Insect Growth Regulators: Methoprene and Stauffer R-20458 in Pupae of the Stable Fly[1] from Treated Breeding Medium[2]

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The application of insect growth regulators (IGR) with juvenile hormone activity to breeding media prevents pupal-adult metamorphoses of stable flies, Stomoxys calcitrans (L.), and house flies, Musca domestica L. (WRIGHT 1972, WRIGHT et al. 1973), if the more sensitive pupal stage is exposed to the chemical (WRIGHT 1970). Recently, we developed methods of determining the residues of 2 of the more active IGR's, methoprene and Stauffer R-20458 ((E)-6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene) in the fly breeding media (BOWMAN et al. 1973, WRIGHT and BOWMAN 1973a, 1973b). The present study was made to determine the quantity of the IGR's in stable fly pupae removed from the treated media.

METHODS AND MATERIALS

Methoprene was supplied by the Zoecon Corp., Palo Alto, Calif., as a 1.45% EC; Stauffer R-20458 was furnished by Stauffer Chemical Co., Mountain View, Calif., as a 50% EC.

Stable fly larval medium (a mixture of ground sugar cane pulp, whole wheat flour, fish protein, sodium bicarbonate and water) was placed in a plastic pan 30.5 X 35.6 X 15.2 cm deep to a depth of 10-13 cm, and about 7200 stable fly eggs from the laboratory colony were added. The normal yield from an untreated pan prepared in this way is 4500-5500 flies. The rearing room was maintained at 25.6 ± 2°C and 50 ± 5% RH. On the 3rd day after the medium was infested, pans were sprayed with 1.0, 0.5, 0.25, 0.10, or 0.05 g AI of R-20458 or with 0.5, 0.25, 0.10, 0.05, or 0.01 g AI of methoprene in a total volume of 100 ml of water.

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1. Diptera: Muscidae.
2. This paper reflects the results of research only. Mention of a proprietary product or an insecticide does not constitute an endorsement or a recommendation by the USDA.
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Bulletin of Environmental Contamination & Toxicology, Vol. 15, No. 5 © 1976 by Springer-Verlag New York Inc.

Samples of 100 pupae each were then removed from the treated media at 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, and 20 days posttreatment and frozen until analyzed for residues. Similar samples taken from untreated medium were handled similarly.

All solvents were CP grade and were redistilled in glass. Silica gel (J. T. Baker Co., No. 3405) and alumina (Fisher Scientific Co., No. A-540) were the adsorbents used for cleanup. The alumina-moisture content was adjusted to 2.0%, and the silica gel moisture was adjusted to 5.0% for methoprene and to 7.0% for Stauffer R-20458. Anhydrous sodium sulfate was used for drying and column adsorbent separation.

The cleanup procedure was adapted from that described by BOWMAN et al. (1973).

The procedure was as follows: The stable fly pupae (150 mg) were homogenized in 50 ml of benzene-methanol (90:10) on a Virtis "23" at a medium speed for 3 min, and the extract was filtered through a plug of sodium sulfate (25 mm diam X 30 mm thick). The solids were homogenized again with 2 additional 50 ml aliquots of solvent, and the combined extracts were evaporated to dryness under vacuum at 50°C. The residue was dissolved in 5 ml benzene for column cleanup.

Cleanup columns were prepared by adding a plug of glass wool, 2 g sodium sulfate, 5 g alumina, 2 g sodium sulfate, 5 g silica gel, and 2 g sodium sulfate to each glass column (10 mm ID X 23 cm L). The column was prewashed with 20 ml benzene, which was discarded.

The sample, in 5 ml of benzene, was added to the column, and three 5-ml benzene washes of the container also were added. Benzene (180 ml) was added to the column, and the 50- to 200-ml fraction was collected for analysis. This fraction was evaporated to dryness under vacuum (50°C) and taken up in hexane for analysis.

A Hewlett-Packard Model 7620 gas chromatograph with a flame ionization detector was used in the quantitative analyses. A 12-ft glass column packed with 5% AN-600 (w/w, Analabs, Inc.) on Gas Chrom Q (80/100 mesh, Applied Science Laboratory) was used under the following conditions: carrier gas (He) 100 ml/min, injector port 225°C, detector 275°C, column 160°C for methoprene and 200°C for R-20458. All samples were injected in 5 µl of hexane, and residues were quantitated by comparing the peak height with a known standard.

At the test conditions, retention times were: 6.5 and 8.0 min for the cis and trans isomers of methoprene and 6.0 and 7.5 min for the cis and trans isomers of R-20458. The efficiency of the extraction and the cleanup procedure was determined by adding known quantities of the compounds to untreated pupa and processing these samples with the same procedure. Recoveries for methoprene were 98% for cis and 95% for trans; for R-20458, they were 69% for cis and 81% for trans. All reported residues have been corrected for recovery.

RESULTS AND DISCUSSION

Table 1 reports residues of methoprene and R-20458 in stable fly pupae recovered from the different treatments. Some dead larvae were observed at 1-day post-treatment in medium treated with methoprene at rates of 0.25 and 0.5 g, but there was no difficulty in obtaining adequate samples of pupae exposed to these 2 concentrations at the later intervals.

The data show that the applications of the IGR's to the surface of fly breeding media was an effective method of exposing developing flies to the chemicals. Previous leaching studies with methoprene and R-20458 revealed that 10 in. of rainfall had little effect on these 2 IGR's and that both remained in the upper 20 mm of the laboratory rearing medium for stable flies (WRIGHT and BOWMAN 1973b).

Residues of methoprene were considerably higher in the stable fly pupae resulting from the various treatments than the residues of R-20458 after similar treatments (Table 1). SCHAEFER and DUPRAS (1973) reported that the half life of technical methoprene in treated field water was ca. 2 h and that no detectable residues of the 10% flowable liquid formulation were found after 24 h though biological activity continued for several days. The half life of R-20458 in water is ca. 10 h (Stauffer Technical Bulletin R-20458).

The residues of the IGR's found in the stable fly pupae in the present study differed little from the residues determined in the treated fly breeding media by WRIGHT and BOWMAN (1973a) for methoprene and by BOWMAN et al. (1973) for R-20458. The stable fly pupae apparently did not accumulate the IGR as larvae since the amount of the IGR that penetrated or was ingested approached the level found in the environment.

The concentration of methoprene or R-20458 needed to inhibit development and emergence of stable flies is less than the amount of the larvicide, stirofos

TABLE I

Residues of methoprene and Stauffer R-20458 in stable fly pupae removed from different levels of treated media at 11 time intervals as determined by gas chromatography

Treatment of medium (g AI/ft^2 surface area)	Total ppm (dry) of IGR in sample of 100 stable fly pupae at indicated day posttreatment										
	3	4	5	6	8	10	12	14	16	18	20
					Methoprene						
0.01	1.5	0.6	0.7	1.0	0.8	1.6	1.5	0.8	1.9	1.2	1.3
0.05	4.9	2.0	1.1	1.1	2.2	1.8	2.4	2.9	5.7	2.1	1.7
0.10	14.2	5.4	5.0	2.3	1.3	10.4	7.3	5.1	10.7	4.3	5.0
0.25	47.6	24.1	21.6	12.8	18.9	18.4	16.4	28.1	30.2	23.2	24.8
0.50	109.6	67.2	97.2	22.0	28.1	43.8	81.5	61.4	--	68.2	68.7
					Stauffer R-20458						
0.05	< 0.4	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.4	< 0.3	< 0.8	< 0.3	< 0.3
0.10	1.1	1.6	0.6	1.3	0.6	< 0.3	< 0.4	< 0.4	< 0.3	< 0.3	< 0.3
0.25	7.9	10.0	1.6	4.1	9.0	1.1	< 0.4	< 0.4	< 0.4	< 0.3	< 0.3
0.50	15.6	18.8	--	24.0	20.7	15.2	--	19.3	20.6	13.6	7.2
1.0	35.6	57.8	22.0	27.2	7.2	13.0	13.5	--	28.7	15.9	--

(Rabon®), that is presently used to control the flies (1-2% with 1 gal/100 ft^2) (BAILEY et al. 1968, MATTHYSSE and MCCLAIN 1973). Moreover, the IGR's do not have extended persistence, are not toxic to sheep (SMALLEY et al. 1974), and do not interfere with the development of parasites in treated stable fly pupae (WRIGHT and SPATES 1972). The method of topical application of these IGR's as pesticides therefore seems to have certain advantages.

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