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Environmental Fate of Dimilin 25-W® in a Central Appalachian Forest

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Diflubenzuron (1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl) urea) (commercial names: Dimilin 25-W,® TH-6040) is an insect growth regulator that interferes with the formation of arthropod endocuticle (Maas et al. 1980) by inhibiting chitin biosynthesis and deposition. In aquatic and agroecosystems diflubenzuron (DFB) causes little or no vertebrate toxicity, is degraded quickly by microorganisms, and does not bioaccumulate (Booth and Ferrell 1977, Maas et al. 1980, Booth et al. 1987). Except for its toxic effects on nontarget aquatic arthropods (Mulla et al. 1975, Willcox and Coffey 1978) its ecological impact seems minimal, and it therefore has a potentially wide range of uses.

The behavior of DFB in forest ecosystems is less well known. Furthermore, many studies on DFB in forests are unpublished and not widely available. The widespread use of DFB in the suppression of forest insect defoliators (Duphar 1984) could lead to potentially harmful ecological effects. For example, indiscriminate reduction of immature Lepidoptera and other mandibulate herbivorous insects upon which forest birds feed could affect breeding bird populations. Lastly, there are no published studies on the dissipation of DFB residues in components of the forest ecosystem.

Here we report the results of one objective in a two year study of the ecological effects of DFB in an oak forest in West Virginia: the environmental fate of DFB in litter, foliage, arthropods, and insectivorous forest birds.

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MATERIALS AND METHODS

The study was conducted at Sleepy Creek Public Hunting and Fishing Area near Berkeley Springs (Morgan Co.), West Virginia. The area consists of two parallel ridges (maximum elevation 658 m) characterized by mature (40 yr old) oak-pine and oak-hickory forests. In 1985 this area was on the leading edge of the southward expanding range of gypsy moth defoliation. Climatic data (rainfall) were taken from two NOAA weather stations: Cacapon (10 km west of study area), and Martinsburg (18 km east of study area). As an estimate of the rainfall at the study site, data from the two weather stations were averaged.

A northeast-southwest row of six plots, each 770 x 770 m (60 ha) in size, was established on an east-facing slope. Treatment status was assigned systematically (to insure interspersion) to plots 2, 4, and 6 after a random start. Plots were separated by at least 150 m to minimize the effects of spray drift. Dimilin 25-W was aerially applied to treatment plots on 8 May 1985 at a rate of 70.75 g/ha (2 oz/acre) by the West Virginia Department of Agriculture. Gypsy moths were mostly first and second instars, and foliage was not quite fully expanded on this date.

Samples for residue analysis were collected on days -3 (prespray) 1, 3, 10, and 21 (postspray) near a central location in each plot (the sample station). On each sampling occassion, the three foliage, three litter, one canopy arthropod, and one litter arthropod samples were taken within a 50 m radius of the sample station. For foliage samples, three mature oaks (Quercus rubra L., Q. velutina Lam., or Q. prinus L.) were randomly selected. From each tree a branch was removed from a height of 11-13 m. Twenty to thirty leaves were removed from each of the three branches for the three foliage samples. The remaining foliage from these and additional branches were searched for canopy arthropods (immature Lepidoptera and Hymenoptera), which were collected and stored in portable containers. Litter samples (a handful of unincorporated material from the forest floor) were also collected at three random locations within the 50 m radius. Litter arthropods (mostly Coleoptera, Chilopoda, Diplopoda, and Araneida) were collected manually and in pitfall traps (without killing fluid). The laboratory procedure required that the arthropod samples consist of at least 5 g wet weight each. This necessitated a great amount of collecting time in the forest and allowed for only two (one litter and one canopy) arthropod samples per plot per sample occasion.

Another objective of the study was to census bird populations on these plots. Therefore we could not collect birds for residue analysis there. Instead, birds were collected (with .410 gauge shotgun) in nearby treated and untreated areas designated by the West Virginia Department of Agriculture. Collected birds were categorized as belonging to one of two foraging guilds: canopy foragers (great crested flycatcher (Myiarchus crinitus), eastern wood-pewee (Contopus virens), black-capped chickadee (Parus atricapillus), tufted titmouse (P. bicolor), blue-gray gnatcatcher (Polioptila caerulea), red-eyed vireo (Vireo olivaceus), warblers (Dendroica spp.), scarlet tanager (Piranga olivacea)); and ground or low foragers (wood thrush (Hylocichla mustelina), ovenbird (Seiurus aurocapillus), rufous-sided towhee (Pipilo erythrophthalmus), chipping sparrow (Spizella passerina), song sparrow (Melospiza melodia), and indigo bunting (Passerina cyanea)). Eight birds from treated and eight from control areas were collected on each sample occasion.

On a sampling day, control samples were collected first. Hands and collecting equipment were washed between plots. Specimens were immediately placed individually in sealed plastic freezer bags and packed in ice in portable coolers. Soon thereafter, specimens were shipped in dry ice containers via jet to Environmental Labs, a contract toxicology laboratory at Brigham Young University.

For residue analysis a modified HPLC procedure (Rabenort et al. 1978, Booth et al. 1987), was used. Litter and foliage residues were extracted by blending with dichloromethane, and arthropod and bird residues were extracted by blending with acetonitrile. Following filtration, the extract was evaporated to dryness. The residue was then cleaned up using a Florisil liquid-partition column followed by a Florisil-Alumina-silica gel column. For the final determination, an HPLC apparatus equipped with a UV detector (254 nm) was used with a μ-Bondapak/C_{18} or column for separations. This method had a detection limit of about 0.03 PPM. All samples were analyzed whole.

RESULTS AND DISCUSSION

DFB residues in samples were estimated using the heights of peaks above background on the chromatographs (Booth et al. 1987). Due to the rather variable signal/noise ratio, standard errors were quite large and some prespray and control samples appeared to contain more DFB than the 0.03 PPM detection limit (Table 1). Nevertheless, the overall trend in the

Table 1. Diflubenzuron residues in samples taken at Sleepy Creek Public Hunting and Fishing Area, Morgan County, West Virginia.

Sample Date*		-3	1	3	10	21
Sample	Treatment**	Residue in PPM***				
Canopy Birds	T	Tr	.12±.04	.21±.09	.05±.01	.09±.02
	U	Tr	Tr	Tr	Tr	Tr
Understory Birds	T	Tr	.20±.03	.06±.02	.04±.003	Tr
	U	Tr	Tr	Tr	Tr	Tr
Foliage	T	Tr	.45±.25	.31±.16	.10±.06	.18±.16
	U	Tr	Tr	Tr	Tr	Tr
Foliage Arthropods	T	Tr	.18±.06	.49±.10	.10±.04	.10±.06
	U	.10±.02	.15±.05	.03±.02	Tr	Tr
Litter	T	Tr	Tr	Tr	Tr	Tr
	U	Tr	Tr	Tr	Tr	Tr
Litter Arthropods	T	Tr	.07±.07	.06±.04	.11±.07	.03±.03
	U	Tr	Tr	.04±.02	.06±.04	Tr

* Days from spray date.
** T - Treated, U - Untreated
*** Mean ±SE of 4 replications for birds, 9 for foliage and litter, 3 for foliage and litter arthropods. Tr - Trace: <0.03 PPM.

samples was that of decreasing residue levels over time in treated plots, and residue levels below the detection limit in untreated plots. With the exception of the understory bird and litter samples, all mean residue levels in treated plots were above the detection limit on day 21, (Fig 1).

Residue levels in birds were highest on days 1 and 3 postspray and dropped thereafter. On day 21 residues in canopy birds were still well above the detection limit, while those in low foraging and ground birds had dropped below the detection limit. Except for day 1, canopy birds appeared to contain higher levels of residue than ground and understory birds (Fig. 1A). Initial residue levels in canopy arthropods on day 1 were followed by an increase on day 3, probably due to

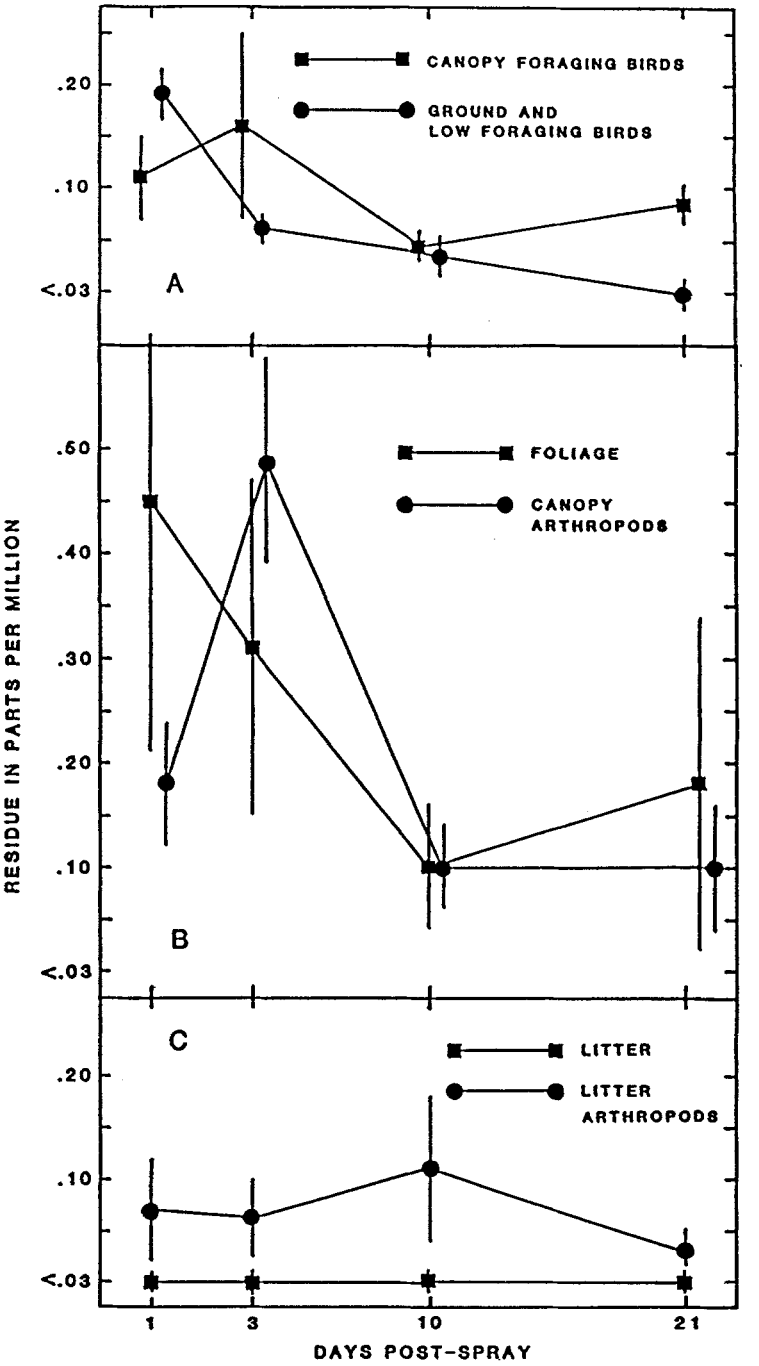

Fig. 1. Dissipation of diflubenzuron residues (means + SE from Table 1) residues in treated plots 1-21 days following spray date at Sleepy Creek Public Hunting and Fishing Area, West Virginia.

consumption of contaminated foliage. Following day 3, residues in canopy arthropods were similar to levels found in the foliage samples. Residues on foliage declined sharply between days 1 and 10, and stabilized between days 10 and 21 (Fig. 1B). The decline between days 1 and 3 was probably not significant. Lastly, overall residue levels were lowest in litter and litter arthropods, with no apparent trend over time (Fig 1C). Litter samples contained no detectable residues.

DFB is effective against immature Lepidoptera only when injested. Since it is also excreted rapidly and metabolized only slightly (Mulder and Gijswijt 1973, Verloop and Ferrell 1977, Still and Leopold 1978), it is likely that treated food must be continually ingested in order for a lethal concentration to be present at the time of moulting. However, DFB is lethal in extremely small quantities. In one study, 0.10 PPM in artificial diet caused 100% mortality in gypsy moth larvae (Granett and Dunbar 1975). We found foliage residues well above this level on day 21. Therefore, although residue levels may drop over time, adequate foliage protection is maintained long after the spray date. In another study, DFB caused 100% mortality of Douglas-fir tussock moth larvae up to seven weeks following application on foliage (Robertson and Boelter 1979).

There appears to be a correspondence in residue levels between canopy birds and canopy arthropods, and likewise, between the generally higher residues in canopy birds and arthropods compared with lower residues in ground and low foraging birds and litter arthropods. Residue levels in birds thus appear to be tracking the residue levels in arthropods that birds may be using as prey. This suggests that the primary mode of forest bird contamination is through ingestion of contaminated arthropods. However, day 21 residue levels in foliage and canopy insects were higher than those in canopy birds, indicating that DFB does not bioaccumulate at higher trophic levels.

Our results are surprising in some respects when compared with other studies. First, we found no consistent relationship between rainfall and the dissipation rate of DFB on foliage. The insignificant decline in residues between days 1 and 3 when there was no rain and the sharp decline between days 3 and 10 when there was 3.45 cm of rain is consistant with other studies. However, there was no decline between days 10 and 21 when 3.51 cm of rain fell. Previous studies (Ganyard 1986, Nigg et al. 1986) indicate that residues on foliage should decrease at rates related to rainfall. Second, we found no detectable residues in

the litter. As DFB is washed off the foliage, the residue in litter should increase (Ganyard 1986). A greater amount of DFB may reach the litter and soil in agroecosystems than in forest ecosystems, perhaps due to the lack of a well-defined canopy in the former. In either case, once in the litter and soil, DFB is broken down quickly by microorganisms (Verloop and Ferrell 1977, Willcox and Coffey 1978, Nimmo et al. 1984, Nigg et al. 1986). Lastly, the fact that residues were still present in birds on day 21 was surprising in light of previous studies (Maas et al. 1980). Although some DFB added to poultry feed for fly control in manure ends up in poultry body fat and liver tissues (Miller et al. 1976), most of the DFB is excreted or metabolized immediately (Opdyke 1976). However, as long as significant residue levels remain on foliage, the possibility of continuous forest bird contamination remains high, via ingestion of herbivorous insects.

In a follow-up study we plan to sample for residues in forest birds and small mammals up to day 60 post spray. In addition, integument, muscle, and digestive tract tissues will be analyzed separately, in order to determine the ultimate fate of DFB in forest birds.

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