A SIX-POSITION ARTIFICIAL FEEDING APPARATUS FOR *CULICOIDES VARIIPENNIS*[1]

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ABSTRACT. The equipment is described and a brief discussion given of its successful use with *Culicoides variipennis* (Coquillett) and mosquitoes. The portable apparatus contains 6 identical feeding compartments in a permanent arrangement. The temperature of the artificial-meal fluid is maintained by circulation of warm water through the jackets of the membrane feeders. Uniformity of the meal is provided by stirring to prevent sedimentation and by preventing fluid evaporation.

Extensive literature has been published on the artificial feeding of fluids to biting flies through membranes (Rutledge *et al.*, 1964). However, for the artificial feeding apparatus constructed for our use with *Culicoides variipennis* (Coquillett) in research on the transmission of bluetongue virus, the causative agent of a disease of sheep and cattle, we incorporated several changes that proved to be significant improvements. Two recent papers (Behim, 1967; Kitaoka and Morii, 1970) have not changed our concepts.

A provision was made for continuous stirring of the fluid being fed to keep it in suspension while it was offered to the flies; uniformity of the meal was further enhanced by preventing fluid evaporation during use. We considered it essential that the feeding apparatus have the capacity to accommodate several tests simultaneously and in a permanent arrangement, thus providing for adequate experimental design. Because our feeding apparatus was constructed with 6 standard, fixed positions for feeding, this provided a reproducible situation that lent itself to later test replication. We made the unit portable so that it could be isolated in an environmental chamber to standardize test conditions. Finally, we designed the equipment specifically for use with *C. variipennis,* a fly whose feeding behavior indicated the need of a system in which the female could crawl a short distance to reach a feeding surface comprising the entire cage ceiling. Thus, the female was not forced to locate a feeding surface that was only a small percentage of the total ceiling area.

The equipment design for the most part makes use of the concepts of previous workers (Rutledge *et al.*, 1964; Behim, 1967). The apparatus contains six identical feeding compartments, which are protected from the environment on all but the front side. Cages of flies are placed in these compartments so that the flies can crawl, or fly, up the slanted surface of each cage to a feeding surface that comprises the entire cage ceiling. The feeding surface is a membrane attached to the lower end of a glass membrane feeder. Warm water circulated through the jacket of the membrane feeder regulates the temperature of the artificial-meal fluid contained in the central well of the feeder.

In the description of this equipment, measurements are not critical except in the relation of parts to one another and in the dimensions of materials that were purchased.

CONSTRUCTION. Our experimental model of the artificial-feeding apparatus (Fig. 1, 2) consists of three major pieces: the wood framework, and the upper and lower plastic assemblies. These three pieces can be simplified and combined so that the apparatus consists of a single plastic assembly. A unit designed for accommodating larger cages of flies would probably have fewer feeding compartments.

[1] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.

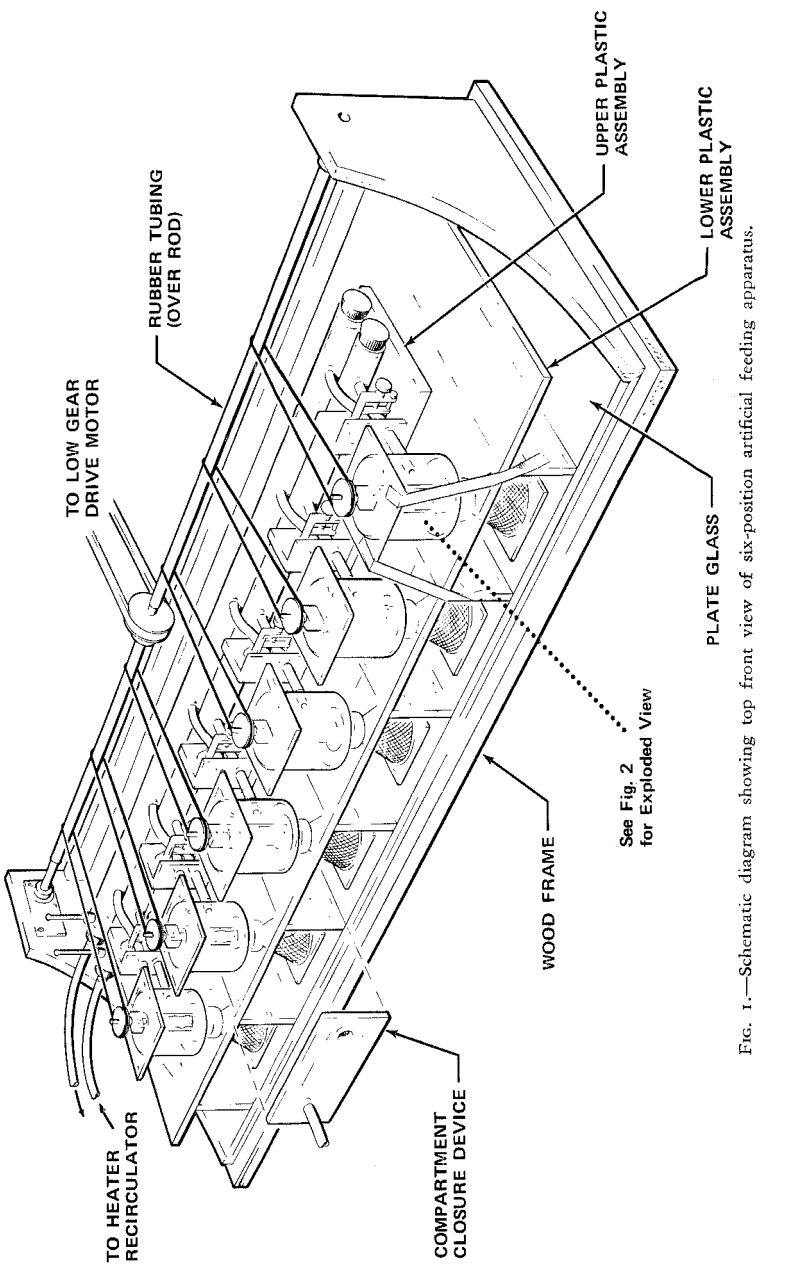

FIG. 1.—Schematic diagram showing top front view of six-position artificial feeding apparatus.

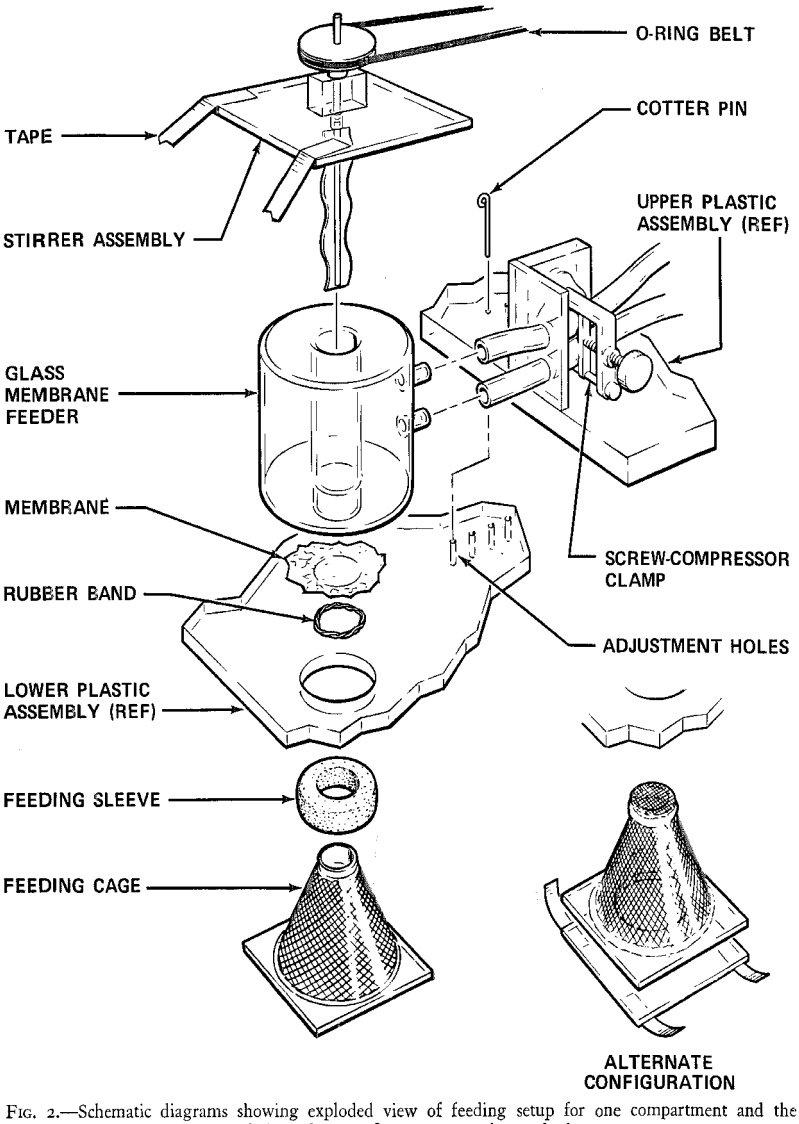

FIG. 2.—Schematic diagrams showing exploded view of feeding setup for one compartment and the regulation of water flow to a membrane feeder.

The wood framework, provided with a cover (not shown), has an observation port (can be omitted in simplified model) in the floor under the feeding compartments and a revolving 12-mm-diameter iron rod at the rear to transfer power for stirring the artificial-meal fluid. The rod is ball-bearing mounted and is encased in a length of rubber tubing (placed by lubrication with glycerin) to provide a good grip for the belts to the stirring rods. A sheet of plate glass, preferred over plastic for some experimental work to provide better visibility through the port, covers the floor of the wood framework and, except for the area over the port, it is separated from it by a sheet of white felt. The glass is held in place by the sides and back of the wood framework and by a narrow strip of wood, of the same thickness as the glass, along the front edge. The two plastic assemblies, an upper and lower, complete the feeding apparatus itself: these complex assemblies, unless otherwise specified, are made of clear plastic sheeting 6 mm thick.

The lower plastic assembly rests on the plate glass and fits snugly against the wood framework at the rear; it consists of a 1065- x 355-mm sheet with 63-mm-high strips cemented underneath for support so that 6 feeding compartments (147-mm wide x 153-mm deep x 63-mm high) are formed over the plate glass and under the main sheet of the assembly even with its front edge. Overhead in each feeding compartment, the main plastic sheet has a 50-mm-diameter hole located equidistant from the sides and 20 mm from the front edge.

The upper plastic assembly contains all the parts for controlling the flow of water, the heat-exchange fluid, to the jackets of the membrane feeders. Its base is a 12-mm-thick sheet of plastic (840 x 100 mm) that is aligned on top of the lower plastic assembly, equidistant from the sides but set back about 140 mm from the front edge of the lower assembly. The upper plastic assembly is held in place by two cotter pins in a series of adjustment holes (can be omitted in simplified model) between its base and the top of the lower plastic assembly. At 153-mm intervals (to position the rubber tubing to the membrane feeders with the center of each feeding hole in the top of the lower plastic assembly), six L-shaped plastic pieces are cemented to the floor of the base with the 3-mm-thick (x 30-mm wide x 60-mm high) arm along the front edge of the base and the sturdier 9-mm-thick (x 40-mm wide x 60-mm high) arm cemented at right angles to it and providing the major support. Each 3-mm arm of the L-shaped piece firmly holds two pieces of 6 mm-ID rubber tubing by compression through two holes that are 6 mm apart and close to the arm's outer edge so that the two tubes can be opened and closed by the screw-compressor clamp mounted to the sturdier arm of each L-shaped piece. The middle of each short piece of tubing is thus held so that when a membrane feeder is attached to the feeder end of the tubing it is always in the same front-to-back position over a feeding hole. The rear portion of the 12 short lengths of rubber tubing, an inlet and an exit for each of the six positions, connects by compression through a hole in one of two 26-mm-ID plastic tubes that are cemented along the floor at the rear of the base of the upper plastic assembly with the holes positioned to receive the rubber tubing. One plastic tube serves as a common inlet and one as a common exit for the water of the circulating heat-exchange system; the upper rubber tube from each feeder and the more removed plastic tube are for water exit. The right end of each plastic tube is stoppered; the left is connected through another stopper to flexible tubes that lead to and from a constant-temperature heater-circulator. Paired thermometers, one at the start of the inlet tube and one at the end of the exit tube, measure the temperature of the circulating water.

The glass membrane feeders (Fig. 1, 2) are similar to those in use by various investigators (Rutledge *et al.*, 1964); they can be made by a scientific glassware com-

pany. Two segments of glass tubing are connected so that the external one forms a jacket around the inner one. The jacket formed by the outer tube has an upper and a lower flat surface and measures 65-mm high and 73 mm in diameter. The jacket at one side is provided with two nipples for the attachment of the rubber tubing carrying the circulating water. The inner tube, 16-mm ID, is open at the top and flush with the upper surface of the jacket; the lower end protrudes about 16 mm beyond the lower flat surface of the jacket. When a membrane is attached to the lower end of the inner tube, a well of a little over 15-ml capacity is formed over the membrane. A membrane is attached by two or more winds of a rubber band.

Two types of feeding cages are used. An open-topped cage (Fig. 1, 2) consists of a plastic-screen cone (35 mesh, Saran plastic screen) that measures about 55 mm in height and 16 and 55 mm in diameter at the open top and closed bottom. The 3-mm-thick bottom, clear if it is desirable to look up through the observation port at the feeding flies, is normally clear-plastic sheeting or a polyester-resin poured plastic; the plastic-screen cone is cemented to a clear-plastic sheet or attached in poured plastic. The upper rim of the cone is fashioned so that a large, internal ledge is not formed that would tend to stop flies crawling upward toward the membrane feeding surface; we constructed ours with a 3-mm-thick, 15-mm-diameter ring of silicone rubber cemented with silicone-rubber cement to the upper cone edge—the ring was cut from 3-mm-thick poured sheeting with two appropriately-sized cork borers. The gauze-topped feeding cage is similar to the open-topped cage: the top (22-mm diameter with a 16-mm hole) is covered with fine-mesh nylon-stocking-type netting cemented in place with silicone-rubber cement; the bottom has a central 45-mm hole than can be covered by taping a 75- x 75-mm piece of plastic to the bottom of the feeding cage.

Because measurements are not exact, a gap of as much as 2 mm exists between the membrane and the rubber rim of the feeding cage when the cage is placed in a feeding compartment under a membrane feeder. When the open-topped cage is used, a feeding sleeve is placed around the upper rim of the feeding cage and positioned over the gap during the feeding procedure. The feeding sleeve is a 12-mm-high ring of polyfoam cut around a 16-mm hole cut in 12-mm sheeting with a cork borer. When a gauze-topped cage is used, 75- x 75-mm shims of plastic of different thickness are used to raise the gauze top so the membrane rests on the gauze.

The artificial-meal fluid is maintained in suspension in each feeder by a stirrer (Fig. 1, 2) that consists of a shaped piece of metal attached to a 1.5-mm-diameter metal shaft through a flexible sleeve that lessens binding of the stirrer as it turns in the well of the membrane feeder. The metal shaft is held centrally along the axis of the well of the membrane feeder because it is fitted snugly through a hole in a small 12-mm-thick piece of plastic cemented to a 75- x 75-mm baseplate that is carefully positioned and then taped onto the flat top of the feeder. A pulley, attached to the top of each shaft, provides seating for a large O-ring belt that turns the stirrer at 36 rpm. A belt for each membrane feeder is looped around the rubber-tubing-encased steel rod mounted at the rear of the wood framework; the steel rod is turned with a V-belt attached to a low-gear motor mounted at the left end of the artificial-feeding apparatus. Adjustment for belt length of the O-rings can be made by the small series of adjustment holes where the upper and lower plastic assemblies are coupled by the two cotter pins; if necessary, any one of the belts can be fixed at one position and tightened by placing layers of masking tape around the steel rod.

A compartment closure device (Fig. 1) is provided so flies in a feeding cage can be anaesthetized. This plastic piece (180 x 75 mm) has two holes. A rubber tube compressed through one hole carries the

CO_2 used for anaesthesia; the other hole is screened and allows the exit of surplus gas.

PROCEDURE. Once the apparatus is set up and the artificial-meal fluid and insects are on hand, very little time or effort is required to put the apparatus into operation. The membranes are attached to the membrane feeders, the feeders are attached to the apparatus, the artificial-meal fluid is placed in the wells of the feeders, the constant-temperature heater-circulator is turned on, and the appropriate screw-compressor clamps are opened to allow the heat-exchange water to circulate into the jackets of the membrane feeders that are being used. If no membranes have developed leaks (it is sometimes best to check these before attachment to the apparatus), the stirrers are placed in the wells and the motor that powers the belts is turned on. Flies anaesthetized with CO_2 are placed into the feeding cages through a small, disposable paper funnel that is inserted into the top of the open-topped cage armed with its feeding sleeve or into the bottom hole of the gauze-topped cage which then has a square piece of plastic taped to the bottom. The feeding cage is placed under the membrane; and either the feeding sleeve is drawn up so that it covers the gap between the top of the open-topped feeding cage and the membrane edge or, for the gauze-topped cage, additional pieces of plastic are placed below the cage until the membrane rests snugly on the gauze. One at a time, each belt is placed in the pulley of its stirrer, and the base piece of the stirrer is taped into place with two strips of tape so that the stirrer turns smoothly. If only two membrane feeders are to be used and with only a few simple steps for the preparation of the artificial-meal fluid, the whole procedure of setting up takes less than 10 minutes.

The flies are removed by placing the compartment closure device over the front of the compartment and anaesthetizing the flies with CO_2. When an open-topped feeding cage is used, any flies still feeding are pushed off into the feeding cage with a fine brush; when a gauze-topped cage is used, anaesthetizing the flies can be avoided but care should be exercised, to avoid pulling attached flies through the netting.

DISCUSSION. The equipment described has been in use at our laboratory since early 1967 and has provided us with a standard method for feeding flies an infective meal of known virus titer in our transmission studies with *C. variipennis* and bluetongue virus. During this time, our methods have been refined and minor changes have been incorporated into the design of the apparatus.

In early work with a preliminary 2-position model of the artificial feeding apparatus, we established that 36.5–37.5° C was a satisfactory temperature for the artificial-meal fluid during feeding. In a short series of four paired tests, we determined that chick membrane was superior to Badruche membrane (a bovine-intestine preparation), since an average of 70 percent versus only 19 percent of the females engorged and all four tests had a higher percentage of females engorge through the chick membrane.

The standard membrane now used for our research is the dried skin of a chick less than 1 day old. Chicks are killed and frozen, and a supply of dry membranes is prepared as needed. Chicks are partially thawed until the skin is soft; then most of the down is plucked, and the wings are clipped off. The skin is slit along one side, pulled off, and spread with pins on cardboard to dry. Excess fat is picked off. Stored dry membranes maintained at nonhumid ambient temperatures can be used for at least 2 weeks; however, with longer storage, they tend to become brittle and are more difficult to use. Membranes are usually dry when they are placed on a feeder; however, especially with older membranes, they are sometimes best placed after wetting them to increase their pliability.

A problem that occurs when *C. variipennis* females are given a blood meal is that occasionally some flies take a clear-fluid

meal. This has happened several times during the past few years when flies were fed on various species of animals. On one occasion, 1 of 18 females that engorged of a total of 23 placed on a sheep took a clear-fluid meal. On another occasion, 4 of 19 females that were blood fed on a rabbit engorged with a clear fluid. Another more striking case occurred with a cow that had been used previously for blood feeding females of *C. variipennis*. Almost immediately after the first flies started to feed, a localized shock to the animal's system occurred; the localized edema was so great that a hard, shallow swelling about 8 inches in diameter occurred before most of the flies could get settled and feed. Therefore, of 27 females that were placed on the cow, 2 blood-fed (eyesight determination), 6 took clear fluid that was tinged pink (a trace of blood), 11 took clear fluid only, and 8 did not feed.

Obviously, and probably depending largely on the exact circumstances, the normal blood meal of a pool feeder such as *C. variipennis* will vary greatly as to blood content. Because of varying amounts of lymph and tissue fluids present in the blood pool, the number of red blood cells per unit volume of the blood meal varies greatly, even though it visually appears that all flies have engorged with equivalent blood meals. That a similar situation may exist for mosquitoes, which are normally not considered pool feeders, was demonstrated when one female of a pool of *Aedes aegypti* (Linnaeus) blood-fed on a rabbit took a fully clear meal.

It has normally been assumed by most investigators that artificial feeding through a membrane will yield a more standardized meal for test flies. However, at least for a pool feeder such as *C. variipennis*, feeding flies a standardized meal through a membrane does not eliminate the occurrence of these clear-fluid meals. Although the appearance of females with a completely clear meal is a rare occurrence with feeding on a live host, it happens fairly often in feeding through a membrane on an artificial-blood fluid. Because the occurrence of females engorged with a clear fluid, together with other females with a mixture of clear fluid and blood, indicates a failure in the artificial system for giving flies a standardized meal, we normally discard tests in which such meals occur. At this time, we are not sure of the reasons for this apparent variable in blood feeding. However, we have determined that clear meals occur with membranes made from the skin of 1-day-old rabbits as well as from chick membranes.

Stirring of the artificial meal to prevent sedimentation proved to be essential. Without stirring, a well-prepared, somewhat sagging membrane would sometimes tighten up, apparently because it had lost some of its permeability when a layer of cells settled on the inner surface. The result was a reduction in blood feeding. Since we did not know whether the fluid or solids portion of the virus-blood mixture in an artificial-meal system contained the major portion of the virus, it was necessary to keep the components in suspension so that the artificial-meal fluid was standardized throughout the feeding period. Another factor that provided more standardization of the meal was that evaporation of fluids from the artificial-meal system was prevented by the stirrer baseplate that covered the cell of the feeder during feeding. This was important when several cages of flies were allowed to feed on the same meal, thus prolonging the use of the artificial meal for several hours. It was assumed for our work with an artificial system that the physical state of the virus-blood relationship did not change during a feeding time of less than 4 hours.

Our use of this apparatus for bluetongue research had dictated some of our procedures and has affected the design of the equipment. Bluetongue is not spread by contact between animals and does not affect man. However, to increase our security precautions, especially in view of our recent involvement with other viruses, we now use the gauze-topped feeding cage for all virus feedings so that no flies can

escape during the blood-feeding procedure. To determine the effect of the gauze intervening between the flies and the membrane on the percentage of females taking a blood meal, we conducted several comparative tests. The data (Table 1) indicate that any reduction in blood-feeding that occurs because of the intervening gauze is negligible.

TABLE 1.—The effect of gauze intervening between the flies (males and females present) and the membrane on the percentage of females taking a blood meal.

Test	Exposure time (hours)	Type of feeding cage	Percentage of females taking blood meal	
			Range	Average
1	2	Open	60–78	68
1	2	Gauze	80–87	83
2	1	Open	80–84	82
2	1	Gauze	77–78	77

We have also fed several species of mosquitoes successfully with this apparatus. The dimensions of the feeding compartments did not allow us to use as large a feeding cage as we have normally used for mosquitoes, so we used cages made from ½-pint ice-cream cartons.

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AN ANIMAL-BAITED TRAP FOR THE COLLECTION OF *CULICOIDES*[1] SPP. (DIPTERA: CERATOPOGONIDAE)

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ABSTRACT. An animal-baited trap to collect *Culicoides* spp. is described. In preliminary trials eight species were collected. The advantages of this trap are that it can operate unattended and it can be suspended at different elevations in the forest canopy.

Animal-baited traps are widely used in studies of the feeding activities of adult *Culicoides* spp. (Diptera: Ceratopogonidae). Snow (1955), Snow and Pickard (1954), Jamnback and Watthews (1963), Kettle and Linley (1967, 1969) and numerous other workers have used man as the bait animal in studies to determine the effect of environmental factors on the feeding habits of these biting midges.

Fallis and Wood (1957), Bennett (1960) and Hair and Turner (1968) used various wild and domestic animals in bait traps in host preference experiments. The animals were restrained and exposed. After a known period of time, an insect-proof cage was placed over the animals to trap the biting insects. DeFoliart and Morris (1967) and Nelson (1965) devised other types of traps in which dry ice was used instead of animals to attract *Culicoides*. These traps were effective, but they had to be attended regularly during the trapping period or needed an artificial attractant. It was desired that an animal trap be designed that could capture *Culicoides*

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