FIELD EVALUATION OF DIFLUBENZURON AGAINST SIMULIUM LARVAE\textsuperscript{1, 2}

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ABSTRACT. Diflubenzuron (WP 25%) was tested in small artificial streamlets against Simulium vittatum Zetterstedt and a mixed population of Simulium spp. at 0.2, 0.1 and 0.02 ppm AI for 1 h of exposure. The granular formulation (0.9%) was tested against a mixed population at 0.04 ppm based on 1 h exposure. The 0.2 and 0.1 ppm gave about 95% control of exposed larvae of S. vittatum 30 m below treatment point. The mixed population responded with 100 and 76% mortality (200 m below treatment) of exposed larvae to the 0.2 and 0.02 ppm concn respectively. The granular formulation produced very little mortality in the mixed population.

At 0.2 ppm/1 h diflubenzuron when tested against a mixed population (S. brunnatum Malloch (86%) and S. argus Williston (14%)) in a moderate-size stream, produced complete mortality of the exposed larvae for the entire length of the stream (4.3 km). Within 3.5 weeks the population rebounded to 56% of the pretreatment numbers. Seven weeks after treatment the population was 64% of the pretreatment numbers.

The field efficacy of diflubenzuron (Dimilin\textsuperscript{®}, TH-6040) has been reported for a number of medically important and nuisance flies (Ables et al. 1975, Mulla and Darwazeh 1976, Ali and Mulla 1977, and Kunz et al. 1977). However, there are no published accounts on the field activity of diflubenzuron against larvae of blackflies. The laboratory bioassay of diflubenzuron against Simulium vittatum Zetterstedt (Lacey and Mulla 1977) indicated that it has potential as a blackfly larvicide but requires a longer period of observation and more detailed analysis of efficacy than the conventional larvicides. Here we present information on the development of assessment methods for the field use and evaluation of diflubenzuron against blackflies, its activity against various Simulium spp., and the recovery of larval populations in treated streams.

METHODS AND MATERIALS

A sampling method similar to that described by Williams and Obeng (1962) which utilizes polyethylene strips as artificial substrates was employed for estimating pre- and posttreatment larval and pupal numbers. The strips measured 36.1 x 1.3 cm (15 x ½ in.) and were attached to wire spikes with staples (Fig. 1). Four strips were placed above the point of treatment and at varying distances downstream by inserting the wire spike into the substratum. Ten days were allowed for colonization of the strips by larvae after which time 2 strips from each sampling site were removed and placed in 70% alcohol for future counting of pre-treatment larvae, pupae, and pupal exuviae. The appropriate amount of diflubenzuron based on the total volume of water flowing through a point for 1 hr, was then added to the stream. The needed quantity of the WP was diluted in 200 ml water and poured into the stream at the point of treatment. One week after treatment, the remaining 2 strips were removed and placed in alcohol for determination of posttreatment numbers of larvae, both maturing (4th-ultimate instar) and younger instars (3rd and younger) and pupae, and pupal exuviae. All of the tests except one were conducted in artificial streamlets (flow rate 3.8–7.7 liters/sec, 0.62 m/sec) in Thousand Palms Oasis, 120 km east of Riverside. Due to the variety of conditions within the oasis it was possible to locate Simulium populations with varying species.
compositions. A relatively short streamlet (40 m long, water temp. 16°C) was inhabited almost entirely by Simulium vittatum Zetterstedt. At this location diflubenzuron WP25 was tested at 0.2 ppm (LC₉₅ in lab as found by Lacey and Mulla 1977) and 0.1 ppm for 1 h exposure.

A warm (22°C) 300-m long streamlet inhabited by S. tectorum Stone and Boreham (66%), S. aureum Fries (25%), S. argus Williston (5%), and S. vittatum (3%) was utilized for testing WP25 at 0.2 and 0.02 ppm/1 h and 0.3% granular formulation (0.04 ppm calculated at 1 h exposure). The granules were wrapped in gauze and left on the stream bottom for one week.

To determine downstream carry of the material and population recovery, a moderate-size stream (flow rate 610 liters/sec; 0.84 m/sec; 16°C) fed with the effluent water from a fish hatchery (Victorville, CA) was utilized. The same pre- and posttreatment sampling techniques were utilized as described above but, in addition, new strips were implanted upon each visit and sampling was continued for an additional 6 weeks at a point 3.5 km below the point of IGR introduction. This stream was inhabited by S. bivittatum Malloch and S. argus, and their repopulation rate was monitored.

The % mortality or reduction of the larvae exposed to the diflubenzuron was calculated with the formula of Mulla (Mulla et al. 1971):

\[
\% \text{ reduction} = 100 - \frac{C_2}{T_1} \times \frac{T_2}{C_2} 100
\]

where: \(C_1\) = avg. no. exposed larvae, pre-
treatment at check station(s), \(T_1\) = avg. no. exposed larvae, pretreatment at each treated station, \(C_2\) = avg. no. maturing larvae, posttreatment at check station(s), \(T_2\) = avg. no. maturing larvae, posttreatment at each treated station.

In certain climatic zones, such as southern California, many Simulium spp. have asynchronous generations. In general, diflubenzuron manifests its activity at the time of ecdysis of larvae; its activity is quite low against pupae. Since level of control was assessed 1 week posttreatment and egg hatch in the interim yielded
larvae which were not subjected to lethal concentrations, these younger instars in
the posttreatment determination were not included in the calculations by the
above formula. Although the density of
the various life stages and forms is given
in the tables, the level of control is only
based on the number of larvae (maturing)
pre-and posttreatment in check (point
above treatment) and the treated stations.

RESULTS AND DISCUSSION

When the S. vittatum larvae were
treated with 0.2 ppm diflubenzuron,
there was a complete elimination of the
target population (data not presented).
The results of a treatment at half this rate
(0.1 ppm) are presented in Table 1. This
treatment yielded 95–100% mortality of
the exposed larvae. Larvae that hatched
during the posttreatment period (7 days)
appeared normal indicating lack of any
residual activity of diflubenzuron. Rearing
of these surviving individuals to the
adult stage yielded normal emergence. It
appears that if control over a longer
stream distance is desired, a higher con-
centration or different formulation of
diffubenzuron may be required to pro-
duce the additional carry.

The 0.2 ppm treatment also totally
eliminated the mixed population of
Simulium. After observing these effects
and knowing that S. tesserum and S.
argus are very sensitive to diflubenzuron
(Lacey and Mulla 1978), the dosage was
reduced by a factor of 10 rather than by
half as in the S. vittatum test to avoid the
possibility of again eliminating the pop-
ulation. Table 2 presents the effects of
treating the stream with 0.02 ppm. Nearly
complete elimination of the exposed lar-
vae, even from natural substrates was ap-
parent for 100 m downstream of the di-
flubenzuron injection site. The lack of
total reduction at the 20 m sampling site
was undoubtedly the result of drifting
larvae from above the treatment point
where the population had actually in-
creased by 14%. The downstream carry
of this treatment was quite limited as the
extent of reduction was only 76%, 200 m
below the treatment point. The lack of
carry was especially apparent 500 m
below the treatment point, where reduc-
tion in the exposed larvae due to treat-
ment was nil.

The high level of control observed with
the 0.02 ppm treatment of the mixed
Simulium population in the upper por-
tions of this warm stream was to be ex-
pected based on laboratory findings
(Lacey and Mulla 1978). Both dominant
species in this test are more susceptible
than the others and also the activity of this
IGR is enhanced at higher temperatures
(Lacey and Mulla 1978).

The concentration for the granular
treatment was calculated on the basis of
0.04 ppm of diflubenzuron for 1 h of
exposure. In reality, the release of insec-
ticides from granules may be prolonged
for several days (Mulla et al. 1968).4 Al-
though, there was an 80% reduction 20 m
below the point of treatment, the 100,
200, and 300 m sites actually underwent a
slight net gain in numbers of larvae (data
omitted). Based on laboratory findings
(Lacey and Mulla 1977), 4 times the
amount of IGR necessary for control at 1 h
exposure would be necessary for the
same level of control with a 24 h exposure
in the lotic environment. This high level
of diflubenzuron was not utilized in an
effort to avoid elimination of larvae in a
stream that was to be used for subsequent
experiments.

The rate of recovery of S. hiaticatum and
S. argus populations after complete elimi-
nation of the exposed larvae is shown in
Fig. 2. The number of young instars (al-
though low) a week after treatment was
undoubtedly the result of posttreatment
hatch. As this cohort matured and
emerged (see pupal curve), a sharp in-
crease in the younger instars after July 16
indicates oviposition by the closed

4 Mulla, M. S., M. F. B. Chaudhury and H.
A. Darwazech. 1968. Release of new mosquito
larvicides into water from granular formul-
ations. World Health Organization mimeo. dox.
WHO/VBC/68.94. 6p.
Table 1. Evaluation of diflubenzuron (WP 25 at 0.1 ppm/h) against larvae of S. oitatum\(^a\)

<table>
<thead>
<tr>
<th>Distance from treatment (meters)</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Mean no/strip</th>
<th>% Reduction exposed larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae (L(_0^b))</td>
<td>Pupal exuviae (E(_0))</td>
<td>Maturing larvae (L(_0^b))</td>
<td>Wupe (P(_0))</td>
</tr>
<tr>
<td>2 above (Check)</td>
<td>117</td>
<td>1</td>
<td>105</td>
<td>0</td>
</tr>
<tr>
<td>20 below</td>
<td>131</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 below</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Thousand Palms Oasis, water temp. 16°C.

\(^b\) 4th and older instars.

\(^c\) 3rd and younger instars.

Table 2. Evaluation of diflubenzuron (WP 25 at 0.02 ppm/h) against mixed population of larvae of S. smirum\(^a\)

<table>
<thead>
<tr>
<th>Distance from treatment (meters)</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Mean no/strip</th>
<th>% Reduction exposed larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae (L(_0^b))</td>
<td>Pupal exuviae (E(_0))</td>
<td>Maturing larvae (L(_0^b))</td>
<td>Wupe (P(_0))</td>
</tr>
<tr>
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<td>175</td>
<td>400</td>
<td>203</td>
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<tr>
<td>100</td>
<td>524</td>
<td>407</td>
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<td>200</td>
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<tr>
<td>300</td>
<td>218</td>
<td>96</td>
<td>24</td>
<td>28</td>
</tr>
</tbody>
</table>

\(^a\) S. tescum 66%, S. aurem 25%, S. argui 6%, S. oitatum 3%, Thousand Palms Oasis, water temp. 22°C.

\(^b\) 4th and older instars.

\(^c\) 3rd and younger instars.
adults. The total population reached a plateau 3.5 weeks after treatment and stabilized at this point over the next 3.5 weeks of sampling. As indicated by the recovery of this multivoltine population, a 2nd treatment prior to the pupation of this cohort would be needed. Additional treatments will be necessary at 2–3 week intervals for sustained control of persistent infestations.

When utilizing slow acting larvicides, it is necessary to observe the population structure and posttreatment changes over a protracted period of time as opposed to the rapid assessment made with the faster acting organophosphate insecticides. Our formula presented above takes into account larval population changes due to mortality or drift in the check as well as in the treated stations. The % reduction calculated by the formula is based on the number of exposed larvae only, excluding young larvae observed before or a week after treatment, as these are the result of posttreatment hatch and hence are not part of the “exposed” population. However, increase in the number of young larvae during posttreatment intervals is an indication of the lack of residual activity of diflubenzuron. Most of these larvae are expected to undergo normal development to the pupal and adult stages.

From these studies it is apparent that the IGR diflubenzuron offer some potential for the control of Simulium vectors. Also a method for the evaluation of slow-acting compounds has been developed. Further studies are needed to elucidate the field efficacy of this and other IGRs against Simulium flies.

References Cited
Kunz, S. E., R. L. Harris, B. F. Hogan and J. L. Wright. 1977. Inhibition of development in a field population of horn flies treated with diflubenzuron. Ibid. 70: 298–300.