COMPARISON OF THREE METHODS FOR COLLECTING ADULT MOSQUITOES ASSOCIATED WITH RICEFIELD AND IRRIGATED PASTURE HABITATS IN NORTHERN CALIFORNIA

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ABSTRACT. A complement of one 0.3 m^3 red box, CDC miniature light trap baited with dry ice (CO_2 light trap) and New Jersey light trap were placed at 4 locations in northern California to evaluate their effectiveness in sampling *Aedes melanimon, Anopheles freeborni* and *Culex tarsalis*. The CO_2 light traps collected the largest numbers of *Cx. tarsalis*. Both of the light traps were equally effective for sampling *Ae. melanimon*. New Jersey light traps were most effective for collecting *An. freeborni*. The highest proportions of gravid and blood-fed *An. freeborni* and *Cx. tarsalis* were collected in red boxes and for *Ae. melanimon* in New Jersey light traps. Parous rates were highest for female *Cx. tarsalis* sampled by CO_2 light traps, for female *An. freeborni* sampled by either CO_2 light traps or New Jersey light traps and for female *Ae. melanimon* sampled by New Jersey light traps. Males and teneral females were more abundant in traps located near breeding sources.

INTRODUCTION

In the Central Valley of California, *Aedes melanimon* Dyar, *Anopheles freeborni* Aitken and *Culex tarsalis* Coquillett are important vectors of arthropod-borne diseases of humans. *Aedes melanimon* and *Cx. tarsalis* transmit endemic arboviruses (Reeves and Hammon 1962, Hardy and Bruen 1974), while *An. freeborni* has transmitted autochthonous human malaria (Bailey 1972). Local mosquito abatement agencies use the New Jersey light trap (Mulhern 1953), the CDC miniature light trap (Sudia and Chamberlain 1962) baited with dry ice, and the 0.3 m^3 red box (Goodwin 1942) for monitoring changes in population abundance of these species in areas where rice is grown (*An. freeborni* and *Cx. tarsalis*) or where land has been converted into large irrigated pastures (*Ae. melanimon*). The present study compares the relative trapping efficiency of each sampling method for collecting the 3 species in a mixed agricultural habitat in northern California and also includes a comparison of the metabolic and parity status of female mosquitoes sampled by each method.

MATERIALS AND METHODS

STUDY AREA. Mosquitoes were collected at 4 residences in Sutter County, California, USA. Two residences, 1.5 km E and 2.0 km SSE of the town of Sutter, were surrounded by ricefields and irrigated pastures that supported preimaginal populations of *Ae. melanimon* (pastures only), *An. freeborni* and *Cx. tarsalis*. The remaining 2 residences were within the incorporated boundaries of Sutter. One residence, 0.5 km N of the center of town, was located within an almond orchard, while the other was within a residential area 0.2 km E of the center of town. All residences were well-shaded and landscaped.

SAMPLING AND PROCESSING OF COLLECTIONS. Mosquitoes were live-trapped by one red box, CO_2 light trap and New Jersey light trap at each residence. Each CO_2 light trap was baited with ca. 2.5 kg of dry ice. New Jersey light traps were operated with a 25-watt incandescent light bulb and fitted with a collection bag from a CDC light trap. At each residence, the New Jersey light trap and CO_2 light trap were operated at fixed locations that were separated by 30 m to reduce intertrap competition (Barr 1963). The red boxes were placed either on well-shaded porches or under vegetation to minimize exposure to direct sunlight.

Six biweekly collections were made between July 10 and September 18, 1974. New Jersey light traps and CO_2 light traps were operated from sunset to sunrise (1900–0800 hr). Adult mosquitoes were collected from red boxes by a battery-powered mechanical aspirator the morning following the night when light traps were operated. Mosquitoes collected by all traps were transferred by mechanical aspirator to 3.8 liter cylindrical screened holding cartons, humidified with moist toweling and transported to the laboratory for processing.

Collections were sorted by sex and species. All females were classified as to metabolic state (Bellamy and Reeves 1963). A maximum of 50 females of each species was dissected in each collection to determine parity by using the dilatation method of Polovodova for *Ae. melanimon* and *An. freeborni* and the tracheation method of Detinova for *Cx. tarsalis* (Detinova 1962). Ovarian dissections were performed according to the methods described by Giglioli (1963). Females of *An. freeborni* and *Ae. melanimon* with single or serial dilatations on a minimum of 20 ovarioles were scored as parous and

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the number of gonotrophic cycles recorded. Teneral females were identified by the presence of the green abdominal coloration and/or meconium (Rosay 1961).

DATA EVALUATION. The mean collection size of males and females, proportion of females in each metabolic state, and the proportion of parous females of each species were compared among trapping methods. Collection data were transformed to $\log_{10}$ (N + 1) and the means compared by Duncan's Multiple Range Test. Intertrap differences in the summed proportion of females in each metabolic state and gonotrophic-age were tested by contingency χ^2. Consistency of intertrap parous rates and metabolic states of females collected near breeding sites and at the residences in Sutter were tested for each species by homogeneity χ^2 (Sokal and Rohlf 1969).

RESULTS

The temporal abundance of *Ae. melanimon* and *An. freeborni* measured at each residence by each trapping method varied sporadically, however the abundance of *Cx. tarsalis* sampled concurrently by each method decreased progressively ($p<0.05$) from July until the termination of sampling in mid-September (Fig. 1). Sampling efficiency of the 3 collection methods varied considerably for each species, and some methods were selective for sampling either males or females (Table 1). Red boxes and New Jersey light traps were equally effective for sampling both sexes of *An. freeborni* and *Cx. tarsalis*. CO_2 light traps were particularly effective for sampling large numbers of female *Cx. tarsalis*. Females of *Ae. melanimon* were equally abundant in New Jersey light traps and CO_2 light traps, while males were collected predominantly by New Jersey light traps. Red boxes were ineffective for sampling either sex of *Ae. melanimon*.

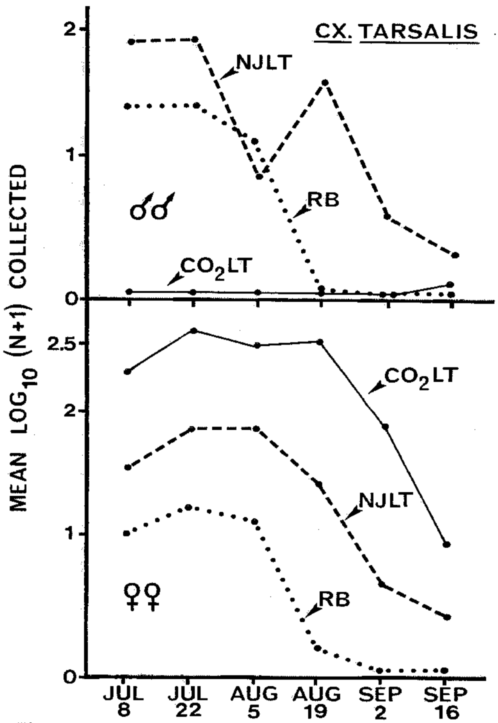

Fig. 1. Abundance of male and female *Culex tarsalis* sampled by 3 methods (n = 4 per sample date). RB = red box, NJLT = New Jersey light trap, CO_2LT = CO_2 light trap.

The number of conspecific males and females sampled by each method were compared (differences tested by ANOVA, data backtransformed to $\bar{X}$w or William's Mean) between residences located near breeding sources and those located in Sutter. Males of *An. freeborni* were more abundant in red boxes

Table 1. Comparison of the mean collection sizes ($\bar{X}$w ± SE) of species sampled by red box (RB), CO_2 light trap (CO_2LT) and New Jersey light trap (NJLT)

Method	*Anopheles freeborni*	*Aedes melanimon*	*Culex tarsalis*
	Mean ± S.E. females per collection[1]		
RB	28.8 ± 8.3 ab[2]	0.04 ± 0.04 b	6.9 ± 3.3 c
CO_2LT	11.1 ± 4.6 b	14.4 ± 4.4 a	249.9 ± 102.3 a
NJLT	74.1 ± 26.9 a	9.8 ± 4.3 a	47.3 ± 16.2 b
	Mean ± S.E. males per collection[1]		
RB	23.6 ± 5.9 a	0.00 ± 0.00 b	11.9 ± 5.4 b
CO_2LT	0.8 ± 0.4 b	0.2 ± 0.1 b	0.8 ± 0.3 c
NJLT	20.7 ± 7.9 a	5.1 ± 2.1 a	48.8 ± 22.1 a

[1] N = 24 per collection method.
[2] Means followed by the same letter are not significantly different in a Duncan's Multiple Range Test, p > 0.05.

near ricefields ($p<0.01$) ($\bar{X}w=75.2 \pm 31.3$ compared to 7.3 ± 2.4). New Jersey light traps operated near ricefields collected significantly ($p<0.01$) more males (188.1 ± 85.9) and females (164.5 ± 57.6) than New Jersey light traps operated in Sutter (7.2 ± 3.6, 16.5 ± 5.1, respectively). Adults of *Ae. melanimon* were equally abundant at all residences and differences in abundance were not apparent for other collection methods for sampling *An. freeborni* and *Cx. tarsalis* near breeding sources.

Tests for homogeneity, excluding female *An. freeborni* sampled by red boxes, did not indicate significant differences in the composition of metabolic states ($\chi^2 = 4.86$, $p>0.05$) and parity ($\chi^2 = 3.64$, $p>0.10$) of females collected in traps operated near breeding sources and those in Sutter. The proportion of blooded (0.41), gravid (0.06) and parous (0.24) *An. freeborni* was significantly greater ($p<0.05$) in red boxes near ricefields than those in Sutter (0.18, 0.02, 0.15, respectively).

Red boxes sampled a significantly greater proportion of blood-fed and gravid *An. freeborni* and *Cx. tarsalis* than either of the other traps (Table 2). Unfed females of both species comprised greater than 0.98 of the total numbers collected by New Jersey light traps or CO_2 light traps. More blood-fed and gravid *Ae. melanimon* were collected by New Jersey light traps and unfed individuals by CO_2 light traps.

Parity was comparable among female *An. freeborni* sampled by New Jersey light traps and CO_2 light traps, but was lowest in red boxes (Table 3). The proportion of multiparous (>1-parous) *An. freeborni* was not that different between both types of light traps and was again lowest in red boxes. Proportionately more parous and multiparous *Ae. melanimon* were sampled by New Jersey light traps compared to CO_2 light traps. The highest proportion of parous *Cx. tarsalis* was sampled by CO_2 light traps and the lowest by New Jersey light traps.

Less than 0.04 of the female *Ae. melanimon* and *Cx. tarsalis* collected by any method were determined to be teneral. The proportion of teneral female *An. freeborni* was higher ($p<0.001$) in red boxes (0.17) than in either of the light traps (0.06). Teneral *An. freeborni* were slightly more common ($p<0.05$) in red boxes near ricefields (0.19) than in Sutter (0.14).

DISCUSSION

Results of this study have illustrated that males and females of the 3 species were collected selectively by the 3 sampling methods we evaluated. New Jersey light trap and CO_2 light trap catches may have been biased as a result of trap location, since both traps were operated at fixed locations and were not rotated to reduce systematic sampling error (Bidlingmayer 1967). Climatological phenomena posed no major problems with sampling as relatively fair conditions prevailed from July through September and wind velocities never exceeded ca. 10 km/hr on each trapping night. Sporadic changes in mosquito relative abundance, particularly of *An. freeborni* and *Ae. melanimon*, measured at all residences are difficult to resolve. Most likely, localized adult population movements and temporal changes in adult recruitment from nearby production sources affected sampling continuity. Preimaginal populations were not sampled routinely to determine temporal changes in larval-pupal densities. The progressive decline in the relative abundance of

Table 2. Composition of metabolic states of species sampled by red box (RB), CO_2 light trap (CO_2LT) and New Jersey light trap (NJLT).

Method	No. females collected	Proportion		
		Empty	Blood-fed	Gravid
		Anopheles freeborni		
RB	692	0.66***[1]	0.30***	0.04***
CO_2LT	265	0.98[2]	0.02	0.00
NJLT	1784	0.99	0.007	0.003
		Aedes melanimon		
RB	1	1.00	0.00	0.00
CO_2LT	346	0.98	0.02	0.00
NJLT	234	0.92**	0.03**	0.05 **
		Culex tarsalis		
RB	116	0.78***	0.13 ***	0.09***
CO_2LT	6008	0.99	0.007	0.003
NJLT	1136	0.98	0.00	0.02

[1] Significance between proportions tested by 2×3 contingency χ^2, ** $p < 0.01$, *** $p < 0.001$.
[2] Proportions not marked by an asterisk (s) are not significantly different, $p > 0.05$.

Table 3. The gonotrophic-age of species sampled by red box (RB), CO_2 light trap (CO_2LT) and New Jersey light Trap (NJLT).

Method	No. females examined	Gonotrophic-age					
		Nulliparous	Parous	P−1[1]	P−2	P−3+	Proportion Parous
			Anopheles freeborni				
RB	574	474	100	86	9	5	0.17 b[2]
CO_2LT	153	92	61	50	11	0	0.40 a
NJLT	344	197	147	115	24	8	0.43 a
			Aedes melanimon				
RB	1	1	0	0	0	0	0.00 c
CO_2LT	243	134	109	101	8	0	0.45 b
NJLT	75	32	43	37	5	2	0.57 a(*)
			Culex tarsalis				
RB	124	70	54	—	—	—	0.44 b
CO_2LT	1229	554	675	—	—	—	0.56 a
NJLT	225	144	81	—	—	—	0.36 c

[1] Parous − 1, − 2, − 3+.
[2] Proportions followed by the same letter are not significantly different in 2 × 2 contingency χ^2, $p > 0.001$, (*) $p < 0.05$.

Cx. tarsalis after mid-summer has been consistent in the Sutter area in recent years.

Red boxes proved to be totally ineffective for sampling adults of *Ae. melanimon*. In agreement with our data, previous studies have shown that large numbers of *Aedes*, particularly females, were best collected by either CO_2 attractant or animal-baited traps (Olson and Grimes 1974, Pennington and Lloyd 1975) or by light trap (Hayes et al. 1973).

Differences in trap selectivity among the different species and sexes were a manifestation of the behavioral responses to the attractant presented by the New Jersey light trap (phototaxis) or CO_2 light trap (chemotaxis + weak phototaxis) (Service 1976). Dry ice (CO_2) significantly increased the relative attractiveness of the CO_2 light traps to female *Cx. tarsalis* with smaller numbers being collected concurrently by New Jersey light traps operated with 25-watt incandescent light bulbs. Apparently female *Cx. tarsalis* exhibit a more sensitive chemotactic response to CO_2 than a phototactic response to incandescent light. Milby et al. (1978) calculated the sampling efficiency of the CO_2 light trap to be 3- to 10-fold greater than that of a New Jersey light trap. By comparison, males of *Cx. tarsalis* were strongly phototactic and were sampled most abundantly by New Jersey light traps. Barr (1960) tested the phototactic responses of male and female *Cx. tarsalis* and found that males are more photosensitive than females as the level of illumination was increased. Unlike female *Cx. tarsalis*, female *An. freeborni* were considerably more phototactic than males and responded strongly to New Jersey light traps. This observation also concurs with that of Barr et al. (1960) and Milby et al. (1978). Overall, CO_2 light traps were relatively ineffective for sampling female *An. freeborni*. Interestingly, our data indicated that both light traps were equally effective for sampling female *Ae. melanimon*.

Historically, natural and artificial shelters in California have provided consistent numbers of blood-fed and gravid female *An. freeborni* and *Cx. tarsalis* (Loomis and Green 1959, Bellamy and Reeves 1963). The proportion of blood-fed and gravid females of both species collected from red boxes at Sutter was in temporal (mid- to late summer) agreement with the proportion of these metabolic states among females of both species collected from red boxes in the San Joaquin River delta (Loomis and Green 1959). In the arid Sierra Nevada foothills of Kern County, California, the proportion of blood-fed and gravid *Cx. tarsalis* collected from large walk-in (1 × 1 × 2 m) red boxes was considerably larger (Reisen et al. 1983). Perhaps geography, habitat or size of the red box influences the utilization of different resting sites and thus, the effectiveness of red boxes to sample female mosquitoes in various metabolic states.

Most (0.98) females collected by CO_2 light traps were unfed and presumably host-seeking. Reisen et al. (1983) showed that the follicular stages of most female *Cx. tarsalis* collected by the light trap were characteristic of females that were host-seeking. In our studies, CO_2 light traps collected a very small proportion (0.02) of either blood-fed or gravid females. Their capture was probably in response to the low photo-attraction of the CDC light trap. New Jersey light traps provided the best sampling method for collecting blood-fed and gravid *Ae. melanimon*.

Dry ice supplied to CDC light traps was very effective for attracting parous female *Cx. tarsalis* and comparable to the New Jersey light trap for attracting parous female *Ae. melanimon*. However, CO_2 light traps were not nearly as effective for attracting parous female *An. freeborni* as were New Jersey light traps. Similar trap comparison studies by Magnarelli (1975) and Feldlaufer and Crans (1979) revealed that parous females of different mosquito taxa also exhibited a variable attractiveness to CO_2-baited and unbaited light traps. Trap-specific parous rates also have been shown to differ between conspecific female populations sampled in different habitats (Bidlingmayer et al. 1974, Magnarelli 1975). Parous rates were higher among female *An. freeborni* collected from red boxes at residences near ricefields than among females collected from red boxes at residences in Sutter.

With the apparent lack of uniform trap attractiveness among parous females of the 3 species sampled in this study, vector surveillance in the Central Valley of California would require the incorporation of several sampling methods. For example, parous female *Ae. melanimon* and *Cx. tarsalis* potentially infected with endemic arboviruses could be effectively sampled by CO_2 light trap collections. However, the concurrent sampling of parous *An. freeborni* potentially infected with human malaria would require the supplemental deployment of either red boxes or less-portable New Jersey light traps. Shelter-collected females of *Cx. tarsalis* have yielded an unusually high recovery rate of arboviruses in California (Reeves and Hammon 1962) and were more sensitive in detecting arboviral activity than females collected by the CO_2 light trap (Sudia et al. 1971). Therefore, shelter-collected female *Cx. tarsalis* should be included for viral testing in California arboviral surveillance programs. We would also recommend that CO_2 light traps be used instead of New Jersey light traps for sampling female *Cx. tarsalis* when population abundances are relatively low as measured by the New Jersey light trap.

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References Cited

Bailey, S. F. 1972. Some ecological aspects of malaria in California. Proc. Calif. Mosq. Control Assoc. 40:87–92.

Barr, R. 1963. Evaluation of some factors affecting efficiency of light traps in collecting mosquitoes. J. Econ. Entomol. 56:123–127.

Barr, R. A., T. A. Smith and M. M. Boreham. 1960. Light intensity and the attraction of mosquitoes to light traps. J. Econ. Entomol. 53:876–880.

Bellamy, R. E. and W. C. Reeves. 1963. The winter biology of *Culex tarsalis* (Diptera: Culicidae) in Kern County, California. Ann. Entomol. Soc. Am. 56:314–323.

Bidlingmayer, W. L. 1967. A comparison of trapping methods for adult mosquitoes: species response and environmental influence. J. Med. Entomol. 4: 200–220.

Bidlingmayer, W. L., B. P. Franklin, A. M. Jennings and E. F. Cody. 1974. Mosquito flight paths in relation to the environment. Influence of blood meals, ovarian stage and parity. Ann. Entomol. Soc. Am. 67:919–927.

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. Wld. Hlth. Org., Geneva, 216 pp.

Feldlaufer, M. F. and W. J. Crans. 1979. The relative attractiveness of carbon dioxide to parous and nulliparous mosquitoes. J. Med. Entomol. 15:140–142.

Giglioli, M. E. C. 1963. Aids to ovarian dissection for age-determination in mosquitoes. Mosq. News 23: 156–159.

Goodwin, M. H. Jr. 1942. Studies on artificial resting places of *Anopheles quadrimaculatus* Say. J. Nat. Malaria Soc. 1:93–99.

Hardy, J. L. and J. P. Bruen. 1974. *Aedes melanimon* as a vector of WEE virus in California. Proc. Calif. Mosq. Control Assoc. 42:36.

Hayes, R. O., C. H. Tempelis, A. D. Hess and W. C. Reeves. 1973. Mosquito host preference studies in Hale County, Texas. Am. J. Trop. Med. Hyg. 22: 270–277.

Loomis, E. C. and D. H. Green. 1959. Ecological observations on *Culex tarsalis* Coquillett and other mosquitoes in the delta region of the Central Valley of California, 1953–1956 (Diptera: Culicidae). Ann. Entomol. Soc. Am. 52:524–533.

Magnarelli, L. A. 1975. Relative abundance and parity of mosquitoes collected in dry ice-baited and unbaited CDC miniature light traps. Mosq. News 35:350–353.

Milby, M. M., E. E. Kauffmann and J. F. Harvey. 1978. Conversion of CDC light trap indices to New Jersey light trap indices for several species of California mosquitoes. Proc. Calif. Mosq. Control Assoc. 46:58–60.

Mulhern, T. D. 1953. Better results with mosquito light traps through standardizing mechanical performance. Mosq. News 13:130–133.

Olson, J. K. and J. E. Grimes. 1974. Mosquito activity in Texas during the 1971 outbreak of Venezuelan equine encephalitis (VEE): II. Virus incidence in mosquito samples from the south Texas plains. Mosq. News 34:178–182.

Pennington, R. G. and J. E. Lloyd. 1975. Mosquitoes captured in a bovine-baited trap in a Wyoming pasture subject to river and irrigation flooding. Mosq. News 35:402–408.

Reeves, W. C. and W. McD. Hammon. 1962. Epidemiology of the arthropod-borne viral encephalitis in Kern County. Univ. Calif. Pub. Health 4:1–257.

Reisen, W. K., M. M. Milby, W. C. Reeves, R. P. Meyer and M. E. Bock. 1983. Population ecology of *Culex tarsalis* (Diptera: Culicidae) in a foothill environment of Kern County, California: temporal changes in female relative abundance, reproductive status, and survivorship. Ann. Entomol. Soc. Am. 76:800–808.

Rosay, B. 1961. Anatomical indicators for assessing the age of mosquitoes: the teneral adult (Diptera: Culicidae). Ann. Entomol. Soc. Am. 54:526–529.

Service, M. W. 1976. Mosquito ecology: Field sampling methods. Applied Science Publishers, London. 583 pp.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA. 776 pp.

Sudia, W. D. and R. W. Chamberlain. 1962. Battery-operated light trap, an improved model. Mosq. News 22:126–129.

Sudia, W. D., R. W. Emmons, V. F. Newhouse and R. F. Peters. 1971. Arbovirus vector studies in the central valley of California. Mosq. News 31:160–168.