lected east of Elgin, Illinois (Cook County, Hanover Township, Section 8, just west of Brenner Road on Shoe Factory Road), July 2, 1975 in a New Jersey light trap. The light trap hung 6 feet above the ground from the northern overhang of a backyard shed. Chickens were kept in the south end of the shed and 2 horses grazed in an area behind it.

The head was entirely female in appearance, with normal antennae and palpi. The terminal abdominal segments and the tarsal claws had male characteristics. Collected along with the above form in the same trap that night were 774 female and 9 male *A. vexans*. This was the highest *A. vexans* count for any of our 10 traps for the 1975 season.

A. vexans is the most abundant mosquito species in the Northwest (Ill.) District. For the past 19 years (1957 through 1975), the time the District has been in operation, a total of 423,119 male and female *A. vexans* mosquitoes have been collected in light traps. The form described here is the first aberrant type of any species collected in our traps to date.

Literature Cited

Minson, Kenneth L. 1969. An *Aedes vexans* gynandromorph. Mosquito News 29:135.

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QUANTITATIVE REARING OF SMALL NUMBERS OF MOSQUITO LARVAE [1]

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The deleterious effects of chemical pesticides on human and wildlife populations have revived interest in the use of entomogenous agents for controlling insects of agricultural and medical importance. A simple and inexpensive, yet readily standardized and reproducible, system would facilitate laboratory assessment of potential insect pathogens. Ignoffo and Boening (1970) described a system for rearing larvae of house flies and phytophagous insects on solidified media held in miniature compartments of disposable clear plastic trays. In this report, we describe a system using a multiple well tray to rear 1 to 2 mosquito larvae/well in a liquid medium developed for use in these traps.

The inexpensive (ca. $0.04 each) disposable clear polystyrene rearing tray (Champion Packages Co., Belvidere, Ill. 61008) has 5 rows of 5 wells/row, each individual well measuring 2.8 x 4.1 x 1.6 cm with a capacity of ca. 15 ml. A Plexiglas® sheet (19 x 26 x 0.6 cm) is provided with 2 plastic dowels positioned at diagonal corners; these dowels pass through preformed holes in the tray lip (Fig. 1). Another tray is inverted over the first to retard evaporation and also provide additional air space for each well. The inverted tray is weighed down with another piece of Plexiglas of the same dimensions as the bottom piece. This stabilized tray unit facilitates handling, counting and observation of the larvae (2 persons can feed and accurately count up to 10,000 larvae/day). Ten similar units are held at 25° C. in a slotted clear Plexiglas storage rack. This minimizes space requirements during use.

Larval diets suitable for many species of mosquitoes reared in large numbers in a single container were described by Gerberg (1970). Finely-ground Laboratory Rabbit Chow (Ralston Purina, St. Louis, Mo. 63188) is the standard larval diet used in our insectary, but it was unsuitable for rearing larvae singly or in pairs in the miniature wells. It was difficult and time-consuming to administer a consistent amount of the dry food into each well, and growth of larvae was not uniform on this diet. After preliminary trials, we decided to use basic growth medium suspension prepared by blending (for 2 min) 0.8 g Laboratory Rabbit Chow pellets, 0.8 g Spur® pellets (Albers, Div. of Carnation Co., Van Nuys, Calif. 91412) and 16 g fresh or frozen alfalfa foliage in 300 ml distilled water. The mixture is autoclaved at 1.4 kg/cm^2 for 45–60 min, cooled, then filtered through coarse glass wool to remove large particulate matter. This suspension has a pH of ca. 7.2 and can be stored at 4° C for 1 wk.

Earlier experiments with different stock suspensions had indicated the necessity of infusing the sterilized medium with a mixture of bacteria that was nutritionally adequate for larval development to the pupal stage and that prior autoclaving of the medium was necessary for maintenance of a controlled flora in the wells. This led to the adoption of a 2 part feeding schedule using (1) a bacteria-inoculated basic growth medium followed by (2) a yeast-supplemented medium. A mixed culture of nonsporeforming bacteria, present in an incubated, unsterilized basic medium suspension, was isolated on nutrient agar plates and used initially in aqueous suspension to inoculate the sterilized basic medium. No steps were taken to identify the bacteria added to the medium but examinations were made at regular intervals to ensure exclusion of sporeforming,

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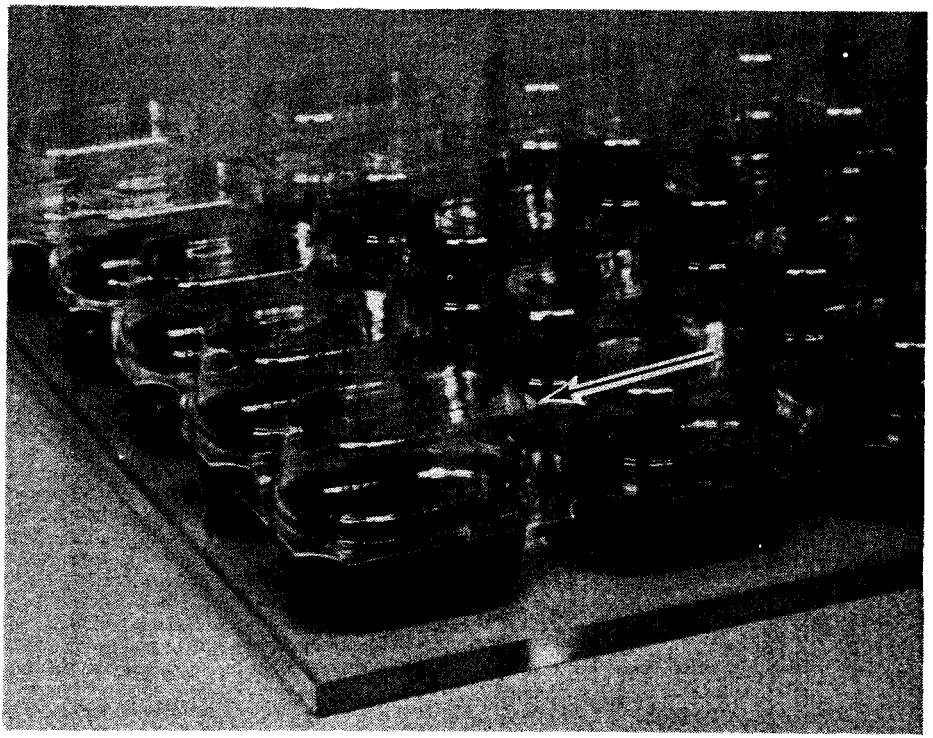

Fig. 1. Part of the miniature multiple well mosquito larva rearing unit. Arrow points to one of two dowels that stabilize the rearing unit. Each well is 4.1 cm in length.

possibly entomopathic, bacteria from the mixed flora. After the initial inoculation, subsequent batches of sterilized basic medium were seeded with fluid containing bacteria from wells that supported growth of healthy larvae and pupae.[2]

The current procedure is to put 50 ml of a previous week's medium containing the mixed flora into each newly prepared lot of sterilized basic medium, adding sterilized distilled water to make a final volume of 1000 ml. The inoculated medium is incubated overnight at 30° C on a shaker to enhance growth of aerobic bacteria. This procedure eliminates scum formation on the water surface, a problem often encountered in rearing immature mosquitoes, and allows usage of nonaerated rearing units. The dilute medium is relatively clear and allows ready observation and counting of early larval stages.

After the first day of larval growth on the bacteria-seeded medium, a supplemental food consisting of basic medium enriched with heat-killed yeast is dispensed into each well. Dried baker's yeast (Red Star Yeast Co., Oakland, Calif. 94607) is added to distilled water, 25 g/liter, and after autoclaving for 20 min at 1.4 kg/cm^2, the autoclave is turned off and allowed to depressurize slowly for an additional 20 min. After cooling, 100 ml of the yeast mixture is added to 900 ml of diluted basic medium to prepare the supplemental food. In preliminary trials, inclusion of yeast in the medium provided on the first day was found to be detrimental to growth of the larvae; however, addition of heat-killed yeast after the first day prevented high larval and pupal mortality and delayed pupation.

The efficacy of this rearing system and the growth medium was determined for *Culex pipiens pipiens* L., *Culex tarsalis* Coquillett and *Aedes taeniorhynchus* (Wiedemann). In each trial, 2 larvae less than 24 hr posthatch were placed into each well of a rearing unit in ca. 0.1 ml water before adding 4 ml of the bacteria-seeded medium with a Cornwall pipette. Thereafter, 0.5 ml of the enriched heat-killed yeast supplement was added daily to each well until the end of the experiment. Table 1 summarizes larval and pupal mortality for the 3 species of mosquitoes reared to the pupal stage on the growth medium. Criteria for gauging the nutritional adequacy of the

[2] This procedure was suggested by T. Clark, United States Department of Agriculture, Fresno, Calif.

Table 1. Mortality and pupation of mosquito larvae reared in miniature wells* at 25° C.

Mosquito species	Experiment number	Number of larvae per experiment	Percent live pupation	Day 50% or > of larvae pupated	Day last larvae pupated or died
Culex tarsalis	1	100	98	10	13
	2	200	95.5	10	15
	3	50	94	9	12
Culex pipiens	1	200	98	8	14
	2	100	97.5	7	12
	3	200	92.5	7	11
Aedes taeniorhynchus	1	100	93	7	17
	2	100	91	7	14
	3	100	91	7	11

* The tray holds 50 larvae (2 larvae/well; 25 wells/tray).

medium were larval motility, larval and pupal mortality, and time to pupation. Under the conditions of the experiments, there was low larval and pupal mortality (<10%), good larval motility, and a pupation rate and time comparable to the same species mass-reared in large containers with daily provision of an equivalent amount of the basic medium/larva in the insectary. Viable microbial counts of the fluid in the large containers and in the miniature wells showed that each system had a flora of ca. 10^8 bacteria/ml.

The pupae were not held for adult emergence in the experiments conducted in the minature well system.[3] However, relatively high emergence rates (>80%) occurred in pupae of the 3 species mass-reared in the insectary on the described medium. Adult survival was similar to that observed in the same species routinely reared in the insectary on the standard diet. Eggs were laid in quantities eqivalent to that of the regular laboratory colonies and the percentage hatch was high, indicating that the medium used in the miniature well system was nutritionally adequate for both immature and adult stages.

Literature Cited

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Ignoffo, C. M. and Boening, O. P. 1970. Compartmented disposable plastic trays for rearing insects. J. Econ. Entomol. 63:1696–1697.

[3] This was necessitated by the presence of arboviruses elsewhere in this Laboratory which presented a possibility of chance transmission by adult mosquitoes.

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RESPONSE OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY EGGS, LARVAE, AND PUPAE TO ADDITIONS OF FLIT MLO TO THEIR BREEDING SITE WATER[1]

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One of the most promising insecticides of recent years for mosquito control is Flit MLO®. The usefulness of this material has been well established for safe mosquito control without danger to non-target fauna and flora after application. Many mosquitoes breed in stock watering ponds and troughs. A study was conducted to determine how effective Flit MLO may be for controlling mosquitoes in such bodies of water.

Aquatic stages of *C. p. quinquefasciatus* mosquitoes were found in ground pools and in barrels of standing water. The first tests were conducted in barrels of water which contained eggs, larvae and pupae. Temperature of the water was 26.7° C. Two barrels of water were used for each test. One was for the tests while the other was a control. Larvae were too numerous to count (TNTC). In addition to larvae, the waters contained 5–6 mosquito egg rafts per dip. One milliliter of Flit MLO was dripped onto the surface of the water in the barrels.

To permit an unobstructed view of the action

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