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EVALUATION OF FIVE TRAPPING SYSTEMS FOR THE SURVEILLANCE OF GRAVID MOSQUITOES IN PRINCE GEORGES COUNTY, MARYLAND

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ABSTRACT. Five mosquito trapping systems were evaluated in a large wildlife research center containing extensive and diverse mosquito-breeding habitat. The systems evaluated included Centers for Disease Control (CDC) New Standard Miniature Light traps with and without CO_2 (dry ice), CDC gravid traps, partially open 1.8-m³ cages, and Fay–Prince traps baited with CO_2. The first 4 trap systems were evaluated for 17 periods, while the Fay–Prince trap was evaluated on 5 trapping dates. Parameters recorded for each species were total catch, percent males, total females, and whether blood-fed/gravid. Fourteen of the 23 species caught yielded over 50 individuals in the 5 trapping systems (range of 71–2,524 specimens per species). Both light and CO_2 were powerful attractants for 12 of the 14 commonly caught species. However, for most species, the majority of captured females were nongravid. Gravid traps caught fewer mosquitoes than did light- or CO_2-baited traps, but the catch consisted of a higher percentage of gravid females. The open cages caught substantial numbers (>100 individuals) of 5 species, and for 2 species, this was the most productive trap. While light- and CO_2-baited traps tended to catch few males or gravid females, the open cages caught an eclectic mixture of males, gravid females, and nongravid females, perhaps representative of the true percentages of each in nature.

KEY WORDS Surveillance, gravid, parous, mosquito trapping, carbon dioxide

INTRODUCTION

Trapping is used to study seasonal trends of mosquito populations and the diseases they transmit. Developing chemicals that increase trap efficacy requires a sound understanding of how mosquito populations vary physiologically and how each type of trap samples the various kinds of mosquitoes. Trap systems are often made up of combinations of attractants. Many mosquito species respond to these attractant combinations, but it is well known that different mosquito species will not respond equally to the individual components. For example, Burkett et al. (1998) found that the addition of light significantly increased the catch of *Anopheles crucians* Wiedemann and *Uranotaenia sapphirina* (Osten Sacken) over CO_2 alone, while no difference in catch was found for *Ochlerotatus canadensis* (Theobald) or *Coquillettidia perturbans* (Walker). Trapping systems are also known to misrepresent nuisance mosquito abundance at trap sites even when catches are enhanced by a general attractant such as CO_2 (Slaff et al. 1983). Thus, the generalized behavior of mosquitoes toward attractants often results in sampling at the community level rather than targeted species level (Service 1976).

Virgin females are of minor importance for disease detection because transovarial transmission of most mosquito-borne diseases is minimal. Thus, gravid traps have been developed specifically to sample the potentially infective component of the mosquito population. While *Culex* spp. are well represented in gravid trap collections (Reiter 1983), species in other genera have different oviposition preferences and largely avoid gravid traps baited with plant infusions. Therefore, traps designed to sample the host-seeking portion of the female population must provide clues about the proportion of gravid or blood-fed individuals in the population. Several trapping systems using light and/or CO_2 have been developed to sample mosquito populations (Sudia and Chamberlain 1962, Fay and Prince 1970). The 3 trapping systems most often used for this purpose are the New Standard Miniature Light trap, with or without CO_2, and the omnidirectional Fay–Prince Trap baited with CO_2 (Kloter et al. 1983, Jensen et al. 1994, Becker et al. 1995).

In this study, we evaluated 5 trapping systems against gravid/blood-fed female mosquitoes in Prince Georges County, MD, namely, the New Standard Miniature Light trap without CO_2 (LTX) and with CO_2 (LTC), the Fay–Prince trap baited with CO_2 (FP), the standard CDC gravid trap (GT), and 1.8-m³ partially open cages. The study was designed to establish what portion of the mosquito complex, i.e., species and their physiological state, responded to the above trapping systems.

MATERIALS AND METHODS

Study area. The study was conducted at a mixed deciduous forest of the Patuxent Research National Wildlife Refuge, Prince Georges County, MD. The Patuxent River runs through the northeast portion of the refuge, producing many permanent marshes and woodland swamps. We selected 5 sites dominated by beeches (*Fagus grandiflora* Ehrhart) mixed with oaks (*Quercus* spp.), maples (*Acer*

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Table 1. The combined total mosquito catch, the catch per trap night, the percentage male and female, and the percentage gravid + blood-fed females of 23 mosquito species for 5 trap types evaluated in 2003.

Trap type	Trap nights	Total mosquitoes	Mosquitoes per night	% Males	% Females	% Gravid
LTX	17	2,891	170.1	13.5	86.5	14.3
LTC	17	5,902	347.2	3.3	96.7	7.1
GT	17	379	22.3	4.0	96.0	71.7
Cage	34	2,643	77.7	20.5	79.5	41.2
FP	5	2,059	411.8	0.6	99.4	2.5
Totals		13,874		8.3	91.7	15.3

spp.), and river birch (*Betula nigra* L.). All 5 sites were located within 30 m of the road and at least 50 m from the Patuxent River or associated wetlands. All sites were separated from one another by at least a kilometer.

Trapping systems. We trapped from Monday to Wednesday at all 5 sites for 17 wk from May 12 to September 17, 2003. New Standard Miniature Light traps (model 1012; John W. Hock Co., Gainesville, FL) were set up on Mondays and Tuesdays. Traps without CO_2 (LTX) were activated during Monday morning, between 0900 and 1100 h and collected on Tuesday morning between 0900 and 1100 h. The previous night's LTX traps were then redeployed with CO_2 (LTC) and fresh batteries. LTC catches were collected on Wednesday between 0900 and 1100 h. Fay–Price (FP) traps (model 112; John W. Hock Co., Gainesville, FL) were also deployed at the 5 sites, placed out at 0900–1100 h Tuesday, taken in at 0900–1100 h Wednesday, for 5 of the 17 wk: once in May, once in June, twice in July, and once in August. The LTC and FP traps were separated by about 30 m at each site. Both traps were hung about 1.5 m above the ground on a tree limb with the CO_2 source suspended above the trap. We used approximately 600 g of dry ice in a 1.9-liter Rubbermaid Victory thermal jug (Wal-Mart; Beltsville, MD) with the spout closed as the CO_2 source for the LTC. The FP traps came with thermal jugs that were configured in the same manner as the light traps. Set up this way (spout closed), these jugs provided about 500 ml CO_2/min at 22.8°C. The average daily temperature was 21.4 ± 0.1°C standard error of the mean. Weather data were obtained from weather station no. 3 at the Beltsville Agricultural Research Center, Beltsville, MD. CDC gravid traps (GT) (model 1712; John W. Hock Co., Gainesville, FL) were deployed between 0900 and 1100 h Monday and serviced between 0900 and 1100 h Tuesday. We used rabbit chow fermented for 1 wk as an oviposition attractant (Beehler and Mulla 1995).

Cages, unbaited with CO_2 or light, were set up at each of the 5 sites and sampled to provide a reasonably unbiased estimate of the adult population (Service 1976). A hand-held mechanical aspirator was used to collect mosquitoes from the 1.8-m^3 dull-tan Saran cages between 0900 and 1100 h Monday and between 0900 and 1100 h Wednesday. We closed the cage after entering to minimize collecting mosquitoes attracted to the cages by the investigator; otherwise, the entrance slit remained open for the week to allow mosquitoes free entry. After collecting from the cage walls, the duff was kicked up to disturb any hiding mosquitoes that were then collected. We stopped collecting when no more mosquitoes were apparent in the cage.

All mosquito catches were processed on the day of collection. We placed the collector bags from traps in the freezer for 15–20 min to kill the insects. Adult mosquitoes were separated and examined under a 10× dissection microscope to determine species, sex, and physiological status. We considered females with any trace of blood in the abdomen as blood-fed and those with visible egg outlines as gravid (Boxmeyer and Palchick 1999). Blood-fed and gravid females were pooled for analysis. All catches were square-root transformed for statistical testing (Zar 1996). *T*-tests were performed on the catch for each species summed over all trap nights where at least 1 individual was caught. A separate analysis of variance (ANOVA) test (function aov in program R v. 1.7.1: available from URL: http://www.r-project.org) with $\alpha = 0.05$ was used to test the differences between LTX, LTC, and FP on the 5 nights when all 3 traps were active for each species/physiological status combination. The data were summed over all 5 sites to provide sufficient counts of analysis and to maintain a balanced experimental design. The dates were then considered replicate samples.

RESULTS

A total of 13,874 mosquitoes in 23 species were collected, 12,720 (91.7%) females and 1,154 (8.3%) males. Of the females, 1,940 (15.3%) were visibly gravid or had a blood meal (Table 1). On a per night basis, total catch was 411.8, 347.2, 170.1, 77.7, and 22.3, respectively, for the Fay–Prince traps, the CDC light traps + CO_2, the CDC light traps alone, the cage traps, and the CDC gravid traps. The percentages of females caught in the above traps were, respectively, 99.4, 96.7, 86.5, 79.5, and 90.0. Blood-fed/gravid females comprised 2.5, 7.1, 14.3, 41.3, and 71.7%, respectively,

Table 2. Average total mosquito trap catches, percentage males in the trap catches, average female trap catches, and percentage gravid females in light traps with or without CO_2 at 5 locations at the Patuxent Research National Wildlife Refuge, Prince Georges County, MD, for up to 17 trapping periods in 2003.

	Trap type[1,2]					
	LTX				LTC	
Species	Total avg. ± SEM	% males	Avg. females ± SEM	% gravid	Total avg. ± SEM	% males
Aedes hendersoni	6.0 ± 4.1	0.0	6.0 ± 4.1 a	63.3	53.2 ± 21.8	0.0
Ae. vexans	38.8 ± 10.4	17.0	32.2 ± 6.9 a	57.1	82.6 ± 7.9	2.4
Anopheles crucians	243.6 ± 159.8	1.2	240.6 ± 159.6 a	5.2	155.0 ± 96.2	0.9
An. punctipennis	24.4 ± 5.9	30.3	17.0 ± 3.8 a	15.3	30.8 ± 13.6	1.3
An. quadrimaculatus	16.0 ± 4.9	0.0	16.0 ± 4.9 a	7.5	15.4 ± 4.3	2.6
Culex erraticus	4.2 ± 1.4	4.8	4.0 ± 1.4 a	5.0	18.0 ± 6.8	0.0
Cx. pipiens	87 ± 13.3	19.5	70.0 ± 10.1 a	27.4	199.0 ± 71.6	6.3
Cx. restuans	24.8 ± 7.6	31.5	17.0 ± 4.4 a	43.5	25.6 ± 11.0	23.4
Cx. territans	2.2 ± 1.1	9.1	2.0 ± 1.0 a	0.0	2.6 ± 0.8	15.4
Coquillettidia perturbans	48.8 ± 31.7	0.8	48.4 ± 31.8 a	3.7	157.2 ± 69.9	0.0
Ochlerotatus canadensis	5.0 ± 1.4	40.0	3.0 ± 0.7 a	13.3	317.0 ± 114.5	1.5
Oc. triseriatus	5.4 ± 2.4	74.1	1.4 ± 0.1 a	14.3	89.0 ± 24.1	0.7
Psorophora ferox	0.2 ± 0.2	0.0	0.2 ± 0.2 a	0	7.2 ± 3.8	2.8
Uranotaenia sapphirina	57.0 ± 15.4	36.1	36.4 ± 10.2 a	8.2	24.2 ± 4.1	36.4

[1] Rows followed by the same letter are not significantly different at P=0.005 using a *t* test
[2] LTX, CDC New Standard Miniature Light trap without CO_2; LTC, CDC New Standard Miniature Light trap with CO_2; GT, CDC Gravid Trap.

of the total catch for the 5 trapping systems (Table 1).

The 14 species caught in meaningful numbers (50 or more specimens total) for the 5 trapping systems are given in Table 2 (species per trap type for 17 trapping periods averaged over 5 sites). These species included *Oc. canadensis* (3,633), *An. crucians* (2,053), *Culex pipiens* L. (2,047), *Cq. perturbans* (1,286), *Cx. restuans* Theobald (1,152), *Oc. triseriatus* (Say) (884), *Aedes vexans* (789), *Ur. sapphirina* (417), *Oc. hendersoni* (Cockerell) (362), *Cx. territans* Walker (325), *An. puntipennis* (Say) (297), *Cx. erraticus* (Dyar and Knab) (217), *An. quadrimaculatus* Say (196), and *Psorophora ferox* (Humboldt) (73). The 11 other species captured constituted less than 2% of the total catch and were *Ae. albopictus* (Skuse) (16), *Oc. grossbecki* Dyar and Knab (15), *Oc. japonicus* (Theobald) (12), *An. perplexens* Ludlow (9), *Culiseta inornata* (Williston) (6), *Orthopodomyia signifera* (Coquillett) (2), *Ae. cinereus* Meigen (2), *An. barberi* Coquillett (1), and *Cs. melanura* (Coquillett) (1).

Comparisons of the CDC light traps with and without CO_2 for the 14 most commonly caught mosquito species are given in Table 2. Females of 9 species were caught in substantially greater numbers in the CDC light traps with CO_2 than in the light traps alone; however, these differences were statistically significant for only 5 species (*Oc. hendersoni, Co. perturbans, Oc. canadensis, Oc. triseriatus,* and *Ps. ferox*). Three species (*An. quadrimaculatus, Cx. restuans,* and *Cx. territans*) had roughly equal numbers in the 2 trap types, while more *An. crucians* and *Ur. sapphirina* females were caught in the light trap alone than in the traps plus CO_2. Thus, the addition of CO_2 provided a significant increase ($P = 0.05$ by *t*-test) in catch for only 5 of the 14 species. Similar numbers of gravid females responded to light traps alone (357) and to light traps + CO_2 (404), while the large excess of nongravid females were attracted to the light + CO_2 (5,301 versus 2,145 attracted to light alone).

On 5 dates, we directly compared the light traps and the light + CO_2 traps with the CO_2-baited Fay–Prince traps. Figure 1 shows the relative trap-catch totals for the 14 species for which greater than 50 specimens were collected. Considerably more *Ur. sapphirina* were caught with light alone than in either of the traps baited with CO_2, with or without light. *Anopheles crucians* displayed a similar pattern, with few individuals attracted to CO_2 alone. Numbers of *An. quadrimaculatus* and *An. punctipennis* were too low for a definite trend to be established; however, *An. puntipennis* seemed to prefer the combination of light and CO_2. All *Anopheles* spp. were poorly attracted to CO_2 alone. The 3 *Culex* spp. showed a range of behaviors, but none exhibited a statistically significant ($P = 0.05$ by ANOVA) preference for 1 trapping system, although fewer gravid females were caught for all 3 species in the CO_2 alone than in the 2 traps with light. Too few *Cx. restuans* and *Cx. erraticus* were caught to establish a definite trend, but *Cx. erraticus* seemed to prefer CO_2 to light. The trap preference of *Co. perturbans* was very similar to that of *Cx. pipiens,* in that, with both species, there was a numerical (but not significant) preference of a combination of light + CO_2 over either factor alone. *Aedes vexans,*

Table 2. Extended.

LTC		GT				Cage			
Avg. females ± SEM	% gravid	Total avg. ± SEM	% males	Avg. females ± SEM	% gravid	Total avg. ± SEM	% males	Avg. females ± SEM	% gravid
53.2 ± 22 b	1.1	0.4 ± 0.2	0.0	0.4 ± 0.2	0.0	1.6 ± 0.5	0.0	1.6 ± 0.5	37.5
80.6 ± 7.1 b	24.1	1.0 ± 0.5	0.0	1.0 ± 0.5	40.0	23.8 ± 3.9	15.1	20.2 ± 3.9	49.5
153.6 ± 96.2 a	3.1	2.0 ± 0.5	30.0	1.4 ± 0.2	71.4	6.8 ± 4.3	20.6	5.4 ± 3.6	44.8
30.4 ± 13.5 a	2.0	0.4 ± 0.2	0.0	0.4 ± 0.2	50.0	1.8 ± 0.3	33.3	1.2 ± 0.3	50.0
15.0 ± 4.5 a	10.7	1.2 ± 1.1	50.0	0.6 ± 0.5	66.7	2.8 ± 2.0	42.9	1.6 ± 1.1	50.0
18.0 ± 6.8 b	1.2	1.2 ± 0.5	16.7	1.0 ± 0.4	40.0	7.8 ± 6.1	37.9	5.6 ± 4.0	32.1
186.4 ± 74.5 a	12.8	29.8 ± 6.8	0.0	29.8 ± 6.6	81.9	76.2 ± 25.5	1.0	75.4 ± 11.3	34.2
19.6 ± 6.9 a	33.7	23.8 ± 6.5	1.7	23.4 ± 6.2	91.5	149.8 ± 40.2	25.4	111.8 ± 21.8	41.0
2.2 ± 0.7 a	36.4	2.0 ± 1.3	0.0	2.0 ± 1.1	30.0	56.4 ± 10.0	11.3	50.0 ± 9.8	54.0
157.2 ± 69.9 a	2.0	1.8 ± 1.2	11.1	1.6 ± 1	12.5	6.0 ± 2.4	30.0	4.2 ± 1.9	52.4
312.4 ± 113.1 b	4.2	5.4 ± 1.9	3.7	5.2 ± 2	11.5	177.4 ± 64.7	25.5	132.2 ± 46.7	38.6
88.4 ± 23.9 b	4.1	2.6 ± 1	0.0	2.6 ± 1	76.9	13.0 ± 3.4	10.8	11.6 ± 2.9	31.0
7.2 ± 3.9 a	0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0	2.0 ± 0.5	0.0	2.0 ± 0.5	30.0
15.4 ± 1.9 a	6.5	0.2 ± 0.2	0.0	0.2 ± 0.2	0.0	1.6 ± 1.3	37.5	1.0 ± 0.7	40.0

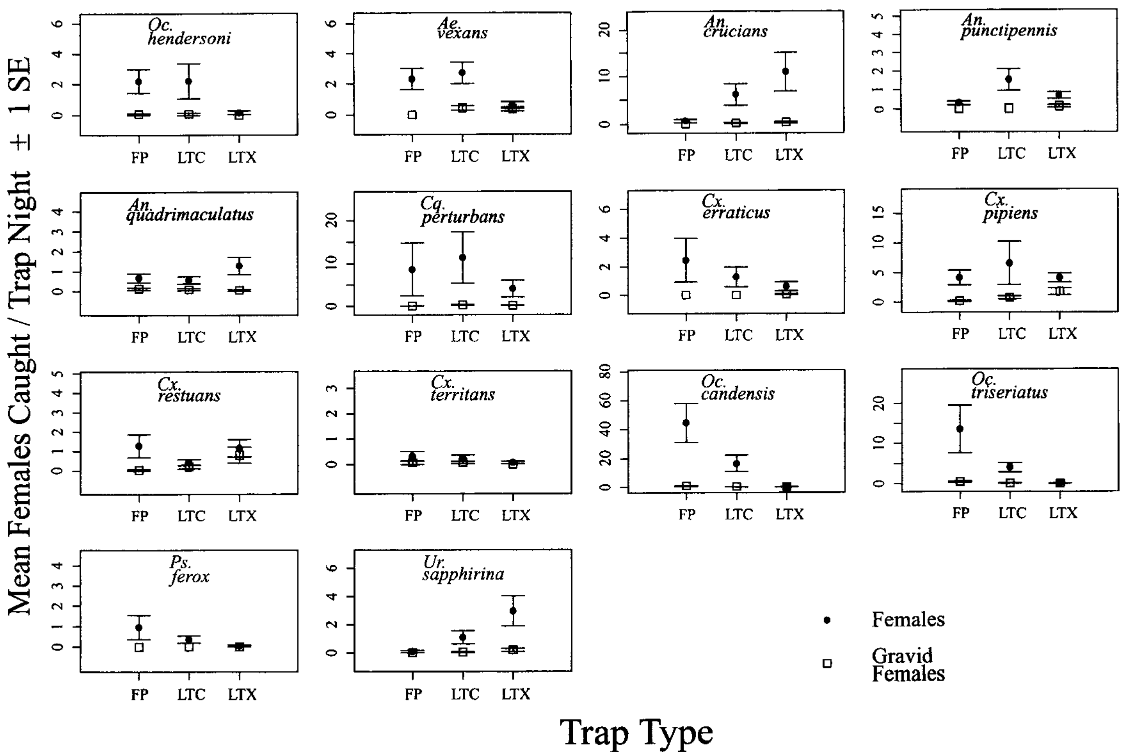

Fig. 1. Mean numbers caught per trap night (± 1 SE) of female mosquitoes and gravid mosquitoes for 14 species caught in CDC New Standard Miniature Light traps (LTX), CDC New Standard Miniature Light traps with CO_2 (LTC), and the omni-directional Fay–Prince traps with CO_2 (FP) at the Patuxent Research National Wildlife Refuge, Prince Georges County, MD in 2003.

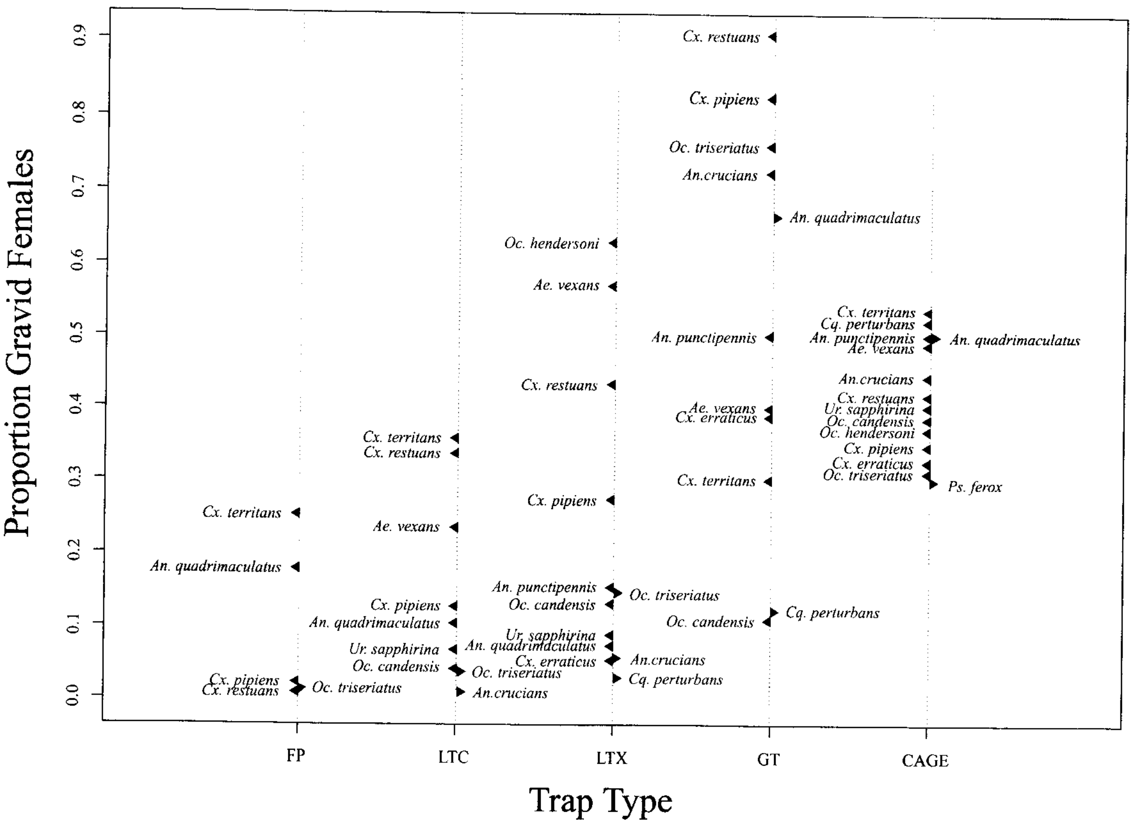

Fig. 2. Proportions of gravid female mosquitoes by trap type for 5 trapping periods (Fay–Prince traps) or 17 trapping periods (all other traps) in 2003 at the Patuxent Research National Wildlife Refuge, Prince Georges County, MD. CDC New Standard Miniature Light traps (LTX), CDC New Standard Miniature Light traps with CO_2 (LTC), and the omnidirectional Fay–Prince Traps with CO_2 (FP), plus CDC gravid traps (GT), and 1.8-m³ cages (CAGE). Some species were slightly adjusted (±3%) for clarity.

whether gravid or not, showed a significant and clear preference for light + CO_2 over light alone, while *Oc. hendersoni* avoided the trap with only light as an attractant. Both *Ae. vexans* and *Oc. hendersoni* showed good attraction to the CO_2 alone. Traps without CO_2 caught few *Oc. canadensis* or *Oc. triseriatus.* Both species seemed to be attracted preferentially to CO_2 alone, but this apparent preference was not statistically significant ($P = 0.05$) by ANOVA.

Catches in the CDC gravid traps and the cage traps are given in Table 2. The gravid traps caught high percentages of gravid females of most species but caught substantial numbers for only *Cx. pipiens* and *Cx. restuans.* The cages were heavily frequented by *Oc. canadensis* (887 total for the 5 sites) and *Cx. restuans* (749), moderately frequented by *Cx. pipiens* (381), *Cx. territans* (282), and *Ae. vexans* (119); lightly so by *Oc. triseriatus* (65), *Cx. erraticus* (39), *An. crucians* (34), and *Co. perturbans* (30); and sparingly (13 or fewer) by the other species. The cages were the most productive traps for *Cx. restuans* and *Cx. territans.* While light- and CO_2-based trapping systems caught relatively few males or gravid females, the cages caught an eclectic mix of males, gravid females, and nongravid females.

Trap type affected the proportion of gravid females captured (Fig. 2). The cages captured gravid and nongravid females in proportions that probably more closely reflected their natural occurrences. Catch in the gravid traps, as designed, was overly represented by gravid females of *Cx. pipiens* and *Cx. restuans* when compared with their abundances in cages. Conversely, the proportion of gravid females in the light + CO_2 traps, and especially the CO_2-baited Fay–Prince traps, was underrepresented. The light trap without CO_2 also tended to underrepresent the percentage of gravid females except for *Oc. hendersoni, Ae. vexans, Cx. restuans,* and *Cx. pipiens.*

DISCUSSION

Passive trapping in the form of artificial resting units has a long history of use in mosquito-control programs for monitoring certain mosquito species such as *Cx. tarsalis* Coquillett and *An. freeborni*

Aitken (Loomis and Sherman 1959). Our study found 5 additional species that were abundantly trapped in open cages. Moreover, the proportion of males, gravid, and nongravid females from the open cages more closely reflected the natural proportion of these sexual and physiological categories than did the captures from other trap types (Service 1976). These results agree in part with those of Reisen and Pfuntner (1987), who found that red walk-in boxes were the most effective of 5 trapping systems for sampling the males of 3 *Culex* species in rural, but not urban, habitats.

It is well known that the addition of CO_2 to light traps will increase the catch of many mosquito species (Newhouse et al. 1966, Becker et al. 1995). Conversely, the addition of light increases the catch of mosquitoes to traps baited with CO_2 and other chemical attractants (Van der Hurk et al. 1997, Rueda et al. 2001). Thus. it seems logical for all mosquito-control programs to use light + CO_2 and/or other attractant chemicals in their monitoring programs. At the same time, financial pressures require federal, state, and local governments to run less expensive but efficient mosquito-control programs. Additional cost and reliable sources of CO_2 must be considered when adding CO_2 to a mosquito trapping program. In rural mosquito abatement districts with limited budgets, the increased catch afforded by the addition of CO_2 to traps may not be worth the extra costs if the same control decisions can be deduced through light trapping alone.

While the addition of CO_2 to the CDC light trap more than doubled the mosquito trap catch in our study, for 9 of the most common 14 species; there was no significant difference in the catch in light traps with or without CO_2. If the 5 species that significantly responded to the addition of CO_2 are removed from the totals, catches in the 2 trap types are similar (2,580 in light traps versus 2,846 in light + CO_2). In fact, much of the observed increase in catch with CO_2 was due to *Oc. canadensis,* a negatively phototactic species that is strongly attracted to CO_2. A cool, wet spring and favorable habitat led to an outbreak of *Oc. canadensis* in our study area. This species accounted for 27% of the total trap catch in the light + CO_2 traps, 54% of the total catch in the CO_2-baited Fay–Prince traps, and less than 1% of the catch in the light traps without CO_2.

Local governments, in responding to citizen complaints, may require mosquito-control programs to apply treatments on the basis of how many mosquitoes were caught in a trap, regardless of species composition within that catch or the trap system used. Hypothetically, a decision trigger for treatment could be based on 25 mosquitoes caught with light alone or with light + CO_2. The catch increase due to the differential attraction of mosquito species to CO_2 might then lead to unwarranted treatment decisions. On the other hand, the addition of CO_2 may be warranted if 1 or more of the 5 species with trap catches statistically boosted by CO_2 are of special concern. For example, *Oc. triseriatus* is a leading vector of La Crosse virus in western North Carolina (Szumlas et al. 1996). Because *Oc. triseriatus* responds poorly to light, programs in La Cross virus-endemic areas should consider using traps baited with CO_2.

Gravid traps are specialty traps designed to catch a high percentage of gravid *Culex* females. Catching gravid females is a high priority in disease-monitoring programs, as these females offer visible proof of a previous blood meal (Reiter et al. 1986). However, parous females, nongravid females who have laid at least 1 egg mass, are also useful for disease surveillance.

The long-standing challenge to mosquito-control programs is that traps or trapping systems accurately sample the nuisance mosquito populations while still fitting within their program budget. When citizens perceive high levels of mosquito biting, they expect the local government to take appropriate action. Often, the first step is trapping of the adult mosquito population and any decision to treat areas for nuisance mosquitoes will hinge on these local trap catches. Because of differences between mosquito species, these traps may catch many individuals of a relatively nonmedically important species and still trigger a treatment decision.

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