FACTORS AFFECTING THE ACTIVITY OF DIFLUBENZURON AGAINST *SIMULIUM* LARVAE (DIPTERA:SIMULIIDAE)[1]

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ABSTRACT. Diflubenzuron at 0.02 ppm AI/l h tested in the laboratory against late instars of field-collected larvae of *Simulium vittatum, S. argus, S. tescorum, S. aureum,* and *S. virgatum* produced differential mortality in these species. *S. vittatum* was the least, while *S. tescorum* was the most susceptible species, responding with 59 and 88% mortality, respectively.

Second and 3rd instars of *S. vittatum* were much more susceptible than the ultimate instars, suffering 3 times more mortality.

There was a positive correlation (r = 0.99) between temperature and extent of mortality; larvae exposed to 0.02 ppm/1 h at 10, 15, 19 and 24°C responded with 18, 35, 53, and 75% preimaginal mortality respectively.

Diflubenzuron was shown to be active *per os.* No contact activity was observed when larvae were exposed in static water.

Rearing water pH and degree of turbidity and larval crowding were not shown to significantly influence the biocidal activity of diflubenzuron.

Studies on factors that affect the efficacy of *Simulium* larvicides are usually directed toward the effects of formulation, concentration, length of exposure, stream type and method of application (Jamnback 1976, unpublished document W.H.O.) Biological and physical factors that affect the activity of test compounds are scarcely investigated.

In earlier studies on diflubenzuron as a blackfly larvicide (Lacey and Mulla 1977b), we investigated the influence of formulation, concentration (conc), and exposure period on the extent of larval mortality and also the influence of conc, length of exposure and egg age on ovicidal activity.

This paper presents information on other factors, both biological and physical, which affect the biological activity of diflubenzuron against blackfly larvae.

METHODS AND MATERIALS

Unless otherwise noted, 30 field-collected late instar larvae (penultimate and ultimate instars) per replicate were exposed to 0.02 ppm AI of diflubenzuron (using WP 25) under varying conditions for 1 hr utilizing the bioassay procedures described by Lacey and Mulla (1977a,b). The modified jar system (Lacey and Mulla 1977a) was employed for studying the effects of rearing water pH and temperature on diflubenzuron activity. In all experiments except the ones determining relative species susceptibility and the contact activity of diflubenzuron, *S. vittatum* Zetterstedt larvae were utilized.

Species susceptibility was investigated by exposing larvae of *S. argus* Williston, *S. aureum* Fries, *S. tescorum* Stone and Boreham, *S. virgatum* Coquillett, and *S. vittatum* to diflubenzuron employing the standard procedure. The effect of larval age on susceptibility was studied by exposing young larvae (2nd and 3rd instars) to diflubenzuron at 0.01 and 0.01 ppm AI and early ultimate instars (histoblast formed but not melanized) to 0.02 ppm.

The contact and ingestion activity of diflubenzuron was studied by exposing late instars of *S. tescorum* (90%) and *S. vittatum* (10%) to 0.025 ppm diflubenzuron for 1 hr in 250 ml of static IGR suspension in glass custard dishes. Control larvae were also left in still water for 1 hr. After the exposure period, the larvae were rinsed twice and transferred to

[1] These studies were partially supported by the World Health Organization.

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bioassay units; they were observed in circulating water for the standard 2 wk period before mortality was assessed. For comparison, late instars were exposed to the same conc of diflubenzuron utilizing standard procedures employing water aeration (i.e. facilitating ingestion of the IGR).

The exposure of larvae to diflubenzuron at 10, 15, 19, and 24°C was accomplished in a temperature controlled cabinet where the air tempreature was varied to obtain the desired water temperature. Variation in the temperature of replicates was less than ± 1°C at each of the temperature regimes. The temperature of fresh exchange water was adjusted to the experimental temperature by storing it in buckets in the cabinet of a given temperature for 12 hr before use.

The effect of rearing water pH on diflubenzuron efficacy was investigated by adjusting the pH with excess NaOH (saturated) or glacial acetic acid 12 hr before water exchange and storing the water at 19°C so that the desired pH was attained at the time of water exchange. This prevented an extreme change in the pH that was observed when the water was adjusted just prior to each exchange of water. Even with this procedure, the high pH water changed from 10 to 9 and the low pH raised from 6 to 6.5 between water exchanges. The copious amounts of buffer solutions that would have been required to maintain the desired pH would not have been practical.

Food dilution and turbidity effects were studied by substituting the standard amount of food (35 mg) with no food and 140 mg during and for 12 hr following the exposure period utilizing the standard bioassay procedure.

Crowding effects were investigated by comparing the standard number of larvae (30) exposed to 0.02 ppm diflubenzuron with 60 and 90 larvae exposed to the same conc.

To study the effects of WP suspension storage on biological activity, 0.1% suspensions stored in deionized water at 2°C for 71–222 days and at 19°C for 175 days were bioassayed against larvae (standard procedure).

Treatment effects were analyzed statistically with either Duncan's Multiple Range Test or t-test after correction for control mortality with Abbott's formula followed by arcsin transformation. Correlation coefficients were determined by linear regression analysis.

RESULTS AND DISCUSSION

Table 1 presents data on species susceptibility to diflubenzuron. *S. vittatum*, proved to be the least susceptible, while *S. tescorum*, the most anthropophilic black fly in southern California (Hall 1972) requiring abatement in some situations (Pelsue et al. 1970) was the most susceptible species. Field activity (Lacey and Mulla 1978) of diflubenzuron against this species indicates that it can be controlled with conc that are lower than those for the widely used temephos.

Table 1. Susceptibility of various *Simulium* spp. to diflubenzuron (.02 ppm/1 h exp./2 wk obs.).[a]

	Mean % Mortality ± S.E.	
	Treatment	Control
S. (Psilozia) vittatum	59 ± 2.39 a	7 ± 3.00
S. (P.) argus	84 ± 5.07 b	14 ± 3.38
S. (Simulium) tescorum	88 ± 2.10 b	6 ± 2.50
S. (Eusimulium) aureum	70 ± 1.73 a	8 ± 4.63
S. (Hemicnetha) virgatum	62 ± 5.78 a	11 ± 8.95

[a] Means in the same column followed by the same letter are not significantly different at the 0.05 level.

The difference in susceptibility of the 2 age groups tested is rather pronounced (Table 2). Even at half the conc, the younger instars responded with more than double the mortality of the ultimate instars. This is in contrast to the results obtained when treating insects with the terpinoid IGRs where the most sensitive

Table 2. Effect of larval age on the susceptibility of *S. vittatum* to diflubenzuron (1 h exp./2 wk obs.).[a]

Age Group	Conc.	Mean % Mortality ± S.E. Treatment	Control
Ultimate instars	0.02	32 ± 2.39 c	0
Young instars[b]	0.01	80 ± 3.09 b	6 ± 2.52
	0.02	90 ± 2.96 a	6 ± 2.52

[a] See footnote Table 1.
[b] 2nd and 3rd instars.

larvae are usually the ultimate instars. Although no age effects have thus far been reported for the JH analogs against *Simulium*, Jamnback and Frempong-Boadu (1966) reported only small differences in the susceptibility of small, medium, and full grown larvae to methoxychlor.

There was no contact activity observed with diflubenzuron (Table 3). It is apparent that for any evaluation of diflubenzuron in the laboratory, a system should be utilized that allows normal feeding by the larvae. A prerequisite for this, at least with species that feed exclusively by filtration, is the presence of a water current produced by air or water recirculation by pumping.

The effects of temperature on diflubenzuron activity are presented in Table 4. There was a strong positive correlation between temperature and degree of preimaginal mortality (r = 0.99). Since feeding rates do not differ at 15–24°C (Lacey and Mulla, unpublished data), this was not suspected as a factor responsible for the differences in mortality at these temperatures. Feeding rates, however, are reduced by 50% at 10°C.

Table 3. Contact vs *per os* activity of diflubenzuron against *S. tescorum* (90%) and *S. vittatum* (10%) (0.025 ppm/1 h exp./2 wk obs.).[a]

Treatment	Mean % Mortality ± S.E.
Contact	9 ± 1.00 a
Per os	93 ± 1.33 b
Control	12 ± 4.33 a

[a] See footnote, Table 1.

Apparently it is the rate of absorption of diflubenzuron through the gut and subsequent lesion formation that are affected by the ambient temperature. Temperature was also reported by Arias (1973) to be an influencing factor in the mortality of larvae of *Culex tarsalis* Coquillett exposed to diflubenzuron.

The mortality observed for each of the temperature regimes has been divided into preimaginal and adult mortality due to the pronounced adult mortality at the time of emergence for the 10°C regime, but not for other temperatures. At higher temperatures, death of adults at emergence from pupae resulting from larvae treated with diflubenzuron is slight (0–10%) and usually not significantly different from controls (Lacey and Mulla 1977b). Since adult mortality in the 10°C regime did not differ significantly between the treated and control, this was probably due to the inability of the adult to fly at this temperature, therefore preimaginal mortality was regarded as a true indication of diflubenzuron activity at the various temperatures.

The effect of rearing water pH on the activity of diflubenzuron is presented in Table 5. Although there appears to be a negative correlation between rearing water pH and extent of mortality, the variability in the replicates is too high for any significance to be shown.

No significant loss of activity was observed in the diflubenzuron WP suspen-

Table 4. Effect of temperature on the biocidal activity of diflubenzuron against *S. vittatum* larvae (0.02 ppm/1 h exp.).[a]

Temperature °C	Mean % preimaginal mortality ± S.E.		Mean % adult mortality ± S.E.	
	Treatment	Control	Treatment	Control
10	18 ± 3.89 a	5 ± 1.45 a	62 ± 12.55 a	54 ± 7.13 a
15	35 ± 2.68 b	5 ± 1.87 a	7 ± 1.71 b	2 ± 0.88 b
19	53 ± 5.46 c	4 ± 4.00 a	0 —— c	0 —— b
24	75 ± 5.33 d	4 ± 0.88 a	5 ± 1.65 b	6 ± 3.84 b

[a] See footnote, Table 1.

sion in water stored at 2 temperatures. The 175 day old 0.1% suspension held at 19°C produced an average of 65% ± 5.03 mortality, while suspensions stored at 2°C for 71–222 days produced mortality ranging from 54 to 61%. Various findings on the stability of diflubenzuron in water have been reported. Metcalf et al. (1975) reported that diflubenzuron is moderately stable in a model ecosystem. However, Schaefer et al. (1975) reported little or no effective residual activity in the natural habitat of *Cx. tarsalis*. Although our studies indicate that the activity of diflubenzuron is maintained after prolonged storage in water, it is not known whether the parent compound or one of its breakdown products is responsible for the biological activity.

The degree of turbidity and crowding that were investigated did not significantly affect the biological activity of diflubenzuron.

It is apparent from our studies that diflubenzuron offers good control potential against species in a wide-range of *Simulium* subgenera. The increased activity against younger larvae indicates that the effective field dosage against highly synchronized *Simulium* populations may be reduced considerably if the cohort is treated when it is still young. The lack of contact activity of diflubenzuron may provide some selectivity in the lotic environments by not seriously affecting non-filter feeding invertebrates. Grazing herbivorous species and predators would only be subjected to a short period of external contact with the IGR, after which the dilution and flushing of diflubenzuron would curtail any further contact.

Table 5. Effects of rearing water pH on the biocidal activity of diflubenzuron against *S. vittatum* (0.02 ppm/1 h exp./2 wk obs.).[a]

pH	Mean % mortality ± S.E.	
	Treatment	Control
6.0	57 ± 0.88 a	16 ± 2.91
8.2	48 ± 6.11 a	10 ± 1.73
10.0	41 ± 6.01 a	11 ± 2.65

[a] See footnote, Table 1.

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ENTOMOLOGICAL EVALUATION OF MALATHION AS A RESIDUAL SPRAY FOR THE CONTROL OF *ANOPHELES CULICIFACIES* IN THE PROVINCE OF HELMAND, SOUTHWEST AFGHANISTAN, 1976[1]

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ABSTRACT. A field trial evaluation of malathion was carried out in the province of Helmand, southwest Afghanistan, in order to evaluate the effectiveness of this insecticide for control of adult anopheline mosquitoes. *Anopheles culicifacies* Giles is one of the main vectors of malaria, with an epidemic potential in the lower Helmand province, that showed a strong resistance to DDT in 1972.

One round of house spraying with malathion 50% w.d.p. 2g/m² was carried out in July 1976, at the peak of activity of *An. culicifacies*. On the basis of the results obtained, it was concluded that malathion is an effective insecticide against *An. culicifacies*.

INTRODUCTION

After the development of DDT resistance in *Anopheles culicifacies* Giles, the main vector of malaria in southwest Afghanistan, in 1972, the number of malaria cases in the lower Helmand province, with a population of 207,334, has steadily increased (Malaria Institute, Kabul, unpublished document). Lower Helmand province had been treated with DDT 75% w.d.p., 1.5 g/m², 18 rounds once a year during 1953–74, and one round of malathion 50% w.d.p., 2g/m² in 1975 for malaria control.

Various entomological observations indicate that *An. culicifacies* persists in this area with high densities during June-September, biting man and animals, particularly during the season when people and animals sleep outdoors. *An. stephensi* starts its activity from about late August. *An. pulcherrimus* is regarded as a suspected vector in this area, and mostly rests in outside shelters.

During the course of preliminary surveys carried out in 1950–52 (Dhir and Rahim 1957) *An. superpictus* was considered to be the main and only vector in northern and mountainous valley of Helmand.

MATERIAL AND METHOD

THE STUDY AREA. Lower Helmand

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