EVALUATION OF TWO NEW INSECT GROWTH REGULATORS AGAINST MOSQUITOES IN THE LABORATORY[1]

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ABSTRACT. Biological activity of two new IGRs was studied under laboratory conditions against 2nd- and 4th-instar larvae and pupae of *Anopheles quadrimaculatus, Aedes aegypti* and *Culex tarsalis*. The IGR S–21149, an oxime [0-(2-(4'-Phenoxyphenoxy)ethyl propionaldoxime], produced an overall mortality or inhibition of emergence of 95% (when 4th instar treated) at 0.0047, 0.0013 and 0.00041 mg/liter in the three species, respectively. The EI_{95} values for the second IGR S–31183, a pyridine compound, [1-(4'-Phenoxyphenoxy)-2-(2'pyridyloxy) propane] were 0.017, 0.0026 and 0.00032 mg/liter for the three species, respectively. Both materials in general were less active against 2nd than 4th-instar larvae, except that the 2nd- and 4th instars of *An. quadrimaculatus* were equally susceptible. Pupae treated at up to 0.1 mg/liter showed no mortality in the pupal or the ensuing adult stages.

INTRODUCTION

In the past decade, several synthetic chemicals which possess insect growth regulating properties have become available for experimentation in the laboratory and under field conditions against a variety of insect species of medical and economic importance. A large number of insect growth regulators (IGRs) have been studied against insects of public health importance, such as mosquitoes, chironomid midges and synanthropic flies (Axtell et al. 1980, Dame et al. 1976, Meyer et al. 1984, Mulla and Darwazeh 1975, Mulla and Axelrod 1983, Mulla et al. 1974, Schaefer and Wilder 1972). A comprehensive review of studies carried out on IGRs for the control of Diptera of public health importance was recently published by Mian and Mulla (1982).

Insect growth regulators have become an important tool for the control of mosquitoes. The juvenoid methoprene has been employed in mosquito control since 1975, and the chitin synthesis inhibitor, diflubenzuron, was registered for mosquito control in noncrop situations in 1985. Thus, in some 15 years of research, only two IGRs have been cleared for use in mosquito control, a situation indicating the need for further evaluation and development of additional IGRs.

The objective of this research was to evaluate the activity of two new insect growth regulators against three species of mosquitoes: *Aedes aegypti* (Linnaeus), *Anopheles quadrimaculatus* (Say) and *Culex tarsalis* (Coquillett) under laboratory conditions. Assessment of activity against a member of each of these three important genera will provide useful information on the potency and spectrum of activity of new materials to be developed for vector control programs.

MATERIALS AND METHODS

Technical materials of two insect growth regulators S-21149, (lot No. 402271) an oxime [0-(2-(4'-Phenoxyphenoxy)ethyl propionaldoxime] and S-31183 (lot 40227) a pyridine compound [1-(4'-Phenoxyphenoxy)-2-(2'pyridyloxy)-propane] provided by Sumitomo Chemical Co., Ltd., Osaka, Japan, were used in the experiments. The tests were carried out against 2nd- and 4th-instar larvae and pupae of *Cx. tarsalis*, *An. quadrimaculatus* and *Ae. aegypti* which were obtained from laboratory colonies at the University of California, Riverside. The *Cx. tarsalis* colony was started in 1984 from OP-resistant larvae collected from sewer effluent in Norco, Riverside County in southern California. For testing, the technical IGRs were dissolved in acetone and serially diluted. Aliquots of the proper strength dilutions were added to 200 ml distilled water in China foam bowls (Dixie Marathon Products, American Can Co., Greenwich, CT 06301) to which 20 early 2nd- or 4th-instar larvae or 20 pupae (<24 hr old) were added. Each material was tested on three different occasions, at 4–7 different concentrations. In each test, each concentration was run in triplicate, and 3 bowls were left untreated as checks.

Soon after treatment, larvae in check and treated bowls were given larval food. *Anopheles quadrimaculatus* and *Cx. tarsalis* were provided with 15 mg/bowl of ground up dog chow and yeast mix (2:1) and *Ae. aegypti* with 20 mg of lab chow and brewer's yeast (3:1). At 3- to 4-day intervals mortality readings were taken and the larvae were provided food each time until they reached the pupal stage. The bowls containing water and test organisms were placed in a room

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where temperature was maintained at 26°C and a photoperiod cycle of 12L:12D. During the test period, there was some evaporation of water and to compensate for this loss, each bowl was refilled to the original volume level every 3–4 days when needed. On each scheduled reading, adult emergence was assessed by counting and removing completely separated exuviae. Adult mosquitoes which underwent incomplete emergence were counted as dead (Mulla et al. 1974). Results of each concentration for each compound tested were subjected to computer log-probit analysis using computer program; the EI_{50} and EI_{95} values (mg/liter) were estimated by means of the linear regression analysis. Overall activity was assessed as the percent inhibition of emergence (EI) based on the starting population. Additionally, the values of mean mortality by stage for each concentration of each compound were obtained and these are included in the tables. Since natural mortality in the checks was low (<8%), this mortality was not taken into consideration when calculating the % EI.

RESULTS AND DISCUSSION

The IGRs S-21149 and S-31183 showed varying levels of activity against 4th-instar larvae (Table 1). The two compounds also showed differential activity against the three species. For example, *An. quadrimaculatus* was the least susceptible to both compounds, but was more susceptible to S–21149 than S–31183. *Aedes aegypti* was next in susceptibility, showing the same susceptibility profile to the two compounds as *An. quadrimaculatus*. *Culex tarsalis* (OP resistant) was the most susceptible species, but was only slightly more susceptible to S–31183 than S–21149. Pupae of each species exposed to concentrations of up to 0.1 mg/liter did not die in this stage or as adults (data not presented). The slope of the regression lines for *Cx. tarsalis* (Table 1) was steeper than for the other two species. It is quite evident that this species is more susceptible than the other two species.

Second-instar larvae were also susceptible to both IGRs (Table 2). *Anopheles quadrimaculatus* was most susceptible to S–21149 followed by the other two species. However, the susceptibility pattern to S–31183 was quite different; *Cx. tarsalis* was most susceptible followed by *Ae. aegypti* and *An. quadrimaculatus*. Both IGRs tested displayed about equal activity (EI_{95}) against the 2nd- and 4th-instar larvae of *An. quadrimaculatus* (see Table 1). However, with 2nd-instar larvae of *Ae. aegypti* and *Cx. tarsalis* the two compounds showed lower levels of activity than against the 4th instars, the latter species was much more tolerant as 2nd instars (Tables 1 and 2). At the EI_{95} level, S–21149 was 22 times less active against 2nd-instar larvae of *Cx. tarsalis* than 4th instars. Likewise, S–31183 was 14 times less active against 2nd-instar larvae of *Cx. tarsalis* than 4th instars. Second-instar *Ae. aegypti* showed two times less susceptibility than 4th instars to both compounds.

Complete inhibition of emergence was produced by S–21149 at the concentrations of 0.0005, 0.0025 and 0.005 mg/liter, respectively, when 4th-instar larvae of *Cx. tarsalis, Ae. aegypti* and *An. quadrimaculatus* were treated. Most mortality occurred in the pupal stage of all species treated as 2nd or 4th instars. There was also some noticeable mortality in the larval stages of *An. quadrimaculatus* and *Cx. tarsalis,* whereas *Ae. aegypti* showed very low larval mortality. Almost all *Ae. aegypti* died as pupae (Table 3). Also, complete inhibition of emergence was produced at 0.0005, 0.005 and 0.025 mg/liter concentration of S–31183 against 4th-instar larvae of *Cx. tarsalis, Ae. aegypti* and *An. quadrimaculatus,* respectively (Table 4). As in S–21149, most of the mortality was in the pupal stage. This compound also caused some mortality in larval stage in *Cx. tarsalis* and *An. quadrimaculatus,* but in *Ae. aegypti* almost all the mortality was in the pupal stage (Table 4) as was the case with the other compound.

Table 1. Activity of the IGRs S-21149 and S-31183 against 4th-instar mosquito larvae.

Species	EI_{50} (mg/liter)	95% fiducial limits	EI_{95} (mg/liter)[a]	Slope
		S-21149		
Anopheles quadrimaculatus	0.00044	0.0003 –0.0006	0.0047	1.6
Aedes aegypti	0.00014	0.0001 –0.0002	0.0013	1.7
Culex tarsalis	0.00011	0.0001 –0.0012	0.00041	2.6
		S-31183		
Anopheles quadrimaculatus	0.0013	0.0009 –0.002	0.017	1.4
Aedes aegypti	0.00033	0.00024–0.0004	0.0026	1.84
Culex tarsalis	0.000085	0.00007–0.0001	0.00032	2.81

[a] 95% fiducial limits omitted.

Table 2. Activity of the IGRs S-21149 and S-31183 against 2nd-instar mosquito larvae.

Species	EI_{50} (mg/liter)	95% fiducial limits	EI_{95} (mg/liter)[a]	Slope
		S-21149		
Anopheles quadrimaculatus	0.00027	0.00016–0.0004	0.0030	1.5
Aedes aegypti	0.00042	0.00031–0.0005	0.0034	1.8
Culex tarsalis	0.0018	0.0013 –0.0023	0.009	2.23
		S-31183		
Anopheles quadrimaculatus	0.00043	0.00026–0.0007	0.0173	1.02
Aedes aegypti	0.00121	0.0010 –0.0015	0.0055	2.49
Culex tarsalis	0.00068	0.00047–0.0009	0.0044	2.02

[a] 95% fiducial limits omitted.

In tests using 2nd-instar of the three species, both compounds produced higher level of larval mortality than in 4th-instar treatments of *An. quadrimaculatus* and *Cx. tarsalis*. Very low or no larval mortality was noted in *Ae. aegypti*. Pupal mortality trends, however, were higher in all the test species (Tables 5 and 6).

From these studies it is clear that most of the mortality in the three species occurs in the pupal stage; thus the mode of action of both compounds is quite similar to the terpenoid type and butyl-substituted phenol IGRs (Mian and Mulla 1982). Furthermore, computer output produced a linear regression profile in which EI_{90} values were calculated for each compound. These values were compared with the data obtained on methoprene and diflubenzuron against *An. quadrimaculatus* (Dame et al. 1976) and *Ae. aegypti* and *Cx. tarsalis* (Hsieh and Steelman 1974). Compound S–21149 was almost as active as diflubenzuron against *An. quadrimaculatus* and *Ae. aegypti*. This compound showed an EI_{90} of 0.0028 mg/liter against *An. quadrimaculatus* and 0.0008 mg/liter against *Ae. aegypti* in our studies, while diflubenzuron had an EI_{90} of 0.004 mg/liter and 0.0007 mg/liter against the two species, respectively. With *Cx. tarsalis*, S–21149 was about three times as active as diflubenzuron (EI_{90} of 0.0003 mg/liter for S-21149 compared to 0.001 mg/liter for diflubenzuron). The compound S-31183 showed the highest activity against *Cx. tarsalis* and was four times more active than diflubenzuron with an EI_{90} of 0.0002 mg/liter. In our studies, the EI_{90} values of S–31183 for *An. quadrimaculatus* and *Ae. aegypti* were 0.0096 and 0.0016 mg/liter

Table 3. Cumulative mortality by stage and inhibition of emergence from 4th-instar larvae treated with the IGR S-21149.

Concentration (mg/liter)	Mean percent cumulative mortality			
	Larvae	Pupae	Adult	% EI[a]
	Anopheles quadrimaculatus			
0.0005	5	35	10	50
0.001	8	43	15	66
0.0025	8	77	3	88
0.005	13	87	0	100
Check	3	3	0	6
	Aedes aegypti			
0.0001	3	38	0	41
0.0005	2	72	0	74
0.001	0	97	0	97
0.0025	0	100	0	100
Check	2	2	0	4
	Culex tarsalis			
0.0001	7	50	5	62
0.00025	15	58	3	72
0.0005	25	73	2	100
Check	2	3	0	5

[a] EI values below 41% at lower concentrations are omitted.

Table 4. Cumulative mortality by stage and inhibition of emergence from 4th-instar larvae treated with the IGR S-31183.

Concentration (mg/liter)	Mean percent cumulative mortality			
	Larvae	Pupae	Adult	% EI[a]
	Anopheles quadrimaculatus			
0.001	7	42	7	56
0.005	12	52	12	76
0.01	8	73	8	84
0.025	30	70	0	100
Check	0	5	0	5
	Aedes aegypti			
0.0005	2	53	2	57
0.001	2	77	2	81
0.0025	0	97	0	97
0.005	0	100	0	100
Check	2	2	0	4
	Culex tarsalis			
0.0001	13	42	10	65
0.00025	7	72	7	86
0.0005	2	98	0	100
Check	3	3	2	8

[a] EI values below 32% at lower concentrations are omitted.

Table 5. Cumulative mortality by stage and inhibition of emergence from 2nd-instar larvae treated with the IGR S-21149.

Concentration (mg/liter)	Mean percent cumulative mortality			
	Larvae	Pupae	Adult	% EI[a]
	Anopheles quadrimaculatus			
0.00025	17	23	12	52
0.001	13	65	3	81
0.005	28	63	5	96
0.01	23	77	0	100
Check	3	3	0	6
	Aedes aegypti			
0.0005	3	52	0	55
0.001	2	63	8	73
0.0025	0	88	2	90
0.005	0	97	3	100
Check	2	2	0	4
	Culex tarsalis			
0.001	8	30	3	41
0.0025	20	33	7	60
0.005	27	53	2	82
0.007	23	75	2	100
Check	5	3	0	8

[a] EI values below 34% at lower concentrations are omitted.

Table 6. Cumulative mortality by stage and inhibition of emergence from 2nd-instar larvae treated with the IGR S-31183.

Concentration (mg/liter)	Mean percent cumulative mortality			
	Larvae	Pupae	Adult	% EI[a]
	Anopheles quadrimaculatus			
0.0005	43	13	0	46
0.001	43	22	2	67
0.005	38	38	0	76
0.01	47	42	3	92
0.025	52	48	0	100
Check	5	3	0	8
	Aedes aegypti			
0.001	0	38	7	48
0.0025	8	67	2	77
0.005	3	80	13	96
0.007	5	95	0	100
Check	0	2	5	7
	Culex tarsalis			
0.0005	20	23	3	46
0.0025	33	48	3	84
0.005	47	53	0	100
Check	5	3	0	8

[a] EI values below 34% at lower concentrations are omitted.

respectively. This IGR was slightly less active than diflubenzuron against these two species.

Both S–21149 and S–31183 were more active against all three species than methoprene, a currently used IGR in mosquito control programs. At the EI_{90} level, the two experimental IGRs were about 100X as active as methoprene against *Cx. tarsalis*, while they were about 700X as active against *Ae. aegypti* on the basis of data of Hsieh and Steelman (1974). The two compounds were about 4X to 1.2X as active as methoprene against *An. quadrimaculatus* (Dame et al. 1976). In general, both these IGRs showed high level of activity, even against the least susceptible species. This wide spectrum of activity indicates that these two IGRs require further evaluation for use in mosquito control programs.

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