

LIGHT, CARBON DIOXIDE, AND OCTENOL-BAITED MOSQUITO TRAP AND HOST-SEEKING ACTIVITY EVALUATIONS FOR MOSQUITOES IN A MALARIOUS AREA OF THE REPUBLIC OF KOREA

DOUGLAS A. BURKETT,¹ WON J. LEE,² KWAN W. LEE,³ HEUNG C. KIM,³ HEE I. LEE,² JONG S. LEE,² E. H. SHIN,² ROBERT A. WIRTZ,⁴ HAE W. CHO,² DAVID M. CLABORN,⁵ RUSSEL E. COLEMAN⁶ AND TERRY A. KLEIN⁷

ABSTRACT. Two field trials for commercially available and experimental mosquito traps variously baited with light, carbon dioxide, octenol, or combinations of these were evaluated in a malarious area at Paekyeon-Ri near Tongil-Chon (village) and Camp Greaves, Paju County, Kyonggi Province, Republic of Korea. The host-seeking activity for common mosquito species was determined using hourly aspirator collections from a human- and propane lantern-baited Shannon trap. The total number of mosquitoes and number of each species captured during the test were compared using 8 × 8 and 5 × 5 Latin square designs based on trap location. Significant differences were observed for the total number of mosquitoes collected in the 8 × 8 test, such that counterflow geometry (CFG) with CO₂ ≥ CFG with CO₂ and octenol ≥ Shannon trap ≥ Mosquito Magnet[®] with octenol > American Biophysics Corporation (ABC) light trap with light, CO₂ (500 ml/min), and octenol ≥ ABC light trap with light and dry ice ≥ ABC light trap with light and CO₂ > ABC light trap with light only. A concurrent 5 × 5 test found significant differences in trap catch, where Mosquito Magnet with octenol > New Jersey light trap ≥ EPAR[®] Mosquito Killer with CO₂ ≥ ABC light trap with light and dry ice > Centers for Disease Control (CDC) light trap (manufactured by John W. Hock) with light and octenol. Significant differences in trap catch were noted for several species including: *Aedes vexans*, *Anopheles sinensis*, *An. yatsushiroensis*, *An. lesteri*, *Culex pipiens*, and *Cx. orientalis*. Traps baited with octenol captured significantly fewer *Cx. pipiens* than those not baited with octenol. Likewise, no *Cx. orientalis* were captured in octenol-baited traps. Host-seeking activity showed a similar bimodal pattern for all species captured. Results from these field trap evaluations can significantly enhance surveillance efforts. Significantly greater numbers of mosquitoes were captured with mosquito traps using counterflow technology (e.g., Mosquito Magnet and CFG traps) when compared to standard light and carbon dioxide-baited traps. Additionally, field evaluations demonstrate that various traps can be utilized for isolation and detection of arboviruses and other pathogens.

KEY WORDS Korea, light traps, *Anopheles sinensis*, mosquito surveillance, attractants

INTRODUCTION

The effectiveness of several commercially available and experimental mosquito traps using various combinations of attractants were evaluated in a rice field habitat in a malarious area near a U.S. military installation (Camp Greaves) and at Paekyeon-Ri, near Tongil-Chon (village), Paju County, in Northern Kyonggi Province, Republic of Korea (ROK). Other mosquito trapping studies have been conducted in the ROK (Self et al. 1973; Lee et al.

1984; Joo and Wada 1985; Shim et al. 1990; Baik and Joo 1991; Lee and Ree 1991; Joo and Kang 1992; Yang and Yu 1992; Shim et al. 1997; Kim et al. 1999; 2000; Strickman et al. 2000). Most of these investigators used New Jersey (NJ) light traps, black light traps, livestock-baited window traps, and human and livestock landing collections. The Korean Ministry of Health and Social Affairs began a peninsula-wide vector surveillance program in 1969 (Lee et al. 1971, Ree et al. 1973). Likewise, the U.S. Army has used NJ light traps to monitor mosquito populations at fixed U.S. military installations since the end of the Korean War, but did not institute an organized ROK-wide vector surveillance program until 1974 (Lee et al. 1984). Miscellaneous and inconsistent mosquito surveillance operations using a variety of commercially available light trap designs and attractants are also conducted throughout Korea (and throughout most of the rest of the world) by different branches of the military, either at the installation level or during field exercises and contingency operations. Surveillance results from these efforts that use an assortment of mosquito traps and attractants cannot be compared and the results are of questionable value.

The poor condition of specimens collected from NJ or other nonselective light traps often results in

¹ Detachment 3, U.S. Air Force Institute for Environment, Safety, and Occupational Health Risk Analysis (AF-IERA), Okinawa, Japan, APO AP 96368.

² Korean National Institute of Health, Department of Viral Diseases, 5 Nokbun-Dong, Eunpyung-Gu, Seoul 122-701, Republic of Korea.

³ U.S. Army, 5th Medical Detachment, 18th Medical Command, Unit 14247, APO AP 96205-0020.

⁴ Entomology Branch, Centers for Disease Control and Prevention, F-22, 4700 Buford Highway, NE, Atlanta, GA 30341-3724.

⁵ Uniformed Services University of Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

⁶ Department of Entomology, U.S. Army Medical, Armed Forces Research Institute of Medical Sciences, APO AP 96546.

⁷ Preventive Services Directorate, 18 Medical Command, Unit 15281, APO AP 96205-0054.

Table 1. Description of mosquito traps and attractant combinations.

Trap type	Human	Light	CO ₂	Octenol ¹	Trial
Shannon trap	Yes	Yes	Yes	No	8 × 8
ABC light trap	No	Yes	No	No	8 × 8
ABC light trap	No	Yes	Yes ²	No	Both
ABC light trap	No	Yes	Yes ³	No	8 × 8
ABC light trap	No	Yes	Yes ³	Yes	8 × 8
ABC counterflow geometry	No	No	Yes ³	No	8 × 8
ABC counterflow geometry	No	No	Yes ³	Yes	8 × 8
Mosquito Magnet [®]	No	No	Yes ⁴	Yes	Both
CDC light trap	No	Yes	No	Yes	5 × 5
New Jersey light trap	No	Yes	No	No	5 × 5
EPAR [®] Mosquito Killer	No	No	Yes ⁵	No	5 × 5

¹ Octenol cartridge.

² Source of CO₂, dry ice.

³ Source of CO₂, gas cylinder (500 ml/min).

⁴ Source of CO₂, propane gas.

⁵ Source of CO₂, gas cylinder (750 ml/min).

inaccurate identification of morphologically similar mosquitoes, which may result in errors when incriminating malaria or arbovirus vectors. Additionally, specimens are generally collected dead and in relatively small numbers, which precludes adequate sample sizes for determining malaria and arbovirus infection rates. This is the 1st published report that evaluates the attractiveness of commonly used modern mosquito traps and attractants for medically important mosquitoes in the ROK. Moreover, few of the previously reported trapping studies in the ROK have differentiated among the morphologically similar *Anopheles* malaria vectors (*An. sinensis* Wiedemann, *An. lesteri* Baisas and Hu, *An. yatsushiroensis* Miyazaki), all of which are implicated in the transmission of malaria in the ROK. Hourly host-seeking activity of the commonly captured mosquitoes also was recorded in this study to identify periods of greatest human exposure.

MATERIALS AND METHODS

Two mosquito trap evaluation field trials were conducted during June 19–30, 2000, from 1900 to 0600 h. Because of time limitations and the electrical requirements for some traps, both an 8 × 8 and a 5 × 5 Latin square design were employed to evaluate the effectiveness of various traps and attractants for mosquitoes. Trap data were not used for days when it rained during the test period. Trap data were transformed to log ($x + 1$) before analysis. Trap, day, and position effects were evaluated using a 3-way analysis of variance (SAS Institute 1995). Mean comparisons were made using the Ryan–Einot–Gabriel–Welsh multiple range test ($\alpha = 0.05$). The positions of each trap were changed nightly so that each trap would occupy every position during each of the test periods. After each trap night, mosquito collections were placed in shipping containers over dry ice and transported to the 5th Medical Detachment Entomology Laboratory, where they were identified using keys specific

to Korean mosquitoes (Lee 1998) and counted. *Anopheles* were separated by species and date of collection and sent to the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, to determine malaria infection rates (by enzyme-linked immunosorbent assay). Culicine mosquitoes were separated by species, placed in cryovials (30/ vial), and then maintained on dry ice. Specimens later were sent to the U.S. Army Medical Research Institute of Infectious Disease for virus isolation. Both of the latter studies will be reported separately.

8 × 8 rice field trapping study. Trials were conducted in approximately 4 ha of terraced rice fields (37°54'N, 126°43'E) adjacent to and northeast of Camp Greaves (U.S. Army installation) in the ROK. Traps were positioned on elevated walkways that separated the terraced rice fields. One hundred meters or more separated the traps. Several of the trap locations were bordered by primary woodland habitat (mixed deciduous and coniferous forest with underbrush). The 8 × 8 trial trap and attractant combinations are shown in Table 1.

Human landing/biting collections were not made during this investigation. As a substitute, human host-seeking/attractant collections were conducted using mouth aspirators from a 6 × 4 × 6-ft white cotton Shannon-type trap (Service 1993) baited with 2 human collectors and a propane lantern (Model 5152D700T, Coleman Company Inc., Wichita, KS) placed about 40 cm from the ground. A 20-cm gap at the bottom of the trap allowed mosquitoes to enter the trap. Two groups of 2 collectors each manned the trap. Groups were rotated throughout the test to reduce collection bias. Mosquitoes landing on the outside or inside of the Shannon trap were aspirated continuously throughout the collection period. Captured mosquitoes were placed in a screen-topped pint carton at hourly intervals. At the termination of each hour/collection period, mosquitoes were placed in a cooler. At the end of the daily collection, each carton was placed

on dry ice and transported to the 5th Medical Detachment, Yongsan, Korea.

Two carbon dioxide (CO₂)-baited Centers for Disease Control (CDC)-type traps (TrapkitDI and TrapkitI, American Biophysics Corp., East Greenwich, RI) were evaluated; 1 with 1.5 kg of dry ice and the other with CO₂ dispensed from a 9-kg compressed gas cylinder. Although the CO₂ flow rate for the dry ice was not measured, Reisen et al. (2000) reported an average CO₂ release rate of 500 ml/min for a 1.5-kg block of dry ice in a similar trap. Locally obtained compressed gas cylinders were used as the source of carbon dioxide for the other ABC light traps. The CO₂ flow rate (500 ml/min) was controlled using regulators, restriction couplings, and filters (FlowkitI, American Biophysics Corp.). Battery power was provided using Powersonic® (PowerSonic Corp., San Diego, CA) 6-V, 10-amp-h rechargeable gel cell batteries to run the fan motor and incandescent light. Traps were hung from tripods constructed from aluminum tent poles so that the light sources were approximately 60 cm from the ground. The ABC traps were used as received from the manufacturer and included a standard CM-47 bulb, and a 3-in.-diameter, 4-bladed fan inserted into a plastic housing and covered with a rain guard. The dry ice-baited ABC light trap had an insulated container above the rain guard to hold the dry ice. To save battery power, both traps were operated with light set to flicker (32.5 Hz). Studies show that the ABC light traps are representative of other typical CDC-type traps. For example, Vaidyanathan and Edman (1997) compared 11 trapping methods and found no significant difference in trap collections between the John W. Hock CDC-type traps (John W. Hock Company, Gainesville, FL) and similarly equipped ABC light traps. They also observed no significant differences in trap collection numbers for ABC traps with bulbs set to steady or flicker.

Two counterflow geometry (CFG) traps were evaluated, 1 with an octenol cartridge (OCT1, American Biophysics Corp.) and the other without. Neither of the traps used a light source, and both were baited with CO₂ from a compressed gas cylinder dispensed as described above for the ABC light traps. Both were operated using 6-V rechargeable batteries as described above. The CFG traps use 2 fans simultaneously that move air in opposite directions. A smaller, 40-mm fan sends a CO₂-enriched attractant plume down and out of a central 5-cm pipe extending medially and slightly beneath the bottom of the trap. Simultaneously, a stronger 80-mm fan creates an updraft through the trap entrance that surrounds the central downdraft pipe, forcing attracted insects into the trap's internal collection chamber. As of this writing, CFG traps, as described by Kline (1999), are not commercially available.

As with the CFG traps, the Mosquito Magnet® (Pro Model, American Biophysics Corp.) uses a

similar counterflow technology to capture insects. Propane gas supplied by 20-lb tanks powered the fan motors, produced heat, and generated CO₂ (a by-product of combustion). Otherwise, the trap was operated (including the OCT1 octenol cartridge) per manufacturer's instructions. The trap hung from a wheeled stand that placed the opening 60 cm above the ground. As with the other traps, the Mosquito Magnet was operated nightly from 1900 to 0600 h and shut off during the day. Collection nets from all the traps were emptied and replaced daily.

5 × 5 trapping study. Trials also were conducted on Camp Greaves (37°53'N, 126°43'E) about 0.5 km from the 8 × 8 study site. Traps were placed adjacent to areas where soldiers congregate (i.e., dining halls, guard gates, recreational areas, and barracks). The 5 × 5 trial evaluated the trap and attractant combinations shown in Table 1. In general, trap sites were not ideal (competing light sources) because they were limited by the electrical outlet requirements for the NJ light trap and EPAR™ Mosquito Killer trap (Model MKS-H, Environmental Products and Research, Blytheville, AR).

The Mosquito Magnet and the ABC light trap with dry ice were operated and specimens were processed as described in the preceding 8 × 8 methods. Similar to the ABC light traps, the CDC light trap (Model 1012, John W. Hock Company) consisted of a CM-47 bulb (steady light) and a 3-in.-diameter, 4-bladed fan inserted into a plastic housing covered with a black plastic rain guard. Insects were collected alive in a mesh collection net and otherwise processed and powered as with the ABC light traps. An octenol cartridge (OCT1) was fastened with a wire to the plastic trap housing of the CDC trap at the same height as the light. No carbon dioxide was used for the CDC light trap treatment.

Mosquitoes and other insects collected in the NJ light trap (John W. Hock, Model 1112) were captured in a pint polypropylene jar containing a 6 × 6-cm piece of dichlorvos-impregnated vinyl strip used as a killing agent. The NJ light trap used a 25-W incandescent lamp and was positioned so the light source was 1.5 m above the ground.

The Mosquito Killer trap also required an electrical outlet to operate the fan, heating element, and electric grid. Light is not used as an attractant, but the trap is otherwise similar in size, shape (19 × 24 in., 19 lb), and color to that of a standard NJ light trap. An internal fan forces mosquitoes and other insects landing on the side of the trap (or alighting close to the trap opening located beneath the rain shield) into and through an electrical grid where the insects are killed and blown into a net at the trap bottom. A fine-mesh nylon net, fastened with an elastic cord around the trap bottom, is not normally used for the EPAR Mosquito Killer, but was supplied by the manufacturer for these trials. Carbon dioxide was supplied using a 9-kg com-

pressed gas cylinder coupled to a manufacturer-supplied single-stage CO₂ regulator and a 100 × 1.5-cm rubber hose. The hose was attached to the side of the trap using Velcro® tape so that the end of the hose was just beneath the rain shield. A perforated plastic disk supplied by the manufacturer was inserted into the rubber hose to control the CO₂ flow rate, which was estimated to be about 750 ml/min. Before the start of the 5 × 5 trials, preliminary trials were conducted using the Mosquito Killer trap operated per manufacturers instructions without using CO₂ for 4 consecutive nights. The trap was turned on at 1745 h and turned off at 0615 h the following morning. Nightly collections were transferred to petri dishes and stored on dry ice, processed, and identified as described above for the 8 × 8 test. Mosquitoes captured in this trap or the NJ light traps were not captured alive and subsequently were not processed for arbovirus studies.

RESULTS

8 × 8 study

A total of 40,764 mosquitoes was collected among rice fields during the trial (8 trap nights). Back-transformed means, P values, and significant differences for the common species collected are shown in Table 2. As noted in the table, significant trap-location and day effects were found for some species. Significant differences in the total number of mosquitoes captured were observed among different traps and attractant combinations ($P < 0.01$) and among species (*An. lesteri* [$P < 0.01$], *An. sinensis* [$P < 0.01$], *An. yatsushiroensis* [$P < 0.01$], *Aedes vexans* (Meigen) [$P < 0.01$], *Culex pipiens* L. [$P < 0.01$], and *Cx. orientalis* Edwards [$P = 0.02$]). Overall, the greatest numbers of anopheline mosquitoes were captured with the Shannon and Mosquito Magnet traps. Conversely, significantly greater numbers of *Ae. vexans* were captured in the 2 CFG traps, followed by the Shannon and Mosquito Magnet traps. The ABC traps baited with CO₂ (compressed gas and dry ice) and the CFG trap with no octenol captured significantly more *Cx. pipiens* than did the other traps. Interestingly, those traps baited with octenol as 1 of the attractants captured significantly fewer *Cx. pipiens*. Likewise, no *Cx. orientalis* were collected in any of the octenol-baited traps. *Ochlerotatus dorsalis* (Meigen) (1), *Anopheles sineroides* Yamada (7), *An. lindesayii japonicus* Yamada (5), *Culex tritaeniorhynchus* Giles (2), *Cx. bitaeniorhynchus* Giles (1), and *Cx. vagans* Wiedemann (2) were collected in insignificant numbers for analysis (totals are given in parentheses). Small numbers of at least 3 species of tabanid flies (species not determined) were captured in the Mosquito Magnet and Shannon traps. Ceratopogonid midges, although not routinely counted, also were captured in some traps.

Table 2. Mosquito species composition and back-transformed nightly means for variously baited commercially available and experimental mosquito traps. Three-way analysis of variance and multiple comparison (Ryan-Einot-Gabriel-Welsh multiple range test) were performed after log (x + 1) transformations. Means within each row having the same letter are not significantly different ($n = 8$ nights; $\alpha = 0.05$).

Species ¹	Magnet + Octenol	CFG + CO ₂	CFG + Octenol	Shannon	ABC + CO ₂	ABC + CO ₂ + Octenol	ABC + dry ice	ABC + light	P value
Total mosquitoes ^{2,3}	531 a	911 a	1093 a	726 a	202 b	110 b	210 b	0.9 c	0.0001
<i>An. lesteri</i>	6.0 ab	2.2 bc	2.9 abc	1.1 a	1.3 bcd	1.3 bcd	0.8 cd	0.0 d	0.0002
<i>An. sinensis</i> ²	93.8 a	30.9 a	29.3 a	87 a	4.8 b	8.2 b	6.0 b	0.1 c	0.0001
<i>An. yatsushiroensis</i> ²	25.7 b	17.5 b	23.6 b	127 a	2.2 c	4.8 c	2 c	0.0 d	0.0001
<i>Ae. vexans</i> ^{2,3}	346 b	681 a	966 a	396 b	108 c	78.0 c	124 c	0.4 d	0.0001
<i>Cx. pipiens</i>	7.8 b	52.4 a	4.5 cd	2.1 cd	29.3 a	3.8 dc	67.4 a	0.2 d	0.0001
<i>Cx. orientalis</i>	0.0 b	0.3 b	0.0 b	0.0 b	1.0 a	0.0 b	0.4 ab	0.0 b	0.02

¹ *An.*, *Anopheles*; *Ae.*, *Aedes*; *Cx.*, *Culex*.
² Significant day effect ($P < 0.05$).
³ Significant trap location effects ($P < 0.05$).

Table 3. Hourly mean (\pm SEM) number of mosquitoes, by species, collected for Shannon trap collection in a rice field habitat ($n = 8$ nights).

Species ¹	1900	2000	2100	2200	2300
Total mosquitoes	0.4 \pm 0.3	196 \pm 53.3	283.8 \pm 103.7	145 \pm 50.5	106 \pm 36.7
<i>An. lesteri</i>	0.0 \pm 0.0	3.3 \pm 2.1	6.4 \pm 3.7	0.8 \pm 0.4	1.5 \pm 0.7
<i>An. sinensis</i>	0.0 \pm 0.0	32.6 \pm 13.8	45.6 \pm 19.9	13.8 \pm 5.0	9.9 \pm 5.3
<i>An. yatsushiroensis</i>	0.0 \pm 0.0	39.5 \pm 14.6	49.8 \pm 17.0	22.0 \pm 7.2	13.1 \pm 4.5
<i>Ae. vexans</i>	0.3 \pm 0.3	119 \pm 40.1	182 \pm 75.9	109 \pm 40.3	81.6 \pm 27.8
<i>Cx. pipiens</i>	0.0 \pm 0.0	1.8 \pm 0.6	0.1 \pm 0.1b	0.0 \pm 0.0	0.0 \pm 0.0

¹ *An.*, *Anopheles*; *Ae.*, *Aedes*; *Cx.*, *Culex*.

Shannon trap

A total of 8,653 mosquitoes was collected in the Shannon trap. Arithmetic means with standard errors for each of the common species are listed by hourly collections from 1900 to 0600 h (Table 3). No mosquitoes were captured between 1800 and 1900 h. Overall, *Ae. vexans* (700 \pm 268) was the most abundant species, followed by roughly equal numbers of *An. sinensis* (176 \pm 68) and *An. yatsushiroensis* (183 \pm 49; Table 3). More (although not significant) *An. lesteri* (18 \pm 7) were captured with the Shannon trap than with the other traps. Conversely, few *Cx. pipiens* or *Cx. orientalis* were collected in the Shannon trap when compared to those traps not baited with octenol as 1 of the attractants. All anophelines and *Ae. vexans* showed similar bimodal flight activities, which peaked during the periods 2000–2200 and 0300–0400 h. Relatively small numbers (mean = 2.9 per night) of *Cx. pipiens* were collected, but more than 50% of those collected were active between 2000 and 2100 h. The mean ($n = 8$ nights) relative percent composition of diel host-seeking activity for the most common species captured at the Shannon trap is shown in Fig. 1.

5 \times 5 study

A total of 5,443 mosquitoes was collected at Camp Greaves during the 5 trap nights. Back-transformed means, *P* values, and significant differences for the common species collected in the 5 \times 5 trials are shown in Table 4. No day effects were found, although significance ($\alpha = 0.05$) due to trap location was found for *An. yatsushiroensis* and *Cx. pipiens*. Overall, the Mosquito Magnet captured 3 times as many mosquitoes as the next most attractive trap (NJ) and more than 13 times as many as the dry ice-baited ABC light trap and Mosquito Killer trap. With the exception of *An. yatsushiroensis*, the NJ light trap did not capture significantly more mosquitoes ($\alpha = 0.05$) than the dry ice/CO₂-baited ABC trap and Mosquito Killer. Analysis using multiple comparison procedures was not done for *An. lesteri* ($P = 0.08$) or *Cx. orientalis* ($P = 0.44$) because of the small numbers that were collected and the lack of significant ($\alpha = 0.05$) differences among trap collections. Numbers too small

to analyze were collected for *An. sineroides* (3) and *Cx. tritaeniorhynchus* (2). Nearly equal numbers (532 and 547, respectively) of mosquitoes were captured in the Mosquito Magnet for both the 5 \times 5 and 8 \times 8 studies. However, perhaps because of competing light sources, the dry ice-baited ABC light trap captured 77% fewer mosquitoes than the dry ice-baited ABC light trap in the 8 \times 8 study. More *An. sinensis* were collected in the Mosquito Magnet, NJ light trap, and the Mosquito Killer than in the ABC light trap with dry ice. The Mosquito Magnet and NJ light trap captured more *An. yatsushiroensis* than the other traps. The dry ice-baited ABC light trap, Mosquito Killer, and Mosquito Magnet captured the most *Cx. pipiens*. When operated with CO₂, the Mosquito Killer captured numbers similar to the dry ice-baited ABC light trap. However, during a 3-night preliminary evaluation conducted before starting the 5 \times 5 trial, the Mosquito Killer trap was used per manufacturer's instructions without CO₂ as a supplementary attractant. During this 3-night period, no mosquitoes and very few other flying insects were captured.

DISCUSSION

Few field studies in the ROK have evaluated mosquito trap collections comparing different trap designs and attractants. A paucity of human cases of mosquito-borne diseases (malaria and Japanese encephalitis [JE]) in the ROK from the 1970s to the mid-1990s provided little impetus for U.S. Forces Korea or the Korean civilian government and research community to reevaluate mosquito trapping methods that remain an important component of their respective vector surveillance programs. As a result, both military and civilian mosquito surveillance programs have remained largely unchanged for decades.

Starting in 1993–94, autochthonous *Plasmodium vivax* malaria reestablished itself in the civilian and military communities in northwestern Kangwon and northern Kyonggi provinces bordering the ROK Demilitarized Zone (DMZ) (Lee et al. 1998, Kho et al. 1999, Ree 2000). The reemergence of malaria, as well as the presence of JE, emphasizes the need to develop efficient and effective mosquito surveillance methods. For malaria, this is especially

Table 3. Extended.

0000	0100	0200	0300	0400	0500	Total
60.8 ± 23.6	56.9 ± 29.1	68.0 ± 35.2	111 ± 40.3	52.0 ± 19.3	0.8 ± 0.3	1,082 ± 359.7
0.8 ± 0.3	0.9 ± 0.5	1.3 ± 0.5	2.1 ± 1.0	1.1 ± 0.5	0.0 ± 0.0	18.0 ± 6.8
6.4 ± 2.4	3.9 ± 0.6	12.6 ± 7.6	33.3 ± 16.9	18.1 ± 9.1	0.1 ± 0.1	176 ± 68.7
10.3 ± 4.8	8.5 ± 3.6	8.9 ± 2.7	22.1 ± 6.7	8.5 ± 3.4	0.1 ± 0.1	183 ± 49.4
43.1 ± 17.0	43.6 ± 25.2	44.9 ± 25.6	53.3 ± 25.7	23.9 ± 11.7	0.5 ± 0.3	700 ± 267.7
0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	2.9 ± 0.8

critical because the ROK had been largely malaria free since the late 1970s; malaria results in high morbidity among civilian and military personnel and adversely affects the economy and wartime readiness; delayed onset of symptoms of 6–18 months for the endemic *P. vivax* strain expedites its spread into malaria-free areas, especially via U.S. soldiers returning to the United States; and improvements in pathogen identification from mosquito pools and trapping technology expands vector surveillance to include disease surveillance and provides useful data for the medical community when applying and prioritizing limited vector control and medical resources.

Mosquito trap data from the ROK during the last 25–30 years provide a useful historical perspective of vector populations, changes in species composition, and control effectiveness. Unfortunately, the nonselective light-baited traps capture specimens in poor and often unidentifiable condition or in numbers insufficient for accurately estimating mosquito populations or evaluating infection rates for arbovirus or malaria parasites. With the exception of the Mosquito Killer and NJ light trap used in the 5 × 5 study, the other traps in this evaluation generally captured specimens alive and in good condition. Therefore, specimens collected in these traps were more easily and rapidly identified and were used for arbovirus isolations and determining malaria infection rates. This is especially important for the identification of emerging or reemerging and unknown arboviruses, because polymerase chain re-

action techniques would only capture those pathogens for which tests are specifically conducted.

Inconsistent reporting for *Anopheles* species captured in older trapping studies makes direct comparison of trapping studies difficult. Relatively large quantities of living, undamaged anopheline specimens preserving key identification characteristics were collected in the traps using counterflow technology (Mosquito Magnet and CFG traps), making this technology more efficient for both vector and nuisance mosquito identification.

Various review articles report that both *An. sinensis* and *An. yatsushiroensis* have been found to be naturally infected with sporozoites and to transmit malaria parasites to humans (Chow 1973, Paik et al. 1988, Chai 1999, Ree 2000). *Anopheles lesteri* also has been collected throughout the Korean peninsula and is often misidentified as the morphologically similar *An. sinensis*. In China, even though *An. lesteri* is less common, it is considered a more important malaria vector than *An. sinensis* (Liu et al. 1986). Some confusion remains in Korea over distinguishing *An. sinensis* and *An. lesteri*. Preliminary taxonomic examination of individual progeny broods showed that the primary morphological characters used to distinguish *An. sinensis* and *An. lesteri* are not always transferred to subsequent progeny. Because characters used to separate the adults of these 2 species are very similar, population densities of *An. lesteri* may be greater and their subsequent importance in malaria transmission more important than estimated by either light trap or human-bait collections. It is critical that the malaria vectors are characterized and their distribution and populations determined as a basic part of understanding the epidemiology of malaria and developing effective malaria vector and disease control strategies in the ROK.

In our field trials, both trap design and attractant were found to be important determinants for capturing total and species-specific numbers of medically important and nuisance species of *Anopheles* and culicine mosquitoes. Historically, large numbers of the primary JE vector, *Cx. tritaeniorhynchus*, do not appear along the Korean DMZ until mid-July. Subsequently, an additional trap study was conducted in September 2000 (published separately) to evaluate many of the same commercially available traps. In other regions of the world, stud-

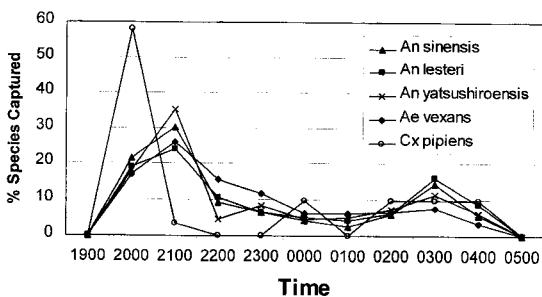


Fig. 1. Relative percent composition of hourly aspirator collections for common mosquito species captured at a human and propane lamp-baited Shannon trap ($n = 8$ nights).

Table 4. Mosquito species composition and back-transformed nightly means for variously baited commercially available mosquito traps. Three-way analysis of variance and multiple comparison (Ryan–Einot–Gabriel–Welsh multiple range test) were performed after $\log(x + 1)$ transformations. Means within each row having the same letter are not significantly different ($n = 5$ nights; $\alpha = 0.05$).

Species ¹	Magnet + Octenol	New Jersey	ABC + dry ice	Killer + CO ₂	CDC + Octenol	<i>P</i> value
Total mosquitoes	547 a	156 b	50.1 b	42.0 b	7.1 c	0.0001
<i>An. lesteri</i>	2.6	0.6	0.0	0.0	0.0	0.08
<i>An. sinensis</i>	48.4 a	23.3 a	1.8 b	8.5 ab	1.2 b	0.002
<i>An. yatsushiroensis</i> ²	13.2 a	15.7 a	1.2 b	2.6 b	1.1 b	0.001
<i>Ae. vexans</i>	407 a	101 b	29.0 b	13.0 b	2.8 c	0.0002
<i>Cx. pipiens</i> ²	3.8 ab	1.1 b	10.4 a	3.9 ab	0.4 b	0.006
<i>Cx. orientalis</i>	0.0	0.0	0.6	0.0	0.0	0.44

¹ *An.*, *Anopheles*; *Ae.*, *Aedes*; *Cx.*, *Culex*.

² Significant trap location effect ($P < 0.05$).

ies have compared and evaluated modern trap designs against mosquitoes and differences were observed in mosquito collections using various modern trap designs and attractant combinations when compared to older established methods (Vaidyanathan and Edman 1997, Kline 1999, Mboera et al. 2000, Reisen et al. 2000). As with those studies, we found that the addition of CO₂ increased the size of trap collections. However, unlike the study of Reisen et al. (2000), our study did not capture significantly fewer mosquitoes in dry ice-baited traps over those baited with CO₂ from compressed gas cylinders. For our study, mosquito representation differed significantly among trap design and attractant combinations, ranging from <1 mosquito per night for the ABC trap using only light as an attractant to >900 per night for the CFG traps. All trap designs in both trials consistently showed *Ae. vexans* as the most abundant mosquito captured during the trapping period. With few exceptions (notably *An. lesteri* and *An. yatsushiroensis*), other light trap studies in the same Korean province (Ree et al. 1973; Frommer et al. 1979; Lee et al. 1984; Kim et al. 1995; Shim et al. 1997; Kim et al. 1999; Kim 2000; Strickman et al. 2000) during a similar time of year found mosquito species composition and relative abundance consistent with those for our study.

With the exception of the Shannon trap, those traps employing counterflow technology (Mosquito Magnet and CFG traps) captured significantly greater numbers of mosquitoes than the variously baited ABC or CDC light traps. This agrees with the studies of Kline (1999) and Mboera et al. (2000), which also found CFG traps to capture significantly more mosquitoes than CO₂-baited CDC traps. Unfortunately, the CFG traps are not yet (and may not become) commercially available.

Some of the results from octenol-baited trap were surprising. Kline (1994) reported that traps baited with octenol and CO₂ are synergistic for many species, but attractive to only a few when octenol is used alone. In the 8 × 8 study, when octenol-baited traps were compared to similarly baited traps with

no octenol, no synergistic effects were observed. Indeed, the opposite occurred for selected species. Although other authors (Becker et al. 1995, Mboera et al. 2000) found no significant difference in *Cx. pipiens* in octenol- versus non-octenol-baited traps, we observed that all octenol-baited traps were repellent and captured significantly fewer *Cx. pipiens* and no *Cx. orientalis* than unbaited traps. No previous report has documented species-specific decreases in trap collections for octenol-baited traps. Although not compared directly, the octenol-baited CDC light trap (no CO₂) in the 5 × 5 trial captured more (7/trap/night) mosquitoes than the unbaited ABC light trap in the 8 × 8 trial, which captured <1 mosquito per night. In another study (Reisen et al. 1999), NJ light traps were found to collect representative samples of several, but not all, medically important mosquitoes. Simultaneous use of CO₂-baited CDC traps was required to capture mosquito species not adequately represented in the NJ traps. In our 5 × 5 study, the unbaited NJ light trap captured significantly more mosquitoes than the dry ice-baited ABC light trap. This is inconsistent with other studies (Acuff 1976, Slaff et al. 1983), which almost invariably found 4 or more times as many mosquitoes (variable by species) collected in CO₂-baited CDC traps.

Although human landing/biting collections were not used in our study per se, human bait was a primary attractant to gather hourly flight information for host-seeking mosquitoes collected at the Shannon trap. Dry ice-baited bed nets or Shannon-like traps have previously been used in Japan and Korea. Sasa and Sabin (1950) and Ree et al. (1969) captured similar species compositions to those in this study, including *Ae. vexans*, *An. sinensis*, *Cx. pipiens*, and *Cx. tritaeniorhynchus*. Although not significantly different, the mean number of *Anopheles* species captured by the Shannon trap was greater than for other traps. Assuming that human-bait and Shannon trap collections are similar, this is not consistent with results of Davis et al. (1995), who captured 1.2 times as many *Anopheles* using light traps when compared to human-bait collec-

tions. The Shannon trap was not effective for all species. Although useful for *Anopheles* species, the human-baited Shannon trap did not effectively attract *Cx. pipiens* or *Cx. orientalis*.

Time of year and weather conditions, among others, are obvious and well-documented factors that play significant roles in mosquito diel flight and host-seeking activities. In Korea, location, even within the same province, also seems to affect flight and biting activity. As an example, hourly human-landing collections reported by Strickman et al. (2000) found a high degree of variability for biting activity in *An. sinensis* at different locations throughout Korea. Interestingly, our study, conducted near Taesung-Dong, showed a similar bimodal biting pattern to that observed by Strickman et al. (2000). The results of an hourly light-trap collection made in Kyungbook Province in July by Joo and Kang (1992) also demonstrated *An. sinensis* host-seeking activity similar to that found in our study. However, results of the study of Joo and Wada (1985) were very different from ours, with peak activity from 1200 to 0400 h for *An. sinensis* for cow-baited light trap collections. Depending on the mosquito species, diel host-seeking and flight variability for vector or nuisance species can complicate control efforts when trying to optimize the timing of ultra-low-volume adulticide applications or other management efforts to correspond with those of peak mosquito activity and susceptibility to insecticidal treatments.

Based on either the total number of mosquitoes, the number of a vector species (*Cx. tritaeniorhynchus*), or both collected in NJ light traps, Yi et al. (1988) developed trap indices for U.S. military installations in the ROK. A modified trap index (25 females/night/trap [May 1 to July 31], and 10 females/trap/night [August 1 to October 15]) based on this study is used by U.S. Army pest management and preventive medicine personnel as a means to justify and implement control measures. Should the U.S. military decide to change trapping procedures based on the results presented in our study and use more efficient Mosquito Magnets or other CFG traps for mosquito and vector surveillance at all or select installations (especially in malarious areas along the DMZ) the trap indices will have to be adjusted. Additional research will be needed to establish realistic and effective trap indices for selected traps. Changing traps to 1 of the more efficient CFG-type traps will not solve the present malaria situation, but would almost certainly improve and the ability to monitor vector populations and possibly provide a significant reduction of mosquito populations in limited areas. The Mosquito Magnet in particular can be placed where needed and is not subject to the restrictions and logistical problems imposed by using NJ light traps or dry ice-baited CDC-type traps. An additional benefit of switching to 1 of the counterflow technology traps includes expanding the current mosquito and vector surveil-

lance program into a more comprehensive disease surveillance program where larger numbers of mosquitoes are collected and where mosquito pools can be tested for arbovirus and malaria infection rates. Taking statistical subsamples from the larger trap collections can attenuate the additional burden placed on identification personnel.

The vector surveillance options currently available for use during military exercises and humanitarian, contingency, or other remote operations are limited. For those surveillance options available, landing/biting collections are controversial and may expose personnel to life-threatening diseases. Mechanical mosquito traps (i.e., NJ light traps) are impractical because of electricity requirements and are otherwise bulky and difficult to pack. Likewise, the wide range of collapsible, and portable battery-operated CDC light or CFG traps are virtually useless in the absence of carbon dioxide as an attractant. Dry ice or compressed gas cylinders are generally not readily available during remote operations. The currently available counterflow technology traps (i.e., Mosquito Magnet), although practical for use in cantonment and nearby training sites, are largely impractical for deployments because of their size and poor shipping ability. Ideally, a portable and effective mosquito trap needs to be developed that incorporates durability, increased portability, and easily obtained and used attractants that collect as many mosquitoes as possible. A collapsible CFG-type trap with the ability to use propane gas as an attractant and power source would be ideal. In general, propane gas and tanks are used worldwide for heating, refrigeration, and other uses and tend to be available worldwide and are less of a logistical problem to transport than compressed CO₂ gas cylinders or dry ice.

This study illustrates both similarities and differences in adult mosquito collections based on both old and new technologies, as well as results from other parts of the world. The unique mosquito fauna of the ROK makes it critical that tests are conducted for local species and not extrapolated for species elsewhere. The benefits of these new technologies are great, including producing live collections for arbovirus isolation, collecting specimens in good condition for increasing ease and reliability of identification, allowing uniformity of collection technique, and collecting large numbers of mosquitoes that may effectively reduce biting populations when traps are employed near human activities. These new technologies need to be employed in highly portable, effective, and efficient traps for inclusion as part of the U.S. military mosquito surveillance and control system. Evaluating new designs and technologies where U.S. soldiers are deployed will increase our knowledge of vectors and methods for implementing vector and disease control strategies.

ACKNOWLEDGMENTS

We are extremely thankful to James Kirkpatrick, Commander, 18th Medical Command, for his support in evaluation of mosquito traps that may serve to enhance the U.S. Forces Korea Malaria Prevention Plan. We thank Wan Y. Kim from the U.S. Army Center for Health Promotion and Preventive Medicine-Pacific (CHPPM-PAC), whose help in logistics and as an interpreter were vital for completing these joint agency field evaluations. We also thank Kotu Phull, Zia Mehr, Anthony Schuster, and others from CHPPM-PAC for their support and critical review. Sincere thanks to Patrick T. Stackpole, Commander, and the other soldiers of Camp Greaves, who provided facilities necessary for these studies. Thanks to Brian Blaylock and Velvan Webb from Public Health Flight at the 51st Aerospace Medical Squadron, Osan Air Base, ROK, and Richard Johnson, Armed Forces Pest Management Board, MD, who kindly provided us with several traps. We would also like to acknowledge Henry McKeithen, USDA-ARS, Gainesville, FL, and Chad McHugh, Air Force Institute for Environment, Safety, and Occupational Health Risk Analysis, who helped run SAS data sets. We are grateful for the assistance of Tae Kyu Kang, Edwin Huertas, Hee Choon Lee, Myong Wa Yi, and other members of the Preventive Services Directorate, 18th Medical Command, for their logistical and technical support. Also, we thank Sonya Schleich, Commander, 5th Medical Detachment, 168th Medical Battalion (Area Support), for providing much needed personnel to perform the study. We greatly appreciate the assistance of Seung Ho Kang and Yong Bum Yi, Laboratory of Medical Zoology, Department of Viral Disease, National Institute of Health, Ministry of Health and Welfare, Seoul, Republic of Korea. Finally, we wish to acknowledge trap donations from American Biophysics Corporation and Environmental Products and Research. Funding for this project was provided by the Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS), Walter Reed Army Institute of Research, and the 18th Medical Command, 8th U.S. Army, Republic of Korea. The mention of trade names or commercial products does not constitute endorsement or recommendation for use by the Department of Defense, Centers for Disease Control and Prevention, or the Korean National Institute of Health.

REFERENCES CITED

- Acuff VR. 1976. Trap biases influencing mosquito collecting. *Mosq News* 36:173-176.
- Baik DH, Joo CY. 1991. Epidemio-entomological survey of Japanese encephalitis in Korea. *Korean J Parasitol* 29:67-85.
- Becker N, Zgomba M, Petric D, Ludwig M. 1995. Comparison of carbon dioxide, octenol and a host-odour as mosquito attractants in the Upper Rhine Valley, Germany. *Med Vet Entomol* 9:377-380.
- Chai JY. 1999. Re-emerging *Plasmodium vivax* malaria in the Republic of Korea. *Korean J Parasitol* 37:129-143.
- Chow CY. 1973. Arthropods of public health importance in Korea. *Korean J Entomol* 3:31-54.
- Davis JR, Hall T, Chee EM, Majala A, Minjas J, Shiff CJ. 1995. Comparison of sampling anopheline mosquitoes by light-trap and human-bait collections indoors at Bagamoyo, Tanzania. *Med Vet Entomol* 9:249-255.
- Frommer RL, Pae CM, Lee TK. 1979. The distribution of adult mosquitoes collected from light traps in the Republic of Korea during 1977. *J Korean Med Assoc* 22:373-381.
- Joo CY, Kang GT. 1992. Epidemio-entomological survey on malarial vector mosquitoes in Kyongbuk, Korea. *Korean J Parasitol* 30:329-340.
- Joo CY, Wada Y. 1985. Seasonal prevalence of the vector mosquitoes of Japanese encephalitis virus in Kyungpook Province, Korea. *Korean J Parasitol* 23:139-150.
- Kho WG, Jang JY, Hong ST, Lee HW, Lee WJ, Lee JS. 1999. Border malaria characters of reemerging *vivax* malaria in the Republic of Korea. *Korean J Parasitol* 37:71-76.
- Kim HC 2000. *January 2000. Light trap mosquito surveillance on U.S. Army installations in the Republic of Korea, May-October 1999.* Available from Department of the Army, 5th Medical Detachment, 168th Medical Battalion, 18th Medical Command, APO AP96205-0020. 39 p.
- Kim HC, Lee KW, Klein TA, Strickman DA. 1999. Seasonal prevalence of mosquitoes collected from light traps in Korea (1995-1996). *Korean J Entomol* 29:181-187.
- Kim HC, Lee KW, Robert LL, Sardelis MR, Chase FE. 1995. Seasonal prevalence of mosquitoes collected from light traps in Korea (1991-1992). *Korean J Entomol* 25:225-234.
- Kim HC, Strickman DA, Lee KW. 2000. Seasonal prevalence and feeding activity of *Anopheles sinensis* (Diptera: Culicidae) in the northwestern part of Kyonggi Province, Republic of Korea. *Korean J Entomol* 30:193-197.
- Kline DL. 1994. Olfactory attractants for mosquito surveillance and control: 1-octen-3-ol. *J Am Mosq Control Assoc* 10:280-287.
- Kline DL. 1999. Comparison of two American Biophysics mosquito traps: the professional and a new counterflow geometry trap. *J Am Mosq Control Assoc* 15:276-282.
- Lee KW 1998. *A revision of the illustrated taxonomic keys to genera and species of female mosquitoes of Korea (Diptera: Culicidae)* Available from Department of the Army, 5th Medical Detachment, 168th Medical Battalion, 18th Medical Command, APO AP96205-0020. 40 p.
- Lee KW, Gupta RK, Wildie JR. 1984. Collection of adult and larval mosquitoes in U.S. Army compounds in the Republic of Korea during 1979-1983. *Korean J Parasitol* 22:102-108.
- Lee JS, Kho WG, Lee HW, Seo M, Lee WJ. 1998. Current status of *vivax* malaria among civilians in Korea. *Korean J Parasitol* 36:241-248.
- Lee KW, Shin HK, Yoon HS, Tonn RJ, Self LS, Cho YS, Ahn SK (World Health Organization). 1971. *Japanese encephalitis vector studies in Korea: light trap surveys WHO/VBC/71.324.* 10 p.
- Lee SK, Ree HI. 1991. Studies on mosquito population dynamics in Cholla-bugdo, Korea (1985-1990). I. Sea-

- sonal and annual fluctuations in population size. *Korean J Entomol* 21:141-155.
- Liu C, Qian H, Gu Z, Pan J, Zheng X. 1986. Quantitative study on the role of *Anopheles lesteri anthropophagus* in malaria transmission. *J Parasitol Parasitic Dis* 4: 161-164.
- Mboera LE, Takken W, Sambu EZ. 2000. The response of *Culex quinquefasciatus* (Diptera: Culicidae) to traps baited with carbon dioxide, 1-octen-3-ol, acetone, butyric acid and human foot odour in Tanzania. *Bull Entomol Res* 90:155-159
- Paik YH, Ree HI, Shim JC. 1988. Malaria in Korea. *Jpn J Exp Med* 58:55-66.
- Ree HI. 2000. Unstable *vivax* malaria in Korea. *Korean J Parasitol* 38:119-138.
- Ree HI, Chen YH, Chow CY. 1969. Methods of sampling populations of the Japanese encephalitis vector mosquitoes—a preliminary report. *Med J Malaya* 23:293-295.
- Ree HI, Self LS, Hong HK, Lee KW. 1973. Mosquito light trap surveys in Korea, 1969-1971. *SE Asian J Trop Med Public Health* 4:328-386.
- Reisen WK, Boyce K, Cummings RC, Delgado O, Gutierrez A, Meyer RP, Scott TW. 1999. Comparative effectiveness of three adult mosquito sampling methods in habitats representative of four different biomes of California. *J Am Mosq Control Assoc* 15:24-31.
- Reisen WK, Meyer RP, Cummings RF, Delgado O. 2000. Effects of trap design and CO₂ presentation on the measurement of adult mosquito abundance using Centers for Disease Control-style miniature light traps. *J Am Mosq Control Assoc* 16:13-18.
- Sasa M, Sabin AB. 1950. Ecological studies on the mosquitoes of Okayama in relation to the epidemiology of Japanese B encephalitis. *Am J Hyg* 51:21-35.
- SAS Institute. 1995. *SAS/STAT user's manual, version 6.03*. Cary, NC: SAS Institute.
- Self LS, Shin HK, Kim KH, Lee KW, Chow CY, Hong HK. 1973. Ecological studies on *Culex tritaeniorhynchus* as a vector of Japanese encephalitis. *Bull WHO* 49:41-47.
- Service MW. 1993. *Mosquito field ecology, field sampling methods* London: Chapman and Hall.
- Shim JC, Shin EH, Yang DS, Lee WK. 1997. Seasonal prevalence and feeding time of mosquitoes (Diptera: Culicidae) at outbreak regions of domestic malaria (*P. vivax*) in Korea. *Korean J Entomol* 27:267-274.
- Shim JC, Yoon YH, Kim CL, Lee WJ, Shin EH, Cho YB. 1990. Population densities of the vector of Japanese encephalitis *Culex tritaeniorhynchus* in Korea. *NIH Rpt Korea* 27:165-172.
- Slaff M, Crans WJ, McCuiston LJ. 1983. A comparison of three mosquito sampling techniques in northwestern New Jersey. *Mosq News* 43:287-290.
- Strickman D, Miller ME, Kim HC, Lee KW. 2000. Mosquito surveillance in the Demilitarized Zone, Republic of Korea, during an outbreak of *Plasmodium vivax* malaria in 1996 and 1997. *J Am Mosq Control Assoc* 16: 100-113.
- Vaidyanathan R, Edman JD. 1997. Sampling methods for potential epidemic vectors of eastern equine encephalomyelitis virus in Massachusetts. *J Am Mosq Control Assoc* 13:342-347.
- Yang KH, Yu HS. 1992. Seasonal abundance and breeding habitats of mosquitoes (Culicidae; Diptera) from Suwon, Korea. *Entomol Res Bull (Korea)* 18:15-24.
- Yi SC, Zorka TJ, Edgecomb RS. 1988. Comparative evaluation of trap index limit to control population. *Korean J Entomol* 18:197-203.