Persistence and Leaching of Juvenile Hormone Analogs in Stable Fly Laboratory Rearing Medium

James E. Wright* and Malcolm C. Bowman[1]

The results of concentration, aging, and leaching of the juvenile hormone analog (JHA), *trans*-8(4′-ethylphenoxy)-2,6-dimethyl-2,3-epoxy-6-octene, showed that the material was stratified in the upper 20 mm of the laboratory rearing medium for stable flies, *Stomoxys calcitrans* (L.), before and after the equivalent of 8 in. of rainfall. There appeared to be a gradual downward movement of the material below the 20-mm level. Also, the material was still present in the upper 20 mm after 22 days. When this JHA and two others, 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)phenoxy]-2-*cis,trans*-octene and isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate, were placed on columns of laboratory medium either as a technical or emulsifiable concentrate (ec) solution, the ec solutions of the first two JHA's were leached more than the technical solution after the equivalent of 10 in. of rainfall.

The environmental stability and persistence of juvenile hormone analogs (JHA) are important factors to be considered in the potential use of these compounds for control of economically important insects. Certain JHA are effective against the stable fly, *Stomoxys calcitrans* (L.), when they are applied directly to laboratory breeding medium or to normal fly breeding areas (Wright and Spates, 1971, 1972; Wright *et al.*, 1973). The JHA disrupt eclosion by preventing the adult from completing metamorphosis within the puparium after external exposure (Wright, 1970, 1972).

Preliminary indications are that certain JHA have a short environmental persistence (Bagley and Bauernfeind, 1972). Also, application must be made at a specific time in the life cycle of an insect for maximum effect as demonstrated with *Platysamia* (= Hyalophora) *cecropia* (L.) and *Galleria mellonella* (L.) (Gilbert and Schneiderman, 1960), with *Pyrrhocoris apterus* (L.) and *Oncopeltus fasciatus* (Dallas) (Riddiford, 1970), with *Tenebrio molitor* L. (Schmialek, 1961), with *Aedes aegypti* (L.) (Spielman and Williams, 1966), with *Rhodnius prolixus* Stol (Wigglesworth, 1969), with the stable fly (Wright, 1970), and with others. However, in the natural rearing conditions of stable flies, several life stages are normally present in the rearing medium and therefore, for maximum effectiveness, the JHA should be present over a long enough period so that it will contact the pupa, which is the more sensitive stadium. Moreover, the location of the JHA in the medium is important because larvae generally pupate near the surface. Thus, the objective of the present study was to determine the distribution and persistence of JHA in laboratory rearing medium exposed and unexposed to leaching. This rearing medium approximates that of the natural rearing medium very closely and previous studies indicate that efficacy of the JHA for prevention of adult emergence in this medium correlates with that obtained under natural field conditions (Wright, 1970; Wright *et al.*, 1973).

MATERIALS AND METHODS

The JHA used were: R-20458 = *trans*-8(4′-ethylphenoxy)-2,6-dimethyl-2,3-epoxy-6-octene, provided by Stauffer Chemical Co., Mountain View, Calif.; RO 7-9767 = 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)phenoxy]-2-*cis,trans*-octene, provided by Hoffmann-LaRoche, Inc., Nutley, N. J.; and ZR-515 = isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate, provided by Zoecon Corp., Palo Alto, Calif.

Effects of Concentration, Aging, and Leaching with Water on the Distribution of R-20458 in Laboratory-Treated Rearing Medium. The rearing medium for stable flies (*ca.* 10-cm deep) in pans (30.5 × 35.6 × 15.2 cm deep) (Wright, 1972) was sprayed uniformly with 100 ml of water containing 1.0 or 5.0% of an emulsifiable concentrate (ec) of R-20458. Duplicate core samples (5.3 cm diameter in metal pipes) were taken at 1, 8, 15, and 22 days posttreatment from the treated medium, frozen immediately after collection, and later shipped under Dry Ice to Tifton, Ga., for chemical analysis.

One set of samples was allowed to thaw; then the medium was carefully removed from the sampling tube, visually divided into three layers of about the same thickness, weighed, and extracted for analysis in a Soxhlet extractor containing a plug of glass wool under an atmosphere of nitrogen for 6 hr with 150 ml of benzene–methanol (9:1 v/v) at a rate of eight solvent exchanges/hr. After it cooled, the extract was percolated through sodium sulfate, and the container and sodium sulfate were washed with 10 ml of benzene. The extract was evaporated to dryness on a 60° water bath under vacuum, and the residue was taken up in benzene for the cleanup step. The effects of aging on the distribution of R-20458 in the top, center, and bottom layers of these two media (treated with the two concentrations) were determined from this first set of samples.

The second set of treated core samples (still in the metal pipes).was allowed to thaw over a funnel containing a plug of glass wool. Then the samples were eluted with 56, 56, 112, and 224 ml of distilled water (equivalent to 0–1, 1–2, 2–4, and 4–8 in. of rainfall), and the separate fractions of aqueous effluent were extracted and analyzed as described. The medium remaining in the tubes after leaching was removed, divided, weighed, and analyzed as described.

Effects of Leaching with Water on the Distribution of Technical and ec Preparations of R-20458, RO 7-9767, and ZR-515 in Stable Fly Medium. Metal pipes, 5.3-cm diameter × 45.7-cm long, were packed with untreated medium (7 days old) at College Station, Tex., frozen, and shipped under Dry Ice to Tifton, Ga. Six of the tubes (untreated controls) were allowed to thaw for 24 hr over funnels plugged with glass wool. Three tubes were treated by distributing 40-mg active ingredient (ai) portions of technical R-20458, RO 7-9767, or ZR-515 in 5 ml of hexane uniformly over the surface of the medium. The surfaces of the medium in the other three tubes were treated in the same manner with 40-mg (ai) ec preparations of the same

Veterinary Toxicology & Entomology Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, College Station, Texas 77840.

[1] Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas; formerly with Agricultural Research Service, U. S. Department of Agriculture, Tifton, Georgia.

Table I. Distribution of R-20458 in Cores (53-mm Diameter × about 60-mm Deep) of Stable Fly Medium from Rearing Pans after Treatment with an ec. Aging for Various Intervals and Leaching with the Equivalent of 8 in. of Rainfall

Sampling interval, days after application	Location of sample[a]	Samples not leached: Wet sample weight, g	Samples not leached: Ppm (wet basis) of R-20458	Samples leached: Wet sample weight, g	Samples leached: Ppm (wet basis) of R-20458
		1.0% application			
	T	25.82	411.0	16.53	463.0
	C	32.28	48.9	27.42	163.0
1	B	37.79	6.0	24.26	76.7
	Total	95.89	129.0	68.21	205.0
	T	17.81	876.0	21.75	296.0
	C	22.05	80.7	23.28	83.3
8	B	37.05	9.6	22.86	27.2
	Total	76.91	231.0	67.89	133.0
	T	14.15	489.0	15.70	179.0
	C	19.70	223.0	19.00	138.0
15	B	15.50	154.0	17.52	106.0
	Total	49.35	278.0	52.22	140.0
	T	12.12	289.0	14.04	294.0
	C	13.82	229.0	15.95	150.0
22	B	16.07	90.9	18.20	15.9
	Total	42.01	193.0	48.19	142.0
		5.0% application			
	T	19.94	3890.0	20.00	1600.0
	C	29.64	241.0	23.68	253.0
1	B	35.52	13.9	24.00	250.0
	Total	85.10	1000.0	67.68	649.0
	T	19.67	3310.0	22.95	1700.0
	C	24.27	486.0	34.65	233.0
8	B	40.19	28.4	28.72	151.0
	Total	84.13	939.0	86.32	595.0
	T	17.70	2320.0	22.01	2500.0
	C	28.82	684.0	26.80	429.0
15	B	19.02	299.0	20.37	457.0
	Total	65.54	1010.0	69.18	1100.0
	T	13.84	2980.0	17.04	2370.0
	C	15.82	1450.0	18.96	976.0
22	B	15.29	442.0	21.73	292.0
	Total	44.95	1580.0	57.73	1130.0

[a] The core of medium was carefully removed from the sampling tube, visually divided into three layers of about the same thickness, weighed, and extracted for analysis. T, C, and B denote the top, center, and bottom layer.

chemicals in 2 ml of distilled water. After each tube stood for 24 hr, it was eluted with 56, 56, 112, 224, and 112 ml of distilled water (equivalent to 0–1, 1–2, 2–4, 4–8, and 8–10 in. of rainfall). All eluates were analyzed separately to detect residues of the chemicals, and the remaining medium in each tube was removed, divided visually into 12 sections of approximately the same size, and analyzed.

Chemical Analysis. Residues of the three chemicals in the medium were determined by gas chromatography as described by Bowman *et al.* (1973) and Wright and Bowman (1973). The aqueous eluates were supersaturated with NaCl and extracted twice with equal volumes of benzene; however, heavy emulsions usually formed and centrifugation was required to separate the layers. The combined benzene extracts were dried by percolating them through a plug of sodium sulfate and then concentrated to about 5 ml by using a 50° water bath and water pump vacuum. The concentrated extracts were carried through the cleanup and analysis as cited.

Table II. Concentrations of R-20458 Found in the Eluates after Leaching of Cores of Rearing Media Treated with ec Preparations and Aged for Various Periods[a]

Water applied, in.	Mg of R-20458 in eluate at indicated day: 1% treatment 1	1% treatment 8	1% treatment 15	1% treatment 21	5% treatment 1	5% treatment 8	5% treatment 15	5% treatment 21
0–1	0.07	0.08	0.02	0.01	2.77	1.50	1.26	0.45
1–2	0.06	0.08	0.04	0.04	2.37	1.53	0.89	0.31
2–4	0.21	0.16	0.07	0.11	3.70	1.94	2.05	2.65
4–8	0.27	0.14	0.08	0.14	1.86	1.31	1.24	1.54

[a] See Table I for analyses of cores after these tests were performed.

RESULTS AND DISCUSSION

Table I reports the distribution and concentration of R-20458 in stable fly medium at specified times after treatment with the ec preparation. The data for residue (total ppm, wet basis) agree with those previously reported for this material by Bowman *et al.* (1973), except for medium treated with a 1% concentration and sampled at 1 day posttreatment. In the present study, the total ppm of residue at that time was 129 instead of 728, as reported earlier (Bowman *et al.*, 1973). This difference was probably caused by an unequal distribution of the chemical due to the spray application used. After both treatments, the greatest amounts of R-20458 were found in the uppermost level of the medium, but the concentrations tended to move downward slowly as the medium aged. Shortly after treatment, the surface of the medium was moist; at 22 days posttreatment, the surface was dry.

Table I also reports the distribution of R-20458 in the medium after leaching with the equivalent of 8 in. of rainfall. More compound was leached out of the cores taken at 1 and 8 days posttreatment (Table II). However, the amount of residue present before and after leaching in samples taken at 22 days should still be adequate to prevent emergence of adult stable flies since an application of only 0.25% concentration of R-20458 on stable fly medium left about 100 ppm of residue at 1 day posttreatment (Wright, 1972; Wright *et al.*, 1973).

The tests described here were made with carefully controlled conditions in the laboratory. The results should reflect relevancy to field application and are certainly indicative of the distribution and persistence of R-20458 because the stable fly rearing medium closely parallels the natural stable fly breeding medium. In field conditions, ultraviolet (uv) light may interfere with the stability of the JHA. For example, Pawson *et al.* (1972) reported that the stability of the JHA 4-[(6,7-epoxy-3,7-dimethyl-2-nonenyl)oxy]-1,2-(methylenedioxy) benzene was greater in the absence of uv light and that exposure to uv light caused more than 90% decomposition of this analog within 96 hr.

The experiment and analysis were performed only once due to the results that readily indicated the JHA remained in the upper 20 mm of the rearing medium (Table I). This confirmed results from the field tests in Nebraska and in Florida where only surface areas were treated and the JHA prevented adult emergence (Wright, *et al.*, 1973). The distribution of the JHA must be in the upper surface of the breeding area to prevent adult emergence because larvae pupate near the surface and the newly ecdysed pupa is the more sensitive stage for the disruption of metamorphosis by the JHA (Wright, 1970).

Table III. Distribution of JHA in a Column of Rearing Medium (5.3 cm Diameter × 45 cm Long) after Treatment with 40 mg (ai) of the Technical Chemical or an ec Preparation Leached with the Equivalent of 10 in. of Water

	R-20458				RO 7-9767				ZR-515			
	Technical		Ec		Technical		Ec		Technical		Ec	
Location of sample in column[a]	Wet sample wt, g	% distribution	Wet sample wt, g	% distribution	Wet sample wt, g	% distribution	Wet sample wt, g	% distribution	Wet sample wt, g	% distribution	Wet sample wt, g	% distribution
1 (top)	51.65	93.13	69.98	85.09	68.50	93.70	69.65	81.11	49.55	90.35	69.78	90.39
2	48.56	5.10	43.59	8.13	64.90	5.32	43.48	8.99	49.00	4.45	64.41	5.21
3	45.61	0.86	40.12	2.22	42.20	0.89	42.62	3.61	42.09	3.16	37.68	1.15
4	30.38	0.84	50.50	1.55	36.41	0.09	40.75	3.84	40.44	1.76	40.79	1.62
5	45.19	0.07	44.02	0.58	37.48	b	36.83	1.76	38.83	0.16	39.95	1.04
6	45.23	b	40.31	0.48	38.10	b	35.38	0.54	41.98	0.05	35.10	0.33
7	47.69	b	36.89	0.32	38.08	b	39.19	0.15	45.05	0.02	38.26	0.20
8	45.05	b	49.38	0.37	37.78	b	47.35	b	44.23	0.03	38.15	0.03
9	51.01	b	36.59	0.20	38.60	b	47.94	b	57.50	b	43.70	0.03
10	45.47	b	40.60	0.03	50.51	b	57.01	b	44.19	b	35.04	b
11	36.57	b	37.93	0.02	47.18	b	48.44	b	40.08	b	43.85	b
12 (bottom)	43.07	b	55.92	b	34.54	b	53.65	b	35.50	b	42.23	b

[a] After leaching, the medium in the column was visually divided into 12 sections of about the same size and then analyzed. [b] None detected.

The results of studies of the effects of leaching with water (10 in.) on the distribution of technical and ec preparations of R-20458, RO 7-9767, and ZR-515 are shown in Table III. None of the aqueous eluates contained any detectable amounts of the chemicals. Also, except with ZR-515, the concentrations of the JHA in the top layers of the media were lower when the ec's were used than when the technical preparations were applied. More ZR-515 than R-20458 or RO 7-9767 was distributed in subsurface layers. The technical materials moved less within the larval medium than the ec preparations; perhaps a low-volume application of high concentrations of a technical material would cause the material to remain near the surface of the medium where the larvae pupate. However, no data are available concerning the effectiveness of or the nature of the residues following such treatment.

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