

Persistence of Diflubenzuron on Appalachian Forest Leaves after Aerial Application of Dimilin

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Using a new method for the analysis of diflubenzuron, the active ingredient in the pesticide Dimilin, the persistence of diflubenzuron on leaves of 20 Appalachian forest trees of 7 species was studied throughout the 1991 season after aerial application of the pesticide. Composites were made from leaf samples of each the upper and lower tree canopies, and replicate subsamples were analyzed for diflubenzuron concentration. The two values closely agreed, confirming composite homogeneity and precision of the analytical method. Growth dilution of diflubenzuron during this study was negligible. A significant loss of diflubenzuron from the foliage, ranging from 20% to 80%, was observed within the first 3 weeks after application. The remaining diflubenzuron was generally found to persist for the rest of the growing season until leaf fall, at which 13 of the 20 trees retained more than 20% of the original pesticide applied. The remaining seven trees, consisting of all five yellow poplars and two black oaks, showed from 5% to 20% diflubenzuron retention, primarily due to a greater initial crop. At leaf fall, the residual diflubenzuron is delivered along with the leaves to the streams and the ground litter underlying the canopy.

INTRODUCTION

The pesticide diflubenzuron (trade name Dimilin) acts as an insecticide by inhibiting the deposition of cuticular chitin, thereby disrupting normal insect growth and development (Haijar and Casida, 1978; Marx, 1977; Mulder and Gijswijt, 1973; Post et al., 1974; Verloop and Ferrell, 1977). Its use in forest ecosystems to combat the gypsy moth has the potential to expose and impact numerous nontarget species that also depend upon chitin production for their growth (Muzzarelli, 1986). Understanding the environmental fate of diflubenzuron in the forest ecosystem should guide and complement nontarget impact studies, thereby expanding the base of knowledge for informed decision making regarding the use of diflubenzuron in this complex and diverse environment.

Previous studies of diflubenzuron persistence on foliage, recently reviewed by Fischer and Hall (1992), have shown that ¹⁴C-labeled diflubenzuron applied to cotton leaf surfaces is resistant to photodegradation (Bull and Ivie, 1978; Schaefer and Dupras, 1976) and is not taken up or metabolized by the leaves (Bull and Ivie, 1978; Mansager et al., 1979). Minimal losses were observed over a 14-day period due to volatilization or weathering, and 23% of the original diflubenzuron was subsequently retained following exposure to 7-8 cm of rain between days 14 and 21 (Bull and Ivie, 1978). On citrus leaves, diflubenzuron was found to persist with no loss over 1 month if conditions were cool and dry or with a 50% loss within the month if conditions were hot and wet (Nigg et al., 1986).

A short-term (21-day) study of diflubenzuron persistence on three Appalachian oak trees in West Virginia was reported using an HPLC analytical method requiring sample cleanup to remove interfering compounds (Martinat et al., 1987). The pesticide was reported to decrease on the leaves to approximately 20% of the application level within 10 days postspray during which a rain in excess of 3 cm fell. An initial loss of 1/3 of the diflubenzuron was observed without a rain even, however. Between 10 and 21 days, although more rain had occurred, no further loss

of diflubenzuron was seen. Relatively large margins of error were observed in the analytical data of this study.

In a research summary report, 25% of the original diflubenzuron residue has been reported to persist on southern Appalachian forest leaves after 63 days (Van den Berg, 1986). Diflubenzuron persistence on foliage has also been suggested by the finding of an increase in diflubenzuron concentration in ground litter the year after application in a coniferous forest, attributed to residual pesticides on needles that fell the previous autumn (Mutanen et al., 1988). To date, no comprehensive diflubenzuron persistence study in a forest ecosystem throughout the growing season, as well as post leaf fall, has been reported.

Critical to a thorough environmental fate study is the availability of a rapid, sensitive, and precise method for analyzing diflubenzuron in complex environmental samples such as extracts of foliage, ground litter, or soil. Methods for analyzing diflubenzuron have been limited in the past due to various factors (Wimmer et al., 1991). However, a newly developed procedure takes advantage of the heat-induced fragmentation of diflubenzuron during gas chromatography (Wimmer et al., 1991). With mass spectrometric detection of the resulting fragments using selected ion monitoring along with deuterated diflubenzuron as an internal standard, the interference from coextracted compounds in leaf extracts is removed without purification of the diflubenzuron. Thus, rapid and sensitive analyses can be done, making a comprehensive environmental fate study feasible.

This paper describes such a study in 1991 of the persistence of diflubenzuron on 20 hardwood trees, 7 different species, in an Appalachian forest environment over 141 days of postspray growing season. The tree sampling method is detailed, and the new GC/mass spectrometric analytical method for diflubenzuron is used. The following paper (Harrady et al., 1993) describes an investigation of the subsequent fate of diflubenzuron when

leaves containing the pesticide fall into a stream ecosystem underlying the tree canopy.

MATERIALS AND METHODS

Materials. All pesticide extraction and storage solvents were Fisher Optima grade. Acetone and methylene chloride recovered from the leaf extractions were redistilled and reused. Water was removed from the latter by decanting and then passing the solvent through anhydrous sodium sulfate; no diflubenzuron was found upon analysis of a 250-mL sample concentrated to 150 μ L. Deuterated diflubenzuron was synthesized as previously described (Wimmer et al., 1991). Commercial diflubenzuron wettable powder formulation (Uniroyal 25WP) was contributed and applied by the West Virginia Department of Agriculture as part of their 1991 diflubenzuron spray program for control of the gypsy moth.

Dimilin Application. Dimilin was applied to a forest spray block (1120 acres) in the West Virginia University Experimental Forest outside Morgantown, WV, using a Twin Beech aircraft flying just above tree-top level. The application rate was 0.03 lb of ai/acre (1 gal/acre) using an aqueous suspension of the wettable powder. In the center of the block, 20 trees (7 different species) 15-30 m apart along an approximately linear transect were labeled for sampling throughout the season. Within 0.5 h after spray and after sampling only trees 19 and 20, a rainstorm occurred which drenched the leaves; therefore, the Dimilin application was repeated the following morning to ensure ample coverage at the start.

Leaf Sampling and Composite Formation. For each sampling time, branches from approximately the four cardinal directions were manually clipped by tree climbers from the upper and lower canopies of each of 20 sample trees. From these branches, 200 representative leaves were removed by hand for each canopy; gloves were changed between trees to prevent cross-contamination. The samples were returned to the laboratory in zip-lock bags where they were stored initially at 4 °C. The leaves were counted as the total leaf area was measured using an automatic leaf area meter (Li-Cor Co., Lincoln, NE) at the USDA—Forest Service Northeast Experiment Station in Morgantown, WV.

The leaves were cut manually using scissors into approximately 1-cm square pieces, and the pieces were well-mixed by shaking in an air-filled polyethylene sample storage bag (30 \times 51 cm) for 2 min to generate a homogeneous composite sample of the 200 leaves. The total composite weight was recorded, and two subsamples of different weights, 8-12 g each, were weighed out into zip-lock bags to extract and analyze for diflubenzuron. All samples were stored frozen at -23 °C in non-frost-free freezers until subsample extraction; diflubenzuron has been extracted from leaves stored frozen for over a year, and no loss of pesticide is observed (M. J. Wimmer, West Virginia University, 1992, unpublished observation). No dry weight of leaves was taken because the normal drying process (heating) would likely decompose at least some of the heat-labile diflubenzuron as well as potentially affect its binding to, thus extraction from, the leaf surface.

Extraction and Analysis of Diflubenzuron from Leaf Subsamples. Extraction of diflubenzuron from each leaf subsample and its subsequent quantification were done as previously described (Wimmer et al., 1991) with one modification: the deuterated diflubenzuron internal standard (25 μ g) was added in the first of three acetone washes, instead of at the end of the workup when yield was being studied. After the three washes were combined, the protonated:deuterated ratio of the two diflubenzuron species was fixed and the yield in subsequent steps was not critical. This is one of the major advantages of isotope ratio methods for quantifying pesticides. The protonated diflubenzuron originated solely from the environmental sample. From the known amount of deuterated diflubenzuron added and the H/D isotope ratio of the resulting diflubenzuron fragments after gas chromatography/mass spectrometry, the amount of the protonated form can be calculated (Wimmer et al., 1991).

The amount of diflubenzuron can be expressed in ng/cm² of leaf area (concentration on the leaf surface)/or μ g/kg (ppb, concentration by nondried leaf weight). To determine the actual

amount of diflubenzuron remaining where leaf growth had occurred, a growth dilution correction was made as follows: the diflubenzuron coverage (ng/cm²) was multiplied by the relevant growth factor determined by dividing the area/leaf at the time point of interest by the initial area/leaf at time zero (the first time point, taken immediately after diflubenzuron application).

Field Weather Data. Weather data were obtained with the cooperation of the Federal Aviation Administration/Department of Transportation and the National Climatic Data Center, NOAA Environmental Data Service, Asheville, NC. The data were recorded at the Morgantown Municipal Airport monitoring station located approximately 13 km southwest of the West Virginia University Experimental Forest spray block and at a similar elevation.

RESULTS

Tree Sampling and Leaf Composites. In determining the environmental fate of diflubenzuron aerially applied as Dimilin to the leaves of Appalachian hardwoods, a sampling protocol that accurately reflects the amount of diflubenzuron on these 60-70-year-old trees during the growing season was necessary. The decision was made to determine the diflubenzuron levels on the upper and lower canopies of each tree by collecting a representative leaf sample from each canopy and averaging the total diflubenzuron over these leaves. After making a homogeneous composite from the leaves, we analyzed subsamples for diflubenzuron and the amount was then extrapolated back to the whole. Therefore, approximately 200 leaves each from the upper and lower canopies were removed from branches cut from the four cardinal directions for each of six time points after Dimilin application.

A key part of the analysis was the development of a procedure for making a homogeneous composite sample from the 200 sample leaves and then properly subsampling the composite for accurate replicate analyses. The leaves were cut into 1-cm square pieces in a composite whose total area was usually 10 000–20 000 cm². The size of the pieces allowed for good mixing within the foliage sample, and yet they were large enough to permit good settling of the leaf material for decanting of the acetone washes (Wimmer et al., 1991). The subsample size was set initially by the maximum volume of acetone desired when used at a rate of 10 mL/g of leaf material. From the composite, the two 8-13-g subsamples taken typically represented 4-10% of the total composite weight. Measuring the amount of diflubenzuron in the foliage subsample is made rapid (no purification required) and precise using the newly-developed GC/mass spectrometric analytical method (Wimmer et al., 1991).

Diflubenzuron Persistence on Leaves. In a study of 20 trees representing 7 tree species in the West Virginia University Experimental Forest, the amount of aerially applied diflubenzuron on the leaves was monitored from 141 days postspray (just prior to leaf fall) to determine its persistence on the foliage. The rainfall and air temperature data for each sampling period (Table I) illustrate that the 1991 growing season at the site received low amounts of precipitation during an unusually long and warm growing season.

Two representative diflubenzuron persistence curves, one for northern red oak (Figure 1A) and one for yellow poplar (Figure 1B), illustrate the utility of the quantitation method. (The poplar had lost its leaves by the 141-day sampling.) For each time point, the two coverage values shown are for the two subsamples taken from each of the 200-leaf composite samples. The excellent agreement seen between the two values supports the homogeneous nature of the composite when subsampled in this manner.

Table I. Weather Conditions during the 1991 Diflubenzuron Persistence Study^a

presampling period ^b	total period rainfall (in.)	period's av max temp (°F)	period's av min temp (°F)
5/14-5/15	0.06	84	63
5/15-5/22	0.00	80	58
5/23-6/03	1.01	86	65
6/04-7/07	2.55	85	62
7/08-8/12	2.38	87	62
8/13-10/02	2.58	81	57

^a Data received from the National Climatic Data Center, NOAA Environmental Data Service, Asheville, NC 28801. ^b Initial diflubenzuron application was on 5/14 followed by rain; final diflubenzuron application was on 5/15. Sampling dates were 5/15, 5/23, 6/04, 7/08, 8/13, and 10/03.

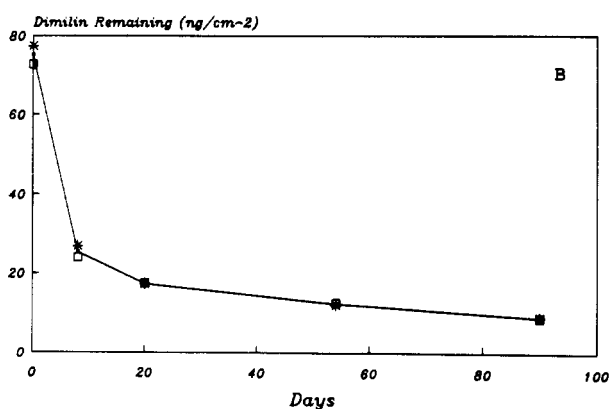
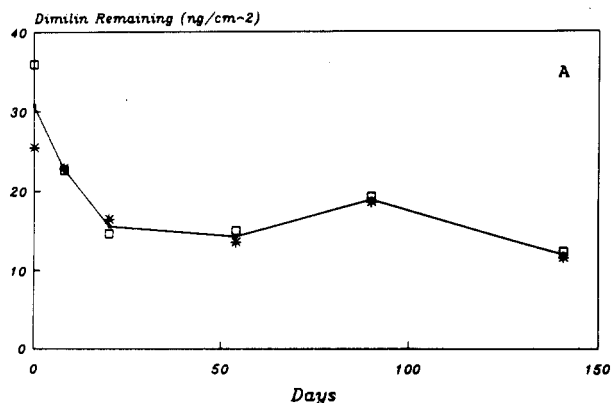


Figure 1. Analysis of diflubenzuron on tree foliage during the growing season. The two coverage values at each time point represent the coverage determined for two subsamples of the leaf sample composite as described under Materials and Methods. (A) Upper canopy of northern red oak; (B) upper canopy of yellow poplar.

Therefore, the amount of diflubenzuron can be precisely measured in a foliage sample using the GC/mass spectroscopic method, with the diflubenzuron level variability resting not on the sample workup and analysis but on the sampling of the tree itself during the season and on the sample handling prior to freezing.

The initial diflubenzuron coverage on the 20 sample trees was found to vary from 2.9 to 75 ng/cm² of leaf area on the upper canopy leaves and from 1.9 to 25 ng/cm² on the lower canopy leaves which, as expected, typically received less diflubenzuron than the leaves above them (Table II). The pattern of coverage went gradually from low to high as the tree number increased along the transect. The effect of a rain event immediately postspray was to wash nearly half the diflubenzuron off the upper leaf canopies of the two trees sampled (Table III). The reapplication of pesticide for the season-long persistence study did not raise the net coverage to an unusual level;

Table II. Initial Diflubenzuron Coverage of the Upper and Lower Canopy Leaves of Appalachian Forest Trees after Aerial Application

tree type, no. ^a	initial diflubenzuron coverage (ng/cm ²) ^b		% lower/upper
	upper canopy	lower canopy	
red maple			
1	2.91 (1.46) ^c	3.80 (0.78)	131
2	4.98 (0.58)	3.82 (0.18)	77
10	6.43 (0.12)	4.64 (0.00)	72
sugar maple			
4	5.14 (0.53)	5.03 (2.81)	68
yellow poplar			
5	9.35 (0.45)	3.38 (0.94)	36
11	6.71 (0.69)	4.40 (0.45)	66
13	13.9 (1.74)	7.34 (1.74)	53
17	39.5 (8.84)	12.9 (0.28)	33
20	75.0 (3.25)	8.09 (2.13)	11
white oak			
9	4.27 (1.99)	1.92 (0.06)	45
chestnut oak			
18	19.1 (0.93)	16.3 (3.10)	86
black oak			
3	11.0 (4.43)	7.30 (1.58)	66
6	9.79 (2.47)	10.4 (2.21)	107
northern red oak			
7	7.88 (3.92)	3.74 (0.11)	47
8	6.48 (0.80)	8.98 (1.25)	139
12	26.6 (5.66)	12.1 (2.28)	46
14	12.5 (1.12)	11.2 (1.05)	89
15	30.7 (7.37)	24.9 (0.01)	81
16	29.2 (0.04)	24.8 (8.20)	85
19	11.6 (0.62)	5.38 (0.88)	46

^a No. refers to the designated number of the individual tree monitored for diflubenzuron throughout the season. ^b Each value represents the average of two subsample values as described under Materials and Methods. ^c Numbers in parentheses are the standard deviations from the two subsample values. Where missing, only one subsample was analyzed.

Table III. Diflubenzuron Coverage on Tree Foliage before and after a Rain Event^a

tree sample	diflubenzuron coverage (ng/cm ²)		% diflubenzuron lost (av)
	postspray	postrain	
19 upper 1	18.7	12.2	47
19 upper 2	23.8	10.1	
20 upper 1	50.2	35.5	40
20 upper 2	65.6	34.8	

^a Composite samples were made from approximately 200 leaf samples of the upper canopies of tree 19 (northern red oak) and tree 20 (yellow poplar), and the two subsamples shown for each were analyzed for diflubenzuron as described under Materials and Methods. Diflubenzuron coverage is expressed as ng/cm² of leaf area. The rain event was a short, drenching thunderstorm.

the evidence for this is that 2-5 times the highest final level is being routinely seen in a 1992 helicopter application, at the same application rate, in the Fernow Experimental Forest near Parsons, WV (M. J. Wimmer, West Virginia University, 1992, unpublished observations).

After an initial significant loss of diflubenzuron, ranging from 20% to 80%, during the first 8 days after application, the remaining diflubenzuron is found to generally persist on leaf surfaces throughout the rest of the growing season, becoming resistant to wind or rain washoff or degradation. The data are presented in two formats: the percent of diflubenzuron remaining, with the average of the two subsample values plotted as a function of time for groupings of like tree species (Figure 2A-D); and the average of the diflubenzuron coverage in ng/cm² for each tree along with the standard deviation over time to illustrate the quality of agreement between subsample values (Table IV). Only a nonmeasurable trace of rain

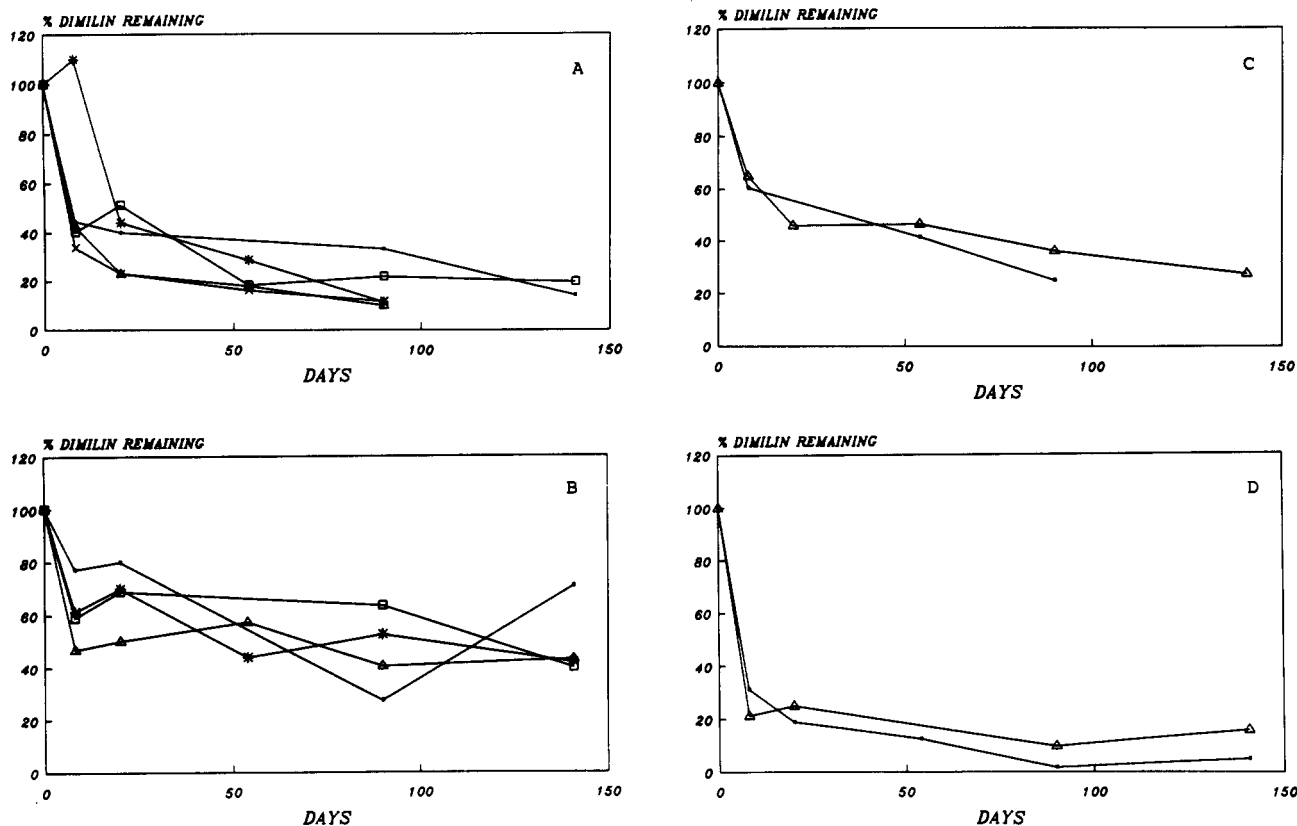


Figure 2. Persistence of diflubenzuron on Appalachian forest trees from application as Dimilin in May to leaf fall. The percentage of the originally-applied diflubenzuron remaining on the upper canopy leaves is plotted as a function of time, averaging the two subsample values for each tree at each time point. Tree numbers refer to those from Tables II and IV: (A) yellow poplar trees 5 (dot), 11 (triangle), 13 (asterisk), 17 (square), and 20 (cross); (B) red maple trees 1 (dot), 2 (triangle), and 10 (asterisk) and sugar maple tree 4 (square); (C) chestnut oak tree 18 (dot) and white oak tree 9 (triangle); and (D) black oak trees 3 (dot) and 6 (triangle).

fell during the first time period (8 days postspray), implying that the initial loss of diflubenzuron seen was due to wind or other physical removal of the dried powder formulation of the pesticide. The initial loss of diflubenzuron from yellow poplar and black oak species appeared consistently greater than that for the other species. Thirteen of the 20 trees retained more than 20% of the original diflubenzuron applied by the time of leaf fall. The remaining seven trees, consisting of the five yellow poplars and the two black oaks, showed from 5% to 20% diflubenzuron retention, primarily due to the greater initial drop.

On three of seven of the northern red oak trees (Figure 3) and one of the yellow poplars (Figure 2A), the diflubenzuron coverage appears to increase between days 0 and 8. The fact that the remainder of the curves look similar to the other trees suggests that the initial values may be low and not representative of the true initial coverage. Two possible causes for this may be either tree sampling variability or decomposition of the pesticide during sample storage and manipulation prior to freezing. Young leaves removed from the trees have been found to be more sensitive to decomposition than older leaves, some turning brown prior to freezing. Preliminary results from our 1992 diflubenzuron leaf persistence study on the Fernow Experimental Forest in West Virginia show levels of diflubenzuron retention similar to the trees in this 1991 study (M. J. Wimmer, West Virginia University, 1993, unpublished observations).

Growth Dilution. In an environmental fate study of a pesticide, two questions can be asked: (a) how long does the total pesticide originally introduced into the environment remain and (b) how does the efficacy of the pesticide, expressed by its concentration in the environmental media of interest, change during its lifetime? The

first question is answered by determining the total amount of pesticide in the environmental media as a function of time, in this case on leaf surfaces during the growing season. The micrograms of diflubenzuron in each leaf composite subsample is measured, converted to a normalized unit of concentration (either ng/cm^2 or $\mu\text{g}/\text{kg}$), and then compared with the initial value.

The problem arises when a significant size change occurs in the environmental media over time, such as the increase in both a leaf's area and weight during the growth season. This change alters the normalization factor which enables comparison with original pesticide levels and results in false, lower-than-actual numbers for the amount of pesticide remaining.

In 1991 at the West Virginia University Experimental Forest, the early onset of warm weather caused most leaf expansion to have occurred by the time of Dimilin application (May 14–15) in this 20-tree study. Growth was accurately monitored by measuring the total area of each leaf sample and dividing that by the actual number of leaves; thus, the area per leaf is averaged over the entire approximately 200-leaf sample. The only trees to show slight growth during the course of this diflubenzuron persistence study were the yellow poplars (Figure 4A–D), and then, only the tail end of their growth curve, if any, was seen. The highest growth dilution factor, 1.2–1.3, does not change the 10–20% range of diflubenzuron persistence discussed above. The maples and oaks did not show any consistent growth pattern, indicating that their growth was essentially complete at the time of spray.

Another factor that will affect the measurement of pesticide persistence is the growth of new leaves that were not present at the time of application and therefore, by definition, have zero diflubenzuron coverage unless from

Table IV. Persistence of Diflubenzuron on the Leaves of Appalachian Forest Trees during the 1991 Growing Season

tree type, no. ^a	diflubenzuron coverage (ng/cm ²)				
	8 days ^b	20 days	54 days	90 days	141 days
Upper Canopy					
red maple					
1	2.3 (1.1) ^c	2.3 (0.2)		0.8 (0.1)	2.1 (0.1)
2	4.2 (0.1)	4.5 (0.8)	5.2 (0.9)	3.7 (0.1)	3.9 (0.4)
10	4.0 (1.1)	4.5 (1.1)	2.8 (0.7)	3.4 (0.2)	2.7 (1.3)
sugar maple					
4	3.0 (0.1)	3.6 (1.4)		3.3 (1.4)	2.0 (0.0)
yellow poplar					
5	4.2 (0.7)	3.8 (0.6)		3.1 (0.1)	1.3 (0.1)
11	2.9 (1.3)	1.6 (0.2)	1.2 (0.9)	0.7 (0.0)	
13	15.2 (0.0)	6.1 (2.1)	4.0	1.6 (1.1)	
17	15.8 (2.5)	20.3 (3.5)	7.3 (0.5)	8.7 (1.7)	7.8 (0.4)
20	25.3 (2.1)	17.3 (0.1)	12.2 (0.4)	8.9 (0.1)	
white oak					
9	2.8 (1.9)	2.0 (0.2)	2.0 (1.4)	1.5 (0.4)	1.2 (0.3)
chestnut oak					
18	11.5 (0.8)		8.0 (1.5)	4.8 (0.9)	
black oak					
3	3.4 (0.6)	2.1 (0.1)	1.4 (0.1)	0.2 (0.0)	0.5 (0.1)
6	2.1 (0.3)	2.4 (0.7)		1.0 (0.0)	1.5 (0.8)
northern red oak					
7	3.9 (0.4)	3.2 (1.5)		5.6 (1.3)	2.5 (0.6)
8	3.1 (0.2)			4.6 (0.4)	3.5 (0.5)
12	17.0 (6.1)	4.9 (1.4)	7.3 (1.4)	8.2	11.1 (2.3)
14	22.5 (13.5)	10.3 (1.6)	3.2 (1.6)	10.6 (1.6)	11.9 (3.4)
15	22.8 (0.2)	15.6 (1.3)	14.3 (1.0)	18.9 (0.5)	12.0 (0.5)
16	35.9 (3.5)	26.0 (3.5)	21.3 (0.3)	14.6 (0.3)	18.5 (2.4)
19	17.4 (4.9)	11.0 (2.0)	10.0 (0.6)	9.4 (1.8)	11.7 (0.9)
Lower Canopy					
red maple					
10	3.2 (1.4)	1.7 (0.3)	0.9 (0.2)	0.8 (0.2)	3.8 (4.6)
yellow poplar					
11	2.0 (0.1)			0.7 (0.2)	
13	5.6 (0.6)	6.6 (1.1)	1.5 (1.1)	1.1 (0.3)	
17	10.8 (3.3)	5.9 (0.7)	4.1 (0.4)	3.8 (0.4)	
20	12.0 (0.6)	3.1 (0.2)	12.1 (7.8)	2.4 (0.4)	
white oak					
9	3.6 (0.6)	2.7 (0.4)	2.2 (0.5)	1.2 (0.6)	2.3 (2.2)
chestnut oak					
18	7.0 (0.6)	8.1 (2.6)	5.0 (2.0)	3.0 (0.3)	
northern red oak					
12	5.7 (0.7)	6.6 (1.2)		5.0 (0.2)	3.7 (0.2)
14	7.0 (0.3)			6.0 (1.5)	5.1 (0.2)
15	12.6 (2.1)	11.8	11.5 (0.8)	16.3 (0.6)	13.7 (1.4)
16	11.8	21.1 (4.4)	9.7	8.9 (0.9)	
19	6.3 (1.0)	7.6 (0.8)	18.9 (0.3)	4.7 (1.1)	2.0 (0.1)

^aNo. refers to the designated number of the individual tree monitored for diflubenzuron throughout the season. ^bThe values for diflubenzuron coverage immediately postspray (day zero) are given in Table II. ^cNumbers in parentheses are the standard deviations from the two subsample values. Where missing, only one subsample was analyzed.

pesticide runoff from adjacent dosed leaves. [Lack of systemic transfer of pesticide to the new leaves is indicated by the finding that diflubenzuron is not taken up by leaves or roots (Bull and Ivie, 1978; Mansager et al., 1979).] In sampling a tree during the growing season, any new leaves contributing to the total will result in a false, low value for pesticide persistence that can not be corrected by growth dilution. In this study, some care was taken to not remove new growth.

DISCUSSION

A comprehensive study of the environmental fate of diflubenzuron aerially applied to an Appalachian forest ecosystem begins by tracing its persistence on tree foliage, a major initial receptor, throughout the growing season. The behavior of diflubenzuron on leaves reported here followed an initial rapid decline within the first 2 weeks, after which the remaining diflubenzuron was generally

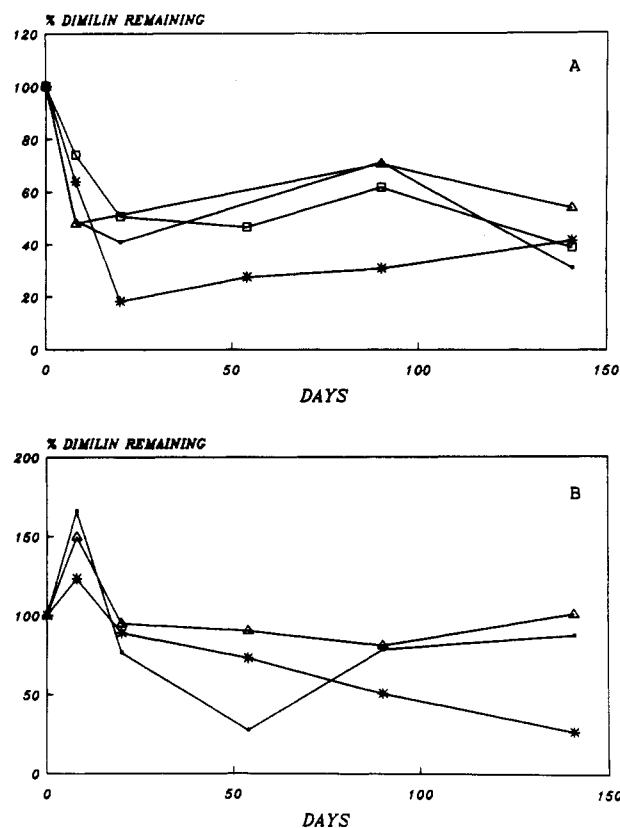


Figure 3. Persistence of diflubenzuron on Appalachian northern red oaks from application as Dimilin in May to leaf fall. The diflubenzuron coverage in ng/cm² is plotted as a function of time, averaging the two subsample values for each tree at each time point. Tree numbers refer to those from Tables II and IV: (A) trees 7 (dot), 8 (triangle), 12 (asterisk), and 15 (square) and (B) trees 14 (dot), 16 (asterisk), and 19 (triangle).

stable on the leaf surface until leaf fall over 4 months postspray. A similar pattern was seen with seven different tree species. A previous short-term study of diflubenzuron persistence on oak reported the same initial drop in the absence of rain (Martinat et al., 1987). Our observation of consistently more loss from yellow poplar and black oak may reflect leaf surface or physical movement differences. Whether or not the pesticide is lost as diflubenzuron or its breakdown products is not known, although our study of ground litter underlying the sample trees suggests an increase in the diflubenzuron level there prior to leaf fall (M. J. Wimmer, West Virginia University, 1992, unpublished observations).

The extraction of diflubenzuron from leaves in the field appears to be complete with acetone shaking, implying a lack of systemic absorption of the pesticide. With a variety of manually dosed fresh and dried leaves, diflubenzuron is extracted in over 95% yield (Wimmer et al., 1991). That diflubenzuron is extracted in virtually 100% yield after weathering on leaves in the field has been established by mortar-and-pestle grinding, in acetone or methylene chloride, leaf material remaining after the acetone wash; no additional diflubenzuron was detected in the several samples analyzed (M. J. Wimmer, West Virginia University, 1992, unpublished observations). Lack of absorption into the leaves of Appalachian hardwood trees is consistent with previous studies on cotton (Bull and Ivie, 1978; Mansager et al., 1979).

That a significant amount of diflubenzuron is found by direct measurement to persist on leaf surfaces throughout the growing season confirms and extends previous foliage studies (see Introduction). The observation is also con-

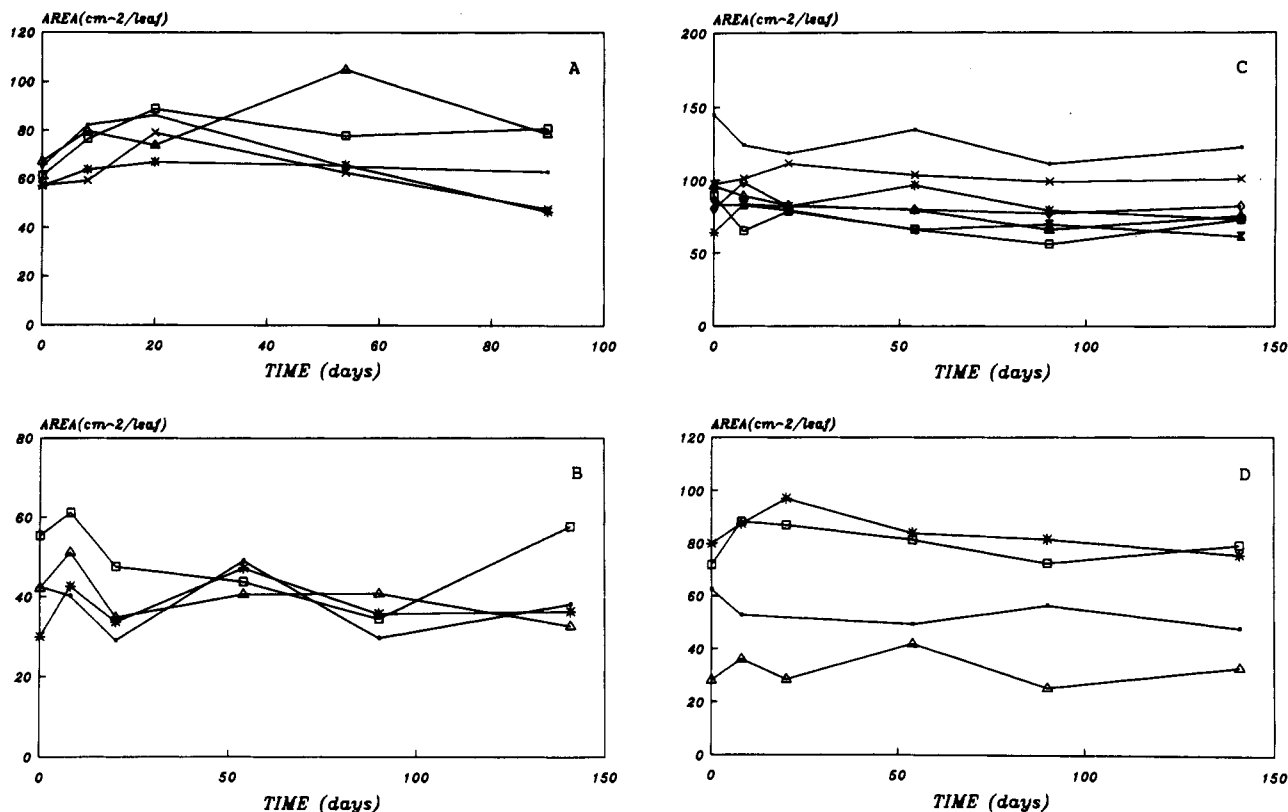


Figure 4. Variation of the upper canopy leaf area from Dimilin application in May to leaf fall. For each curve, the area per leaf averaged over the approximately 200-leaf sample is plotted as a function of time. Tree numbers refer to those from Tables II and IV: (A) yellow poplar trees 5 (cross); 11 (dot), 13 (triangle), 17 (asterisk), and 20 (square); (B) red maple trees 1 (dot), 2 (triangle), and 10 (asterisk) and sugar maple tree 4 (square); (C) northern red oak trees 7 (dot), 8 (hourglass), 12 (asterisk), 14 (square), 15 (cross), 16 (diamond), and 19 (triangle); and (D) chestnut oak tree 18 (dot), white oak tree 9 (triangle), and black oak trees 3 (asterisk) and 6 (square).

sistent with a technical report on the pesticide in which its "rainfastness" is described (Uniroyal, 1989). A bioassay for toxicity toward gypsy moth (*Lymantria dispar*) was used to test greenhouse oak seedlings for diflubenzuron efficacy after being sprayed with Dimilin 25WP at a rate of 1, 2, and 4 oz/acre (0.25-1 oz of ai/acre, in the same range as the present study) followed by simulated rainfall of 1-5 in. Mortality of the gypsy moth test species did not decline significantly from the initial 100% with any treatment combination, indicating retention of diflubenzuron at a fully toxic level even after 5 in. of rainfall. The actual amount of pesticide retained on the leaves in the Uniroyal study was not indicated.

Continuing the comprehensive study of diflubenzuron's environmental fate, the level of diflubenzuron in ground litter under the 20 trees sampled in this study is currently being investigated by our laboratory both prior to and post leaf fall. The following paper (Harrahy et al., 1993) reflects on what happens to the pesticide remaining on leaves when these leaves fall into a stream environment underlying the canopy. A second diflubenzuron persistence study has been initiated on the Fernow Experimental Forest near Parsons, WV. This study furthers the research reported here, especially in that (a) the persistence study is coupled to a thorough study of the nontarget organism impacts by diflubenzuron, including canopy, terrestrial, and aquatic invertebrates and salamanders, to investigate food chain effects and (b) the application of diflubenzuron occurred at a more normal time, i.e., when most of the leaves had reached less than 50% expansion, so the study of the growth dilution along with the chemical fate of the pesticide will be possible.

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LITERATURE CITED

- Bull, D. L.; Ivie, G. W. Fate of Diflubenzuron in Cotton, Soil, and Rotational Crops. *J. Agric. Food Chem.* 1978, 26, 515-520.
 Fischer, S. A.; Hall, L. W., Jr. Environmental Concentrations and Aquatic Toxicity Data on Diflubenzuron (Dimilin). *Crit. Rev. Toxicol.* 1992, 22, 45-79.

- Haijar, N. P.; Casida, J. E. Insecticidal Benzoylphenyl Ureas: Structure-Activity Relationships as Chitin Synthesis Inhibitors. *Science* 1978, 200, 1499-1500.
- Harrary, E. A.; Wimmer, M. J.; Perry, S. P.; Faber, D. C.; Miracle, J. E.; Perry, W. R. Persistence of Diflubenzuron (Dimilin) on Appalachian Forest Leaves in Stream Water. *J. Agric. Food Chem.* 1993, following paper in this issue.
- Mansager, E. R.; Still, G. G.; Frear, D. S. Fate of [¹⁴C]-Diflubenzuron on Cotton and Soil. *Pestic. Biochem. Physiol.* 1979, 12, 172-182.
- Martinat, P. J.; Christman, V.; Cooper, R. J.; Dodge, K. M.; Whitmore, R. C.; Booth, G.; Seidel, G. Environmental Fate of Dimilin 25-W in a Central Appalachian Forest. *Bull. Environ. Contam. Toxicol.* 1987, 39, 142-149.
- Marx, J. L. Chitin Synthesis Inhibitors: New Class of Insecticides. *Science* 1977, 197, 1170-1172.
- Mulder, R.; Gijswijt, M. J. The Laboratory Evaluation of Two Promising New Insecticides Which Interfere with Cuticle Deposition. *Pestic. Sci.* 1973, 4, 737.
- Mutanen, R. M.; Siltanen, H. T.; Kuukka, V. P. Residues of Diflubenzuron and Two of Its Metabolites in a Forest Ecosystem After Control of the Pine Looper Moth, *Bupalus piniarius*. *J. Pestic. Sci.* 1988, 23, 131-140.
- Muzzarelli, R. Chitin Synthesis Inhibitors: Effects on Insects and on Nontarget Organisms. *Crit. Rev. Environ. Control* 1986, 16, 141-146.
- Nigg, H. N.; Cannizaro, R. D.; Stamper, J. H. Diflubenzuron Surface Residues in Florida Citrus. *Bull. Environ. Contam. Toxicol.* 1986, 36, 833-838.
- Post, L. C.; deJong, B. J.; Vincent, W. R. 1-(2,6-Disubstituted benzoyl)-3-phenylurea Insecticides: Inhibitors of Chitin Synthesis. *Pestic. Biochem. Physiol.* 1974, 4, 473.
- Schaefer, C. H.; Dupras, E. F. Factors Affecting the Stability of Dimilin in Water and the Persistence of Dimilin in Field Waters. *J. Agric. Food Chem.* 1976, 24, 733-739.
- Uniroyal Chemical Co., Inc., Middlebury, CN. Dimilin Product Information: Rainfastness; 1989.
- Van den Berg. Dissipation of Diflubenzuron Residues After Application of Diflubenzuron WP-25 in a Forestry Area in North Carolina and Some Ecological Effects. Internal Report No. 56637/47/1986; Duphar B. V. WEESP: Holland, 1986.
- Verloop, A.; Ferrell, C. D. Benzoylphenyl Ureas — A New Group of Larvicides Interfering with Chitin Deposition. *Pesticide Chemistry in the 20th Century*; Plimmer, J., Ed.; ACS Symposium Series 37; American Chemical Society: Washington, DC, 1977; p 237.
- Wimmer, M. J.; Smith, R. R.; Jones, J. P. Analysis of Diflubenzuron by Gas Chromatography/Mass Spectrometry Using Deuterated Diflubenzuron as Internal Standard. *J. Agric. Food Chem.* 1991, 39, 280-286.

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