Degradation of Fungicides in Turfgrass Systems

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Executive Summary

Increasing public concern over the fate of organic pesticides has necessitated research describing the degradation and potential accumulation of these compounds. This USGA funded study strives to increase understanding of the fate of fungicides that reach the turf canopy, thatch and soil. This project is broken into three tasks with specific objectives of describing sorption coefficients for fungicides in the turfgrass canopy, determining the importance of microbial populations in controlling the degradation of fungicides on turfgrass leaves, and characterizing the long term fate of fungicides that reach the thatch and soil environment.

The work done this first year of the three year project has primarily focused on the field portion of task one which investigates the hypothesis that the chemical/physical structure of the leaf controls initial sorption of fungicides. Pesticide which is tightly sorbed to the turfgrass leaf may not be available for microbial degradation or movement into the thatch and soil. Concentrations of fungicides on *Penncross* creeping bentgrass clippings were measured experimentally over a period of four weeks allowing determination of half lives and dissipation patterns of parent compounds. Data was averaged from two identical experiments initiated on 12 May and 25 August 1995. Other areas of progress include developing laboratory procedures for all tasks and obtaining three of the four radiolabeled fungicides needed to meet our objectives. To extend the applicability of our work, four fungicides which represent four families of chemicals are being used in this project: chlorothalonil (Daconil 2787), metalaxyl (Subdue), triadimefon (Bayleton) and vinclozolin (Curalan).

Loss of fungicide was clearly shown in turfgrass clippings from creeping bentgrass plots. Concentrations of metalaxyl, triadimefon, and vinclozolin fell below detectable limits within 13, 10, and 17 days respectively. In both experiments chlorothalonil was detectable at low levels from clippings collected at the termination of the experiment on day 27. The average peak fungicide concentrations extracted from turfgrass clippings were 38.16 $\mu g\ g^{-1}$ (dry clipping weight) for metalaxyl, 112.46 $\mu g\ g^{-1}$ for triadimefon, 549.65 $\mu g\ g^{-1}$ for chlorothalonil and 370.35 $\mu g\ g^{-1}$ for vinclozolin. The half lives of parent fungicides were 4.4 days, 1.1 days, 3.2 days and 2.6 days for metalaxyl, triadimefon, chlorothalonil, and vinclozolin respectively. High initial concentrations of fungicides coupled with rapid loss from turfgrass clippings indicates that dissipation in the field is not limited by sorption. In conclusion, when fungicides are applied at recommended rates pesticide loading is unlikely on the live turfgrass plant.

Introduction

The use of fungicides on golf course greens and tees is a common turfgrass management practice employed by superintendents to control fungal pathogens. Agnew and Lewis (1993) found that fungicides accounted for up to 76% of the total pesticide application in a year. Increasing public concern over the fate of organic pesticides has necessitated research describing the degradation and potential accumulation of these compounds. Prolonged persistence of applied pesticides in the environment can result in groundwater and surface water contamination. Dissipation of fungicides from the environment as a result of degradation precludes the possibility of contamination but degradation must not be so rapid that the effectiveness of the fungicide is undermined. Ideally an applied fungicide should persist in the environment only long enough to have the desired effect.

Degradation can proceed via biological transformation, photodecomposition, chemical breakdown or plant enzymatic action. Sorption of pesticides can attenuate microbial degradation. Pesticides which are tightly sorbed to the turfgrass leaf may not be available for microbial degradation or movement into the thatch and soil. When studying plant litter, Dao (1991) indicated a strong correlation between the amount of lignin as a function of total carbon and sorption of pesticides. Dell et al. (1994) reported sorption coefficients 7 to 12 times higher in thatch than in soil. Sorption may limit degradation in the thatch due its high lignin content. The turfgrass leaf differs in that it has not undergone degradation and has a lower percentage of lignin. One objective of our work is to determine the sorption properties of the turfgrass leaf. We hypothesize that the biochemical-physical structure of the leaf controls the initial sorption of fungicides. Previous work in our lab Frederick, (1994) has shown accelerated degradation of vinclozolin after multiple field applications throughout a summer. Fungicide in solution could stimulate the development of an adapted microbial population resulting in the increased degradation found on the turfgrass leaf. We hypothesize that a microbial population capable of rapid transformation of the fungicide develop on the leaf surface. This population is derived from the general soil population.

One significant sink for pesticides applied to turfgrass is clippings. Stahnke et al. (1991) showed that as much as 95% of applied pendimethalin was retained by the grass canopy. Frederick, et al. (1994) found that in soil the limits of detection of chloroneb were reached after 42 days. Grass clipping, however, retained over 50% of chloroneb initially applied at the termination of the 60 day experiment. Greens are generally mown daily to produce the manicured playing surface that golfers demand, which results in large quantities of clippings.

This project consists of three tasks. Task one is to describe sorption coefficients for fungicides in the turf canopy. We hypothesize that the biochemical-physical structure of the leaf controls the initial sorption of fungicides. Fungicide sorption to the turfgrass leaf is a significant area of chemical retention and controls many subsequent reactions. Task one includes both a laboratory and a field study. In the lab sorption isotherms will be constructed for *Penncross* creeping bentgrass clippings at five fungicide concentrations supplemented with radiolabeled material. Data will be fit to the Fruenlich equation to calculate sorption coefficients.

$$X_m = K_f C_e^n$$

Here X_m = amount of pesticide sorbed (Mmol/Kg), C_e = pesticide concentration at equilibrium (Mmol/L), and K_f and n are constants that indicate the type and strength of the sorption reaction. The field study is described below. The current work has focused on task one. Laboratory methods for all tasks have been developed.

The objective of task two is to determine the importance of microbial populations in controlling the degradation of fungicides on the turfgrass leaf surface. The first hypothesis states that close contact between the soil surface and the turf canopy allows for a microbial population to develop on the leaf similar to that found in the soil. Degradation studies will be performed with radiolabeled fungicides on sterile and non-sterile clippings. Previously untreated clippings will be incubated in biometer flasks and evolved $^{14}C\text{-}CO_2$ will be trapped. At the termination of the experiment the clippings will be fractionated into water soluble and non-polar residues determining the amount of bound materials. CO_2 evolution data will be fit to kinetic models to quantitatively asses the differences in treatments. (Assaf and Turco, 1994) The second hypothesis states that repeated application of fungicide to the plant canopy can result in the development of a microbial population capable of rapid degradation and transformation of fungicides. To test this, a study similar to hypothesis one will be conducted with turfgrass clippings taken from plots that have had fungicide applied either zero, two, or eight times in one season.

Task three works towards characterizing the long term fate of fungicides that reach the thatch and soil environment. We hypothesize that long incubation times will lead to significant degradation and incorporation of portions of the fungicide into the thatch and soil. This covalent reaction will make the return of the chemicals to the soil solution unlikely. We will document the extent of microbial degradation of fungicides using radiolabeled chemicals and procedures similar to task two. This will address concerns about fungicide loading and will provide a framework to interpret degradation on the turfgrass leaf.

Objective:
The objective of the current work, task one, is to determine the character of the turfgrass leaf as a sorption material for fungicide.

Hypothesis:
The biochemical-physical structure of the leaf controls the initial sorption of fungicides. Fungicide sorption to the turfgrass leaf is a significant area of chemical retention and controls many subsequent reactions.

Materials and Methods:
Four fungicides which represent four families of chemicals are being used in this project: chlorothalonil (Daconil 2787), metalaxyl (Subdue), triadimefon (Bayleton) and vinclozolin (Curalan). The physical properties of these fungicides are given in Table 1.

The field study was performed at the Purdue University Agronomy Research Farm, West Lafayette, Indiana on *Penncross* creeping bentgrass growing in Chalmers silty clay loam soil with 4.7% organic matter, 26% clay and pH of 7.1. Three four week experiments were performed on 20 plots 1.5 m by 3.0 m with 0.5 m borders. The plots were arranged in a randomized block design with four replications for each fungicide and a control. Fungicides were applied to the plots on 12 May (experiment one) and 25 August (experiment two) 1995. Data from an experiment initiated on 16 June were deemed invalid due to an analysis problem and not used. Fungicides were delivered at the equivalent of 1629.14 L ha^{-1} (4 gallons / 1000 ft^2) using a backpack CO_2 sprayer. Metalaxyl was applied at 1.53 kg a.i. ha^{-1} (2 fl. oz. / 1000 ft^2), triadimefon at 1.51 kg a.i. ha^{-1} (2 dry oz. / 1000 ft^2), chlorothalonil at 17.51 kg a.i. ha^{-1} (11 fl. oz. / 1000 ft^2), and vinclozolin at 3.08 kg a.i. ha^{-1} (2 dry oz. / 1000 ft^2).

The highest recommended fungicide rates were used to mimic a plausible scenario. The actual amount of fungicide delivered to the plots was determined by placing three or four glass petri dishes, 9 cm in diameter, on each plot during chemical application (Day 0). Fungicide was extracted off the plates by rinsing three times with acetonitrile and filtering with 25 mm nylon Titan® HPLC syringe filters (0.45 µm). The fungicide solution was then dried under N_2 gas and resuspended in 5.0 mL or 1.0 mL of acetonitrile. Fungicide was also extracted off a fourth plate (stored at 4^0C) from each plot of experiment two and resuspended in 10.0 mL. This second extraction was performed because initial fungicide concentrations tended to exceed our detection capabilities. The amount of fungicide applied to each plot was then calculated by determining the average amount of fungicide collected on the plates and scaling it to the amount applied to the whole plot.

Plots were mown at 0.64 cm (1/4 inch) twice a week for four weeks after each fungicide application with a walk mower. In all experiments fungicides were applied on Fridays. Mowings occurred on Mondays and Thursdays. Plots were mown twice a week to allow enough growth to meet the clipping weight demands of the laboratory procedures. Experiment two had an additional mowing eight hours after fungicide application. After each plot was mown, the mower basket was emptied and recoverable clipping were transferred into preweighed sterile 710 mL Whirlpak® sample bags. Clippings (about 1 cm in length) were refrigerated in 400 mL beakers covered with cheesecloth to slow decomposition until analysis. All initial extractions were done within 48 hours of mowing. The bags of clippings were weighed and a subsample was removed and dried at 60^0C for 24 hours to determine moisture content. Three subsamples of approximately one gram (wet clippings) were weighed into 50 mL Teflon centrifuge tubes from each of the four replications. Fungicides were extracted by shaking clippings in 7 mL hexane for one hour. Extracts were poured through syringe filters into glass test tubes (20 x 150 mm). Clippings were rinsed three times with 3 mL of hexane and rinseate was combined. Hexane was chosen because it exhibited both a high fungicide extraction efficiency and a low chlorophyll extraction efficiency relative to other solvents tested. Hexane was then removed with a stream of N_2 gas and the residue was resuspended in 1 mL of acetonitrile.

For experiment two, stored clippings from mowing one (eight hours after fungicide application) were reextracted and resuspended in 7 mL acetonitrile.

Samples were analyzed for fungicide concentration on a Varian model 5000 liquid chromatograph using a Spherisorb 10 ODS 2 (250 by 4.6 mm) column. Mobile phase consisted of 75:25 acetonitrile:water with a flow rate of 1.0 mL min^{-1}. Chemicals were detected using a bilson Holochrome UV/VIS detector (220 nm). Fungicide concentrations were quantified against external standards of metalaxyl (purity: 99%), triadimefon (purity: 99%), chlorothalonil (purity: 98%), and vinclozolin (purity: 99%) (Chem Service, West Chester, PA). Method blanks were tested periodically throughout the experiment. All solvents were HPLC grade.

Concentration values from three subsamples from four replications were averaged to give one value for fungicide concentration for each sampling plot.

Results and Discussion:
Rapid loss of fungicide was clearly shown in turfgrass clippings from creeping bentgrass plots. Table 2 lists first order decay constants and half lives of the four fungicides from the two field experiments. Decay constants, k (day^{-1}), are taken from the equation $X = A\ exp^{-kt}$ where X = concentration ($\mu g\ g^{-1}$) of fungicide on clippings at time t, A = concentration ($\mu g\ g^{-1}$) of fungicide detected at first mowing, and t = time (days). K values are the slope of graphs in figure 1. The half life of extractable fungicide was calculated from $t_{1/2} = \ln 2 / k$. Perceived variations (Figure 1) in the amount of concentration data results from the fungicide concentrations dropping below detection limits and being evaluated as zero.

Concentrations of metalaxyl, triadimefon, and vinclozolin fell below detectable limits within 13, 10, and 17 days respectively. In both experiments chlorothalonil was detectable at low levels from clippings collected at the termination of the experiment on day 27. This correlates with the fungicides' design. Chlorothalonil is a contact fungicide while metalaxyl, triadimefon, and vinclozolin are systemic fungicides. The half life of extractable parent fungicides in the field ranged from 0.89 days for triadimefon to 5.84 days for metalaxyl. These values are slightly lower than those calculated from decay constants reported by Frederick, et al. (1994) in a laboratory degradation study which included triadimefon and vinclozolin. This expected result is due to environmental effects such as direct exposure to sunlight, rain, and continual contact with the diverse soil microbial population. Fungicide half lives had no correlation with either water solubility or Log K_{ow}. Rapid loss of parent fungicide from turfgrass clippings indicates that dissipation in the field is not prevented by sorption. The next phase of this project will be to determine sorption coefficients of the fungicides on turfgrass leaves.

The average peak fungicide concentrations on turfgrass clippings observed either at the first or second sampling were 38.16 $\mu g\ g^{-1}$ (dry clipping weight), 112.46 $\mu g\ g^{-1}$, 549.65 $\mu g\ g^{-1}$ and 370.35 $\mu g\ g^{-1}$ for metalaxyl, triadimefon, chlorothalonil and vinclozolin. Fungicide concentrations extracted from the initial mowings were highly variable. Frederick (1994) reported similar variability when conducting degradation studies on vinclozolin, triadimefon, and chloroneb. One possible explanation for higher concentrations of fungicides extracted from turfgrass clippings collected at the second mowing is leaf orientation. The upright nature of the creeping bentgrass leaf may cause more fungicide to reach the lower part of the plant at the time of application. This portion of the leaf would be collected as clippings during the second mowing after a period of growth. We are planning to determine if there is a correlation between the dry weight of clippings removed from the whole plot at each mowing and fungicide concentration.

The first mowing in experiment one occurred three days after fungicide application. Questions were raised about the validity of estimating initial chemical concentrations extracted off clippings that were exposed to environmental conditions for three days. In the second experiment, turf plots were mown eight hours after fungicide application to determine the magnitude of this effect. The initial concentrations and variability were similar between the two experiments. Explanations include the possibility that time in the field has little effect or that higher concentrations in the turfgrass environment as a whole eight hours after spraying is causing the tops of the leaves to have similar concentrations as the lower portions subject to greater exposure and mown three days later.

Table three shows that application efficiencies of fungicides to the plots varied greatly between chemicals but remained relatively consistent over both experiments approximately three months apart. Percent fungicide applied was calculated by taking the amount of fungicide applied to the total area, a number experimentally derived from the concentration of fungicide extracted off of the petri dishes, divided by the amount of fungicide theoretically applied in accordance with the recommended rates. The efficiency of chlorothalonil delivery, 7.7% and 9.4% in experiments one and two is unexpectedly low. Experiments intended to isolate potential lab induced errors are planned. Sorption of chlorothalonil to plastic and rubber portions of the spray apparatus is suspected.

The planned degradation studies and sorption isotherms necessitate ^{14}C-CO_2 labeled fungicides. Metalaxyl, triadimefon, and vinclozolin have been graciously donated by Ciba-Geigy, Bayer Corporation, and BASF Corporation. We are attempting to have radiolabeled chlorothalonil made although the success of the organic synthesis is currently unclear.

In conclusion, when fungicides are applied at recommended rates pesticide loading is unlikely on the live turfgrass plant. Initial concentrations of fungicides on clipping were high but dissipated rapidly. The average half life of all chemicals tested was under five days. Care in handling and disposing of turfgrass clippings may be warranted for short periods of time after fungicide application.

Tables and Figures:

Table 1. Physical Properties of Fungicides†

Fungicide	Molecular weight	Melting temperature	Water solubility	Log K_{ow}‡
	g	°C	μmol mL^{-1}	
metalaxyl	279.35	71	25.41	N/A
triadimefon	293.8	82	885.1	2.39
chlorothalonil	265.9	250	2.3	2.69
vinclozolin	286.1	108	3495.0	3.01

† From Agrochemicals Handbook (Hartley and Kidd, 1987) or Merck Index (Windholz, 1983)

‡ From Dell et al. (1994) Values were experimentally determined.

Table 2. Rates of loss of parent fungicide on creeping bentgrass clippings.

Experiment	Fungicide	k (day^{-1})	r^2	1/2 - Life† (day)	Average‡ 1/2 - Life (day)
1	metalaxyl	0.200	0.919	3.465	4.350
2	metalaxyl	0.119	0.604	5.841	
1	triadimefon	0.503	0.607	1.379	1.076
2	triadimefon	0.785	0.835	0.883	
1	chlorothalonil	0.251	0.951	2.763	3.163
2	chlorothalonil	0.187	0.688	3.699	
1	vinclozolin	0.273	0.841	2.535	2.629
2	vinclozolin	0.254	0.798	2.729	

†Chemical has dissipated to half the concentration extracted from clippings after first mowing.

‡Average of experiments one and two.

Table 3. Fungicide application efficiencies.

Fungicide	Experiment 1	Experiment 2
metalaxyl	55.8 %	65.4 %
triadimefon	91.2 %	90.6 %
chlorothalonil	7.7 %	9.4 %
vinclozolin	55.2 %	74.2 %

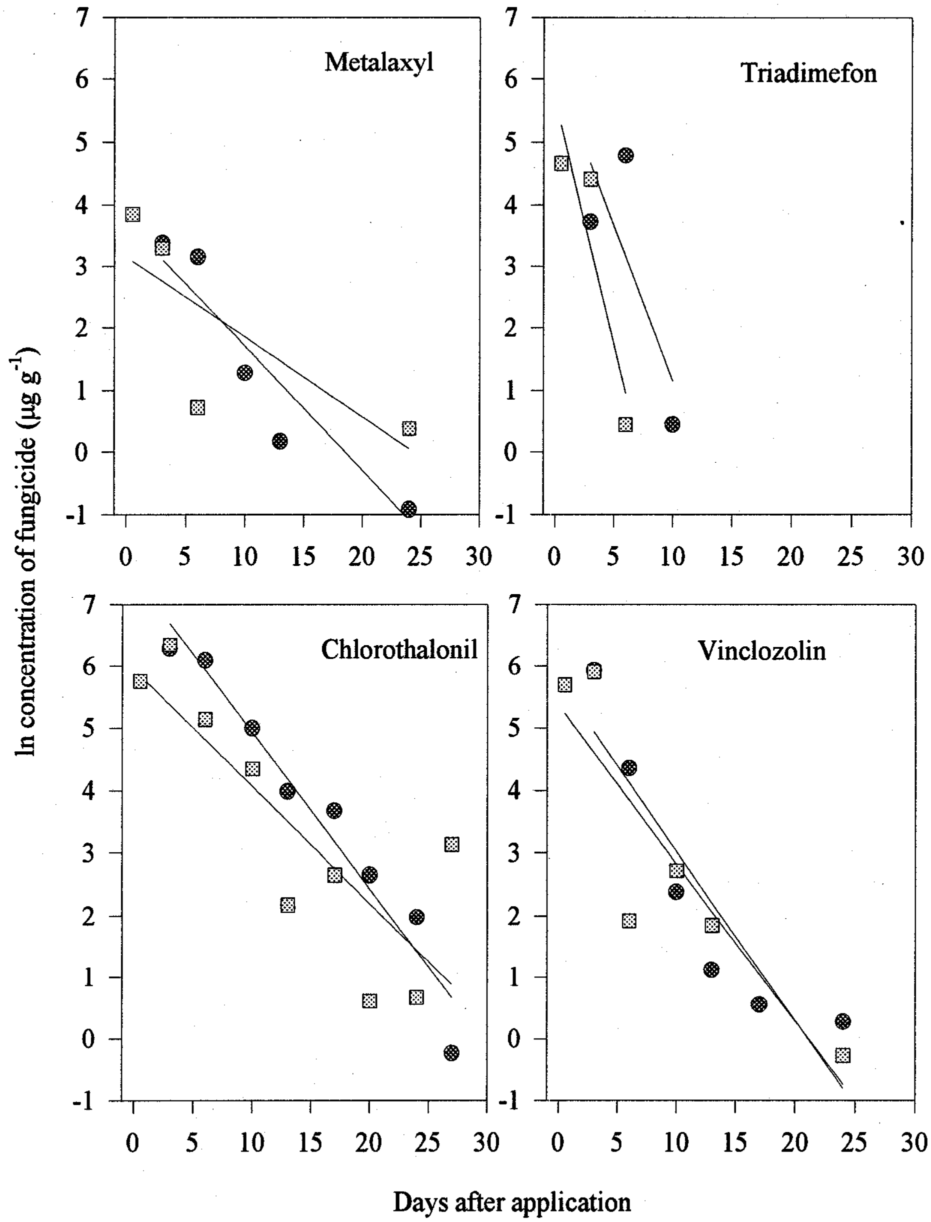

Figure 1. Concentration of four fungicides as a function of time in well maintained creeping bentgrass. Each point on the graphs is an average of three subsamples from each of four replicates.

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