after transfer. Three replicates of each treatment with each species was conducted.

An analysis of data, Chi Square and Fisher's Exact Tests, failed to detect any statistically significant differences ($P \leq 0.05$) in the average percent mortality of *Ae. aegypti* versus *Ae. taeniorhynchus*. The number of times mosquitoes were transferred did not significantly affect mortality of either species. *Aedes aegypti* showed 6.7% mortality on one transfer and 8.3% mortality on two transfers. *Aedes taeniorhynchus* showed no mortality on one transfer and only 1.7% mortality on two transfers.

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## THE EFFECT OF DIFFERENT METHODS OF OVIPOSITION INDUCEMENT ON EGG FERTILITY RATES IN A *SIMULIUM DAMNOSUM* THEOBALD COMPLEX SPECIES (DIPTERA: SIMULIIDAE)

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Most gravid females of the species of African Simuliidae so far tested will oviposit in captivity, but the conditions required vary with the species (Raybould and Grunewald 1975). Lewis et al. (1961) found that gravid *Simulium damnosum* s.l. can be induced to lay eggs by immersion in water or decapitation. These methods have since been used successfully with all members of the *S. damnosum* complex investigated, although for convenience some workers have crushed the head rather than removing it entirely. Although most females can be induced to oviposit in the laboratory with these techniques, many eggs laid by inseminated females fail to develop (Wenk and Raybould 1972).

New, effective techniques for inducing oviposition using artificial twilight conditions have recently been developed (Cupp et al. 1981, Simmons and Edman 1981, 1982). These methods simulate natural conditions more closely than the earlier techniques. Simmons and Edman (1982) reported that most eggs laid by *S. damnosum* s.l females stimulated by their technique were fertile. They speculated that the more natural oviposition stimuli may have produced higher egg fertilization rates.

Since it is important to obtain maximum egg fertility rates for laboratory colonization, investigations were carried out on the *S. damnosum* complex species *Simulium squamosum* (Enderlein) to compare the proportion of fertile eggs laid following two different methods of oviposition inducement: the Simmons and Edman (1982) technique and the immersion method. The latter was chosen for comparison rather than decapitation because it does not damage the fly.

The *S. damnosum* complex females used were caught at Tsatsadu Falls, near Hohoe in the Volta Region of Ghana, when the biting population present was probably entirely *S. squamosum*.

Biting females were allowed to feed to repletion on man in the field and tubed separately in 4.5 × 1.5 cm plastic (not glass) tubes with push-in caps. They were kept cool before and during transportation. In the laboratory each fly was maintained in a separate tube, fed on sugar solution and protected from attack by ants (Raybould et al. 1982). Three or occasionally four days after feeding, each fly was induced to oviposit by one of the following methods:

THE IMMERSION METHOD. To determine whether the time of oviposition inducement affected egg fertility rates, about half the flies were stimulated during the day and the other half in the evening at about 1930 hr under artificial lighting. Each female was placed in a separate 4.5 × 1.5 cm plastic tube lined with a rolled up piece of fine silk-netting and filled with water to a depth of about 1 cm. The tube was tilted and rotated in such a way as to immerse the fly and induce it to oviposit on the netting, but not on the inside of the lid or the bottom of the tube. The netting with the eggs attached was placed in a container of well aerated water until the next day when the number

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of developing and undeveloped eggs was counted under a dissecting microscope. The parent fly was dissected and the number of retained eggs counted.

THE SIMMONS AND EDMAN (TWILIGHT) METHOD. Gravid females were stimulated after dark at about 1930 hr. The apparatus used was based on that of Simmons and Edman (1982), but was made up of ready-made rather than especially built components. Nevertheless, the basic features remained the same in that agitated water and dim light were provided to simulate twilight conditions in natural oviposition-sites. The method can therefore by aptly termed the "twilight method."

The apparatus (Fig.1) comprised two plastic or glass aquaria, each measuring 30 × 20 × 20 cm, placed end to end and filled with water to a depth of about 9 cm. A piece of cardboard, extending down to the water level, was placed between the two aquaria. One of the aquaria was used as the oviposition chamber. To provide oviposition substrates, fine nylon-netting was fixed to the end-wall of the chamber nearest the cardboard plate and corks were floated nearby. (Although the flies will oviposit directly on the oviposition chamber wall, as they did in Simmons and Edman's original apparatus, the use of netting facilitates egg removal.) Water agitation and current against the corks was produced by pumping air through 0.5 cm diam plastic tubing. The oviposition chamber was fitted with a cover. An aeration stone producing fine air bubbles was placed in the second chamber at the end adjacent to the cardboard plate.

Before oviposition inducement was attempted, the main light was switched-off and the two chambers were illuminated by flashlight. The flashlight was placed horizontally at water-level and shone through the second chamber first (Fig. 1). The presence of the opaque cardboard plate allowed the water in the oviposition chamber to be illuminated only from below. Each fly was stimulated to oviposit separately so that the parent of each egg batch could be identified and the number of retained eggs related to the number laid. Individuals that flew to the top of the chamber were returned to the corks with an aspirator and any that floated in the water were scooped-up on a cork. Eggs were laid both on the corks and the nylon-netting. Postoviposition procedures were essentially the same as with the immersion method.

Relatively few flies were stimulated to oviposit on the same day. This avoided the possibility of conditions on any particular occasion exerting an undue influence on the results. Whenever possible, the different techniques were both tested on each occasion.

Although a few flies drowned, escaped or refused to oviposit, most individuals laid all or most of their eggs when stimulated either by immersion or by the twilight method. Mean results for all ovipositing flies are given in Table 1. A few flies retained many eggs giving a mean of between 9 and 12% retained eggs for the various inducement techniques. Individual egg batches showed a wide range in fertility irrespective of the inducement technique used. The fertility rates of eggs laid following the different methods of inducement were: 38% for daytime immersion, 36% for immersion at night and 71% for the twilight method. The Simmons and Edman technique resulted in a fertility rate almost twice as high as the immersion method and results with the latter were not affected by the time of day at which immersion was carried out. The differences observed between the two main techniques is highly significant (student's *t*-test for higher (d) numbers: $d = 4.6432$; $P < 0.001$). Our results suggest that the Simmons and Edman technique of oviposition inducement is better than the immersion method for normal use in the colonization of *S. damnosum*

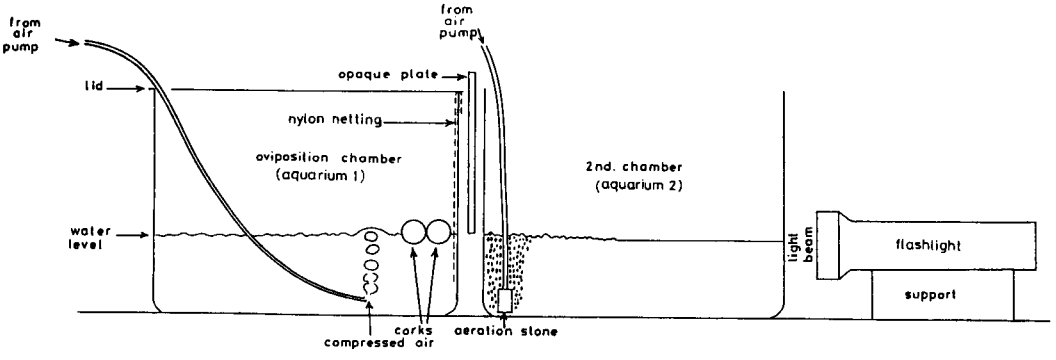


Fig. 1. Simplified version of the Simmons and Edman apparatus for inducing oviposition in *Simulium*.

Table 1. Fate of *Simulium squamosum* eggs laid by females induced to oviposit by various procedures. Data from 40 females for each treatment.

| Procedure | Eggs retained | | | Eggs laid | | Fertility | | |
|---|---|---|---|---|---|---|---|---|
| | Mean no. | Range | Mean %* | Mean no. | Range | Mean no. | Range | Mean %+ |
| Daytime immersion | 35 | 0–471 | 9 | 345 | 86–891 | 130 | 0–713 | 38 |
| Night-time immersion | 48 | 0–547 | 12 | 343 | 80–653 | 124 | 0–475 | 36 |
| Twilight method | 47 | 0–524 | 11 | 382 | 114–690 | 270 | 6–645 | 71 |

*Percentages of total eggs produced.
+ Percentages of eggs laid.

s.l., although the assumption that other cytospecies would give results similar to *S. squamosum* remains to be proven. The method of Brenner et al. (1980), which has been successfully used for *S. damnosum* s.l. (Cupp et al. 1981), also requires testing and might well give results similar to the Simmons and Edman technique since it is based on somewhat similar principles.

A minor problem with the Simmons and Edman apparatus is that after oviposition the corks (unlike the nylon-netting) cannot be suspended in gravity rearing troughs without causing splashing, unless a sliver is sliced from the surface. This difficulty can be overcome by covering the corks with easily removable materials such as fine netting.

Although we only tested the Simmons and Edman method in the evening after dark, it can be used during daylight hours in a darkened room (Dr. K.R. Simmons, personnel communication). Whether results are the same throughout the day still requires testing, but no change occurred with the immersion method.

The twilight method is convenient for colonization work when many females can be left to oviposit overnight, but it is less practical for obtaining egg batches from individual flies. For the latter purpose only one fly can be stimulated at a time and inducing several females to oviposit in succession may require hours of work.

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