Effects of the Insect Growth Regulator Diflubenzuron on Non-Target Aquatic Populations in a Louisiana Intermediate Marsh[1]

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ABSTRACT

A study was conducted in a Louisiana coastal marsh to determine the ecological impact of the insect growth regulator diflubenzuron on populations of non-target aquatic organisms.

Six applications of diflubenzuron (28 mg AI/ha) over an 18-mo period caused statistically significant differences in the population density of aquatic organisms when treated and untreated populations were compared. Populations of 5 taxa (nymphs of *Trichocorixa louisianae* Jaczewski and *Buenoa* spp., Coenagrionidae naiad spp., *Berosus infuscatus* LeConte adults, and *Hyalella azteca* (Saussure)) were significantly ($P < 0.01$) reduced while populations of 15 taxa (*Physa* sp., *Caenis* sp. and *Callibaetis* sp. naiads, Noteridae larvae, *Hydrovatus cuspidatus* Kunze adults, *Hydrovatus* sp. larvae, Dytiscidae (tribe Bidessini) larvae, *Mesovelia mulsanti* Jaczewski adults, *Trichocorixa louisianae* adults, larvae of Chironomidae, Ephdridae, Dolichopodiae, and Tabanidae, and the fishes *Gambusia affinis* (Baird and Girard) and *Jordanella floridae* (Goode and Bean) showed significant ($P < 0.05$) increases after exposure to diflubenzuron. The analysis of data on the 27 remaining taxa collected indicated no statistically significant ($P > 0.05$) difference when the treated and untreated populations were compared.

Several studies have indicated the susceptibility of various mosquito species to diflubenzuron (Jakob 1973, Hsieh and Steelman 1974, Lowe et al. 1975, Steelman et al. 1975, Mulla and Darwazeh 1975a, Rathburn and Boike 1975, Schaefer et al. 1975), but few comprehensive studies on the effects of mosquito control agents on non-target aquatic organisms have been conducted. Available information generally consists of incidental observations made during studies of the effects of Insect Growth Regulators (IGR) on mosquitoes (Steelman and Schilling 1972, Steelman et al. 1975, Pelsue et al. 1974, Miura and Takahashi 1975, Mulla and Darwazeh 1975b, Mulla et al. 1975). However, laboratory studies to determine the effects of IGR's on non-target organisms have been reported by Miura and Takahashi (1974a), Miura and Takahashi (1974b), and Miura et al. (1975).

This paper reports the results of a study conducted in a Louisiana coastal intermediate marsh to determine the impact of the IGR, diflubenzuron, on populations of several aquatic organisms.

Materials and Methods

Two adjoining 20-ha plots were selected in an intermediate marsh (salinity less than 3 parts/thousand) habitat in hydrologic unit VIII of the chenier plain zone (Chabreck 1972) near Grand Chenier in Cameron Parish, Louisiana. The southern portion of each plot was of slightly higher elevation while the northern portion was permanent marsh with large open water areas characterized by little or no emergent vegetation.

The water depth ranged from 0–26.7 cm with an avg of 16 cm during the 18-mo study period and varied with rainfall and drying conditions. Drainage control structures on the canals within the marsh afforded some stability to the marsh conditions. Water salinity values obtained during the study ranged from 1.7–3.5 parts/thousand. The pH of the marsh water averaged 6.65 with a range of 3.55–7.65.

The distribution of plant species in the study sites is shown in Table 1. A gradation in water depth occurred from south to north with the shallow flooded areas on the south end covered with emergent vegetation such as *Paspalum vaginatum*, *Bacopa monnieri*, and *Spartina patens* and the deeper (open) water area to the north characterized by no emergent vegetation although *Sagittaria graminae* was submerged during a portion of the year.

The treated plot received an aerial application of diflubenzuron on the following dates: June 11, 1974; Sept. 20; 1974, Jan. 21, 1975; Apr. 17, 1975; June 13, 1975; and Sept. 15, 1975. A Cessna Ag Wagon aircraft equipped with a spray system using 6 D-4 nozzles was used to apply diflubenzuron at a rate of 28 gm AI/ha.

Samples were collected at ca. 2-wk intervals throughout the 18-mo study. A sampling device was constructed of 3.18 mm (1/8") sheet metal and resembled a 0.0283-m^3 (1 ft^3) box open at both ends and was designed to sample 0.09 m^2 (1 ft^2) of marsh. Angle iron (12.7 mm) was used to reinforce the upper edges of the device and a band of angle iron was welded to the outside 7.6 cm above the bottom. The lower edges were sharpened to facilitate cutting through the sod and substratum. The lower band of angle iron insured the uniform implantation of the device to a depth of 7.6 cm into the soil.

Ten 0.09-m^2 samples were taken randomly from

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Table 1.—Distribution of plant species recorded in the vegetation transect (south to north) through the center of the study plots in Oct. 1975.

	% species occurring within the designated area														
	South						Meters								North
Plant	0–30	30–60	60–90	90–120	120–150	150–180	180–210	210–240	240–270	270–300	300–330	330–360	360–390	390–420	420–450
Cynodon dactylon (Linnaeus)	60	50	60	50	50										
Spartina patens (Aiton)	35	48	30	50	50						60	50		60	
Sagittaria graminae Michaux						20	20	25	80	70	15		10		25
Paspalum vaginatum Swartz						70	70	25		30	15	10	35		50
Zizaniopsis miliaceae (Michaux) Doll and Ascherson								30	20		5				
Eleocharis walteri												30	35	5	
Phragmites communis Trinius													5	10	20
Juncus roemerianus Scheele			10					5					5		
Bacopa monnieri (Linnaeus) Persoon							5								
Cyperus erythrohizos Muhlenburg															5
Other species[a]	5	2				10	5	15			5	10	10	25	

[a] *Alternanthera philoxeroides* (Martius) Grisebach, *Eleocharis* sp., *Polygonum* sp., *Sagittaria falcata* Pursh, *Scirpus olneyi* Gray, *Scirpus robustus* Pursh, *Scirpus validus* Vahl, *Stetaria geniculata* (Lamark) Beauvois.

each of the plots and consisted of 5 taken in open water areas and 5 in areas having abundant emergent vegetation. Samples were taken by randomly placing the sampler into the marsh to a depth of 7.6 cm. The water depth was measured inside the sampler and the vegetation within the sample pulled up and washed in the sample water. The top 2.54 cm of debris and mud was then agitated to facilitate collection of benthic organisms and the entire water sample was immediately removed from the sampler by means of a rectangular one-liter can and filtered through a 100-mesh (40.8/cm) bag. The resulting mass of debris and organisms was placed in 95% ethyl alcohol and transported to the laboratory. Additionally, water and soil temperature and pH were measured at the site on each collection date.

The statistical design used in this experiment was a 29×2×2 factorial arrangement of treatments in a completely randomized design. There were 29 dates, 2 areas (treated vs. control), 2 subareas (emergent vegetation vs. open water) and 5 random samples per subarea. The analysis of variance for the date, area, and subarea was determined along with those of all possible interactions. This design resulted in 290 samples collected in each of the treated and control areas. A standard transformation ($\sqrt{x+1}$) was performed on the data in order to reduce the possibility of error resulting from skewed population distributions. The efficiency of the experiment was determined by calculating the least significant difference (LSD) for each source of variation within the analysis.

Results and Discussion

The macroscopic aquatic fauna collected in this Louisiana intermediate marsh included 3 phyla, 6 classes, 17 orders, and more than 46 families and 75 genera collected from the test site over an 18-mo study period. A severe drought caused the entire marsh in the area, including the test plots, to dry completely from early July–late Aug., 1974. No aquatic samples were taken during this period.

A highly significant ($P < 0.01$) reduction was detected in the following non-target aquatic organism populations after 6 applications of diflubenzuron when compared with untreated populations: *Hyalella azteca* (Saussure) (Amphipoda: Talitridae); Coenagrionidae naiads (Odonata); *Trichocorixa louisianae* Jaczewski (Hemiptera: Corixidae) nymphs; *Buenoa* spp. nymphs (Hemiptera: Notonectidae); and *Berosus infuscatus* (LeConte) (Coleoptera: Hydrophilidae) adults (Table 2).

The *H. azteca* populations in both habitats of the treated plot were significantly reduced (Fig. 1) with the greater reduction in population numbers occurring in the vegetative habitat (73%) as compared to 36% in the open water habitat.

Throughout the 18-mo study the Coenagrionidae naiads showed a highly significant ($P<0.01$) preference for the habitat that contained emergent vegetation in both plots (Table 2). This vegetative habitat provided considerable protection from the treatments in that the population numbers were reduced by 26% while a reduction of 52% occurred in the open water habitat.

During the 18-mo study *T. louisianae* nymphs were significantly ($P<0.01$) reduced by the diflubenzuron applications. Greater nymph mortality occurred in the open water habitats (62%) than in the vegetative habitats (2%). No statistical difference ($P>0.05$) was shown between the 2 habitats of the untreated plot. Miura and Takahashi (1975) and Miura et al. (1975) reported "slight" nymphal mortality in Corixidae populations when irrigated pastures and experimental ponds were treated with diflubenzuron. Steelman et al. (1975) reported no

Table 2.—Mean numbers of aquatic organisms significantly ($P<0.01$) reduced after exposure to 6 applications of diflubenzuron (28 gm AI/ha/application) over an 18-mo period in a Louisiana intermediate marsh.

		$\overline{X}$ no. organisms/0.09 m²					
		Untreated			Treated		
Species		Plants vs.	Open	$\overline{X}$	Plants vs.	Open	$\overline{X}$
Hyalella azteca	A&I[a]	5.42	3.88	4.65[c]	1.48	2.50	1.99
Coenagrionidae	I	1.83[b]	1.28	1.56[c]	1.36[b]	0.61	0.99
Trichocorixa louisianae	I	5.57	13.76[b]	9.69[c]	5.46	5.23	5.34
Buenoa spp.	I	0.30	0.28	0.29[c]	0.10	0.09	0.09
Berosus infuscatus	A	0.88	1.10	0.99[c]	0.65	0.34	0.49

[a] A = Adults, I = Immature.
[b] Significantly ($P < 0.01$) higher when comparing plants vs. open.
[c] Significantly ($P < 0.01$) higher than treated.

significant reduction in nymphal populations of Corixidae in rice fields.

Of great interest is the lack of synchrony of the *T. louisianae* reproductive cycles in the 2 plots (Figure 2). The largest numbers of nymphs were collected in the treated and control plots on Apr. 3, 1975 and May 13, 1975, respectively. This difference of 6 wk is striking considering that the environmental influences on these 2 populations, which were only 600 m apart, were similar except for the IGR in the treated plot. The results were 2 non-coincident generation peaks which were probably influenced by the diflubenzuron treatments.

The diflubenzuron applications caused a highly significant ($P<0.01$) reduction in the *Buenoa* spp. nymph population (69%) during the 18-mo study (Table 1). No significant ($P>0.05$) habitat preference was shown in the control plot. The effects of the treatments were equal on the organisms in both the habitat containing emergent vegetation and the open water habitat.

Miura and Takahashi (1974a) reported suppression of reproduction in selected Notonectidae with no eggs or nymphs being observed in artificial containers for more than 2 mo after treatment. Miura and Takahashi (1975) and Miura et al. (1975) ob-

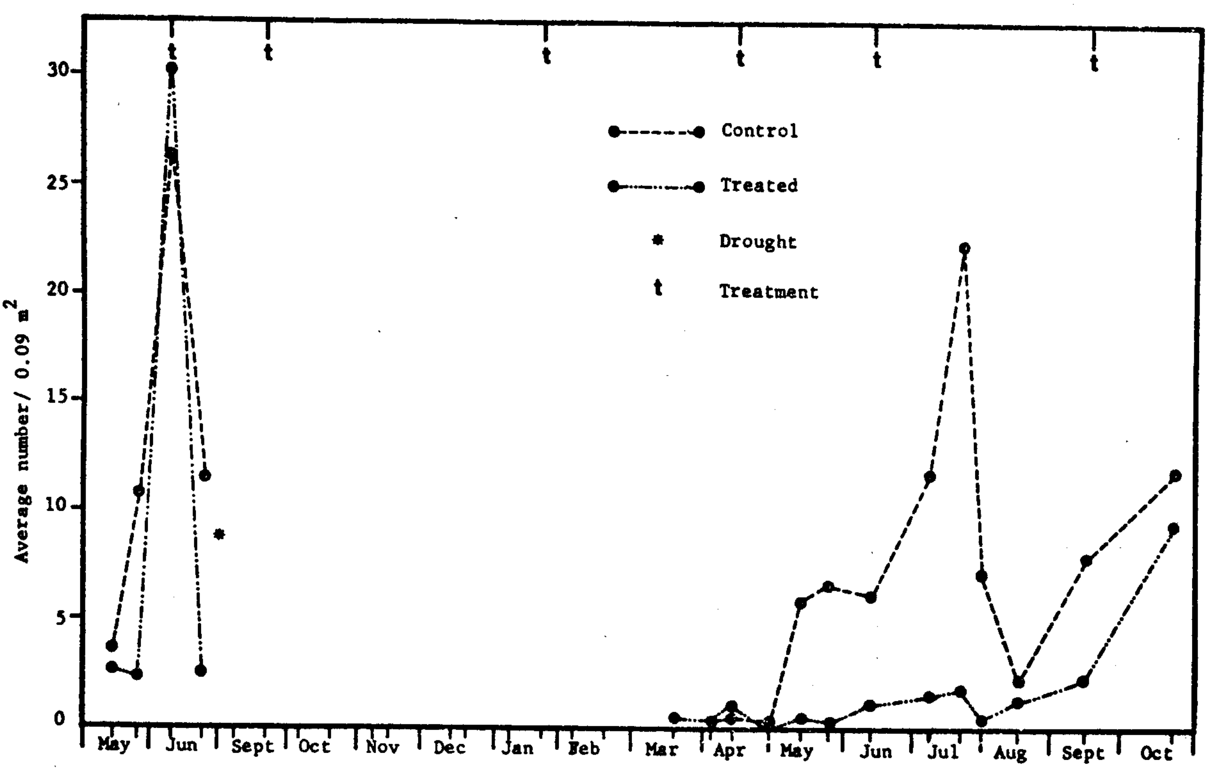

FIG. 1.—Avg number of *Hyalella azteca* collected in treated and control plots in a Louisiana intermediate marsh habitat, May 14, 1974–Oct. 26, 1975.

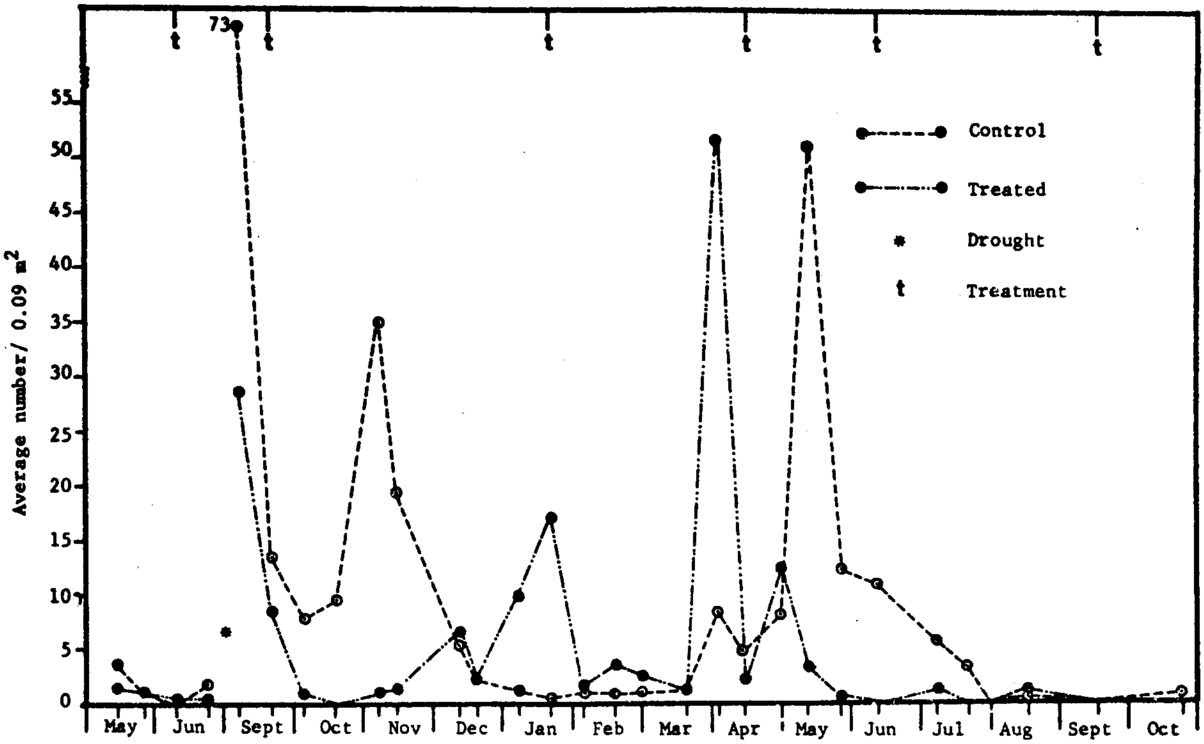

FIG. 2.—Avg number of *Trichocorixa* sp. nymphs collected in treated and control plots in a Louisiana intermediate marsh habitat, May 14, 1974–Oct. 26, 1975.

served "slight" nymphal mortality in Notonectidae when irrigated pastures and experimental ponds were treated and Steelman et al. (1975) reported no significant ($P>0.05$) reduction in nymphal populations of Notonectidae in rice plots.

Adult *Berosus infuscatus* occurred in high numbers ($12+/0.09m^2$) in the control plot following the drought of 1974 but remained at less than 1.0 adult/ 0.09 m^2 throughout 1975. No adults were present at the time of the 1st treatment although *Berosus* spp. larvae were present in both the treated and control plots on that date. The population of adults in the control plot increased during the months following the initiation of treatments while the population in the treated plot remained low and did not reach the level of that in the control plot until ca. 30 days after the last treatment. The numbers of adults in the treated plot may reflect the possible effects on late instar larvae or pupae of these beetles.

Berosus infuscatus adults exhibited no significant ($P>0.05$) preference for either habitat (Table 1), but there was a significant ($P<0.01$) difference between the populations in the treated and control plots. This difference may have been due to a reduction in the emergence of adults from treated *Berosus* spp. larvae although the larval populations of *Berosus* spp. showed no effect of the diflubenzuron treatment.

Reports of the effect of diflubenzuron on *B. infuscatus* adults could not be found in the literature. The only statistical analysis on any Hydrophilidae population affected by diflubenzuron was reported by Steelman et al. (1975) in which a highly significant ($P<0.01$) reduction in *Tropisternus* spp. adult populations in rice plots was observed.

Several aquatic organisms had significant ($P<0.05$ and <0.01) population increases after the 6 diflubenzuron applications when population numbers from the treated and untreated plots were compared (Table 3). These were: *Physa* sp. (Basommatophora: Physidae); adults of *Hydrovatus cuspidatus* Kunze (Coleoptera: Dytiscidae), *Mesovelia mulsanti* Jaczewski (Hemiptera: Mesoveliidae), and *T. louisianae* (Hemiptera: Corixidae); naiads of *Caenis* sp. (Ephemeroptera: Caenidae) and *Callibaetis* sp. (Ephemeroptera: Baetidae); larvae of Noteridae (Coleoptera), *Hydrovatus* sp., and Bidessini (Coleoptera: Dytiscidae); Chironomidae, Ephydridae, Dolichopodidae, and Tabanidae (Diptera); and the fishes *Gambusia affinis* (Baird and Girard) (Cypriniformes: Poeciliidae) and *Jordanella floridae* Goode and Bean (Cypriniformes: Cyprinodontidae).

The population increases of certain organisms could be attributed to greater survival due to the diflubenzuron treatments which reduced populations of predators that prey on these species. Statistical analyses of these data showed that the populations of 4 out of the 5 aquatic organisms that were significantly reduced by the 6 treatments showed significant ($P<0.05$ to $P<0.01$) negative correlations with certain species that had significant ($P<$ 0.01) population increases. Thus, as the population numbers of certain species were decreased, a direct increase in population numbers occurred in certain other species.

Significant ($P<0.05$ to $P<0.01$) negative correlations occurred between *Buenoa* spp. nymphs and Chironomidae larvae; Coenagrionidae naiads and larvae of Chironomidae and Dolichopodidae; *Berosus infuscatus* adults and larvae of Chironomidae, Noteridae, and Dytiscidae and naiads of *Callibaetis* sp.; and *Trichocorixa louisianae* nymphs and naiads of *Caenis* sp. and *Callibaetis* sp. and larvae of *Hydrovatus* sp., Bidessini and Ephydridae. These data show that as the populations of certain species (in this case known as probable predators) were reduced by the diflubenzuron treatments, a statistically significant increase occurred in certain prey populations. This influence of chemical treatments on predator-prey relationships was reported by Steelman et al. (1975) wherein significant reductions in the number of *Tropisternus* sp. adults were significantly related to an increase in the number of Baetidae naiads. Data supportive of the predator-prey relationships shown by the present study have been published. The backswimmer (*Buenoa* spp.) has been reported to feed on small crustacea, chironomid and mosquito larvae and fish fry (Usinger 1956). The naiads of Coenagriondiae feed on many aquatic organisms (Smith and Pritchard 1956). The *Berosus* (Hydrophilidae) adults are largely herbivorous but a few have been reported to be predaceous (Leech and Chandler 1956). The corixids are primary converters of plant material (Usinger 1956); however, certain species have been reported to act as typical predators feeding on small midge and mosquito larvae (Griffith 1945).

Population increases in predatory fish and insects could be a direct result of an abundance of prey. More *Gambusia* and *Jordanella* were collected in the treated plot than in the control plot. Washino and Hokama (1967) reported the following insect orders to comprise a significant proportion of the diet of *Gambusia affinis:* Ephemeroptera, Odonata, Hemiptera, Coleoptera, and Diptera. The fact that several of the organisms having significant population increases are known to be important food items of *Gambusia* suggests the possibility that the fish were responding to an abundance of food rather than to any direct effect of diflubenzuron.

After analysis of the data the following aquatic organisms indicated no statistically significant ($P>0.05$) reduction or increase due to the 6 diflubenzuron treatments: adult *Buenoa* sp. (Hemiptera: Notonectidae), *Berosus exiguus* (Say) (Coleoptera: Hydrophilidae), *Tropisternus lateralis* (Fabricius) (Coleoptera: Hydrophilidae), *Enochrus blatchleyi* (Fall) (Coleoptera: Hydrophilidae), *Laccophilus proximus* (Say) (Coleoptera: Dytiscidae), *Liodessus affinis* (Say) (Coleoptera: Dytiscidae), *Hydrocanthus* sp. (Coleoptera: Noteridae), *Suphisellus* sp. (Coleoptera: Noteridae), *Celina angustata* Anbe (Coleoptera: Dytiscidae), *Onychylis nigrirostris* (Boheman) (Coleoptera: Curculionidae), and *Listronotus appendiculatus* (Say) (Coleoptera: Curculionidae); adult and larvae *Lissorhoptrus* sp. (Coleoptera Curculionidae); adult and nymph *Belostoma* sp. (Hemiptera: Belostomidae); mixed stages and ages *Taphromysis louisianae* (Banner) (Mysidacea: Mysidae), *Palaemonetes paludosus* (Gibbs) (Decapoda: Palaemonidae), *Cambarellus* sp. and *Procambarus clarki* (Girard) (Decapoda: Astacidae); larval *Berosus*

Table 3.—Mean numbers of aquatic organisms significantly ($P<0.01$) increased after exposure to 6 applications of diflubenzuron (28 gm AI/ha/applications) over an 18-mo period in a Louisiana coastal marsh.

Species		$\bar{X}$ no. organisms/0.09 m^2					
		Untreated			Treated		
		Plants	Open	$\bar{X}$	Plants	Open	$\bar{X}$
Physa sp.	A&I[a]	0.61[b]	0.17	0.39	0.80[b]	0.61	0.71[c]
Caenis sp.	N	0.06	0.12[b]	0.09	0.28[b]	0.06	0.17[d]
Callibaetis sp.	N	0.59	0.65	0.62	1.50[b]	0.49	0.99[c]
Noteridae	I	1.23[b]	0.39	0.81	1.38[b]	0.77	1.08[c]
Hydrovatus cuspidatus	A	0.35[b]	0.08	0.21	0.76[b]	0.20	0.48[c]
Hydrovatus sp.	I	0.37[b]	0.06	0.22	0.67[b]	0.09	0.38[d]
Bidessinae	I	0.77[b]	0.12	0.45	1.50[b]	0.24	0.87[d]
Mesovelia mulsanti	A	0.08	0.03	0.06	0.24[b]	0.09	0.17[c]
Trichocorixa louisianae	A	3.25	13.17[b]	8.21	5.40	18.72[b]	12.06[c]
Chironomidae	I	7.12	32.14[b]	19.63	10.98	32.68[b]	21.83[d]
Ephydridae	I	6.30[b]	1.00	3.65	5.93[b]	2.70	4.31[c]
Dolichopodidae	I	0.32[b]	0.04	0.18	0.96[b]	0.12	0.54[c]
Tabanidae	I	0.04	0.08	0.06	0.17	0.12	0.14[d]
Gambusia affinis	A&I	1.32[b]	0.61	0.99	1.17	1.50[b]	1.33[c]
Jordanella floridae	A&I	0.01	0.04[b]	0.02	0.0	0.13[b]	0.07[d]

[a] A = Adult, I = Immature, N = Nymphs or Naiads.
[b] Significantly ($P<0.01$) higher when comparing plants vs. open.
[c] Significantly ($P<0.01$) higher than untreated.
[d] Significantly ($P<0.05$) higher than untreated.

sp., *Tropisternus* sp. and *Enochrus* sp. (Coleoptera: Hydrophilidae); *Laccophilus* sp. (Coleoptera: Dytiscidae); Muscidae and Stratiomyiidae (Diptera); and naiads of *Pachydiplax* and *Belonia* sp. (Odonata: Libellulidae), *Anax* sp. (Odonata: Aeschnidae), and nymphs of *Mesovelia* sp. (Hemiptera: Mesoveliidae).

These data indicate that the 6 applications of diflubenzuron (28 mg AI/ha) over the 18-mo period differentially affected species inhabiting this intermediate marsh community. However, the effects of the drought that occurred in thousands of ha of marsh land from early July–late Aug., 1974 caused a more severe impact on the aquatic organisms than did the diflubenzuron treatments. The area of marsh land that would receive treatment for mosquito control is small since a relatively small proportion of this ecosystem (probably less than 25%) actually breeds mosquitoes. Data obtained during this study support the following conclusions: (1) Diflubenzuron applied to mosquito breeding areas of marsh caused reductions in populations of some non-target species, but none of the organisms affected were completely eliminated from the ecosystem; and (2) the untreated marsh areas would provide populations of aquatic organisms that could repopulate the treated areas.

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