ported again and Abate was applied at 0.05 lb. (30 gals. emulsion) per acre. Another Abate spray was applied at the same rate on June 24, and another on July 12, each time after dip counts showed first stage larvae developing. A local shower, not recorded at the site of the weather station, was reported after the July 12 application. This, plus a heavy rain on the 10th, resulted in flood conditions, and larvae appeared only 3 days after the spray. It was decided to use DDT again on the 15th. By July 21, first stage larvae were found again, following heavy rain on the 18th, and thereafter the Abate schedule was continued. After the Abate application of 0.05 lb. per acre on the 21st of July, the next record of first stage larvae was on August 9. In order to determine if a higher concentration would be effective over a longer period, the dosage was increased for the next two aplications to 0.1 lb. per acre (August 9 and 19), and then increased again to 0.5 lb. per acre for the next application, which happened to be the last that was needed that season. The earlier, increased dosages apparently did not result in any longer period of effectiveness, but it may be that the comparatively heavy dosage of 0.5 lb. per acre on August 30 was responsible for prolonging the larvae-free period to the point where no more sprays were necessary.

DISCUSSION. As pointed out above, direct comparison from one year to the next could not be made because of a lack of comparable data in 1964. However, the number of times spraying was considered necessary somewhere in the marsh because of reappearance of larvae was fewer in 1965, with the Abate program than in 1964 with the DDT program, even though the amount of rainfall was greater in 1965.

All that could be concluded was that a program of Abate treatments seemed to be a very promising substitute for DDT.

FIELD EVALUATION OF TWO MOSQUITO LARVICIDES, ABATE AND DURSBAN, AGAINST *ANOPHELES QUADRIMACULATUS* AND ASSOCIATED *CULEX* SPECIES

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INTRODUCTION. For many years, TVA has been using DDT as a mosquito larvicide. The usual application has been by helicopter at the rate of 0.1 pound DDT per acre, increased to 0.3 pound in densely canopied areas. In 1966, two promising new larvicides, Abate and Dursban, were field tested as potential substitutes for DDT. These chemicals were chosen because preliminary reports from various sources indicated that they had the qualities suitable for effective low volume discharge rates required for helicopter application.

MATERIALS AND METHODS. All field evaluations were conducted along shoreline areas of Dogwood or Redbud Lake (Beech River Watershed Development Authority) located east of Lexington, Tennessee, in Henderson County. Several shoreline segments characterized by quiet, shallow water populated by decaying annual vegetation and thriving water-tolerant plants (*Eleocharis, Juncus, Scirpus, Ludwigia,* small willow, etc.) were used intermittently as representative larval mosquito populations developed.

Typically, evaluation plots were estab-

lished along a 200-foot line parallel to the shoreline by setting wooden stakes at 20-foot intervals. The center stake represented the line of flight which was perpendicular to the line of stakes. Any departure from this arrangement will be discussed elsewhere in this report.

Pretreatment and 24-hour posttreatment larval counts were taken from 20 dips at each plot. Larvicidal coverage at each plot, calculated in terms of milligrams per acre, was sampled by the number and size of droplets recovered on glass slides exposed in floating paper pie plates at the time of treatment. The mass median diameter of droplets was 78–100 microns for Abate and 69–107 microns for Dursban.

Oil base sprays were prepared by dilution of the stock solution with Amsco HT solvent. The Abate stock was 43 percent emulsifiable concentrate (4 lbs./gal.) purchased on the open market; the Dursban was a 63 percent nonemulsifiable formulation (6 lbs./gal.) supplied by the Dow Chemical Company for study purposes only. The sprays were prepared on the basis of a discharge rate of 144 c.c./ac. Proper concentrations were obtained by altering the amount of stock solution.

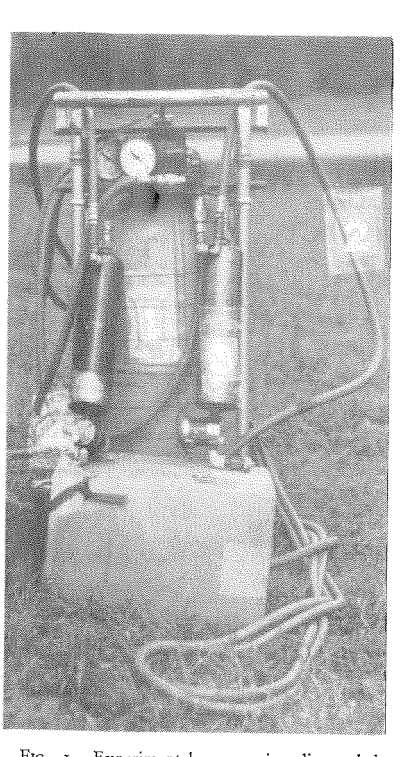

FIG. 1.—Experimental spray rig, dismantled. (1) Air cylinder, (2) Air hose, (3) Spray cylinder, (4) Solenoid and discharge valve.

Helicopters used for routine larviciding were fitted with experimental spray equipment designed for use in this study. Figure 1 shows the dismantled apparatus which consists of an air cylinder and a pair of rubber hoses leading from the air cylinder to a pair of 800 c.c. cylinders containing the spray. The cylinder air, under 150 lbs./in.^{2} pressure, was released at 50 lbs./in.^{2} by a pressure regulator valve. Each spray cylinder was electrically activated by solenoid valves and equipped with an 80015 Spraying Systems nozzle. The equipment was mounted (Fig. 2) by strapping the air cylinder in the passenger seat of the helicopter, securing the air hoses to the booms, and clamping the spray cylinders in place near the ends of the booms separated from each other by a distance of 22 feet 4 inches. Spray nozzles were directed forward and parallel to the ground.

Evaluation plots were treated by having the helicopter fly at a speed of 50 mph at an altitude of 30 feet. This combination delivered the desired 144 c.c./ac. of spray material based on a swath width of 100 feet. A total of 15 treatments was run, 7 with Abate and 8 with Dursban. Observations on nontarget organisms included one rather detailed study and several superficial appraisals. The nontarget organism studies are discussed in a separate section of this report.

APPLICATION RATES AND LARVAL MORTALITY

(1) *Abate.* Preliminary runs in 1965 indicated that Abate, discharged at rates sufficient to recover 100 mg./ac. or more by the glass slide technique, gave 100 percent anopheline control in adjacent

FIG. 2.—Side view of helicopter with experimental spray rig mounted. Legend same as in Fig. 1.

plastic containers. This warranted the current field evaluation. Results obtained from the seven tests conducted with Abate in 1966 are given in Table 1. The significant column in this table is the one headed "Best 100' Swath" since this was target size. The plots were established at widths of 200 feet with the intention of revealing the distribution pattern since it was realized that drifts would occur and normal curves would seldom be obtained. However, it was observed that larval kill sometimes exceeded even the 200-foot staked limits. Therefore, because of thin distribution, the actual concentration of larvicide on the water surface was much

TABLE 1.—Summary of Abate field evaluation results, 1966.

Test No.	Date	Rate of Discharge Based on 100' Swath (lb./ac.)	Effective Swath Width* (ft.)	Corrected Rate of Application** (lb./ac.)	Percent Mortality			
					Best 100' Swath		200' Staked Plot	
					Anopheles	Culicines	Anopheles	Culicines
1	7-19	0.022	185	0.012	99	88	88	76
2	7-26	0.022	250	0.0088	100	81	94	71
3	8-2	0.015	230	0.0065	100	95	98	77
4	8-2	0.01	220	0.0045	99	87	98	83
5	9-13	0.004	160	0.0025	100	82	98	78
6	9-13	0.004	50+	0.004	99	80	..	..
7	9-20	0.004	150	0.0027	99	58	68	58

* Based on anopheline larval kill of 90% or better.
** Rate of application calculated by correcting baseline 100' swath width to effective swath width.

less than the planned concentration which was based on 100-foot swath widths. Actual effective swaths as determined by 90 percent anopheline kill are shown in a separate column. This required a corrected concentration column based on the actual swaths obtained. As experimenting progressed, these figures came to be as meaningful as the rate of application in working out the optimum concentration.

Tests 1–5 with Abate were standard tests with concentration the significant variable. Test No. 4, Table 1, showed that an application rate of 0.01 lb./ac. gave almost as good control as did higher rates in test Nos. 1–3. When this was corrected to take into account the 220-foot swath width obtained, it is seen that the actual rate was only 0.0045 lb./ac. Test No. 5 shows that a discharge rate of 0.004 lb./ac. resulted in 100 percent and 82 percent mortality among *Anopheles* and *Culex*, respectively. The application rate corrected on the basis of the 160-foot swath width actually obtained in this test was only 0.0025 lb./ac. With such low dosages giving excellent *Anopheles* and adequate *Culex* control, it seems evident that an application rate of 0.004 lb./ac. is sufficient to assure control over a 100-foot swath width, with bonus benefits resulting from extra wide coverage ranging from 160 to 250 feet depending upon conditions.

Tests 6 and 7 were conducted after the 0.004 lb./ac. figure had been agreed upon as the optimum from data available. Changes in equipment to narrow the swath width were not desirable at this late date, even though indications are that with a narrower swath the dosage might be reduced even more. Test No. 6 was conducted to evaluate a 0.004 lb./ac. treatment under conditions more nearly typical of operational patterns. Here a linear run was made parallel to the shoreline with a single pass over a rather narrow (50′) band of vegetation. Pretreatment and posttreatment sampling of 50 dips in a 100-foot treated section showed 99 percent and 80 percent kill, respectively, of *Anopheles* and *Culex*. Test No. 7 was devised to show results obtained with routine operational equipment rather than the experimental rig previously described. Coverage was good (150′ swath) and control of *Anopheles* was 99 percent. The poor showing against *Culex* reflects extremely low numbers of culicine larvae in the pretreatment and posttreatment sampling. Residual effectiveness of Abate was checked in test No. 1. Eight days after treatment the larval population had recovered to pretreatment numbers. The pretreatment count on July 19 was 3.2 larvae per dip, whereas the posttreatment residual count on July 27 was 4.5 larvae per dip.

The mosquito population was dominated by *A. quadrimaculatus* and *C. erraticus* throughout this study. Superficial examination of arthropods, fish, and frogs in pretreatment and posttreatment dipping procedures indicates no apparent acute effect by Abate on this segment of the fauna at any of the concentrations used.

In summary, Abate, applied at the rate of 0.004 lb./ac. based on a 100-foot swath, gave excellent *Anopheles* control and adequate *Culex* control without acute harm to nontarget organisms. Additional effectiveness ranging up to a total width of 250 feet was obtained. No evidence of residual effectiveness was noted.

It might be feasible to alter discharge equipment to lessen the swath and perhaps the application rate of Abate. However, it seems that the application rate of 0.004 lb./ac. over a wide swath might provide a desirable hedge against a too small "margin of error."

(2) *Dursban*. A total of 8 tests was conducted with Dursban. Results are recorded in Table 2. Application rates ranged from a high of 0.021 lb./ac. in test No. 1 to a low of 0.001 lb./ac. in test Nos. 6–8. Five tests (Nos. 1–4 and No. 6) were conducted in the standard manner as previously described. Test No. 4 shows that Dursban at a rate of 0.004 lb./ac. gave 97 percent and 100 percent control of *Anopheles* and *Culex*, respec-

TABLE 2.—Summary of Dursban field evaluation results, 1966.

Test No.	Date	Rate of Discharge Based on 100′ Swath (lb./ac.)	Effective Swath Width * (ft.)	Corrected Rate of Application ** (lb./ac.)	Percent Mortality			
					Best 100′ Swath		200′ Staked Plot	
					Anopheles	Culicines	Anopheles	Culicines
1	8-10	0.021	120	0.0175	99	84	92	76
2	8-10	0.01	280	0.0036	100	98	98	90
3	8-17	0.005	280	0.0018	100	83	95	64
4	9-7	0.004	190	0.0021	97	100	95	97
5	9-7	0.004	50+	0.004	99	96	99	96
6	8-17	0.001	195	0.0005	93	61	84	39
7	8-24	0.001	...	.....	87	83	46	86
8	8-24	0.001	50+	0.001	99	53	99	53

* Based on anopheline larval kill of 90% or better.
** Rate of application calculated by correcting baseline 100′ swath width to effective swath width.

tively. This is as good as the higher rates used in test Nos. 1–3. An effort to further reduce the dosage to 0.001 lb./ac. (test No. 6) reduced the effectiveness against *Anopheles* only slightly (93% mortality); however, the effectiveness against *Culex* was reduced considerably (61% mortality).

As was the case with Abate, wide swaths were obtained with Dursban (up to 280′). This made a corrected, actual application rate necessary to help arrive at an optimum concentration. A review of Table 2 indicates that a discharge rate of less than 0.004 lb./ac. tended to lose effectiveness, especially against culicines.

Linear runs (a single pass over a segment of the shoreline) in test Nos. 5 and 8 at 0.004 lb./ac. and 0.001 lb./ac., respectively, gave results similar to those obtained in the same concentrations in the standard runs at these rates (test Nos. 4 and 6). Tests Nos. 7 and 8 were devised to show what effect the use of only one nozzle would have toward reducing the swath. The amount of Dursban was doubled while the discharge rate of the single nozzle was maintained. Results were essentially the same as those obtained in test No. 6 when the same rate (0.001 lb./ac.) was delivered by both nozzles. Unfortunately, the exact swath could not be determined since test No. 8 was a shoreline run covering only about 50 feet of habitat and test No. 7 was complicated by excessive wind.

Larval counts for residual effectiveness were made in Dursban test Nos. 1, 2, and 6. In test No. 1 (0.021 lb./ac.) pretreatment counts on August 10 averaged 3.0 larvae per dip; 6 days later on August 16, the count was 0.2 larva per dip. In test No. 2 (0.01 lb./ac.) pretreatment counts on August 10 averaged 3.0 larvae per dip; posttreatment counts 6 days later showed only 0.3 larva per dip; and 14 days after treatment on August 24 the larval population had rebounded to the pretreatment level. In test No. 6 (0.001 lb./ac.) pretreatment counts averaged 2.8 larvae per dip on August 24; 7 days later the larval count was 2.6 larvae per dip.

Dursban, at a rate of 0.004 lb./ac., gave excellent control against both *Anopheles* and *Culex*. At lower rates, effectiveness was lost. Higher rates were unnecessary, apparently detrimental to nontarget organisms, and there is some indication that culicine control regressed with increasing rates, notwithstanding more residual effectiveness.

NONTARGET ORGANISMS. Abate had no noticeable effect on nontarget organisms at any of the application rates tested. Although no detailed studies were made on this aspect with Abate, an "eyeball" inventory was taken on two occasions 24 hours after treatment. In test No. 4, con-

ducted on August 2, 1966, the posttreatment inventory revealed the following organisms to be present in representative numbers apparently not harmed by the application of Abate at the rate of 0.01 lb./ac.: water striders, all stages; surface mites; adult and larval dytiscids; adult hydrophilids; damselfly and dragonfly naiads; Collembola; and heleid larvae. In test No. 5, conducted on September 13, 1966, the posttreatment inventory after using 0.004 lb./ac. Abate included: adult hydrophilids; water striders, all stages; damselfly and dragonfly naiads; syrphid larvae; tabanid larvae; and sarcophagid larvae.

"Eyeball" inventories on nontarget organisms in Dursban tests were conducted on August 17, 1966, at rates of 0.001 and 0.005 lb./ac. Here, there was no noticeable effect on macroscopic organisms including minnows, tadpoles, and arthropod fauna (water striders, hydrophilid adults, dytiscid larvae and adults, dragonfly and damselfly naiads, tabanid larvae, syrphid larvae, etc.). However, at 0.01 and 0.021 lb./ac. in tests conducted earlier, a noticeable die-off of practically all arthropods was observed.

In a detailed study conducted in conjunction with Dursban test No. 5 on September 7, 1966, at 0.004 lb./ac., nontarget organisms were not noticeably affected. In this study, 17 sampling stations were set up in a criss-cross pattern along the path of a treatment. Surface samples by dipping, aquatic net samples, and bottom drag samples were taken before and 24 hours after treatment at each station. Indicator organisms recovered are shown in Table 3. From these data, it appears that Dursban applied at rates of 0.005 lb./ac., or less, was relatively safe to nontarget organisms. However, at higher rates it appears that caution should be used. More defined work is needed before a reliable opinion can be rendered.

SUMMARY AND CONCLUSIONS. Field evaluations of Abate and Dursban, based on 7 and 8 tests, respectively, during the summer of 1966 indicate that either insecticide is suitable as a substitute for DDT in the TVA larvicidal program. The optimum rate of application for both chemicals was determined to be 0.004 lb./ac. on the basis of these tests. Abate appeared to be more selective than Dursban for *Anopheles* but was less effective against culicine mosquitoes. Dursban was more effective against culicines, but,

TABLE 3.—Pretreatment and 24-hour posttreatment inventory of nontarget organisms by three sampling methods, Dursban test no. 5 (0.004 lb./ac.).

Method	Organism	Number of Specimens		
		Pretreatment	24-Hr. Posttreatment	Percent Reduction
Surface Dips	Water striders	122	104	14.1
	Water spiders	30	23	23.3
	Miscellaneous	13	9	3.1
Dip Net	Minnows	9	17	None
	Snails	63	57	9.5
	Tabanid larvae	15	16	None
	Aquatic bugs	2	5	None
	Dragonfly naiads	3	7	None
	Mayfly naiads	3	17	None
	Stratiomyiid larvae	2	2	None
	Chironomid larvae	48	90	None
Bottom Scraping	Chironomid larvae	223	135	39.4
	Heleid larvae	1	11	None
	Mayfly naiads	1	8	None
	Snails	1	9	None

being less selective, nontarget organisms were more susceptible, especially at higher rates. Both larvicides were extremely effective against *Anopheles*. Dursban appeared to have a residual effect lasting from a week to 10 days in some cases (0.01 and 0.021 lb./ac.), whereas no residual effect was observed for Abate.

Although application rates reported herein are based on expected effective swaths of 100 feet, wider swaths were usually attained. This might be a desirable condition since it would allow a margin of error even at the extremely low dosages used. However, it appears feasible to continue evaluation toward a narrower swath or alternate right, left, or two-nozzle spray equipment to concentrate spray when required by field conditions.

STEMPELLIA MAGNA (KUDO) (NOSEMATIDAE: MICROSPORIDIA) IN *CULEX RESTUANS* THEOBALD FROM VIRGINIA

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A new geographic record of the microsporidian *Stempellia magna* is reported. Late instar *Culex restuans* larvae collected on June 23, 1966 at Falls Church, Virginia had a 35 percent incidence of infection. Later collections of mixed species from the same site showed infections only in *C. restuans*. *Anopheles stephensi* larvae reared in water from the site produced a frank infection, with mature spores, in a single larva and several larvae with early developmental stages of the parasite indicating *per os* transmission.

INTRODUCTION. In 1920, Kudo described a new microsporidian parasite, *Thelohania magna,* from larvae of *Culex pipiens* collected near Urbana, Illinois. The next year heavy infections of this parasite were reported from *Culex territans* collected at Warren, Pennsylvania (Kudo, 1921). In 1925 Kudo placed this species in the genus *Stempellia*. *Stempellia magna* was not reisolated until 1962 when Kudo collected it from *Culex restuans* and *C. pipiens* on the campus of Southern Illinois University at Carbondale, Illinois. *Stempellia magna* was also reported from *Culex restuans* at the Gettysburg Battlefield, Gettysburg, Pennsylvania, by Wills and Beaudoin (1965). Thus the distribution and host records of *Stempellia magna* are few, with the parasite recorded from two states and three mosquito species. This species is the only member of the genus *Stempellia* that has been described from mosquitoes. The other species, *S. mutabilis* Léger and Hesse, was described from an ephemerid in France.

METHODS AND MATERIALS. On June 23, 1966 a larval collection of *Culex restuans* was made at Falls Church, Virginia. The larvae were brought into the laboratory and placed in a white enamel pan for observation. All dead and moribund larvae were removed from the pan daily, smeared on microscope slides, air-dried, fixed in absolute methanol for one minute, and stained for 30 minutes with a 1:20 ratio of Giemsa stock solution and tap water. On July 5, four live larvae and five live

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