yet been established from tissues of *C. salinarius*.

Summary. The first successful primary tissue cultures from the mosquito, *Culex salinarius* are described together with the types of cell growth and the techniques utilized. Comparison is given of the types of growth obtained from the various developmental stages of the mosquito. From larval tissues, vesicles developed and were maintained in culture for periods up to four months. From pupal tissue, primary monolayer cell-sheets attached to the flasks developed repeatedly and were maintained for periods from two to six weeks.

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OBSERVATIONS ON LOW VOLUME SPRAYING

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The concept of low volume appeared in the operating techniques of the spray planes of this District in 1955. Airplane spraying at the rate of one quart per acre started in that year. Fifteen years later applications are at the rate of 0.5 to 1.0 fluid ounce per acre.

The change has brought about considerable savings. The cost of the solvent saved in a year can be in the order of 5¢ per acre, or $5,000.00 for each 100,000 acres! Other savings, more difficult to isolate, are in the operation of the airplanes and their service vehicles. At one time we operated two, 2,000-gallon tank trucks, one for each plane. Each tanker carried water, insecticide, gasoline and oil. There were transfer pumps, recirculating pumps and gasoline pumps. There were measuring tanks and mixing tanks. Also, there were tank trucks stuck trying to reach landing strips near the scene of operations.

Fifteen years later we have a small trailer pulled by a ½ ton pick-up truck. The trailer carries insecticide solution in a 200-gallon plastic tank. An insecticide-resistant transfer pump can fill both planes in just minutes. A 100-gallon supply of gasoline is in stainless steel tanks. An explosion proof pump services the planes quickly.

New techniques were developed during the years as application rates were reduced, and we now accomplish more, with fewer men, doing less work. What we thought was to be an Utopian operation was spoiled when we awakened to the fact that we did not know our rate of application at any given time. We knew rates *before* application and *after* application but not *during* application. Sometimes a good kill was obtained in an area, but, in the next area treated, just a few miles away, there would be a poor kill.

Pressure appeared to vary for no apparent reason. Better pressure gauges showed clearly that pressure did vary. Precision pressure regulators were tried in place of the "pop-off" valve type so common on agricultural spray rigs. These helped the situation, when new. They were expensive, subject to corrosion, and failed without warning. Finally, flow meters were installed. The first meters were mounted so that they could be observed but not adjusted by the operator.

At once our great trouble was located; we had no control over the flow of the insecticide. The spray rig could be set to deliver a relatively accurate amount over a period of time, but at any given instant the flow rate was without control. Fluctuations within the system might average out to approximately the desired value, but they also might result in over or under treatment of areas.

During the later phases of developing LV air spraying we were also working on LV ground spraying. Lack of control of flow-rate also occurred with ground LV spraying but was more acute due to the smaller amounts of insecticide involved. One solution seemed to be to place a control valve in reach of the operator. Meters and controls were relocated so that constant observation was made easy and instant correction possible. The work involved in regulating flow rates of about 2 to 3 gallons per hour became a burden to the equipment operators. Supervision and maintenance became a nightmare.

When the application rate is such that spray material is being used at the rate of 20–30 gallons per hour or more, an error of 1 or 2 gallons per hour may seem to be of little concern, but when application rates are less than 5 gallons per hour, an error of 1 gallon per hour is critical. Under field conditions a 1-gallon-per-hour error may very easily occur. Precision equipment, mounted on a bouncing truck, near a one-cylinder engine, or a vibrating aircraft engine, rapidly becomes junk.

Over-treatment wastes insecticide, increases the cost of operation, and can injure non-target organisms.

Under-treatment also should be avoided, since if control is not achieved, it is not only a complete waste, but the public loses confidence.

Table 1, calculated for Spraying System D4–23 nozzles using technical Cythion, presents data indicating the magnitude of error due to poor control of flow rate in a system.

The monetary loss only of over-treatment is indicated.

In order to keep as many of the advantages of LV as possible, but considering the smaller percentage errors obtainable with larger volumes, it was decided to work with diluted concentrates. For ground work we now dilute so that a rate of 8 gallons per hour provides the desired pounds per acre of the insecticide. An approximate metric equivalent of 8 gallons per hour is 505 ml. per minute. The ground equipment includes four low volume foggers and four Buffalo Turbines modified as "Mist'ers" (Thompson 1969).

It is not correct to say that one part concentrate plus one part solvent will make a total of two parts since many of the solvents that can be used are soluble within the insecticide solution. All mixing must begin with a known volume of concentrate. Then the solvent may be

TABLE 1.—Output of D4–23 nozzles with pressure varying by two pounds.

P.S.I.	G.P.M.	G.P.H. Desired	G.P.H. Actual	Cythion Lbs./Hour	Lbs./Hour Difference	Cost/Hour /Nozzle @ $7.00/Gal.
25	0.130		7.80	75.66		
26	0.132		7.92			
27	0.134	8.04		78.79		
28	0.136		8.16			
29	0.138		8.28	80.32	1.53	$10.71

added, but not by volume. The solvent must be added to the concentrate until a predetermined volume is obtained.

Some difficulty was encountered during low volume fogging with solutions containing "additives." Some of the "additives" are materials to keep the insecticide in solution in otherwise poor solvents. The additives encountered have caused excessive gum formation in the fog nozzle. The gum is most difficult to remove and can even prevent the completion of the work scheduled for one night. There is no need to use such additives when a suitable solvent is used. Such problems do not occur with a mist applicator.

The equipment and techniques used during the development of LV spraying did not provide the constant flow needed to insure even application of insecticide at all points of the run. Constant adjustment of the needle valve was required to keep the flow meter on the mark. It was discovered that a constant pressure throughout the entire system is required to maintain a uniform flow. A restricting orifice should be located at the point of discharge. Some nozzles do not have a restricting orifice, others have an orifice that, under operating conditions, does not function as a restriction. The operating pressure must be considered when selecting the orifice. The deviation should be small, as near the desired flow as possible.

The system must include provisions to furnish a steady pressure and a constant flow. A gas pressure system may be used in place of a pump. A small air compressor or bottle of compressed nitrogen can be used to maintain a constant pressure in an insecticide tank. Nitrogen is dry and inert, making an excellent propellant for the unstable or more corrosive insecticides. A small air compressor is inexpensive and convenient. Air or gas pressure has provided the most trouble-free liquid propulsion system for our purposes.

Our equipment is calibrated in milliliters per minute. The metric system was found to be best for our needs. A large 1,000 ml. graduate is ideal to catch and measure the effluent. A stop-watch is most convenient; however, any watch with a sweep-second hand will suffice. All liquids should be tested at several points from the lowest to the highest probable ambient temperatures. Tests should be with the exact equipment to be used in actual application (Thompson 1970). Tests should be made at not greater than 2 degree intervals to insure proper evaluation of the flow. Some solutions will exhibit a plateau in the curve of flow rate versus temperature at constant pressure.

A flow meter capable of being read with reasonable accuracy is a necessary part of the system being tested. The flow meters in ground use are Brooks "Sho-Rate 150." The 6" long scale is divided into 15 equal parts, each being subdivided into tenths. Brooks "Sho-Rate 50 size 8" are used in the airplanes.

Table 2 presents data obtained from a low-volume fogger during routine operating procedures. Pressure regulators were not altered and pressure gauges showed no apparent changes. The flow meter indicator was varied only 2/15 of the calibrated 6" scale by adjusting a needle valve.

These data indicate how easy it is to over-treat. I have found no way other

TABLE 2.—Data obtained from field equipment in daily use.

Flow Meter Reading	* Actual G.P.H.	Actual Lbs. Cythion/Hour	**Cost/Hour	Excess Cost/Hour
9.0	9.9	67.22	$48.50	$8.12
8.0	8.24	55.95	$40.38	
7.0	7.3	49.57	$35.77	

* Solution containing tech Cythion, 7 gallons made up to 10 gallons with suitable solvent.
** Calculated on base of $7.00 per gallon for tech. Cythion.

than a flow meter to provide continuous and instantaneous information concerning the flow of insecticide leaving the nozzles of a system. It is my belief that, after a few simple tests, few applicators will apply low volume sprays without a flow meter to monitor the output.

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HATCHING OF FLOOD-WATER MOSQUITOES IN SCREENED AND UNSCREENED ENCLOSURES EXPOSED TO NATURAL FLOODING OF LOUISIANA SALT MARSHES

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Many researchers have studied the effect of age and the number of floodings on the hatching of eggs of floodwater mosquitoes in controlled laboratory experiments, but only a few long-term controlled studies have been made in the field. For example, in a recent investigation, Breeland and Pickard (1967) reported the production of nine species of fresh water *Aedes* and *Psorophora* in screened and unscreened enclosures exposed to artificial flooding. Small numbers of *Psorophora confinnis* (Lynch Arribálzaga), *P. cyanescens* (Coquillett), *P. ciliata* (F.), and *P. howardii* Coquillett were still being produced in a screened enclosure after 23 floodings occurring in 4 years. However, two species, *P. ferox* (Humboldt) and *Aedes atlanticus* Dyar and Knab, did not appear in the screened enclosures until the 18th and 21st floodings, respectively, which took place during the fourth year. It may have been coincidental that these two species appeared in the screened enclosures at the same time populations were building up in the unscreened enclosures and in the general area because the ecology of the area was gradually changing to a woodland type habitat more conducive to their breeding.

We wished to find out whether it was possible to use the natural habitats to hatch out all the eggs of our most important species in southwestern Louisiana (*Aedes sollicitans* (Walker), *A. taeniorhynchus* (Wiedemann), and *P. confinnis*) over a period of time. Therefore, several salt marshes were selected for study that had consistently produced broods of these species.

MATERIALS AND METHODS. The vicinity of Big Lake, Louisiana was chosen for the study. Big Lake is connected to the Gulf of Mexico by the Calcasieu ship channel; hence, the areas surrounding the lake are subjected to tidal action, possess salt-marsh vegetation, and often produce enormous broods of salt-marsh mosquitoes and smaller broods of *Psorophora confinnis*.

Eight plots, each 10 feet square (Figure 1) were laid out in two salt-marsh areas, GL-I and GL-II, and walled to a height of about 18 inches above the ground and to a depth of about 6 inches beneath the surface of the soil. The walls were of cedar siding with redwood stock at the corners, and all joints were sealed to ex-

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