EFFECTS OF THE INSECT GROWTH REGULATOR DIMILIN® OR TH-6040 ON MOSQUITOES AND SOME NONTARGET ORGANISMS [1]

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ABSTRACT. Granular and wettable powder formulations of the insect growth regulator Dimilin® or TH-6040 {1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)urea} having novel growth modifying properties were evaluated in replicated ponds against *Culex tarsalis* Coquillett, chironomid midges and some commonly associated nontarget organisms. The material was applied at the rates of 0.25 (0.08 ppm) and 0.05 (0.016 ppm) lb/acre active ingredients. Decline in the population of 3rd and 4th instar mosquito larvae was apparent from 2–8 days after treatment but was not observed 11 days posttreatment; no appreciable decline was noticed in 1st instars as these resulted from continuous oviposition. Adult emergence from treated larvae isolated in floating units was almost completely inhibited up to at least 11 days posttreatment.

Marked decline in the population of nektonic chironomid larvae was not detected in the treatments during the 15-day duration of the experiment. Emergence of chironomids, however, was depressed up to 8–15 days after treatment.

Among the nontarget organisms studied, mayfly, *Baetis* sp., naiads were depressed slightly but recovered to normal levels soon after treatment. Cladocera (*Daphnia* sp.) were moderately depressed by the WP but not by the granular formulation. The copepods *Cyclops* sp. and *Diaptomus* sp. were affected for a short time. All affected nontarget organisms recovered as did the target organisms 11–15 days after treatment. The ostracods (*Cypricercus* sp. and *Cyprinotus* sp.) were not affected by the treatments, nor were diving beetle larvae and adults and odonate naiads during the duration of these experiments.

INTRODUCTION

Recently a variety of compounds manifesting growth and developmental inhibitory effects in mosquitoes and chironomid midges have been subjected to experimental research (Jakob 1973, Jakob and Schoof 1972, Mulla et al. 1974a, b, Schaefer and Wilder 1973). Some of these compounds are effective only when larvae of certain stages are exposed. Late 4th instars are the most sensitive stage to the juvenile hormone type of compounds (Schaefer and Wilder 1973).

A new urea type compound (Van Daalen et al. 1972, Wellinga et al. 1973) showing insect growth regulating properties by inhibiting chitin formation (Post and Vincent 1973) was recently studied by Mulla et al. (1974 a, b) and Jakob (1973) against mosquitoes, houseflies, and midges. This compound, known as Dimilin or TH-6040 (Thompson-Hayward Company, Kansas City, KS 66110), unlike the juvenile hormone type of compounds, produced most of the mortality in the larval mosquitoes. It also caused mortality in the larval stages of the midges, although this mortality was spread over a long period. This compound was found to have exceptional activity against both mosquitoes and midges (Mulla et al. 1974 a, b).

The current studies were initiated to assess under field conditions the effects of 2 formulations of this compound on mosquitoes, midges, and some dominant aquatic nontarget organisms.

MATERIALS AND METHODS

Two formulations of Dimilin® or TH-6040 {1-(4-chlorophenyl)-3(2,6-difluorobenzyl)-urea} were applied at the rate of 0.025 and 0.05 lb AI/acre (0.08 and 0.016 ppm, respectively) to experimental ponds (12′ x 24′) where the water level was maintained at 12–15″ deep by float valves. The ponds were filled with water from a reservoir. The wettable powder formulation (25%) was suspended in 100 ml

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water and sprayed onto the water surface with a polyethylene squeeze bottle. The granular formulation (0.5% on Florex 30/60) was applied evenly by hand. Two ponds were used per each dosage and check. The experiment was conducted during August and September 1974, when mean maximum and minimum water temperatures were 95 and 62° F, respectively.

Mosquito larvae and some nontarget organisms were sampled by a dipper. One dip was taken next to each of 5 artificial straw bundle sampling sites in each pond (Fanara and Mulla 1974). The 5 samples (4 from corners and 1 from the center) were composited and concentrated in a measuring cup provided with 150-mesh stainless steel cloth affixed to a cutout section 0.5 in. above the bottom. The sampled organisms remained in the bottom of the cup in a small amount of water and the excess water was drained through the fine screen. The sample after concentration was transferred to plastic vials, preserved by addition of 95% ethyl alcohol, and examined under a stereoscopic microscope in the laboratory. Mosquito and midge larvae, mayfly naiads, cladocerans, ostracods, and copepods were recovered in good numbers by this procedure.

A tow net mounted on a metal sled was also used for sampling planktonic organisms (Hurlbert et al. 1970). The sled was pulled by rope through the water twice lengthwise 5 ft from the edge of the pond. The lower edge of the net traveled about 3 in. above the bottom of the pond and the upper edge was 3–6 in. below the water surface. The diameter of the net opening was 6 in. at the mouth, and the cloth had 192 strands/in. Organisms collected by the net were washed down into the collection vial by pulling the net vertically up through the water. Excess water was drained through the 150-mesh screen of the collection vial, the sample transferred to a plastic vial and Formalin added to make the formaldehyde concentration 3% in the vial. The sample was thus preserved and examined microscopically. Copepods and cladocerans were recovered in large numbers by this method.

Benthic chironomid midge larvae were sampled by taking two 6″ x 6″ bottom mud samples by a scraper in each pond. Each mud sample was washed through a 50-mesh screen sieve and the residue on the screen containing midrange and mature larvae was transferred to cups. The larvae were floated from the residue by adding saturated solution of Mg SO_4. The larvae were counted and grouped into chironomine and tanypodine midges under a 5X magnifying lamp. Emergence of chironomid midges was assessed by placing 2 emergence cylinders (diameter ca. 15″) fitted with a collection chamber at the top (Mulla et al. 1974b) in each pond. The emergence cylinders were moved to a new spot every 48 hours.

Inhibition of emergence of mosquitoes was assessed by placing 20 4th instar *Culex tarsalis* Coquillett in each of 2 floating isolation units per pond (Mulla et al. 1974a). These isolated individuals were followed until emergence. Mortality in each stage was determined and the final inhibition of emergence (EI) was calculated by the formula

$$\% \text{ inhibition of emergence (EI)} = 100 - \frac{T}{C}(100), \text{ where}$$

T = emergence or survival in treatments

C = emergence or survival in checks

Organisms sampled and studied were: chironomid midges, mostly *Tanytarsus* sp. and *Pentaneura* sp.; mayflies, mostly *Baetis* sp.; copepods, mostly *Cyclops* sp. and *Diaptomus* sp.; cladocerans, mostly *Daphnia* sp.; and ostracods, mostly *Cypricercus* sp. and *Cyprinotus* sp.

RESULTS AND DISCUSSION

MOSQUITOES. The density of 2nd, 3rd, and 4th instars was markedly reduced by the treatments with TH-6040, the reduction, however, taking place slightly later in the ponds treated with the WP formulation (Table 1). Most of the larvae present in the treated ponds were 1st instars during the posttreatment samplings of 2, 4, and 8 days. Young larvae hatching from eggs had not had a chance to be affected by the treatments. In the samples taken 11 and 15 days post-treatment, the proportion of 2nd-4th instars increased, indicating decline in the efficacy of the treatments. This is further evidenced by the appearance of pupae in the treated ponds 11 days post-treatment.

Since TH-6040 acts as an antimolting agent or molting toxin (Mulla et al. 1974a, Post and Vincent 1973), it affects the number of larvae in later instars. Reduction in the 1st instar population was negligible as continuous oviposition contributed to a relatively constant level of 1st instars.

Although pupae appeared in the treated ponds 11 days post-treatment, it is not certain whether these transformed into normal adults. This could not be proven because no 4th instars were available in sufficient numbers on the 8th day after treatment for assessment of emergence by the isolation unit technique (see below).

Evaluation of emergence from natural populations of larvae confined in isolation units showed almost complete inhibition of emergence from the sampled populations up to 8 days post-treatment in all treatments (Table 2). On the 8th day post-treatment there were insufficient numbers of 4th instars to be isolated for the assessment of emergence, and this absence of mature larvae and pupae is good indication of control. This is also indicated by the data in Table 1. From the appearance of pupae 11 days post-treatment, it is hypothesized that most of the pupae prevailing will probably produce normal adults in another 2 or 3 days. It thus appears that TH-6040 inhibits emergence of *C. tarsalis* for at least 11 days, if not more, at the rates used here. There is little or no difference in efficacy of the two rates.

CHIRONOMID MIDGES. The number of

Table 1. Effect of 2 formulations of the IGR TH-6040 on the composition by stages of larvae of *C. tarsalis* in experimental ponds sampled by dippings from artificial sampling sites.

Formulation (%)	Rate lb/A	Avg. no. of larvae and pupae/5 dips sample pre- and post-treat (days)																	
		Pretreat			2[a]			4[a]			8[a]			11			15		
		1–2	3–4	P	1–2	3–4	P	1–2	3–4	P	1–2	3–4	P	1–2	3–4	P	1–2	3–4	P
0.5 G	0.025	48	26	2	70	12	1	78	4	0	27	31[b]	0	46	15	3	45	27	2
	0.050	45	20	6	75	7	3	43	1	0	23	11[b]	0	56	13	1	51	23	4
25 WP	0.025	43	59	6	44	27	0	56	15	0	18	5[b]	0	28	23	1	68	29	1
	0.050	59	41	3	77	32	2	42	13	0	31	6[b]	0	30	26	1	70	13	2
Check	. .	79	45	4	126	25	5	70	11	1	39	21	1	29	22	3	24	20	1

[a] 1–2 instar category were mostly comprised of 1st instar population.
[b] Third-stage larvae mostly.

Table 2. Mortality and inhibition of emergence of *C. tarsalis* from isolated larvae in ponds treated with the IGR TH-6040.

Formulation	Post-treat (days)	Avg. (%) cumulative mortality at indicated rates (lb/A)[a] 0.025 L	P	A	(%) EI[c]	0.050 L	P	A	(%) EI[c]
0.5 G	2	77	23	0	100	100	0	0	100
	4	70	30	0	100	[b]	..	..	..
	8	[b]	..	..	..	[b]	..	..	..
25 WP	2	100	0	0	100	95	0	0	95
	4	90	10	0	100	90	10	0	100
	8	[b]	..	..	..	[b]	..	..	..
Check	2	2	0	0	2	..	..	..	..
	4	2	0	0	2	..	..	..	..
	8	5	5	0	10	..	..	..	..

[a] Two isolation units containing 20 4th-stage larvae/unit were placed in each pond at indicated days after treatment.
[b] Not enough 4th-stage larvae present in the ponds for isolation units.
[c] EI = Inhibition of emergence.

midge larvae sampled by dips from the straw bundles was quite stable over a 15-day post-treatment sampling period. Some reduction in the density of chironomine larvae occurred on the 11th day post-treatment. No reduction, however, was discernible on the tanypodine larval population. These findings are not in agreement with results obtained from the treatment of a warm water lake with TH-6040, where monitoring of the midge larvae continued over a longer period than here (Mulla, unpublished data). In these studies, there was a gradual reduction in the larval populations. It appears that reduction in the chironomid larvae takes longer than 2 weeks, the maximum duration of this experiment.

Adult chironomid midge emergence was quite low from all ponds (treated as well as checks). Emergence was markedly suppressed up to 11–15 days by the WP treatments, but inhibition of emergence was for only 4–8 days in the granular treatments. Evidence from lakes treated with 0.1 lb/acre of TH-6040 WP shows this material to yield complete control of most chironomine and tanypodine midges from 4–8 weeks or longer (Mulla, unpublished data).

NONTARGET INSECTS. Mayfly naiads (*Baetis* sp.) were reduced in numbers slightly and only for a short time in the treated ponds on the 2nd and 4th day of sampling (Table 3). Their numbers, however, appeared to be within the natural fluctuation limits equal to the check ponds or the pretreatment levels during all other sampling periods. In general it appears that neither rate nor formulation of TH-6040 caused drastic reduction in the mayfly population during the experimental period. Mayfly naiads, as we have found them over the years, are one of the most susceptible organisms to many insecticides. A related species of mayflies in laboratory tests was found to be quite susceptible to TH-6040 (Miura and Takahashi 1974).

Diving beetles (Dytiscidae and Hydrophilidae) were not seemingly affected by the treatments during the 2-week experimental samplings. Their populations were stable and showed trends similar to those in the check ponds. Similarly, large numbers of odonate naiads prevailed in both treated and untreated ponds. To detect subtle impact of an insect growth regulator on these two groups of insects, studies of longer duration are deemed desirable.

CRUSTACEA. Both formulations of TH-6040 at both rates caused a short-term reduction in copepod populations when

Table 3. Effect of the IGR TH-6040 on mayflies[a] in experimental ponds sampled by dippings from artificial sampling sites.

Formulation (%)	Rate lb/A	Avg. no. of naiads/5 dips pre- and post-treat (days)					
		Pre	2	4	8	11	15
0.5 G	0.025	18	11	7	20	8	18
	0.050	14	16	6	17	17	23
25 WP	0.025	11	4	5	4	9	23
	0.050	41	8	3	19	11	14
Check	...	19	14	14	13	16	13

[a] Baetidae, *Baetis*, sp.

these were sampled by the dip method (Table 4). The reduction was much more marked with the WP treatments than with the granular treatments. Populations in the check ponds remained high, mostly equal to the pretreatment level. In all the treated ponds, copepod population started to increase on the 11th or 15th day of post-treatment samplings.

It appears that the effect of TH-6040 on copepods is quite similar to that on mosquito larvae. There is a reduction in the population starting 2–4 days post-treatment and the population increased again 11 days post-treatment. Recovery under these field conditions was more rapid than that reported by Miura and Takahashi (1974) in artificial containers.

There is little or no effect of TH-6040 on the ostracod population. It appears that ostracod density underwent normal fluctuations and showed a relatively constant trend during the duration of the experiment. Similar results were obtained by Miura and Takahashi (1974).

Cladoceran and copepod populations were also assessed by the tow net method. Cladocera were severely affected by all treatments (Table 5). Their density almost reached zero 4 days post-treatment and remained so until the 11th day after treatment, showing considerable recovery on the 15th day of sampling after treatment. It seems that TH-6040 affects Cladocera to a greater extent than the target organisms, mosquitoes and midges.

Copepods, assessed by the tow net method, were also affected by all treatments of TH-6040 except the lower dosage of the granular formulation. Recovery, however, was rapid; numbers reached or surpassed pretreatment levels on the 11th day post-treatment. Recovery of both Cladocera and Copepoda was faster in our studies than in outdoor artificial containers treated with this compound (Miura

Table 4. Effect of the IGR TH-6040 on some crustacea in experimental ponds sampled by dippings from artificial sampling sites.

Formulation (%)	Rate lb/A	Avg. no. of crustacea/5 dips pre- and post-treat (days)											
		Copepoda[a]						Ostracoda[b]					
		Pre	2	4	8	11	15	Pre	2	4	8	11	15
G 0.5	0.025	17	10	6	1	4	9	10	10	12	4	9	9
	0.050	26	14	8	10	8	9	12	9	11	10	7	8
WP 25	0.025	17	9	2	1	7	18	23	10	18	8	10	8
	0.050	27	10	3	1	10	6	10	9	6	7	5	13
Check	...	19	26	12	23	24	18	11	17	15	13	16	18

[a] Includes both cyclopoid and calanoid copepods, mostly *Cyclops* sp. and *Diaptomus* sp.
[b] Mostly *Cyprinotus* sp. and *Cypricercus* sp.

Table 5. Effect of the IGR TH-6040 on some crustacea in experimental ponds as sampled by plankton tow net.

Formulation (%)	Rate lb/A	Avg. no. of crustacea/tow net sample pre- and post-treat (days)									
		Cladocera[a]					Copepoda[b]				
		Pre	4	8	11	15	Pre	4	8	11	15
G 0.5	0.025	0	0	0	3	25	61	8	32	201 (1644)[c]	70 (443)[c]
	0.050	250	2	0	5	76	26	2	0	11 (618)	10 (46)
WP 25	0.025	12	0	1	3	48	6	0	0	16 (1227)	13 (420)
	0.050	11	0	0	3	45	7	0	2	9 (902)	8 (444)
Check	...	452	168	48	511	95	134	65	50	671 (238)	125 (23)

[a] Mostly *Daphnia* sp.
[b] Includes both cyclopoid and calanoid copepods, mostly *Cyclops* sp. and *Diaptomus* sp.
[c] Immature stages, including nauplius 2–6, given in parenthesis.

and Takahashi 1974). Due to the nature of the two test conditions, this difference is to be expected.

From these studies it can be concluded that rates as low as 0.025 and 0.05 lb/acre of the IGR TH-6040 can result in good mosquito control for 8–15 days. These same dosages also suppress populations of Cladocera and Copepoda. The duration of effect on these nontarget aquatic organisms is variable, depending on the group of organisms. All affected organisms, however, were found to recover within 11–15 days after treatment, as did the target organisms.

Literature Cited

Fanara, D. M. and M. S. Mulla. 1974. Population dynamics of larvae of *Culex tarsalis* Coquillett and *Culiseta inornata* (Williston) as related to flooding and temperature of ponds. Mosq. News. 34:98–104.

Hurlbert, S. H., M. S. Mulla, J. O. Keith, W. E. Westlake and M. E. Düsch. 1970. Biological effects and persistence of Dursban in freshwater ponds. J. Econ. Entomol. 63:43–52.

Jakob, W. L. 1973. Developmental inhibition of mosquitoes and the housefly by urea analogue. J. Med. Entomol. 10:452–455.

Jakob, W. L. and H. F. Schoof. 1971. Studies with juvenile hormone-type compounds against mosquito larvae Mosq. News. 31:541–543.

Miura, T. and R. M. Takahashi. 1974. Insect development inhibitors. Effects of candidate mosquito control agents on nontarget aquatic organisms. Environ. Entomol. 3:631–636.

Mulla, M. S., H. A. Darwazeh and R. L. Norland. 1974a. Insect growth regulators: Evaluation procedures and activity against mosquitoes. J. Econ. Entomol. 67:329–332.

Mulla, M. S., R. L. Norland, T. Ikeshoji and W. L. Kramer. 1974b. Insect growth regulators for the control of aquatic midges. J. Econ. Entomol. 67:165–170.

Post, L. C. and W. R. Vicent. 1973. A new insecticide inhibits chitin synthesis. Naturwissenschaften. 60:431–432.

Schaefer, C. H. and W. H. Wilder. 1972. Insect developmental inhibitors: a practical evaluation of mosquito control agents. J. Econ. Entomol. 65:1066–1071.

Van Daalen, J. J., J. Meltzer, R. Mulder and K. Wellinga. 1972. A selective insecticide with a novel mode of action. Naturwissenschaften. 59:312–313.

Wellinga, K., R. Mulder and J. J. Van Daalen. 1973. Synthesis and laboratory evaluation of 1-(2,6-disubstituted benzoyl)-3-phenylureas, a new class of insecticides. I. 1-(2,6-dichlorobenzoyl)-3-phenylureas. J. Agr. Food Chem. 21:348–354.