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BIOLOGICAL ACTIVITY OF JUVENILE HORMONE ANALOGUES AGAINST LARVAE OF *CULEX PIPIENS PIPIENS* TESTED IN SMALL-SCALE FIELD TRIALS

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ABSTRACT. Five synthetic materials with juvenile hormone activity has been tested in small-scale field trials under subtropical climatic conditions. Their inhibiting effects on morphogenesis, as well as their persistence, were investigated. Emulsifiable concentrates (50% AI) and granular formulations (5% AI) were used. Ro 8–9801 was the most active compound tested and caused inhibition of adult emergence in both formulations.

INTRODUCTION. Increase of mosquito resistance to conventional insecticides has turned world-wide attention to the use of juvenile hormone mimics in the control of this vector. At present, various juvenile hormone analogues are known. Their effect on mosquitoes under laboratory conditions has been demonstrated by Spielman and Williams (1966), Spielman and Skaff (1967), Nair (1967), Sacher (1971), Jakob and Schoof (1971, 1972), Jakob (1972), Bransby-Williams (1972). The first outdoor experiences with juvenile hormone mimics have been obtained by Wheeler and Thebault (1971) and Schaeffer and Wilder (1972).

Small-scale field trials with juvenile hormone analogues were conducted in middle Italy over a period of 3 months. The primary objectives of these tests were to determine the morphogenetic activity and persistence of the candidate compounds. The results obtained are presented here.

MATERIALS AND METHODS. Test No. 1 (morphogenetic activity). The trials were conducted in the vicinity of Grosseto, Tuscany. The experimental area was established in the neighborhood of a farm and a rice-field. Forty-liter plastic basins were situated in 4 rows and placed under cages (Fig. 1). Each cage consisted of a light metal frame covered with gauze and was fixed with 3 small stakes to the ground. All manipulations inside the cages could be performed through two terminal openings which were fastened by using clothes pegs. To protect the larvae from strong midday sunshine, each cage was partly covered with a piece of jute sacking. Each basin was filled with 30 liters of

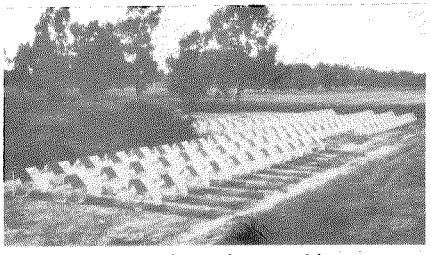

FIG. 1. Experimental area with cages.

sewage water, and the water level was controlled daily and replenished when needed. Each variant was run in 3 plots except the untreated control variant when 8 plots were used.

Infestation: Field-collected egg-rafts of *Culex pipiens pipiens* were reared out of doors in plastic bowls. A mixed population of approximately 400 second, third and fourth instar larvae was used for the first introduction. In addition, 4 artificial infestations were carried out at an interval of 9 days. In each case, approximately 100 second larval instars were introduced into each basin.

Treatment: The following compounds were tested.

Ro 8–3165 2- [{(cis/trans-6,7-epoxy-3,7dimethyl) oxy} methyl] -1,4-benzodioxan (Roche)
Ro 8–9801 6,7-Epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene (Stauffer R-20458)
Ro 10–6425 Isopropyl-11-methoxy 3,7,11-trimethyl-2cis/trans-4-trans-dodecadienoate (Zoecon ZR-515)
Ro 11–2602 2,6-di-tert.butyl-4-(α,α-dimethylbenzyl)-phenol (Monsanto MON-0585)
Ro 20–3600 6,7-Epoxy-3,7-dimethyl,1-1 [3,4(methylendioxy)-phenoxy] -2-nonene (Roche)

The compounds were applied weekly at concentrations of 1 ppm and 0.3 ppm. Moreover, 1 ppm treatment was carried out at a 3-weeks interval. The first treatment was performed 1 day after the first introduction of larvae. Within the experimental period 7 treatments were accomplished with an interval of 1 week and three treatments with an interval of 3 weeks. Water and air temperature and relative air humidity were continuously recorded.

Test No. 2 (persistence)

Special "floating-boxes" were developed for this test (Fig. 2). Each box containing approximately 40 fourth instars larvae of *Culex pipiens pipiens* was placed into a plastic basin filled with 30 litres of water from a local brook and treated with a granulate formulation. The fish-food "Biorell" was used as food for larvae (0.5 g/box). The first assessment was carried out at the moment when more than 90% of the adults had emerged in the untreated plots. Afterwards, the boxes were rinsed with tap water and new fourth instars larvae were introduced. Each box was then put again into the corresponding basin. The second control was carried out after another two weeks.

RESULTS AND DISCUSSION. The evaluation of both tests was based on the total number of emerged adults from treated plots as compared with the number obtained in untreated plots, which was assumed to have 100% emergence. Table 1 summarizes the results of Test No. 1. They show that the emulsifiable concentrate of Ro 8–9801 was the most active compound in inhibiting adult emergence. Ro 10–6425 was slightly less effective. The activity of granular formulations was superior to the emulsifiable concentrates. This effect might be due to the possible direct contact of the larvae with the granules. In addition, we believe that repeated treatments with granules may result in a certain accumulation of active material in the water. It is assumed that at the moment of the next treatment the amount of active ingredient from the previous treatment is higher in case of granules than in case of EC-treatment.

Table 2 shows the results of the persistency test. The first evaluation 2 weeks after the treatment indicated a total inhibition of adult emergence in case of Ro 20–3600 and Ro 8–9801 at a dosage of 1 ppm. Ro 20–3600 was the only compound which showed any effect at 1 ppm after another 2 weeks.

Poor persistency of emulsified compounds with juvenile hormone activity to microbial degradation, sunlight and high temperatures has been reported by Schae-

TABLE 1. Biological activity of juvenile hormone analogs against larvae of *Culex pipiens pipiens* (in percent inhibition of adult emergence).

Compound	Conc. ppm	Interval (weeks)	Number of treatment	Normal adults *	Inhibition of adult emergence **
Check	..	..	..	643	..
Blank	1	1	7	749	..
	0,3	1	7	559	..
		Emulsifiable concentrates			
Ro 8–3165	1	1	7	19	97
	0,3	1	7	453	30
	1	3	3	273	58
Ro 8–9801	1	1	7	0	100
	0,3	1	7	9	99
	1	3	3	204	69
Ro 10–6425	1	1	7	0	100
	0,3	1	7	92	86
	1	3	3	292	55
Ro 11–2602	0,76	1	7	84	87
	0,23	1	7	211	67
	0,76	3	3	276	57
Ro 20–3600	1	1	7	139	79
	0,3	1	7	397	39
	1	3	3	226	65
		Granules			
Ro 8–9801	0,3	1	7	0	100
	1	3	3	0	100
Ro 10–6425	0,3	1	7	11	98
	1	3	3	44	93
Ro 20–3600	0,3	1	7	0	100
	1	3	3	2	99,7

* absolute figures.
** corrected by Abbott's formula.

TABLE 2. Persistence of juvenile hormone analogs formulated as granulates and tested against 4th larval instar of *Culex pipiens pipiens* (in percent inhibition of adult emergence).

			Evaluation after 2 weeks		Evaluation after 4 weeks	
Compound	Conc. ppm	Number of treatments	Normal adults *	Inhibition of adult emergence **	Normal adults *	Inhibition of adult emergence **
Check	..	..	70	..	45	..
Blank	1	1	110	..	36	..
Ro 8–9801	1	1	0	100	45	0
	0,3	1	18	80	52	0
Ro 10–6425	1	1	2	98	42	0
	0,3	1	1	99	71	0
Ro 20–3600	1	1	0	100	30	27
	0,3	1	6	93	56	0

* absolute figures.
** corrected by Abbott's formula.

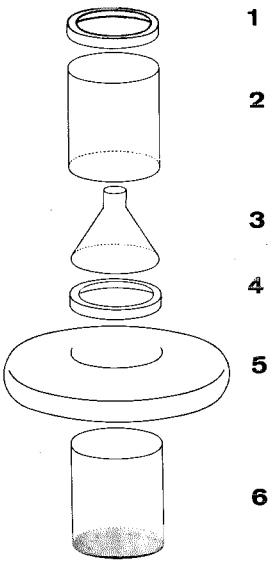

FIG. 2. Floating box for out of doors tests with mosquitoes. 1. cover-lid with a gauze, 2. box without a bottom, 3. plastic tunnel, 4. cover-lid without a gauze, 5. plastic ring, 6. box with a gauze bottom into which the target larvae are introduced scale 1:9.

fer and Wilder (1972). Dunn and Strong (1973), however, published results concerning slow-release polymer formulation of ZR-515, which was shown to be effective for almost 2 months. In our results with sinking granular formulations applied into shallow water containers, full activity was registered over a period of 3 weeks (Table 1).

Compound Ro 11–2602, which is not actually a juvenile hormone analogue, was not as effective as Ro 8–9801 and Ro 10–6425. The different mode of action of Ro 11–2602 could be observed. Many unmelanized, dead pupae were present in the plots, which is in agreement with the results of Jakob and Schoof (1972). This effect may be caused through interference of tyrosine metabolism pathway involved in cuticle darkening (Schaefer and Wilder, 1972).

Under certain circumstances juvenile hormone mimics are promising substances for practical control of mosquitoes. However, it is desirable that the persistence of the compounds will be increased. Further studies with different types of formulations will be carried out.

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