house mosquitoes so that they can access the honey-soaked FTA cards, these traps can be used for many other aspects of mosquito biology and control. A line or barrier of CO_2-baited traps using a passive bottom entry could be used to collect and kill large numbers of mosquitoes, providing protection to people within the barrier (van den Hurk et al. 2012). The passive trap is also an excellent tool to collect live, pristine mosquitoes. Captured mosquitoes do not pass through a motorized fan, which potentially can damage them. Once within the trap, they are temporarily housed within a humid, protective box. Undamaged, pristine mosquitoes make identification easier, and are useful for teaching mosquito taxonomy and as a source of reference specimens. Obviously, if the objective is to collect live mosquitoes, any form of insecticide-treated cards or bait would have to be omitted. Instead, the box trap can be provided with food such as honey, or a sugar–blood mix could potentially be used to blood feed mosquitoes while in the field for collection of eggs.

The PBT arbovirus surveillance system could potentially be used to detect a range of pathogens vectored by hematophagous arthropods. This system

could readily be employed for the surveillance of other pathogenic arboviruses, such as WNV, JEV, and Rift Valley fever virus. Detection of dengue or chikungunya viruses in diurnal *Aedes* spp., such as *Ae. aegypti* (L.) and *Ae. albopictus*, likely would require a different trapping system incorporating visual lures or additional olfactants such as lactic acid (Hoel et al. 2007). Furthermore, the highly clustered, premise-level distribution of *Ae. aegypti* may not make CO_2-baited traps practical, and simple inexpensive sticky ovitraps (Chadee and Ritchie 2010) may be a more practical solution. Other mosquito-borne pathogens, such as malaria and filarial worms could also be detected, although whether mosquitoes expectorate these protozoan or metazoan parasites while sugar feeding remains to be explored. With modifications, the PBT and FTA card system could be used to collect small dipterans, such as *Culicoides* (Ritchie et al. 1994) and *Phlebotomus* (Beavers et al. 2004) that typically are collected with CO_2-baited traps. With the emergence of novel arboviruses such as Schmallenberg virus (Śmietanka et al. 2012), and the expansion of vector-borne pathogens such as *Leishmania* (Dujardin et al. 2008) and blue tongue virus (Wilson and Mellor 2009), cheap and effective arbovirus surveillance systems are essential for facilitating informed management strategies. Finally, the simplicity of this system make it ideal for use in developing countries, providing that a suitable source of CO_2 is available.

Acknowledgments

We thank property owners in Cairns and Vero Beach, FL for allowing us to set mosquito traps on their land. This work was funded by Pathfinders Proof-of-Concept grant to S. Ritchie and a grant from Queensland Health to A. van den Hurk.

References Cited

Allan, S. A. 2011. Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits. J. Vector Ecol. 36: 59–67.

Beavers, G. M., H. A. Hanafi, and E. A. Dykstra. 2004. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for *Phlebotomus papatasi* (Diptera: Psychodidae) in southern Egypt. J. Am. Mosq. Control Assoc. 20: 130–133.

Chadee, D. D., and S. A. Ritchie. 2010. Efficacy of sticky and standard ovitraps for *Aedes aegypti* in Trinidad, West Indies. J. Vector Ecol. 35: 395–400.

Cooperband, M. F., and R. T. Cardé. 2006a. Comparison of plume structures of carbon dioxide emitted from different mosquito traps. Med. Vet. Entomol. 20: 1–10.

Cooperband, M. F., and R. T. Cardé. 2006b. Orientation of *Culex* mosquitoes to carbon dioxide-baited traps: flight manoeuvres and trapping efficiency. Med. Vet. Entomol. 20: 11–26.

Díaz-Fleischer, F., J. Arredondo, S. Flores, P. Montoya, and M. Aluja. 2009. There is no magic fruit fly trap: multiple biological factors influence the response of adult *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae) individuals to MultiLure traps baited with BioLure or NuLure. J. Econ. Entomol. 102: 86–94.

Dujardin, J. C., L. Campino, C. Cañavate, J. P. Dedet, L. Gradoni, K. Soteriadou, A. Mazeris, Y. Ozbel, and M. Boelaert. 2008. Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. Emerg. Infect. Dis. 14: 1013–1018.

Hall, R. A., N. A. Prow, and A. T. Pyke. 2011. Ross River virus, pp. 349–359. *In* D. Liu (ed.), Molecular detection of human viral pathogens. CRC, Boca Raton, FL.

Hall-Mendelin, S., S. A. Ritchie, C. A. Johansen, P. Zborowski, G. Cortis, S. Dandridge, R. A. Hall, and A. F. van den Hurk. 2010. Exploiting mosquito sugar feeding to detect mosquito-borne pathogens. Proc. Natl. Acad. Sci. U.S.A. 107: 11255–11259.

Hoel, D. F., D. L. Kline, S. A. Allan, and A. Grant. 2007. Evaluation of carbon dioxide, 1-octen-3-ol, and lactic acid as baits in Mosquito Magnet Pro traps for *Aedes albopictus* in north central Florida. J. Am. Mosq. Control Assoc. 23: 11–17.

Johansen, C. A., B. L. Montgomery, J. S. Mackenzie, and S. A. Ritchie. 2003. Efficacies of the mosquitomagnet and counterflow geometry traps in north Queensland, Australia. J. Am. Mosq. Control Assoc. 19: 265–270.

Kemme, J. A., P. H. Van Essen, S. A. Ritchie, and B. H. Kay. 1993. Response of mosquitoes to carbon dioxide and 1-octen-3-ol in southeast Queensland, Australia. J. Am. Mosq. Control Assoc. 9: 431–435.

Kline, D. L. 2006. Traps and trapping techniques for adult mosquito control. J. Am. Mosq. Control Assoc. 22: 490–496.

Kline, D. L. 2007. Semiochemicals, traps/targets and mass trapping technology for mosquito management. J. Am. Mosq. Control Assoc. 23: 241–251.

Kramer, L. D., L. M. Styer, and G. D. Ebel. 2008. A global perspective on the epidemiology of West Nile virus. Annu. Rev. Entomol. 53: 61–81.

Pyke, A. T., I. L. Smith, A. F. van den Hurk, J. A. Northill, T. F. Chuan, A. J. Westacott, and G. A. Smith. 2004. Detection of Australasian Flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays. J. Virol. Methods 117: 161–167.

Ritchie, S. A., P. H. van Essen, J. A. Kemme, B. H. Kay, and D. Allaway. 1994. Response of biting midges (Diptera: Ceratopogonidae) to carbon dioxide, octenol, and light in southeastern Queensland, Australia. J. Med. Entomol. 31: 645–648.

Ritchie, S. A., A. T. Pyke, G. A. Smith, J. A. Northill, R. A. Hall, A. F. van den Hurk, C. A. Johansen, B. L. Montgomery, and J. S. Mackenzie. 2003. Field evaluation of a sentinel mosquito (Diptera: Culicidae) trap system to detect Japanese encephalitis in remote Australia. J. Med. Entomol. 40: 249–252.

Ritchie, S. A., A. F. van den Hurk, P. Zborowski, T. J. Kerlin, D. Banks, J. A. Walker, J. M. Lee, B. L. Montgomery, G. A. Smith, A. T. Pike, et al. 2007. Operational trials of remote mosquito trap systems for Japanese encephalitis virus surveillance in the Torres Strait, Australia. Vector Borne Zoonotic Dis. 7: 497–506.

Ritchie, S. A., P. Zborowski, D. Banks, I. Walsh, and J. Davis. 2008. Efficacy of novel updraft traps for collection of mosquitoes in Cairns, Australia. J. Am. Mosq. Control Assoc. 24: 520–527.

Schreck, C. E., H. K. Gouck, and K. H. Posey. 1970. An experimental Plexiglas mosquito trap utilizing carbon dioxide. Mosq. News 30: 641–645.

Silver, J. D. 2007. Mosquito ecology: field sampling methods. Springer, the Netherlands.

Śmietanka, K., A. Zietek-Barszcz, B. Miechowicz, and M. P. Polak. 2012. Schmallenberg virus-A new threat to animal health in Europe. Med. Weter. 68: 139–142.

Thomas, D. B., T. C. Holler, R. R. Heath, E. J. Salinas, and A. L. Moses. 2001. Trap-lure combinations for surveillance of *Anastrepha* fruit flies (Diptera: Tephritidae). Fla. Entomol. 84: 344–351.

van den Hurk, A. F., N. W. Beebe, and S. A. Ritchie. 1997. Responses of mosquitoes of the *Anopheles farauti* complex to 1-octen-3-ol and light in combination with carbon dioxide in northern Queensland, Australia. Med. Vet. Entomol. 11: 177–180.

van den Hurk, A. E., B. L. Montgomery, P. Zborowski, N. W. Beebe, R. D. Cooper, and S. A. Ritchie. 2006. Does 1-octen-3-ol enhance trap collections of Japanese encephalitis virus mosquito vectors in northern Australia? J. Am. Mosq. Control Assoc. 22: 15–21.

van den Hurk, A. F., S. A. Ritchie, and J. S. Mackenzie. 2009. Ecology and geographical expansion of Japanese encephalitis virus. Annu. Rev. Entomol. 54: 17–35.

van den Hurk, A. F., S. Hall-Mendelin, C. A. Johansen, D. Warrilow, and S. A. Ritchie. 2012. Evolution of mosquito-based arbovirus surveillance systems in Australia. J. Biomed. Biotechnol. doi:10.1155/2012/325659.

Van Essen, P. H., J. A. Kemme, S. A. Ritchie, and B. H. Kay. 1994. Differential responses of *Aedes* and *Culex* mosquitoes to octenol or light in combination with carbon dioxide in Queensland, Australia. Med. Vet. Entomol. 8: 63–67.

Wilson, A. J., and P. S. Mellor. 2009. Bluetongue in Europe: past, present and future. Phil. Trans. R. Soc. B: Biol. Sci. 364: 2669–2681.

Received 16 May 2012; accepted 22 August 2012.