P. ferox (Humb.). Fairly common in Hampton Roads area and well distributed throughout State.

P. horrida (D. & K.). Rare. Woodstock, Aug. 4, 1904, F. C. Pratt, U.S.N.M. det. by H. G. Dyar.

P. howardii (Coq.). Fairly common.

P. varipes (Coq.). Rare. Princess Anne Co. near Norfolk Airport, July 20, 1943. Four females. Biting. H. P. Nicholson. Camp Lee, July 20, 1943. Single female, biting. E. J. Gerberg.

Culiseta:

C. inornata (Will.). Fairly common especially in Cape Henry area. Larvae hard to locate.

C. melanura (Coq.). Fairly common and well distributed.

Uranotaenia:

U. sapphirina (O.-S.). Common in lakes and reservoirs and in water chestnut areas of Potomac. Thousands taken in traps in Northern Virginia.

SIMPLE FIELD AND LABORATORY TESTS OF LARVICIDES

H. L. FELLTON

The ultimate criterion of the effectiveness of a larvicide is its ability to kill mosquito larvae. A larvicide, properly applied in sufficient quantity to breeding places, should result in effective control of mosquito breeding. When this does not occur, it indicates that something is wrong with the larvicide. Larvicides being used in control operations should be constantly checked, particularly in the field, to determine their effectiveness.

Paris Green

Paris green which conforms to proper specifications as to chemical structure and particle size is known to be toxic to anopheline larvae when applied in very small amounts. Laboratory experiments have shown clearly that amounts of paris green so small as to escape visibility under the hand lens may poison all larvae in a container (Barber). From other laboratory experiments it may be assumed that the minimum lethal dose for fourth instar **A. quadrimaculatus** larvae is less than 0.0000004 gms of paris green.

An approximation of the relative toxicity of different paris green dusts may be obtained as follows: A larva is placed in a Petri dish containing water and observed under a hand lens. When it is feeding well, a very small amount of paris green is carefully applied to the water surface near the larva. As soon as it has ingested a small particle, it is removed by pipette to a second dish, washed, and put into a third where the time of its survival is noted.

Pan toxicity tests may be made to ascertain effectiveness. Ten or more second, third and fourth instar larvae are introduced into a pan partially filled with water, and the larvicide in the same paris green-diluent mixture and at the same rate of application as that to be used in the field is dusted over the surface of the water. An effective paris green should give a complete kill within two hours.

Dosages of larvicide determined by laboratory means are not always effective under field conditions, where the spread of the dust may be obstructed by scum, algae, floatage, and other factors. An effective field dose may be determined by sampling the larval population at specific points in

breeding areas before and after application of known dosages of the larvicide. A light treatment should be tried first and the dosage gradually increased until it is found to be effective. Checking of mortality should be performed on the day following the application.

Petri dishes or other containers partially filled with water and with a known number of larvae in each may be placed at various points in the breeding area shortly before applying the larvicide. The dishes can be examined after the dusting and the precentage of mortality determined.

Petroleum Oil

Petroleum oils do not, as many people believe, kill mosquito larvae and pupae by suffocation. The volatile components of the oil exert direct toxic effects on the tissues of the larvae and pupae which result in their death. The evidence indicates that volatility is directly correlated with toxicity (Freeborn and Atsatt). Oils of low boiling range and high volatility exert a direct toxic action within a very short time. A high boiling, nonvolatile, viscous oil, on the other hand, may slowly cause death within a longer period.

A larvicidal oil should approximate the following specifications as nearly as possible:

Type of Oil:	Light distillate fuel or Diesel
Gravity (A.P. 1.)	27-33
Flash Point	130° or higher
Viscosity S. U. (a) 100° F.	35-40
Spreading Coefficient	16 dynes/cm or higher

Distillation:

10%:	430° - 450° F.
50%:	510° - 550° F.
90%:	630° F. or higher

Blends made from fractions of widely divergent volatility and viscosity are likely to be ineffective and uneconomical.

Toxicity:

The toxicity of larvicidal oils can be determined in the field by sampling the larval population of a breeding area before and after spraying, as has been described for paris green. It should be remembered that factors other than the toxicity of the oil may influence the kill.

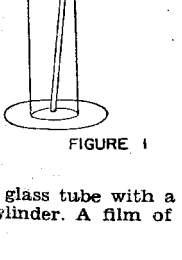

FIGURE 1

The easiest and most simply performed field or laboratory test to determine toxicity is to introduce at least ten (preferably 50) larvae and some pupae into a jar, pan, or other container partially filled with water and then apply a thin surface film of the oil being tested. The time elapsing before the larvae and pupae are dead, or the fact that the larvae and/or pupae are not all killed, indicates the relative toxicity of the oil.

In another test, described by Herms and Gray, the larvae and pupae are placed in a tall glass cylinder not over two inches in diameter, filled with clean water almost to the top. A glass tube which has been connected by means of rubber tubing to a source of water supply is inserted to the bottom of the cylinder (figure 1). Several glass containers filled with water and a glass tube with a rubber suction bulb at one end are placed adjacent to the cylinder. A film of the oil to be tested is put into the water surface in the

cylinder, with as little disturbance as possible. After 30 seconds (using a stop-watch, if available), provided all the larvae and pupae have been in contact with the oil, fresh water is introduced gently through the glass tube to float off the oil, which is finally absorbed with blotting paper or similar material. The larvae and pupae are transferred by means of the glass tube with suction bulb to a jar of clean water. They should then be kept under observation to determine the time of death.

Repeat the experiment, increasing the time of contact by half minute increments up to a total of five minutes. If an oil will not kill within a total contact time of five minutes, it is probably insufficiently toxic to be of use for mosquito control. (Contact time should not be confused with the elapsed time after initial contact with the oil film).

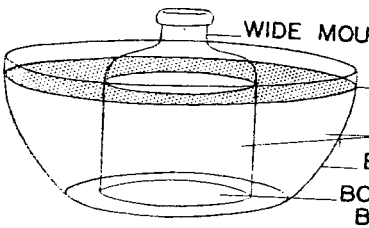

FIGURE 2

The following test as described by W. A. L. David, should result in a mortality of at least 50% when **Aedes aegypti** is the test insect, or at least 90% when **Anopheles quadrimaculatus** is used. The test should be conducted at 77° F.

Take a china or enamel hand bowl about 12 inches in diameter and thoroughly clean it with water (hot water may be used with advantage but avoid the use of soap). Nearly fill the bowl with clean water, bringing the temperature to 77° F. From a bottle having a short neck about 1 inch in diameter remove the bottom at such a level that the mouth of the neck is about 2 inches above the water surface (figure 2).

When the bottle prepared as described is standing in the bowl, put larvicide on the water surrounding the neck. The bowl should be covered with a complete film right up to the edge; if this is not the case, the apparatus was insufficiently washed. For a bowl 12 inches in diameter, use 0.5 ml. larvicide, i.e., enough to form a film about 10 u (u= one-one thousandth mm.) thick.

By following the above technique it is possible to get the larvae under the only spread film without any possibility of accidentally oiling them.

Allow the larvae to remain under the oil film for 30 minutes, and then remove them to a clean beaker by running the pipette through the neck of the. Remove those at bottom first and if necessary any larvae sticking in the surface film may be caused to sink by touching with a glass rod. Set the beaker aside (preferably at about 77° F.) and observe the larvae after 24 hours. If larvae show no sign of swimming movement after gently touching with a glass rod, they may be considered dead.

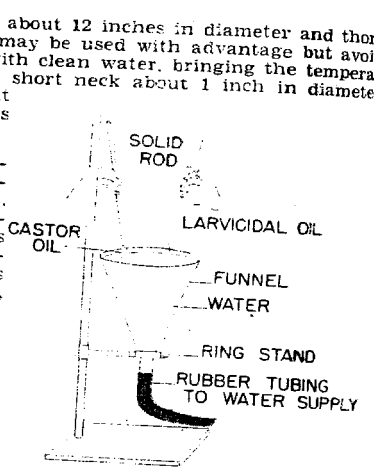

FIGURE 3

(A "control" of equal number of larvae should be prepared and handled in the same manner except exposure to the larvicide. Fifty test larvae will yield more statistically significant data.)

Tests similar to those described above can be used to determine the toxicity of pyrethrum-oil emulsion and other surface acting liquid larvicides.

Spreading Coefficient

The spreading coefficient of larvicidal oil is of fundamental importance since it is an index of water surface coverage, i.e., whether the oil will spread rapidly and evenly so as to produce a thin, uniform, unbroken film on the entire water surface or whether it will remain in thick localized patches. The spreading coefficient of a larvicidal oil should not be less than 16 dynes per cm.

A simple qualitative determination of this property may be obtained by observing carefully the spreading action of the oil when it is applied to actual breeding places, or when a drop or two of the oil is allowed to fall on water in a container of some sort.

A more quantative determination may be made by utilizing castor oil, which has a spreading coefficient of about 16 to 20 dynes per cm. A 6-8 inch diameter glass funnel is fixed in an upright position with the stem connected by rubber tubing to a water supply (figure 3). The apparatus should be set up over a sink or some other receptacle to dispose of the waste water.

Water should be introduced through the rubber tubing and allowed to overflow from the funnel for a short time until a completely clean surface is obtained. The water is then turned off and the funnel tilted slightly and straightened again so as to bring the water surface to about one-eighth inch below the rim of the funnel.

A clean glass rod held in one hand is dipped into the sample to be tested while a rod in the other hand is dipped into castor oil. A drop from each rod is lowered on to the water surface simultaneously. The spreading of the oils is observed by looking along the water surface horizontally.

If both oils occupy an approximately equal area of water surface, their spreading coefficients are about equal (16-20 dynes/cm). If the castor oil spreads over the greater portion of the water surface, the larvicidal oil has a spreading coefficient below 16-20 dynes/cm; if the difference in spread is very great, the larvicide cannot be regarded as satisfactory. The larvicidal oil has a spreading coefficient greater than 16-20 dynes/cm. and is satisfactory when it occupies most of the water surface.

Specific Gravity

Since the oil must form a surface film on the water, the specific gravity of the oil should not approach too closely that of the water. A specific gravity of 0.83 to 0.86 (20° C/4°C) is satisfactory although it may be somewhat higher. A hydrometer can be used to read the specific gravity directly. When a hydrometer is not available gently drop a layer of oil about one-eighth inch thick on the surface of the water in a glass vessel. Stir and allow to stand for about 15 minutes. If the water is clean and the oil/water interface is sharp, the oil may be judged satisfactory.

Viscosity

The viscosity, or resistance of the oil to flow, is of importance since it is concerned with the passage of the oil through the respiratory openings and along the tracheal tubes of the larvae and pupae. If too viscous, penetration is inhibited or retarded. In addition, it is more difficult to spray the thick

heavy oils of higher viscosity. A viscosity range of 35 to 40 (Saybolt Universal at 100°F) is most satisfactory.

Ring the outlet tube of an ordinary 25 to 50 cc. pipette about 2 inches below the bulb with a piece of gummed paper. Prepare a mixture of 60 volumes glycerine (95% pure) and 40 volumes distilled water. Measure the glycerine and water separately and then combine; do not measure 60 volumes glycerine and then add enough water to make 100 volumes.) This mixture has a viscosity of 45* Saybolt Universial at 100° F. Bring the mixture to 70° by cautiously warming over a small flame or cooling if necessary. Draw mixture into pipette, fix the pipette vertically in a clamp and take the time in seconds for the solution to flow from the graduation mark down to the gummed paper ring. Repeat the test and take the average time. If possible, use a pipette with outflow of 30-60 seconds. Clean out pipette, dry, and having brought larvicidal oil to 70° F. run it through in the same way. The time taken should not exceed that for the glycerine/water mixture.

*Viscometer reading by William Spicer, Georgia Institute of Technology.

References

1. Fellton, H. L. Larvicides and their application. Mosquito News, 3(3): 96-104. September, 1943.

2. Barber, M. A. Paris green (aceto arsenite of copper) and other stomach poisons as larvicides against mosquito larvae. (in) A symposium on Human Malaria (Amer. Assoc. Adv. Sci. Pub. 15). 1941.

3. Freeborn, S. B. and R. F. Atsatt. The effects of petroleum oils on mosquito larvae. Jour. Econ. Entom., 11, 299-307. 1918.

4. Herms, W. B., and H. F. Gray. Mosquito Control. Commonwealth Fund, 1940.

5. David, W. A. L. Simple tests for estimating the suitability of mineral oils as mosquito larvicides. Bul. Entom. Research, 33, 195-203. 1942.

MALARIA IN EGYPT

Time (March 13, 1944, p. 28), commenting on the introduction of **Anopheles gambiae** into upper Egypt from the Sudan, reports that the malaria death rate in Qena and Aswan provinces has been unusually high. There is a "prospect that **A. gambiae** may continue its progress north and breed in the myriad ponds and pools of Northern Egypt . . ."