ATTRACTIVENESS OF SELECTED OVIPOSITION SUBSTRATES FOR GRAVID *CULEX TARSALIS* AND *CULEX QUINQUEFASCIATUS* IN CALIFORNIA

WILLIAM K. REISEN AND RICHARD P. MEYER

Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720

ABSTRACT. Eight potential oviposition attractants were evaluated for gravid *Culex tarsalis* and *Cx. quinquefasciatus* under insectary, outdoor cage and field conditions. With the possible exception of a steer manure infusion under insectary conditions, none of the media consistently attracted large numbers of gravid *Cx. tarsalis* females. Reiter medium, steer manure infusion, cattle feces and Bermuda sod were attractive to gravid *Cx. quinquefasciatus*. Catch size at CDC gravid traps and oviposition traps were sensitive to the number of competitive oviposition sites and trap placement.

INTRODUCTION

Sampling the blood fed component of a mosquito population will increase the probability of recovering pathogens during a field surveillance program. Thus, collections of resting female mosquitoes which included blood fed and gravid individuals produced higher arbovirus isolation rates than collections of unfed females attracted to light, CO_2 or vertebrate baits (Reeves et al. 1961). Unfortunately, searches for resting females in natural or manmade shelters are labor-intensive and typically yield far fewer specimens than attractive methods such as CO_2-baited traps (Reisen et al. 1983, Reisen and Pfuntner 1987).

Reiter (1983) described a gravid female trap for collecting *Culex quinquefasciatus* Say in Memphis, Tennessee. Operationally, the "CDC gravid trap" was far more efficient in collecting adult mosquitoes than were searches of natural resting sites by an experienced collector (Reiter et al. 1986). In Florida the catch of gravid *Culex* females (mostly *Cx. nigripalpus* Theobald) was increased when isopropyl alcohol was added to the hay infusion oviposition medium and a CDC style trap with a light bulb was suspended over the medium (Ritchie 1984). While these methods were effective for sampling the *Cx. pipiens* complex and *Cx. nigripalpus*, their ability to collect *Cx. tarsalis* Coquillett has not been evaluated. Maw and Bracken (1971) introduced a sod-baited trap to monitor *Culex* oviposition activity in Canada, and recently this trap was found to be effective for sampling *Cx. tarsalis* in Canada (Brust 1990).

The State of California Encephalitis Surveillance Program tests a large number of host-seeking *Cx. tarsalis* females collected by CO_2-baited traps for arbovirus infection (Emmons et al. 1988). Since the parity rates for *Cx. tarsalis* collected by CO_2-baited traps are usually less than 40% (Nelson et al. 1978, Reisen et al. 1983), 60% or more of the mosquitoes tested have never imbibed a blood meal and thus would not be infected with horizontally transmitted arboviruses such as western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. In addition, the recent recognition of SLE as a public health problem in the greater Los Angeles metropolitan area (Emmons et al. 1985) has necessitated the monitoring of virus infection rates in *Cx. quinquefasciatus* and *Cx. stigmatosoma* Dyar, neither of which are sampled efficiently by CO_2-baited traps in residential habitats (Reisen and Pfuntner 1987, Reisen et al. 1990).

The objectives of the present research were to evaluate the effectiveness of the Reiter (1983), Ritchie (1984), Maw and Bracken (1971) and other oviposition substrates in attracting gravid *Cx. tarsalis* and to determine their possible utility for arbovirus vector surveillance. Since these techniques were developed primarily to sample members of the *Cx. pipiens* complex, comparative observations also were made on *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Laboratory evaluations: Two laboratory strains of mosquitoes from Kern County, California, were used in insectary cage evaluations: 1) *Cx. tarsalis* colonized from Breckenridge Road in the Sierra-Nevadas in 1980, and 2) *Cx. quinquefasciatus* colonized from Bakersfield in 1985. Oviposition attractants were offered to mosquito colonies under insectary conditions (temp. = 25 ± 2°C, photoperiod = 16L:8D) approximately 5 days after blood feeding. Solutions were placed in red-colored dishes (diam. = 10 cm) which were allocated randomly to 2 or 4 of the 4 corners of the screened holding cages (0.5 × 0.5 × 0.5 m). The number of egg rafts oviposited in each dish after a 2-day exposure period was used as an index of the comparative attractiveness of each compound for gravid females.

The solutions tested and the rationale for their selection were as follows:

1) Tap water: Bakersfield tap water normally used as an oviposition substrate during colony maintenance was used as a control.

2) Larval water: Conspecific larval rearing water with exuviae, remaining 4th instar larvae and pupae, and surplus food removed by straining was used, since the gravid females of some culicines oviposited more readily on water in which conspecific larvae had been reared than on tap water controls (Reisen and Siddiqui 1978, Asman et al. 1983).

3) Pupal water: Water from conspecific pupal emergence containers were strained to remove dead adults, pupae and pupal exuviae. Previously, Andreadis (1977) had described an oviposition attractant of pupal origin.

4) Field water: Surface water from which *Cx. tarsalis* larvae were collected was strained to remove debris and larvae. This water was considered a natural control, since gravid females previously had been attracted to this substrate as indicated by the presence of larvae.

5) Reiter medium: The Reiter (1983) medium was modified to consist of 30 g of hay (both dried Timothy and fresh alfalfa were tried), 2 g of brewer's yeast and 2 g of lactalbumen added to 9 liters of tap water and aged from 5 to 15 days. Our quantities were 75, 500 and 500%, respectively, of the concentrations of hay, yeast and lactalbumen recommended by Reiter (1983).

6) Ritchie medium: The Ritchie (1984) medium consisted of 2 parts Reiter medium added to 1 part isopropyl alcohol.

7) Leaf infusion: A mulberry leaf litter infusion aged from 1 to 3 weeks was used to simulate flooded debris frequently found in river overflow pools commonly exploited by *Cx. tarsalis* (Reeves and Hammon 1962).

8) Alfalfa infusion: Alfalfa pellets infused in tap water and aged for 3 days were used to simulate flooded pasture also frequently utilized as a breeding site by *Cx. tarsalis* (Reeves and Hammon 1962).

9) Manure infusion: A dried steer manure infusion in tap water aged from 1 to 3 weeks was used to simulate the dairy sumps commonly used as breeding sites by *Cx. quinquefasciatus* (Bohart and Washino 1978).

Freshly hatched egg rafts with the apical droplet still intact were added to selected oviposition media. Osgood (1971) suggested that the fatty acids associated with the apical droplet of the egg served as an oviposition pheromone for *Cx. tarsalis*.

Outdoor cage evaluations: Since the proximity of the oviposition substrates in the insectary cage may have confounded their evaluation, the attractiveness of tap water, Reiter medium, Ritchie medium, leaf infusion and manure infusion for gravid *Cx. quinquefasciatus* also were compared outdoors in a large Quonset hut cage (Terwedow et al. 1977). Females from the Bakersfield colony and emerging from pupae collected in the Bakersfield area were held 3–7 days until sexually mature, blood fed on a restrained chick, held 5 days until gravid and then released into each Quonset hut in late afternoon. One liter of each oviposition substrate was presented in a dark brown wash tub randomly allocated to each of the four corners of the outdoor cage. The number of egg rafts oviposited on each substrate was recorded over a 5- to 8-day period. Since some solutions such as the Ritchie medium may be attractive at long range, but repellent at short range to ovipositing females, CDC gravid traps were operated over each media during one test.

Field evaluations: CDC gravid traps (Reiter 1983) baited with various oviposition substrates were operated from late afternoon to early morning at localities in Kern County supporting *Cx. tarsalis* and/or *Cx. quinquefasciatus* populations. CDC traps (Sudia and Chamberlain 1962) operated without lights and baited with dry ice were operated concurrently to verify the presence of female populations of each species. Walk-in red boxes (Meyer 1985) also were used at some localities.

The Kern Mosquito Abatement District operates 35 oviposition traps baited with cow feces to monitor *Cx. quinquefasciatus* abundance in Bakersfield. White plastic buckets (27-liter capacity) were filled with ca. 20 liters of water, baited with fresh cow feces wrapped in muslin, covered and allowed to age for 6 weeks prior to use. Lids of the buckets then were opened 5 cm to permit access to ovipositing females. During September–October 1987, we compared the number of egg rafts laid in the oviposition traps in a single night with the numbers of gravid females collected concurrently at a CDC gravid trap.

Sod-baited oviposition traps (Maw and Bracken 1971) were evaluated at Poso Creek in the foothills of the Sierra Nevadas. Single traps were positioned adjacent to wooded riparian habitat or in pasture habitat, baited with commercially produced Bermuda sod (30 × 30 × 4 cm) and then flooded to a depth of 8–10 cm. Traps were operated for 6 days mid-month from July to October 1988. Egg rafts were collected on alternate days, returned to the laboratory for hatching and the larvae reared to 2nd instar for identification. Concurrent with the monthly operation of the oviposition traps, 12 CO_2-baited traps were operated for 3 consecutive nights to monitor host-seeking female abundance.

RESULTS

Laboratory cage evaluations: With the exception of the steer manure infusion, gravid female *Cx. tarsalis* did not oviposit on any of the substrates more frequently than on tap water when tested under insectary conditions (Table 1). The addition of intact, freshly hatched egg rafts did not significantly improve the attractiveness of tap water (Exp. 1, 5, 8) or field water (Exp. 4, 8). The previous presence of either larvae (Exp. 2, 9, 11) or pupae (Exp. 6) did not impart attractiveness to tap water for gravid females. Field water gave mixed results, being significantly more attractive than larval rearing water (Exp. 2) or alfalfa infusion (Exp. 4), but equally attractive as tap water (Exp. 7, 8). Both the Reiter (Exp. 3, 5, 9, 10) and Ritchie (Exp. 9, 10) media were significantly less attractive than larval or tap water. The alfalfa infusion was less attractive than field water (Exp. 4) or tap water (Exp. 5), while the leaf infusion was less attractive than tap water (Exp. 10) or manure infusion (Exp. 11). In 4 trials, steer manure infusion was significantly more attractive than leaf infusion, larval water or tap water (Exp. 11).

Similar results were obtained with *Cx. quinquefasciatus* (Table 1). Again the Reiter and Ritchie media were decidedly less attractive than tap water (Exp. 12, 13). Steer manure infusion was more attractive than leaf infusion, larval water or tap water (Exp. 14).

Outdoor cage evaluations: A laboratory assessment of the Reiter medium may not have provided a fair evaluation, since the odors may have been too concentrated in the confines of the insectary. Subsequently, selected media were evaluated for attractiveness to laboratory and field strains of *Cx. quinquefasciatus* under outdoor cage conditions (Table 2). The Reiter medium performed best when used as bait for the CDC gravid female trap (Exp. 2); however, this medium was significantly less attractive than either leaf (Exp. 1) or steer manure (Exp. 2–5) infusions. Similar to results in insectary cages, the manure infusion was the most attractive of the compounds evaluated under outdoor cage conditions.

Field evaluation: The comparative effectiveness of the CDC gravid trap to collect gravid *Cx. tarsalis* and *Cx. quinquefasciatus* were evaluated in rural and residential habitats in Kern County during 1984–87 (Table 3). Gravid traps baited with Reiter medium, alfalfa infusion, field water, larval water or solutions with egg rafts collected markedly fewer gravid *Cx. tarsalis* females than did concurrently sampled walk-in red boxes. Solutions did not differ markedly in attractiveness.

Table 1. Attractiveness of oviposition substrates to colonized strains of *Culex* mosquitoes under insectary cage conditions.

Exp.	Oviposition media tested (% total rafts)[1]	Total rafts[2] (n)	Chi square[3]
	Culex tarsalis		
1	TW (51) vs. TW + rafts (49)	466 (5)	0.1 ns
2	FW (68) vs. LW (32)	697 (3)	88.9**
3	RM (34) vs. AI (66)	481 (5)	48.7*
4	FW + rafts (32) vs. FW (45) vs. AI + rafts (5) vs. AI (18)	22 (1)	8.2*
5	AI (18) vs. RM (11) vs. TW (32) vs. TW + rafts (39)	28 (1)	5.7 ns
6	PW (47) vs. TW (53)	257 (3)	0.8 ns
7	FW (49) vs. TW (51)	595 (3)	0.3 ns
8	FW (35) vs. FW + rafts (13) vs. TW (9) vs. TW + rafts (43)	475 (2)	118.8*
9	RM (0) vs. IM (0) vs. LW (40) vs. TW (60)	506 (2)	548.8**
10	RM (2) vs. IM (0) vs. LL (5) vs. TW (93)	147 (2)	365.3**
11	SM (57) vs. LL (3) vs. LW (19) vs. TW (20)	1272 (4)	793.9**
	Cx. quinquefasciatus		
12	RM (0) vs. IM (0) vs. LW (20) vs. TW (80)	95 (1)	163.4**
13	RM (1) vs. IM (0) vs. LL (18) vs. TW (81)	73 (2)	132.2**
14	SM (50) vs. LL (20) vs. LW (12) vs. TW (18)	117 (4)	39.3**

[1] Media evaluated included tap water (TW), field water (FW), larval water (LW), Reiter medium (RM), alfalfa infusion (AI), pupal water (PW), Ritchie medium (IM), leaf infusion (LL) and manure infusion (SM). For media + rafts, 10 recently hatched rafts were added.

[2] Total rafts oviposited during all tests; n = number of tests with oviposition cups allocated randomly to cage corners.

[3] Test for departure from equal attractiveness; ** $P < 0.01$, * $P < 0.05$, ns $P > 0.05$.

Carbon dioxide-baited traps collected considerably more specimens than either the gravid trap or the red box (Table 3).

Few gravid *Cx. quinquefasciatus* were collected in rural habitats using gravid traps baited with Reiter medium, leaf infusion or steer manure infusion (Table 3). Concurrently operated CO_2-baited traps collected disproportionately more specimens than could be explained by mortality losses within a gonotrophic cycle during 1986, but not 1987. In 1987, manure infusion collected 1.9 times more females than did Reiter medium, although this difference was not significant when tested by a *t* test ($P \geq 0.05$).

Few female *Cx. quinquefasciatus* were collected with Reiter medium during 1986, even at a residence near a breeding source created by run-off from a meat packing plant where 214 females were collected during a single night by a CO_2-baited trap (Table 3). Trap catches improved markedly when traps were operated in residential settings away from major breeding sites, and CO_2-baited trap catches were low (1.7 females/trap night). Only 3 *Cx. tarsalis* were collected by CO_2-baited trap, and none were collected in gravid traps operated at these residential sites.

During September–October 1987, a mean (SE) of 38.7 (6.6) gravid *Cx. quinquefasciatus* females were collected per CDC gravid trap

Table 2. Attractiveness of oviposition substrates to gravid *Cx. quinquefasciatus* females from the Bakersfield 1985 colony and females emerging from field-collected pupae under outdoor cage conditions.

Exp.	Oviposition media (% total)[1]				Total rafts	Chi square[2]
		Bakersfield colony (ca. 500 females)				
Media:	RM	IM	LL	TW		
1	21	1	75	2	138	200.2**
		Field-collected pupae (ca. 3,000 females/cage)				
Media:	RM	SM	LL	TW		
2[3] rafts	23	44	33	nd	288	20.3**
UF F	21	41	38	nd	39	2.2 ns
GR F	11	78	11	nd	83	73.6**
M	8	71	21	nd	24	13.7**
3	1	67	32	0	117	140.4**
4	0	76	23	1	193	289.1**
5	5	75	17	3	272	370.2**

[1] Test media are described in the methods section and abbreviations listed in Table 1, footnote 1. In Exp. 3 the media was placed in the oviposition pans 3 days before the females were released. In the remaining experiments the media were added just prior to the release of the mosquitoes.

[2] Tests for departure from equal attractiveness with rows; ** $P < 0.01$, * $P < 0.05$, ns $P > 0.05$.

[3] Percentage of egg rafts oviposited on the media and numbers of unfed females (UF F), gravid females (GR F) and males (M) collected by CDC gravid female trap during 3 consecutive nights.

Table 3. Sampling effectiveness of the CDC gravid trap baited with different oviposition media for *Culex tarsalis* and *Cx. quinquefasciatus* in Kern County, California.

Mo/Yr[1] (TN)	Oviposition media[2] (gravids/TN)	CO_2 trap (unfeds/TN)	Walk-in red box (gravids/ sample)
	Cx. tarsalis—rural habitat		
Jun. 1984 (1)	RM (4) vs. AI (1)	850	14
Jun. 1984 (1)	FW (3) vs. FW + 15 egg rafts (0)	1,220	25
Jun. 1984 (1)	LW + larvae (4) vs. FW (1) vs. FW + 50 egg rafts (1)	1,830	41
Sep.–Oct. 1987 (8)	RM (0) vs. SM (0)	22.7	1
	Cx. quinquefasciatus—rural habitat		
Nov. 1986 (2)	RM (0) vs. LL (0) vs. SM (1)	25.0	2
Sep.–Oct. 1987 (8)	RM (3.5) vs. SM (6.7)	13.3	0
	Cx. quinquefasciatus—residential habitat		
Nov. 1986 (5)	RM (0.2) vs. IM (1.0) vs. TW (0.2)	3.2	nd
Nov. 1986 (1)	RM (0) vs. LL (13) vs. TW (6)	214	nd
Oct. 1987 (14)	RM (19.9) vs. SM (17.3)	1.7	nd

[1] TN = number of replicate trap nights operated.

[2] Media abbreviations follow Table 1.

night at 35 residences in Bakersfield: significantly more than the 10.0 (2.1) rafts deposited per oviposition trap night ($P < 0.05$). Only 1 female *Cx. tarsalis* was collected by CDC gravid trap; and, therefore, most egg rafts in the ovitrap were presumed to be *Cx. quinquefasciatus*. Unexpectedly, the number of gravid *Cx. quinquefasciatus* females collected per gravid trap night was not correlated with the number of egg rafts oviposited per night in the oviposition trap ($r = 0.22$, $P > 0.05$).

During 1988, 80 *Cx. tarsalis* egg rafts (3.3 per oviposition trap night) were collected in a sod-baited oviposition trap located at the edge of a pasture, while 5 rafts were collected in a trap located adjacent to wooded riparian habitat (Table 4). Traps were most effective during July and August when the pasture was not well irrigated and competitive oviposition sites were scarce. The number of *Cx. tarsalis* rafts in the ovitrap was considerably less than the number of host-seeking females collected concurrently at 12 CO_2-baited traps (mean = 84.1 females/trap night).

In contrast to *Cx. tarsalis*, ovitraps positioned near to the wooded riparian habitat collected 143 *Cx. quinquefasciatus* egg rafts during July, September and October; considerably more than the 89 rafts collected concurrently in the trap positioned near the pasture (Table 4). Considering attrition due to daily mortality, the number of rafts collected near riparian habitat during September—October (11.5 rafts/night) was comparable to the mean number of females collected per CO_2 trap night (15.5 females/night).

DISCUSSION

Gravid *Cx. tarsalis* were not attracted strongly to any of the solutions or traps evaluated under laboratory or field conditions. The strong odors produced by fermentation of the Reiter and Ritchie media were unattractive to gravid *Cx. tarsalis* which typically breed in ephemeral and relatively unpolluted surface water habitats (Reeves and Hammon 1962). However, the strongly contagious dispersion pattern frequently observed for egg rafts and early instar larvae in nature (Stewart et al. 1983) suggests the presence of an oviposition attractant. Previously, Gerhardt (1959), Ikeshoji and Mulla (1970) and others have suggested that this attractant is associated with soil or vegetation present in dried breeding sites and is liberated shortly after inundation. Oviposition activity typically declines with breeding site maturation (Fanara and Mulla 1974, Mulla 1990), perhaps indicating that the initial oviposition attractant denatures over time. The alfalfa pellet and mulberry leaf infusions as well as the field larval water did not show marked attractiveness to gravid females. Bermuda sod in outdoor oviposition traps positioned adjacent to pasture worked best, but the number of egg rafts recovered was less than expected from the collection of host-seeking females. For example, if female survivorship was conservatively 0.7 per day (Nelson et al. 1978) and the duration of the gonotrophic cycle was 3 days, then during August when 95.5 females were collected per CO_2-baited trap night, 32.7 rafts should have been recovered from the oviposition trap per night presuming that both traps sampled equal proportions of the available host-seeking and ovipositing females, respectively. However, during August the oviposition trap near the pasture collected only 6.0 rafts per night, a disappointing 18.3% of what was expected.

Other studies have described (Osgood 1971) and biochemically characterized (Starratt and Osgood 1972) an oviposition pheromone associated with the apical droplet of the egg. However, in the present study the addition of up 50 fresh egg rafts to field water in which larvae were breeding did not increase the number of gravid females trapped per night.

Culex quinquefasciatus which exploits peridomestic artificial container and eutrophic surface water breeding habitats was attracted to

Table 4. Numbers of *Culex* egg rafts oviposited in sod-baited traps and females collected in CO_2-baited traps at Poso Creek, 1988.

	No./trap night[1]					
	Cx. tarsalis			*Cx. quinquefasciatus*		
	Ovitrap			Ovitrap		
Month	Pasture	Riparian	CO_2	Pasture	Riparian	CO_2
Jul.	5.5	0.2	85.5	2.0	0.8	0.6
Aug.	6.0	nd	95.5	1.3	nd	1.4
Sep.	1.5	0.6	99.5	10.8	18.7	23.8
Oct.	0.3	0.0	55.8	2.0	4.3	7.3

[1] Traps positioned adjacent to pasture and wooded riparian habitat.

several of the solutions evaluated in both rural and residential habitats. Encouraging results were obtained using eutrophic solutions such as Reiter medium, steer manure infusion, and cattle feces and relatively oligotrophic solutions such as recently flooded Bermuda sod. Catch size was extremely variable and seemed to be influenced strongly by the abundance of competitive oviposition sites and trap placement as well as population abundance and age structure. For example, in rural habitats with competitive oviposition sites, disproportionately fewer gravid females were collected in CDC gravid traps baited with Reiter and steer manure infusion media than were collected host-seeking at CO_2-baited traps. However, in residential Bakersfield, trap catches were reversed with disproportionately more females collected in oviposition traps. Similar results also were obtained in 2 residential communities in the Los Angeles basin with differing availability of peripheral and peridomestic breeding sites (Reisen et al. 1990). The importance of trap placement was indicated strongly by the collection of nearly twice as many *Cx. quinquefasciatus* rafts in a sod-baited oviposition trap placed adjacent to wooded riparian habitat than in a trap positioned away from vegetation near pasture.

In conclusion, our attempts to develop an oviposition or gravid female trap to collect *Cx. tarsalis* were decidedly unsuccessful. The aggregation of egg rafts in nature strongly indicates the presence of an oviposition attractant. However, none of the solutions tested were able to attract large enough numbers of females to replace CO_2-baited traps as the method of choice to sample *Cx. tarsalis*. Future research should target the development of a gravid trap to sample this important arbovirus vector and thereby improve the efficiency of arbovirus surveillance.

Conversely, *Cx. quinquefasciatus* were sampled effectively by several oviposition attractants, especially in residential environments. Surveillance would be improved by using CDC gravid traps baited with either Reiter medium or steer manure infusion than by either CO_2-baited traps or oviposition buckets. Gravid female traps were advantageous operationally, since females could be identified easily and then tested for arboviruses. Carbon dioxide-baited traps collected comparatively few individuals (most of which were empty and nulliparous), while oviposition traps required extra processing time to hatch and rear larvae for identification.

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