BIOLOGICAL ACTIVITY OF DIFLUBENZURON AND THREE NEW IGRs AGAINST *SIMULIUM VITTATUM* (DIPTERA:SIMULIIDAE)[1]

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ABSTRACT. Three urea analogues, diflubenzuron (1-(4-chlorophenyl)-3-(2,6-diflu orobenzoyl) urea), Bay SIR 6874 (1-[3,5-dichloro-4-(4-nitrophenoxy) phenyl]-3-(2-chlorobenzoyl)-urea), Bay SIR 8514 (1-(4-trifluoromethoxyphenyl) - 3 - (2 - chlorobenzoyl) urea) and a JH analogue Stauffer MV-678 (2-methyl-9-(p-isopropylphenyl)-2,6-dimethylnonane), were compared for activity at 0.02 ppm AI/1h against late instars of *Simulium vittatum*. Mean % mortalities of 70, 100, 86 and 45% were observed respectively. Some abnormalities produced in larvae by the urea analogues are discussed.

Bay SIR 6874 produced mean mortalities in 72-96 h-old *S. vittatum* eggs of 15 and 50% at 0.1 and 0.2 ppm/24 h respectively. Diflubenzuron produced 100% mortality in eggs of the same age at 0.2 ppm/24 h.

The IGR diflubenzuron exhibits larvicidal activity against several anthropophilic and pestiferous black fly species (Lacey and Mulla 1977b, 1978ab). Against certain species, such as *Simulium tescorum* Stone and Boreham, it appears to be as efficacious as some of the conventionally utilized organophosphorous black fly larvicides. Recent development of other urea type of IGRs with favorable mammalian and environmental features may offer additional control alternatives.

This paper presents a comparison of the activity of diflubenzuron with 2 new urea type and a juvenile hormone (JH) analogue having insect growth regulating properties.

METHODS AND MATERIALS

Thirty field-collected penultimate and ultimate instar larvae of *S. vittatum* Zetterstedt per replicate were exposed to 0.02 ppm AI of either diflubenzuron (25% WP), Bay SIR 6874 0.1% technical in acetone, Bay SIR 8514 as 25% WP diluted in water, or Stauffer MV-678 (2-methoxy-9-(p-isopropylphenyl)-2,6-dimethylnonane) as 50% EC. Chemical description and structures of the 3 urea type IGRs are shown in Fig. 1. These IGRs were evaluated in the laboratory utilizing the bioassay unit and procedures described by Lacey and Mulla (1977a,b). Most of the mortality was assessed 2 wk after exposure, except that death at eclosion was recorded on a daily basis. At the termination of the experiment all unemerged pupae were removed and examined for mortality.

Field-collected eggs of *S. vittatum* (ca. 72-96h-old) were exposed to diflubenzuron at 0.2 ppm AI (25% WP diluted and suspended in water) and Bay SIR 6874 at 0.1 and 0.2 ppm AI (0.1% technical in acetone serially diluted in water) in stender dishes for 24 h utilizing the method described in Lacey and Mulla (1977b).

Treatment effects were statistically analyzed with Duncan's Multiple Range Test after arcsin transformation.

RESULTS AND DISCUSSION

The results of exposing *S. vittatum* larvae to the indicated formulations of the 4 IGRs for 1 h are presented in Table 1. The mortality observed with Bay SIR 6874 was significantly higher than any of the other 3 IGRs. Since the mortality response was 100%, an LC_{90} value may be

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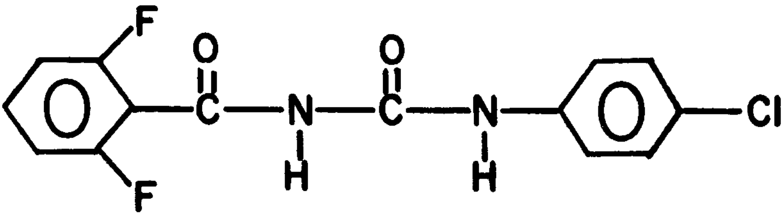
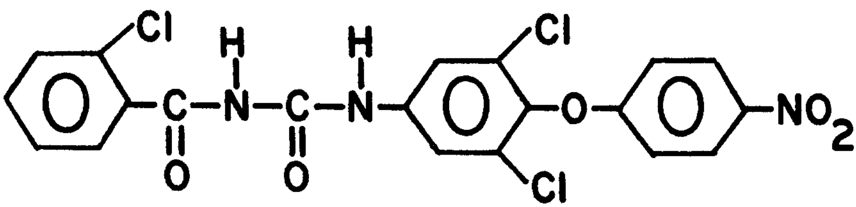
Diflubenzuron



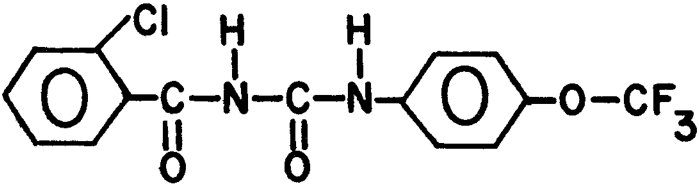
1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea

BAY SIR 6874



1-[3,5-Dichloro-4-(4-nitrophenoxy)phenyl]-3-(2-chlorobenzoyl)urea

BAY SIR 8514



1-(4-Trifluoromethoxyphenyl)-3-(2-chlorobenzoyl)urea

Fig. 1. Chemical description and structural formulae of 3 urea type insect growth regulators (IGRs) evaluated against black fly larvae.

Table 1. Effects of diflubenzuron, Bay SIR 6874, Bay SIR 8514 and MV-678 on *S. vittatum* larvae treated with 0.02 ppm AI/h[a]

Material and Formulation	Mean % mortality ± S. E.[b] in or at Larvae & Intermediate	Pupae	Eclosion	Total	Mean Emergence (%)
diflubenzuron 25WP	59	4±2.59 a	7±2.87 a	70±5.27 c	30±5.27 c
Bay SIR 6874 25WP	99	0 a	1±0.75 a	100 d	0 d
Bay SIR 8514 25WP	81	3±0.95 a	2±1.03 a	86±3.66 c	15±3.66 c
MV-678 50 EC	3	35±6.99 b	7±2.39 a	45±6.29 b	55±6.29 b
Control	4	1±0.75 a	2±1.03 a	7±2.93 a	92±2.12 a

[a] From Mojave River, Victorville, Calif.

[b] Means in the same column followed by the same letter are not significantly different at the .05 level.

considerably lower than 0.02 ppm/1 h. Diflubenzuron and Bay SIR 8514 produced significantly higher mortalities than the JH analogue MV-678. It should be pointed out that the extent of the mortality can vary significantly by using different formulations in the treatments.

The urea type IGRs induce mortality in the treated larvae at the time of molting. When ultimate instars of *S. vittatum* larvae are exposed to the urea analogues, mortality mostly occurs during molting to the pupal stage with the production of larval-pupal-intermediates (Fig. 2). A spectrum of intermediate forms is observed, some individuals retain larval appearance while others resemble pupae more. Younger instars succumb to lower conc (Lacey and Mulla 1978a) usually at the next insterstadial molt (Fig. 3). These larvae undergo a number of abnormal developmental changes. The head capsule as well as the rest of the old integument may be nearly shed, or the process of molting may only be slightly initiated. Some larvae may die between molts from being unable to close the cephalic fans. In these individuals the fans accumulate excess particulate matter making them appear larger. A small and usually insignificant number of individuals (0–10%) die at eclosion, either by remaining stuck to the pupal exuvium or by being unable to clear the water surface, apparently due to wing malformation. Most individuals that have pupated normally go on to emerge successfully. A low percentage of normally formed pupae die before eclosion.

Larvae exposed to the JH analogue (MV-678), however, did not suffer mortality at the time of molting. Most of the mortality in this population occurred during the pupal stage that appeared normal. The suppression of pupation and production of abnormal individuals in treatments with methoprene as reported by Garris and Adkins (1974) and McKague and Wood (1974) were not significant in our studies. The conc and/or period of exposure reported by these authors were considerably greater and these may have been contributing factors in the production of morphogenetic aberrations.

Our findings differ somewhat from those observed when mosquito larvae are exposed to the JH analogue methoprene. Arias and Mulla (1975) reported a significant amount of death at eclosion working with *Culex tarsalis* Coquillett but reported no death of normally formed pupae. No supernumerary instars as reported by Spielman and Skaff (1967) in *Aedes aegypti*.

The effects of Bay SIR 6874 and diflubenzuron on *S. vittatum* eggs are presented in Table 2. Although more active as a black fly larvicide, this compound was far less efficacious as an ovicide than diflubenzuron. Normal appearing unhatched larvae were observed through the chorion in eggs that were killed by both of these compounds.

From these studies it is apparent that the 2 Bay SIR urea analogues as well as

Fig. 2. Larval-pupal intermediates produced in a population of ultimate instar larvae treated with 0.02 ppm diflubenzuron. Similar intermediates are produced with the other urea type IGRs.

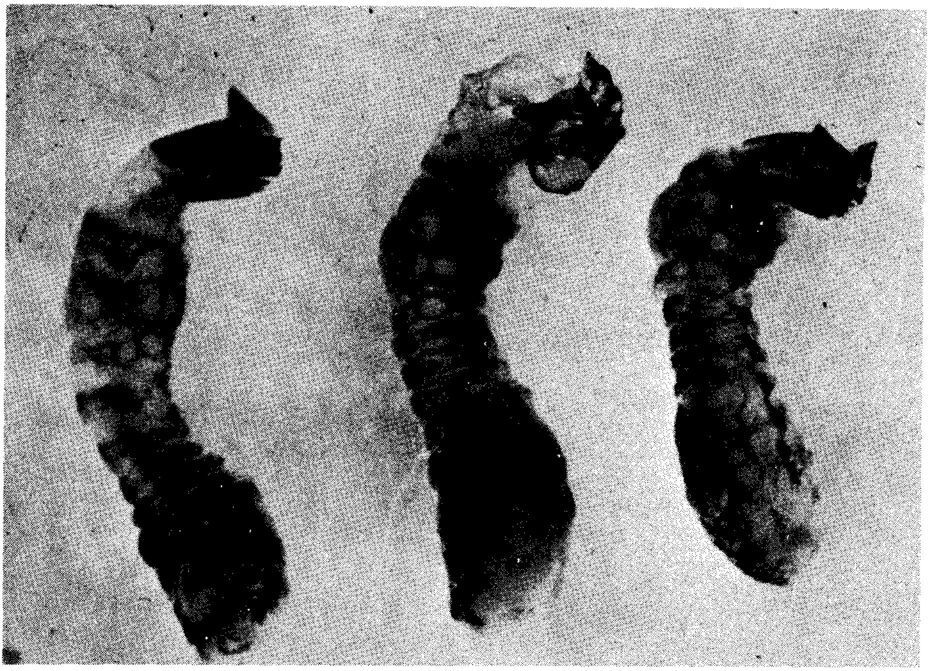


Fig. 3. Molting abnormality in penultimate instar larvae exposed to diflubenzuron. Larva at left head capsule partially shed, in larva at right ecdysis not apparent, in larva at center head capsule almost completely shed.

Table 2. Ovicidal activity of Bay SIR 6874 and diflubenzuron on 72-96h-old eggs of *S. vittatum* (24 h exp.)[a]

Treatment	Mean % mortality ± S. E.[b]
Bay SIR 6874 (0.1 ppm)	15±1.20 b
(0.2 ppm)	50±3.28 c
diflubenzuron (0.2 ppm)	100 d
Control	0 a

[a, b] See footnotes Table 1.

diflubenzuron offer promising potential for the control of *Simulium* vectors. These 3rd generation insecticides having good specificity are just as effective as the currently utilized second generation compounds for the abatement of black flies.

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