LABORATORY COLONIZATION AND MASS-PRODUCTION PROCEDURES FOR *CULICOIDES GUTTIPENNIS*

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The minute, bloodsucking flies of the genus *Culicoides* (Diptera:Ceratopogonidae) have not received detailed study as have most biting flies. This can probably be explained by the fact that the size of these "no-see ums" not only makes them difficult to work with but also accounts for their being virtually unnoticed as facultative parasites of livestock. Basic studies on their life history and habits and vector potential have been greatly hindered due to insufficient laboratory reared material. There have been only two previous reports on colonization of this genus. *Culicoides nubeculosus* (Mg.) was maintained in a laboratory in Britain from 1947 until it deteriorated in 1953 (Downes, 1950). Jones (1957; 1960) reported on the establishment of *Culicoides variipennis sonorensis* in the laboratory from material collected in Edwards County, and Kerrville, Texas. In the 1960 paper Jones gave mass-production procedures for *C. variipennis sonorensis* established in 1957 at Kerrville, Texas. A routine procedure for propagation of 1,000 adults per day was described.

A colony of *C. guttipennis* was established in our laboratory during April 1965 as one phase of a study on arthropod vectors of infectious synovitis in poultry. Since its establishment in April, each generation has produced progressively larger numbers of adults.

METHODS AND MATERIALS: ADULT MAINTENANCE. Adults are maintained in constant temperature cabinets at 80° F.±2° and 85 percent RH ± 10 percent in semi-darkness (less than 1 ft. candle) at all times except while being attended.

Adult holding cages (Figure 1) are constructed of ½ gal. cardboard food containers, fine-mesh nylon stocking, transparent polyethylene and cork stoppers. A number of holes are cut in the sides of the cardboard container. A 2-in. front view window cut about 2 in. from the top of the cage is covered with transparent polyethylene taped in place. A ½ in. polyethylene covered hole cut near the top rim on one side provides an entrance for a beam of light used to attract the females to the top of the cage during blood feeding procedures. Two other ¾ in. holes located about 2 in. from the bottom (at any position) are entrances for egg-collecting vials and are stoppered during blood feedings. One other hole stoppered with a small cork provides an entrance for adding newly emerged adults to the parent cage. From 10–15 minute holes are bored in the side of the cage and allow for the entrance of capillary tubes containing liquid diets. These holes are covered by a small piece of tape when capillary tubes are removed during blood feeding. The top of the carton is covered with tightly stretched, fine-mesh nylon stocking which is held firmly with rubber bands and glue.

Adults are afforded a number of different diets. Rabbit blood is offered the adults daily from the time of emergence. The feeding procedure is similar to that described by Jones (1960). It differs, however, in that in our laboratory the females feed through the stockinged cage top on a closely shaven belly of a rabbit and there is no loss of adults through anesthesia or escape (See Jones (1960) for description of rabbit stanchion). Split

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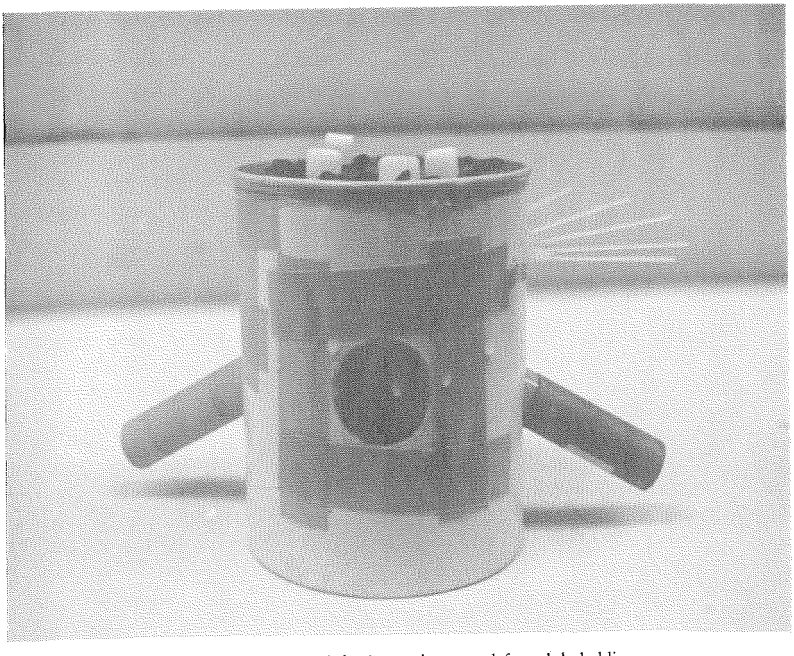

FIG. 1.—Modified cardboard food containers used for adult holding cages.

raisins and sugar cubes are placed on the stockinged top of the adult-holding cage between blood meals. A fourth diet constituent is the adult house fly diet described by LaBrecque and Gouck (1963). This is mixed with an equal weight of water and is administered in capillary tubes through the side of the cage. A 10 percent honey water solution is fed likewise.

EGG COLLECTING. When the first adults in a cage reach 4 days of age, two-dram shell vials containing filter paper and "artificial stump water" described below are inserted through the side of the cage. The filter paper is rolled to fit the inner surface of the vial and the vial is filled about ½ full with water which has been allowed to stand on decaying leaves and organic matter for a week or more. The water has attained a characteristic brown color and has a sour smell. It resembles water frequently found in hollow trees and stumps in nature.

The egg vials are removed daily, or every other day depending on the egg production of the females, and placed upright in a 5 x 5 x 8 in. plastic box containing a small amount of distilled water. They remain in this humid condition until they begin to hatch, following which the filter paper and water contents of the vials are transferred to larval rearing chambers.

LARVAL REARING CHAMBERS. Larval rearing is conducted at 87° F. ± 3° in complete darkness except for the light that diffuses through the emergence cups. Four-gallon aquaria are used as larval rearing chambers. The sides are covered on the outside with black polyethylene, or painted, to exclude all light. The top is likewise covered with black polyethylene which is taped in place. Two holes are cut in the top of the chambers. One is about ⅛ in. in diameter and is the entrance for an air hose used to break up scums; the other is the size of a pint

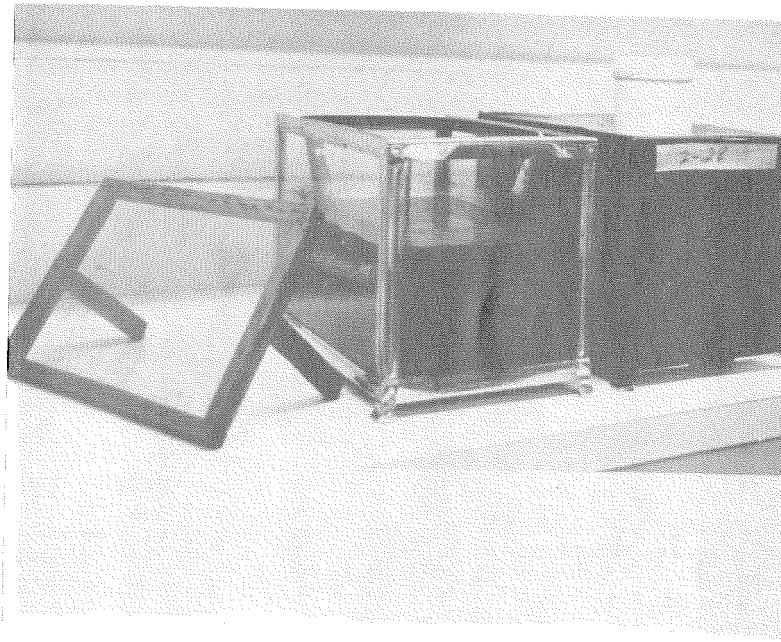

FIG. 2.—Equipment and steps in preparing a larval rearing chamber.

cardboard carton. The pint cardboard carton used as an adult collecting chamber is modified so that the bottom of the carton and top panel of the lid is replaced with 40 mesh saran screening (Source: National Filter Media Corp., 1717 Pixwell Ave., New Haven 14, Conn.). A slit ⅛ in. wide is cut across the bottom screen. The emerging adults are attracted to the lighter screened area, where some light diffusion occurs, and crawl through the slit into the container. The cups are then removed and transferred to a refrigerator for cooling of adults for a short time, after which the adults are easily transferred to the adult holding cages.

LARVAL MEDIA. The larval media consist of decaying leaf-mold collected in hardwood forest in several areas near Blacksburg, Virginia. The most satisfactory debris is that at least 1 year old. Bottoms and gullies where deep leaf accumulations existed afforded the best collecting sites of leaf mold. The newly-fallen leaves were raked back and the decaying humus collected for use. Work on artificial diets has been initiated, but a completely satisfactory diet has not yet been developed.

From 1 to 1½ gal. of leaf mold is placed in each aquarium and held at the bottom with a frame covered with screen (Figure 2). Two gallons of distilled water are then added and the aquarium is placed at 87° F.±3° for 1–3 days before newly-hatched larvae are introduced. The evaporation rate is low and generally no additional water is added. The aquaria contents can be used for a second generation of larvae, but this practice can result in unsatisfactory conditions for the second batch.

RESULTS AND DISCUSSION. The current adult production of our *C. guttipennis* colony is over 1,000/day. Recent egg pro-

duction has been so great that far more are collected than needed to maintain the current status of the colony. Maintenance requires the services of a well trained individual for 2-3 hours/day.

The generalized life cycle of *C. guttipennis* in the laboratory is about 25±4 days—egg 2–3, larva 12+, pupa 3, prefeeding 1 or less, premating less than 2 days, preoviposition 4–5.

DIETS. The adult diet used has proven very successful and accounts for an average adult longevity of about 12 days. Both sexes feed readily on the liquid diets given in capillary tubes. The other constituents have also provided greater longevity.

The exact diet of larvae has not yet been determined but it is hoped that present investigations will reveal this shortly. From our field and laboratory observations, it appears that the larvae can be scavengers (i.e., feed on dead earthworms, dead insects or other organisms present as well as other organic matter), predators (i.e., feeding on live mosquito larvae of *Orthopodomyia signifera* (Coq.) and *Aedes triseriatus* (Say), or larvae of Helodidae (Coleoptera)), or under extremely crowded conditions, cannibalistic. Their frequent habit of swimming slowly through algal and bacterial mats in laboratory rearing chambers might suggest that this, too, accounts for at least a portion of their diet. For food in our laboratory rearing procedures, larvae are limited to bacterial and algal growths, a few small stray invertebrate organisms in the leaf mold, and the leaf mold itself. Each 4 gal. aquarium is generally capable of supporting about 1,500 larvae.

MATING. Temperature and humidity as well as proper lighting are especially important in stimulating mating. Mating is generally initiated while both sexes are in flight, but on occasions the male may dislodge a female from the side of the holding chamber and mating occurs as they fall to the floor of the cage. The conditions previously mentioned have proven successful in stimulating flight and mating. Excessive illumination causes uncoordinated flight and is unfruitful in stimulating mating. Newly engorged females are especially attractive to males.

OVIPOSITION. Oviposition occurs within the special oviposition vials along the air-water interface. The eggs are laid singly as the female slowly creeps along on the filter paper in a fairly straight line near the water's edge. Dissections of gravid females reared in the laboratory show that they are capable of laying from 175–350 eggs in a single gonadotropic cycle.

LARVAL AND PUPAL DEVELOPMENT. With proper food each of the four larval instars is completed in about 3 days and pupation occurs after about 12 days. Adults emerge above the surface of the water from a dorsal pupal slit as the floating pupae reach 3 days of age. Surface scums are especially detrimental to pupae, due to the need for atmospheric air by pupae. Also, scums interfere extensively with adult emergence.

SUMMARY AND CONCLUSIONS. A colony of *Culicoides guttipennis* has been maintained in the laboratory for almost a year and has completed 12–15 generations. Numbers have steadily increased each generation to a current level of above 1,000/day. A limited knowledge of the laboratory biology has been determined. Further studies are in progress.

It is our opinion that one of the most important factors in our colonization procedures was to supply the proper illumination, humidity and temperature to induce mating. The current production of our colony could possibly have been reached many months ago had we attained maximum mating of adults.

These rearing procedures probably can be applied to other tree-hole breeding *Culicoides* and result in successful establishment of other colonies of this genus.

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