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TOLERANCE OF THE PLANARIAN *DUGESIA TIGRINA* (TRICLADIDA: TURBELLARIA) TO PESTICIDES AND INSECT GROWTH REGULATORS IN A SMALL-SCALE FIELD STUDY

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ABSTRACT. Two insect growth regulators, methoprene and a benzyl-1,3,benzodioxole (J-2931), had no detrimental effects on *Dugesia tigrina* under field conditions. Three other compounds, resmethrin, temephos, and cyromazine, had only minimal effects. Asexual multiplication among these planarian predators exceeded 68% when combined with *Culex quinquefasciatus* larvae and methoprene at different concentration levels. Also, this combined treatment with *D. tigrina* and methoprene resulted in high level (98.9%) reduction of *Cx. quinquefasciatus* populations through the 7-wk field study.

The brown planarians, *Dugesia tigrina* (Girard) and *Dugesia dorotocephala* (Woodworth), have demonstrated their effectiveness in reducing different species of mosquito larvae (Legner and Medved 1974, Yu and Legner 1976, Meyer and Learned 1981). *Dugesia tigrina* increased 112% in 4 wk in used automobile tires placed in the field in July 1993 and provided 95% population reduction of *Aedes albopictus* (F. R. S. Nelson, unpublished report). The tolerance of these predators has never been investigated in the field with compounds used in mosquito larvicidal programs. Nelson et al. (1986) reported that the predatory and asexual reproductive potential of the 2 *Dugesia* species are comparable. A knowledge of their susceptibility to pesticides and insect growth regulators is necessary to evaluate the applicability of these potential biocontrol agents in an integrated larvicidal program (Levy and Miller 1978). In laboratory tests, *D. tigrina* has shown a high level of tolerance to several pesticides used in larvicidal programs, at low and high dosages (Nelson et al. 1986, 1988). This study investigated the tolerance of *D. tigrina* to low and high concentrations of pesticides and insect growth regulators (IGRs) under field conditions.

The planarians (*D. tigrina*) used were originally procured from Carolina Biological Supply Co., Burlington, NC. They were reared in our laboratory in 17 × 17 × 6.8-cm trays containing a medium of aged pond water and 2 small rocks as resting substrate. The planarians were fed biweekly on small pieces of previously frozen and thawed beef liver for *ca.* 2 h and then transferred to fresh rearing medium. Temperatures were maintained at 22–24°C. The compounds used were: Scourge®, 18% resmethrin + 54% piperonyl butoxide (Holiman Equipment Inc., Jackson, MS); temephos (4E) (American Cyanamid Co., Kansas City, MO); a benzyl-1,3,benzodioxole (J-2931) (99.5%) (Western Regional Research Center, ARS, USDA Berkeley, CA); cyromazine (95.6%) (CEIBA-GEIGY, Greensboro, NC); and methoprene (89.25%) (Zoecon Corp., Dallas, TX). *Culex quinquefasciatus* Say larvae were taken from a colony maintained in this laboratory for several years.

The study was conducted in 2 phases between June 13 and the last week of July 1991. In phase 1, 100 mature *D. tigrina* (8–10 mm long), were placed in each of 10 1-liter glass dishes with bottoms completely covered with small rocks of uniform size. Each dish was subsequently transferred to a 33-liter plastic refuse container in the field. Twelve liters of pond water were added to each container and the planarians were allowed to acclimatize for 24 h to conditions within the containers prior to the addition of the test compounds dissolved in acetone, followed by thorough mixing. Planarians in 5 of the containers were exposed to 12 liters of 10-ppm solution of temephos, J-2931, resmethrin, cyromazine, or methoprene. Those in the remaining 5 containers were held as untreated controls. Planarians were exposed for 3 days (4 replicates). At the end of each test the containers were transported to the laboratory and the number of live *D. tigrina* were determined. These data were used to evaluate any effect of each of the compounds tested.

In phase 2, the procedures were similar to phase 1, with the following exceptions: 16 test containers were used, each with 20 mature *D. tigrina.* In addition, fallen leaves, twigs, and other debris were added to simulate a natural habitat for the planarians. The test consisted of 4 replicates each of 0.05, 1.00, or 10.0 ppm methoprene plus planaria-only treatment (untreated planarians). Initially, and weekly thereafter, 20 2nd-instar *Cx. quinquefasciatus* larvae were added to each container. Approximately 1 gram of a 3:1 mixture of finely ground rabbit food and brewers yeast (40 mesh size) was supplied to each container each time *Cx. quinquefasciatus* larvae were added. The containers were monitored 3–4 times per

Table 1. Effects of different concentrations of methoprene on *Dugesia tigrina* after 7-wk continuous field exposure.

Dosages (ppm)	Number planarians per replicate				% increase
	1	2	3	4	
0.05	49	25	13	33	50.0
1.00	21	27	39	48	68.8
10.00	33	19	48	26	57.5
0.00	44	20	23	31	47.5

week for 7 wk. Newly formed pupae were removed, counted, and held for emergence to determine the efficacy of the combined treatments against *Cx. quinquefasciatus.*

The size of the *Dugesia* population was determined at weekly intervals by removing 4 samples of rock from different areas of each container. Counts were made of the number of planarians attached to the rocks and then they were immediately returned to the containers. The water temperature was recorded daily in the planaria-only containers. After 7 wk of continuous exposure, the containers were transported to the laboratory and the number of surviving planarians was determined as previously described. The data were subjected to an analysis of variance (ANOVA).

The results of 72-h posttreatment counts indicated that all *D. tigrina* exposed continuously to J-2931 and methoprene survived. With the other compounds, (resmethrin, temephos, and cyromazine) the percent survival was slightly less (99%, 99%, and 93%, respectively). No asexual reproduction was observed among the planarians in the treatment and control containers during the experiment.

Shown in Table 1 are the results of the 7-wk field tests with methoprene and *D. tigrina.* Asexual multiplication occurred at all concentration levels and in the planaria-only treatment. Population increase among the methoprene-treated *Dugesia* ranged from 50 to 68.7%. No significant differences were detected ($P > 0.05$) between the treatment means. The integrated treatments with *D. tigrina* and methoprene provided 98.9% reduction among the *Cx. quinquefasciatus* populations; the planaria-only treatment provided a 97.1% reduction. Sunlight and high temperatures in the field contribute to rapid degradation of the IGR methoprene in water (Schaefer and Dupras 1973). All the newly formed pupae collected in the combination treatments of methoprene and planaria did not emerge into adult *Cx. quinquefasciatus,* which can be attributed to the morphogenetic activity of methoprene. Also, no pupa appeared in the methoprene–planaria containers in the final week of the study. The remaining portion of the *Cx. quinquefasciatus* population was reduced by *D. tigrina* as compared with the planaria-only treatment.

The IGR methoprene was tested at the approximate concentration used in mosquito larviciding programs and also at higher concentrations that *Dugesia* may encounter in the field from larvicidal residues. Methoprene had no effect on the survival or feeding activity of the mosquito larva predator *D. tigrina* and probably had some stimulating effects on their asexual reproductive potential. It is possible that the copious amount of mucus secreted by *D. tigrina* could have aided in counteracting the toxic effects of the methoprene treatments. Available data suggest the necessity for further trials with *D. tigrina* and methoprene and other selected compounds evaluated in integrated mosquito larvicidal programs. An established population of *D. tigrina,* as treatment only or in combination with methoprene, should continue to increase with availability of or an increase in mosquito prey density and provide substantial suppression of the mosquito population, as indicated in this study.

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