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Part I

INJECTION METHOD FOR DRAIN CHANNELS

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It has been the goal of the Orange County Mosquito Abatement District to keep improving the control of mosquitoes and chironomid midges in flood channels. A few years ago, when control of these channels started, the only known way was the use of liquid larvicides. Due to the width of the channels, a two-man operation, one spraying and one driving, was necessary. A high pressure spray rig, mounted on $\frac{3}{4}$ ton truck with four-wheel drive was required. Channels with bottoms wider than 40 ft. (our widest channel is 300 ft.) required one operator to spray while walking the bottom edge of the channel. Another way was to spray from both sides of the service road. All of this took a lot of time, especially when material ran out, since the operator would then have to leave the channel to find a source of water before he could mix a new tank full of spray.

After a few years of treating flood channels using this method, the District switched over to the use of granulated insecticides. International Harvester Scouts,

a Thiokol Swamp Spryte and an air boat equipped with compressed air spray units, using the Orange County Compressed Air Granule Gun, were used for applying the granules. This method of treating channels proved to be much faster and saved time because the operator could carry enough granules to carry him through the day without stopping.

Orange County has been confronted in the past several years with an ever increasing midge problem. In some locations, we have found that chironomid midges are a greater nuisance than mosquitoes, especially in the past two or three years, due to the great influx of population. We have found this problem to be acute near our flood channels and large rain-filled depressions throughout the lower elevation and the coastal area of Orange County. Farm lands which previously had produced dry lima beans and tomatoes have been replaced with homes located next to these breeding sources.

Street run-off water from the residential and commercial areas finds its way to the

network of drainage ditches and flood channels which service our urban area of some 320 sq. miles with a population of over 1,000,000 people. The drainage water is high in nutrients and creates a very favorable environment for midge production throughout the year except during our infrequent flood flows during the winter months of January, February and March. The volume of street run-off water is sufficient throughout the remaining 9 months to maintain a constant flow to a depth of 6 inches to several feet in most channels. Midge larval populations will run from 8 to 150 per sq. ft. of bottom mud. Mud samples are taken by a specially designed 4" x 4" nylon net. Larval counts are made in the field by using a grid scaled pan. Duplicate mud samples taken by our vector ecologist, using glycerine for separation of the very small midge larvae, show from 50 to over 800 midge larvae per sq. ft. Using these actual figures for the 107 channels running 250 miles in length, one can readily estimate the tremendous numbers of adult midges which can be produced.

During the 1968 season, both Baytex and Abate granules were used with reasonable success for *Chironomus* sp. but with very little success on *Tanytus* sp. Excellent conditions for the production of midges occurred in 1969 immediately following the winter floods. During March and April from 20 to 56 service requests were received per day. With the midges being a mosquito-like insect, the mosquito control operator's hardest job was to convince the requester that the midges were not mosquitoes and could not bite. True, the midge is a nonbiting insect but the physical annoyance and mental agony it afflicts on the resident can be greater than dozens of mosquitoes. Homes located within a quarter mile of the breeding sources had thousands of midges resting on the outside walls, under the eaves and in doorways. They entered the home through the slightest crack in the door or open window especially at night since they are attracted to artificial light.

Throughout this period when hordes of midges were invading residential areas, our District was able to maintain and actually improve public relations only because our mosquito control operators had the training and the patience to explain the problem to each requester and to advise on what the District was doing to help alleviate the nuisance.

In April, we first experimented with Dursban (4# emulsifiable concentrate) by treating a flood channel at the rate of 0.05 lb. per acre using the injection method. A 4-hour re-inspection was so phenomenal that pictures were taken to show the masses of red *Chironomus* sp. larvae that came up from the bottom mud to float down stream. From this point, we set plans in motion for power injection with Dursban at half-mile intervals at periods of 7 to 10 days. After two months of repetitive treatments, midge populations were reduced 60 to 70 percent. We feel these results were so outstanding we are now laying plans to expand this program throughout the District in 1970.

In the tests that were made during the summer of 1969, Dursban was used to make a standard spray solution containing 0.05 lb. per gallon. This spray solution was applied at 40 pounds pressure using a Spraying Systems No. 22 Gun Jet Spray Gun with a solid stream nozzle No. 0002 discharging at the rate of 0.2 GPM. The channels were measured for total length and width to figure total acreage of breeding source area. A work sheet is made up on each channel giving the acreage between street crossing bridges and the number of minutes and seconds to inject the spray solution at each bridge. The injection time required per acre is shown in Table 1. One gallon of emulsion spray is required to treat one acre at 0.05 lb. The treatment time would be 5 minutes with the No. 0002 nozzle. Using a stopwatch, we inject the amount of emulsion needed corresponding to the amount of acreage in that half-mile section.

To sample the channels, three test stations over a one-half mile section were

TABLE I.—Injection time per acre.

For Equivalent Dosage Rate.....of 0.05 lb. Toxicant/Acre
 Or Equivalent Dosage Rate.....of 0.018 ppm for 12" depth
 Using Standard Spray Emulsion...of 0.05 lb. Toxicant/Gallon
 At Discharge Rate.....of 0.2 Gallon/Minute
 and 0.5 Gallon/Minute

| Area Below Inj. Point Acres | Pounds Required Toxicant | Gallons Required Spray | Spray Time Required | | | |
|-----------------------------------|--------------------------------|------------------------------|--------------------------|----|--------------------------|----|
| | | | 0.2 gal/min Min - Sec | | 0.5 gal/min Min - Sec | |
| 0.1 | 0.005 | 0.1 | 0 | 30 | 0 | 12 |
| 0.2 | 0.010 | 0.2 | 1 | 0 | 0 | 24 |
| 0.3 | 0.015 | 0.3 | 1 | 30 | 0 | 36 |
| 0.4 | 0.020 | 0.4 | 2 | 0 | 0 | 48 |
| 0.5 | 0.025 | 0.5 | 2 | 30 | 1 | 0 |
| 0.6 | 0.030 | 0.6 | 3 | 0 | 1 | 12 |
| 0.7 | 0.035 | 0.7 | 3 | 30 | 1 | 24 |
| 0.8 | 0.040 | 0.8 | 4 | 0 | 1 | 36 |
| 0.9 | 0.045 | 0.9 | 4 | 30 | 1 | 48 |
| 1.0 | 0.05 | 1.0 | 5 | 0 | 2 | 0 |
| 2.0 | 0.10 | 2.0 | 10 | 0 | 4 | 0 |
| 3.0 | 0.15 | 3.0 | 15 | 0 | 6 | 0 |
| 4.0 | 0.20 | 4.0 | 20 | 0 | 8 | 0 |
| 5.0 | 0.25 | 5.0 | 25 | 0 | 10 | 0 |
| 6.0 | 0.30 | 6.0 | 30 | 0 | 12 | 0 |
| 7.0 | 0.35 | 7.0 | 35 | 0 | 14 | 0 |
| 8.0 | 0.40 | 8.0 | 40 | 0 | 16 | 0 |
| 9.0 | 0.45 | 9.0 | 45 | 0 | 18 | 0 |
| 10.0 | 0.50 | 10.0 | 50 | 0 | 20 | 0 |

staked with markers in order to insure reasonable accuracy of location. One of the test stations was just below the injection point to get a sampling of maximum kill, a second station at the middle of the half-mile section, and a third at the end to determine the total kill rate. Two mud samples were taken from each station and the average midge larval count recorded. Before the channel was injected, a pretreatment count was recorded for each of the stations. Three days after the injection a post-treatment midge larval count was recorded. The percent of kill at each station and an overall percentage kill for the entire channel was figured.

Midge larval samples taken early in the summer of 1969 showed an average 75 percent-100 percent kill on *Chironomus* sp. after the first injection. No reoccurrence of *Chironomus* sp. larvae was observed

for the rest of the season. The *Tanytus* sp. midge larvae were not effectively controlled during the early part of the summer by this treatment. As continuous injections were made every 10 days, however, control increased from 10 percent to an average kill of 65 percent by the end of June. This average percent kill remained about the same through the remainder of the summer and proved to be adequate to relieve the homeowners of the gross nuisance from adult midges. Later, we extended our injection cycle to 14 days with good success. We are considering lengthening our cycle to 3 weeks during the 1970 season.

It has been our experience during the 1969 season that the use of Dursban at a dosage rate of 0.05 lb. per acre applied every 14 days to flood channels by our injection method at one-half mile intervals has adequately controlled both *Chi-*

ronomus sp. and *Tanytus* sp. midge larvae. Since this injection method requires only one spray operator, the man hours required to control our 250 miles

of flood channels will be reduced by approximately 50 percent as compared to our previous area spray method requiring two spray operators.

SOME EFFECTS OF FUMAGILLIN ON *ANOPHELES STEPHENSI*

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Fumagillin (2,4,6,8-decatetraendioic acid 4-(1,2-epoxy-1,5-dimethyl-4-hexenyl)-5-methoxy-1-oxaspiro (2.5) oct-6yl ester), an antibiotic produced by *Aspergillus fumigatus* (Aspergillales, Aspergillaceae), is effective in the treatment of infections of *Endamoeba histolytica* (Amoebida, Endamoebidae) in man and *Nosema apis* (Microsporida, Nosematidae) in the honey bee (Killough *et al.*, 1952; Katznelson and Jamieson, 1952). This indicated that the compound might be useful for the control of nosema disease in laboratory colonies of anopheline mosquitoes. During the evaluation we observed a number of side effects on our test insect, *Anopheles stephensi*.

MATERIALS AND METHODS

EXPERIMENT 1. First stage larvae of *Anopheles stephensi* were reared in 400-ml beakers containing 200 ml water and a commercial formulation¹ of bicyclohexylammonium fumagillin at 0, 5, 10, 20 and 40 ppm of the antibiotic. There were 5 replicates per treatment with 25 larvae per beaker. The larvae were reared on ground "D. & G." brand dog biscuits at

24.3 ± 1.4° C. Water lost through evaporation was replenished from time to time. Pupae were counted and removed from treatment on the day of pupation. Adults were counted, dissected in physiological saline solution and examined microscopically to determine the incidence of nosema disease on the day of emergence.

EXPERIMENT 2. This experiment was similar to experiment 1 except that the larvae were transferred to beakers of untreated water after 24 hours' exposure to fumagillin. There were 4 replicates of each treatment.

RESULTS

In experiment 1 the mean larval period was lengthened in a dose-dependent manner: 8.0, 11.6, 13.3, 15.0 and 17.2 days at 0, 5, 10, 20 and 40 ppm fumagillin, respectively. Interpolation gives an estimate of 29.1 ppm as the dosage required to double the larval period. In experiment 2 the larval period averaged 1.1 times longer in treated larvae, but dose-dependence was uncertain. Mean pupal periods were not determined, but some periods of 4 and 5 days were observed in experiment 1 at higher concentrations. The normal maximum pupal period is 3 days.

Continuous larval exposure produced dose-dependent larval and pupal mortality

¹"Fumadil B," Abbott Laboratories. This formulation contains bicyclohexylammonium fumagillin together with protective and buffering agents.