environmental persistence and contamination are very small. Indeed, both bioassay and gas chromatography have shown that the half-life of 10 ppm MON-0585 in soil was approximately two days. The chemical has, however, sufficient hydrolytic and photostability to control mosquito larvae in the field. Preliminary field tests indicate that MON-0585 gives complete control of mosquito larvae at approximately 1.0 lb./A.

SUMMARY. MON-0585 is a specific, low toxicity material for control of mosquito larvae at their breeding sources. It is a new type of material which exhibits a different mode of action against immature mosquitoes. The compound appears to have a relatively low order of environmental persistence and a potential for minimal environmental contamination.

ACKNOWLEDGMENT. The author thanks Mr. A. B. Gee for technical assistance.

References

Bower, W. S. 1968. Juvenile hormone: Activity of natural and synthetic synergists. Science 161:895–897.

Bower, W. S., Fales, H. M., Thompson, M. J. and Uebel, E. C. 1966. Juvenile hormone: Identification of an active compound from balsam fir. Science 154:1020–1021.

Spielman, A. and Williams, C. M. 1966. Lethal effects of synthetic juvenile hormone on larvae of the yellow fever mosquito, *Aedes aegypti*. Science 154:1043–1044.

Srivastava, U. S. and Gilbert, L. I. 1968. Juvenile hormone: Effects on a higher dipteran. Science 161:61–62.

Wigglesworth, V. B. 1969. Chemical structure of juvenile hormone activity: Comparative tests on *Rhodnius prolixus*. J. Insect Physiol. 15: 73–94.

Williams, C. M. 1967. Third generation pesticides. Sci. Amer. 217:13–17.

AUTOMATIC DEVICES FOR RECORDING OVIPOSITION PERIODICITY OF MOSQUITOES IN THE LABORATORY

PHILIP S. CORBET,[1] R. E. BELLAMY AND R. H. KEMPSTER

Research Institute, Canada Department of Agriculture, Belleville, Ontario

INTRODUCTION. It is often necessary to know the temporal pattern of oviposition before other aspects of this activity can be studied critically. We describe here two devices that can be used to obtain such information, relatively simply, for most species of mosquito that will oviposit in the laboratory.

In devising a method of recording the temporal pattern of oviposition, we proceeded differently for (1) species that normally lay on a moist substrate close to water (e.g., *Aedes aegypti* (L.)), and for (2) those that normally lay on the surface of free water (e.g., *Culex pipiens* L.). In each case our device periodically presents, and withdraws, an oviposition surface; but in one design (for *A. aegypti*) the substrate is withdrawn without the mosquitoes remaining on it, and in the other design it is withdrawn with the mosquitoes still on it and then immediately rendered less suitable for oviposition. These two devices will be described separately. Both are electrically driven and regulated by an electric clock[2] set to provide impulses hourly on a 24-hour cycle.

THE SUNSET STRIP (*Aedes aegypti*) (Figures 1–3). This apparatus is fitted to the outside of a modified cage and is exposed by opening a trapdoor in the cage wall; it can therefore be installed, operated, and removed with minimal disturbance to mosquitoes in the cage.

[1] Present address: Department of Biology, University of Waterloo, Waterloo, Ontario.

[2] 24-hr. dial Model 8682 Remind-O-Timer.

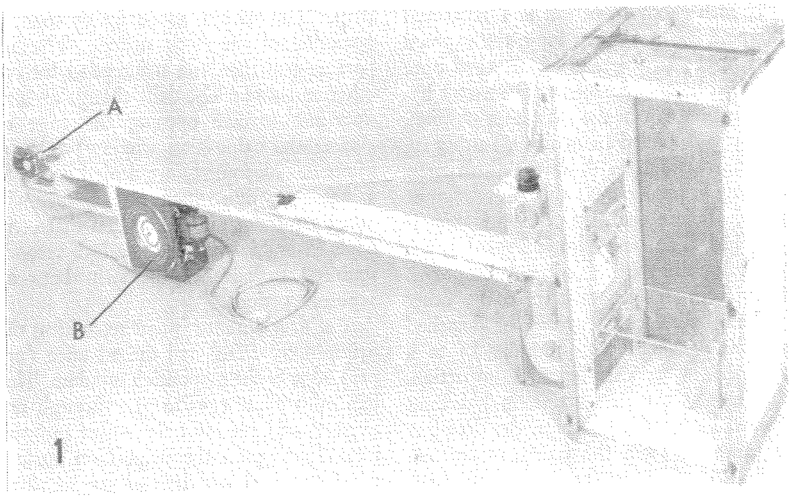


FIG. 1.—The Sunset Strip attached to a cage and ready for use. The cage is about 60 cm high. A, electric motor; B, electric clock.

The mosquitoes oviposit on a slightly curved surface of rough, brown-paper towelling [3] kept at uniform wetness (Figure 2, J). A strip of towelling 10-cm wide is drawn from a roll below (Figure 2, A), passing through a water bath (Figure 2, B) kept at a constant depth by a 500-ml reservoir flask (Figure 2, F), and then around a drum where mosquitoes gain access to it through the "oviposition window" (dimensions 3 x 8 cm) (Figure 2, J). At the end of each exposure period, the part of the strip that has been exposed is drawn upwards beyond the window, and covered by a clear plastic film,[4] 10-cm wide, dispensed from a roll above (Figure 2, E). The resulting two-layered strip is eventually drawn out of the enclosed part of the device and along a horizontal platform (Figure 2, C) where the eggs (4 or more hours after having been laid) can be viewed and counted through the plastic film. The film prevents eggs from being detached or displaced if the strip is accidentally touched; and it prevents stray mosquitoes from ovipositing on the paper towelling.

A drag-line [5] running the length of the platform is attached to the free end of the double strip by a spring clip (Figure 2, D) gripping a plastic bar that prevents the strip from creasing or tearing. The drag-line runs to a pulley attached to an electric motor [6] (Figure 1, A) controlled by the electric clock (Figure 1, B). At the end of each exposure period the motor is activated; it turns the pulley for 30 seconds, advancing the paper 4.4 cm, and then switches itself off by 3-way switching between the clock and pulley. Throughout each exposure period an indelible pencil [7] (Figure 2, H) rests on one upper corner of the exposed part of paper towelling. As the strip begins to move, between periods, this pencil is raised by a solenoid (Figure 2, G) and is not lowered again until the strip stops. The marks left on the strip enable exposed areas to be identified afterwards. Figure 3 shows the wiring diagram for the Sunset Strip.

[3] Peerless, cut to 10-cm width.
[4] Handi-Wrap, cut to 10-cm width.
[5] 8-lb. test monofilament fishing line.
[6] I.R.P.M. Synchron.
[7] Dixon Blue Anadel 1950 Colored Copying.

If each exposure period is 1 hour and the platform is 135 cm long, the exposed part of the strip has to be detached once every 24 hours. As soon as the motor has drawn the strip to the 25th hour-position, the strip is pulled manually a further 35 cm—far enough to draw the three most recently exposed areas out of the apparatus. Before the section of the strip is detached, the plastic film is stapled to the paper towelling beneath it to prevent one layer from moving relative to the other and thus displacing eggs. The detached strip is anchored with thumbtacks to a rigid board for carrying and inspection. As soon as the detached strip has been removed from the platform, the drag-line is unwound from the pulley, untwisted, and attached to the free end of the next strip. The slack is taken up by manual operation of the clock switch. The apparatus will then run unattended for another 24 hours. The daily change (which does not involve opening the cage or closing the oviposition window) takes 6 minutes. Mosquitoes are denied access to a stationary oviposition surface for only about 1 minute, an interruption that compares favorably with the usual 30 seconds required to advance the strip between exposure periods.

A critical distance is that separating the oviposition surface and the advance (upper) edge of the oviposition window. This gap must allow eggs to pass through untouched and yet must not admit mosquitoes standing on the oviposition surface when this is drawn beyond the window.

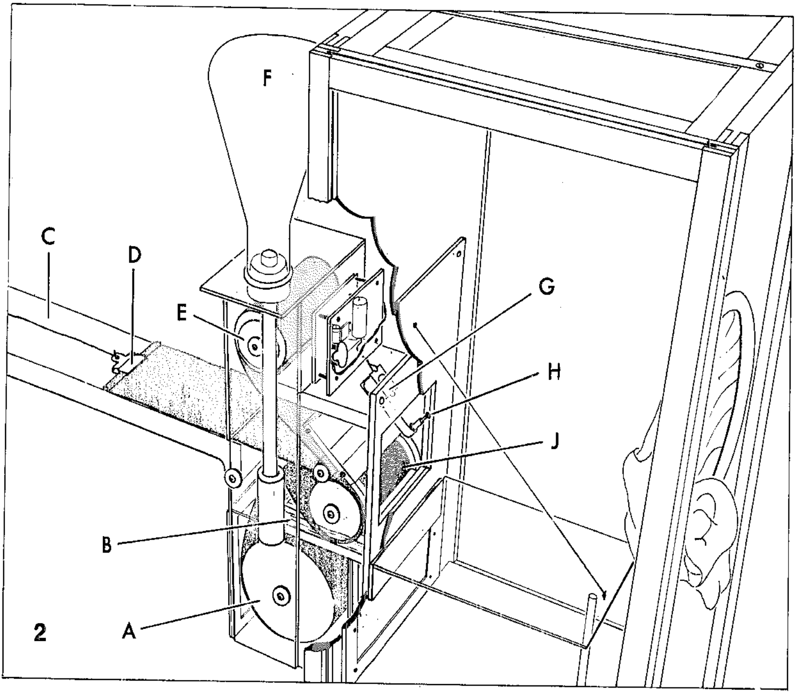


FIG. 2.—The Sunset Strip: cut-away diagram of attachment to cage. A, roll of paper towelling; B, water bath; C, horizontal platform supporting two-layered strip; D, spring clip; E, roll of plastic film; F, reservoir flask; G, solenoid; H, indelible pencil; J, towelling surface exposed in oviposition window.

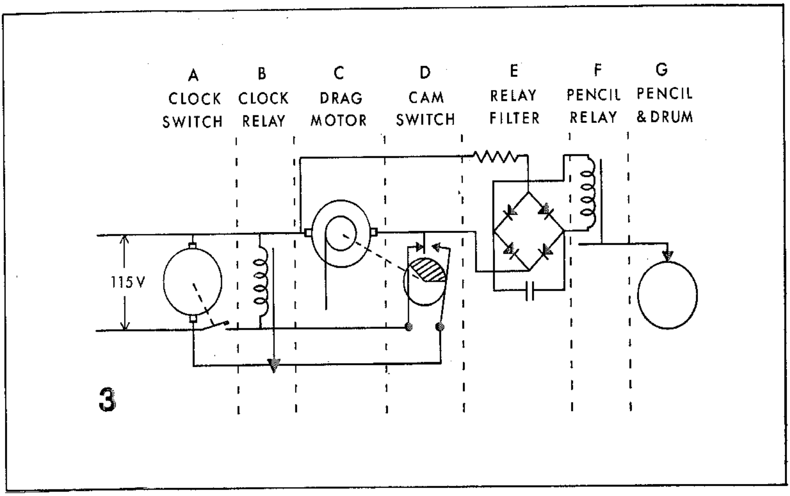


FIG. 3.—Wiring diagram for the Sunset Strip.

To allow for different thicknesses of paper, sizes of mosquitoes, and densities of eggs, this clearance is made adjustable by screws in elongated holes; for moderate numbers (300) of synchronously gravid *A. aegypti* we have found that a clearance of 1.5 mm between strip and window-edge is satisfactory for an oviposition window of dimensions 3 x 8 cm.

As described here, the Sunset Strip offers no exposed water surface to the insects. It will be for each investigator to determine whether or not such a surface should be provided and, if so, in what form. Because the presence of an exposed water surface affects the number of eggs laid by *A. aegypti* on moist paper (Goma, 1964) and also the diel pattern of oviposition (O'Gower, 1963), we carried out tests to compare oviposition performance on the Sunset Strip (condition A) with that on moist paper (of the same area and composition as that used in the Sunset Strip) adjacent to an exposed water surface in a bowl (condition B). Though performance differed slightly and consistently in A and B, it did not do so to an extent that would have affected most studies on oviposition periodicity.

Tests were conducted in a 12:12 light: dark regime at 81° F. and 70 ± 5 percent R.H. The *numbers of eggs* laid in A and B were closely similar during the first 20 days of egg-laying: 26,127 (A) *versus* 26,991 (B)—a difference of only 1.63 percent. Oviposition began on the same day in each condition, but the median number of eggs was laid about 28 hours later in A. After day 20, when oviposition had almost ceased, an oviposition bowl (as in B) was added to A; a short burst of oviposition followed, revealing that females in A had previously withheld about 3.2 percent of eggs. The *periodicities* in A and B were similar in general, with the peak falling in the same hour and containing 30–40 percent of the eggs laid daily (except when oviposition had fallen to a low level at the end of a batch). The periodicities differed slightly in two respects: in A the progressive increase in oviposition before the peak was smoother and more consistent; and in B slightly more of the oviposition occurred during daylight. These small differences were caused mainly by the *presence* of an exposed water surface: the diel pattern of laying on the Sunset Strip became intermediate between patterns typical of A and B when a bowl of water was placed close to the oviposition window, but only if the bowl was left uncovered. The number of eggs laid in the water itself (148/9,707 or 1.4 percent) was too small to account for the effect.

These experiments show (1) that periodicities, as measured by the Sunset Strip and by a paper-lined bowl of water, differ slightly; (2) that this difference is too small to affect seriously the use of the Strip as an experimental tool; and (3) that the difference can be made smaller by placing an open water receptacle close to the oviposition window. In our strain of *A. aegypti* the number of eggs laid on the water itself in such a situation was insignificant for experimental purposes. But strains of this species can differ in this regard (Wood, 1963) and if this number were to be prohibitively large for a given experiment the investigator might have to use the Carousel instead.

The Carousel *(Culex pipiens)* (Figures 4–7). This employs the principle of the roundabout: oviposition containers, peripherally disposed on a circular platform, pass successively beneath a stationary cage of mosquitoes (Figures 5 and 6).

A 115-v 13-w motor, coupled through reduction gearing, pulleys and a "V" belt, turns the platform 1/26 of a circumference at the end of each hour. A selection switch (Figure 5, B) offers: manual operation; step A; step B; or automatic (clock) control. A "stop" position between each selection allows any setting to be chosen independently. A 4-spoke rotary switch under the platform, activated by pins that engage the spokes, stops the platform at the correct intervals when steps A, B or automatic are selected. A limit switch stops the machine when 24 containers have been exposed.

Mosquitoes lay eggs on a water surface presented beneath the "oviposition window"—a circular hole (diameter 8 cm tapering to 6.2 cm) in the floor of their cage (Figure 6, D). At preset intervals the lower platform rotates enough to remove one oviposition container and expose another, an operation completed in 5 seconds. After exposure, the oviposition container is automatically closed when it passes beneath a plastic overlay that comes to rest on top of it. Mosquitoes on the oviposition surface at the time of transition are usually carried along in the mov-

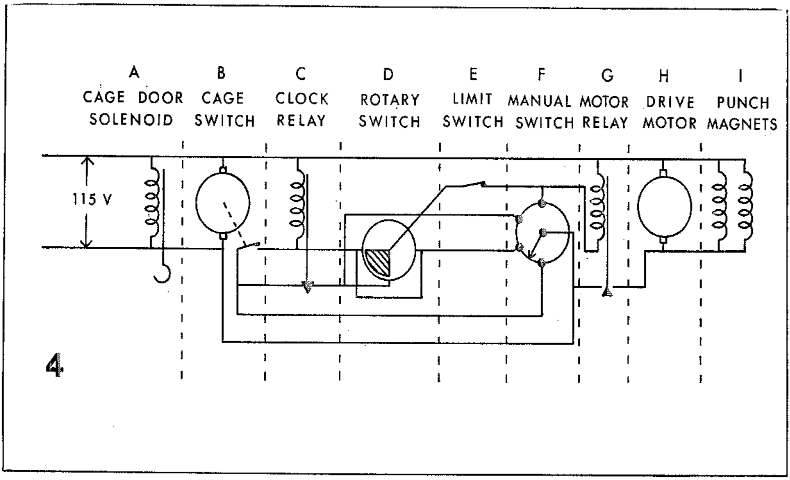
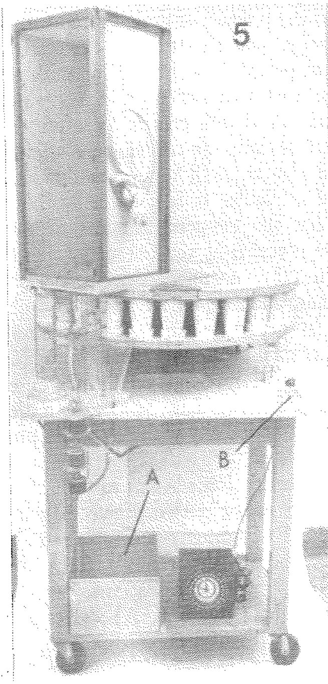


Fig. 4.—Wiring diagram for the Carousel.



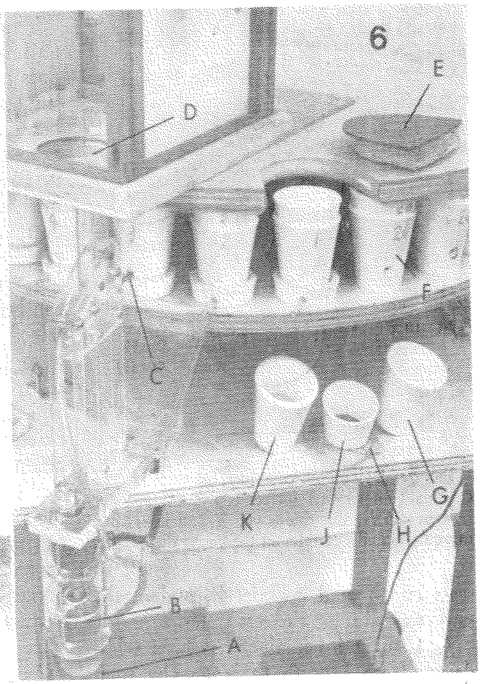
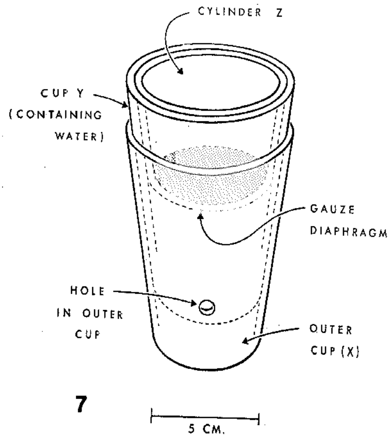


FIG. 5.—The Carousel ready for use. A, receptacle for water drained from cups; B, selection switch.
FIG. 6.—The Carousel: close-up of mechanism. A, counterweight; B, electromagnets; C, stainless steel cannula; D, oviposition window; E, trapdoor in upper platform; F, nested styrofoam cups; G, cup Y; H, gauze diaphragm; J, cylinder Z; K, cylinder Z nested in cup Y.

ing container (and are therefore withdrawn from the ovipositing population, a possible shortcoming of this device for certain kinds of investigation). Given this situation, it becomes necessary that the mosquitoes so confined, some of which may have been ovipositing at the time of transition, are discouraged from ovipositing further, because the time-segregation might no longer be reliable were they allowed to do so. In our device this is achieved by removing the water (though not the mosquitoes or laid eggs) from the container immediately after it has been withdrawn from beneath the cage. For this to be done the container must be of special design.

Each oviposition container consists of two, nested styrofoam cups [8] (Figure 6, F; Figure 7). The lower, outside cup (X) is fixed to the lower platform and has a hole (diameter 1 cm) near the bottom of the cup (Figure 7). The upper cup (Y) sits in cup X and contains a tapered cylinder Z, itself part of a cup, that secures a piece of nylon gauze (20 mesh/cm) which thus forms a horizontal diaphragm or sieve, about halfway up the oviposition container. Cup Y and cylinder Z have to be nested precisely in cup X so that the whole, composite container is of a standard height. For experimental purposes the water level should be standardized in each container. Accordingly, when the Carousel is being loaded before an experimental run, each oviposition container is assembled and filled with water; then a large inverted cup with a central plunger is pressed down on top of the container. This

[8] Poly-Maid.



7

FIG. 7.—Oviposition container, assembled. The cannula (not shown) pierces cup Y through the hole in cup X; this drains cup Y and leaves any mosquitoes and egg rafts on the gauze diaphragm.

seats cup Y and cylinder Z, and at the same time displaces excess water.

As soon as a container has been withdrawn from beneath the cage (by rotation of the lower platform) at the end of an exposure interval, a stainless-steel cannula (14 gauge, 10-cm long) (Figure 6, C) is driven through the hole (Figure 7) in cup X so as to puncture cup Y below the gauze diaphragm. The water in cup Y then flows through the cannula into a receptacle below (Figure 5, A), or, if there is leakage, is caught in a saucer beneath the cup and delivered to the receptacle via a trough. In this manner, 45 seconds after the transition (i.e., 50 seconds after the end of an exposure period) any mosquitoes and eggs in the container find themselves stranded on the moist diaphragm which, being nylon, reduces substrate moisture. Experiments designed specifically to determine this showed (1) that the draining procedure neither prevents females in the container from completing rafts already started nor from beginning and completing rafts an hour or more after draining has occurred; and (2) that draining substantially reduces the incidence of laying among females so treated.

Thus the practice of draining represents an improvement over existing devices but does not provide the investigator with assurance that oviposition in a container has been prevented completely after the exposure period; for this to be obtained, manual clearing still represents the only method available. It is noteworthy, however, that the temporal patterns of oviposition revealed by the Carousel (operating in the manner described) are consistent, and resemble closely those obtained by manual clearing (M. G. Maw, 1970, personal communication). Thus for many types of investigation the Carousel will provide results that characterize a diel oviposition periodicity with acceptable precision.

When the platform begins to turn after the next exposure period, electromagnets (Figure 6, B) of the cup-puncturing mechanism withdraw the cannula from the drained cup; when the platform stops, a counterweight (Figure 6, A) drives the cannula into the next cup.

As described here, with exposure periods of 1 hour, the Carousel has to be emptied once daily. The maximum continuous run is 24 hours and there are 26 cups. Before an experiment is started, cup No. 26 (a dry blank) blocks the hole in the cage floor. The experiment begins as cup No. 1 is automatically moved under the hole; 24 hours later cup No. 25 (also a blank) moves into place beneath the cage and the machine automatically stops. If the experiment has to be continued, the hole in the cage floor is closed manually by activating a sliding door, the cage is quickly moved to a standby platform containing an oviposition container, and the door in the floor is opened. During this interim arrangement it is, of course, necessary that the lighting conditions remain unchanged and that the cage is not jarred. (The sliding door is held open by a solenoid and in the event of an electrical failure springs shut automatically.)

Whenever necessary (e.g., when reloading after a 24-hour run) the manual control of the platform movement can be used without disturbing the clock's preset cycle.

The platform can be turned through its regular stops, and each cup replaced through the opening left by removal of a trapdoor (Figure 6, E) in the upper platform. Cup X bears a number that can be related to the time of exposure, and Y is labelled appropriately as it is removed. When the cups have been replaced, the automatic control is again chosen and the clock takes over. Since the cage has to be left on the standby platform for 1 hour, the automatic control has to expose cup No. 2 as soon as the cage is returned to the Carousel. This means that (if desired) reloading can occur at the same time each day.

If experimental requirements permit, cup Y, cylinder Z and the gauze diaphragm can be used again after removal of eggs and mosquitoes, and after washing (the puncture in cup Y can be patched with tape of a kind that does not contaminate the water in the container). It must be emphasized, however, that to do this in some kinds of investigation might introduce an experimental error if, by laying in a container, females were to render it either more or less attractive as an oviposition site. The findings of Osgood (1971b) reveal the need to allow for chemical factors that may influence oviposition intraspecifically in this way. Cup X is not in direct contact with mosquitoes at any time; it is subject to possible contamination with the water on which eggs have been laid only if there is leakage around the cannula when cup Y is drained. To compensate for, and detect, possible error due to this cause, a given cup on the wheel can be exposed at different times of the day on successive runs.

The Carousel is designed primarily to study mosquitoes that oviposit on the surface of water. However, with minor modifications, it can be used for most species of aquatic insects (including *Aedes aegypti*) that will lay in cages. When such modifications are being devised, it should be remembered that the oviposition surface should be rendered less suitable immediately after the end of an exposure period. For species of *Aedes,* that may lay on moist gauze, the work of Knight and Baker (1962) would have close relevance to such an operation.

The Carousel differs from a device described by Logen and Harwood (1965) in the following respects. In the Carousel the cage stays still and the oviposition containers rotate instead of *vice versa;* the Carousel has 26 instead of 13 oviposition containers; in the Carousel (alone) females that are carried along in an oviposition container at the time of transition are discouraged from ovipositing subsequently; and the Carousel makes provision for emptying and recharging the oviposition containers without breaking the continuity of hourly observations.

Although it is expected that the Carousel will be the device of choice for studies of mosquitoes that lay eggs on the water surface, recent observations by Osgood (1971a) reveal the likelihood that the Sunset Strip, perhaps modified to offer an horizontal oviposition substrate, might also be available for this purpose.

Summary. Two electrically driven devices are described that automatically segregate successive time-samples of eggs laid by mosquitoes in cages. One device is suitable for species that oviposit mainly on moist surfaces near water (e.g., *Aedes aegypti* (L.)), and the other is suitable for species that oviposit on the water surface itself (e.g., *Culex pipiens* L.). The devices are designed for studies of oviposition periodicity.

Acknowledgments. We thank Messrs. H. E. Bisdee, H. Hurst and C. F. Nicholls for assistance during this study, and Dr. C. E. Osgood for valued comments on the manuscript. Mr. T. Stovell prepared the photographs, and Mr. Nicholls the drawings.

References Cited

Goma, L. K. H. 1964. Laboratory observations on the oviposition habits of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus). Ann. Trop. Med. Parasitol. 58:347–349.

Knight, K. L. and Baker, T. E. 1962. The role of the substrate moisture content in the selection of oviposition sites by *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.). Mosq. News 22:247–254.

Logen, D. and Harwood, R. F. 1965. Oviposition of the mosquito *Culex tarsalis* in response to light cues. Mosq. News 25:462–465.

O'Gower, A. K. 1963. Environmental stimuli and oviposition behaviour of *Aedes aegypti* var. *queenslandensis* Theobald (Diptera, Culicidae). Anim. Behav. 11:189–197.

Osgood, C. E. 1971a. Wet filter paper as an oviposition substrate for mosquitoes that lay egg-rafts. Mosq. News 31:32–35.

Osgood, C. E. 1971b. An oviposition pheromone associated with egg-rafts of the mosquito *Culex tarsalis* Coquillett. J. Econ. Entomol. 64:1038–1041.

Wood, R. J. 1963. Oviposition in DDT-resistant and susceptible strains of *Aedes aegypti* (L.): egg-laying on open-water surfaces. Bull. Entomol. Res. 53:785–790.

THE PUPAL STAGE OF *CULICOIDES FURENSOIDES* WILLIAMS (DIPTERA: CERATOPOGONIDAE); A FIRST DESCRIPTION

ALAN H. COCHRANE

Department of Entomology, College of Agriculture, Cornell University, Ithaca, New York 14850

Abstract. The pupal stage of *Culicoides furensoides* Williams is described from a single specimen collected at the Malloryville Bog, Tompkins County, New York. The affinities of the pupal stage of this species with other species of the *furens* group in which it is placed are discussed.

Adult *C. furensoides* were first recovered by Williams (1955) with an emergence cage placed over a *Sphagnum* mat at the edge of a pond at Bryant's Bog, Cheboygan County, Michigan, July 22, 1954. The first New York State record is credited to W. W. Wirth who collected a male of this species at Braddock Bay, Monroe County, New York, June 12, 1963.

During the period of July 8, 1970, to July 23, 1970, the author collected 18 females using a light trap placed at the Malloryville Bog, Tompkins County, New York. The following summer one pupa was isolated from the *Sphagnum* mat at this bog. This paper provides the first description of the pupal stage of *C. furensoides*.

MATERIALS AND METHODS

On July 21, 1971, a sample of *Sphagnum* taken at the Malloryville Bog yielded one *C. furensoides* pupa after being washed, using a set of three nested brass gauze sieves, 10, 40, and 80 mesh, and then being subjected to magnesium sulphate flotation (Kettle and Lawson, 1962). The specimen was transferred to moist absorbent cotton in a clear plastic box measuring 2 cm. x 2 cm. x 1½ cm. for rearing. The adult, a female, emerged July 22, 1971.

The adult and pupal exuvia were mounted on the same slide under separate coverslips. The specimens were first cleared in a saturated solution of phenol in absolute alcohol, passed through a graded series of phenol-balsam, and then mounted in pure, paper-filtered, Canada balsam. The respiratory horns and operculum were dissected from the intact pupal pelt for close examination.

A 10 x 10-square grid micrometer disc was used to make the drawings. Terminology for the body tubercles is that of Carter *et al.* (1920), with the exception of the dorsomedian (*d.m.*) tubercle which was first used taxonomically by Lawson (1951). Many of the body tubercles, including the *d.*, *v.m.*, and *v.l.*, *d.a.s.m.*, *d.p.m.*, and *v.p.m.* tubercles, were drawn in correct relationship to one another.