

# OPERATIONAL AND SCIENTIFIC NOTES

## LABORATORY EVALUATION OF TOLERANCE OF *DUGESIA DOROTOCEPHALA* TO DIFFERENT DOSAGES OF DIFLUBENZURON<sup>1</sup>

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### INTRODUCTION

TH-6040 [1 - (chlorophenyl)-3(2,6-difluoro-benzoyl)-urea] also known as Dimilin® has been determined as an inhibitor of chitin synthesis in a wide range of mosquito species (Jakob 1973, Hsieh and Steelman 1974). The importance of St. Louis encephalitis and canine filariasis stimulated greater interest in biological agents for control of mosquito vectors of these diseases. Miura and Takahashi (1975) reported that diflubenzuron had little or no deleterious effects on nontarget organisms and showed potential for long-lasting control of asynchronous populations of immature mosquitoes at low practical rates. However, Mulla and Tsai (1978) found that inoculation rates with *Dugesia dorotocephala* (Woodworth) exceeding 2,000/m<sup>2</sup> of water surface were lethal to *Tapilia zillis* (Gervais) fish fry in experimental indoor or outdoor aquaria. Previous studies on the predatory capabilities of *D. dorotocephala* demonstrated that this species is an effective predator against *Culex peus* (Speiser), *Cx. tarsalis* (Coquillett), and *Cx. quinquefasciatus* (Say), at inoculation rate of 25 *Dugesia*/m<sup>2</sup> of water surface (Yu and Legner 1976). Nelson (1979) showed that sectioned *D. dorotocephala* have comparable predatory and reproductive potential as unsectioned *Dugesia* in reducing larval population of *Cx. quinquefasciatus*. Legner, Tsai and Medvid (1976) found *Dugesia* compatible with a wide variety of water qualities but later work (Levy and Miller 1978b) determined that chloride concentration at 1500 ppm had hazardous effects on predatory potential. Levy and Miller (1978a) also found *D. dorotocephala* refractory to relatively high dosages of some insect

growth regulators and pesticides, thus enhancing their potential for use in integrated mosquito control programs.

Although some attention has been directed towards studies on predation and asexual reproduction, very little has been done on larvicidal effects on these 2 processes or on the predator survival. Thus, this study was conducted to determine the immediate and latent effects of diflubenzuron on *D. dorotocephala* survival and effects on predation at concentrations used for larval mosquito control.

### MATERIALS AND METHODS

Larvae used in the current study were 3rd or 4th instars originated from stock culture maintained in the laboratory. *Dugesia dorotocephala* were from a recently established laboratory culture. All tests (with the exception of predatory experiments kept at room temperature) were conducted at ambient temperatures (22–26°C) and each replicated 3 times. Sectioned *Dugesia* tested were less than 2 wk old.

The initial experiments were conducted in 20 mm diam glass culture dishes, each contained 2 small stones as resting substrate for the planaria. Twenty mixed sectioned *D. dorotocephala* (Nelson 1979) were exposed to 100 *Cx. quinquefasciatus* larvae in one liter of 0.1, 0.005, 0.01, 0.004, or 0.0025 ppm diflubenzuron solutions (25% wp). The surviving regenerates were counted and recorded after 192 hr. Culture dishes containing pond water and similar numbers of planaria only were used as controls. After 192 hr exposure, 20 *Dugesia* were randomly selected from each concentration level, rinsed 3 times with pond water to remove any chemical residue that may remain on the exoskeleton, and transferred to each of ten 6.5 mm glass dishes (10 planaria/dish) containing 75 ml pond water. Similar numbers of planaria were also transferred from the chemical-free dishes to monitor post-treatment effects. Observations were made at the end of 360 hr to ascertain if there were any delayed effects on *Dugesia* due to 192 hr treatment exposure. Planaria unable to glide and remaining motionless when probed under a dissecting microscope were rated as dead.

<sup>1</sup> Tricladida: Planariidae.

In a subsequent test, 20 mixed sectioned *Dugesia* were exposed to 100, 3rd or 4th instar mosquito larvae at similar concentrations (0.1 to 0.0025 ppm). Larval predation was assessed every 48 hr for 192 hr and those that pupated were removed from treatment dishes and replaced by an appropriate number of similar instar larvae.

## RESULTS AND DISCUSSION

**SURVIVAL EFFECTS.** Data collected indicate that *D. dorotocephala* exposed 192 hr to diflubenzuron suffered no observable deleterious effects when compared with controls. All *Dugesia* survived the different concentration levels tested with a 3% cumulative population increase in the treatments and 12% in the controls. Similarly, no noticeable adverse effects on *Dugesia* behavior or asexual reproduction were detected during the extended 360 hr post-exposure observations. The post-exposure population showed an accumulated increase of 78% and controls 72%.

**PREDATORY EFFECTS.** All concentrations had minimal or no visible effects on *Dugesia* during the 192 hr exposure to the insecticide. The accumulated predation levels were 93% (0.1 ppm), 88% (0.05 ppm), 97.7% (0.01 ppm), 94.7% (0.004 ppm) and 98% (0.0025 ppm). The lower predation at 0.05 ppm was probably due to abnormal temperatures during the earlier portion of this experiment rather than the effects of the treatment. Predation rates in the controls varied from 91 to 100%. With the exception of one replicate, a greater number (ca. 80%) of prey was consumed 48 hr post-inoculation. In a comparative study Nelson (1979) recorded a consumption rate of 91% with untreated sectioned *Dugesia*, 48 hr post-treatment.

Tolerance tests with *Dugesia* (Levy and Miller 1978a) using a single concentration of diflubenzuron and other larvicides produced neither mortality, immediate nor delayed effects. It seems likely that the biocontrol potential of *Dugesia* would not be impaired by a wide group of pesticides used in integrated or larviciding mosquito program.

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## THE OPERATIONAL RESPONSE TO THE 1980 OUTBREAK OF EASTERN EQUINE ENCEPHALITIS IN SOUTHEASTERN GEORGIA

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The following paper is a chronological account of the actions taken by the Mosquito Control departments of Glynn and Chatham counties in response to an outbreak of EEE in 2