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Field Studies on the Impact of a New Benzoylphenylurea Insect Growth Regulator (UC-84572) on Selected Aquatic Nontarget Invertebrates

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Since the discovery of the benzoylphenylurea (BPU) insect growth regulator (IGR) diflubenzuron, several new BPU analogs inhibiting chitin synthesis have been successfully tested against a variety of dipterous vector and nuisance insects, particularly mosquitoes and chironomid midges (Ali and Lord 1980; Ali and Stanley 1981; Schaefer et al. 1978). Recently, a new BPU, UC-84572, tested in the laboratory against seven species of mosquitoes and two species of midges, proved highly toxic with LC_{90} values against mosquitoes ranging from 0.53 ppb (Culex nigripalpus) to 11.64 ppb (Anopheles albimanus) (Ali and Nayar 1987). This new BPU analog was 4 to 13 times more active against five species of mosquitoes and 2 to 4 times against the midges, than diflubenzuron (Ali and Nayar 1987).

The superior insecticidal activity of UC-84572 in the laboratory warranted investigation of its effectiveness in the field. The IGR was applied at different rates to assess control of natural populations of chironomid midges in outdoor ponds. Reported here is the impact of UC-84572 on selected nontarget invertebrates coexisting with the midges. Diflubenzuron (Dimilin(R) 25% wettable powder) was also applied to the ponds as a standard in this study because of its existing large data-base and to compare the adverse effects of the two IGRs on beneficial pond biota.

MATERIALS AND METHODS

The ponds utilized in this study are located at the University of Florida's Central Florida Research and Education Center at Sanford. Each pond is 6x4 m and holds 40-45 cm deep water with the water level in each pond maintained constant by a float valve. The water supply to the ponds is from an Artesian source with adequate natural pressure to maintain the desired water depth.

On May 6, 1985, a 10 EC (emulsifiable concentrate containing 10.8% active ingredient) formulation of UC-84572 (supplied by Union Carbide Agricultural Products Co., Inc., NC) was applied to the ponds at 1, 5, and 10 ppb of the active ingredient (AI), while Dimilin(R) (25 WP) was applied at 10 ppb AI. Each treatment rate of UC-84572 and Dimilin(R) was applied to three ponds (replicates) in a randomized block design; three ponds were left untreated to serve as controls. Separate spray bottles (500-ml) were used for each IGR treatment(s). The required amount of each formulation for a pond was thoroughly mixed with 300 ml tap water in the bottle and applied evenly to the pond surface.

At predetermined intervals after the treatments and on the day of treatments, samples of zooplankton and of free-swimming invertebrates were collected. A zooplankton nylon net (50 cm long, 20 cm diam mouth, and 125 µm pore) mounted on a metal sled was used. A sample was collected by releasing the net from one side of the pond (widthwise) and gently pulling it through the water column by a rope from the opposite side. Two zooplankton samples were collected along the width of each pond on each sampling date. The organisms collected in the net were transferred into bottles and preserved (in formalin). The free-swimming invertebrates were collected by using a 500-ml dip sampler. Five dip samples from each pond (one from each corner and one from the pond middle) were collected, composited, and filtered through a 125 µm Nitex screen. The residue on the screen containing invertebrates was transferred into labeled bottles and preserved.

Air and water temperatures during the study period were measured with a remote recording thermograph. The water temperature was taken by placing the thermister at a fixed location in one pond.

In the laboratory, the macrofauna in the zooplankton and dip samples was visually separated. The remaining material in each sample was then examined under a dissecting microscope (30X magnification). Subsamples of some zooplankton samples (Ali and Mulla 1978) were examined where necessary; the invertebrates were identified (Edmondson 1963) and counted.

Reductions of different invertebrate taxa due to the treatments were assessed by the formula given in Mulla et al. (1971). This formula corrects population reductions in the treated ponds by adjusting with the corresponding simultaneous natural population fluctuations in the control ponds. The pretreatment and periodic posttreatment population data were also compared by analysis of variance and Duncan's Multiple Range Test; the data were transformed to log (n+1) to improve homoscedasticity.

RESULTS AND DISCUSSION

A diverse group of invertebrate fauna was collected in this study. Of the invertebrates collected, populations of Ephemeroptera (*Baetis* sp.), Coleoptera (mostly Dytiscidae, Hydrophilidae, and Haliplidae), Copepoda (*Cyclops* spp. and *Diaptomus* spp.), Cladocera (*Moina* spp., *Alona* sp., *Macrothrix* spp., and *Simocephalus* spp.), and Rotifera were quantitatively sufficient at the time of treatments to elucidate the treatments effects on their populations. Population densities of other invertebrate groups, such as Hemiptera (mostly Corixidae and Notonectidae), Odonata (mostly Anisoptera), Collembola, Hydracarina, Ostracoda, Gastropoda, Oligochaeta, and

Nematoda remained either too low throughout the study period, or were absent from some ponds at the time of treatments to determine any treatment effects.

Nymphs of Baetis sp. were sensitive to UC-84572 and to diflubenzuron (Table 1). At 1 ppb, UC-84572 caused a maximum of 24% reduction of Baetis sp. in the 3 wk posttreatment period, while the 5 and 10 ppb concentrations caused a maximum of 75 and 92% reductions of the mayfly, respectively. Diflubenzuron at 10 ppb induced a maximum of 56% reduction in the mayfly populations within 1 wk posttreatment. The populations of Baetis sp. recovered completely within 2 to 4 wk posttreatment in all treated ponds.

Coleoptera larvae were reduced by UC-84572 at all three rates of treatment (Table 2); the reductions ranged from 41 to 78, 24 to 92, and 41 to 87% at 1, 5, and 10 ppb of UC-84572, respectively. These reductions, however, occurred within 2 wk after treatment and in subsequent observations (21-50 d posttreatment), Coleoptera larvae in the ponds receiving UC-84572 showed a trend similar to that occurring in the control ponds. The larval reductions of Coleoptera in the diflubenzuron treated ponds were apparently of a lesser magnitude than in the UC-84572 treated ponds; however, adult Coleoptera were reduced up to 93% at 10 ppb concentration of diflubenzuron and >80% with 1 to 10 ppb of UC-84572.

Nauplius and adult Copepoda (mostly Cyclops spp.) were adversely affected by UC-84572 and diflubenzuron (Table 3). Reductions of copepod populations during the study period ranged from 57 to 99, 49 to 99, and 77 to 99% at 1, 5, and 10 ppb of UC-84572, respectively. The copepod populations recovered significantly 35 d after treatment in the ponds receiving UC-84572. Diflubenzuron caused 39 to 94% reductions of the copepods during the study period.

Populations of Cladocera (predominantly Moina spp.) remained unaffected at 1 ppb of UC-84572. At the higher rates, UC-84572 caused 71 to 97% reductions (5 ppb) in 1 wk and 31 to 99% reductions (10 ppb) within 2 wk after the treatments (Table 4). Diflubenzuron also caused up to 99% reduction of the Cladocera during the 2 wk after treatment, with complete recovery of their populations occurring during the 22 to 50 d of the posttreatment period.

Rotifera were not affected by any concentration of UC-84572 or by diflubenzuron (data not included). Their mean densities ranged from 19,000 to 149,000, 10,600 to 154,000, and 6,500 to 350,000/250 L in the ponds receiving UC-84572, diflubenzuron, and in the control ponds, respectively.

The impact of UC-84572 and diflubenzuron on Hemiptera, Odonata, Collembola, Hydracarina, Ostracoda, Gastropoda, Oligochaeta, and Nematoda could not be discerned due to the reasons already given. However, the general population increases (data not included) of many of these invertebrates during the posttreatment observations indicated that they were probably not affected (adversely) by UC-84572 or by diflubenzuron at the application rates used.

Table 1. Effects of various rates of insect growth regulators (IGRs) UC-84572 (10 EC) and diflubenzuron (Dimilin(R) 25 WP) on nymphs of the mayfly, *Baetis* sp., in experimental ponds[a] at the aquatic research facility of the University of Florida's Central Florida Research and Education Center at Sanford, Florida (May-June 1985).

IGRs	Treatment rate (AI ppb)	Mean[b] no./5 dips[c] pre-, and posttreatment (days)								Significance
		Pretreat	2	7	14	22	35	42	50	
UC-84572	1	59b	38b	57b	70b	37b	151a	38b	97ab	*
UC-84572	5	90ab	16b	30b	94ab	96ab	128ab	68b	390a	*
UC-84572	10	69ab	8b	7b	88ab	60ab	147a	87ab	147a	*
Diflubenzuron	10	49ab	15b	47ab	67ab	52ab	106a	135a	71a	*
Control	--	76ab	53ab	97ab	109ab	11c	133ab	39bc	182a	*

[a]Ambient water temperature 22-32°C; air temperature 18-37°C.
[b]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other when subjected to analysis of variance and Duncan's Multiple Range Test; data were transformed to log (n+1).
* Significant at 5% level.
[c]400 ml water/dip.
10 EC = 10.8% AI emulsifiable concentrate. 25 WP = 25% AI wettable powder. AI = active ingredient.

Table 2. Effects of various rates of insect growth regulators (IGRs) UC-84572 (10 EC) and diflubenzuron (Dimilin(R) 25 WP) on larval and adult Coleoptera[a] in experimental ponds[b] at the aquatic research facility of the University of Florida's Central Florida Research and Education Center at Sanford, Florida (May-June 1985).

IGRs	Treatment rate (AI ppb)	Mean[c] no./5 dips[d] pre-, and posttreatment (days) Pretreat	2	7	14	22	35	42	50	Significance
					Larvae					
UC-84572	1	28a	3b	4b	2bc	2bc	0d	1cd	0d	**
UC-84572	5	22a	3b	1bc	2bc	3bc	1bc	1bc	0c	**
UC-84572	10	28a	5b	2bc	2bc	2bc	1c	1c	0c	**
Diflubenzuron	10	21a	20ab	11ab	5b	1c	1c	1c	1c	**
Control	--	25a	12a	14a	3b	1c	0c	1c	0c	**
					Adult					
UC-84572	1	19a	11ab	6abc	7abc	12ab	5bc	3bc	1c	*
UC-84572	5	7a	5a	2ab	3ab	6a	3ab	3ab	1b	*
UC-84572	10	14a	3ab	8ab	5ab	12a	10a	2ab	1b	*
Diflubenzuron	10	14a	12a	6ab	7ab	6ab	1c	4abc	1c	*
Control	--	7abc	2cd	11ab	13a	8abc	7abc	2bcd	1d	*

[a]Dytiscidae, Hydrophilidae and Haliplidae Coleoptera.

[b]Ambient water temperature 22-32°C; air temperature 18-37°C.

[c]Means in a row followed by the same letter are not significantly different (P>0.05) from each other when subjected to analysis of variance and Duncan's Multiple Range Test; data were transformed to log (n+1). *,** Significant at 5% and 1% levels, respectively.

[c]400 ml water/dip.

10 EC = 10.8% AI emulsifiable concentrate. 25 WP = 25% AI wettable powder.

AI = active ingredient.

Table 3. Effects of various rates of insect growth regulators (IGRs) UC-84572 (10 EC) and diflubenzuron (Dimilin(R) 25 WP) on Copepoda[a] in experimental ponds[b] at the aquatic research facility of the University of Florida's Central Florida Research and Education Center at Sanford, Florida (May-June 1985).

IGRs	Treatment rate (AI ppb)	Mean[c] no./250 liters pre-, and posttreatment (days)								Significance
		Pretreat	2	7	14	22	35	42	50	
UC-84572	1	363,467a	844c	144c	37,400b	25,211b	27,500b	25,000b	37,933b	**
UC-84572	5	178,833a	44,000abc	33c	633bc	300bc	1,889abc	3,889ab	26,511ab	*
UC-84572	10	199,922a	1,344cde	89e	78de	33e	967bcd	7,378abc	9,378ab	**
Diflubenzuron	10	317,233ab	11,289ab	5,956b	21,978ab	21,722ab	13,989ab	30,778a	38,856a	*
Control	--	71,344	41,200	13,056	46,967	38,911	27,522	11,344	20,622	NS

[a]Only nauplius and adult *Cyclops* spp. until day 14 posttreatment; mean numbers shown under days 22-50 posttreatment include 0-15% nauplius and adult *Diaptomus* spp.
[b]Ambient water temperature 22-32°C; air temperature 18-37°C.
[c]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other when subjected to analysis of variance and Duncan's Multiple Range Test; data were transformed to log (n+1).
*,** Significant at 5% and 1% levels, respectively. NS = not significant.
10 EC = 10.8% AI emulsifiable concentrate. 25 WP = 25% AI wettable powder. AI = active ingredient.

Table 4. Effects of various rates of insect growth regulators (IGRs) UC-84572 (10 EC) and diflubenzuron (Dimilin(R) 25 WP) on Cladocera[a] in experimental ponds[b] at the aquatic research facility of the University of Florida's Central Florida Research and Education Center at Sanford, Florida (May-June 1985).

IGRs	Treatment rate (AI ppb)	Mean[c] no./250 liters pre-, and posttreatment (days): Pretreat	2	7	14	22	35	42	50	Significance
UC-84572	1	50,756a	30,333a	7,378ab	9,056ab	3,089b	1,256b	4,022b	3,389b	*
UC-84572	5	36,456a	322b	900b	22,156a	22,211a	26,744a	28,589a	10,889a	**
UC-84572	10	80,844a	33c	56c	6,678b	5,189ab	13,978ab	22,778ab	14,167ab	**
Diflubenzuron	10	89,400a	222b	89b	12,400a	11,089a	28,689a	16,933a	7,122a	**
Control	--	84,956	22,321	7,111	10,233	3,533	7,644	2,422	7,722	NS

[a]Predominantly (>95%) *Moina* spp. until day 14 posttreatment; mean numbers shown under days 22-50 posttreatment included *Alona* sp. (0-10%), *Macrothrix* spp. (0-32%), and *Simocephalus* spp. (0-15%).
[b]Ambient water temperature 22-32°C; air temperature 18-37°C.
[c]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other when subjected to analysis of variance and Duncan's Multiple Range Test; data were transformed to log (n+1).
*,** Significant at 5% and 1% levels, respectively. NS = not significant.
10 EC = 10.8% AI emulsifiable concentrate. 25 WP = 25% AI wettable powder. AI = active ingredient.

It is evident from this study that field applications of UC-84572 at rates up to 10 ppb AI or higher (to control midges or mosquitoes) would cause simultaneous adverse effects on some nontarget aquatic invertebrates, such as nymphal Ephemeroptera, larval and adult Coleoptera, Cladocera, and Copepoda. Previously studied BPUs, including diflubenzuron, Bay SIR-8514, and UC-62644 used at field rates comparable to the ones employed in this study had also adversely affected such nontarget organisms (Ali and Lord 1980; Ali and Stanley 1981; Apperson et al. 1978; Miura and Takahashi 1974). These adverse effects, however, are temporary and usually short-lived as shown in the present study and in other previous studies. No displacement or replacement of any animal group was noted in the ponds indicating that UC-84572 did not cause any long-term or permanent alterations in the aquatic food-chain. Thus, the superior activity of UC-84572 over diflubenzuron against target nuisance and vector Diptera (mosquitoes and midges) merits its further development and this new IGR has an excellent potential for their control.

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