EFFECTIVENESS OF FIVE METHODS FOR SAMPLING ADULT *CULEX* MOSQUITOES IN RURAL AND URBAN HABITATS IN SAN BERNARDINO COUNTY, CALIFORNIA

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ABSTRACT. The comparative effectiveness of NJ light traps, CO_2-baited traps, walk-in red boxes, high-intensity light traps and gravid traps for sampling adult *Culex* mosquitoes for arbovirus surveillance was compared at rural dairy and residential urban habitats near Chino, California. *Culex quinquefasciatus* was the most abundant of the 3 species present comprising 94.9% of the 23,159 mosquitoes collected, followed by *Cx. peus* (4.2%) and *Cx. tarsalis* (0.9%). Female mosquitoes of all species were most abundant in CO_2-baited traps at both urban and rural environments. Males were collected most effectively by walk-in red boxes in rural, but not urban, environments. More parous females were collected by CO_2-baited traps at urban than rural habitats; however, 70–79% of all females were nulliparous and had never blood fed. These data point out the potential insensitivity of the current sampling methodology in monitoring mosquito arbovirus infection rates.

INTRODUCTION

St. Louis encephalitis virus (SLE) recently has been recognized as a public health problem in the greater Los Angeles metropolitan area, where 25 human cases have been confirmed since 1983 (Emmons et al. 1984, 1985, 1986, 1987). In addition, SLE virus was isolated on 40 occasions from mosquitoes including *Culex tarsalis* Coquillett (35 isolates), *Cx. quinquefasciatus* Say (3) and *Cx. peus* Speiser (2). Quantitative descriptions of the abundance and possible epidemiological involvement of these 3 *Culex* species potentially were obscured by the relative effectiveness of sampling methods which were developed to monitor the abundance and arbovirus infection rates of *Cx. tarsalis* in agricultural communities. In the present surveillance program, mosquito abundance is monitored by New Jersey light traps (Mulhern 1942), while virus infection rates are determined by testing females collected by a variety of CO_2-baited, CDC-style traps (Sudia and Chamberlain 1962). Although the effectiveness of these methods for sampling mosquitoes was described in the Central Valley by Milby et al. (1978), Meyer et al. (1984) and others, the utility of these and newly described methods such as the CDC gravid female trap (Reiter 1983) have not been evaluated for sampling *Culex* mosquitoes in southern California.

The objectives of the present research were to evaluate the effectiveness of 5 different methods to collect *Culex* mosquitoes in rural and urban habitats in southern California. Emphasis was placed on estimating the relative abundance, sex ratio, metabolic status and age structure of mosquitoes collected by each method to assess their utility in arbovirus surveillance.

MATERIALS AND METHODS

Description of Study Area: The Chino area of San Bernardino County, California, was selected for study because: 1) waste water from the dairy industry produces large *Cx. peus* and *Cx. quinquefasciatus* populations, and 2) the urban sprawl of the greater Los Angeles metropolitan area has resulted in the recent construction of residential areas in close proximity to these rural mosquito-breeding sources. Rural study sites were situated near confirmed mosquito-breeding sources created by the impounding and spreading of dairy waste water. Urban sites were located in residential areas situated 0.5–3.0 km away from the nearest dairy sump.

Sampling Methods: Sampling devices were positioned along tree lines or near farm houses at rural sites, and in shrubbery in front and/or back yards or hung from the eaves of houses at urban sites. Five devices were compared:

1. NJ light trap. A standard New Jersey style light trap fitted with a 25 watt bulb (Mulhern 1942) was positioned from 1.5 to 2.0 m above the ground and operated from dusk to dawn.
2. CO_2-baited trap. A CDC style trap modified as described by Pfuntner (1979) and baited with ca. 1 kg of dry ice was operated without light from dusk to dawn. Carbon dioxide-baited traps were hung from trees or from the eaves of residences about 1.5–2.0 m above the ground.
3. High intensity light trap. Battery-powered traps (Pfuntner 1979) which were fitted with a high intensity, 12 volt automobile tail light bulb and hung 1.5–2.0 m above the ground were operated from dusk to dawn. For *Cx. peus* (A. R. Pfuntner, unpublished data) and *Cx. tarsalis* (Barr et al. 1960), trap attractancy increases as a function of increasing illumination.
4. Red box. All mosquitoes resting inside a walk-

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in red box (2 x 2 x 1.3 m) fitted with a screened door (Nelson 1966, Meyer 1985) were collected by a battery-powered aspirator during early morning. Red boxes were positioned with the door facing west to prevent morning sunlight from entering the box.

5. Gravid trap. CDC gravid female traps (Reiter 1983) were baited with about 1 liter of barn wash water and were operated from dusk to dawn. Barn wash water was dipped from impounded dairy barn effluent and all mosquito immatures removed prior to use. Gravid traps were placed on the ground under vegetative canopy or adjacent to houses. In addition, gravid traps baited with barn wash water, the Reiter (Reiter 1983) and Ritchie (Ritchie 1984) media were operated concurrently at one rural and 2 urban sites to assess the relative attractiveness of each oviposition substrate.

Study Design and Mosquito Processing: The 5 sampling devices were placed at fixed locations in each of 3 rural and 3 urban localities and were operated on 4 occasions from July 22 to October 6, 1986 (n = 120 total collections). Mosquitoes were collected alive and transported to the laboratory where they were anaesthetized, sorted to species, sex, female metabolic status (unfed, blood fed or gravid) and counted. A representative subsample of up to 15 females per species per sample were frozen at −5°C for later dissection. Frozen females were later thawed and the reproductive system excised into physiological saline. Follicular maturation was classified by the system of Christophers (1911) as modified by Mer (1936). Parity was determined by the presence of dilatations and by the degree of tracheole coiling (Detinova 1962). Spermathecae and the bursa copulatrix were examined for the presence of sperm.

Autogeny: Since autogeny can confound the interpretation of parity data, pupae were collected from dairy sumps near rural adult sampling devices to determine the frequency of autogeny. Adults were allowed to emerge under laboratory conditions (25 ± 2°C), maintained for >5 days on 10% sucrose and then dissected to determine follicular maturation. Females with follicles matured to ≥Stage IV were considered to be autogenous.

Statistics: The numbers of each species and sex collected per trapping occasion were analyzed separately by a factorial analysis of variance (ANOVA) with sampling devices and habitat types as main effects and replicate collection dates nested within localities within habitats (Sokal and Rohlf 1981). Three missing values due to trap failures were estimated interpolatively prior to analysis. When the trap x habitat interaction effect was significant ($P < 0.05$), trap means within each habitat were compared by a posteriori multiple range test (Duncan 1955).

RESULTS

Abundance: A total of 23,159 male and female mosquitoes were recovered from the 117 collections made at urban and rural habitats near Chino. *Culex quinquefasciatus* was the most abundant species comprising 94.9% of the specimens collected, followed by *Cx. peus* (4.2%) and *Cx. tarsalis* (0.9%). Most specimens were collected by CO_2-baited trap (84.9%), followed by red box (9.7%), NJ light trap (2.0%), high intensity light trap (1.8%) and gravid trap (1.5%). More mosquitoes were collected in rural localities situated near dairy breeding sites (85.4%) than at residential urban localities (14.6%). Effective sampling devices collected disproportionately greater numbers of both sexes of all 3 *Culex* species at rural than urban sites as indicated by the significance ($P < 0.05$) of the interaction term in all ANOVAs.

Red boxes positioned along tree lines in rural habitats were most effective in sampling males of all 3 species (Table 1). The brighter bulb in the high intensity light trap collected more *Cx. peus*, but not *Cx. quinquefasciatus* or *Cx. tarsalis* males in rural habitats than the NJ light trap. Red boxes were ineffective in sampling both males and females in urban backyard environments.

Carbon dioxide-baited traps positioned along tree lines in rural habitats were most effective for collecting female *Cx. quinquefasciatus* and *Cx. tarsalis*, but *Cx. peus* were more abundant at red boxes in such habitats (Table 1). The CO_2-baited traps collected from 2.2 (*Cx. peus*) to 115.6 (*Cx. quinquefasciatus*) times more females than NJ light traps suggesting that females responded more sensitively to a CO_2 chemotaxis than a phototaxis. New Jersey and high intensity light traps collected comparable numbers of females of all 3 species. Gravid traps baited with barn wash water collected the fewest females of all sampling methods.

The use of either the Reiter (1983) or Ritchie (1984) media in the gravid female trap did not increase collection size. The mean number of females (% gravid)—males of *Cx. quinquefasciatus* attracted to each media per trap night, respectively, were Reiter = 5.0 (13%) − 4.3, Ritchie = 2.7 (0%) − 4.3 and barn wash water = 16.0 (10%) − 14.0. Only 2 unfed female *Cx. peus* and no *Cx. tarsalis* were collected during the 9 trap nights. Of interest was the collection of comparable numbers of females (71) and males (68) in the gravid traps.

Table 1. Number of adult *Culex* mosquitoes collected per sample by each of 5 methods operated near Chino, San Bernardino County, California, 1986.[1]

Culex species	Sex	Habitat	Sampling method					
			NJLT	CO2T	WIRB	HILT	OVT	Mean
peus	M	Rural	1.7c	0.0c	24.5a	7.5b	0.1c	6.6a
		Urban	2.0a	0.0a	0.3a	1.2a	0.6a	0.8b
	F	Rural	1.7b	4.7b	28.1a	3.7b	0.1b	7.4a
		Urban	2.8a	5.2a	0.3a	1.6a	0.6a	2.2b
quinquefasciatus	M	Rural	17.3b	0.0b	71.7a	11.2b	4.3b	20.4a
		Urban	1.0a	0.4a	0.3a	1.1a	4.1a	1.4b
	F	Rural	11.8b	1475.4a	72.6b	9.6b	13.7b	306.1a
		Urban	3.3b	259.0a	1.1b	0.8b	7.5b	54.4b
tarsalis	M	Rural	0.1b	0.1b	0.8a	0.1b	0.0b	0.2a
		Urban	0.0a	0.0a	0.0a	0.1a	0.0a	0.0b
	F	Rural	0.3b	14.3a	2.0b	0.3b	0.0b	3.2a
		Urban	0.0a	0.1a	0.0a	0.0a	0.1a	0.1b

[1] Sampling devices: NJLT = NJ light trap, CO2T = CO_2 baited trap, WIRB = walk-in red box, HILT = high intensity light trap, OVT = gravid female trap. Trap means within habitats and habitat means followed by the same letter were not significantly different ($P > 0.05$) when compared by Duncan's multiple range tests or ANOVA, respectively.

Female Reproductive Status: Overall, 12% of 276 *Cx. peus* females dissected were parous (Table 2). Although significantly more females were collected by red boxes at rural than nonrural sites, few of these females were inseminated (37%) and most were unfed (79%). Females collected in rural CO_2-baited traps were older than females resting in red boxes, since 96% were inseminated; however, few were parous (7%). The oldest females were collected by urban CO_2-baited traps with 35% parous and 12% sacculate indicating recent oviposition. Surprisingly, gravid traps collected few females and none of those dissected were gravid.

Of 509 *Cx. quinquefasciatus* females dissected, 21% were parous (Table 2). *Culex quinquefasciatus* females were generally older than *Cx. peus* females with >80% of females from all sampling devices inseminated and >17% parous. Insemination and parity rates of females collected at rural sites were not significantly different ($P > 0.05$) from females collected at urban sites. The percentage of gravid females collected by gravid traps was not significantly different than the percentage of gravid females collected by red boxes, suggesting that the gravid traps may have been attractive to mosquitoes searching for diurnal resting rather than oviposition sites.

Of 199 *Cx. tarsalis* females dissected, 31% were parous. Most dissected females were collected by CO_2-baited trap (66%) or red box (27%) at rural sites (92%).

Autogeny. A subsample of the *Cx. quinquefasciatus* and all of the *Cx. peus* and *Cx. tarsalis* females collected were dissected to determine autogeny status. Autogenous egg development was not detected for *Cx. peus* (0/12 dissected) or *Cx. quinquefasciatus* (0/125 dissected) and only 10 (13%) of 75 *Cx. tarsalis* had Stage V follicles indicating autogenous egg development. Thus, most parous *Culex* females in the present study had taken a previous blood meal.

DISCUSSION

Sampling Utility and Effectiveness: The 5 sampling devices studied differed considerably in their operational utility and effectiveness in sampling the 3 species of *Culex*. The NJ light trap used for mosquito population monitoring throughout California (Loomis and Meyers 1960) sacrificed collection size and possibly sampling sensitivity for operational utility, collecting significantly fewer females of each species in rural and urban habitats than CO_2-baited traps. New Jersey light trap attractiveness varied among species with *Cx. peus* most readily sampled, followed by *Cx. tarsalis* and *Cx. quinquefasciatus*. Although the ratio of NJ light trap to CO_2-baited trap catch sizes have been shown previously to vary among mosquito species over time and space (Milby et al. 1978, Meyer et al. 1984), CO_2-baited traps invariably collected more female mosquitoes than did NJ light traps. Processing NJ light trap catches was complicated by the sorting of the mosquitoes from the

Table 2. Reproductive status of female *Culex* collected from Chino, San Bernardino County, California, 1986.

Sampling method[1]	Habitat	No. dissected	Percentage of total					
			Inseminated	Unfed	Blooded	Gravid	Parous	Sacculate
			Culex peus					
NJLT	Urban	16	88	81	0	19	25	13
	Rural	12	25	100	0	0	8	8
CO2T	Urban	34	97	100	0	0	35	12
	Rural	28	96	100	0	0	7	0
WIRB	Urban	1	0	100	0	0	0	0
	Rural	135	37	81	2	17	4	3
HILT	Urban	13	69	77	8	15	15	0
	Rural	29	41	93	0	7	24	14
OVT	Urban	7	0	100	0	0	0	0
	Rural	1	0	100	0	0	0	0
Total	Urban	71	79	92	1	7	25	8
	Rural	205	45	87	1	12	7	4
			Culex quinquefasciatus					
NJLT	Urban	3	100	100	0	0	67	0
	Rural	37	97	86	3	11	19	0
CO2T	Urban	106	99	97	3	0	25	6
	Rural	105	99	98	2	0	17	4
WIRB	Urban	5	80	80	0	20	20	20
	Rural	109	81	53	37	10	19	6
HILT	Urban	0						
	Rural	42	95	98	0	2	19	0
OVT	Urban	41	95	80	10	10	24	20
	Rural	61	97	80	10	10	20	0
Total	Urban	155	97	92	5	3	25	10
	Rural	354	92	80	14	6	19	3

[1] Same abbreviations as in Table 1.

other phototactic insects. Conversely, CO_2 traps required little presorting with few other insects present except for *Culicoides* spp. attracted to the carbon dioxide.

New Jersey light traps did sample male mosquitoes and required considerably less collection effort during routine use than did CO_2-baited traps. The NJ light traps powered by AC current, timers and specimen kill jars can be operated daily, with the trap visited periodically for specimen pick-up (Loomis and Meyers 1960). Conversely, CO_2-baited traps can only be operated for a single night (dusk to dawn) increasing variability due to inclement weather, must be serviced twice per run and do not sample males. Males of all 3 species were most readily collected by red boxes in rural environments. Thus, the combination of CO_2-baited traps and red boxes would provide the most effective sampling system for *Culex* females and males, respectively; however, increased collection effort would increase the cost per collection and limit the number of sites which could be sampled concurrently. Males were not collected abundantly at urban sites by any of the methods evaluated which may reflect the juxtaposition of sampling to breeding sites and/or the influence of increased background illumination on urban light trap catches (Barr et al. 1963).

Mosquito Bionomics: Comparisons of trap catch sizes and female reproductive status between rural and urban habitats indicated several interesting ecological trends. Larval surveys in the Chino area have indicated that *Cx. peus* primarily breed in highly eutrophic dairy sumps (A. R. Pfuntner, unpublished data). Host-seeking females apparently disperse rapidly from breeding sites after emergence and oviposition,

since disproportionately few host-seeking or blooded females were collected at CO_2-baited traps or red boxes in rural environments, respectively. A similar paucity of female *Cx. peus* at CO_2-baited traps was noted previously (Meyer 1985). In the present study, there was no significant difference between CO_2-baited trap catch size in rural and urban habitats, but the proportion of females with sacculate dilatations (an indication of recent oviposition) was significantly higher in urban than rural environments indicating rapid dispersal after oviposition. The CO_2-baited traps may not have adequately sampled the host-seeking component of the *Cx. peus* population. Trap height (1.5–2.0 meters) and/or the CO_2 release rate (estimated to be 1.0–0.5 liters/min from the dry ice sublimation rate) in the present study may have been inappropriate to sample *Cx. peus* which feeds frequently on passeriform birds (Tempelis and Washino 1967).

The proportions of inseminated and parous *Cx. quinquefasciatus* did not differ markedly among sampling methods or habitats. The consistently high proportion of inseminated females indicated that teneral females may not rest in rural red boxes and/or dispersal to urban sites occurred soon after emergence. Alternatively, females may have been sedentary and the *Cx. quinquefasciatus* collected at urban sites may have originated from breeding sources such as underground catch basins. In agreement with the latter premise, proportionately more females collected in urban sites were sacculate than were females collected in rural sites.

Arbovirus Surveillance: Sampling methods which collect proportionately more parous or gravid females can be more effective in detecting the presence of horizontally maintained arboviruses such as SLE (Reeves et al. 1961). In the present study, from 70 to 79% of the specimens collected by CO_2-baited trap were unfed and nulliparous. Nulliparity rates observed during the present study were slightly higher than those reported recently for host-seeking *Cx. quinquefasciatus* and *Cx. tarsalis* in a marsh in nearby Orange County (Barr et al. 1986: 67 and 65%, respectively). Collectively these data indicated that most females collected by the CO_2-baited trap were unfed nullipars which would never have had the opportunity of contacting a viremic host. The collection of mostly unfed nullipars reduces the efficiency of the statewide arbovirus surveillance program and requires the testing of large numbers of mosquitoes to monitor arbovirus infection rates (e.g., 195,705 mosquitoes were tested in 4,417 pools during 1985 to yield 28 and 30 isolates of western equine encephalomyelitis and SLE viruses, Emmons et al. 1986).

Alternative sampling methods such as the CDC gravid female trap (Reiter 1983) reportedly collect females which have imbibed and digested a blood meal and thus theoretically should be more effective in detecting the presence of arboviruses (Reiter et al. 1986). Unfortunately, during the present study, few gravid females were collected by the gravid traps using the barn wash water, Reiter or Ritchie oviposition attractants. In fact, for *Cx. quinquefasciatus* the mean number of gravid females collected per sample in red boxes (3.7) was significantly greater than the number concurrently collected per gravid trap night (0.4). The failure of the gravid traps to collect suitable numbers of gravid *Culex* females for virus monitoring was unexpected in light of the encouraging results previously reported [Reiter et al. (1986): 142.3 females/trap night, 95% gravid; Ritchie (1984): 405.2 females/trap night, 57% gravid] and will be the subject of future research.

ACKNOWLEDGMENTS

We thank Drs. R. P. Meyer and W. C. Reeves, University of California, Berkeley, for critically reading the manuscript and the citizens of Chino for providing access to their property for sampling. The support of the Board of Trustees of the West Valley Vector Control District is gratefully acknowledged. This research was funded by research grant AI-3028D from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 5-S07-RR-05441 from the National Institutes of Health, and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California.

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