Persistence of Diflubenzuron on Appalachian Forest Leaves in Stream Water

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The persistence of diflubenzuron on Appalachian forest leaves placed in stream water was examined using a new gas chromatographic/mass spectrometric method for analyzing the pesticide. Leaves came from trees aerially sprayed with Dimilin in the spring and left to weather during the growing season. The rain exposure minimizes loss of pesticide when the treated leaves are first immersed. After diflubenzuron coverage was measured, leaf samples were placed in a headwater stream and residual diflubenzuron was monitored as a function of time. During July and August, the amount of diflubenzuron on white oak decreased significantly (by 36% and 23%, respectively) within the first 48 h of stream incubation, reaching less than 10% of the original concentration within 3 weeks. In the December studies with yellow poplar, red maple, and white oak leaves, the rate of loss of diflubenzuron was slow. After 54 days in the stream, yellow poplar and red maple leaves retained 45% and 40%, respectively, of the original diflubenzuron and white oak showed no significant loss. In laboratory experiments mimicking the December field conditions, no significant loss of diflubenzuron was seen from yellow poplar leaves. In view of the persistence of diflubenzuron on hardwood leaves observed throughout the growing season to leaf fall, at low stream temperatures, nontarget aquatic organisms that consume these fallen leaves may be exposed to the pesticide for a significant period of time.

INTRODUCTION

Diflubenzuron (trade name Dimilin) is an insect growth regulator that inhibits synthesis of the cuticular chitin of arthropods (Post et al., 1974). Because of its unique mode of action, diflubenzuron is being used extensively to suppress the gypsy moth (*Lymantria dispar L.*) in forest ecosystems, particularly in the Appalachian region. Due to its potential impact on nontarget organisms and thus food chains in these complex ecosystems, diflubenzuron's fate in the environment is of concern.

Studies on the persistence of diflubenzuron on Appalachain forest trees have shown that significant residues can remain on the leaves throughout the growing season (May through September). In a 1991 study of 20 trees representing seven species in the West Virginia University Experimental Forest, after an initial drop of 20–80% within the first 3 weeks, significant retention of diflubenzuron was seen throughout the remainder of the growing season (Wimmer et al., 1993). At leaf fall, more than 20% of the originally applied diflubenzuron remained on northern red oak, white oak, chestnut oak, red maple, and sugar maple, while 6–20% of the original level was retained by black oak and yellow poplar. These results were consistent with a previous 21-day study of three oak trees in West Virginia, in which diflubenzuron was reported to decrease on leaves to approximately 20% of the application level within 10 days postspray, after which, despite rain, no further loss was seen (Martinat et al., 1987). The toxicity to gypsy moths of Dimilin-treated oak seedlings was reported not to decline after a simulation of up to 5 in. of rainfall (Uniroyal, 1989). Broadcast use of diflubenzuron in forest environments, therefore, results in its introduction with leaf fall to underlying headwater streams.

Aquatic insect communities in headwater streams within forests are generally dominated by species that function as shredders, scrapers, and collectors (Merritt and Cummins, 1984; Vannote et al., 1980). These organisms rely heavily upon the organic matter of fallen leaves as a food source in fall and winter. Studies have shown that many of the aquatic arthropods are sensitive to Dimilin, particularly as they proceed through that part of their growth cycle that requires chitin deposition for proper molting, the process blocked by this pesticide (Hansen and Garton, 1982; Harrahy, 1992; Rodrigues and Kaushik, 1986; Fischer and Hall, 1992). Duration of exposure has been shown to be an important factor in determining mortality (Cunningham, 1986; Grosscurt and Jongsma, 1987). Thus, knowledge of the persistence of diflubenzuron on foliage after normal aerial application to leaves in the spring and subsequent leaf fall into headwater streams would be useful in assessing pesticide impacts on nontarget aquatic arthropods.

The persistence of diflubenzuron on foliage once Dimilin-coated leaves enter a stream has not been thoroughly studied (Fischer and Hall, 1992). Swift et al. (1988) showed that diflubenzuron persisted in artificial leaf packs in a second-order stream for 4 months beginning in November. In their study, however, tulip poplar leaves manually sprayed with Dimilin wettable powder were used and more than 60% of the diflubenzuron was lost initially from the leaves when they were placed in the water. The residual level of diflubenzuron was determined to be toxic to shredders in a laboratory bioassay, although both shredders

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and collectors were well represented in the leaf packs throughout the stream study.

The usefulness of past environmental fate studies of diflubenzuron is limited by the imprecision in analytical methods used for quantifying the pesticide in complex samples such as leaf and litter extracts. A procedure for analyzing diflubenzuron by gas chromatography/mass spectrometry was recently developed (Wimmer et al., 1991). This method takes advantage of the heat-induced breakdown of diflubenzuron during gas chromatography by using deuterated diflubenzuron as an internal standard. The greater sensitivity and selectivity of mass spectrometry permits rapid analysis of complex leaf extracts without derivatization or purification of the pesticide.

This paper describes in-stream field experiments more closely mimicking the natural situation to determine the persistence of diflubenzuron on leaves in stream water. Dimilin was applied aerially to leaves on trees as a water suspension of wettable powder under normal field application conditions. The diflubenzuron was allowed to weather on the leaves over the growing season, and after monitoring their diflubenzuron coverage, leaves from three tree species (white oak, yellow poplar, and red maple) were used for the in-stream and laboratory studies reported.

MATERIALS AND METHODS

Materials. Commercial grade Dimilin (Uniroyal 25% WP) was provided by Alan Miller, West Virginia Department of Agriculture. 1-(2,6-Difluorobenzoyl)-3-[4-chloro(2,6-dideutiero)-phenyl]urea (deuterated diflubenzuron) was synthesized as previously described (Wimmer et al., 1991). All pesticide extraction and sample storage solvents were Fisher Optima grade. Acetone and methylene chloride recovered from extractions were redistilled and reused, with the latter dried over anhydrous sodium sulfate. All volatile solvent transfers were done in well-ventilated exhaust hoods. Methanol and water used for HPLC were HPLC grade from MCB Manufacturing Chemists, Inc.

Leaf Source for Stream Studies. A section of forest owned by the Westvaco Corporation (Ripley, WV) near Elkins, WV, and a section of the West Virginia University Experimental Forest were aerially sprayed with Dimilin at an application rate of 13.6 g of ai/acre (0.03 lbs of ai/acre) from a Twin Beech aircraft. The Dimilin was allowed to weather on the trees during the season, and leaves were removed at various times for diflubenzuron residue analysis (Wimmer et al., 1993). The frozen composite samples left over from the residue analyses were used in the stream persistence studies, as described under Results. Measurements have shown diflubenzuron coverage to be stable over the storage time period under the frozen condition (non-frost-free freezer, −23 °C) in which the leaves were stored (M. J. Wimmer, West Virginia University, 1992, unpublished observations.

Stream Persistence Studies. Field persistence studies were conducted in Laurel Creek, a second-order stream within the West Virginia University Experimental Forest, in July, August, and December of 1991. Composite leaf samples that had been cut into 1-cm square pieces and mixed to homogeneity were divided into subsamples weighing 9–11 g each. This size of each replicate leaf sample yielded results in replicate diflubenzuron coverage values that are generally in close agreement (Wimmer et al., 1993), evidence of the homogeneity of the composite sample subsampled in this way. The subsamples were placed in 15-cm-long chambers made of 7.2-cm-diameter poly(vinyl chloride) (PVC) pipe; 1-mm Nitex mesh was hot-glued to one end and secured with an elastic band around the other end. This arrangement allowed containment of the leaves and exclusion of large shredder organisms and debris.

The chambers were placed parallel to the flow and secured with rocks. Each was located in a similar stream environment, in the same riffle area approximately 30 cm below the water surface. At each sampling time point (see Results), duplicate chambers were removed from the stream, placed in plastic freezer bags in a cooler without additional refrigeration, and brought to the laboratory for immediate extraction as described below. Temperature, pH, velocity, alkalinity, and hardness data were collected for Laurel Creek during the August and December persistence tests.

Laboratory Stream Water Study. Nine to eleven grams of composite leaves as described in Table I were placed in each of 12 3.8-L glass jars containing 1000 mL of stream water (Laurel Creek) in a Sherer growth chamber. Plastic and other nonglass containers adsorb hydrophobic diflubenzuron from water (M. J. Wimmer, E. A. Harrahy, and S. A. Perry, West Virginia University, 1992, unpublished observations). Vigorous aeration was provided by forcing compressed air through plastic pipet tips placed below the surface of the water, a process which also circulates the chamber water. Lighting was provided on an 8-h-on, 16-h-off schedule using 40-W fluorescent bulbs placed 18 in. above the chamber. The temperature was held at 8 °C (±1 °C). Duplicate leaf and water samples were collected for residue analysis at the indicated times (see Results).

Extraction and Chemical Analysis. Diflubenzuron was extracted from the leaf samples and analyzed according to the method of Wimmer et al. (1991). Briefly, each wet leaf sample was transferred from its PVC pipe holder into a 500-mL Erlenmayer flask immediately after returning to the laboratory. The integrity of the leaf pieces remained, even after 54 days in the stream. The diflubenzuron was removed from the leaf surface by shaking in acetone three times after the addition of 25 μg of deuterated diflubenzuron as the internal standard. The organic solvent was removed from the pooled washes by rotary evaporation, leaving the extract in a suspension with a few milliliters of water from the leaves.

After transfer to 15-mL glass conical centrifuge tubes, the diflubenzuron was extracted into methylene chloride by vortexing with 2–3 mL of solvent per tube. The layers were separated by a quick benchtop centrifugation, and any residual water in the bottom organic layer was removed by passing the methylene chloride extract, carefully transferred with a pasteur pipet, through anhydrous sodium sulfate. This extraction was repeated once, the extracts were combined, and the solvent was evaporated to dryness. The residue was taken up in 1.2 mL of acetonitrile and stored at 4 °C prior to analysis by gas chromatography/mass spectrometry (GC/mass spec).

The more complex extracts of leaf samples incubated beyond a few days in stream water in the laboratory required partial cleanup prior to GC/mass spec analysis. This was done by high-performance liquid chromatography (HPLC) on a 5-μm C-18 Resolve column (8 × 100 mm) from Waters Division of Millipore with a μBondapak C-18 Guard Pak precolumn also from Waters. The instrumentation used was a Perkin-Elmer Series 3B with a Sigma 10B data station and a LC 235 diode array detector. The column was run with a solvent of 65% methanol in water at a flow rate of 1 mL/min and was monitored at 260 nm. Under these conditions, diflubenzuron elutes between 4 and 6 min.

The diflubenzuron peak was collected in one pool from three 100-μL injections of the sample extract in acetonitrile. Methanol in the eluting solvent was removed by rotary evaporation, holding the sample at 10–15 °C. The resulting water suspension was extracted with methylene chloride as described above. The methylene chloride layer was carefully withdrawn with a Pasteur pipet, any remaining water was removed by passing through anhydrous sodium sulfate, and the solution was taken to dryness in a pear-shaped flask on a rotary evaporator. The residue was taken up in 200 μL of acetonitrile to slightly concentrate the sample for increased sensitivity, and the sample was stored in a glass, sealed vial at 4 °C prior to GC/mass spec analysis.

Diflubenzuron in water samples was extracted in a separatory funnel with three washes of methylene chloride (60 mL/L of water sample) after measuring the water volume and adding 25 μg of deuterated diflubenzuron internal standard. After the methylene chloride washes were passed through anhydrous sodium suflate, the solvent was removed by rotary evaporation. The residue was taken up in 1.2 mL of acetonitrile and stored at 4 °C prior to GC/mass spec analysis.

GC/mass spec analyses were performed on a Finnigan 4500 System gas chromatograph and mass spectrometer equipped with an Incos data station at the West Virginia University Mass Spectrometry Center as previously described (Wimmer et al., 1991).

Table I. Spray Date, Tree Sampling Date, and Initial Diflubenzuron Residue Data for Leaves Used in the Persistence Studies

persistence study initiated	tree species	spray date	tree sample date	initial diflubenzuron concn (μg/kg)
July 1, 1991	white oak	June 26, '90	Aug 13, '90	1755 (U)
				940 (L)
Aug 5, 1991	white oak	June 26, '90	July 3, '90	1579 (U)
				3053 (L)
Dec 10 1991	white oak	June 26, '90	July 26, '90	655
			Aug 13, '90	940
	yellow poplar	May 15, '91	July 8, '91	596
	red maple	May 15, '91	Oct 17, '91	227
March 6, 1992	yellow poplar	May 15, '91	July 18, '91	326

Statistical Analyses. Analysis of variance was conducted to determine significance of differences in the observed effects, testing diflubenzuron measurements by orthogonal contrast, a standard statistical method for comparing treatments in a multitreatment experiment. The total diflubenzuron loss or loss rate (i.e., the addition of individual observations) was tested for significance of difference between summer and winter persistence studies. White oak data for the July, August, and December trials were each pooled and transformed by an arc sine square-root transformation, the standard statistical operation when analyzing percentage data. Percentages greater than 100 were standardized to 100%. A p value less than 0.05, signifying a 95% chance that a difference exists, was considered significant.

Physical Water Data. Temperature of the stream water was recorded every 2 h with a Ryan TempMentor digital thermograph. The pH was measured with an Accumet Model 900 bench top pH meter. A Marsh McBirney Model 201D flow meter was used to measure stream velocity. Alkalinity and hardness were determined with Hach kits.

RESULTS

Source of Dosed Leaf Material for Stream Studies. Stream persistence studies of leaves mimicking the natural situation after leaf fall require a source of leaf material with a known, stable amount of diflubenzuron coverage at the start. A recent study has found that, after spring pesticide application, a significnt amount of diflubenzuron persists up to the time of leaf fall on Appalachian forest leaves (Wimmer et al., 1993). The use of this rain-exposed leaf material for our stream studies avoids the problem associated with using leaves manually dosed with Dimilin wettable powder, namely the initial immediate loss of more than half of the diflubenzuron from the leaf surface when first placed in the stream (Swift et al., 1988). Not applying Dimilin to leaves in an organic solvent avoids the possibility that pesticide binding to the leaf surface is different than that of a water suspension naturally weathered on. Furthermore, out study closely mimicks conditions involved in the normal use of Dimilin for pest control, making the information from this research more relevant to pest management decisions.

The original Dimilin application dates and the dates of sampling individual trees for leaves used in the stream persistence studies are shown in Table I. Dimilin had weathered on the trees for at least 1 month prior to leaf collection (or for two rain events in the case of the August study), after which the pesticide has been found to be generally resistant to removal by rain and other weathering forces (Martinat et al., 1987; Wimmer et al., 1993).

Field Studies. The amount of diflubenzuron remaining on stream-incubated leaves in the July and August persistence studies showed a rapid decrease within the first 48 h after placement in Laurel Creek (Figure 1). In July, the amount of diflubenzuron remaining on the white oak leaves decreased to 36% of the original within this time period, and in August, the amount remaining had

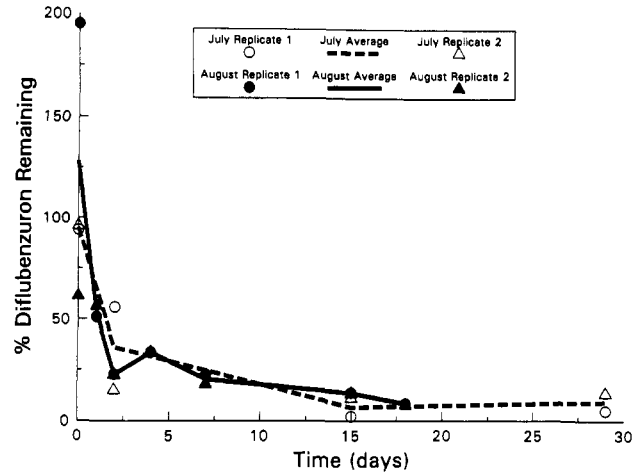

Figure 1. Persistence of diflubenzuron on white oak leaves incubated in stream water. The percent remaining of the diflubenzuron on the leaves at the time of placement in the stream (Table I) is plotted as a function of time in the water. The first time point shown was taken at 5 min. Values of replicate samples are indicated: July 1991 study (– – –) and August 1991 study (—).

decreased to 23%. By 15 days, most of the diflubenzuron had disappeared, with only 7% and 14% of the original remaining for July and August, respectively. Except for the 5-min time point of the August study, there is close agreement between the values of diflubenzuron coverage on the duplicate leaf samples taken from the stream at each time point. The leaves used in the two studies came from the same white oak tree; although the foliage had been taken from the tree at different times after the diflubenzuron spray application (Table I), no major difference is seen in the rate of loss of pesticide in the stream in July compared to that in August.

Diflubenzuron was found to persist significantly longer on the leaves during the December study than during the summer months. Following 54 days of incubation in the stream, 78% of the original diflubenzuron remained on the white oak leaves (Figure 2A), 45% remained on the yellow poplar leaves (Figure 2B), and 40% remained on the red maple leaves (Figure 2C). As above, the agreement between replicate samples for each time point is close for each tree species. The difference in diflubenzuron persistence between summer and winter was confirmed by statistical analysis, which showed the loss of diflubenzuron from white oak leaves in the summer study to be significantly different from that in winter (p = 0.0013).

Comparison of physical stream data from the three studies (Table II) reveals an expected major difference in stream water temperature between August and December (no temperature readings were taken in July). The winter temperature averaged 17 °C lower than that in August. The pH values were the same in the two summer studies but averaged 0.7 unit lower in December when alkalinity and hardness were halved.

Laboratory Study. To check the finding of diflubenzuron persistence on leaves in the December stream study, an experiment was conducted under laboratory conditions mimicking the December field conditions. Not only did diflubenzuron remain on the leaf material measured but also, by containing the stream water in a laboratory incubator, it was possible to measure diflubenzuron released from the leaves as diflubenzuron into the water. Metabolites of the pesticide were not measured. The diflubenzuron application history of the yellow poplar leaves used in this study is described in Table I (March 6, 1992).

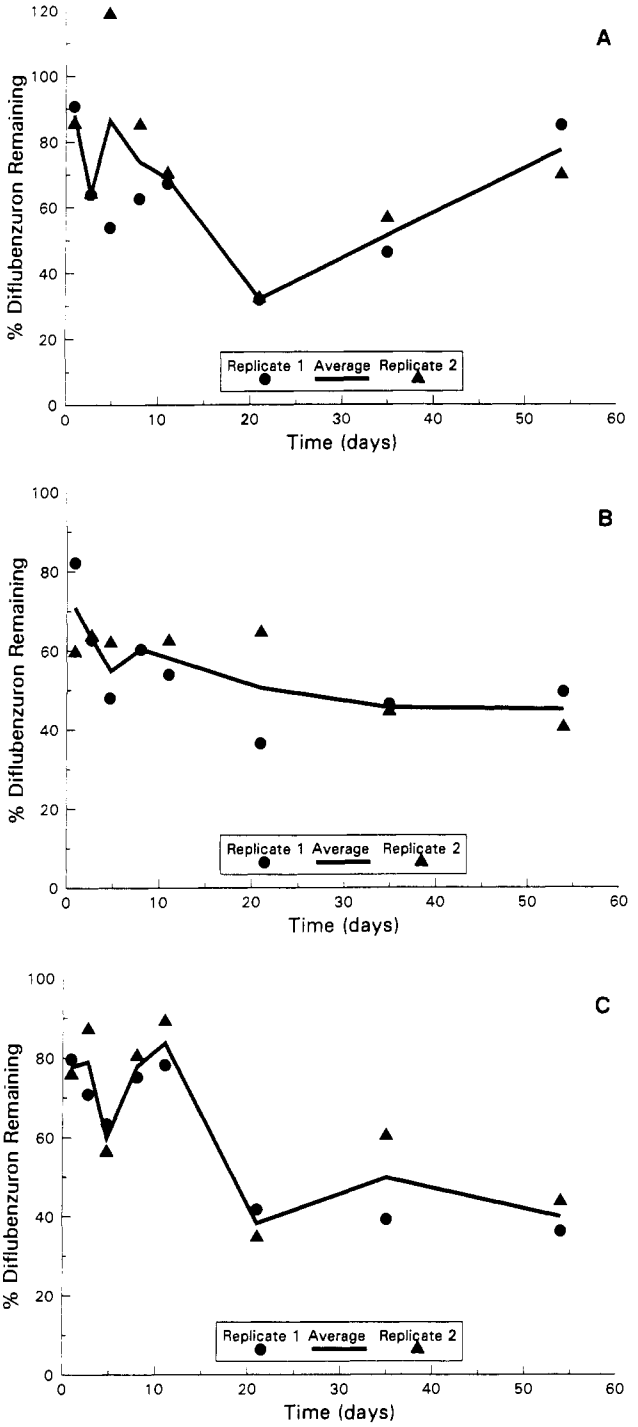

Figure 2. Persistence of diflubenzuron on Appalachian forest leaves incubated in stream water beginning December 1991. The percent remaining of the diflubenzuron on the leaves at the time of placement in the stream (Table I) is plotted as a function of time in the water. Values of replicate samples are indicated: (A) white oak, (B) yellow poplar, and (C) red maple.

Diflubenzuron residue on the yellow poplar leaves did not significantly decrease in the laboratory persistence study over a 50-day time period (p = 0.38) (Figure 3). Although large margins of error exist for two of the seven time points, 70–100% of the original pesticide coverage was consistently found, lending support to the December field results. Analysis of the chamber water for diflubenzuron further supports the retention of the pesticide by the leaf material in the laboratory study; the levels seen were either below the detectability limit (approximately 0.5 μg/kg in the incubation water) or too low to be accurately quantified.

Table II. Water Quality Variables in Laurel Creek during Field Persistence Studies[a]

	temp (°C)	pH	velocity (m/s)	alkalinity (mg $CaCO_3$/L)	hardness (EDTA) (mg of $CaCO_3$/L)
July		7.4 (7.3–7.65)			
Aug	19 (18–20)	7.4 (7.2–7.5)	0.04 (0.03–0.05)	32 (20–40)	52 (40–70)
Dec	2 (0–7)	6.7 (6.5–7.0)	0.51 0.36–0.63)	16 (10–50)	23 (20–30)

[a] All values are means with the ranges in parentheses.

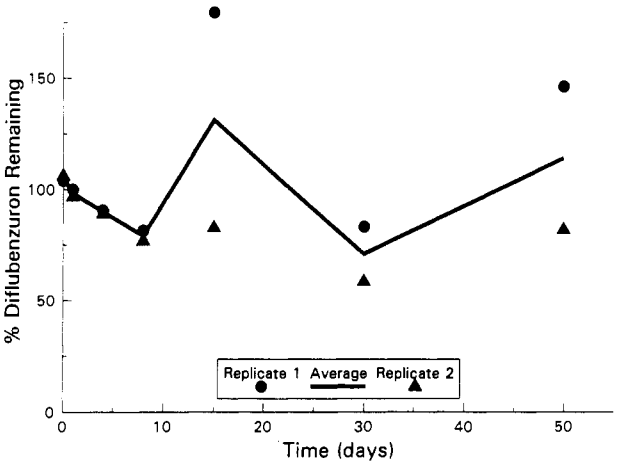

Figure 3. Persistence of diflubenzuron on yellow poplar leaves incubated in contained stream water in the laboratory mimicking December field conditions. The percent remaining of the diflubenzuron on the leaves at the time of placement in the water (Table I) is plotted as a function of incubation time. Values of replicate samples are indicated.

DISCUSSION

The results of our December stream field study extend the research of Swift et al. (1988). In their study (begun in November), in which a water suspension of diflubenzuron wettable powder was manually sprayed to dose the leaf material, immediate loss of over 60% of the diflubenzuron was seen upon introduction of the leaves into the stream. Our study was done under more natural conditions using three species of rain-weathered leaves that had been dosed with diflubenzuron by a normal aerial application of Dimilin wettable powder. The initial loss of diflubenzuron when such leaves are placed in a stream is lessened (0–30%), as predicted by Swift et al. In both studies, the diflubenzuron left after the initial immersion was found to persist on the leaf material for more than 2 months.

The white oak winter persistence curve (Figure 2A) shows significant variability over time, decreasing to approximately 30% in 21 days and then increasing back to 80% at 54 days. To a smaller degree, this also shows in the curve for the red maple (Figure 2C). This variability is likely due to the different microenvironments experienced in the stream bed, even though care was taken to minimize these factors. The reason for the value of more than 100% diflubenzuron persistence in the 5-min time point of the August study (Figure 1) is likely the lack of precision in this particular measurement, reflected in the wide margin of error observed between the duplicate values.

The efficiency of extraction of diflubenzuron from leaves is not expected to be a problem. We have established that all diflubenzuron is removed from 1-cm square leaf pieces by acetone shaking after weathering of leaves in the field. This was done by grinding leaf pieces remaining after the

acetone wash, in acetone or in methylene chloride, and finding no detectable levels of diflubenzuron in the several samples tested (M. J. Wimmer, West Virginia University, 1992, unpublished observations). The reason for using acetone instead of methylene chloride for the initial extraction is to break up any aqueous coating on the leaf surface that might prevent dissolution of all the diflubenzuron. In studies on cotton, the pesticide has been found to remain on the leaf surface and not to be taken up by leaves or roots (Bull and Ivie, 1978; Mansager et al., 1979). There is the unlikely possibility that some diflubenzuron may be left on stream-conditioned leaves after the acetone wash; if this is the case, the change in the conclusion of this study would be that the persistence of diflubenzuron is greater than that reported herein. During the applicable winter season, that persistence is already substantial.

In the laboratory study (Figure 3), the 15- and 50-day time points suffer from wide differences in the replicate samples, resulting in an uneven persistence curve. Overall, however, the amount of diflubenzuron remains in the 70–100% range, and this persistence on the leaf material is confirmed by a lack of diflubenzuron in the corresponding chamber incubation water.

The decrease in the rate of loss of diflubenzuron from leaves in our December field study compared with the results in July and August may be attributable to a variety of factors, several of which have been reported in the literature. The major factor is likely the lower water temperatures, which have been reported to increase diflubenzuron stability (Schaefer and Dupras, 1976; Ivie et al., 1980) and possibly aid in its retention on foliage (Nigg et al., 1986). Furthermore, one expects a decrease in the rate of diflubenzuron biodegradation at lower temperatures when it takes place through microbial metabolism in the stream environment.

The stability of diflubenzuron in water has been found to decrease with increasing pH when coupled to high temperatures (37–38 °C). The half-life of diflubenzuron in distilled water at 37 °C was reported to be approximately 2 and 7 days at pH 10 and 6, respectively (Ivie et al., 1980); however, at pH 4, no degradation was seen after 56 days. In tap water, the half-life at 38 °C at pH 10 was also found to be 2 days (Schaefer and Dupras, 1976), while that at pH 7.7 was 8 days. At lower temperatures (24 and 10 °C), little loss of diflubenzuron was reported at either pH 10 or 7.7 over a 9-day period (Schaefer and Dupras, 1976).

The pH of Laurel Creek decreased from an average of pH 7.4 to 6.7 between the August and December field studies. In view of the temperatures involved (19 and 2 °C, respectively), the difference in pH is unlikely to be a major factor in the difference in diflubenzuron persistence observed unless microorganism activity or leaf-surface-binding mechanisms are altered as a result of the pH change.

Reports on diflubenzuron breakdown by microorganisms vary. Four fungal isolates from soil were reported to rapidly degrade diflubenzuron with half-lives from 7 to 18 days (Seuferer et al., 1979). In that study, however, diflubenzuron broke down in the uninoculated control with a half-life of 27 days. Also, analysis of the pesticide was reported to be done by gas chromatography; diflubenzuron is known to break down in the heat of the GC (Corley et al., 1974; Tamiri and Zitrin, 1987; Wimmer et al., 1991). In a 91-day study of [^{14}C]diflubenzuron breakdown in soil, as the percentage of ethanol-soluble radioactivity gradually decreased from 93% to 29% of the total, the percentage of this ^{14}C persisting as diflubenzuron appeared to stabilize at 50% by day 42 (Mansager et al., 1979). No evidence for the degradation of radiolabeled diflubenzuron by a cell suspension of the gram-negative soil bacterium *Pseudomonas putida* was found after incubation for 6 h at 30 °C (Metcalf et al., 1975). No difference in diflubenzuron loss comparing incubations in boiled and unboiled sewage water was observed (Schaefer and Dupras, 1976), and the losses seen in both (up to 42% in 24 h) were attributed to adsorption by organic matter.

Postharvest diflubenzuron residues in agricultural soil were found to be persistent during the subsequent winter and spring months but to decline rapidly with the onset of high summer temperatures (Bull and Ivie, 1978). Radiolabeled diflubenzuron was found to be relatively resistant to biodegradation when taken up by organisms of a model terrestrial–aquatic ecosystem (Metcalf et al., 1975). In alga, snail, and mosquito, 74–96% of the radioactivity was retained as the parent compound; the only significant metabolism occurred in fish.

The leaf material in our field studies became colonized primarily with fungi, whereas in the laboratory study, immersed leaves also supported algal growth, possibly as a result of the Gro-Lux lighting and the lack of fresh water replacement (Tara Dubey, West Virginia Tech, 1992, unpublished observations). A physical difference was readily observable, namely a sliminess in the laboratory samples not seen in the field foliage. This difference in laboratory leaf material over time necessitated a cleanup step for the leaf extracts, unlike those from the field, implying a clear difference in microorganism populations in the two environments. Nonetheless, consistent with the algal literature discussed above, we observed that algal growth did not seem to enhance the breakdown of diflubenzuron on submerged leaf surfaces. No analysis of leaf colonization by microorganisms was done to compare our July, August, and December field samples. One cannot rule out, therefore, that microorganism differences between summer and winter may contribute to the difference in diflubenzuron persistence observed in the two seasons.

Physical loss of leaf material is unlikely to be a factor in the rapid summer disappearance of diflubenzuron on the submerged foliage. Such loss was minimized by excluding large shredding insects (>1 mm) from the PVC chambers, and no evidence of major shredding activity was observed in any of the samples. The summer–winter difference in diflubenzuron loss was not due simply to difference in rates of being washed off the leaves by water flow, as Laurel Creek had higher average velocities during the December study (0.51 m/s) than during the August study (0.04 m/s).

A difference in the rate of photodegradation of diflubenzuron to account for the more rapid July/August diflubenzuron loss from stream-incubated leaves is not plausible because all of the individual leaf samples were held in PVC tubing and therefore generally unexposed to UV light. Photodegradation is reported to be significant when irradiating methanol solutions of the compound at 254 nm (Metcalf et al., 1975). Exposure of diflubenzuron films on glass to intense, direct sunlight resulted in 12% loss after 9 h (Schaefer and Dupras, 1976). The same authors report that in water, little degradation occurs as a direct result of the sunlight itself. On cotton leaves, less than 5% loss of diflubenzuron was seen over 4 weeks of exposure to sunlight (Bull and Ivie, 1978). When technical grade [^{14}C]diflubenzuron was exposed to sunlight on thin silica gels, more than 98% of the remaining radioactivity was unchanged diflubenzuron after up to 4 weeks.

The leaves used in this report had been treated with the wettable powder formulation of Dimilin. Application of this formulation has been shown to produce more persistent residues on vegetation than application of other formulations (Schaefer and Dupras, 1977). Significant levels of diflubenzuron were found on leaves collected just

prior to abscission from areas treated with this formulation of Dimilin (Wimmer et al., 1993). These leaves will deliver the residual diflubenzuron to the underlying streams at leaf fall, where it may persist over the winter as our study suggests.

The life histories of many stream organisms are timed to make maximum use of leaf detritus as a food base (Hynes, 1970). Winter-growing stream species represent a variety of functional feeding groups and include many shredders, scrapers, and collectors (Cummins, 1984). Shredders play a significant role in the comminution of leaf material so that it becomes available to collectors (Cummins, 1974). *Pteronarcys proteus* (Plecoptera: Pteronarcyidea), a shredder (Merritt and Cummins, 1984), was not sensitive to diflubenzuron when fed treated leaves (Harrahy, 1992), although the low number of molts during the study period may have influenced the results. However, heptageniid mayflies, which function as collectors, are sensitive to diflubenzuron at concentrations as low as 0.6 μg/kg in water (Harrahy, 1992). Studies have shown that other winter-growing species are sensitive to diflubenzuron (Hansen and Garton, 1982; Rodrigues and Kaushik, 1986). The results of our winter persistence studies suggest that nontarget aquatic organisms may be exposed to diflubenzuron for at least 2 months. Because duration of exposure to diflubenzuron has been shown to be an important factor in determining mortality (Cunningham, 1986; Grosscurt and Jongsma, 1987), the introduction of Dimilin-treated leaves to headwater streams at leaf fall may result in adverse effects on the functioning of these ecosystems, an unknown factor that merits further research.

In conclusion, diflubenzuron weathered on leaves during the growing season is shown to persist at significant levels on the foliage after nearly 2 winter months within a stream environment. Such persistence appears to be independent of leaf type, with red maple, white oak, and yellow poplar showing similar results.

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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.

Corley, C.; Miller, R. W.; Hill, K. R Determination of *N*-(4-Chlorophenyl)-*N'*-(2,6-difluorobenzoyl)urea in Milk by High-Speed Liquid Chromatography. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 1269–1271.

Cummins, K. W. Structure and Function of Stream Ecosystems. *BioScience* **1974**, *24*, 631–641.

Cunningham, P. A. A Review of Toxicity Testing and Degradation Studies Used to Predict the Effects of Diflubenzuron (Dimilin) on Estuarine Crustaceans. *Environ. Pollut.* **1986**, *40*, 63–86.

Fischer, S. A.; Hall, L. W., Jr. Environmental Concentrations and Aquatic Toxicity Data on Diflubenzuron (Dimilin). *Crit. Rev. Toxicol.* **1992**, *22*, 45–79.

Grosscurt, A. C.; Jongsma, B. Mode of Action and Insecticidal Properties of Diflubenzuron. In *Chitin and Benzoylphenyl Ureas*; Wright, J. E., Retnakaran, Eds.; Junk Publishers: Boston, MA, 1987.

Hansen, S. R.; Garton, R. R. The Effects of Diflubenzuron on a Complex Laboratory Stream Community. *Arch. Environ. Contam. Toxicol.* **1982**, *11*, 1–10.

Harrahy, E. A. The Effects of Diflubenzuron (Dimilin) on Selected Non-Target Aquatic Invertebrates and the Persistence of Diflubenzuron on Leaves in Water. M.S. Thesis, West Virginia University, Morgantown, WV, 1992.

Hynes, H. B. N. The Ecology of Stream Insects. *Annu. Rev. Entomol.* **1970**, *15*, 25–42.

Ivie, G. W.; Bull, D. L.; Veech, J. A. Fate of Diflubenzuron in Water. *J. Agric. Food Chem.* **1980**, *28*, 330–337.

Mansager, E. R.; Still, G. G.; Frear, D. S. Fate of [^{14}C]-Diflubenzuron on Cotton and in 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.

Merritt, R. W.; Cummins, K. W. *An Introduction to the Aquatic Insects of North America*, 2nd ed.; Kendall/Hunt Publishing: Dubuque, IA, 1984.

Metcalf, R. L.; Lu, P. Y.; Bowlus, S. Degradation and Environmental Fate of 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea. *J. Agric. Food Chem.* **1975**, *23*, 359–364.

Nigg, H. N.; Cannizzaro, 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.

Rodrigues, C. S.; Kaushik, N. K. Laboratory Evaluation of the Insect Growth Regulator Diflubenzuron Against Black Fly (Diptera: Simulidae) Larvae and Its Effects on Nontarget Stream Invertebrates. *Can. Entomol.* **1986**, *118*, 549–558.

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*, 733–738.

Schaefer, C. H.; Dupras, E. F. Residues of Diflubenzuron [1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea] in Pasture, Soil, Vegetation, and Water Following Aerial Applications. *J. Agric. Food Chem.* **1977**, *25*, 1026–1030.

Seuferer, S. L.; Brayner, H. D.; Dunn, J. D. Metabolism of Diflubenzuron by Soil Microorganisms and Mutagenicity of the Metabolites. *Pestic. Biochem. Physiol.* **1979**, *10*, 174–180.

Swift, M. C.; Smucker, R. A.; Cummins, K. W. Effects of Dimilin on Freshwater Litter Decomposition. *Environ. Toxicol. Chem.* **1988**, *7*, 161–166.

Tamiri, T.; Zitrin, S. Gas Chromatography Mass Spectrometry of Some Thermally Labile Urea Pesticides. *Biomed. Environ. Mass Spectrom.* **1987**, *14*, 39–42.

Uniroyal Chemical Co., Inc. Dimilin Product Information: Rainfastness; 1989.

Vannote, R. L.; Minshall, G. W.; Cummins, K. W.; Sedell, J. R.; Cushing, C. E. The River Continuum Concept. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 130–137.

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.

Wimmer, M. J.; Smith, R. R.; Wellings, D. L.; Toney, S. R.; Faber, D. C.; Miracle, J. E.; Carnes, J. T.; Rutherford, A. B. Persistence of Diflubenzuron on Appalachian Forest Leaves after Aerial Application of Dimilin. *J. Agric. Food Chem.* **1993**, preceding paper in this issue.

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