Sampling Gravid *Culex quinquefasciatus* (Diptera: Culicidae) in Tanzania with Traps Baited with Synthetic Oviposition Pheromone and Grass Infusions

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ABSTRACT The effectiveness of traps baited with (*5R,6S*)-6-acetoxy-5-hexadecanolide (the synthetic oviposition pheromone) and grass infusions in sampling a population of gravid *Culex quinquefasciatus* Say was conducted in Muheza, Northeast Tanzania. A counterflow geometry (CFG) trap baited with pheromone and set outdoors, adjacent to a pit latrine building, collected more gravid *Cx. quinquefasciatus* than a CDC trap baited with pheromone and operated without light. Inside pit latrine buildings, significantly more gravid *Cx. quinquefasciatus* were collected in a CFG trap-baited with pheromone or grass infusion than in traps baited with tap water. CFG traps baited with either grass infusion or pheromone and set outdoors, away from known breeding sites, caught significantly more gravid *Cx. quinquefasciatus* than traps baited with tap water. CFG traps baited with pheromone + grass infusion caught significantly more gravid *Cx. quinquefasciatus* than CFG traps baited with either grass infusion or pheromone. In both cases, the proportion of gravid mosquitoes increased as traps were moved away from a natural emergence site. More gravid *Cx. quinquefasciatus* were collected in a pheromone-baited CFG trap than were egg rafts deposited in a jar with pheromone-treated water. It is concluded that CFG traps baited with oviposition attractants can be used effectively to sample gravid *Cx. quinquefasciatus.*

KEY WORDS *Culex quinquefasciatus,* gravid trap, Tanzania

Culex quinquefasciatus SAY, the principal vector of bancroftian filariasis in the urban areas of the East African coast, breeds in polluted water, mainly in wet pit latrines, septic tanks, and soakage pits. (*5R,6S*)-6-acetoxy-5-hexadecanolide (the synthetic oviposition pheromone, henceforth termed 'pheromone') significantly affects oviposition site selection by this mosquito in East Africa (Otieno et al. 1988, Mboera et al. 1999). Recent studies in Muheza, Tanzania, also have indicated that volatiles from soakage pits and grass infusions attract *Cx. quinquefasciatus*, *Cx. cinereus* Theobald, and *Cx. tigripes* Grandpré & de Charmoy to oviposition sites, particularly when pheromone was tested jointly with either soakage pit water or grass infusions (Mboera et al. 1999).

Oviposition attractants show great promise for surveillance (Reiter 1983, 1986, Millar et al. 1994) and, possibly, control (Otieno et al. 1988) of *Cx. quinquefasciatus.* With the specific oviposition mediating compounds such as (*5R,6S*)-6-acetoxy-5-hexadecanolide (Laurence and Pickett 1985) and skatole (Millar et al. 1992), mosquito populations can be sampled and perhaps manipulated by regulating their oviposition behavior. Gravid females of *Cx. quinquefasciatus* are able to distinguish between sites with and without an oviposition pheromone at distances of up to 10 m (Otieno et al. 1988). However, gravid females did not oviposit immediately in water carrying the pheromone alone in both Kenya (Otieno et al. 1988) and Tanzania (Mboera et al. 1999). Although grass infusion-baited traps have proven their usefulness in sampling gravid *Culex* mosquitoes in the United States (Reiter 1983, Ritchie 1984), reports of sampling gravid mosquitoes in Africa are not available. Our objectives in the work reported here were to study the effect of grass infusion and pheromone and their combination in the vicinity of and away from known larval habitats to avoid the effect of competitive odors; to assess the efficiency of traps baited with the 2 oviposition stimuli in sampling gravid mosquitoes; and to compare the efficiency of ovitraps versus gravid mosquito traps when baited with pheromone.

Materials and Methods

Study Area. All experiments were conducted in Muheza (5° 10′ S, 38° 46′ E) in northeast Tanzania. The area lies at an altitude of 214 m above sea level and is ≈40 km to the west of the coastal town of Tanga. Annual rainfall averages 1,000 mm, with the main rainy season in March–May and a 2nd less pronounced season in November–December. The mean annual temperature is 26°C, with cooler months between June and September and warmer months between October and May. The major developmental sites of *Cx. quin-*

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quefasciatus are pit latrines and soakage pits. The latter are artificial clay pits filled with water and organic debris.

Chemicals. Pheromone solutions were prepared as described previously by Dawson et al. (1990). Blank effervescent tablets were spread out on a clean piece of paper and 0.1 ml of the mosquito oviposition pheromone preparation in hexane (200 mg/ml) was placed on each of the tablets using a Hamilton precision syringe (Hamilton, Reno, NV). The pheromone dose delivered by the tablet is 5 mg with the remainder being inactive isomers (Dawson et al. 1990). The tablets were left to dry for a few minutes at room temperature before use. Grass infusion was prepared by fermenting 2 kg of *Digitaria* sp. grass cuttings in 10 liter of tap water for 5 d at room temperature. The solution was filtered through fine netting and frozen until needed.

Experimental Protocol. *Comparative Efficiency of Pheromone-Baited CFG and CDC Traps.* The counterflow geometry (CFG) trap (Kline 1999; power source was a 6-V sealed rechargeable battery, American Biophysics, East Greenwich, RI) was compared with the widely used Centers for Disease Control (CDC) trap (Sudia and Chamberlain 1962; 6-V sealed rechargeable battery, John Hock, Gainesville, FL) baited with pheromone. The CFG trap has been introduced only recently as odor-baited trap for the collection of mosquitoes. Briefly, it operates as follows: through a central tube, odor-laden air is blown out downward (Fig. 1). The odor tube is surrounded by a wider tube, through which air is being sucked into the trap by an updraft fan. This air is led into a wide container for holding mosquitoes that were attracted to the trap. The design of the tubes (with air being blown out and air being sucked in, respectively) is such that odor-laden air is carried away from the trap by the wind before it can be drawn into the trap. The CFG trap operates without a light. Because it has been shown that a CDC trap with light collects significantly fewer *Cx. quinquefasciatus* than traps without light (Mboera 1999), the lamp from the CDC trap used in these experiments was removed. The pheromone-treated tablet was dissolved in 800 ml of tap water. An open glass vial containing 4 ml of this pheromone solution was attached near the entrance of the CDC trap or it was fixed in a small plastic tube that was attached at the odor entrance port on the lateral wall of the CFG trap. The 2 traps were hung 2 m apart on wooden poles on either side of a latrine building 50 cm from the outside wall. Traps were left overnight. The CFG trap was hung 20 cm from the ground, whereas the CDC trap was hung with the shield 1 m from the ground. The traps were operated between 1800 and 0800 hours for 8 d and alternated between the sites every experimental day.

Pheromone or Grass Infusion Versus Tap Water. White plastic jars filled with 800 ml of tap water were treated with either a tablet impregnated with pheromone or hexane (control). The jars were covered with black netting material to avoid mosquitoes ovipositing on the solution. CFG traps were hung above the jars

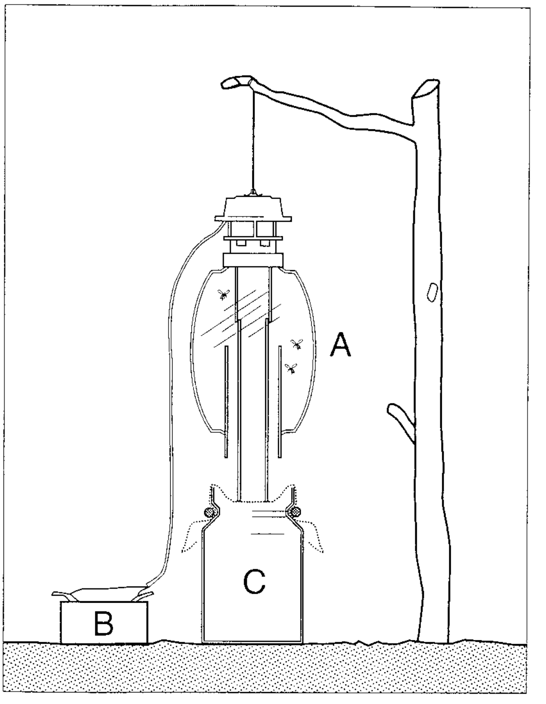

Fig. 1. Counterflow Geometry (CFG) trap baited with an oviposition attractant. (A) CFG trap. (B) Power source (12-V battery). (C) Plastic jar containing an oviposition attractant.

with their lowest part just above the netting material covering the jar (Fig. 1). Pairs of traps were set 1 m apart inside 2 pit latrine buildings and operated between 1800 and 0800 hours for 8 d. Traps were alternated daily between the 2 sites.

In another experiment the response of *Cx. quinquefasciatus* to CFG traps baited with grass infusion was compared with a trap baited with tap water indoors. In a similar setting as described in the experiment, 2 CFG traps were either baited with 800 ml of grass infusion or tap water in a white plastic jar covered with a black netting material and were hung 1 m apart inside the latrine building. The grass infusion and water used in the jars of each trap was replaced each night of trap operation. The traps were operated between 1800 and 0800 hours for 8 d with the treatments alternated between the 2 sites.

Response to Pheromone Versus Grass Infusion Outdoors Adjacent to a Larval Habitat. White plastic jars filled with 800 ml of the treatments were covered with a black netting material and placed under the CFG traps. The traps, with the lowest part touching the netting material, were hung outside a latrine building, 50 cm from the latrine wall, and were placed 2 m apart. The experiment was conducted between 1800 and 0800 hours for 8 d with the treatments alternated daily between the 2 sites.

Response to Pheromone, Grass Infusion or Water, Away from Known Larval Habitat. Three CFG traps were set outdoors in an area away from known *Cx. quinquefasciatus* larval habitats. The nearest human habitation was 200 m away. The treatments (pheromone, grass infusion, and tap water) were put in white plastic jars covered with a black netting material to avoid oviposition. The traps were hung from wooden poles 10 m apart, with their lowest parts touching the netting material. The traps were operated between 1800 and 0800 hours. The 3 treatments were alternated among the 3 sites every day for 6 d.

In another series of experiments, CFG traps baited with pheromone, grass infusion, or pheromone + grass infusion were compared. Two CFG traps, one baited with 800 ml of grass infusion and another with 800 ml of tap water treated with pheromone, were compared with 800 ml of grass infusion treated with pheromone. The traps with the lower most end over jars containing the treatments were placed in an open compound, 200 m from the nearest known larval habitat of *Cx. quinquefasciatus.* In a three-choice arrangement, the traps were alternated between the 3 positions every trapping day and operated between 1800 and 0800 hours for 6 d.

Pheromone-Baited Ovitrap Versus Pheromone-Baited CFG Trap. In this experiment, pheromone-baited CFG traps were used as gravid mosquito traps, whereas pheromone-baited white plastic jars (4 liter) were used as ovitraps. The jars were similar to those used under the CFG traps (Fig. 1). The 4 treatments were as follows: (1) an operating CFG trap (trap-on) baited with pheromone, (2) a CFG trap not operating (trap-off) but baited with pheromone, (3) an unbaited operating CFG trap, and (4) a jar containing pheromone. The jar in treatment 1 was covered with black mosquito netting to prevent oviposition, whereas the jars in treatment 2 and 4 were left uncovered. The treatments were placed 10 m apart in a straight line (10 m from the nearest pit latrine) and were alternated between the 4 sites every day. The experiment was run between 1800 and 0800 hours for 4 d.

Data Analysis. Mosquitoes were sorted to species, sex, and physiological status (i.e., unfed, blood-fed, or gravid). Egg rafts were sorted by shape and taken to the laboratory where they were reared separately to the adult stage when they were identified to species morphologically (Edwards 1942, Gillett 1972). All data were $\log(x + 1)$ transformed and means of treatments and controls were compared using Student *t*-tests (Snedecor and Cochran 1989, pp. 268–272). Data were backtransformed for presentation. Means in factorial experiments were subjected to analysis of variance. A significant *F*-test was followed by a least significant difference test to group like treatment means.

Results

Comparative Efficiency of Pheromone-Baited CFG and CDC Traps. A CFG trap baited with pheromone and set outdoors adjacent to a pit latrine building, collected significantly more gravid *Cx. quinquefasciatus* than a CDC trap ($P < 0.0001$), with mean ± SD values of 20.4 ± 1.2 and 2.2 ± 1.9 gravid mosquitoes per trap day, respectively. Therefore, all other experiments were done with CFG traps.

Table 1. Geometric mean catch per day of gravid *Cx. quinquefasciatus* collected by a CFG trap baited with A, synthetic oviposition pheromone; B, grass infusion versus a CFG trap baited with tap water (control) set indoors; and C, a CFG trap baited with pheromone versus grass infusion set outdoors adjacent to a pit latrine building

Experiment	Treatment	Mean ± SD	*n*
Indoors			
A	Pheromone	140.3 ± 1.9a*	1314
	Control	57.2 ± 1.7b	527
B	Grass infusion	194.0 ± 1.4a**	1632
	Control	43.1 ± 1.5b	370
Outdoors			
C	Pheromone	40.4 ± 1.4a	344
	Grass infusion	26.5 ± 1.7a	244

n, total number of gravid mosquitoes collected; SD = standard deviation. Means followed by a different letter are significantly different (*, $P < 0.01$; **, $P < 0.001$).

Pheromone or Grass Infusion Versus Tap Water. Inside a latrine building, significantly more gravid *Cx. quinquefasciatus* were collected in a CFG trap baited with pheromone or grass infusions than in the control ($P < 0.01$) (Table 1). The proportion of gravid females attracted to the pheromone-baited trap was 63.8% (n = 2,059). With grass infusion the proportion of gravid *Cx. quinquefasciatus* was 65.8% of the total females (n = 2,480) collected.

Response to Pheromone Versus Grass Infusion Outdoors, Adjacent to a Larval Habitat. Outdoors near a pit latrine building, the mean number of gravid *Cx. quinquefasciatus* caught in the CFG trap baited with pheromone was not significantly different from that collected in the trap baited with grass infusion ($P > 0.05$) (Table 1C). Of the females caught by CFG traps baited with pheromone (n = 444) and grass infusion (n = 346), 77.4 and 70.5%, respectively, were gravid.

Response to Pheromone, Grass Infusion or Water, Away from Known Larval Habitat. When CFG traps were set outdoors away from known larval habitats, the mean number ±SD of gravid *Cx. quinquefasciatus* caught in traps baited with pheromone, grass infusion, or tap water was 71.1 ± 1.7, 94.2 ± 1.9, and 6.32 ± 1.9, respectively. The numbers of gravid mosquitoes caught in traps baited with pheromone and grass infusion were not significantly different ($P > 0.05$). Significantly fewer gravid mosquitoes were collected in traps containing tap water ($P < 0.05$). Of the mosquitoes caught in a pheromone or grass infusion baited trap, 94.0 and 93.9% were gravid, respectively. When traps baited with pheromone or grass infusion were compared with a trap baited with pheromone + grass infusion, significantly more gravid *Cx. quinquefasciatus* were caught in a trap baited with the pheromone + grass infusion than in the other 2 traps (Table 2). Of the female *Cx. quinquefasciatus* caught in the traps, 96.9, 94.7, and 97.7% in a trap baited with pheromone, grass infusion, and the combination of the 2, respectively, were gravid mosquitoes.

Table 2. Geometric mean catch per day of gravid *Cx. quinquefasciatus* in a CFG trap baited with either grass infusions, a synthetic oviposition pheromone or their combination in an area away from known larval habitats

Treatment	Mean ± SD	*n*
Pheromone	24.7 ± 1.4a	157
Grass infusion	30.7 ± 1.4a	195
Pheromone + Grass infusion	77.7 ± 1.2b	475

n, total number of gravid mosquitoes collected. Means not followed by the same letter are significantly different at $P < 0.05$.

Pheromone-Baited Ovitrap Versus Pheromone-Baited CFG Trap. The number of gravid *Cx. quinquefasciatus* collected in a pheromone-baited CFG trap-on was greater than the number of egg rafts deposited in a jar under the nonoperating trap or number of egg rafts in the jar containing pheromone in the absence of the trap. Significantly fewer gravid mosquitoes were collected in the unbaited operating trap than in the pheromone-baited CFG trap-on (Table 3).

Discussion

Counterflow geometry traps baited with pheromone and set outdoors were more efficient in collecting gravid *Cx. quinquefasciatus* than CDC traps baited with pheromone. The CFG trap, which uses a counterflow concept, has 2 fans, one dispersing the odor (by pumping it outside the trap) and another stronger fan sucking the mosquitoes into the trap as they are flying up the odor trail. This is not the case with the CDC trap, which operates with a single fan that disperses the odor down and away from the trap entrance.

Significantly more gravid *Cx. quinquefasciatus* were caught in traps baited with either pheromone or grass infusion than in traps baited with tap water. Because the trap collects responding mosquitoes by suction, before they are able to contact and sample the water in the plastic jar, the compounds must be perceived by olfaction for the mosquitoes to respond. These experiments clearly demonstrated that the compounds used are volatile attractants for gravid *Cx. quinquefasciatus.* In a separate study (Mboera et al. 1999), *Cx. quinquefasciatus* deposited more egg rafts in grass infusion or pheromone than in tap water. Similar observations on the effect of pheromone have been reported by Otieno et al. (1988). Strong attraction of *Cx. quinquefasciatus* to hay infusions, similar to our grass infusions, also has been reported by Beehler et al. (1994), and it appears that in the field the oviposition behavior of *Cx. quinquefasciatus* is mediated by both the pheromone and semiochemicals from the organically enriched water. Other mosquito species also use water-borne chemicals for oviposition (Allan and Kline 1995), but the combined use of a pheromone and the water-borne chemicals appears to be limited to some *Culex* species.

Table 3. Geometric mean number of gravid *Cx. quinquefasciatus* caught in either a pheromone-baited or unbaited CFG trap and number of egg rafts deposited in pheromone-treated water in the presence or absence of the CFG trap

Treatment	Mean ± SD gravid mosquitoes	Mean ± SD egg rafts	*n*
Pheromone, trap-on	45.0 ± 1.1a		182
Unbaited trap-on	2.7 ± 1.7c		12
Pheromone, trap-off		11.3 ± 1.4b	46
Pheromone, no trap		14.2 ± 1.1b	56

n, total number of gravid mosquitoes or egg rafts collected. Means in the same column followed by a different letter are significantly different at $P < 0.05$.

Proportionally fewer gravid females were collected inside pit latrine buildings than adjacent to a pit latrine building or away from known larval habitats. However, in both cases the mean number of gravid mosquitoes responding to either grass infusion or pheromone-baited traps was greater than that responding to water-baited traps. The collection of nongravid *Cx. quinquefasciatus* in the vicinity of latrines probably was a result of the fact that traps were placed near mosquito emergence sites and collected newly emerged mosquitoes. The traps, being near emergence sites for mosquitoes, are likely to collect any mosquito flying near them and hence increase the chance of mosquitoes of different physiological status and sex being caught.

In both the indoor and outdoor situations, the number of gravid *Cx. quinquefasciatus* collected in the CFG trap baited with pheromone did not significantly differ from that collected in the trap baited with grass infusion. In the 2 situations, both near or away from known larval habitats, a trap baited with grass infusion or pheromone caught significantly more gravid *Cx. quinquefasciatus* than tap water, showing that gravid mosquitoes in search of a suitable breeding site respond to semiochemicals.

Because gravid traps baited with pheromone collected more gravid mosquitoes than the corresponding number of egg rafts deposited in ovitraps, the pheromone must be perceived by olfaction and the number of egg rafts deposited may not reflect the number of gravid mosquitoes visiting an oviposition site. The gravid mosquito trap caught a larger proportion of gravid *Cx. quinquefasciatus* than the corresponding number of egg rafts deposited in the ovitrap, because as the mosquitoes approached the bait they were sucked into the trap by the fan before they either deposited their egg rafts or left the site. It also may be true that the trap fan actively dispersed a larger plume of pheromone than that dispersed passively by diffusion from the jars. Consequently, many more mosquitoes may have intercepted volatile stimuli from this plume and were attracted.

We conclude that CFG traps baited with oviposition attractants effectively sample gravid *Cx. quinquefasciatus* and that a combination of pheromone and grass infusion is better than either of the 2 alone. Moreover, pheromone-baited CFG traps are more likely to detect gravid mosquitoes than pheromone-baited ovitraps. Survey and monitoring with pheromones and other oviposition attractants are practiced worldwide against a broad array of insect pests, and with the availability of the pheromone and grass infusion, these techniques may provide integral parts of the available intervention measures in the control programs of bancroftian filariasis in Africa.

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