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SCIENTIFIC NOTE

EFFICACIES OF THE MOSQUITOMAGNET™ AND COUNTERFLOW GEOMETRY TRAPS IN NORTH QUEENSLAND, AUSTRALIA

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ABSTRACT. We conducted three trials near Cairns, Australia, to compare the numbers of mosquitoes collected with the standard Encephalitis Vector Surveillance (EVS) and Centers for Disease Control (CDC) light traps with the new MosquitoMagnet™ (MM) and counterflow geometry (CFG) traps with the use of a 4 × 4 latin square experimental design. The MM was generally equal to the performance of the CDC and CFG traps, ranking ahead of or equal to one or both traps in 2 of 3 trials, although there were no significant differences in the performances of the MM, CDC, and CFG traps. The EVS trap ranked last in all trials. The MM, being self powered and self baited via combustion of propane for up to 20 days without requiring a propane refill, would be suitable for collection of adult mosquitoes in remote areas that do not have access to dry ice.

KEY WORDS Surveillance, arbovirus, flavivirus, mosquito trap, MosquitoMagnet™

Japanese encephalitis (JE) virus was 1st detected in the Torres Strait islands in northern Queensland in 1995 when three human cases were serologically confirmed (Hanna et al. 1995, 1996) and has subsequently been detected in the Torres Strait in all but 1 year (1999) (Mackenzie et al. 2002). The regular activity of JE virus in the Torres Strait and its appearance on mainland Australia in 1998 (Hanna et al. 1999) are cause for concern, given the widespread distribution of potential vectors and vertebrate hosts on mainland Australia. In addition to JE virus, Murray Valley encephalitis (MVE) and Kunjin (KUN) viruses are other flaviviruses enzootic in northern Australia (Mackenzie et al. 1998) that occasionally cause human disease. Members of the *Culex sitiens* subgroup (including the morphologically similar *Culex annulirostris* Skuse, *Culex palpalis* (Taylor), and *Culex sitiens* Wiedemann [Lee et al. 1989]) appear to be the most important vectors of JE, MVE, and KUN viruses in Australia (Mackenzie et al. 1994, Ritchie et al. 1997).

Sentinel pigs and chickens are used to monitor for JE and MVE/KUN virus activity, respectively, in Australia (Hall et al. 1990, Shield et al. 1996, Broom et al. 1997). Unfortunately, the use of sentinel animals for surveillance of virus activity in Cape York and the Torres Strait is expensive. The estimated cost of establishing, rearing, and monitoring a herd of 5 pigs in remote Cape York can reach as high as $AUS16,000 (Richard Mason, Australian Quarantine Inspection Service [AQIS], personal communication). Furthermore, pigs are amplifying hosts of JE virus and contribute to JE virus activity, thereby posing a risk to the community. For these reasons, AQIS has listed the development of new methods of JE surveillance as high priority research.

Processing mosquitoes for detection of JE virus RNA by sensitive polymerase chain reaction amplification of large pools (Johansen et al. 2002) may be safer, less expensive, and more timely than detection of seroconversions in sentinel animals because infected mosquitoes could potentially be found before they have transmitted virus to sentinel animals. However, the remoteness of Cape York and islands of the Torres Strait poses logistical problems.

Methods of adult mosquito collection for arbovirus surveillance in Australia currently rely on opportunistic sampling with Encephalitis Vector Surveillance (EVS) and Centers for Disease Control (CDC) light traps baited with dry ice (Sudia and Chamberlain 1962, Rohe and Fall 1979). Both trap types are run overnight and are powered by 1.5-V D cell or 6-V batteries, respectively. The need to change or recharge batteries and rebait the trap with dry ice makes these traps impractical for use in a routine surveillance program in remote areas.

New mosquito traps developed by American Biophysics Corporation (East Greenwich, RI) may overcome some of the difficulties associated with surveillance in remote areas. The counterflow geometry (CFG) trap contains a fan that causes a CO_2-enriched plume of gas to exit the trap down a central pipe. This pipe is surrounded by a larger pipe in which an updraft is created by a fan, drawing mosquitoes attracted to the CO_2 into the interior of the trap (Kline 1999). In one study, the CFG trap collected significantly more mosquitoes than a standard professional (ABC-PRO) American Biophysics trap that uses both light and CO_2 as attractants (Kline 1999). Whereas the CFG trap is powered by

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a rechargeable 12-V battery, the MosquitoMagnet™ (MM) (by the same counterflow geometry technology) is powered by propane gas. The propane combusts to produce CO_2, heat, and water, and a thermoelectric generator uses excess heat from the combustion process to generate electricity to power the trap. Thus, the trap is self powered, produces its own attractant, and allows continuous collection of mosquitoes for ca. 3 wk without servicing. In this paper, we compare the effectiveness of the MM and CFG traps with CDC (Model 512, John W. Hock Co., Gainesville, FL) and EVS traps in northern Queensland, Australia.

Three trials were used to compare the efficacies of the MM, CFG, CDC, and EVS traps (Model E67 PR101, Australian Entomological Supplies, Pty., Ltd., Coorabell, New South Wales). The Edmonton sewage treatment plant was the site of trials I and II (February 1–9 and 12–21, 2001). Located 21 km southwest of Cairns (16°57′S, 145°45′E) in northern Queensland, the site was adjacent to sugarcane farms and a tidal salt marsh. The 4 traps were positioned along a fence line (running west–east) to the north of the sewage treatment plant. Trial III was conducted at Yorkey's Knob sewage treatment plant (March 6–10, 2001), approximately 25 km north of Cairns. Sugarcane farms and extensive areas of tidal salt marsh, mangroves, and *Melaleuca* swamps were the dominant types of habitat. The 4 traps were placed along the western fence line (running north–south) adjacent to the sewage ponds.

The MM produced CO_2 during combustion of propane, whereas the CFG, CDC, and EVS traps were baited with a 1-kg block of dry ice. All 4 traps were supplemented with octenol (at a release rate of approximately 4.5 mg/h), shown to increase collections of some species of mosquitoes when used in conjunction with CO_2 (van Essen et al. 1994, Ritchie and Kline 1995). The CO_2 release rate was estimated by weighing the dry ice at the start and finish of each trap night and extrapolating with release rates described by van Essen and colleagues (1994), who determined that sublimation of 45 g/h of solid CO_2 was equivalent to 387.8 ml/min of CO_2 gas. In trials I and II, dry ice was held inside a Styrofoam-insulated 2-liter "billycan." However, a 2-liter thermos (Willow Ware Australia Pty., Ltd., Melbourne, Australia) was used to hold the dry ice for the CFG trap in trial III to minimize leakage and improve delivery of CO_2.

The MM was powered by a 9-kg propane gas cylinder sufficient to power and bait the trap for 20 days, as determined by measuring propane consumption. A 12-V rechargeable battery was used to power the CFG trap. In addition to CO_2 and octenol, the CDC and EVS traps had 6.3-V, 150-mA and 12-V, 50-mA incandescent bulbs, respectively, as attractants. The larger light bulbs used in CDC traps were blacked out with permanent marker pen ink to reduce light, minimizing the collection of moths. CDC and EVS traps were each powered by a 6-V rechargeable gel cell battery and 2 D cell batteries, respectively.

A 4 × 4 latin square design was used to compare the efficacies of the 4 trap types (Cochran and Cox 1957). During each trial, traps were placed ca. 50 m apart in a line perpendicular to the prevailing wind. Traps were run between 3:30 p.m. and 9:00 a.m. The following groups were analyzed: the genera *Culex, Ochlerotatus, Verrallina,* and *Mansonia, Cx. sitiens* subgroup mosquitoes, and total mosquitoes. For each group, the number of mosquitoes and species per trap were log $(n + 1)$ transformed, then 2-way analysis of variance (ANOVA) (SigmaStat for Windows Version 2.03) was used to compare treatments (trap type and location). Kruskal–Wallis 1-way ANOVAs on ranks were also performed. Tukey's pairwise multiple comparison test was used to compare means in both instances.

The release rate of CO_2 from the MM was ca. 529.1 ml/min. In contrast, the release rates for solid CO_2 were 413.4 ml/min ± 35.3 ml/min and 389.2 ml/min ± 20.2 ml/min from the insulated billycans and thermos, respectively. There was no significant difference in the release rates of CO_2 between the insulated billycans and thermos (*t*-test, $P = 0.256$).

Twenty-one, 19, 24, and 15 taxonomic units were collected in the MM, CDC, CFG, and EVS traps, respectively, in 1 or more of the trials (Table 1). *Culex gelidus* Theobald, members of the *Cx. sitiens* subgroup, *Mansonia uniformis* (Theobald), *Verralina funerea* (Theobald), and *Verralina carmenti* Edwards were the most abundant taxonomic units collected in the MM, CDC, and CFG traps. *Culex gelidus* were less abundant in the EVS trap than in the other 3 traps.

In general, the MM outperformed the CDC, CFG, and EVS traps in trial 1 (Table 2); the MM collected the greatest mean number of mosquitoes per trap per day, as well as the most *Culex* spp., *Cx. sitiens* subgroup mosquitoes, *Verrallina* spp., and *Mansonia* spp. The MM performed significantly better than the EVS trap with collections of *Culex* spp., *Cx. sitiens* subgroup mosquitoes, *Verrallina* spp., and total number of mosquitoes, although it was not significantly better than the CDC or CFG traps. Although the MM did not collect as many *Ochlerotatus* spp. as did the CDC and CFG traps, there was no significant difference among the numbers of *Ochlerotatus* spp. collected in any of the 4 trap types. There was no significant difference ($P > 0.05$) in the number of species collected in each trap or at each trap position during the 1st trial (results not shown). Furthermore, there was no significant difference, for most categories of species, in the number of mosquitoes collected at different trap positions during the 1st trial. Exceptions to this included *Cx. sitiens* subgroup mosquitoes, in which significantly fewer mosquitoes were collected in traps at position 4 than at position 3 (14.5 ± 9.5 and 89.0 ± 80.6, respectively; $P < 0.05$), and *Verrallina* spp., which were collected in greater num-

Table 1. Mosquito species collected in 4 trap types,[1] February–March 2001.

Species	MM		CFG		CDC		EVS	
	n	% of total	*n*	% of total	*n*	% of total	*n*	% of total
Anopheles bancroftii Giles	0	0.0	0	0.0	1	<0.1	0	0.0
Anopheles faurauti Laveran s.l.	97	2.4	71	2.7	58	1.2	28	3.1
Coquillettidia crassipes (Van der Wulp)	4	0.1	3	0.1	8	0.2	3	0.3
Culex bitaeniorhynchus Giles	0	0.0	1	<0.1	0	0.0	0	0.0
Culex gelidus Theobald	1,260	31.1	678	25.8	1,474	30.7	92	10.3
Culex pullus Theobald	0	0.0	3	0.1	2	<0.1	0	0.0
Culex sitiens subgroup	993	24.5	558	21.2	1,788	37.2	241	26.9
Mansonia septempunctata Theobald	1	<0.1	12	0.5	4	0.1	7	0.8
Mansonia uniformis (Theobald)	394	9.7	185	7.0	306	6.4	176	19.6
Mimomyia spp.	1	<0.1	0	0.0	0	0.0	0	0.0
Ochlerotatus alternans (Westwood)	19	0.5	42	1.6	16	0.3	12	1.3
Ochlerotatus aurantius aurantius (Theobald)	1	<0.1	0	0.0	4	0.1	0	0.0
Ochlerotatus kochi (Donitz) s.l.	36	0.9	126	4.8	166	3.5	100	11.2
Ochlerotatus lineatopennis (Ludlow)	6	0.1	13	0.5	5	0.1	2	0.2
Ochlerotatus littlechildi Taylor	0	0.0	0	0.0	2	<0.1	0	0.0
Ochlerotatus normanensis (Taylor)	0	0.0	1	<0.1	1	<0.1	0	0.0
Ochlerotatus notoscriptus (Skuse)	10	0.2	29	1.1	38	0.8	14	1.6
Ochlerotatus tremulus (Theobald)	0	0.0	2	0.1	0	0.0	0	0.0
Ochlerotatus vigilax (Skuse)	73	1.8	33	1.3	135	2.8	41	4.6
Ochlerotatus (*Macleya*) spp.	1	<0.1	2	0.1	0	0.0	1	0.1
Ochlerotatus spp.	1	<0.1	1	<0.1	0	0.0	0	0.0
Tripteroides magnesianus (Edwards)	8	0.2	3	0.1	0	0.0	0	0.0
Uranotaenia nivipes (Theobald)	1	<0.1	4	0.2	0	0.0	0	0.0
Uranotaenia pygmaea Theobald	0	0.0	1	<0.1	0	0.0	0	0.0
Verrallina carmenti Edwards	337	8.3	352	13.4	114	2.4	27	3.0
Verrallina funerea (Theobald)	651	16.1	407	15.5	529	11.0	121	13.5
Verrallina lineata (Taylor)	154	3.8	98	3.7	155	3.2	31	3.5
Unidentifiable spp.	1	<0.1	2	0.1	0	0.0	0	0.0

[1] MM, MosquitoMagnet®; CFG, counterflow geometry; CDC, Centers for Disease Control; EVS, encephalitis vector surveillance.

bers at position 4 than at positions 1 and 2 (19.5 ± 20.2, 5.0 ± 3.6. and 5.5 ± 3.1, respectively; $P < 0.05$).

During trial II, there was no significant difference in the numbers of *Ochlerotatus, Verrallina* and *Mansonia* spp. collected in any of the mosquito trap types. The MM and CDC traps collected significantly ($P < 0.05$) more *Culex* spp. and total mosquitoes than the EVS trap, but not the CFG trap (Table 2). However, the CDC trap alone was significantly better than the EVS trap at collecting *Cx. sitiens* subgroup mosquitoes. No significant differences ($P > 0.05$) were observed in the number of species collected with each trap type or at each position, and no positional effects were observed on the numbers of mosquitoes collected during the 2nd trial (results not shown).

Few significant differences were observed in the numbers of mosquitoes collected in the various trap types during trial III. There was only 1 instance when a mosquito trap performed significantly better than other traps, i.e., when the CFG trap performed significantly better than the MM trap for collections of *Ochlerotatus* spp. In addition, the CFG trap collected significantly more species of mosquitoes ($P < 0.05$) than did the MM, CDC, or EVS traps (results not shown). No positional effects were observed between different trap positions.

When the performance of each mosquito trap was ranked from 1 to 4 (where a rank of 1 indicated best performance and a rank of 4, the poorest), on average the MM performed equally well as or better (although not always significantly better) than other traps (Table 3). The only instance when the MM performed significantly worse than other mosquito traps was during trial III, when it performed significantly worse than the CFG trap at collecting *Ochlerotatus* spp. In most instances, the EVS trap performed the worst of the 4 traps in each trial.

Results of this study indicate that the MM is generally equal to or superior to the mosquito traps (CDC and EVS) currently used routinely in Australia. In most instances, the MM was ranked 1st or 2nd in performance behind the CDC or CFG traps. Notably, there were no significant differences in the numbers of *Culex* mosquitoes (including the likely vectors of JE virus such as *Cx. annulirostris, Cx. sitiens*, and *Cx. gelidus*) collected in the MM, CDC, and CFG traps in any of the 3 trials, and in 2 of the trials, the MM collected significantly more *Culex* mosquitoes than did the EVS trap. These differences were unrelated to trap position effects.

Table 2. The number of mosquitoes (mean ± SD) collected per trap per night in different mosquito trap types in Cairns, Queensland, in February–March 2001.

Trial	Species	Trap type[1]			
		MM	CDC	CFG	EVS
I, Feb 1–9	*Culex* spp.[2]	99.2 ± 54.3a	69.0 ± 85.6ab	46.8 ± 47.7ab	13.8 ± 11.4b
	Culex sitiens subgroup	73.5 ± 48.5a	62.8 ± 78.1ab	35.0 ± 25.0ab	12.8 ± 12.0b
	Ochlerotatus spp.	4.8 ± 3.8a	12.5 ± 10.5a	11.2 ± 8.5a	2.0 ± 1.4a
	Verrallina spp.	23.0 ± 18.9a	5.2 ± 2.2ab	8.8 ± 5.0ab	4.2 ± 2.7b
	Mansonia spp.	51.5 ± 25.8a	37.0 ± 38.3a	31.8 ± 27.0a	24.2 ± 9.1a
	Total mosquitoes	187.8 ± 63.6a	126.8 ± 140.7ab	101.2 ± 77.8ab	42.0 ± 16.5b
II, Feb 12–21	*Culex* spp.	414.0 ± 253.1a	736.5 ± 950.5a	131.2 ± 65.7ab	47.8 ± 42.5b
	Culex sitiens subgroup	154.5 ± 77.3ab	373.0 ± 361.1a	64.2 ± 20.2ab	38.2 ± 39.4b
	Ochlerotatus spp.	23.0 ± 21.6a	47.5 ± 24.2a	21.5 ± 17.8a	14.0 ± 12.2a
	Verrallina spp.	230.2 ± 159.1a	151.8 ± 121.4a	133.5 ± 132.6a	31.8 ± 15.7a
	Mansonia spp.	45.0 ± 42.2a	23.2 ± 14.0a	10.8 ± 3.6a	21.0 ± 13.9a
	Total mosquitoes	721.8 ± 450.9a	1,002.5 ± 862.3a	299.2 ± 99.6ab	112.5 ± 33.7b
III, March 6–10	*Culex* spp.	49.8 ± 48.5a	11.5 ± 20.4a	132.0 ± 128.6a	25.2 ± 33.3a
	Culex sitiens subgroup	20.0 ± 29.0a	11.2 ± 20.5a	40.2 ± 27.9a	9.2 ± 9.6a
	Ochlerotatus spp.	7.5 ± 3.4a	33.8 ± 23.0ab	51.0 ± 31.4b	26.0 ± 19.0ab
	Verrallina spp.	34.0 ± 37.6a	23.8 ± 21.2a	72.7 ± 45.4a	9.0 ± 7.7a
	Mansonia spp.	2.2 ± 1.0a	2.0 ± 2.6a	6.8 ± 5.0a	3.0 ± 2.9a
	Total mosquitoes	102.0 ± 41.5a	77.0 ± 35.8a	280.8 ± 205.1a	72.5 ± 45.2a

[1] Means in the same row followed by the same letter were not significantly different ($P > 0.05$); Tukey's multiple comparison test applied to log(n + 1) transformed data. MM, MosquitoMagnet™; CDC, Centers for Disease Control; CFG, counterflow geometry; EVS, encephalitis vector surveillance.

[2] Comprises all *Culex* mosquito species.

Table 3. The mean performance of 4 trap types during mosquito collections in Cairns, Queensland, in February–March 2001.

Trial	Species	Trap type[1]			
		MM	CDC	CFG	EVS
I, Feb 1–9	*Culex* spp.[2]	1.75a	2.12ab	2.25ab	3.88b
	Culex sitiens subgroup	2.25a	2.00a	2.00a	3.50a
	Ochlerotatus spp.	3.12ab	1.38a	1.62a	3.88b
	Verrallina spp.	1.12a	2.75ab	2.62ab	3.50b
	Mansonia spp.	1.75a	2.25a	3.25a	2.75a
	Total mosquitoes	1.50a	2.25ab	2.50ab	3.75b
II, Feb 12–21	*Culex* spp.	1.50a	1.50a	3.00ab	4.00b
	Culex sitiens subgroup	2.00ab	1.50a	2.75ab	3.75b
	Ochlerotatus spp.	3.25a	1.00a	2.75a	3.00a
	Verrallina spp.	1.25a	2.00a	2.75a	4.00a
	Mansonia spp.	1.75a	1.88a	3.25a	2.62a
	Total mosquitoes	1.50a	1.50a	3.00ab	4.00b
III, Mar 6–10	*Culex* spp.	1.75ab	4.00a	1.25b	3.00ab
	Culex sitiens subgroup	2.00a	3.50a	1.62a	2.88a
	Ochlerotatus spp.	3.75a	2.50ab	1.50b	2.25ab
	Verrallina spp.	3.00a	2.50a	1.25a	3.25a
	Mansonia spp.	3.00a	3.25a	1.25a	2.50a
	Total mosquitoes	2.25a	3.00a	1.50a	3.25a

[1] Means in the same row followed by the same letter were not significantly different ($P > 0.05$); Tukey's multiple comparison test applied to log(n + 1) transformed data. MM, MosquitoMagnet™; CDC, Centers for Disease Control; CFG, counterflow geometry; EVS, encephalitis vector surveillance. Performance ranked from 1 to 4, where 1 indicates the trap that collected the most mosquitoes and 4 indicates the trap the collected the least.

[2] Comprises all *Culex* species.

One exception related to collections of *Ochlerotatus* spp. mosquitoes, when the MM collected fewer than did the CDC and CFG traps, although differences were significantly different on only 1 occasion. However, relatively low numbers of *Ochlerotatus* spp. mosquitoes were collected during the 3 trials, and further experiments with larger populations of *Ochlerotatus* spp. mosquitoes are required to investigate this anomaly further. The relatively poor performance of the EVS trap was not surprising because a previous study showed the EVS trap generally collects fewer mosquitoes (including *Cx. annulirostris* and *Ochlerotatus vigilax*) than does the CDC light trap (Ritchie and Kline 1995).

Other recent studies have also shown that traps designed with the use of counterflow geometry technology compare favorably with other traps used to collect adult mosquitoes. Kline (1999) showed that the CFG trap (baited with CO_2 and octenol) collected significantly more mosquitoes than did the standard professional (ABC-PRO) American Biophysics trap. The CFG trap also collected significantly more *Anopheles gambiae* Giles s.s. and *Culex quinquefasciatus* Say than did the CDC trap (with or without a light) in Tanzania (Mboera et al. 2000). However, in both of these studies, the CO_2 was delivered from a pressurized gas cylinder via a regulator as recommended by the manufacturer, as opposed to block dry ice in insulated containers. It is possible that the delivery of CO_2 from dry ice may have affected the performance of the CFG trap in this study.

The performance of the MM, particularly with regard to *Culex* species, indicates that it is an attractive trap for MVE, KUN, and JE virus surveillance. Because the MM can function for up to 20 days before requiring a propane refill, it has particular application for remote areas such as Cape York Peninsula, the Torres Strait islands, or New Guinea, which are difficult and expensive to access on a regular basis. Large collections of dead mosquitoes could be processed for viral RNA by polymerase chain reaction (Kramer et al. 2001, Johansen et al. 2002). The MM will be field tested with mosquitoes infected with JE or MVE virus in the laboratory before it can be incorporated into a surveillance program for these viruses in remote areas.

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