

SORPTION AND DEGRADATION OF SELECTED FUNGICIDES IN THE TURFGRASS CANOPY

WILLIAM V. SIGLER^{1,2}, ZACHARY REICHER¹, CLARK THROSSELL¹,
MARIANNE BISCHOFF¹ and RONALD F. TURCO^{1*}

¹ Department of Agronomy, Purdue University, West Lafayette, Indiana, U.S.A.; ² Present address: Institute for Terrestrial Ecology, Swiss Federal Institute of Technology (ETH), CH-8952 Schlieren, Switzerland

(* author for correspondence, e-mail: rturco@purdue.edu, fax: 76 549 63210)

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Abstract. Microbial degradation of fungicides on leaf surfaces after repeated applications to turfgrass was investigated. Prior and current work in our laboratory has identified two characteristics of the turfgrass leaf system that may contribute to the enhanced degradation of fungicides after repeated application to turfgrass: (1) The leaf surface is rich in microorganisms ($\sim 10^8$ g⁻¹ dr wt leaf), and (2) Leaf surface microorganisms may respond to repeated fungicide applications in a manner consistent with the phenomena of enhanced biodegradation. Field studies were conducted on 'Penncross' creeping bentgrass with four fungicides representing three chemical families applied either two or eight times in one growing season. Biodegradation was estimated using data from both a field study and a parallel laboratory study that followed the fate of ¹⁴C-labelled fungicides. For the laboratory incubations, the locations of the residual ¹⁴C fungicides were estimated using a sequential extraction protocol that fractionated the materials into three pools: available, retained and bound. Data from both the field and laboratory study refuted our hypothesis that enhanced biodegradation would develop following repeated applications of the fungicides onto the leaf surface. Our studies support a conclusion that a two-stage physical sorption process leads to plant incorporation and this controls most of the fungicide's fate. Thus, our data suggest that microbial activity plays a less important part in the process than would be indicated by considering the size of the microbial population on the leaves.

Keywords: degradation, iprodione and vinclozilin, metalaxyl, plant-canopy, triadimefon

1. Introduction

Fungicides are used in the turfgrass industry to improve plant health by providing protection from pathogenic fungi. High disease pressure from fungal populations can result from the intense management of golf course playing surfaces, weather conditions, and geographical location. While single fungicide applications are used to suppress turfgrass pathogens, chemical applications can be repeated many times per year on golf courses in the U.S.A. (McCarty, 2001). The non-target and toxicological effects of fungicides have been investigated and reviewed (Vyas, 1986; Frederick *et al.*, 1994; Sigler *et al.*, 1999, 2000), and subsequently, concerns have developed about the environmental impact of frequent fungicide applications on



the resident microbial population, and how the response of the population may alter the efficacy of the chemical.

In row-crop agriculture, pesticides are applied directly to bare soil or have a high probability of encountering bare soil. In the turfgrass setting, the majority of the applied pesticide is intercepted by the leaf surface. For example, Stahnke *et al.* (1991) showed that a dense turf canopy would retain 95% of the herbicide pendimethalin, indicating the turfgrass canopy can be a major sink for applied chemicals. This is not surprising if we consider the surface area of the turfgrass and the underlying soil. Assuming a putting green is mowed at 0.4 cm and possesses up to 6.6×10^{11} shoots ha^{-1} of 0.31 cm width (Beard, 1973), the surface area of leaves would be 1.7×10^{16} $\text{cm}^2 \text{ ha}^{-1}$ or 1.7×10^{11} ha ha^{-1} . The large surface area of turfgrass makes it a potential site for fungicide interactions and necessitates an investigation of the role of the turfgrass canopy in the mediation of fungicides.

Since the turfgrass leaf area is large it provides a significant site for interaction and must be considered as a key component in determining the environmental fate of an applied fungicide. Using bioassay methods, Liu and Hsiang (1996) reported that between 46.2 and 59.9% of applied benomyl ([1-[butylamino]carbonyl]-1H-benzimidazol-2-yl]carbamic acid remained bound to turfgrass leaves and was unavailable for fungicidal activity after a 1 hr incubation with turfgrass clippings. Turfgrass leaf sorption coefficients have been calculated for several fungicides. Iprodione ($K_{oc} = 392$), metalaxyl ($K_{oc} = 30$), and triadimefon ($K_{oc} = 171$) were shown to exhibit sorptive behavior on turfgrass clippings with similar magnitude to soil reactions (Taylor, 1996). Although leaf K_{oc} values were generally at the lower end of published values for soil K_{oc} (iprodione: 500–1300, metalaxyl: 29–287, and triadimefon: 73–345)* the adsorptive capacity of fungicides to the leaf underscores the significance of fungicide binding to surfaces other than soil.

Once applied to the turfgrass canopy, it is desirable for a fungicide to impact the target organism, but also to have limited persistence. A chemical that possesses a long environmental half-life and persists in a given environment will pose an increased risk of off-target movement (Alexander, 1994). Given the total surface area involved, a need exists to develop an understanding of the decay rates of a fungicide on turfgrass leaves as a measure of persistence. Studies in our laboratory have shown the decay rates of chloroneb and vinclozilin on turfgrass leaves to be 0.01 and 0.09 d^{-1} , respectively (Frederick *et al.*, 1994). This translates to a half-life of 69.3 and 7.7 d, respectively. In comparison, chloroneb and vinclozilin half-lives in soil were reported to be 2.66 and 0.81 d, respectively (Frederick *et al.*, 1994). Higher rates of fungicide dissipation in soils are assumed to be caused by an increased level of microbial degradative activity. The persistence of parent material on leaf surfaces warrants further investigation into the dynamics of the leaf surface as a potential site for fungicide biodegradation.

* From Summary of Pesticide Properties and Potential for Surface and Subsurface Losses. United States Golf Association. URL <http://www.usga.org/gren/table3.html>

Although the characteristics of leaf surface/fungicide interactions have been sparingly reported, microbial degradation of fungicides in soil has been investigated. Martin *et al.* (1990) found that a single application of iprodione to sterilized soil resulted in a lack of appreciable degradation while additions to a similar unsterilized soil resulted in degradative activity, supporting the idea of biotic degradation of the fungicide. Vinclozilin degradation in soil is at least partially controlled by *Pseudomonas* and *Bacillus* sp. (Goleveva *et al.*, 1991), which have also been reported to be significant members of the turfgrass phyllosphere community (Austin *et al.*, 1978). Repeated applications to soils have been reported to enhance the rate of fungicide degradation when compared to single applications. Increased degradation rates of vinclozilin and iprodione (Walker *et al.*, 1986; Walker, 1987; Slade *et al.*, 1992), metalaxyl (Bailey and Coffee, 1986), benomyl (Yarden *et al.*, 1985), and mancozeb (manganese ethylene bisdithiocarbamate) (Doneche *et al.*, 1983) have been reported to result from multiple applications. The authors implied that enhanced biodegradation resulted from the adaptation of microbial populations that possessed the ability to degrade the fungicides. Subsequent applications were met with increasingly active degrading populations, which lead to an increased rate of degradation.

Previous work in our laboratory suggested that a similar pattern of degradation enhancement might occur on the leaf surface (Frederick, *et al.*, 1996). Although soil is a significant site for processes that determine a pesticide's fate (Dell *et al.*, 1994), the nature of the microbial populations on the turfgrass canopy could predispose it to enhanced biodegradative activities commonly observed in soil. Our work in this study suggested the number of bacteria per gram of turfgrass leaves is comparable to that of soil ($\sim 10^7$ – 10^8 g⁻¹), but the increased bulk density of soil equates to a much larger bacterial population per unit volume (Sigler, 1999). However, the hypothesis of a possible enhancement in fungicide biodegradation in the turfgrass canopy is yet untested. By evaluating the rate of microbial degradation in addition to possible binding and sequestering of fungicide materials on the leaf surface, the importance of the turfgrass canopy as a pesticide sink and degradation site can be understood. The three objectives included: (1) establish the size of leaf surface resident microbial population, (2) establish the potential of the turf canopy to become enhanced for biodegradation of fungicides within one growing season and (3) identification of physical factors modulating the fate of fungicides in the turfgrass canopy.

TABLE I
Physical properties of fungicides used in this study

Fungicide	Family	Molecular weight ^a (g)	Water solubility ^b (mg L ⁻¹)	Log K _{ow}	Log K _{oc}
Iprodione	Dicarboximide	330.1	13	3.10 ^b	1.48 ^b
Metalaxyl	Acylalanine	279.3	7100	1.52 ^b	1.53–1.84 ^b
Triadimefon	Triazole	293.7	260	3.18 ^b	2.28–2.73 ^b
Vinclozilin	Dicarboximide	286.1	1000	3.01 ^c	2.00–2.86 ^c

^a The Merck Index, 1997.

^b J. H. Montgomery, 1993.

^c C. Tomlin, 1994.

2. Materials and Methods

2.1. FUNGICIDES

Four fungicides representing three families of chemicals were used in this study, as they represent important and widely used classes of chemicals for the turfgrass industry (Table I). Iprodione (3-[3,5-dichlorophenyl]-N-isopropyl-2,4-dioximidizolidine-1-carboximide) and vinclozilin (3-[3,5-dichlophenyl]-5-ethenyl-5-methyl-2,4-oxazolidine-2,4-dione) represent the dicarboximide family while metalaxyl (methyl-N-[2-methoxyacetyl]-N-[2,6-xylyl]-DL-alaninate) and triadimefon (1-[4-chlorophenoxy]-3,3-dimethyl-1-[1H-1,2,4-triazole-1-yl]-2-buta-none) represent the acylalanine and triazole families, respectively.

2.2. FIELD SITE DESCRIPTION

The field portion of the study was conducted at the Purdue University Agronomy Research Center, West Lafayette, Indiana on 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) grown on Chalmers silty clay loam. Plots were fertilized with 116 kg N ha⁻¹ and irrigated as needed to avoid water stress. Plots measured 1.5 × 4 m with a 1 m border separating each plot. They were arranged in a randomized complete block design with three replications.

2.3. DETERMINATION OF BACTERIAL POPULATIONS ON GRASS LEAVES

Grass clippings were collected from different locations in stands of Creeping bentgrass using sterile scissors (dipped in methanol and flamed). Ten individual samples were taken from the terminal 1 cm of the grass and combined, generating two composite samples. The grass was generally 3 cm in height when it was clipped. The fresh leaves were placed into sterile plastic bags and transferred to the lab

for processing. Moisture content was determined by drying a subsample at 60 °C for 24 hr. To determine the size of the extractable microbial population resident on the leaves, a modification of a method used to recover bacteria from soil was used. Duplicate samples of approximately 0.5 g (wet wt) were transferred into sterile glass vials and suspended in 10 mL of sterile saline (Zuberer, 1994). The tubes were shaken for 45 min and 0.5 mL of the solution was passed through a 0.2 μ black polycarbonate filter. The filters were stained with a solution of 0.03% acridine orange for 30 secs and washed with 2 to 3 mL of sterile saline solution. The stained filters were fixed to glass slides using emersion oil and observed with a Zeiss epifluorescence microscope (Carl Zeiss Inc., Thornwood, NY). Eight fields per filter were counted and the counts averaged. Counts were expressed per gram dry weight of leaf.

2.4. FUNGICIDE TREATMENTS

Each fungicide was applied to individual plots on either a two or eight week application interval for sixteen weeks beginning in early June. Triadimefon and iprodione were applied at 3.08 kg formulated product ha⁻¹. Metalaxyl was applied at 1.53 kg formulated product ha⁻¹. All fungicides were applied in 1 L of water using a CO₂-pressurized hand-sprayer calibrated to apply 1629 L ha⁻¹. Each pass with the sprayer over the turfgrass plot emptied approximately 250 mL of fungicide mixture and several passes were made over each plot at different angles to ensure even distribution of the fungicide. Misapplication to non-target plots was prevented using a windscreen built from plastic sheeting and PVC pipe. The screen was approximately 1 m high and was placed around the control plots before chemical applications were made to the adjoining plots.

2.5. FUNGICIDE DISSIPATION IN THE TURFGRASS CANOPY

Clippings were collected from the entire plot area at 1, 3, 7, and 11 days following each fungicide application. These four samplings following each treatment are defined collectively as one sampling cycle. Clipping harvests were performed using a push-type reel mower set to cut at 0.64 cm. Recovered clippings were placed into pre-weighed, sterile plastic bags and stored on ice until laboratory analysis (performed within 2 hr). The mower was washed with water between samplings of differently treated plots to prevent fungicide cross-contamination among plots and samples. Bags containing the clippings were weighed and three portions of approximately 2 g (wet weight) from each clipping set were removed at random from bags and dried at 60 °C for 24 hr to determine moisture content. All fungicide recovery values throughout this study were reported on a plant dry weight basis.

2.6. RESIDUE RECOVERY

Fungicide residues were recovered from the leaf with an organic solvent extraction. Triplicate samples of approximately 0.6 g of wet clippings from each plot were weighed into 50 mL glass screw-top vials. Ten mL of pesticide-grade iso-octane (Fisher Scientific, Fair Lawn, NJ) was added to each vial followed by one hour of shaking. After shaking, the extract was drawn off the samples, transferred into screw-top vials and if necessary, stored at $-20\text{ }^{\circ}\text{C}$. Extracts were concentrated by evaporating iso-octane from the vials under a stream of nitrogen followed by resuspension of the residues in 1 mL iso octane. Metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2, 4-triazin-5-one) (Chem Service, West Chester, PA) was added as an internal standard to each sample at a concentration of $1.0093\text{ }\mu\text{g mL}^{-1}$. Samples were analyzed on a Hewlett Packard 5890 gas chromatograph equipped with a splitless capillary injector ($200\text{ }^{\circ}\text{C}$) and a nitrogen-phosphorous detector ($220\text{ }^{\circ}\text{C}$). The fungicides were separated in a DB-5 silica capillary column ($0.53\text{ mm} \times 15\text{ m}$) (J & W Scientific, Folsom, CA). The oven temperature was programmed from 125 to 200 at $7.5\text{ }^{\circ}\text{C min}^{-1}$ with an additional ramp to 280 at $15\text{ }^{\circ}\text{C min}^{-1}$. Varian Star Workstation software (Varian Inc., Walnut Creek, CA) was used to acquire data that was compared against five external standard concentrations. Two injections were made for each extract and the results averaged.

2.7. ESTIMATING ENHANCED FUNGICIDE DEGRADATION

Field dissipation studies, as described above, account for losses by both biotic and abiotic mechanisms. As a result, it is difficult to account for losses mediated by biological processes. We estimated the occurrence of any enhancement in biodegradation (EB) in a separate but parallel study. The potential EB of the fungicides was assessed using a subset of the clippings recovered on day 1 during each sampling cycle. By the end of the experiment, the surface population had been exposed to chemicals eight different times. Previous work had suggested that an adaptation response would be detectable one day after treatment. Moreover, we wanted to focus on the ability of the standing microbial population to degrade the chemicals. Three 5 g samples of wet grass clippings from each of the replicated field plots were incubated in 200 mL screw-top jars with $0.009\text{ }\mu\text{Ci }^{14}\text{C}$ ring-labeled fungicide as was applied in the field treatments. ^{14}C -labeled fungicide was added to the clippings and mixed with a sterile spatula. As in the dissipation study, the EB in plots treated on a two and eight week interval were assessed.

A vial containing 10 mL of 0.5 N potassium hydroxide was placed into each jar to capture evolved $^{14}\text{C-CO}_2$ and the jars were incubated in the dark at $25\text{ }^{\circ}\text{C}$. As a negative control to test the degradation potential associated with non-treated clippings, each of the three radiolabeled fungicides was also added to replicate samples of clippings harvested from control plots.

In all cases, 1 mL aliquots of the KOH trap solution were sampled at 3, 7, 11, and 15 days after the radiolabel application. The aliquot was added to a 22 mL

liquid scintillation vial and mixed with 15 mL of Ecolume (ICN, Costa Mesa, CA) scintillation cocktail. Vials were stored in the dark at room temperature for 24 hr prior to analysis with a Packard 1600 TR Liquid Scintillation Analyzer (Packard Instrument Co., Meridien, CT). The KOH solution was replaced at each sampling to prevent saturation with CO₂.

On 16 July, (beginning of the fourth, two-week fungicide application cycle), we switched from screw-top jars to 250 mL Bellco Biometer flask (Bellco Glass Co, Vineland, NJ). Each biometer consisted of a 250 mL Erlenmeyer fitted with a sidearm and liquid reservoir. The clippings were placed in the flask and the Teflon plug that was used to stopper the Erlenmeyer portion was fitted with a column filters containing Ascarite (Thomas Scientific) and magnesium perchlorate (Fisher Scientific, Fair Lawn, NJ) to trap CO₂. This column was shutoff with a valve except when the KOH was exchanged. KOH was placed in the liquid reservoir of the flask. A stainless steel needle tipped with a 2.5 cm Teflon tube was placed through the plug on the liquid reservoir. The Teflon tube always rested below the surface of the KOH. When the KOH was replaced, the column was opened to the atmosphere and the headspace was refreshed with CO₂-free air. This modification was done to allow an improved air transfer within the system and this procedure was utilized throughout the remainder of the experiment. Due to this change, there were only two samplings for ¹⁴C-CO₂ samplings in the third sampling cycle.

2.8. QUALITATIVE ASSESSMENT OF LEAF SURFACE PROCESSING OF FUNGICIDES

Following the radiolabel assessment (two weeks), leaf samples were removed from the biometer flasks (or small jars) and placed in plastic bags and frozen at -20 °C. The samples were then freeze-dried to preserving the fungicide's physical location and to ease handling. Each freeze-dried clipping sample (replicated three times) was subjected to the following fractionation sequence to characterize the fate of radiolabel material remaining in and on the clippings:

Step 1 *Pre-extraction oxidation – Total Radiolabel Remaining*

To determine the total amount of radiolabel remaining in and on the turf-grass leaf surface, triplicate 100 mg portion of each freeze-dried clipping sample were weighed and combusted at 800 °C for 45 s in a Packard 307 (Packard Instrument Co., Meridien, CT) biological oxidizer. Evolved ¹⁴CO₂ was trapped in 10 mL Carbosorb E and 10 mL of scintillation cocktail (Permafluor E+) was added to each sample and ¹⁴C was analyzed in the same liquid scintillation counter previously mentioned.

Step 2 *Extraction – Extractable Radiolabel*

To determine the amount of extractable ¹⁴C recoverable from the leaf surface, a second subsample of each freeze dried sample was weighed into an extraction vial and shaken with pesticide grade ethyl acetate (Fisher

Scientific, Fair Lawn, NJ) for 1 hr to remove respective fungicide and/or metabolites from the leaf surface.

Step 3 *Post-extraction oxidation – Retained Radiolabel*

A final oxidation to determine the amount of non-extractable fungicide remaining associated with the leaf surface was conducted by combusting the clippings following step 2. Subtracting the value of counts derived from the post-extraction oxidation (step 3) from that of the pre-extraction oxidation (step 1) yielded the total amount of radioactivity (amount of parent material or metabolite) extractable from the leaf surface.

2.9. SHORT-TERM FUNGICIDE ADSORPTION TO LEAF MATERIAL

A second field study was performed to examine the rate at which short-term binding of fungicides to leaf surfaces occurs in the field. Metalaxyl and triadimefon were both used in the study. However, due to the association of iprodione with extracted chlorophyll, vinclozilin (also in dicarboximide family) was used in its place to facilitate GC analysis. A one-time fungicide application was made to creeping bentgrass plots using the protocol for the dissipation study (see above). Vinclozilin was applied at a rate of 3.08 kg formulated product ha⁻¹. Clippings were harvested from the plots at 4, 6, 12, 24, and 48 hr after treatment, however in contrast to the method of collection described above, clippings were then harvested from a different area of each plot at each harvest time. With this collection format, the sample of clippings collected at 48 hr was exposed to the fungicide for 24 hr longer than the sample taken at 24 hr, and for 36 hr longer than sample taken at 12 hr, etc. Clipping samples were placed on dry ice, weighed, sub-sampled for moisture content and extracted for fungicide concentration within two hours of sampling. Extractions and detection were performed as indicated in Section 2.6.

3. Results

3.1. BACTERIAL POPULATIONS RESIDENT ON GRASS LEAVES

Data on the general leaf surface bacterial counts indicated a population of bacteria can be extracted from the leaves. For creeping bentgrass the average count (standard deviations, n = 2) was 1.19×10^8 cells g⁻¹ ($\pm 5.9 \times 10^7$ cells g⁻¹). These numbers were similar to bacterial counts collected in an earlier study using Kentucky bluegrass where average count were 1.01×10^8 cells g⁻¹ ($\pm 2.3 \times 10^7$ cells g⁻¹). Morris *et al.*, (1998) using a milder extraction procedure, found similar bacterial levels in the phyllosphere.

3.2. FUNGICIDE DISSIPATION STUDY

The in-field transformation reactions for the different combinations of time and application frequencies (8 treatments or 2 treatments) were analyzed by calculating a first-order reaction rate (slope) for each time and frequency combination. For the measurements of the effects of the repeated application of chemicals, an analysis of variance (ANOVA) was conducted using the main effects of replication ($n = 3$), chemical, and application frequency and testing differences in reaction rates. The analysis was conducted using a General Linear Model (GLM) within Minitab (ver. 13). Triadimefon, metalaxyl, and iprodione half-lives in the turfgrass canopy were found to be 3.3, 3.7, and 3.6 d, respectively. No significant differences were found with any combination of time, frequency of application for any chemical ($p = 0.05$) or pattern of chemical application (every two weeks versus every eight weeks). This refutes our hypothesis that repeated exposure of the microbial population to the fungicide will induce a change in the mineralization response of the cells resident on the leaf surface.

Data in Figure 1 shows the typical patterns of loss for the fungicides triadimefon, metalaxyl, and iprodione after grass was treated for the eighth time on a two-week application interval over a total of sixteen weeks. As statistical analysis indicated, no difference between the treatments and we have limited our discussion to these data as they reflect a system that most typifies management conditions on a golf course.

3.3. FUNGICIDE BIODEGRADATION STUDY

In an effort to better understand the behavior of the fungicides and differentiate loss mechanisms caused by abiotic responses from those driven by biodegradation, an *in vitro* ^{14}C -biodegradation study was conducted simultaneously to the dissipation study described above. We observed little evolution of $^{14}\text{C}\text{-CO}_2$ from any of the fungicide/clipping incubations suggesting that minimal microbial mineralization took place. The effects of repeated application of chemicals was assessed through an analysis of variance conducted using the main effects of replication ($n = 3$), chemical, and application frequency on the rate of $^{14}\text{C}\text{-CO}_2$ evolution. Analysis was conducted using a General Linear Model (GLM) within Minitab (version 13). Only 1–2% of any of the applied fungicides was mineralized to $^{14}\text{C}\text{-CO}_2$ during any of the eight sampling cycles and the differences were not significant. This observation further supports a conclusion of little microbial enhancement in fungicide mineralization on the leaf surface.

3.4. QUALITATIVE ASSESSMENT OF LEAF SURFACE INTERACTION WITH FUNGICIDE

Based on pre- and post-extraction ^{14}C oxidations and extraction, our results indicated that on average less than 36% of the ^{14}C -fungicide/metabolites was extractable

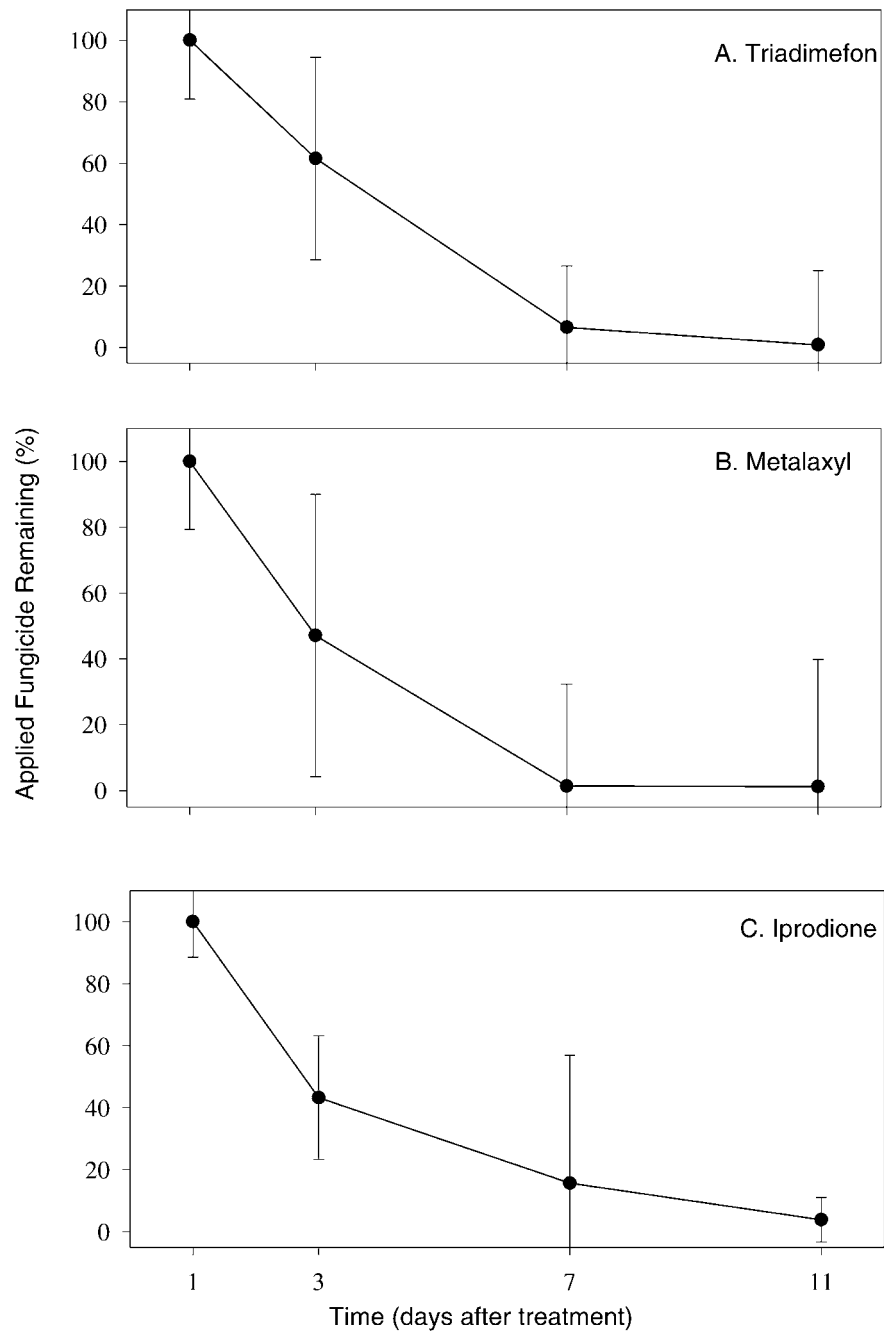


Figure 1. Dissipation rates of fungicides from the *Agrostis palustris* canopy; (A) triadimefon, (B) metalaxyl, and (C) iprodione. Error bars represent the standard error of triplicate measurements. Concentrations corresponding to 100% were Triadimefon 203 (sd = 38); Metalaxyl 203 (sd = 43) and Iprodione 2106 (sd = 229) $\mu\text{g g}$ dry weight of leaves, respectively.

TABLE II

Distribution of the three studied fungicides as determined by measurement of radioactivity in three extraction pools following ^{14}C -fungicide/clipping incubations

	Triadimefon	Metalaxyl	Iprodione
	(%)		
Total	100 ^a	100	100
Bound	66±3.7	59±3.0	64±5.4
Extractable	34±3.3	41±5.4	36±6.6

^a The numbers are the percentage of radioactivity found (\pm sd) in the different fractions as compared to the total radioactivity detected (100%). Values and errors were calculated as averages of triplicate samples.

(Table II) after two weeks of incubation. Approximately two-thirds of each applied fungicide became unextractable after application and suggests the plants surface is strongly sorptive towards the chemicals. Although the lack of ^{14}C - CO_2 from any of the three fungicides indicates little mineralization, it does not completely preclude the possibility that some transformation of the applied fungicides was occurring in the system. In an effort to characterize the extract solution, approximately 200 μL of extract was spotted onto a silica thin layer chromatography plate (Whatman Inc., Clifton, NJ). Following separation, plates were developed for 1 hr in a tank containing 100 mL of ethanol/toluene (90–10%). ^{14}C emissions representative of either parent fungicide or metabolites were analyzed with a thin layer chromatography plate reader (Raytest Isotopenmessgerate GmbH, Germany). The loss of peak areas for the parent fungicide was variable but lacked a pattern that would indicate an enhanced degradation rate. Prior work in our laboratory had shown that pesticide degradation rates increased following repeated application in a system-undergoing enhancement (Turco and Konopka, 1990). In addition to the parent compound peak, we recovered 1, 3, and 2 additional peaks from triadimefon, metalaxyl, and iprodione samples, respectively. However, an exact separation and identification of the metabolites was prevented by the presence of co-extracted plant materials that created a green smear across the plate. The green material was suspected as chlorophyll. A number of attempts were made to separate the radiolabel from the green materials but were all unsuccessful. Therefore, no further characterization of the extraction phase was attempted.

3.5. SHORT-TERM FUNGICIDE ADSORPTION TO LEAF MATERIAL

The extractability of metalaxyl, triadimefon, and vinclozilin from the turfgrass leaf surface declined in a two-step fashion over the 48 hr period following application

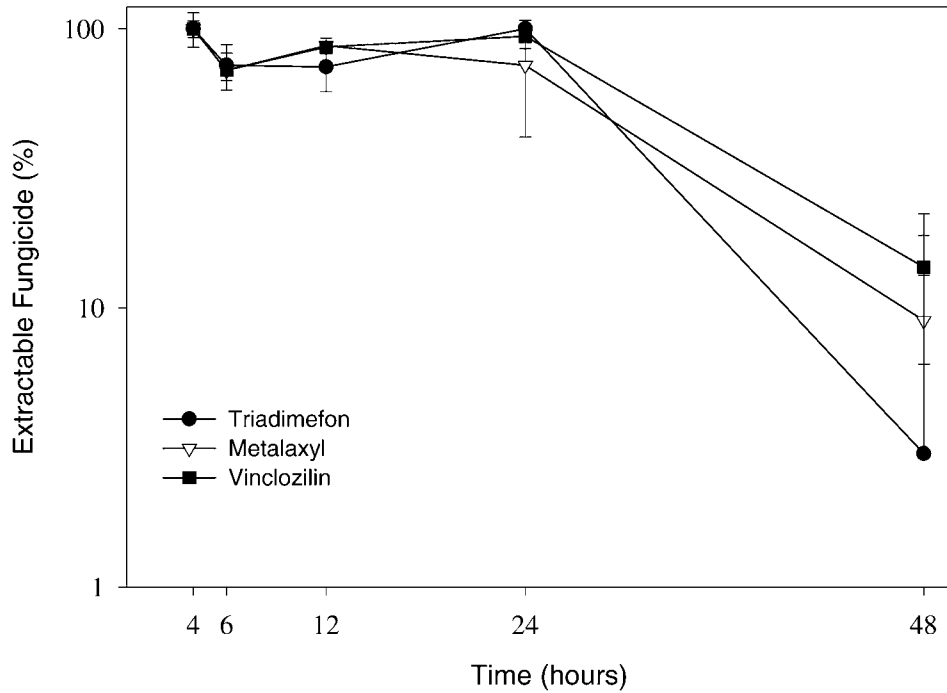


Figure 2. Extractable triadimefon, metalaxyl, and vinclozilin (%) recovered from *Agrostis palustris* leaves harvested at 4, 6, 12, 24, and 48 hr post application. Error bars represent the standard error of triplicate measurements.

(Figure 2). The extractability of the three fungicides remained high for 24 hr. Between 24 and 48 hr, the extractable amounts of metalaxyl, triadimefon, and vinclozilin decreased by 97, 65, and 80% of applied amounts, respectively. This suggests that under field conditions, drying is an important part of the adsorption process. The average clipping dry weight sampled from each of the separate plots was stable throughout the 48 hr experiment (data not shown). Thus, dilution of the fungicides by the growth of the plant between samplings did not effect the measured concentrations of the extracted fungicides. This assessment was possible because of the structure of the experiment were separate plots of turfgrass were treated at the same time but sampled at different times up to 48 hr.

4. Discussion

4.1. CHEMICAL LOSS AND BIODEGRADATION

While some changes in the dissipation rates were evident throughout the study, the lack of a significantly increased degradation rate with increased application frequency indicates that no enhanced biodegradation of applied fungicides was occurring within the canopy during the growing season. Work by Karpouzas and Walker (2000) has shown the enhanced biodegradation response to be present some 24 months after its initial occurrence. Our data indicated that the dissipation rates on turfgrass receiving triadimefon, metalaxyl, and iprodione applications were similar, regardless of the application frequency of either 2 or 8 week intervals. Since our work showed that the leaf surface is inhabited by a large microbial population and that both metalaxyl and iprodione undergo microbial degradation in soils following application (Bailey and Coffee, 1986; Walker *et al.*, 1987; Walker, 1986; Martin *et al.*, 1990, 1991; Slade *et al.*, 1992), we hypothesized that the same process could occur on the leaf surface because of the high microbial numbers. However, our results indicate that application frequency did not affect the abilities of the resident populations to degrade the chemical.

Triadimefon, metalaxyl, and iprodione half-lives in the turfgrass canopy were generally similar to those previously reported by Frederick *et al.* (1994; 1.98 d for triadimefon) and Taylor (1996; 4.6 and 1.6 d for the half-lives of metalaxyl and triadimefon, respectively). Although the observed half-lives suggest a high microbial activity, our work with ^{14}C -labelled fungicides indicates that leaf surface conditions were unfavorable for microbial mineralization as little conversion to CO_2 was observed. As a consequence, our work also suggests shows that leaf environment did not allow for microbial (we did not monitor growth) adaptation towards fungicides as would be the case in enhanced biodegrading system, as significant changes in the response of the system were not seen. Although no enhanced mineralization (as indicated by $^{14}\text{C}\text{-CO}_2$) was observed, metabolites did form but the rate of formation appear to also be unaltered by the repeated chemical applications. While we cannot differentiate changes controlled by the bacterial population from those controlled by the plant, we now suggest that retention in or on the leaf surfaces is the major reaction pathway.

We suggest that abiotic processes are more significant than biotic processes in controlling the fate of these chemicals on the leaf surface because of the speed at which fungicide loss occurred. Photodegradation is also a possible mechanism of chemical loss as triadimefon (Nag and Dureja, 1997), metalaxyl (Sulkul *et al.*, 1992), and vinclozilin (Schwack *et al.*, 1995) all are photoreactive. In the current study, photodegradation reactions would be limited in our *in vitro* incubations as these were conducted in the dark. However, light-catalyzed reactions might have played a role in the field dissipation of applied fungicides. Additionally, the lack of $^{14}\text{C}\text{-CO}_2$ production following incubation and the degree of interaction between

the extracted plant material and the radiolabeled fungicides further support our conclusion that in spite of the large resident populations, the leaf surface community is inactive towards the chemicals.

4.2. SURFACE RETENTION

An irreversible binding to the leaf surface or uptake into the leaf is thought to drive the sorption events that control fate of the applied chemicals after 24 hr in the environment. Work by Lickfeldt and Branham (1995) supports a conclusion that turfgrass leaves are a strong sorbent for organic compounds and that partitioning is the active process in retention. Differences in the leaf K_{oc} values for metalaxyl ($K_{oc} = 30$), triadimefon ($K_{oc} = 171$) (Taylor, 1996), and vinclozilin ($K_{oc} = 128$) suggest that the latter two fungicides have a higher potential for binding to organic surfaces. However, our results indicate that after 24 hr contact with the leaf surface, the amount of fungicide retention appears to become uncoupled from the initial sorption value. The longer time to achieve irreversible binding in the field, as compared to our laboratory studies, possibly reflects the application method employed. In the laboratory the materials were applied without carrier materials while in the field formulated chemicals were utilized.

This bi-phasic nature of fungicide sorption to leaf tissue implies that there exists a period of potential for environmental exposure followed by a rapid reduction in risk. The drastic level of immobilization of fungicides illustrated in Figure 2 and our laboratory studies may help to explain the limited microbial mineralization and degradation that occurred in the study. From our field results, we conclude that the highly sorptive nature of the leaf surface for metalaxyl, triadimefon, and vinclozilin limits the availability of the fungicide to microbial processes. This suggests that degradation by resident leaf microflora needs to occur within approximately 24 hr of application. In our study, we were unable to find an increased degradation rate during this timeframe regardless of our attempts to adapt the population with frequent fungicide applications. This suggests that the limiting factor in fungicide degradation may be the lack of fungicide availability for surface microorganisms. While we have shown that some chemical transformation is taking place on the leaf surface, we maintain that most of the fungicide became tightly bound in/to the turfgrass leaf surface and was unavailable for microbial utilization although the degree of plant-mediated transformation was not directly assessed.

The environment of the leaf surface may also decrease biological activity of microorganisms through factors such as the unfavorable water balances, exposure to DNA-damaging UV light (Jagger, 1991), and limited availability of substrate, in this case fungicide. In conclusion, surface reactions between the plant and chemicals appear to be dominant in controlling the fate of the applied fungicides, especially given the extremely large leaf-surface area that is typically found in turfgrass systems.

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