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Diflubenzuron Application to Citrus and Its Impact on Invertebrates in an Adjacent Pond

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In the past two decades, the insect growth regulator (IGR), diflubenzuron (Dimilin®), has shown excellent activity against economically important terrestrial insects (Flint et al. 1977; Granett and Dunbar 1975). This IGR is highly effective against aquatic dipterans, such as Chaoboridae, Chironomidae, and Culicidae, at field rates ranging from 2.5 to 16 ppb (Ali et al. 1978; Apperson et al. 1978; Mulla et al. 1975). Diflubenzuron is also a promising control agent of the citrus rust mite (Phyllocoptruta oleivora) inhibiting its molting in the 2nd nymphal stage at dosages of 0.04-0.3 g AI/liter (lab. bioassay); at 0.15 g AI/liter, the IGR gave excellent control of citrus rust mite in the field (McCoy 1978).

Diflubenzuron, when applied directly to water for control of aquatic dipterans, simultaneously reduces some invertebrate populations for short periods of time (Ali and Lord 1980; Ali and Mulla 1978). The purpose of this study was to assess adverse effects on invertebrate populations in a pond located amid citrus trees and receiving air-drifted diflubenzuron from surrounding citrus area commercially treated for the control of citrus rust mite.

MATERIALS AND METHODS

Two natural ponds located in a citrus grove in Winter Garden, FL, were selected. One somewhat circular pond, exposed to diflubenzuron air-drifted from an adjacent citrus treatment area, was ca. 2 ha at the surface (Fig. 1). The pond center was at 81° 33' 12" E longitude and 28° 26' 35" N latitude, and three-fourths of its border was lined by citrus trees (orange var. "Valencia"). The pond was connected to a lake (ca. 70 ha) by a canal. The exposed pond contained two different depth zones; 1 m deep along the periphery and 2-3 m deep in the middle. Water was clear, with ca. 60% of the bottom area visible. The 1 ha, oblong control pond, located 0.4 km NE of the exposed pond and diflubenzuron treated area, was parallel to and connected at its NW end to the lake. The control pond was up to 1 m deep, with pond bottom visible throughout.

Decomposed and decaying plant materials formed a 10-15 cm thick mat on the bottom of both ponds. The deep area of the exposed pond had >15 cm deep detritus and muck on the bottom. In shallow areas of

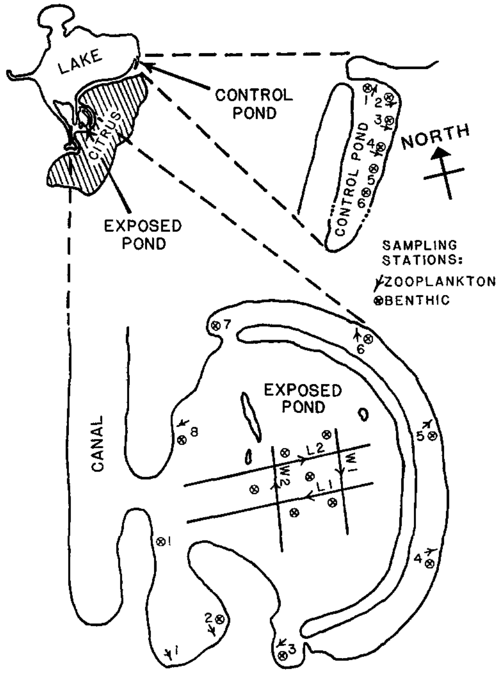

Figure 1. Diagrammatic sketch of the exposed and control ponds in relation to diflubenzuron treatment to adjacent citrus area. Approximate sites of zooplankton and benthic samplings in each pond are shown.

the exposed pond and in the entire control pond, rooted vegetation (predominantly water lily, Nymphaea adorata, spike rush, Eleocharis obtusa, and hat pin, Eriocaulon spp.) prevailed. The deep area of the exposed pond contained patches of floating vegetation.

Diflubenzuron was applied on July 15, 1986 (8:35 - 9:10 A.M.), to 0.8 ha of citrus immediately surrounding the exposed pond, under wind speeds of 0.8-1.6 km/h, with gusts up to 10 km/h from the ESE, generally from the grove toward the exposed pond. An FMC 9100 CP airblast sprayer, with nine nozzles on each side, each equipped with a no. 4 ceramic disc backed with 3-hole whirl plate was used. Details of methodology of diflubenzuron application and the prevailing environmental conditions during the experiment were given in Nigg and Stamper (1987). Briefly, after initial checking and calibration for delivery of 4,677 L/ha or 32.2 L/tree, the sprayer tank was filled to 3,875 L mark with water, and 19 L of spray oil (FCC-435) at 0.5% (v/v) was added. A total amount of 1.81 kg diflubenzuron (Dimilin® 25 WP) was used to make 3.63 L of slurry added to the spray tank. The mixture was constantly agitated in the tank for 10 min prior to and during the application. The sprayer delivered 91 L/min at 2.82 km/h resulting in a rate of 0.56 kg AI/ha. Nozzles were positioned to deliver 15% of the spray mixture to the upper portion, 65% to the middle portion, and 20% to the lower portion of a tree.

Quantitative samples of zooplankton, benthic invertebrates, and emergence of adult insects from the exposed and control ponds were collected at 4 and 1 day prior to the treatment, and at 1, 3, 7, 14, 28, and 56 days posttreatment. Benthos and adult emergence were not sampled 1-day and 56-days posttreatment, respectively.

Eight sites in shallow areas and 6 sites in the deep area of the exposed pond were established for bottom sampling; zooplankton samples were collected at 7 shallow and 4 deep sites (Fig. 1). In the control pond, 6 sites for bottom and 4 for zooplankton samplings were selected. The sites in both ponds were spaced to cover different areas of each pond.

The bottom of shallow areas of exposed and control ponds was sampled by using a 55 cm long nylon net (0.5 mm pore) fitted onto a 20 x 20 cm metal frame with a firm handle. To collect a bottom sample, the operator approached a site by carefully wading through an adjacent area, causing minimal disturbance, and gently placed the net vertically on the bottom and dragged the net toward himself for a distance of ca. 40 cm along the bottom. A gentle downward force had to be applied while dragging the net against the bottom. The net was then lifted out of water in an upward sweeping motion. The material collected in the net was transferred into a labeled container. Bottom samples from the deep area of exposed pond were collected with a 15 x 15 x 15 cm Ekman dredge.

Zooplankton samples from shallow sites of the exposed pond and from the control pond were collected with a 50-cm long conical nylon net (20 cm diam. mouth and 125 μm pore). To collect a sample, the net was held submerged and parallel to the pond bottom and released from one end and pulled through the water column with a gentle, uniform speed from the other end for a distance of 6 m, while keeping it submerged and without touching the bottom. The net was taken out of the water in an upward sweeping motion to concentrate the captured fauna. The collection was transferred into a labeled bottle containing formalin. Zooplankton samples from the deep area of the exposed pond were taken by towing the plankton net behind a motor boat driven at a speed of 10 km/h. Four zooplankton samples from two predetermined parallel rows were taken (Fig. 1); each row along the length and width measured 50 and 30 m, respectively. While sampling, the net remained 5-10 cm below water surface and was pulled out of water in an upward sweeping motion. The collected material was transferred into a labeled bottle containing formalin. Zooplankton sampling at each site preceded bottom sampling.

Adult emergence from the exposed and control ponds was sampled by using 20 submerged metal-cone traps (Ali 1980). One short trap (60 cm diam. and 36 cm high) was used at each site in control pond and at shallow sites in exposed pond. Six tall traps (60 cm diam. and 60 cm high) were used in the deep area of exposed pond.

Daily maximum and minimum water temperatures were read from a max.-min. thermometer placed in each pond at a fixed location.

In the laboratory, each benthic sample was washed through a 0.5 mm pore sifter on the same day it was collected and preserved. These samples were examined entirely by taking small portions in a white enamel tray with a grid. The invertebrates visible to the naked eye were removed, counted, and identified. Adult insects collected in each trap were also counted and identified.

Samples of zooplankton were processed in the laboratory according to the method described in Ali and Mulla (1978). A minimum of three 2-ml subsamples was microscopically examined under 30X and the organisms were identified and counted.

Pretreatment (average of 4-days and 1-day pretreatment samples) and periodic posttreatment population levels of each taxon in exposed and control ponds were analyzed by ANOVA and Duncan's Multiple Range Test. Any reductions of invertebrates in exposed pond were also verified by the formula given in Mulla et al. (1971).

RESULTS AND DISCUSSION

The qualitative composition of fauna in both ponds was generally similar (Table 1). Among zooplankton, the copepods *Cyclops* spp., *Diaptomus* spp., and their nauplii predominated in shallow areas of exposed pond and in control pond. The deep area of exposed pond supported mostly *Cyclops* spp. (Table 1). Cladocerans consisted of 12 genera, with *Alona* sp. and *Macrothrix* sp. being the most abundant in shallow areas of exposed pond and *Alona* sp. and *Bosmina* sp. occurring in relatively high densities in control pond. *Bosmina* sp. was the major cladoceran in deep area of exposed pond. The densities of Hydracarina were higher in shallow areas of the exposed pond and in the control pond as compared to the deep area of the exposed pond. Nymphs of mayfly, *Caenis robusta*, and larval and pupal Chironomidae and Ceratopogonidae occurred in plankton samples in shallow areas of exposed and control ponds.

Both ponds supported low densities of benthic invertebrates (Table 2). Nymphal *C. robusta*, larval and pupal Chironomidae and Ceratopogonidae, nymphal Odonata, larval Trichoptera, larval and adult Coleoptera, nymphal and adult Hemiptera, the decapods, *Procambarus alleni* and *P. fallax*, the amphipod, *Hyalella azteca*, Gastropoda, and Oligochaeta were present in benthos of the shallow areas of the exposed pond and of the control pond. In the deep area of exposed pond, only larval *Chaoborus* sp. and Chironomidae occurred in the benthos in negligible numbers.

A statistical comparison of the pre-, and periodic posttreatment population levels of *C. robusta*, Ceratopogonidae, Chironomidae, Hydracarina, *Cyclops* spp., *Diaptomus* spp., and their nauplii, *Alona* sp., *Macrothrix* sp., other Cladocera (*Bosmina* sp., *Ceriodaphnia* sp., *Diaphanosoma* sp., *Ilyocryptus* sp., *Sida* sp., and *Simocephalus* sp.), Ostracoda, Oligochaeta, Nematoda, and Rotifera in the exposed pond revealed no adverse effects on these organisms due to the treatment. Instead, population levels of

Table 1. Invertebrate population (mostly zooplankton) densities in shallow and deep areas of an exposed pond and in a control pond surrounded by citrus trees commercially treated with diflubenzuron (Dimilin® 25 WP) at 0.56 kg AI/ha for the control of citrus rust mite in a Florida citrus grove, Winter Garden, July-September 1986.

Invertebrates	Mean[a] no. inverts./sample[b] pre-, and posttreatment (days)						
	Pretreat	1	3	7	14	28	56
				Shallow Areas			
Caenis robusta N	39a	66a	29a	36a	49a	30a	37a
Ceratopogonidae L,P	224ab	625a	364ab	271ab	249ab	159b	184b
Chironomidae L,P	2,345ab	2,756a	1,149b	1,761ab	2,152ab	1,946ab	1,956a
Hydracarina	2,830ab	3,148ab	1,619b	2,068ab	3,209a	2,768ab	1,797ab
Cyclops spp.	4,867a	8,018a	7,219a	6,179a	5,065a	7,238a	6,714a
Diaptomus spp.	1,200abc	2,000a	2,060bc	2,488abc	1,274ab	2,024abc	1,048c
Nauplii	10,375ab	14,673a	13,958ab	12,970a	12,065ab	11,500b	10,881ab
Alona sp.	4,134a	4,982a	3,782a	4,083a	5,006a	3,762a	3,143a
Bosmina sp.	405a	458ab	131b	244ab	339ab	571ab	1,048ab
Ceriodaphnia sp.	363a	357a	652a	500a	310a	506a	1,815a
Diaphanosoma sp.	628a	839a	1,149a	2,482a	1,542a	2,357a	2,625a
Ilyocryptus sp.	824ab	1,512a	877bc	1,089abc	696abc	845bc	351c
Macrothrix sp.	2,033abc	1,387c	2,149bc	1,643abc	2,786abc	4,357ab	4,708a
Sida sp.	63a	113a	149a	36a	83a	208a	173a
Simocephalus sp.	122ab	108b	131b	156ab	120ab	637a	526ab
Other Cladocera	24a	24a	756a	488a	262a	327a	143a
Ostracoda	10,941a	11,006a	6,552a	8,155a	8,750a	10,482a	7,464a
Oligochaeta	619a	518a	243a	363a	494a	429a	363a
Nematoda	9,020a	8,054a	9,836a	11,833a	11,679a	11,089a	6,369a
Rotifera	13,330a	13,929a	10,054a	15,750a	8,786a	15,518a	9,946a

Table 1. Cont'd.

Invertebrates	Mean[a] no. inverts./sample[b] pre-, and posttreatment (days)						
	Pretreat	1	3	7	14	28	56
				Deep Area			
C. robusta N	0b	13a	3ab	0b	3ab	0b	--
Chironomidae L,P	1a	1a	7a	8a	0a	8a	--
Hydracarina	14ab	22ab	36a	35a	6b	20ab	--
Cyclops spp.	4,135c	11,932ab	9,911b	13,454ab	17,172a	15,417a	--
Diaptomus spp.	0a	0a	0a	5a	2a	3a	--
Nauplii	4,262f	10,084e	10,892d	14,010c	16,958b	18,723a	--
Bosmina sp.	114c	531a	371a	309ab	158bc	108c	--
Other Cladocera	2b	5b	10ab	22a	4b	43a	--
Ostracoda	24c	127a	52b	49b	89a	40bc	--
Nematoda	23ab	3bc	51a	32a	41a	5c	--
Rotifera	2,395c	3,829b	5,463b	4,668b	901d	50,753a	--

Table 1. Cont'd.

Invertebrates	Mean[a] no. inverts./sample[b] pre-, and posttreatment (days)						
	Pretreat	1	3	7	14	28	56
				Control Pond			
C. robusta N	5a	63a	42a	42a	32a	21a	11a
Ceratopogonidae L,P	13a	6a	33a	18a	4a	3a	11a
Chironomidae L,P	263a	471a	292a	555a	481a	369a	189a
Hydracarina	708a	594a	387a	834a	689a	1,137a	105b
Cyclops spp.	1,922bc	6,281a	3,885ab	1,948b	2,927b	1,948bc	1,094c
Diaptomus spp.	131a	844a	844a	438a	292a	63b	83b
Nauplii	15,339bc	22,875ab	41,385a	33,125abc	25,708ab	12,365bc	11,573c
Alona sp.	802ab	1,500a	1,073ab	1,198a	542ab	958ab	292b
Bosmina sp.	1,063ab	1,375ab	2,302a	2,094ab	885ab	115c	240bc
Ceriodaphnia sp.	47ab	188a	83ab	146ab	21ab	10ab	0b
Diaphanosoma sp.	167a	469a	615a	240a	156a	115a	83a
Ilyocryptus sp.	286a	94a	63a	73a	63a	94a	31a
Macrothrix sp.	10b	281a	63b	21a	10b	0b	0b
Sida sp.	0a	31a	0a	21a	0a	0a	0a
Simocephalus sp.	1a	95a	22a	1a	1a	32a	10a
Other Cladocera	0b	344a	94b	31b	0b	0b	0b
Ostracoda	849b	3,406a	1,042ab	1,323ab	1,854ab	2,073ab	938ab
Oligochaeta	26abc	251a	21bc	167abc	104ab	198abc	10c
Nematoda	4,396abc	8,156a	2,781bc	4,396abc	3,083abc	5,385ab	1,563c
Rotifera	6,021ab	12,406a	9,552ab	9,500ab	6,521ab	5,115b	5,292b

[a]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other as analyzed by ANOVA and Duncan's Multiple Range Test; the data were transformed to log (n+1).
[b]175 liters. L = Larva; P = Pupa; N = Nymph.
Water temp: 24-34°C (exposed pond) and 26-33°C (control pond).

many of these organisms increased during the posttreatment period, generally following the trends in the control pond (Table 1).

In benthic samples, populations of C. robusta, Chironomidae, Ceratopogonidae, Odonata, Trichoptera, Coleoptera, Hemiptera, P. alleni and P. fallax, H. azteca, Gastropoda, and Oligochaeta did not show any significant (5% level) changes after the diflubenzuron treatment in the surrounding area (Table 2). The densities of most

Table 2. Invertebrate population (mostly benthic) densities in shallow and deep areas of an exposed pond and in a control pond surrounded by citrus trees commercially treated with diflubenzuron (Dimilin® 25 WP) at 0.56 kg AI/ha for the control of citrus rust mite in a Florida citrus grove, Winter Garden, July-September 1986.

	Mean[a] no. inverts./sample[b] pre-, and posttreatment (days)					
Invertebrates	Pretreat	3	7	14	28	56
			Shallow Areas			
Caenis robusta N	1.7a	1.3a	1.5a	1.9a	1.0a	2.6a
Ceratopogonidae L,P	36.6a	49.8a	79.9a	28.4a	31.6a	23.4a
Chironomidae L,P	22.3a	37.4a	28.8a	49.4a	31.3a	48.5a
Odonata N	2.4a	2.5a	4.1a	2.7a	2.7a	3.4a
Trichoptera L	0.3a	0.3a	0.3a	0.6a	0.3a	1.1a
Coleoptera L	1.3a	1.9a	0.4a	1.1a	1.4a	1.6a
Coleoptera A	0.0a	0.5a	0.3a	0.1a	0.1a	0.0a
Hemiptera N,A	0.9a	0.5a	1.0a	0.4a	0.7a	0.8a
Procambarus spp.	1.8a	1.1ab	0.4b	1.8a	0.4b	0.8ab
Hyalella azteca	7.4a	2.5a	4.8a	5.6a	7.9a	16.3a
Gastropoda	5.3a	5.9a	3.5a	6.8a	6.0a	8.0a
Oligochaeta	2.3a	1.0a	0.4a	3.0a	2.6a	5.5a
			Deep Area			
Chaoborus sp. L	1.0a	1.0a	---	---	---	---
Chironomidae L	1.0a	1.5a	---	---	---	---
			Control Pond			
C. robusta N	1.3ab	3.2a	2.2a	3.2a	0.8ab	0.3b
Ceratopogonidae L,P	3.3a	0.7a	5.7a	4.2a	1.7a	0.2a
Chironomidae L,P	18.5b	28.5b	69.3a	30.7ab	27.2ab	31.7ab
Odonata N	1.9a	1.9a	3.5a	2.1a	0.8a	2.2a
Trichoptera L	0.2a	0.2a	0.4a	0.4a	0.5a	1.8a
Coleoptera L	2.6a	0.2a	1.2a	0.4a	0.6a	0.8a
Coleoptera A	0.7a	0.0a	0.0a	0.8a	0.0a	0.5a
Hemiptera N,A	0.0a	0.0a	0.0a	0.2a	0.2a	0.0a
Procambarus spp.	2.8a	3.7a	2.3a	2.5a	2.5a	2.0a
H. azteca	0.5a	5.2a	3.0a	10.0a	0.8a	3.0a
Gastropoda	2.9a	3.8a	4.8a	7.0a	6.8a	6.8a
Oligochaeta	6.5a	0.8a	0.3a	0.3a	3.2a	2.2a

[a]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other as analyzed by ANOVA and Duncan's Multiple Range Test; the data were transformed to log (n+1

[b]Approx. 0.125 m^2. A = Adult; L = Larva; P = Pupa; N = Nymph. Water temp: 24-34°C (exposed pond) and 26-33°C (control pond).

of these invertebrates, however, were considerably low in both ponds at the pre-, and posttreatment periods. The adult emergence data (Table 3) also revealed no significant suppression of Chironomidae and C. robusta in the exposed pond.

Table 3. Adult insect emergence from shallow and deep areas of an exposed pond and from a control pond surrounded by citrus trees commercially treated with diflubenzuron (Dimilin® 25 WP) at 0.56 kg AI/ha for the control of citrus rust mite in a Florida citrus grove, Winter Garden, July - August 1986.

Insects	Mean[a] no. adult emergence/trap[b] pre-, and posttreatment (days)					
	Pretreat	1	3	7	14	28
			Shallow Areas			
Chironomidae[c]	5.27ab	7.38a	2.25bc	1.14c	3.25abc	7.25ab
Other insects[d]	0.52a	0.88a	0.25a	0.14a	0.38a	0.38a
			Deep Area			
Chironomidae[c]	1.11a	0.50a	0.33a	0.33a	0.66a	0.16a
Other insects[d]	0.33a	0.00a	0.16a	0.00a	0.00a	0.00a
			Control Pond			
Chironomidae[c]	4.61a	3.33a	2.33a	1.00a	5.33a	4.00a
Other insects[d]	0.72a	0.50a	0.16a	0.16a	0.16a	0.33a

[a]Means in a row followed by the same letter are not significantly different ($P>0.05$) from each other as analyzed by ANOVA and Duncan's Multiple Range Test; the data were transformed to log $(n + 1)$.
[b]0.3 m^2. [c]Mostly Chironomini, Tanytarsini, Cricotopus spp.
[d]Predominantly Caenis robusta.
Water temp: 24-34°C (exposed pond) and 26-33°C (control pond).

The percent reductions data (not included) showed minor reductions of invertebrates, such as Copepoda and Cladocera during the posttreatment period, but these small reductions were most likely due to: short life cycles of these organisms, seasonal population changes, and possible sampling deficiencies. A number of previous studies (e.g., Ali and Lord 1980; Ali and Mulla 1978; Miura and Takahashi 1974) had shown immediate general declines of Copepoda, Cladocera, and Amphipoda at diflubenzuron concentrations of <12 ppb. Such general declines were not observed in the present study.

This study has revealed no apparent adverse effects of diflubenzuron on zooplankton and benthic invertebrates in an exposed pond located amid the treatment area. The main reason for the lack of any ill-effects of diflubenzuron in this study may be the extremely low or undetectable amounts of the IGR in the exposed pond (Nigg and Stamper 1987, unpublished data). The largest residue of 197 ppt of diflubenzuron in the exposed pond occurred at site 2 (zooplankton, Fig. 1), 2-days post-application, with levels returning to trace amounts (<27 ppt) by day 14 post-application. Elsewhere in the pond, trace amounts (<27 ppt) of diflubenzuron were found only incidentally (Nigg and Stamper 1987). At site 2, populations of Copepoda and Cladocera did not decline after the treatment (data not included).

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REFERENCES

Ali A (1980) Diel adult eclosion periodicity of nuisance chironomid midges of central Florida. Environ Entomol 9:365-370

Ali A, Lord J (1980) Impact of experimental insect growth regulators on some nontarget aquatic invertebrates. Mosq News 40:564-571

Ali A, Mulla MS (1978) Effects of chironomid larvicides and diflubenzuron on nontarget invertebrates in residential-recreational lakes. Environ Entomol 3:631-636

Ali A, Mulla MS, Pfuntner AR, Luna LL (1978) Pestiferous midges and their control in a shallow residential-recreational lake in southern California. Mosq News 38:528-535

Apperson CS, Schaefer CH, Cowell AE, Werner GH, Anderson NL, Dupras Jr EF, Longanecker DR (1978) Effects of diflubenzuron on Chaoborus astictopus and nontarget organisms and persistence of diflubenzuron in lentic habitats. J Econ Entomol 71:-521-527

Flint HM, Smith RL, Forey D, Horn B (1977) Diflubenzuron: evaluation for control of the pink bollworm, cabbage looper, and cotton leafperforator in a field cage test. J Econ Entomol 70:237-239

Granett J, Dunbar DM (1975) TH-6040: laboratory and field trials for control of gypsy moth. J Econ Entomol 68:99-102

McCoy CW (1978) Activity of dimilin on the developmental stages of Phyllocoptruta oleivora and its performance in the field. J Econ Entomol 71:122-124

Miura T, Takahashi RM (1974) Insect development inhibitors: effects of candidate mosquito control agents on nontarget aquatic organisms. Environ Entomol 3:631-636

Mulla MS, Majori G, Darwazeh HA (1975) Effects of the insect growth regulator Dimilin or TH-6040 on mosquitoes and some nontarget organisms. Mosq News 35:211-217.

Mulla MS, Norland RL, Fanara DM, Darwazeh HA, McKean DW (1971) Control of chironomid midges in recreational lakes. J Econ Entomol 64:300-307

Nigg HN, Stamper JH (1987) Diflubenzuron drift into a pond during application to citrus. Unpubl Rept, Univ Fla, CREC, Lake Alfred, 17 pp

Received April 6, 1988; accepted July 1, 1988.