A SIMPLE APPARATUS FOR MAINTAINING BLACK FLY ADULTS (SIMULIIDAE) IN THE LABORATORY[1,2]

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ABSTRACT. A newly developed apparatus for maintaining *Simulium* adults is described in which the emphasis is placed upon good air conditioning and the maintenance of high humidity in the holding chamber. Improvements on the safety caps and the use of polystyrene, plastic material and nylon gauze with special hygroscopic qualities led to a maximum survival time of 4 weeks in different species. The advantages of the apparatus are its simplicity of construction, its potential for use in areas lacking electricity and the possibility of maintaining *Simulium* adults in different stages of development, such as blood digestion, egg development and oviposition, under controlled conditions.

INTRODUCTION

Black flies have been the subject of many entomological and parasitological investigations due to their ability to act as vectors of filariae, in particular *Onchocerca volvulus,* the causative organism of human onchocerciasis.

The maintenance of adult simuliids during development of the parasite is crucial in any investigation of vector-host relationships. However, laboratory maintenance of adult simuliids poses many problems. Inducing blood feeding and blood ingestion of adult females is difficult. Black flies are susceptible to desiccation and also to extreme humidity which can result in fungal infections. An additional problem is providing adequate oviposition stimulus, and then the transfer of the females from the oviposition chamber to a holding pot/cage.

Various techniques for maintaining adults have been described in the literature. Matsuo et al. (1978) maintained *Simulium ochraceum* Walker, and Zuluaga and Yuill (1979) maintained *Simulium mexicanum* Bellardi in plastic cups. Glass tubes were used by Davies (1953) and glass beakers by Wenk (1965). Cardboard cups or cartons were found to be useful due to their hygroscopic qualities (Simmons and Edman 1982, Zuluaga and Yuill 1979). In brief, these authors maintained the darkness, temperature and relative humidity of the adult black flies by placing the insect-holding chambers within larger closed containers. It is known that a high relative humidity is necessary for adult survival, but very few authors (Rühm 1973, Simmons and Edman 1978, 1981, 1982; Lok et al. 1980, Ham and Bianco 1983) give exact levels of the relative humidity, i.e. 80–95%. Until now, no studies have been carried out on the humidity conditions required for optimal maintenance of adult Simuliidae under laboratory conditions. Thus an apparatus in which the relative humidity can be controlled and maintained at any level was designed and is described in this paper.

MATERIAL AND METHODS

The maintenance apparatus consists of three parts: air supply (tubes, branches and compressor), regulation and enrichment of water vapor, and the maintenance chamber (Fig. 2). Holes bored in a polystyrene block serve as the basis of the maintenance chamber (Raybould and Mhiddin 1974). In a block of PS 30 SE polystyrene (Fa. Schwenk, Ulm, F.R.G.), measuring 170 × 340 × 40 mm, 30 holes of 25 mm diameter were bored with a brass cork borer. White polyvenylchloride (PVC) stoppers are fitted into the holes at top and bottom (Fig. 1). In addition to a hole covered with gauze, each top stopper has a second hole housing a cotton wool

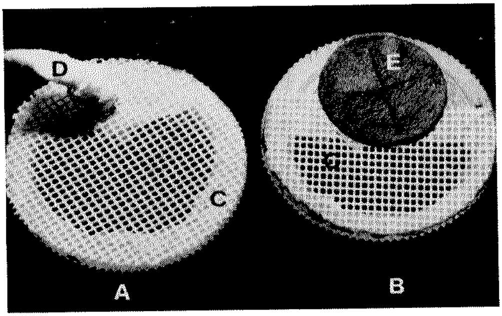

Fig. 1. Plastic lids for closing the holes in the polystyrene blocks. A. Top stoppers, B. Bottom stoppers, C. Fine netting (nylon gauze), D. Cotton wick and E. Cross-cut rubber disk.

[1] This paper is dedicated to Professor Dr. P. Wenk on the occasion of his 60th birthday.

[2] Published with the assistance of a grant from the S. C. Johnson Co., Racine, Wisconsin and funds from The Marco Giglioli Memorial Fund.

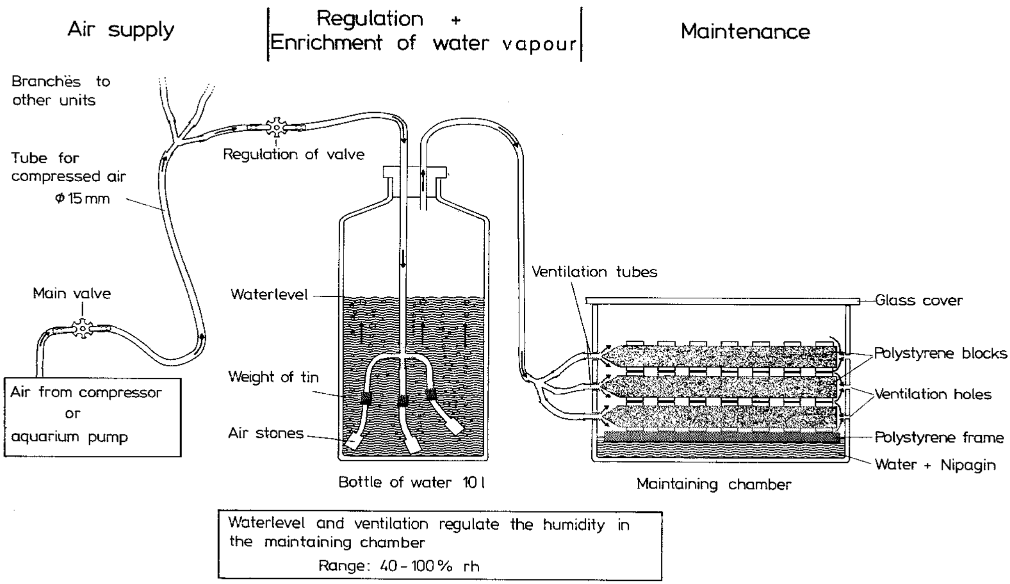

Fig. 2. Diagram of the apparatus for maintaining adult black flies.

wick, which is moistened with a 2 percent sugar feeding solution. The bottom end of the hole was closed with a similar plastic lid, with one hole covered with a fine gauze and a second covered by a rubber disc with a cross-cut in the middle (Fig. 1). Fine ribbing on the plastic lid ensures a tight seal keeping it in place. An inlay of filter paper, which is easily replaceable, prevents the holes from becoming dirty with digested blood. This entire apparatus can be washed and re-used several times on the completion of a series of tests. A small aspirator is used to remove the black flies via the cross-cut of the rubber.

To ensure a high air humidity three polystyrene blocks are placed on top of each other in a closed plastic aquarium 375 × 200 × 240 mm (Fig. 3). To maintain an overall humidity, there is 20–30 mm of water in the chamber. The addition of methyl-4-hydroxybenzoate (Nipagin®, Merck) prevents growth of fungi. A polystyrene frame prevents direct contact of the three polystyrene blocks with the water. The aquarium is closed by a glass cover sealed with vaseline and darkened by self-adhesive black plastic foil around the basin and a black cloth on the top (Fig. 3); darkness has been shown to significantly increase the length of survival of the adults (Figueroa et al. 1977).

The ventilation, constant relative humidity and circumvallation of air inside the chamber is achieved by a system of tubes, regulation valves and a wide-dimension, 10 liter washing bottle providing the physical method of air-stream enrichment (Figs. 2 and 3). Dry air from a compressor or small commercial aquarium pump passes through a defined quantity of water in the wash bottle and is thus humidified before flowing through three ventilation tubes into the maintenance chamber. The arrangement of the tubes ensures similar humidity and ventilation through the holes of each block. The damp air leaves the chamber through holes on the opposite side. The humidity in the chamber is directly related to the amount and speed of the air and the level of water in the wash bottle. Thus, a known quantity of water, constant air speed and temperature will maintain the relative humidity at a specified level. Correction or alteration of the humidity can be made within half an hour by noting and changing the water level in the bottle.

The humidity and temperature is monitored by the electrode of an electronic hygroscope (Rotronic Hygroskop BT with DMS 100H, Rotronic AG Zürich, Switzerland), projecting through a hole in the outside wall of the maintenance chamber (Fig. 3). The manufacturer of the hygroscope gives a standard deviation (s.d.) of ± 2% relative humidity (RH). In this study the s.d. of RH between the measuring point in the chamber and the holes of the different

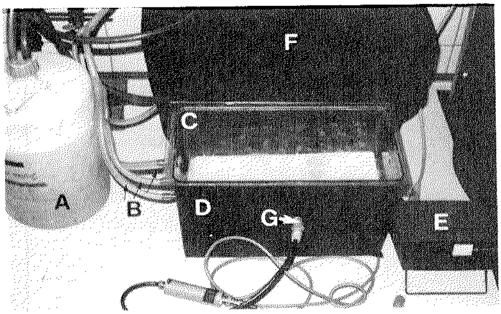

Fig. 3. Maintaining apparatus. A. Wash bottle (10 liters), B. Tubes for air conditioning, C. Glass cover, D. Self-adhesive PVC, E. Measuring equipment for temperature and humidity (hygroscope), F. Black cloth for darkening and G. Electrode of hygroscope.

blocks was at 100% ± 8%, at 90% ± 1.5% and at 80% ± 2.1%. A simple aquarium pump and a small transformer can also be used as a source of air and can be connected to a car battery for use of the apparatus in the field or when electricity is not available.

For transportation of the apparatus the glass cover can be replaced by a hinged lid, and the water by a wet cloth. A second polystyrene frame closed the maintenance block and prevents vibration and movements within. The maintenance chamber can be easily separated from the rest of the apparatus by removing the tubes. Transportation of the darkened maintenance chamber for several hours does not lead to a higher mortality of the flies, as long as the air-conditioning is subsequently reconnected. Various experiments were carried out under controlled conditions using four palaearctic species of Simuliidae, namely *Simulium ornatum* s.l. Meigen, *Simulium lineatum* Meigen, *Simulium noelleri* Friedrichs and *Simulium tuberosum* Lundström and one African species, *Simulium damnosum* s.l. In order to demonstrate the usefulness of the apparatus male and female flies were separated and kept in different maintenance chambers throughout the experiments. Preliminary trials with the teneral flies showed that the survival rate decreased rapidly at humidity levels below 80%. The temperature established during the experiments corresponded to the average natural temperature found in the field in early summer. During the experiments only the humidity levels were altered, the simuliids were maintained at 80, 90 and 100% RH at the temperature ranging from 17.5 to 18.5° C. The survival rates for the most favourable humidity level were plotted (n=50 flies/curve). As the purpose of this paper is only to introduce the apparatus, the results of all experiments will be published in detail elsewhere.

RESULTS AND DISCUSSION

Keeping the flies in a darkened environment restricts their movement and thus reduces damage although they continue blood digestion and egg development. Raybould and Mhiddin (1974) observed losses of flies through ill-fitting lids; however, in this case the well-fitting lids and the cross-cut rubber prevent the escape of flies. The hygroscopic quality of the polystyrene, fine nylon gauze and the PVC lids combined with the air conditioning prevented the flies from becoming wet through condensation, as observed by Raybould and Mhiddin (1974) and Matsuo et al. (1978), even at 100% RH.

In agreement with Janisch (1921), we found that the washbottle-humidification method did not result in the building up of different grades of humidity in layers as is the case if Zwölfer's salt solution method (Zwölfer 1932) or if the vessel method is used. Control measurement in the holes of each of the three blocks (top, middle and bottom) showed that an adjusted, defined humidity can be maintained for 8 to 48 hr with constant temperature. The air conditioning and addition of methyl-4-hydroxybenzoate to the water of the washbottle (and even to the food) prevents the growth of fungi. Very little effort is needed to maintain the apparatus as the humidity is easily adjusted and kept constant, and due to its simplicity the system is not very susceptible to mechanic breakdown.

Figures 4A and 4B illustrate both interspecific and intraspecific (♀♀ versus ♂♂) survival rate differences. Females always lived longer than males. In the experiment with *S. damnosum* s.l., owing to technical restrictions, the two sexes were collected and studied together. In addition, it was possible to perform one experimental trial only, thus, the curve shown in Fig. 4B may not be representative of the optimal survival rate for *S. damnosum* s.l, *S. ornatum* (♂♂ and ♀♀) demonstrated the longest survival rate with an average lifespan of 11,2 days at 90% RH and 18°C. *S. lineatum,* also with potential to survive up to 3 wks, exhibited an average of 8.8 days.

After 7 days Ham and Bianco (1983) observed survival rates after an infection by *O. volvulus* of 83 and 93% for *S. ornatum* and 52 and 56% for *S. lineatum.* In our experiments the survival rates for *S. ornatum* and *S. lineatum* carrying no filarial infections after 7 days were 52 and 56%, respectively. Further experimentation revealed a larger difference in survival rates between early and late generations of the same

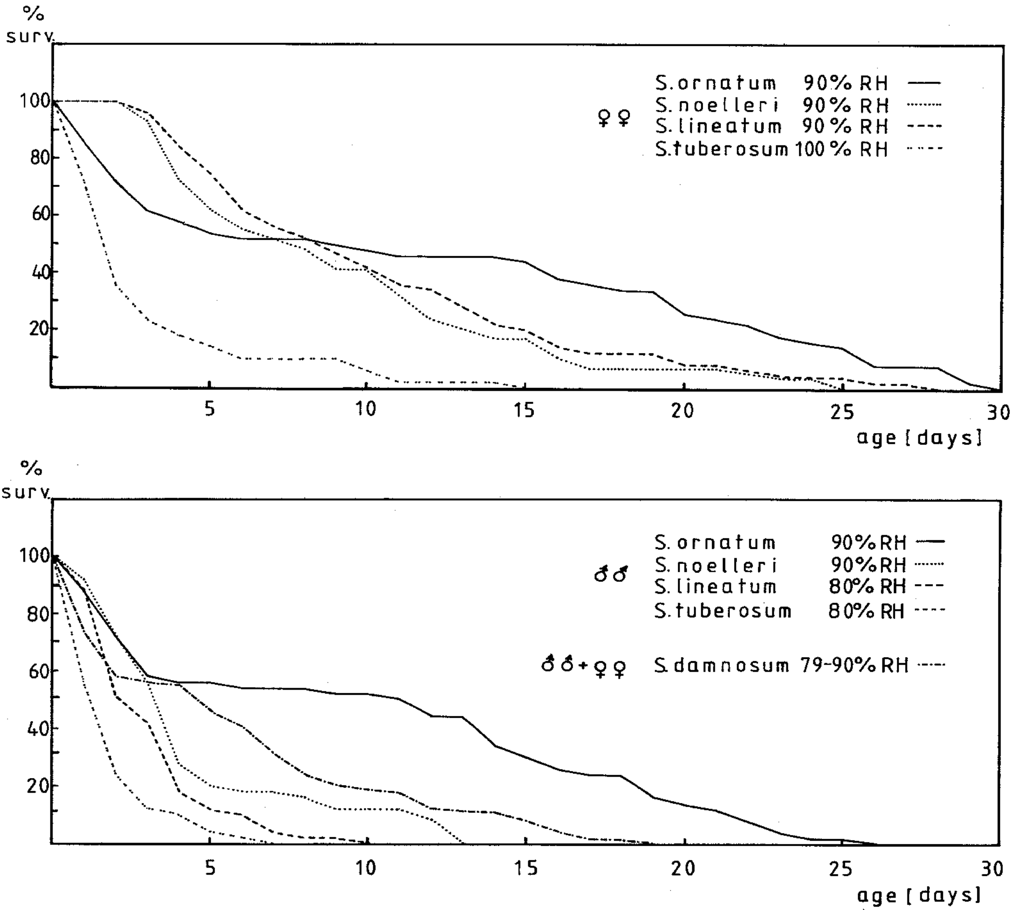

Fig. 4. Best survivorship curves comparing the different species of the spring generation at 18° C and adequate humidity. A: Females, B; Males and *S. damnosum* males and females.

species during the year. In the present study the apparatus has been used only to investigate the effects on survival rate of changing the air humidity. For general studies where the flies must survive as long as possible, there is no need to maintain an exact humidity or temperature, but instead a specific range (e.g. for *S. damnosum* s.l. 80–95% RH and 21–28° C.). Thus the described apparatus is a simple but effective tool to maintain and to study adult *Simulium* in the laboratory.

ACKNOWLEDGMENTS

We wish to express our thanks to Mr. H. Renner, Institute of Tropical Medicine, University of Tübingen, for his instructions of basic techniques in the development of the maintenance apparatus. Special thanks are due to Dr. J. N. Raybould, WHO Onchocerciasis Control Programme, Akosombo, Ghana, for sending *S. damnosum* s.l. egg batches and Dr. S.E.O. Meredith, WHO Onchocerciasis Control Programme, Ouagadougou, Upper Volta, for reviewing the manuscript.

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ERRATUM

Mosquito News, vol. 44, no. 2

Davidson, E. W. Microbiology, pathology and genetics of *Bacillus sphaericus:* Biological aspects which are important to field use, pp. 147–152.

On page 149, left column, paragraph 1, lines 20–27 should read:

The ability of *B. sphaericus* to recycle in the larval cadaver has two important implications in the use of this organism; first it provides a potential source of spores which may infect reinfesting larvae after field treatment (Des Rochers and Garcia 1984), and second, recycling in bioassay containers may produce erratic results in these assays.