TESTS WITH A NEW GRANULAR PARIS GREEN FORMULATION AGAINST *AEDES, ANOPHELES,* AND *PSOROPHORA* LARVAE

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Since Barber and Hayne (1921) first published on paris green as a mosquito larvicide 36 years ago, almost all of the research on this material has been directed toward making the paris green more floatable so as to improve its efficiency in killing surface-feeding anopheline larvae. Griffitts in 1927 published on a moist sand-paris green formulation and showed conclusively that this formulation was very effective against larvae of *Aedes taeniorhynchus, Aedes sollicitans,* and *Culex quinquefasciatus* under field conditions (Griffitts, 1927).

Several papers were published during the nineteen thirties on the use of paris green against culicines (for more complete reviews of the literature on this subject see Herms and Gray, 1940; Barber, 1941; Covell, 1941; and Bishopp, 1949). But there apparently has been no real effort to develop improved formulations of paris green for use against culicine larvae since Griffitts' contribution 31 years ago.

The research reported here is indirectly related to experiments conducted by King and McNeel in Pinellas County, Florida in the thirties (King and McNeel, 1938). Using the simple method of applying a suspension of paris green in water from sprinkling cans, these workers reported excellent kills of *Aedes taeniorhynchus* and *Psorophora confinnis* at dosages as low as one pound per acre. Thus King and McNeel confirmed the earlier work of Griffitts with paris green against culicines, but this promising line of investigation apparently was not continued beyond the nineteen thirties.

In the summer of 1956, Mr. Claude Strickland of St. Petersburg, Florida, who was director of mosquito control in Pinellas County when King and McNeel did their work there, recommended that paris green be tried as a larvicide to control an unusually large brood of salt-marsh *Aedes* that occurred in Pinellas County that year. Since approximately 5000 acres of marsh were to be treated in the space of a few days, hand application of larvicides obviously could not be used, and granular parathion applied by aircraft was used as an emergency measure. Following this work in Pinellas County in 1956, Mr. John A. Mulrennan, Director, Bureau of Entomology, Florida State Board of Health, requested that paris green be tested by the Entomological Research Center against salt-marsh mosquito larvae.

Paris green in suspension in Triton X-100 and water was included as one treatment in small-plot larvicide tests against salt-marsh *Aedes* conducted in cooperation with the Indian River Mosquito Control district in the fall of 1956. Results of these preliminary tests with paris green (not published) were very encouraging, and in 1957 efforts were devoted toward developing a granular formulation.

METHODS. Since paris green is insoluble in most solvents, the problem of formulating was very different from the usual method of dissolving the insecticide in acetone or other solvents for absorption by granular bases. This problem was solved by using vermiculite as the granular base and sticking the paris green to the base with a water-miscible sticker.

Experimental formulations utilizing various stickers were evaluated in the laboratory to determine the effectiveness of the stickers in holding the paris green on the vermiculite and the effectiveness of the best of these formulations against larvae of *Aedes taeniorhynchus.* Biological tests were randomized and replicated and were conducted in metal containers of one square foot size. Dosage was in pounds per

acre and was varied either by volume or concentration. The more promising formulations were tested both in brackish water (salinity about 20/1000) and in fresh water.

All field tests were conducted with the formulation considered best by laboratory evaluation, and which for convenience of identification is designated ERC Larvicide No. 1.

FORMULATING PROCEDURE. The procedure for making this formulation is as follows. The granular base is prepared by removing dust-size particles from No. 4 grade vermiculite with a 30-mesh screen. The sticker is an oil emulsion containing by volume 60 percent of 40W motor oil, 30 percent water, and 10 percent Triton NP-56.* It is best to dissolve the Triton in hot tap water before emulsifying the oil.

In formulating 10 pounds or more of granular paris green, the vermiculite is placed in a tumbler (a cement mixer works well) and the emulsion is sprayed as a fine mist over the vermiculite as the mixer is in motion. A nozzle pressure of 40–50 p.s.i. is required to spray the emulsion. It is important that the emulsion be sprayed over the vermiculite; if poured or dripped, lumping will occur. After blending the sticker and vermiculite thoroughly, the paris green is added in small amounts until a uniform blend is obtained. The finished formulation is spread and air-dried for 24 hours to remove excess moisture. Mechanical drying has not been tried to date but probably could be used.

A formulation containing 15 percent by weight of paris green requires four gallons of emulsion per 100 pounds of formulation. The 15 percent material weighs approximately 15 pounds per cubic foot after excess moisture is removed.

When this formulation is applied to the water surface, the vermiculite will float for several hours but paris green begins immediately to be released and settles slowly to the bottom. However, some of the released paris green will remain on the water surface for several days in laboratory containers if not disturbed. Thus the formulation appears to meet the requirements both for surface-feeding and subsurface-feeding larvae.

TEST RESULTS: LABORATORY TESTS. Paste, mucilage, powdered and liquid starch, motor oil, and flour paste all made what appeared to be good formulations but all failed in biological tests, presumably because sufficient paris green was not released in water.

In fresh water, a 10 percent paris green formulation with methyl cellulose solution as the sticker killed 100 percent of third instar *Aedes taeniorhynchus* at dosages as low as 0.1 lb./acre of paris green. In brackish water (salinity 21.3/1000) this same formulation killed only 78 percent at a dosage of 0.5 lb./acre and gave correspondingly lower kills at lower dosages.

Of all stickers tested, oil in combination with a wetting agent, such as Triton or sodium lauryl sulphate, performed best in killing larvae both in fresh and saline waters; several of these formulations were completely effective at dosages ranging from 0.3 to 0.5 lb./acre of paris green.

FIELD TESTS: All field testing of the granular paris green formulation to date has been in small plots in Indian River County, the formulation being applied with a hand shaker. Due to the early termination of salt-marsh mosquito breeding in Florida in 1957, no opportunity was available for tests by airplane application during the 1957 season. Therefore the effectiveness of this larvicide when applied by aircraft is not known at this time.

In September, 1957 several old ditches in the salt marsh were partitioned with earth dikes to make plots of 300 to 500 square feet in area. Approximately 5000 third and fourth instar larvae of *Aedes taeniorhynchus* from another area of the salt marsh were placed in each plot. The plots were naturally stocked with a small population of *Anopheles bradleyi*. After allowing several hours for the *Aedes* larvae

* Rohm & Haas Co., Philadelphia, Pa.

to become "settled" in the plots, ERC Larvicide No. 1 containing 15 percent (W/W) paris green was applied with a hand shaker at dosages of 0.15, 0.30, and 0.60 lb./acre of paris green. All treatments were applied at a gross volume of four pounds per acre; this was accomplished by diluting the smaller dosages with untreated vermiculite.

Water in the plots was 8 to 20 inches deep and there was a moderate vegetational cover consisting primarily of salt grass, *Distichlis spicata* (L.) Greene, and woody glasswort *Salicornia perennis* Mill. Results are shown in Table 1.

TABLE 1.—Test results with granular paris green against larvae of *Aedes taeniorhynchus* (Wied.) and *Anopheles bradleyi* King, September, 1957 *

Paris Green Lbs./Acre	Average No. Larvae per Dip				Reduction —24 Hrs.	
	Pretreatment		24 Hrs.			
	Aedes	*Anopheles*	*Aedes*	*Anopheles*	*Aedes*	*Anopheles*
0.60	14.5	.95	0.1	.00	99.3	100
0.30	10.1	.95	0.9	.00	91.1	100
0.15	4.7	.38	0.7	.03	86.2	92.3
Check	3.3	.55	2.2	.45	33.3	18.2

* Replications: Treated plots 2; check 1.

The reduction of larvae in the check plot (Table 1) was more apparent than real. In the case of *Anopheles* the apparent reduction was due to the small initial population. At pretreatment inspection, the *Aedes* were concentrated near one end of the check plot and at post-treatment inspection they were noted to be widely dispersed, causing a reduction in the average number per dip. The many dead larvae found in all plots except the check further supported the effectiveness of the treatments.

The second field test against *Aedes taeniorhynchus* was conducted October 3, 1957 on a recently constructed hydraulic fill. This fill was on mangrove marsh in which the mangrove trees were not cleared before filling. After filling, the mangroves shed their leaves, which formed a ground cover very favorable for oviposition by *Aedes taeniorhynchus*. Subsequent rains hatched the eggs in numerous small pools on the fill, some of which were modified to form small plots for a larvicide test.

Sixteen plots, each 200 square feet in area and averaging 10 inches in depth, were arranged in four blocks, making the test a standard randomized-blocks design with four treatments and four replications. All treatments were applied at a gross volume of three pounds per acre at dosages of 0.15, 0.30, and 0.45 lb./acre of paris green.

Results of this test are shown in Table 2. These results confirmed the results against *Aedes taeniorhyncus* in the first

TABLE 2.—Tests results with granular paris green larvicide against *Aedes taeniorhynchus* (Wied.), October, 1957 *

Paris Green Lbs./Acre	Average No. Larvae per Dip			Percent Reduction	
	Pretreatment	4 Hrs.	24 Hrs.	4 Hrs.	24 Hrs.
0.45	83.7	0.7	1.0	99.1	98.8
0.30	58.1	11.6	6.5	80.0	88.9
0.15	82.7	50.3	28.0	39.2	66.1
Check	67.7	71.1	71.4	—	—

* Four replications.

test and show that a dosage of 0.45 to 0.60 pounds of actual paris green per acre was adequate to give good control of *Aedes taeniorhynchus* under the conditions of these tests.

Table 3 shows results of tests with ERC Larvicide No. 1 against third and fourth instar larvae of *Psorophora confinnis*. The table is a summary of similar tests replicated in time between September 18, and October 23, 1957, all in the same area.

TABLE 3.—Test results with granular paris green against *Psorophora confinnis* (L.-Arr.), 1957 *

Paris Green Lbs./Acre	Average No. Larvae per Dip			Percent Reduction	
	Pretreatment	4 Hrs.	24 Hrs.	4 Hrs.	24 Hrs.
0.60	11.7	—	0.8	—	92.9
0.45	7.0	1.0	0.5	85.7	92.6
Check	4.1	4.5	4.3	—	—

* Three replications.

Plots were located in roadside ditches and varied in size between 1000 and 3000 square feet. Water depth ranged from 1 to 13 inches and all plots contained a moderate to very heavy cover of pangola grass, *Digitaria decumbens* Stent. A gross volume of 3 lbs./acre of formulation was used in all tests; a 20 percent formulation was used to obtain the 0.60 pound dosage.

Data in Table 3 indicate that there was no significant difference in the average kill at 0.45 and 0.60 lb./acre. With the 0.45 lb. dosage, the percent reductions in 24 hours for the individual plots were 86 percent, 100 percent, and 98 percent; for the 0.60 lb. dosage, the reductions were 99 percent, 85 percent, and 100 percent. In one plot receiving the 0.45 lb. dosage, the kill was 100 percent in four hours after treatment, and the average number of dead larvae per dip was about the same as for live larvae before treatment. The higher and more rapid kills of *Psorophora confinnis* seemed to be correlated with plot conditions of clear water, moderate to sparse vegetative cover, and a firm bottom. However, sufficient testing has not been conducted to make this observation positive.

DISCUSSION. The data obtained in the field tests again confirm the earlier reports of Griffitts (1927) and King and McNell (1938) that paris green larvicide can be used effectively against a number of culicine species. The failures reported with its use against culicines (see Covell, 1941) could very well have been due to the formulation used. The literature on paris green as an anopheline larvicide leaves little doubt that most dust formulations give poor results against culicine larvae.

Considering the many problems attendant on the use of organic compounds as larvicides, especially the potential hazards to fish and other beneficial forms of life and the ever-increasing "resistance" problem, it would appear that thorough investigation of paris green, as well as other inorganics, offers one of the most promising approaches for the future in control of mosquitoes with chemicals. The literature leaves no doubt that paris green was used as an anopheline larvicide for more than 30 years with a good margin of safety to man, domestic animals, fish and other animals. Also, to the authors' knowledge, no "resistance" to this chemical by mosquitoes was reported in its long history as a larvicide.

In the tests reported in this paper, there was no opportunity to observe effects of the granular formulation on fish, but aquatic Coleoptera, Hemiptera, Odonata, amphibians and other observed forms of life appeared not to be affected at the dosages used.

Calibration tests are being conducted with a Stearman airplane for dispensing the granular formulation by air. The light

weight of the material presents some problems when using standard dusting equipment on aircraft. Research to improve the present formulation by increasing the bulk density and eliminating the drying process is being continued.

Due to the presently limited experience with this formulation, only general suggestions may be made regarding its use at this time. Since paris green kills only when consumed by the larvae, good coverage of the breeding area is required. Although 91 percent of first instar *Aedes taeniorhynchus* were killed in one laboratory test, it is felt that better results might be expected against the more active, later instar larvae. Pupae, of course, are not affected, and there appears to be little or no residual effect at dosages used to date.

Calibration tests with aircraft presently indicate that the gross volume to ensure good coverage and adequate swath when applied by airplane might be at least four to six pounds per acre. Three to four pounds per acre appear to be an adequate volume when applied by hand. A dosage of approximately 0.5 lb. per acre of paris green appears adequate for the species studied and may easily be obtained with any desired volume by adjusting the concentration of paris green in the formulation. At these rates, the cost per acre for materials should compare favorably with the least expensive larvicides now in use.

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TOP THIS ONE!

Recently I was out on the range with Clarence Blair, field man for the Kamloops Mosquito Control Committee, who in past years has plodded many a weary mile with a knapsack sprayer on his back, treating the rangeland pools. This year, however, he stopped his Land Rover beside a good-sized slough, drew out a schoolboy catapult, leaned out of the window, spotted "Tossits" accurately around the margin of the pool, and moved on to the next in a matter of a few moments. It seems to me that this is a wrinkle with a wide application, that should be reported where it will do most good.—L. COLIN CURTIS, Research Officer (Agriculture), Dominion Entomological Laboratory, Kamloops, B. C.