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Environmental Persistence and Impact of Diflubenzuron in a Forest Aquatic Environment Following Aerial Application

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Abstract. Dimilin® WP-25 (diflubenzuron) was applied at a rate of 70 g active ingredient (AI) in 10, 5, and 2.5 L/ha to three spray blocks in a mixed boreal forest near Kaladar, Ontario, Canada. Water, sediment, and aquatic plants were collected from two ponds and a stream at intervals up to 30 days post-treatment for analysis of diflubenzuron (DFB) residues. The duration of detectable residues was different for each substrate, but in all cases was less than two weeks. Zooplankton and benthic invertebrate populations were monitored for up to 110 days post-spray in two ponds in the high volume rate block and in control ponds. Significant mortality occurred in two groups of caged macroinvertebrates (amphipoda and immature corixidae) 1 to 6 days after the ponds were treated with Dimilin. Three taxa of littoral insects (*Caenis*, *Celithemis* and *Coenagrion*) were significantly reduced in abundance in the treated ponds 21 to 34 d post-treatment, but recovered to pre-treatment levels by the end of the season. Of the six remaining groups studied, only one (immature corixidae), may have been slightly affected by treatment. Zooplankton (cladocera and copepoda) populations were reduced 3 days after treatment and remained suppressed for 2–3 months.

Diflubenzuron (DFB) [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea], the active ingredient of Dimilin® WP-25 (a wettable powder formulation) is an effective control agent for several forest insect pests (Retnakaran and Wright 1987; Retnakaran *et al.* 1988). It acts through disruption of the synthesis of new cuticle at moults and is therefore arthropod specific (Mulder and Gijswijt 1973; Deul *et al.* 1978). Laboratory and field studies have shown that DFB can have adverse impacts on aquatic invertebrate communities, especially crustacean zooplankton (Ali and Lord 1980; Ali and Mulla 1978a, 1978b; Apperson *et al.* 1978; Hansen and Garton 1982a, 1982b; Lacey and Mulla 1978; Swift *et al.* 1988). EC_{50} values for cladocerans, one of the most sensitive groups of crustaceans, are reported to be in the range of 1.5 to 15 μg/L (Hansen and Garton 1982a; Julin and Sanders 1978; Miura and Takahashi 1974).

The initial residue levels and persistence of chemical pesticides in nontarget aquatic environments have a direct significance on the severity of impact on aquatic organisms. Therefore, the fate and persistence of DFB in pond and lake environments have been a subject of interest for some years (Apperson *et al.* 1978; Booth 1976; Booth and Ferrell 1977; Booth *et al.* 1987; Martinat *et al.* 1987; Mian and Mulla 1982; Schaefer and Dupras 1976; Smith *et al.* 1985). Several of these studies have shown that DFB normally persists for only about 2 to 3 days. However, persistence is related to water quality and climatic conditions, and low concentrations can persist for 7 weeks or longer in pond waters under low pH and temperatures (Ivie *et al.* 1980). Data are sparse in the literature on the persistence and impact of DFB entering Canadian aquatic environments under the climatic conditions and physical/chemical characteristics normally encountered during operational spraying of insecticides for forest insect control.

The present investigation had two objectives: 1) to determine residue levels and persistence of DFB in forest pond and stream water, sediment, and aquatic plants following spray application using the maximum operational dosage rate (70 g AI/ha) for Canadian forestry and three volume rates of application (10, 5 and 2.5 L/ha). 2) to determine the post-treatment biological effects of the chemical on nontarget arthropod communities in the contaminated ponds. The latter study was conducted only in the pond environment, because sprayed insecticide residues are known to persist longer in stagnant pond waters (Sundaram *et al.* 1986). Thus biological effects were determined under the "worst case scenario" that might be encountered in operational spray situations. Furthermore, the highest volume rate of 10 L/ha was chosen for the impact study, because large volume rates are known to provide high residues at the forest floor level, and by extension maximum residues in the aquatic environment (Sundaram *et al.* 1987).

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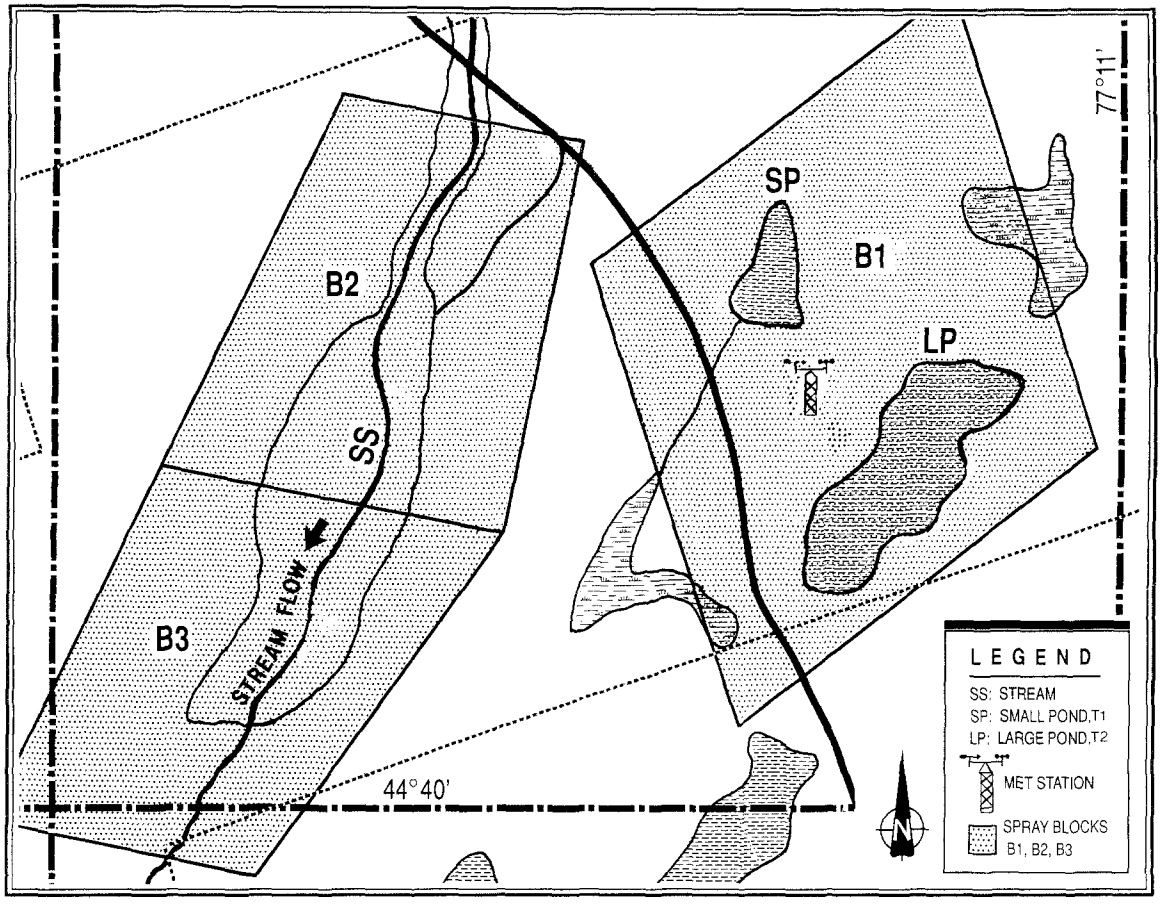

Fig. 1. Location of sampling stations in Dimilin spray blocks B1, B2 and B3

Methods

Study Site

Three spray blocks (B1, 25 ha; B2 and B3, 15 ha each; Figure 1) were selected on privately owned forest land in southern Ontario (44°40'N and 77°11'W). The terrain of B1 was primarily rocky, and contained slopes and depressions and several small ponds. Blocks B2 and B3 were adjacent to each other, with a 20 m buffer between, in a low land area about 3 km away from block B1. Their topography was flat and swampy. A small perennial stream (SS) ran lengthwise through blocks B2 and B3. The ground was covered with a variety of weeds and grass patches.

Two treatment ponds, T1 and T2, were located near the centre of spray block B1. Two other ponds (C1 and C2), located about 1 km south of B1, served as controls for zooplankton and littoral invertebrate studies, respectively. All were shallow beaver ponds. Some physical and chemical characteristics of the ponds and the stream are given in Table 1.

Spray Application

Dimilin WP-25 was mixed with variable amounts of water to provide a constant dosage of 70 g AI/ha in three volume rates (Table 2) and was applied to the three blocks between 0645 and 1215 h (EDT) on 5 June 1986. Block B1 was sprayed first using a volume rate of 10 L/ha, followed by B2 and B3 using 5 and 2.5 L/ha respectively. The aircraft used was a Piper Pawnee Brave (PAC 6-300) equipped with four Micronair AU4000 atomizers, with a blade angle setting of 35°. The aircraft speed was 160 km/h at a spray height of 20 m above tree canopy. The swath width was 45 m. A 6 m high portable weather station was erected in the vicinity of the sampling sites (Figure 1) in Block B1, to record the meteorological conditions during the time of spray application. Relevant details are given in Table 2.

Substrate Sampling and Residue Analysis

Samples of water, sediment and aquatic plants [manna grass (*Glyceria borealis*) in B1, and water smartweed (*Polygonum natans*) in B2 and B3)] were collected, processed and stored at intervals of time before and after spray application according to established procedures (Sundaram 1987), except that water was sampled from the top 5 cm layer instead of the 1 cm layer as reported previously. The samples were transported in a freezer at −20°C to the pesticide laboratory in Sault Ste. Marie, Ontario, for residue analysis.

The DFB was extracted from the substrates, cleaned and analyzed by high performance liquid chromatography (HPLC) according to the method of Sundaram and Nott (1989). The minimum detection level (MDL) for water was 0.05 μg/L and the mean recovery (fortification levels of 1.0, 0.50 and 0.10 μg/L) was 84 ± 8%. For other substrates the MDL was 0.05 μg/g (fresh weight) and the average recovery (fortification levels of 1.0, 0.50 and 0.10 μg/g fresh weight) was 90 ± 7%. Residue data reported in this paper are corrected for extraction efficiency. Pre-spray samples and samples from the control ponds did not show any detectable levels of DFB, nor did they show any impurities having interference peaks corresponding to DFB.

Caged Invertebrates

Juvenile scuds (Amphipoda), water boatman nymphs (Corixidae), and phantom midge larvae (*Chaoborus* sp.) were collected 3 days

Table 1. Physical and chemical characteristics of the ponds and stream in Dimilin spray blocks B1, B2, and B3

	Ponds			Stream	
	B1 (10 L/ha)			B2 (5L/ha)	B3 (2.5L/ha)
Characteristics	Control[a]	T1	T2	SS2[b]	SS3[b]
Area (av.) (m^2)	3800	3400	15300	—	—
Depth (max.) (m)	1.10	1.35	0.75	0.85	1.2
Width (av.) (m)	—	—	—	3.2	2.1
Discharge (L/s)	—	—	—	22	17
Gradient (m/km)	—	—	—	1.7	1.2
Water Quality:					
pH	6.21	5.79	6.22	7.00	6.99
Turbidity (JTU)[c]	33	60	12	25	25
Conductance[d]	29.1	23.7	20.1	144	144
Alkalinity[f]	19.9	6.8	6.0	135	135
Hardness[g]	10.1	10.0	5.0	37.5	37.5
CO_2 (mg/L)	14.6	18.6	7.5	21.8	22.4
NO_x (mg/L)	0.011	0.014	0.013	0.011	0.017
NH_3 (mg/L)	0.112	0.113	0.028	0.025	0.014
Cations (mg/L)[h]	5.73	4.90	3.52	28.2	28.0
Anions (mg/L)[k]	0.87	1.27	2.70	2.76	2.68
SiO_2 (mg/L)	1.60	0.43	2.56	10.6	12.0

[a] Data are for control ponds C1 only
[b] SS2 refers to the stream sampling location in block B2, and SS3 to the stream sampling location in B3
[c] Turbidity expressed in Jackson turbidity units
[d] Specific conductance expressed in μmho/cm
[f] Total alkalinity expressed in mg/L as $CaCO_3$
[g] Hardness expressed in mg/L as $CaCO_3$
[h] Total cations (Na^+, K^+, Mg^{++}, and Ca^{++})
[k] Total anions (Cl^- and SO_4^{--})

Table 2. Meteorological conditions and formulation compositions for aerial spray trials of Dimilin in Blocks B1, B2 and B3

Parameters	Block B1	Block B2	Block B3
Time of application (EST)	0645	0745	1215
Average wind speed (km/h)	7.2	5.7	6.6
Wind direction	NE	NE	NE
Average temperature (°C)	13.6	13.4	10.6
Relative humidity (%)	84.9	86.6	85.7
Cloud cover	9/10	9/10	8/10
Precipitation	Nil	Nil	Very light
Tank mix Composition[a]			
Dimilin WP-25	2.8	5.6	11.2
Water	96.2	93.4	87.8
Tracer dye[b]	1.0	1.0	1.0

[a] All values are expressed in percentage wt/volume
[b] A tracer dye was added to the tank mix to facilitate droplet analysis on spray cards (data to be published elsewhere)

before the application from T1 and placed in floating cages in T1, T2, and the control pond. Fifty individuals of each taxon were included at each site, distributed among 15 cages (five groups of ten individuals of each taxon). The cages consisted of 1 L plastic buckets (11 cm diameter) open on the top and screened on the bottom. Two openings (5 × 10 cm) were cut on the sides of each bucket and screened to allow a circulation of water. The buckets were inserted into sheets of styrofoam and moored to the edges of the ponds. Mortality counts and observations of pupation rate were recorded once daily up to 9 days after the application. Additional *Chaoborus* larvae, that were collected before the application and held at the field laboratory station, were placed in floating cages at each site 2 days after the treatment. Fewer individuals (30 in T1, 25 in T2, 15 in control) were available for this second group. The survival of these additional specimens was assessed to determine the effects of residual DFB on *Chaoborus* larvae. The percent mortality of caged invertebrates in the two treated ponds was compared to the percent mortality in the control pond on each day using a G-test of independence at $\alpha = 0.05$.

Littoral Invertebrates

The abundance of aquatic macroinvertebrates in the littoral zones of the study ponds was estimated from sweep net collections. Five sweep samples were taken from each pond on each sample date.

The sweep samples were collected with a D-frame net (800 μ mesh) extended 1.5 m out from the shore of the pond and drawn back along the bottom toward the edge of the pond. Each sweep sampled 0.44 m^2 of the bottom. The abundance of macroinvertebrates was expressed as mean number per m^2 of five replicates from each site.

The abundance data were ln(x + 1) transformed before statistical analysis (Green 1979) and tested for equality of variances (Brown and Forsythe 1974a). At least 85% of the data sets satisfied the test for homogeneity of variances and no further transformation was considered necessary. Changes in abundance of macroinvertebrates over time in the treated ponds were tested against changes in abundance in the control pond by two-way analysis of variance (2-way ANOVA). The time (sampling period) and site (treatment, control) interaction was used as an error term to determine if the response of littoral invertebrates to the treatment was significant ($p < 0.05$) (Green 1979). A significant interaction indicated that changes in abundance of macroinvertebrates over the season were different in treatment and control ponds. When the interaction term was significant, a reduction in abundance at a treated site concurrent with either an increase or a non-significant decline in abundance in the control was considered to be strong evidence of a treatment effect. A significant interaction resulting from an increase at a treated site concurrent with either a decline or a non-significant increase in the control was not considered to be an indication of impact. Differences in abundance between sample times were tested using a Tukey multiple comparisons procedure at $\alpha = 0.05$ (Day and Quinn 1989).

Zooplankton

Zooplankton were collected from 3 sites in each study pond using a 12 L capacity Schindler-Patalas plankton trap. Depths (m) at the sampling stations were:

Pond	East	Center	West
T1	1.1	1.5	1.4
T2	0.7	0.8	1.0
C	0.8	1.2	0.8

Three samples were taken from just below the water surface at each site on each sampling date; 5 and 2 days before, and 1, 3, 5, 9, 21, 34, 68 and 110 days after treatment. Water samples were strained through a collection bucket fitted with 64 μ mesh Nitex monofilament bolting cloth. The concentrated zooplankton samples were preserved in 4% formalin. In the laboratory, zooplankton were counted in a gridded dish under a 40X dissecting microscope. Cladocera were identified to the family level. The identification of copepods was restricted to differentiating between nauplius, copepodid and adult stages.

Heterogeneity of population variances could not be eliminated by transformation of the zooplankton data. Consequently, the Brown-Forsythe statistic (F*), rather than the ANOVA F statistic, was used for analysis of variance (Brown and Forsythe 1974b), and separate variance t-tests (Welch tests) with Bonferroni probabilities were used rather than Tukey tests for multiple comparisons (Milliken and Johnson 1984). The results of these tests were interpreted in the same way as for littoral invertebrates.

Results and Discussion

Persistence of DFB in a Pond Environment

Water: The concentrations (μg/L) of DFB found in the top 5 cm of water collected at intervals of time from the treated ponds are given in Table 3. From the data, it is apparent that the smaller pond (T1) received a higher initial concentration (13.8 μg/L) of the chemical than the larger pond (T2; 5.90 μg/L). Variations in spray deposits at forest floor level have been reported in earlier studies (Himel *et al.* 1987; Sundaram *et al.* 1985). Several factors including non-uniform swath distribution, meteorological conditions, spray cloud interception by the tree canopy, etc., have been known to cause such variations in deposits in aerial spray trials.

The dissipation of DFB in the pond waters followed an exponential decay pattern, as described by equations (1) to (8):

$$X = X_0 e^{-kt} \quad (1)$$

$$\ln X = \ln X_0 - kt \quad (2)$$

$$\ln (X/X_0) = -kt \quad (3)$$

$$\log_{10} (X/X_0) = -kt/2.303 \quad (4)$$

$$X = X_0 \text{ (when } t = 0) \quad (5)$$

$$X = 0 \text{ (when } t = \infty) \quad (6)$$

$$DT_{50} = (2.303 \log_{10} 2)/k \quad (7)$$

$$DT_{90} = (2.303 \log_{10} 10)/k \quad (8)$$

In the above equations, X_0 represents the initial DFB concentration and k is the dissipation rate constant (the greater the value of k, the faster the dissipation).

The data from Table 3 were converted into percentages of the initial concentrations and fitted into equation (1). Logarithmic transformation and linear regressions were performed and the values of k, DT_{50} and DT_{90} were computed (Table 4).

Dissipation occurred faster in T1, which received higher deposits of DFB, than in T2. After a period of 2 days, levels of DFB in T1 were only slightly higher than in T2. The faster initial rate of dissipation in T1 could be due to increased dilution because of the greater depth of this pond (1.30 m compared to 0.80 m for T2). Schaefer and Dupras (1976) and Mian and Mulla (1982) found that Dimilin WP-25 underwent rapid and homogeneous distribution in simulated ponds. Another factor that could have enhanced the dilution effect in T1 is the tendency of DFB to adsorb onto particulate matter (Booth *et al.* 1987). T1 had a higher turbidity (60 JTU) than T2 (12 JTU). An even distribution of DFB residues would be more rapidly achieved by mixing of the suspended particles in T1. Finally, the initial rapid dissipation in T1 could be due to degradation by microbial organisms in the turbid waters (Mian and Mulla 1982).

The slower rate of dissipation of DFB in T1 after 2 days could also be due to the turbid nature of the water. Because the chemical is adsorbed to particulates (Booth *et al.* 1987), it would be less available to hydrolytic and photolytic degradations. In addition, photolysis would be less prominent in deeper and murkier waters. Nevertheless, the chemical degraded to a level below the detection limit (0.05 μg/L) within 20 d in both ponds.

DFB was detected in ponds T1 and T2 for more than 2 weeks (Table 3). This is longer than the 2 to 3 days reported in several other studies in the literature (Booth 1976; Booth and Ferrell 1977; Booth *et al.* 1987; Schaefer and Dupras 1976), but shorter than the more than 7 weeks reported by Ivie *et al.* (1980). The results of the present study lend support to the suggestion that the persistence of DFB in natural waters is significantly influenced by such factors as the pre-

Table 3. Average concentrations of diflubenzuron (DFB) in pond and stream waters following aerial applications of Dimilin to Blocks B1, B2 and B3

Time after application	Average (n = 3) concentrations (μg/L) of DFB			
	Block B1[a]		Block B2[a]	Block B3[a]
	Pond T1	Pond T2	Stream SS2[b]	Stream SS3[b]
Pre-spray	N.D.[c]	N.D.	N.D.	N.D. (0.26)[e]
1 h	13.82 ± 1.17	5.90 ± 0.21	3.25 ± 0.41	1.59 ± 0.61
3 h	9.67 ± 0.81	5.87 ± 0.90	2.22 ± 0.27	1.81 ± 0.44
6 h	5.99 ± 0.43	6.09 ± 0.63	0.71 ± 0.34	1.61 ± 0.51
12 h	6.28 ± 0.99	4.22 ± 0.21	0.15 ± 0.07	0.52 ± 0.33
1 d	4.31 ± 0.46	2.76 ± 0.20	N.D.	0.13 ± 0.03
2 d	3.36 ± 0.23	2.06 ± 0.32	N.D.	N.D.
3 d	1.84 ± 0.47	1.40 ± 0.28	N.D.	N.D.
5 d	0.63 ± 0.32	0.44 ± 0.15		
7 d	0.47 ± 0.40	0.23 ± 0.06		
10 d	1.02 ± 0.62	0.45 ± 0.18		
15 d	0.22 ± 0.07	0.11 ± 0.05		
20 d	N.D.	N.D.		
30 d	N.D.	N.D.		

[a] Volume rates of application were 10 L/ha for B1, 5 L/ha for B2 and 2.5 L/ha for B3
[b] SS2 refers to the stream sampling location in block B2, and SS3 to the stream sampling location in B3
[c] N.D. = not detected; limit of detection was 0.05 μg/L
[e] Pre-spray water samples collected 0.5 h pre-treatment from B3 contained low levels of DFB due to contamination from upstream. The upstream block (B2) was sprayed about 4.5 h before B3. Samples collected 6 h pre-treatment from B3 did not contain measurable residues of DFB

Table 4. Disappearance rate constants of DFB for pond and stream waters following aerial applications of Dimilin to Blocks B1, B2 and B3

Sampling Site	k[a]	DT50[b] (days)	DT90[c] (days)	S[d] (%)
Pond T1[e]	0.0702	0.4	1.4	85.9
Pond T2[e]	0.0228	1.3	4.2	97.2
Stream (SS2)[f]	0.1602	0.2	0.6	97.5
Stream (SS3)[f]	0.0960	0.3	1.0	97.8

[a] Decay constant
[b] Dissipation time for 50% of the initial concentration
[c] Dissipation time for 90% of the initial concentration
[d] Coefficient of determination
[e] Ponds T1 and T2 were situated in block B1 for which the volume rate of application was 10 L/ha
[f] Stream sampling site SS2 was located in block B2 which received a volume rate of 5 L/ha, and SS3 was in block B3 which received 2.5 L/ha

vailing climatic conditions and the physical/chemical characteristics of the receiving water.

Sediment: DFB concentrations in the sediments of ponds T1 and T2 are presented in Table 5. Residue levels in both ponds declined to the level of detection within 3 days of spraying. No reliable determination could be made of the DT_{50} or DT_{90} values for DFB in pond sediments, but it appears that DFB does not accumulate or persist in sediment to any greater extent than has been demonstrated previously (Apperson *et al.* 1978).

Aquatic Plants: DFB concentrations in manna grass are presented in Table 6. The maximum level measured was 0.36 μg/g for plants collected 1 day post-treatment from T1. The maximum concentration in T2 was much lower (0.14 μg/g immediately after application), corresponding to the lower residues in water in this pond (Table 3). Manna grass concentrated DFB residues by a factor of about 26–43 (maximum concentration in water/maximum concentration in plant). However, residues in plants declined to non-detectable levels in 7–10 days, 10 days sooner than residues in water. These observations are in agreement with those of Booth and Ferrell (1977) and Metcalf *et al.* (1975), who showed that DFB degrades relatively rapidly in aquatic vegetation.

Persistence of DFB in a Stream Environment

Water: Concentrations in stream water were much lower than in pond water (Table 3). Also, the residues disappeared very rapidly from the stream compared to the ponds, reaching levels below the limit of detection (0.05 μg/L) in 1 to 2 days.

The disappearance of DFB residues from stream water followed an exponential model (Table 4). DFB residues declined more rapidly in B2 (initial concentration of 3.25 μg/L) than in B3 (initial concentration of 1.59 μg/L) (Tables 3 and 4). The reason for this difference is not clear, but the fact that SS3 was downstream from SS2 is probably at least partly responsible. Stanley and Trial (1980) have shown that the rate of disappearance of carbaryl from streams following aerial spraying is independent of both initial concentration and stream size.

Table 5. Average concentrations of diflubenzuron (DFB) in pond and stream sediments following aerial applications of Dimilin to Blocks B1, B2 and B3

Time after application	Average (n = 3) concentrations (μg/g) of DFB			
	Block B1[a]		Block B2[a]	Block B3[a]
	T1	T2	SS2[b]	SS3[b]
Pre-spray	N.D.[c]	N.D.	N.D.	N.D.
1 h	N.D.	N.D.	N.D.	N.D.
3 h	T[e]	0.16 ± 0.06	T	N.D.
6 h	0.13 ± 0.05	T	T	N.D.
12 h	0.11 ± 0.04	T	N.D.	N.A.[f]
1 d	0.24 ± 0.08	T	N.D.	N.D.
2 d	0.12 ± 0.06	0.14 ± 0.04	N.D.	N.D.
3 d	T	N.D.	N.D.	N.D.
5 d	N.D.	N.D.	—	—
7 d	N.D.	N.D.	—	—

[a] Volume rates of application were 10 L/ha for B1, 5 L/ha for B2 and 2.5 L/ha for B3
[b] SS2 refers to the stream sampling location in block B2, and SS3 to the stream sampling location in B3
[c] N.D. = not detected; limit of detection was 0.05 μg/g
[e] T = trace; 0.05 to 0.10 μg/g
[f] N.A. = not analyzed

Table 6. Average concentrations of diflubenzuron (DFB) in aquatic plants (manna grass (*Glyceria borealis*) in block B1, and water smartweed (*Polygonum natans*) in B2 and B3) following aerial applications of Dimilin

Time after application	Average (n = 3) concentration (μg/g) of DFB			
	Block B1[a]		Block B2[a]	Block B3[a]
	T1	T2	SS2[b]	SS3[b]
Pre-spray	N.D.[c]	N.D.	N.D.	N.D.
1 h	0.29 ± 0.11	0.14 ± 0.06	T[e]	T
3 h	0.24 ± 0.08	0.11 ± 0.05	T	T
6 h	0.27 ± 0.13	T	T	T
12 h	0.31 ± 0.09	T	T	N.A.[f]
1 d	0.36 ± 0.08	T	T	T
2 d	0.33 ± 0.07	T	T	T
3 d	0.34 ± 0.09	T	N.D.	T
5 d	0.13 ± 0.04	T	N.D.	N.D.
7 d	T	N.D.	N.D.	N.D.
10 d	N.D.	N.D.	N.D.	N.D.
15 d	N.D.	N.A.	N.A.	N.A.

[a] Volume rates of application were 10 L/ha for B1, 5 L/ha for B2 and 2.5 L/ha for B3
[b] SS2 refers to the stream sampling location in block B2, and SS3 to the stream sampling location in B3
[c] N.D. = not detected; limit of detection was 0.05 μg/g
[e] T = trace; 0.05 to 0.10 μg/g
[f] N.A. = not analyzed

Sediments and Aquatic Plants: Only trace levels of DFB (0.05 to 0.10 μg/g) were detected in stream sediments and plants from B1 and B2 (Tables 5 and 6).

Effects of DFB on Nontarget Aquatic Invertebrates

Caged Invertebrates: Mortality of caged *Chaoborus* larvae exceeded 60% in both treated ponds, but was not significantly different (G-test $p > 0.05$) from control mortality on any date (Figure 2). Because of the high mortality of *Chaoborus* in the control pond, treatment effects cannot be separated from caging effects. Of the *Chaoborus* that died, 58% died as larvae and 42% died as pupae in the control pond, 31% as larvae and 69% as pupae in T1, and 45% as larvae and 55% as pupae in T2, indicating that DFB did not inhibit pupal development in the treated ponds. In the second group of *Chaoborus* larvae that were caged in the control and treatment ponds 2 days after the application, there was no evidence of treatment effects from residual DFB on the larvae in T1, and mortality in the control and T1 ponds did not exceed 15% (Table 7). Mortality of *Chaoborus* in the

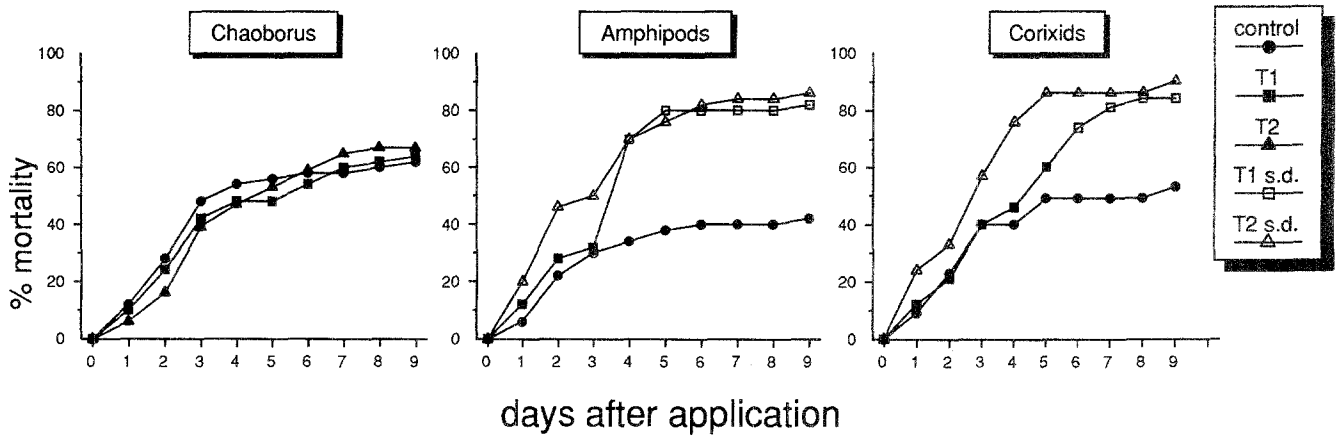

Fig. 2. Mortality of caged invertebrates in treated and control ponds. Open symbols are significantly different from control ($p < 0.05$); solid symbols are not ($p > 0.05$)

Table 7. Percent mortality of *Chaoborus* larvae placed in cages 2 days after the Dimilin application to treatment ponds T1 and T2[a]

	Days afer application						
	3	4	5	6	7	8	9
Control	10	10	15	15	15	15	15
T1	0	0	3	10	10	10	13
T2	23*	28*	43*	48*	57*	71*	71*

[a] Numbers followed by an (*) are significantly different from the control on that day (G-test of independence $p < 0.05$)

second group from T2 was significantly higher (G-test $p < 0.05$) than control mortality on every date, and exceeded 70% by 9 days after treatment. This mortality in T2 indicates that DFB residues persisted in T2 at levels toxic to *Chaoborus* larvae for up to 8 days.

Mortality of caged amphipods and corixids was approximately twice as high in the treated ponds (82–90%) as in the control pond (42–53%). The mortalities of caged amphipods in T2 and T1 were significantly higher than control by 1 day and 4 days post-treatment, respectively (G-test $p < 0.05$; Figure 2). Caged corixid larvae in T2 also showed significant mortality by 1 day post-treatment, but mortality in T1 was not significantly higher than control until 6 days post-treatment (G-test $p < 0.05$).

Mortality of caged amphipods and corixids occurred more quickly and attained a slightly higher level in T2 than in T1, despite the lower concentrations of DFB measured in T2 after the application (Table 3). The reason for this discrepancy is unclear, but may involve the bioavailability of the insecticide to the invertebrates in the floating cages. The concentrations of DFB were higher in water samples collected from T1, but because this pond was more turbid than T2 (Table 1), much of the insecticide may have been strongly adsorbed to suspended particulates and less available to the insects. Coats et al. (1989) reviewed the toxicity of synthetic pyrethroid insecticides to aquatic organisms and found that in several studies, the toxicity of pyrethroids to fish and aquatic invertebrates was significantly reduced in the presence of suspended particulate matter.

Littoral Invertebrates: The abundance and composition of littoral macroinvertebrates collected in sweep samples were extremely variable among sites and over time. During the sampling period, a total of 25 species of insects, and several species of amphipods, gastropods, pelecypods, oligochaetes, and hydracarinids were collected (unpublished data). Those present in both treated and control ponds, and collected in sufficient numbers for analysis, included the mayfly *Caenis* sp., the dragonflies *Celithimis* sp. and *Sympetrum* sp., the damselfly *Coenagrion* sp., the water boatmen *Sigara* sp. and unidentified immature corixids, the backswimmer Notonectidae (mostly *Notonecta* sp.), various beetle larvae (grouped under Coleoptera, including mostly *Agabus* sp., *Dineutus* sp., *Graphoderus* sp. and *Tropisternus* sp.) and midge larvae (Chironomidae).

Abundance data for these invertebrate groups (mean number per m^2, untransformed data) are presented in Table 8. The results of 2-way ANOVA on Table 8 are for $\ln(x + 1)$ transformed data and indicate significant differences in patterns of invertebrate abundance between treated and control ponds over the season. The impact column of Table 8 indicates whether these differences are likely due to treatment. A more detailed description of the response of each group to the application follows.

Caenis sp. was significantly reduced in T1 (ANOVA $p < 0.01$) and T2 (ANOVA $p < 0.05$) after treatment. The abundance of *Caenis* in the control was also reduced during the same period, but the decline was not as great as in the treated ponds, resulting in a significant interaction term (i.e. changes in abundance over the season were significantly different in treated and control ponds). The reduction in *Caenis* was greater in T1 than in T2 (no mayflies were found between 21 and 68 days post-treatment in T1), but by 110 days after treatment, *Caenis* abundance in both treatment ponds was similar (Tukey $p > 0.05$) to pre-treatment levels.

Celithemis sp. also showed significant differences in patterns of abundance between the treated and control ponds (ANOVA $p < 0.01$). Numbers of *Celithemis* in T1 were comparatively low during the entire season and declined to 0 by 21 days after treatment. The abundance of *Celithemis* in the control increased significantly between 9 and 21 days post-treatment (Tukey $p < 0.05$). The abundance of *Celithemis* in

Table 8. Abundance (mean no./m² ± 1 SE) of littoral invertebrates in control (C) and treated (T1, T2) ponds before and after aerial applications of Dimilin to Block B1

Taxon	Site	Pre[a]	Post3	Post9	Post21	Post34	Post68	Post110	ANOVA[b]	Impact[c]
Caenis sp.	C	147.7 ± 39.8	30.4 ± 7.5	4.6 ± 2.9	2.8 ± 1.7	2.3 ± 1.3	10.6 ± 2.8	149.0 ± 69.0		
	T1	16.6 ± 6.4	12.9 ± 4.5	0.9 ± 0.6	0	0	0	7.8 ± 4.5	**	y
	T2	48.8 ± 26.9	46.0 ± 16.0	12.9 ± 5.7	0.5 ± 0.5	0	2.3 ± 1.0	34.0 ± 11.9	*	y
Celithemis sp.	C	24.8 ± 11.1	14.7 ± 4.9	5.1 ± 3.9	22.5 ± 4.7	8.3 ± 4.3	2.8 ± 1.3	5.5 ± 2.1		
	T1	3.7 ± 1.2	2.3 ± 1.0	1.4 ± 0.6	0	0.5 ± 0.5	1.4 ± 0.9	2.3 ± 1.5	**	y
	T2	8.7 ± 2.2	10.6 ± 3.3	7.4 ± 1.8	1.8 ± 1.3	0.9 ± 0.6	6.9 ± 1.6	5.1 ± 2.0	**	y
Sympetrum sp.	C	3.2 ± 1.6	5.5 ± 2.8	0.5 ± 0.5	0	0	0	0		
	T1	0	0	0	0	0	0	0		
	T2	41.9 ± 18.6	20.7 ± 3.0	1.8 ± 1.1	0	0	1.4 ± 0.9	0	**	n
Coenagrion sp.	C	8.3 ± 1.6	4.6 ± 1.9	0	1.4 ± 0.6	3.7 ± 2.1	10.6 ± 2.2	51.1 ± 9.0		
	T1	4.6 ± 2.6	4.1 ± 2.2	2.8 ± 1.1	0.5 ± 0.5	0	6.4 ± 1.5	16.7 ± 5.2	*	y
	T2	19.8 ± 5.0	21.2 ± 4.4	3.2 ± 1.2	0.9 ± 0.6	0	12.4 ± 3.9	25.8 ± 7.9	***	y
Corixidae	C	2.7 ± 1.7	2.7 ± 1.1	0.9 ± 0.9	1.4 ± 0.9	8.3 ± 3.0	0	0		
	T1	67.6 ± 20.9	77.3 ± 15.9	9.2 ± 2.9	0	1.4 ± 0.9	15.2 ± 2.6	0.5 ± 0.5	***	y
	T2	2.8 ± 1.3	4.6 ± 2.6	0.5 ± 0.5	0	1.8 ± 0.9	0	0		
Sigara sp.	C	2.8 ± 1.8	0.5 ± 0.5	0.9 ± 0.6	0.5 ± 0.5	0.5 ± 0.5	0	1.8 ± 1.3		
	T1	3.7 ± 1.4	13.8 ± 3.0	8.7 ± 2.2	8.3 ± 3.3	3.2 ± 1.6	2.3 ± 0.7	3.6 ± 1.6	NS	n
	T2	0.9 ± 0.9	0	0	0.5 ± 0.5	0.5 ± 0.5	0	0		
Notonectidae	C	3.2 ± 1.2	5.5 ± 1.6	0.5 ± 0.5	1.8 ± 1.3	0.9 ± 0.6	2.3 ± 1.3	0.5 ± 0.5		
	T1	0.5 ± 0.5	3.7 ± 1.6	1.4 ± 0.6	0.9 ± 0.6	0.5 ± 0.5	0.9 ± 0.6	1.8 ± 1.1		
	T2	22.5 ± 5.2	17.9 ± 6.5	0.9 ± 0.6	0.9 ± 0.6	0.5 ± 0.5	1.8 ± 1.3	5.5 ± 1.4	**	n
Coleoptera	C	5.1 ± 1.5	8.3 ± 2.6	0	0.5 ± 0.5	0.9 ± 0.6	0.5 ± 0.5	1.4 ± 0.6		
	T1	20.7 ± 7.2	36.8 ± 7.7	14.3 ± 4.2	17.5 ± 3.1	23.5 ± 6.0	14.3 ± 2.0	1.4 ± 0.6	***	n
	T2	7.9 ± 2.7	7.4 ± 1.7	0.5 ± 0.5	1.8 ± 1.1	0.5 ± 0.5	0.5 ± 0.5	3.2 ± 1.6		
Chironomidae	C	198.7 ± 43.1	82.3 ± 17.7	41.9 ± 8.0	97.5 ± 28.3	113.2 ± 70.2	59.8 ± 9.3	156.9 ± 42.5		
	T1	71.8 ± 28.8	110.9 ± 19.5	75.4 ± 36.3	63.9 ± 15.8	57.0 ± 14.6	134.0 ± 30.3	663.0 ± 207.0	**	n
	T2	100.7 ± 34.7	107.2 ± 15.2	23.5 ± 7.3	35.9 ± 7.8	74.5 ± 10.0	27.6 ± 5.4	61.6 ± 27.6		

[a] Pre = 5 days pre-treatment; Post (X) = number of days (X) post-treatment

[b] Results of significance test of time X site interaction term in 2-way ANOVA (see text for details); NS $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[c] Indication of treatment effects based on comparisons with control (see text for details); y = treatment effect (significant interaction results from a reduction in abundance in the treated pond coincident with either an increase or a non-significant decline in abundance in the control pond; n = no treatment effect (significant interaction results from an increase in abundance in the treated pond coincident with either a decline or a non-significant increase in abundance in the control pond

T2 declined between 9 and 21 days after treatment, in contrast to the increase in the control over this period. Populations in both treatment ponds had returned to pre-treatment levels by 68 days post-treatment (Tukey $p > 0.05$).

There was little evidence of a treatment effect on *Sympetrum* sp. in T2. This dragonfly species did not occur in T1. The abundance of *Sympetrum* in T2 was reduced to 0 by 21 days post-treatment, but paralleled the decline of this species in control. The significant interaction (ANOVA $p < 0.01$) occurred largely because of an increase in abundance in T2 at 68 days post-treatment while *Sympetrum* remained absent in samples from the control pond to the end of the season. Although the significant decline in abundance of *Sympetrum* in T2 between 4 days pre-treatment and 9 days post-treatment (Tukey $p < 0.001$) suggests a response to the insecticide, a non-significant decline in abundance at the control site during the same period (Tukey $p > 0.05$) suggests otherwise.

Changes in abundance of *Coenagrion* sp. in the treated ponds indicated a reduction in numbers that may have been in response to DFB. Differences in abundance between the treatment and control ponds were significant (ANOVA $p < 0.05$ for T1, $p < 0.001$ for T2). Numbers of *Coenagrion* in the control increased between 9 and 34 days post-treatment, while abundance in the treated ponds was reduced to 0. By 68 days post-treatment, numbers in all three ponds had returned to pre-treatment levels (Tukey $p > 0.05$). Numbers of *Coenagrion* in the control pond were significantly higher 110 days after treatment than during the pre-treatment period (Tukey $p < 0.01$). The abundance in both treated ponds increased between 4 days pre-treatment and 110 days post-treatment, but not significantly (Tukey $p > 0.05$), suggesting a possible delayed recovery of *Coenagrion* in the treated ponds.

Trends in abundance of immature Corixidae did not differ significantly between the control pond and T2 (ANOVA $p > 0.05$), but were significantly different between the control pond and T1 (ANOVA $p < 0.001$). The significant interaction between the control and T1 sites occurred largely because of a significant increase in the abundance of corixids in T1 between 34 and 68 days post-treatment (Tukey $p < 0.001$), coincident with a significant decline in the control pond (Tukey $p < 0.001$). This difference is not likely related to the DFB application. The patterns of abundance in T1 and the control were also different between 3 and 9 days after treatment. Corixids declined significantly in T1 during this period (Tukey $p < 0.001$), but not in the control (Tukey $p > 0.05$). The sharp decline in abundance in T1 may be attrib-

uted to treatment. The significant increase in abundance later in the season suggests that this effect was short-term, however.

There were no indications of impact on the remaining groups of littoral insects. Changes in abundance of *Sigara* sp. over the season in treated and control ponds were similar (ANOVA $p > 0.05$). Significant differences in patterns of abundance between treated and control ponds for Notonectidae in T2, Coleoptera in T1, and Chironomidae in T1, resulted from increases in the treated ponds concurrent with decreases or smaller increases in the control pond, and do not indicate treatment effects.

In summary, the Dimilin application appears to have resulted in significant but temporary effects on a few species of littoral insects. Of nine taxa collected in sufficient numbers for meaningful comparisons to control, *Caenis*, *Celithemis* and *Coenagrion* were significantly reduced after the application, immature corixids in T1 may have been affected, and the remainder showed little or no evidence of significant treatment effects. The aquatic insect reductions tended to be slightly greater and of longer duration in T1, corresponding to higher DFB residues in that pond, but this difference was not pronounced or consistent. The reductions in abundance were short-term, with numbers returning to pre-treatment or control levels (Tukey $p > 0.05$) by 68 to 110 days after treatment. Treatment effects were generally not apparent until 21 to 34 days after the application. This delayed toxic response can be related to DFB's mode of action on invertebrates. Dimilin inhibits the synthesis of chitin and therefore causes mortality only when the molting process occurs, including larval molts and adult emergence of aquatic macroinvertebrates (Nebeker *et al.* 1983).

Our data are in agreement with the results of several other studies that have demonstrated that field applications of DFB at 28 to 280 g/ha can cause reductions of nontarget aquatic arthropods in lakes and ponds, with recovery of affected taxa requiring up to several weeks (Ali and Lord 1980; Ali and Mulla 1978a, 1978b; Farlow *et al.* 1978; Hanson and Garton 1982a, 1982b; Larson and Bridson 1987; Mian and Mulla 1982; Miura and Takahashi 1974; Nebeker *et al.* 1983).

Zooplankton

There were significant differences in the patterns of abundance of cladocera between the treated and control ponds (ANOVA $p < 0.0001$; Figure 3). Numbers of Daphnidae and Bosminidae in the control pond increased significantly between 5 days before and 3 days after treatment (Bonferroni t-test $p < 0.01$ for Daphnidae and $p < 0.05$ for Bosminidae; Figure 3). Over the same period, populations of these organisms were significantly reduced in T1 (Bonferroni t-test $p < 0.05$ for Daphnidae; Figure 3) and T2 (Bonferroni t-test $p < 0.01$ for Daphnidae and $p < 0.001$ for Bosminidae; Figure 3) and remained at very low levels until 68–110 days post-treatment. Cladocera populations were also reduced in the control pond, but not until 9–21 days after treatment for Daphnidae (Bonferroni t-test $p < 0.05$; Figure 3) and Bosminidae (Bonferroni t-test $p < 0.01$; Figure 3), respectively.

Cladocera populations normally undergo seasonal fluctuations characterized by a rapid increase in numbers during early summer followed by a decline in mid-late summer (Pennak 1978). This pattern was observed in the control pond, but was disrupted in T1 and T2. A second population pulse may or may not occur in the autumn (Pennak 1978). Daphnidae and Bosminidae both exhibited a late summer population pulse 68–110 days after treatment (Figure 3).

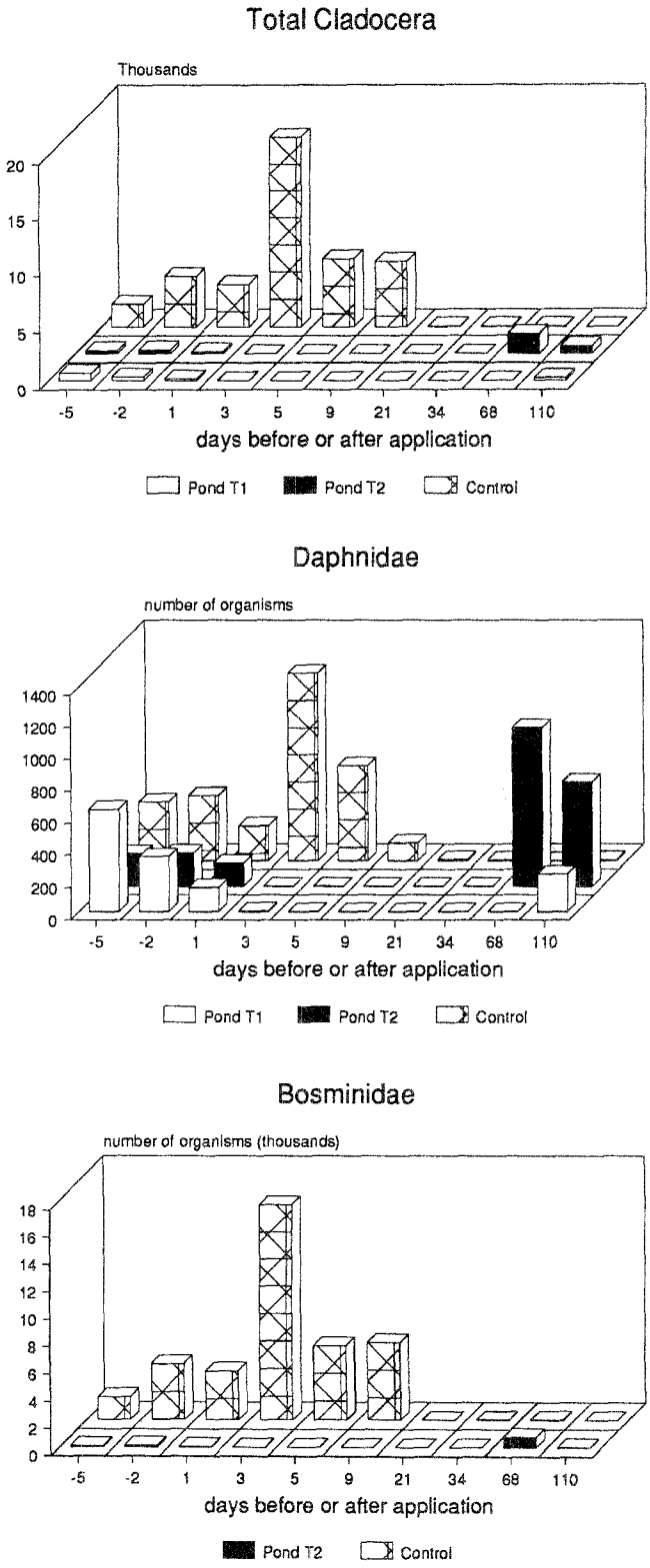

Fig. 3. Population trends of cladocera in treated and control ponds

This pulse, which was not evident in the control, was larger in T2 than in T1, and may have been secondarily related to the Dimilin treatment. A reduction in the numbers of macroinvertebrates and zooplankton in the treated ponds during mid-summer may have resulted in an increase in available food (plankton, algae, bacteria, protozoans) or reduced predation pressure later in the season. By 68 days post-treatment, the direct effects of Dimilin would no longer be important since residues had dropped to very low levels by about 20 days post-treatment (Fig. 3).

Patterns of abundance of copepods in the control and treated ponds differed significantly (ANOVA $p < 0.0001$; Figure 4). This difference cannot be positively attributed to treatment, however, because the significant decline in T1 from 3–34 days post-treatment (Bonferroni t-test $p < 0.05$; Figure 4) and in T2 from 1–34 days post-treatment (Bonferroni t-test $p < 0.05$; Figure 4), were accompanied by similar reductions in the control (Bonferroni t-test $p < 0.05$; Figure 4). Copepod abundance in the control 3 days post-treatment was not significantly different from the pre-treatment level and is an exception. A similar pattern of significantly reduced abundance in all ponds from 3–34 days post-treatment was observed for copepod nauplii, when these were considered separately (Bonferroni t-test $p < 0.05$; Figure 4). In this case, the control data from 21 days post-treatment are the only exception.

A treatment effect on adult copepods and copepodids was more obvious. Although populations declined in all study ponds over the season, the reductions occurred earlier in the treated ponds than in the control pond (Figure 4). Populations were significantly reduced in T1 by 3 days post-treatment (Bonferroni t-test $p < 0.01$; Figure 4) and in T2 by 5 days post-treatment (Bonferroni t-test $p < 0.01$; Figure 4), but not until 21 days post-treatment in the control (Bonferroni t-test $p < 0.05$; Figure 4).

In the control pond, high numbers of copepod nauplii were present in the spring, but numbers declined over the summer as these moulted into copepodids (Figure 4). Numbers of copepodids and adults also declined, but not until later in the season (Figure 4). This pattern was disrupted by the Dimilin treatment. In the treated ponds, relatively few nauplii were successful in moulting into copepodids and consequently the decline in numbers of adults and copepodids occurred earlier (Figure 4).

As with cladocera, a second population pulse of copepods was observed in the treated ponds 68–110 days post-treatment (Figure 4) and was probably the result of the same factors, specifically increased food availability and reduced predation.

Published studies have shown that Dimilin applications can suppress populations of microcrustacean zooplankton, that cladocera are affected more than copepods, and that the time for recovery of these animals ranges from one week to three months depending on the treatment rate and species affected (Ali and Mulla 1978a, 1978b; Apperson *et al.* 1978; Miura and Takahashi 1974, 1975; Mulla *et al.* 1975). These observations were confirmed in the present study. Effects on copepods were less severe than on cladocera and were of shorter duration. Copepod populations recovered by 68 days post-treatment; cladocera recovered by day 68 in T2, but not until day 110 in T1, where DFB residues were higher.

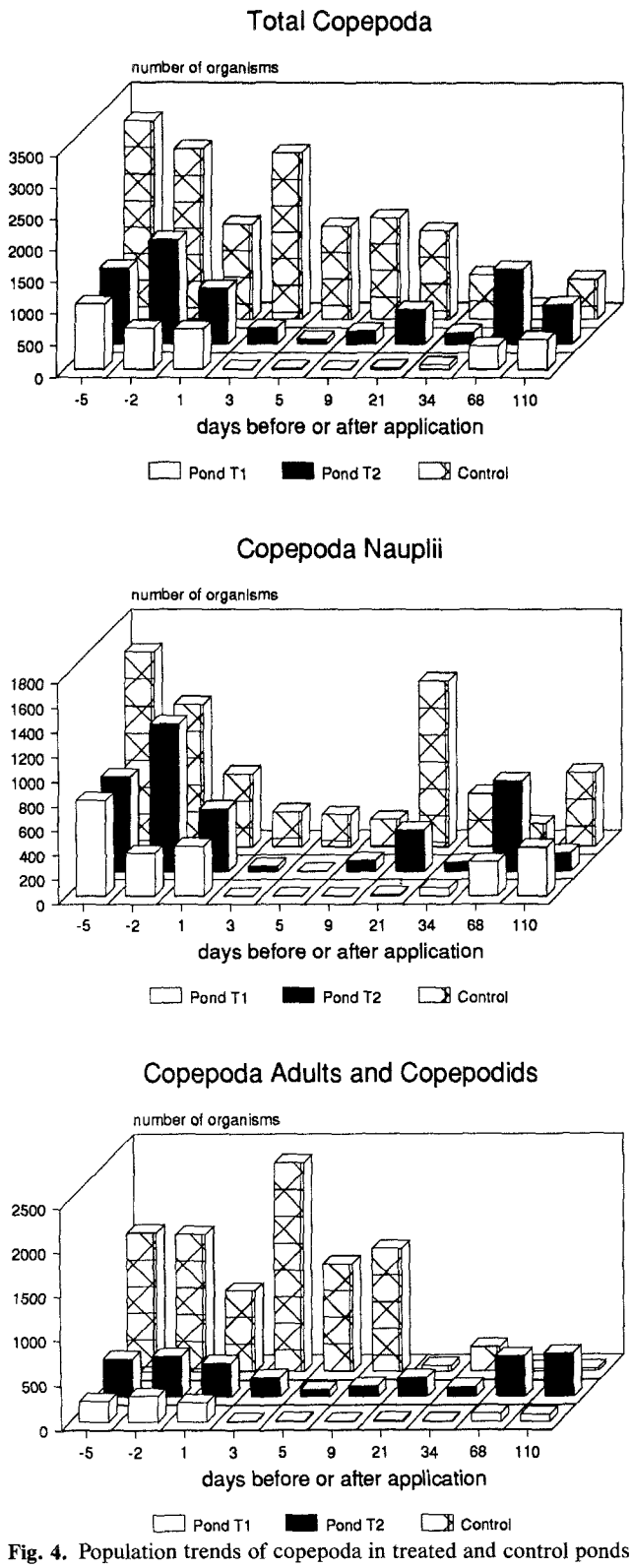

Fig. 4. Population trends of copepoda in treated and control ponds

Conclusions

Direct overspraying of ponds with Dimilin at 70 g/ha will result in appreciable residues in water which may persist for 2 to 3 weeks. The abundance of some macroinvertebrates

may be significantly reduced, but the effects are likely to be sporadic, inconsistent, and temporary. Zooplankton populations, particularly cladocera, will also be suppressed. Results from this and other impact assessments indicate that reductions in macroinvertebrates and zooplankton should not persist beyond 2 to 3 months. These impacts can be reduced by avoiding direct contamination of water during forest spraying.

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