FIELD EVALUATION OF DIFLUBENZURON AGAINST *SIMULIUM* LARVAE[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 (0.5%) 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. bivittatum* 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®, 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 38.1 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 pretreatment 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 instars) 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

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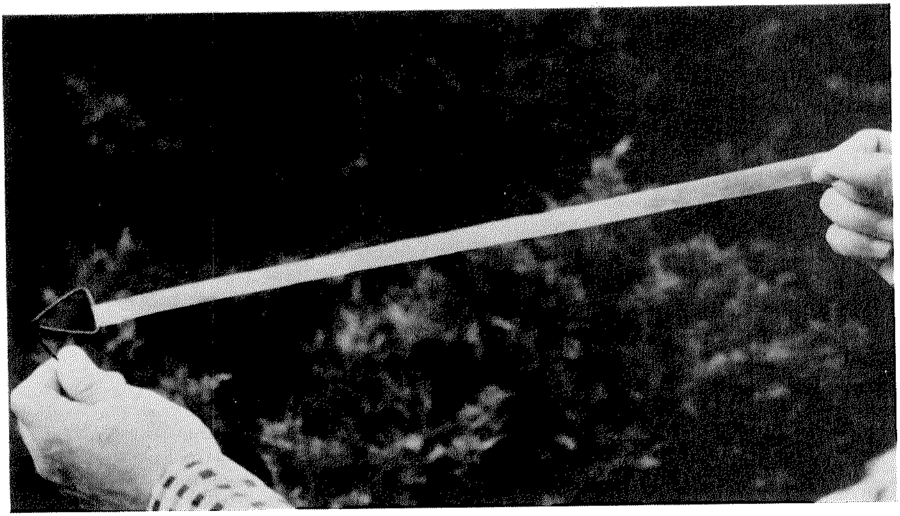

Fig. 1. Polyethylene strip sampling substrate to be fixed in the bottom of a stream.

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 (LC_{95} in lab as found by Lacey and Mulla 1977) and 0.1 ppm for 1 h exposure.

A warm (22°) 300-m long streamlet inhabited by *S. tescorum* Stone and Boreham (66%), *S. aureum* Fries (25%), *S. argus* Williston (6%), and *S. vittatum* (3%) was utilized for testing WP25 at 0.2 and 0.02 ppm/1 h and 0.5% 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):

$$\% \text{ reduction} = 100 - \frac{C_1}{T_1} \times \frac{T_2}{C_2} 100,$$

where: C_1 = avg. no. exposed larvae, pretreatment at check station(s), T_1 = avg. no. exposed larvae, pretreatment at each treated station, C_2 = avg. no. maturing larvae, posttreatment at check stations(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 concentration or different formulation of diflubenzuron may be required to produce the additional carry.

The 0.2 ppm treatment also totally eliminated the mixed population of *Simulium*. After observing these effects and knowing that *S. tescorum* 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 population. Table 2 presents the effects of treating the stream with 0.02 ppm. Nearly complete elimination of the exposed larvae, even from natural substrates was apparent for 100 m downstream of the diflubenzuron 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 increased 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 300 m below the treatment point, where reduction in the exposed larvae due to treatment was nil.

The high level of control observed with the 0.02 ppm treatment of the mixed *Simulium* population in the upper portions of this warm stream was to be expected 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 insecticides from granules may be prolonged for several days (Mulla et al. 1968).[4] Although, 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. bivittatum* and *S. argus* populations after complete elimination of the exposed larvae is shown in Fig. 2. The number of young instars (although low) a week after treatment was undoubtedly the result of posttreatment hatch. As this cohort matured and emerged (see pupal curve), a sharp increase in the younger instars after July 16 indicates oviposition by the eclosed

[4] Mulla, M. S., M. F. B. Chaudhury and H. A. Darwazeh. 1968. Release of new mosquito larvicides into water from granular formulations. World Health Organization mimeo. doc. WHO/VBC/68.94. 6p.

Table 1. Evaluation of diflubenzuron (WP 25 at 0.1 ppm/1 h) against larvae of *S. vittatum*[a]

Distance from treatment (meters)	Mean no./strip: Pretreatment: Larvae (L_1)[b]	Pupae (P_1)	Pupal exuviae (E_1)	Posttreatment: Maturing larvae (L_2)[b]	pupae (P_2)	exuviae (E_2)	larvae (L_y)[c]	% Reduction exposed larvae
2 above (Check)	117	1	2	105	1	0	98	—
20 below	131	1	2	0	0	0	3	100
30 below	20	1	0	1	0	1	3	95

[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/1 h) against mixed population of larvae of *Simulium*[a]

Distance from treatment (meters)	Mean no./strip: Pretreatment: Larvae (L_1)[b]	Pupae (P_1)	Pupal exuviae (E_1)	Posttreatment: Maturing larvae (L_2)[b]	Pupae (P_2)	Exuviae (E_2)	Young larvae (L_y)[c]	% Reduction exposed larvae
5 above (Check)	899	175	93	400	203	682	116	—
20 below	524	467	296	15	74	664	65	94
100	204	124	90	3	42	142	13	97
200	218	133	96	24	28	231	8	76
300	265	85	82	162	27	148	16	0

[a] *S. tescorum* 66%, *S. aureum* 25%, *S. argus* 6%, *S. vittatum* 3%, Thousand Palms Oasis, water temp. 22°C.
[b] 4th and older instars.
[c] 3rd and younger instars.

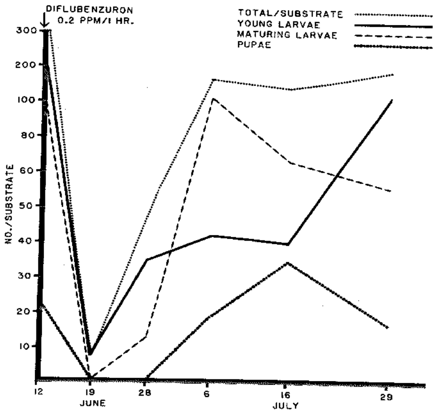

Fig. 2. The recovery of *S. bivittatum* and *S. argus* after treatment with 0.2 ppm/1 h of diflubenzuron.

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.

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