DIMILIN EFFECTS ON LEAF-DECOMPOSING AQUATIC FUNGI ON THE FERNOW EXPERIMENTAL FOREST, WEST VIRGINIA

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Abstract: Dimilin was applied to two watersheds on the Fernow Experimental Forest on May 16, 1992, as part of a study to evaluate its effect on non-target organisms. Data were obtained on the occurrence, conidial production, and leaf litter colonization of aquatic hyphomycetes 5 days prior to and 2, 10, 25, and 55 days following application in the two treated watersheds and two control watersheds. Two days after application, conidial numbers were higher in one treated watershed, while they remained relatively constant in the other treated watershed. Northern red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh.) leaf bags placed in the weir ponds and streams of the treated watersheds prior to Dimilin application and retrieved 2 days after application were colonized by greater numbers of fungal taxa than bags retrieved later. This pattern suggests the possibility of an increasing influence of this pesticide on the occurrence and the litter decomposition activities of this group of aquatic fungi.

INTRODUCTION

Rapid deterioration of the quality of oak forests due to invasion of the gypsy moth (*Lymantria dispar* L.) has led to the widespread use of Dimilin (Diflubenzuron) as a method to control this introduced insect pest. Dimilin is a chitin synthetase inhibitor and affects immature insects during molting. It is ingested as the insect larvae feed on treated foliage. However, other non-target organisms also may be affected negatively.

Aquatic hyphomycetes dominate the assemblages of aquatic fungi associated with decaying leaves in lotic systems (Bärlocher and Kendrick 1974, Suberkropp and Klug 1976, Trisca 1970). Decomposition of leaf detritus and its exploitation by other members of stream detrital communities are largely dependent upon the activities of aquatic hyphomycetes, since hyphomycetes have the enzymatic capability to digest the structural polymers that comprise most dead plant tissue (Bjarnov 1972, Monk 1976). Thus, any change in the physicochemical or biological environment of a stream will influence aquatic hyphomycete activity. Consequently, concerns exist about how Dimilin might affect aquatic hyphomycetes.

The present paper compares aquatic fungal occurrence data in two watersheds treated with Dimilin and two other watersheds maintained as controls. Conidia in stream water samples were enumerated and fungal species colonizing northern red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh.) leaves placed in the four watersheds were identified.

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METHODS

Site Descriptions

Four small watersheds (1, 4, 7, and 13) on the USDA Forest Service's Fernow Experimental Forest, near Parsons in Tucker County, West Virginia, were used for this study. The Fernow Experimental Forest is located in the Allegheny Mountain section of the unglaciated Allegheny Plateau (79° 0' 11" W, 39° 0' 05" N). Elevations range from 530 to 1100 m; slopes range from 10 to 60 percent. Mean annual precipitation is 147 cm, and the mean annual temperature is 9°C. Soils are derived from sandstone and shales of the Hampshire formation. All four watersheds were logged heavily between 1905 and 1910. However, since then their disturbance histories have been quite different.

All the trees on watershed 1 (30.1 ha), except for cull trees and trees less than 2.4-cm dbh, were harvested from May 1957-June 1958. A "logger's choice" method was used such that no specific road construction, harvesting, or skidding guidelines were required. Seventy-four percent of the original basal area was cut, with 24,527 bd ft ha^{-1} removed (Kochenderfer and others 1990). The current stand is 34 years old and is dominated by yellow-poplar (*Liriodendron tulipifera* L.), chestnut oak (*Q. prinus* L.), and red maple (*A. rubrum* L.). In 1986, watershed 1 was treated with Dimilin at a rate of 0.067 kg active ingredient ha^{-1} with a fixed-wing aircraft. This treatment was imposed to measure the persistence of Dimilin in stream water, sediment, organic matter, and throughfall (Jones and Kochenderfer 1987).

Watershed 4 (38.7 ha) has had negligible disturbance since 1910, except for salvage removal of dead American chestnut (*Castanea dentata* (Marsh.) Borkh.) during the 1940s following the chestnut blight (*Cryphonectria parasitica* (Murrill) Barr). Currently, the dominant stand is about 90 years old, though scattered residual trees left from the turn-of-the century logging are estimated to be 175 to 200 years old. The dominant overstory species are sugar maple, red maple, and American beech (*Fagus grandifolia* Ehrh.).

Watershed 7 (24.2 ha) was harvested in two halves in the 1960s. The upper 12.1 ha, comprising 49 percent of the basal area (80.2 m ha^{-1}), were clearcut from November 1963 to March 1964. This half was maintained barren of vegetation with herbicides, principally 2,4,5-T, until October 1969. The lower half of watershed 7 was clearcut (76.9 m ha^{-1}) from October 1966 to March 1967 and also maintained barren until October 1969 (Patric and Reinhart 1971; Kochenderfer and others 1990). Today, the overstory vegetation is dominated by black birch (*Betula lenta* L.), red maple, and sugar maple.

Less is known about the history of watershed 13 (14.2 ha) than of the other watersheds because it became an active research watershed only in 1984. A light selection cut is believed to have been performed in the 1960s, although the volume of wood or percent basal area removed is not known. The dominant overstory vegetation is yellow-poplar, northern red oak, and sugar maple. Stand age is approximately 65 years.

Dimilin Application

For the current study, watersheds 1 and 13 were treated with Dimilin and watersheds 4 and 7 were maintained as untreated controls. The Dimilin was applied at a rate of 0.03 kg active ingredient ha^{-1} on the morning of May 16, 1992. The application was made using a Bell 206 Jet Ranger helicopter. The spraying was done using protocols to ensure that the Dimilin application was confined to the treated watersheds (USDA Forest Service 1991).

Sampling Methods

Aquatic hyphomycete distributions in the four watersheds were studied by membrance filtration of stream water samples (Iqbal and Webster, 1973) and by leaf bag incubation in both streams and weir ponds (Musil and Shearer, 1982). For membrane filtration, 250-ml water samples were collected from each stream 5 days prior to and 2, 10, 25, and 55 days following Dimilin application. Ten samples were collected from each stream on the first three sampling dates; because of low water levels, only five samples were collected from each stream on the last two sampling dates.

Samples were filtered through Millipore 0.45-μm membrane filters in the field at the time of collection. In the laboratory, each filter was treated with 0.1 percent cotton blue and heated in lactic acid at 50-60°C to render it sufficiently transparent for conidial enumeration and identification. Filters were examined microscopically at 100x, and conidia were counted. Taxa were recorded for 40 noncontiguous fields-of-view (36.2 mm^2) on each filter.

For leaf bag incubation, mesh bags containing leaves from northern red oak and sugar maple were prepared in the manner described by Musil and Shearer (1982) and Chamier and others (1984). The leaves were collected in October 1991 shortly after leaf abscission, air-dried, and stored until leaf bags were prepared. Each bag contained approximately 2.0 g of dried leaves of either red oak or sugar maple. One set of four bags of each leaf type was tied to a perforated brick. These were placed in the streams and weir ponds of the four watersheds on May 11, 1992. Bags of each leaf type were retrieved on each of four visits following application of Dimilin (i.e., after 2, 10, 25, and 55 days). Upon retrieval, leaf bags were placed in plastic zipper-lock storage bags, partially filled with stream water, and returned to the laboratory. Leaves were removed from the mesh bags, gently washed in sterile distilled water, and cut into 1 x 1-cm squares. A few squares were examined microscopically to determine the presence of hyphomycetes. The remaining squares were incubated in aerated chambers filled with sterile distilled water, as described by Shearer and Webster (1991). After 24 hours, the water was filtered through a membrane filter, and conidia were identified by microscopic examination of the filter paper after treatment with cotton blue and lactic acid, described previously. Fungal specimens recovered from leaf bags were studied as living material and/or fixed and stained with cotton blue and mounted in lactophenol. Fungal species were identified using keys and description provided in Ainsworth and others (1973), Barnett and Hunter (1972), Ellis (1971), Nilsson (1964), Ingold (1975), and Subramanian (1983). Nomenclature used in this paper follows that given by these authors.

Data Analysis

Separate data sets were compiled for aquatic hyphomycetes recorded as conidia filtered from water samples and for those occurring on leaves. The four streams were compared using coefficient of community (CC) indices (Mueller-Dombois and Ellenberg 1974). The equation for this index, which is based solely on the presence or absence of taxa, is

$$CC = 2c/(a+b) \qquad (1)$$

where a = total number of taxa in the first stream being considered, b = total number of taxa in the second stream, and c = number of taxa common to both streams. The CC value ranges from 0.0 (when no taxa are common to both streams) to 1.0 (when all taxa are common to both streams).

RESULTS

Water Filtration

Two days after the Dimilin application (May 18), the number of conidia in samples from watershed 1 was approximately twice that recorded 5 days prior to treatment (May 11) (Table 1). However, the number of conidia recorded in samples from the other treated watershed decreased from May 11 to May 18. In both treated watersheds, conidial numbers generally increased on subsequent sampling dates. Conidial numbers recorded from control watersheds 4 and 7 also declined from May 11 to May 18 and remained below the pretreatment levels throughout the subsequent sampling dates.

Numbers of fungal taxa recorded on May 18 did not differ significantly between the treated and control watersheds (Table 2). Fewer taxa generally were observed in June and July when compared to the May sampling periods for both treatment and control watersheds. This decline may be the result of the lower water levels or higher water temperatures common in these headwater streams during the growing season.

Table 1. Occurrence of aquatic hyphomycetes as indicated by the presence of conidia filtered from water samples prior to and after the Dimilin application to watersheds 1 and 13, with watersheds 4 and 7 serving as controls. Data are numbers of conidia per 1000 ml of water.

	Sampling Date				
Watershed	May 11	May 18	May 27	June 11	July 12
1	797	1660	359	304	407
13	684	485	262	314	347
4	860	498	414	213	543
7	1834	584	231	110	343

Table 2. Numbers of aquatic hyphomycete taxa recorded from filtered water samples prior to and after the Dimilin application to watersheds 1 and 13, with watersheds 4 and 7 serving as controls.

	Sampling Date				
Watershed	May 11	May 18	May 27	June 11	July 12
1	30	29	19	17	15
13	18	16	20	14	16
4	27	24	24	14	19
7	23	25	14	14	14

Overall, 108 fungal taxa were recorded by means of membrane filtration of water samples from all four watersheds on the five sampling dates. This total included 79 fresh water forms, or Ingoldian hyphomycetes; 24 terrestrial geofungi; and 5 aeroaquatic fungi, which typically occur in marshy environments. Only 25 taxa were common to all four watersheds.

The CC indices calculated from pooled filtration data for all sampling dates (Table 3) indicate that watersheds 1 and 4 were the most similar streams (CC = 0.687), even though watershed 1 was treated with Dimilin and watershed 4 was not treated. Watersheds 7 and 13 were the least similar (CC = 0.556). The average CC value for all possible combinations of streams was 0.638.

Table 3. Community coefficient indices calculated from water filtration data.

Watershed Comparisons	Coefficient of Community
1-13	0.672
1-4	0.687
1-7	0.631
4-7	0.630
4-13	0.654
7-13	0.556

Leaf Bag Colonization

Like the filtration data, leaf bag colonization data (Table 4) are quite variable. In general, more aquatic hyphomycete taxa were associated with red oak leaves than with sugar maple leaves in both the streams and the weir ponds. In most instances, the highest numbers of taxa for both leaf types were recorded on the first sampling date. The maximum number (28) was recorded for red oak in the weir pond of watershed 7 on June 11, whereas the minimum number (4) was recorded for the same leaf type in the weir pond of watershed 13, also on June 11. Overall, numbers of taxa recorded from the two types of ecological situations (i.e., weir ponds and streams) were remarkably similar.

A total of 64 fungal taxa colonized red oak leaves. Forty-six of these taxa were freshwater hyphomycetes. Fifteen species of terrestrial geofungi and 3 species of aeroaquatic hyphomycetes also were recorded. Nine taxa were present only in the treated watersheds, whereas 12 species were restricted to the control watersheds.

Colonization of sugar maple leaves was similar to that of red oak leaves. Sixty-five fungal taxa were recorded. Fifty-one of these taxa were fresh water hyphomycetes, 11 were terrestrial geofungi, and 3 were aeroaquatic hyphomycetes. Nineteen taxa were found only in treated watersheds, while 7 were restricted to the control watersheds. *Anguillospora crassa* and *Flagellospora curvula* were the only taxa recorded from both filtered water samples and leaf bags, with 100 percent consistancy.

Coefficient of community indices calculated from leafbag colonization data are summarized in Table 5. The highest CC value was 0.666, which was recorded for pairwise combinations of watersheds 1 and 4, 1 and 7, 4 and 7, and 1 and 13. Four of these involved red oak and three involved sugar maple. The lowest CC value (0.303) was recorded for sugar maple in watersheds 1 and 13.

Table 4. Aquatic hyphomycete colonization of red oak and sugar maple leaf bags.

Watershed	Leaf type	Stream						Weir pond				
		May 11[a]	May 18	May 27	June 11	July 12	Mean	May 18	May 27	June 11	July 12	Mean
		Number of taxa										
1	Sugar maple		6	8	15	3	8.0	13	6	15	7	10.3
	Red oak		17	8	6	14	11.3	16	5	7	8	9.0
		19										
13	Sugar maple		23	12	6	8	12.3	27	6	11	15	14.8
	Red oak		19	19	12	17	16.8	21	9	4	18	13.0
		14										
4	Sugar maple		15	10	14	16	13.8	10	10	10	9	9.8
	Red oak		19	5	10	12	11.5	12	14	13	15	13.5
		13										
7	Sugar maple		16	13	15	9	13.3	16	13	11	12	13.0
	Red oak		14	16	21	12	15.8	15	5	28	9	14.3
		14										

[a]Present on natural substrates prior to Dimilin application.

Table 5. Coefficient of community (CC) indices calculated from red oak (RO) and sugar maple (SM) leaf bag colonization data obtained on four dates following Dimilin application.

	Sampling Date							
	May 18		May 27		June 11		July 12	
Watershed Comparisons	RO	SM	RO	SM	RO	SM	RO	SM
1-13	0.622	0.451	0.387	0.583	0.545	0.514	0.514	0.303
1-4	0.666	0.555	0.416	0.518	0.451	0.486	0.500	0.416
1-7	0.666	0.611	0.666	0.551	0.380	0.500	0.600	0.384
4-7	0.666	0.500	0.533	0.666	0.509	0.540	0.562	0.666
4-13	0.625	0.528	0.540	0.528	0.571	0.437	0.594	0.594
7-13	0.577	0.490	0.540	0.592	0.434	0.514	0.564	0.410
Average CC Value	0.637	0.522	0.513	0.573	0.481	0.498	0.555	0.462

DISCUSSION

Filtration data indicate that conidial numbers in watershed 1 increased following the Dimilin application, but a similar increase did not occur in watershed 13. In fact, numbers of conidia decreased from May 11 to May 18 for all watersheds except watershed 1. Although the decrease in watershed 13 was less than that observed in the two control watersheds, there is not enough of a difference to suggest that the application of Dimilin caused any sudden increase or decline in fungal occurrence in the treated watersheds.

Generally higher numbers of fungal taxa were observed in leaf bags from treated watersheds than in those from control watersheds. This difference was most obvious on May 18 and was more apparent in the weir ponds of treated watersheds than in streams of the same watersheds. Dimilin may have a greater opportunity to accumulate in weir ponds and thus influence growth and sporulation of litter-decomposing hyphomycetes. Ongoing investigations on residual analysis of Dimilin do indicate persistence of Dimilin in litter and soil within the treated area (Wimmer 1994).

Overall, numbers of fungal taxa recorded from filtration and leaf bag colonization in treated watersheds on May 18 were comparable to or even higher than those recorded on subsequent sampling dates. This pattern demonstrates the tolerance of these fungi to biochemical change. It seems likely that decreases in fungal occurrence and numbers of conidia during June and July may be related to the low streamflow and resultant elevated temperatures.

The possible effects of Dimilin on fungal occurrence can be assessed in two ways. The first way is by examining the direct utilization of residual Dimilin in litter and soil (Wimmer 1994). In an earlier study (Dubey 1992), five species of aquatic hyphomycetes (*Clavariopsis aquatica, Heliscus lugdunensis, Lemonniera aquatica, Lunulospora curvula* and *Tetracladium marchalianum*) showed increased growth rates with increased Dimilin concentrations. However, direct effects of insecticides, such as Dimilin, on aquatic microorganisms probably are modified by a number of factors, including the extent to which the insecticide is water soluble, the contact time between the insecticide and the fungal mycelium, the nutritional status of the environment in which the fungus interacts with the insecticide, the amount of fungal cell material present, and the initial insecticide concentration applied.

The second way to examine the effects of Dimilin involves monitoring changes that may occur in the biochemical environment of treated watersheds as a result of reduced defoliation as gypsy moth larvae are killed after the application of the insecticide. For example, reduced defoliation may result in increases in the watershed's buffering capacity, pH, and Ca and Mg status, and decreases in streamflow and NO_3 and NH_4 compared to conditions of greater defoliation (Downey and others 1994). Low streamflow rates also favor the production of allochthonous coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM), which are basically produced by the enzymatic leaf processing action of fresh water hyphomycetes. CPOM and FPOM serve as major food sources for many aquatic macroinvertebrates. Downey and others (1994) recorded a relatively high density of aquatic macroinvertebrates from mountain streams in Virginia where an abrupt crash in gypsy moth populations due to disease prevented the occurrence of a significant defoliation during the 1992-93 growing seasons. This finding illustrates the relationship between aquatic hyphomycetes and Dimilin -- Dimilin kills gypsy moth larvae, thereby minimizing defoliation, permitting CPOM and FPOM production, and contributing to increased macroinvertebrate activity and success.

CONCLUSIONS

In the present study, Dimilin application was not shown to exhibit any clear evidence of a direct influence on conidial production in treated watersheds two days after treatment. Fungal colonization data for northern red oak and sugar maple leaves suggest that fungal growth and decomposition activities in the treated watersheds were similar to or slightly greater than those occurring in the control watersheds. If aquatic hyphomycetes are affected by Dimilin, these effects are manifested in an indirect rather than a direct manner.

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