Journal of the American Mosquito Control Association, 11(1):39–44, 1995

OVIPOSITION RESPONSES OF *CULEX TARSALIS* AND *CULEX QUINQUEFASCIATUS* TO AGED BERMUDA GRASS INFUSIONS

JUN ISOE, JEFFREY W. BEEHLER, JOCELYN G. MILLAR[1] AND MIR S. MULLA

Department of Entomology, University of California, Riverside, CA 92521

ABSTRACT. Fermented infusions of organic matter are commonly used as baits in traps for gravid female mosquitoes. However, infusions are dynamic, and their effects on mosquito oviposition as their chemical and microbial constituents change over time are not well documented. Bermuda grass infusion fermented for periods of 0–63 days was stimulatory to gravid *Culex quinquefasciatus.* In contrast, only 5–25-day-old infusion was stimulatory to *Culex tarsalis.* Standard-aged infusion (7 days old) was as effective or better than infusion of any other age for *Cx. tarsalis,* whereas *Cx. quinquefasciatus* exhibited a distinct preference for 2–4-wk-old infusion. The results are discussed in terms of mosquito species' oviposition site preferences and in terms of mosquito surveillance programs.

INTRODUCTION

The chemical and microorganismal composition of waters in natural mosquito breeding sites is dynamic, depending on factors such as the microorganisms present, time since flooding, available nutrients, suspended and dissolved solids, and temperature. The degree of attractiveness of natural breeding sites to gravid mosquitoes correspondingly changes over time. For example, *Culex salinarius* Coq. was stimulated to oviposit in fresh and 1-day-old wheat straw infusion, but after a week the infusions were no longer attractive (Murphey and Burbutis 1967). Maw (1970) found that artificial pools supplemented with capric acid were initially repellent to gravid *Culex restuans* Theobald, then over time became attractive, and then lost their attractiveness. Maw (1970) concluded that these changes were probably due to changes in bacterial activity. Brust (1990) demonstrated that *Culex tarsalis* Coq. and *Culex restuans* laid more egg rafts in pools augmented periodically with fresh sod than in pools to which sod was added only once. Beehler and Mulla (1993) demonstrated that oviposition in seasonal ponds increased for the first 3 wk after flooding, then decreased. They further showed that ponds supplemented with rabbit chow received more *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex tarsalis* egg rafts than those with no supplementation, and that overall, attractiveness of ponds with or without supplementation decreased over time.

In laboratory studies, Kramer and Mulla (1979) examined the effects of aging lab chow and chicken manure infusions on the oviposition responses of *Cx. quinquefasciatus* and *Cx. tarsalis.* The 2 *Culex* species behaved quite differently. Five-to-20-day-old lab chow infusions were repellent to *Cx. quinquefasciatus,* whereas still older infusion was no different in attractancy than distilled water. *Culex tarsalis,* on the other hand, was repelled by all ages of the chow infusion. The chicken manure infusion was attractive to *Cx. quinquefasciatus* for extended periods of time. In contrast, *Cx. tarsalis* was first repelled by the infusion, and then the infusion became neutral compared to distilled water over time.

Seasonal abundance and distribution of mosquitoes have often been monitored using artificial pools and/or traps for gravid females baited with infusions of fermented organic matter of known age (Hoban and Craig 1981; Frank and Lynn 1982; Strickman 1983, 1988; Brust 1990). In many other studies monitoring seasonal abundance of mosquitoes, the ages of the infusions used as trap baits were not mentioned. Neglecting to take the age of infusions into account may constitute a significant source of variability in the results of these studies.

Hay or grass infusions are commonly used in mosquito surveillance programs (Reiter 1983, 1986; Reiter et al. 1991). Reiter (1986) stated that infusions should be prepared by a standard protocol, because rapid changes in the attractancy of the infusions may occur as their microbial fauna and chemical compositions change with time. However, to our knowledge, a thorough investigation to determine how the attractancy changes with time has not been carried out with grass infusions, despite their extensive use as baits in traps for gravid mosquitoes. The objective of this study was to examine the effect of age of

[1] Author to whom correspondence and reprint requests should be addressed.

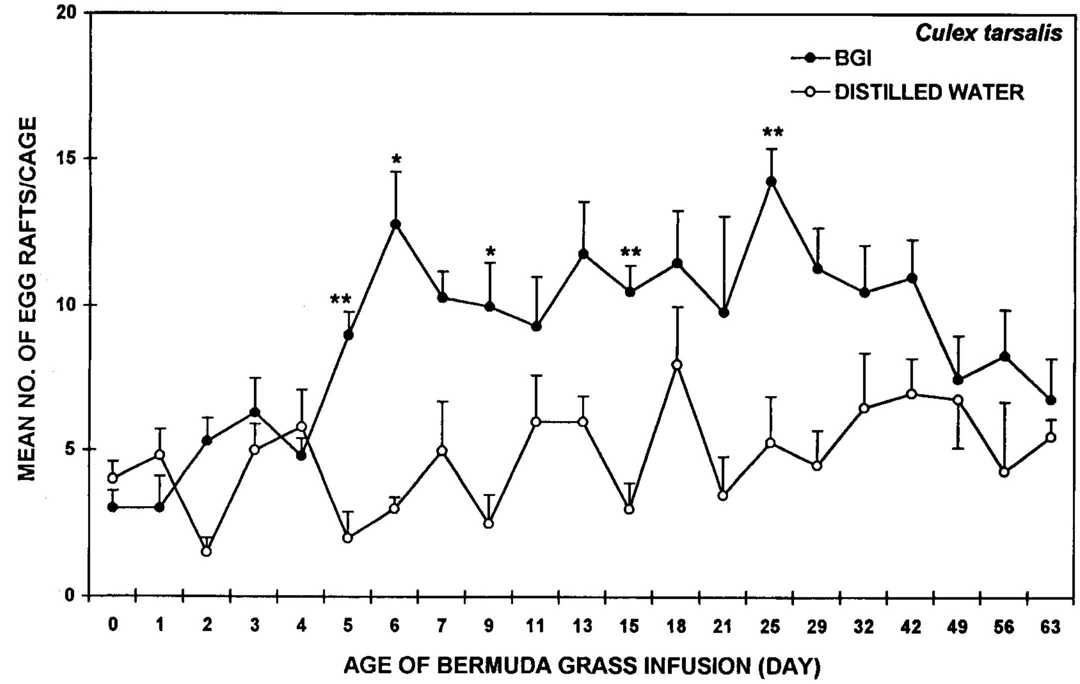

Fig. 1. Oviposition responses (egg rafts laid) of *Culex tarsalis* gravid females to Bermuda grass infusions of different ages versus distilled water controls. Each test was replicated 4 times with 20 mosquitoes/replicate. * = $P < 0.05$, ** = $P < 0.01$.

Bermuda grass infusions on oviposition activity by *Culex tarsalis* and *Cx. quinquefasciatus.*

MATERIALS AND METHODS

Mosquitoes: Culex tarsalis and *Cx. quinquefasciatus* mosquitoes were used in the experiments described below. The *Cx. tarsalis* colony was started from egg rafts collected at field sites near Oasis, CA, in November 1992. Colony maintenance of *Cx. tarsalis* has been previously described (Isoe and Millar 1995). Seven days after bloodfeeding, mosquitoes were deprived of sucrose solution for 24 h, then used in bioassays.

The colony of *Cx. quinquefasciatus* was initiated from egg rafts collected in Orange County, CA, in 1991. Rearing procedures for this species were as described by Kramer and Mulla (1979). Briefly, mosquitoes were maintained at 25 ± 3°C and 50 ± 10% RH. Lighting was provided by 2 fluorescent tubes (80 W) with L:D 12:10 and 1-h dusk and dawn photoperiod provided with an incandescent light (15 W). Larvae were reared in enamel pans, and fed every day with a 3:1 mixture of dog chow and brewer's yeast. Adult mosquitoes were held in screen cages (23 × 23 × 32 cm) and provided with 10% sucrose solution. Adults were bloodfed on 1-wk-old white Leghorn chicks (UCR animal use protocol #A-9105063) at approximately 7 days postemergence. Mosquitoes were used in bioassays about 7 days postbloodfeeding as described above for *Cx. tarsalis.*

Bermuda grass infusion: Fermented Bermuda grass (*Cynadon dactylon* Linn.) infusion (BGI) was prepared by mixing 27 g lactalbumen hydrolysate and brewer's yeast (both from U.S. Biochemical Corp., Cleveland, OH), 450 g dry Bermuda grass cuttings, and 100 liters of irrigation canal water in a 250-liter fiberglass tub. The tub was covered and kept outdoors in the shade at the Aquatic and Vector Control Research Facility on the agricultural experiment station grounds at the University of California, Riverside, from June 3 to August 4, 1993. Samples (400 ml) of continuously fermenting infusion were taken on 21 different days, from day 0 to day 63 (see Figs. 1 and 2). Each sample was filtered through a mesh screen (100 mesh size) to remove large debris, then transferred to a plastic bag and frozen (−20°C) until bioassayed. A large sample (2 liter) of the 7-day-old infusion was frozen in a brown glass bottle (3.87 liter) for use as a positive control. Various ages of 50% diluted BGI (diluted with distilled water) were tested against distilled water or standard 7-day-old 50% diluted BGI for oviposition response by *Cx. tarsalis* and *Cx. quinquefasciatus.* Each experiment contained 4 replicates (20 gravid females/replicate).

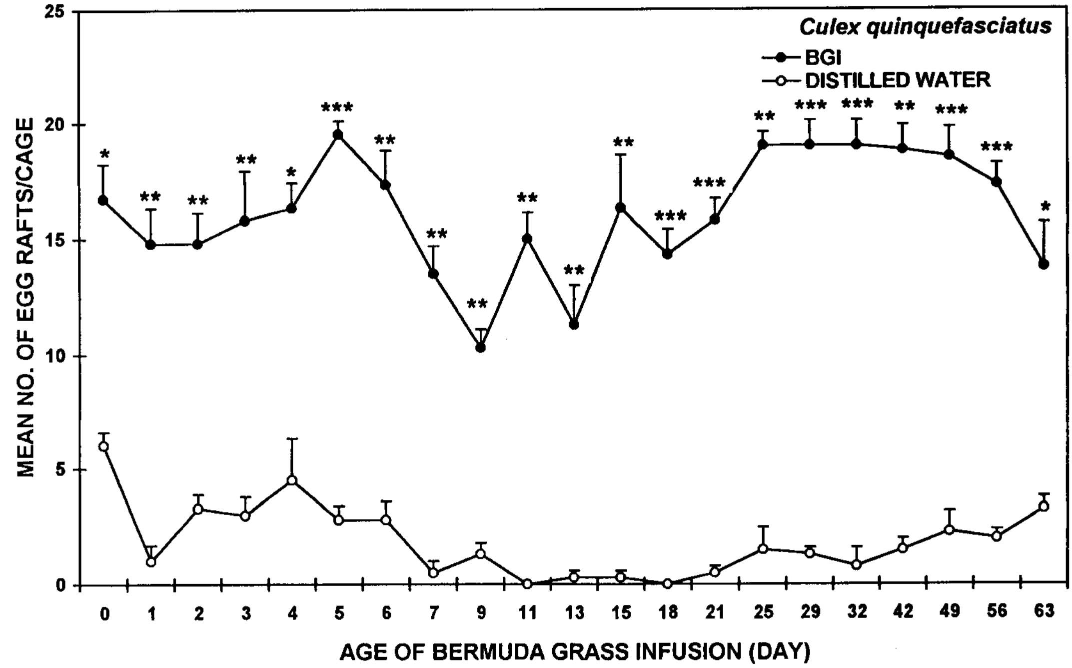

Fig. 2. Oviposition responses (egg rafts laid) of *Cx. quinquefasciatus* gravid females to Bermuda grass infusions of different ages versus distilled water controls. Each test was replicated 4 times with 20 mosquitoes/replicate. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Oviposition responses of Culex tarsalis *and* Cx. quinquefasciatus *to aged Bermuda grass infusion:* BGI samples of various ages were tested for their effects on *Cx. tarsalis* and *Cx. quinquefasciatus* oviposition behavior, using standard egg raft counting bioassays. Bioassays were conducted in an environmentally controlled chamber at 26 ± 1°C and 50 ± 10% RH. The photoperiod was maintained at 12:10 L:D with fluorescent lights. One hour dusk and dawn periods were provided with a 15-W incandescent light. Bioassays were conducted in rectangular cages (23 × 23 × 30 cm) made from wooden frames with glass tops and screen sides. Each bioassay cage was provided with 2 80-ml waxed paper cups, one in each back corner, containing 60 ml of test or control stimuli. Gravid females (20/cage, 4 replicates) were introduced into the cages at 1700 h and allowed to oviposit overnight. Bioassays were evaluated at 0800 h the next morning by counting the numbers of egg rafts laid in each oviposition cup. Gravid females were used once, then discarded.

Statistical analysis: In all paired comparisons, paired *t*-tests were used to determine if there were significant differences between numbers of egg rafts deposited in treatments and/or controls. Regression was used to determine the overall effect of age of BGI versus distilled water or standard 7-day-old BGI on oviposition by mosquitoes.

RESULTS

Oviposition responses by 2 Culex *species to aging BGI:* Oviposition responses by *Cx. tarsalis* and *Cx. quinquefasciatus* to various ages of BGI compared to distilled water controls are shown in Figs. 1 and 2, respectively. *Culex tarsalis* generally responded better to BGI from 5 to 25 days old than to distilled water controls. Infusion less than 5 days old or more than 25 days old was not significantly better as an oviposition medium than distilled water.

Culex quinquefasciatus females laid significantly more egg rafts in all 21 ages of BGI (0–63-day-old BGI) than in the distilled water controls. In some cases (day 11 and day 18), no egg rafts were laid in the distilled water controls, whereas large numbers of egg rafts were laid in the corresponding BGI treatments.

The effects of age of BGI on oviposition responses by mosquitoes were analyzed by multiple regression (Table 1). The regression indicated that age of BGI had no significant effect on oviposition preference by *Cx. tarsalis* toward BGI ($F = 1.54$; df = 2,18; $R^2 = 0.16$; $P < 0.05$). That is, the overall level of attractancy of BGI to *Cx.*

Table 1. Regression analyses,[1] including coefficients, partial *t* values, and *P* values, for the relationship between age of Bermuda grass infusion and percent egg rafts deposited in treatment cups for *Culex tarsalis* and *Culex quinquefasciatus*.

Source	Coefficient	Partial-*t*	*P* value
Intercept			
Cx. tarsalis	58.8		
Cx. quinquefasciatus	82.6		
Day			
Cx. tarsalis	0.8	1.6	0.2
Cx. quinquefasciatus	1.0	4.9	0.0001
Day^2			
Cx. tarsalis	−0.2	1.7	0.1
Cx. quinquefasciatus	−0..02	5.0	0.0001

[1] R^2 for *Cx. tarsalis* regression = 0.16, R^2 for *Cx. quinquefasciatus* regression = 0.58.

tarsalis did not change significantly over time. Furthermore, significantly more egg rafts were laid over time in BGI than the distilled water controls, indicating that over the entire course of the study, BGI was more stimulatory than the distilled water controls.

In contrast, the ages of BGI treatments were positively correlated with oviposition by *Cx. quinquefasciatus* (F = 12.41; df = 2,18; R^2 = 0.58; $P < 0.01$), indicating that mosquito oviposition preferences toward BGI changed over time. However, even at day 63, BGI was much more stimulatory than the distilled water controls.

Experiments were also conducted to compare the effects of 7-day-old "standard" BGI (Reiter 1986) with BGI of other ages on oviposition activity by mosquitoes. *Culex tarsalis* preferred 7-day-old BGI to younger or older ages of BGI (Fig. 3A), with infusions of intermediate age being no different than the 7-day-old infusion. Thus, for this species, 7-day-old infusion is as good as or better than infusion of any other age.

Culex quinquefasciatus also preferred 7-day-old BGI to very young ages of BGI (Fig. 3B). However, 13- and 29-day-old BGI received significantly more egg rafts than 7-day-old BGI, indicating a clear preference for infusion somewhat older than that preferred by *Cx. tarsalis,* and somewhat older than the recommended age (Reiter 1986).

DISCUSSION

There was a distinct progression in the chemical and microbial content of BGI as it aged. The infusion, which smelled "grassy" when first mixed, became progressively more turbid and foul-smelling with the passage of time, as various microorganisms proliferated and produced volatile and nonvolatile metabolic by-products.

In order to compare aliquots of BGI taken from the same batch of infusion over a 63-day period, it was necessary to freeze the aliquots to halt microbial action and limit further changes in the samples. Although the detailed effects of freezing on the microbial constituents have not been investigated, our experience over a number of years indicates that infusions stored frozen for several years remain highly attractive and stimulatory to mosquitoes when used in laboratory experiments or oviposition traps. Consequently, freezing the infusion aliquots for storage probably had minimal effects on their mosquito oviposition-mediating properties.

The oviposition responses of *Cx. tarsalis* and *Cx. quinquefasciatus* to various ages of Bermuda grass infusion were somewhat different. *Culex tarsalis* generally preferred BGI of ages ranging from 5 to 25 days old to distilled water, with the standard 7-day-old infusion being as good or better than infusion of other ages. The responses exhibited by *Cx. quinquefasciatus* were much stronger, with this species being stimulated by all ages of BGI tested. In particular, Beehler et al. (1994) showed that solutions of lactalbumen hydrolysate, one of the ingredients of BGI (at the same concentration as was used in preparing BGI), significantly increased oviposition by this species. Consequently, it was not unexpected that *Cx. quinquefasciatus* would prefer freshly prepared infusion to distilled water.

In contrast to *Cx. tarsalis, Cx. quinquefasciatus* females exhibited a distinct preference for infusions older than the 7-day-old standard infusion. These results may reflect the subtle differences in habitat preferences between these 2 closely related species. That is, *Cx. tarsalis* prefers relatively clean water for its oviposition sites, whereas *Cx. quinquefasciatus* prefers more polluted and turbid waters containing significant amounts of organic matter (Gjullin and Johnsen 1965).

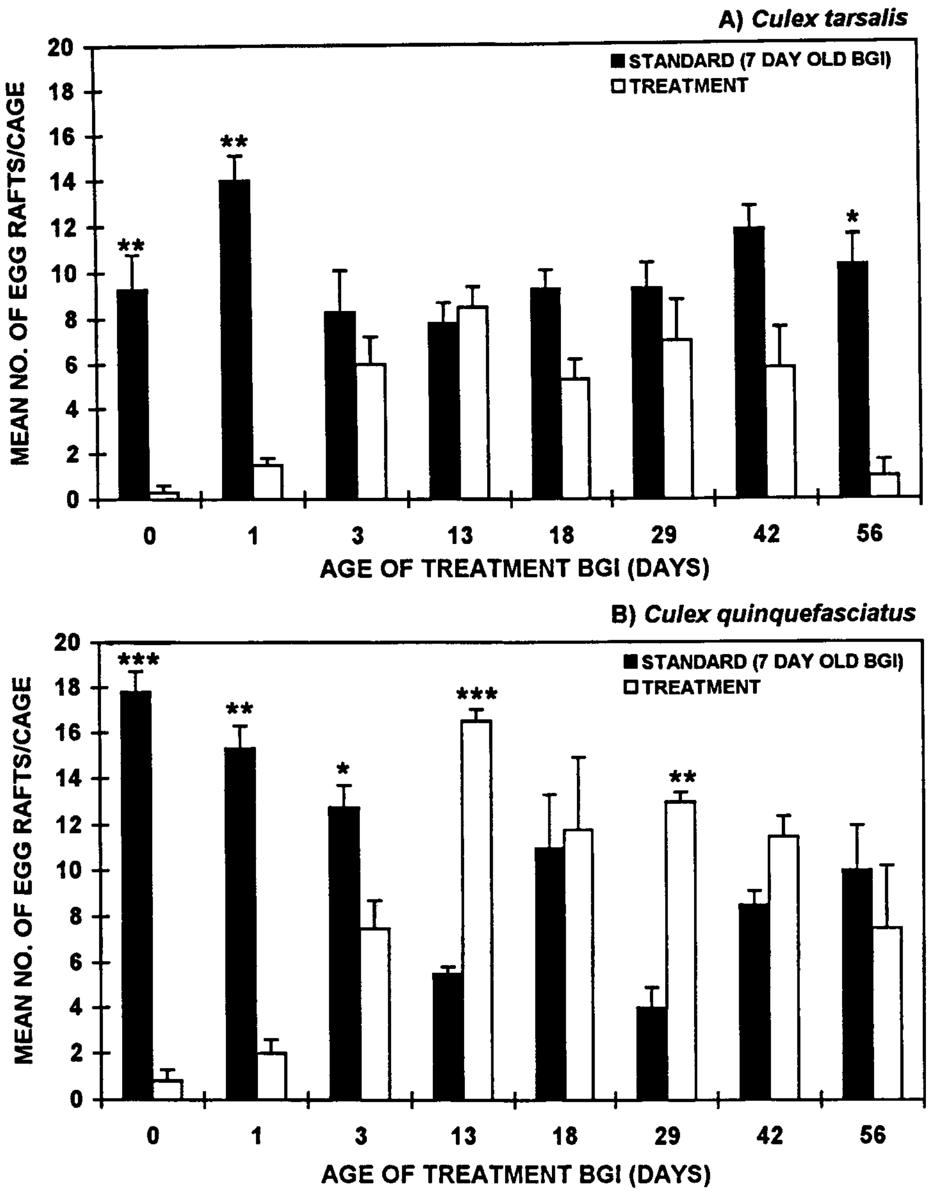

Fig. 3. Comparison of oviposition responses by female *Culex tarsalis* (A) and *Culex quinquefasciatus* (B) to standard-aged (7-day-old) Bermuda grass infusion versus infusion aged for periods from 0 to 56 days. Each experiment was replicated 4 times with 20 mosquitoes/replicate. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

The results of comparisons between 7-day-old standard BGI and various ages of BGI illustrate the importance of using infusions of known and standardized age for mosquito monitoring programs. Clearly, the age of an infusion has a significant effect on its biological activity as a gravid mosquito bait, and the effects seen are different for different species. In particular, *Cx. tarsalis*, a species of importance to monitoring programs because of its role as a vector of encephalitis viruses, responds poorly to older infusions. This, coupled with differences in the response of *Culex* species to the commonly used CDC gravid female trap, may explain why *Cx. tarsalis* often represent only a small fraction of the mosquitoes caught in gravid female traps, even when they are known to be locally abundant from CO_2 or other trap catches.

In summary, the age of Bermuda grass infusions has been shown to significantly affect the outcomes of oviposition by *Cx. tarsalis* and *Cx. quinquefasciatus,* with infusions of different ages varying from being highly stimulatory to virtually inactive in comparison to distilled water. This suggests that infusions of the optimal age range should be used as trap baits in mosquito surveillance programs. However, the situation may be further complicated by the inherent batch-to-batch variability of infusions, which manifests itself, among other ways, in infusions with somewhat different odors. It is entirely conceivable that different batches of infusion, prepared from the same nominal set of ingredients but with different microbial contaminants, may vary in their effects on mosquito oviposition. To our knowledge, the effects of batch-to-batch variability of infusions have been neither rigorously tested nor quantified. This inherent variability, coupled with the changes in infusions with age, points out the need for developing synthetic baits of known, reproducible, and standardized chemical composition and biological activity.

ACKNOWLEDGMENTS

We thank the University of California Systemwide Mosquito Research Program for financial support for Jun Isoe and Jeffrey Beehler.

REFERENCES CITED

Beehler, J. W. and M. S. Mulla. 1993. The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes. Proc. Calif. Mosq. Vector Control Assoc. 61:121–124.

Beehler, J. W., J. G. Millar and M. S. Mulla. 1994. Protein hydrolysates and associated bacteria as oviposition attractants for the mosquito *Culex quinquefasciatus*. Med. Vet. Entomol. 8:381–385.

Brust, R. A. 1990. Oviposition behavior of natural populations of *Culex tarsalis* and *Culex restuans* (Diptera: Culicidae) in artificial pools. J. Med. Entomol. 27:248–255.

Frank, J. H. and H. C. Lynn. 1982. Standardizing oviposition traps for *Aedes aegypti* and *Cx. quinquefasciatus*: time and medium. J. Fla. Anti-Mosq. Assoc. 53:22–27.

Gjullin, C. M. and J. O. Johnsen. 1965. The oviposition responses of two species of *Culex* to waters treated with various chemicals. Mosq. News 25:14–16.

Hoban, B. and G. B. Craig, Jr. 1981. Effectiveness of the ovitrap for monitoring *Culex* populations. Proc. Ind. Vector Control Assoc. 5:10–16.

Isoe, J. and J. G. Millar. 1995. Characterization of factors mediating oviposition site choice by *Culex tarsalis*. J. Am. Mosq. Control Assoc. 11:21–28.

Kramer, W. L. and M. S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8:1111–1117.

Maw, M. G. 1970. Capric acid as a larvicide and an oviposition stimulant for mosquitoes. Nature 227: 1154–1155.

Murphey, F. J. and P. P. Burbutis. 1967. Straw infusion attractiveness to gravid *Culex salinarius*. J. Econ. Entomol. 60:156–161.

Reiter, P. 1983. A portable battery-powered trap for collecting gravid *Culex* mosquitoes. Mosq. News 43: 496–498.

Reiter, P. 1986. A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. J. Am. Mosq. Control Assoc. 2:219–221.

Reiter, P., M. A. Amador and N. Colon. 1991. Enhancement of the CDC trap with hay infusions for daily monitoring of *Aedes aegypti* populations. J. Am. Mosq. Control Assoc. 7:52–55.

Strickman, D. 1983. Preliminary report of seasonal oviposition by *Culex quinquefasciatus* in San Antonio, Texas. Mosq. News 43:226–230.

Strickman, D. 1988. Rate of oviposition by *Culex quinquefasciatus* in San Antonio, Texas, during three years. J. Am. Mosq. Control Assoc. 4:339–344.