Degradation of Fungicides in Turfgrass Systems

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Introduction:

The study of microbial degradation of fungicides in turfgrass systems is important in order to understand the complete environmental fate of xenobiotic materials. This ongoing project investigates degradation as it may occur in the turfgrass canopy.

The turf leaf surface is an important sink for fungicides. It has been shown that a dense turf canopy can intercept over 95% of applied pesticides. Thus the turf canopy is a potentially important site for the degradation of xenobiotic materials including fungicides.

A previous progress report (May, 1997) highlighted the following results of this study which was initiated on June 3, 1996:

Review of last progress report (November,1996-May, 1997):

Part 1. Fungicide dissipation results:

The concentration of fungicides in the extracted samples from the turf canopy was being determined using gas chromatography. Some manipulation of the samples was required in order to keep the concentrations of the detected fungicides within the limits of the standard solution concentrations. The first several samples that were going to be analyzed were those from the first and fifth two-week application cycles. These samples were chosen because they represent the first and fifth applications of fungicides that are applied every two weeks and the first and second applications of fungicides applied every eight weeks. It was thought that differences in degradation rates attributable to application frequency would most likely be observed at these sampling dates.

Part 2. Fungicide degradation results:

Oxidation of the degraded clipping samples showed that the majority (75%) of the applied radiolabeled fungicide was bound in the turfgrass leaves and probably unavailable for microbial degradation. The exact mechanism by which the fungicide became bound to the turf clippings was not clear. Determining the amount of residual parent material and metabolites formed was conducted using thin layer chromatography of ethyl acetate extracts of the degraded clippings. Because of the binding of the fungicide to the clippings, the amounts extracted from the leaf tissue was low. This kept the amount of radioactivity in the extracts spotted onto thin layer chromatography plates at a low level. Thus, plate counter-detection was impaired, and to a large extent, not useful in determining the presence of metabolite/parent compound- relationships. Few of the plate counter tracer graphs showed adequate amounts of activity that would allow for accurate quantification. This information, however, supports our conclusion that little of the fungicide is available in the environment. Most of the material appears to be retained in the leaf tissue.

Progress since May, 1997:

The following discussion will update the research that has been conducted since the previous progress report was submitted.

Methods:

Part 1. Fungicide dissipation:

The fungicide dissipation data acquisition is nearing completion. To date, approximately 1850 of the 2000 total samples have been analyzed. Methods for the determination of the concentration of fungicides in the extracts have been previously described (November1996 progress report).

Part 2. Fungicide degradation:

A.) Quantifying the environmental fate of fungicides:
Throughout this research project, we have discovered that fungicides and their metabolites can encounter three fates in the turf canopy:

1.) microbial mineralization through which microorganisms metabolize the fungicide to CO_2,
2.) binding to the leaf surface which renders the fungicide/metabolite unavailable for biological conversion,
3.) fungicide/metabolite remains in the unbound and free pool, available for biological transformation.

In order to quantify the amount of fungicide/transformation product that was present in the three possible phases (mineralized, bound to leaf material, and free/available), a mass-balance of the amount of material in each phase was determined (Table 1). The results of the mass-balance give a relative indication of the absolute environmental fate of each of the applied fungicides.

B.) Short-term sorptive properties of fungicides and leaf tissue:
It has already been determined that very little microbial mineralization of iprodione, metalaxyl, and triadimefon takes place in the turfgrass canopy. The poor environment that the canopy affords to microbial activity has been postulated as the cause for this inactivity. Thus, the lack of microbial mineralization in the canopy results in the persistence of parent fungicide material on or in the leaf.

In order to assess the extent of the fungicide parent material becoming bound to leaf tissue, a study was carried out to examine the short-term binding properties of fungicides to leaf

surfaces. Metalaxyl and triadimefon were both used in the study, however, due to the adsorptive nature of iprodione, vinclozilin (dicarboximide family) was used in its place to facilitate easier GC analysis. Applications of the fungicides were made to creeping bentgrass plots similar to those used in the degradation study. The fungicides were applied at 2 oz of formulation/1000 ft^2 in 1 L of water. Clippings were harvested from the plots at 4, 6, 12, 24, and 48 h after the fungicide application. Clipping removal was done such that six separate sections of each plot yielded one sample of clippings corresponding to each of the six sampling times. In this collection format, the sample of clippings collected at 48 hours was exposed to the fungicide for 24 h longer than the sample taken at 24h, and for 36 h longer than sample taken at 12 h, and so on. Clipping samples were transported to the laboratory on dry ice, weighed, and sub-sampled for moisture content and fungicide extraction. Extractions were performed with the same method as the dissipation study samples, as was the GC analysis.

Results:

Part 1: Fungicide dissipation in the turfgrass canopy

Analysis of the extracted fungicides showed that the dissipation rates on plots receiving fungicide applications were similar, regardless of frequency of application. Figure 1 shows the averages of all eight sampling cycles for iprodione, metalaxyl, and triadimefon. Although small changes in the dissipation rates were evident among the eight sampling cycles, the general trend of all sampling cycles was not conclusive support for the concept of enhanced biodegradation. The similarity of the dissipation curves suggests that little change in the loss mechanism of the fungicides is taking place.

Part 2: Fungicide degradation:

A.) Quantifying the environmental fate of fungicides:

An example of the mass-balance of fungicide fates is depicted in Table 1. Pre-extraction oxidation is used to determine the amount of radiolabel (^{14}C-labeled fungicide) that is extractable from the leaf surface of the degraded clippings. After shaking with ethyl acetate to remove the extractable fungicide, a post-extraction oxidation was performed to determine the amount of fungicide that is non-extractable and thus not available for microbial degradation. Based on pre and post-oxidations, results showed that, on average, only 36% of the fungicide/metabolites were extractable. This means that almost two-thirds of the applied fungicide remained bound to the leaf surface, unavailable for microbial degradation or loss into the environment. The poor extraction efficiency in addition to a presumed complexing of the fungicides with extracted plant material, such as chlorophyll, is assumed to have resulted in the limited detection and quantification of metabolites using thin layer chromatography.

B.) Short-term sorptive properties of fungicides and leaf tissue:

Results of the 48-hour sorption study are displayed in Figure 2. The two-phased nature of fungicide extractability over time suggests variability in adsorption to the leaf surface. The extractability of the three fungicides over time remained relatively unchanged for the first 24 h. The reason for the variation in recoverable fungicide for the first 24-h period is unknown. It is possible that either diurnal cycles of metabolism unique to the plant are at work or simply uncontrollable sampling error occurred. Changes do however occur in the extractable amounts of all three fungicides after the 24-hour sampling. In the 24 hour period between the 24-hour sampling and the 48-hour sampling, the extractable amounts of triadimefon, metalaxyl, and vinclozilin decreased by 97, 65, and 80% of applied amounts, respectively. Irreversible binding to the leaf surface or uptake into the leaf is thought to drive this phenomenon. The drastic immobilization of fungicide can partially explain the limited microbial mineralization that resulted from the degradation study and also the inefficient extractions of the degraded clipping samples. Thus the highly sorptive nature of turfgrass leaf tissue is important in sequestering iprodione, metalaxyl, and triadimefon and limiting the amount of free chemical in the environment. Dilution of the fungicides by the growth of the plant between samplings was determined not to effect the concentrations of the extracted fungicides. The average clipping dry weight sampled off of each plot was consistent throughout the 48-hour experiment (Figure 3).

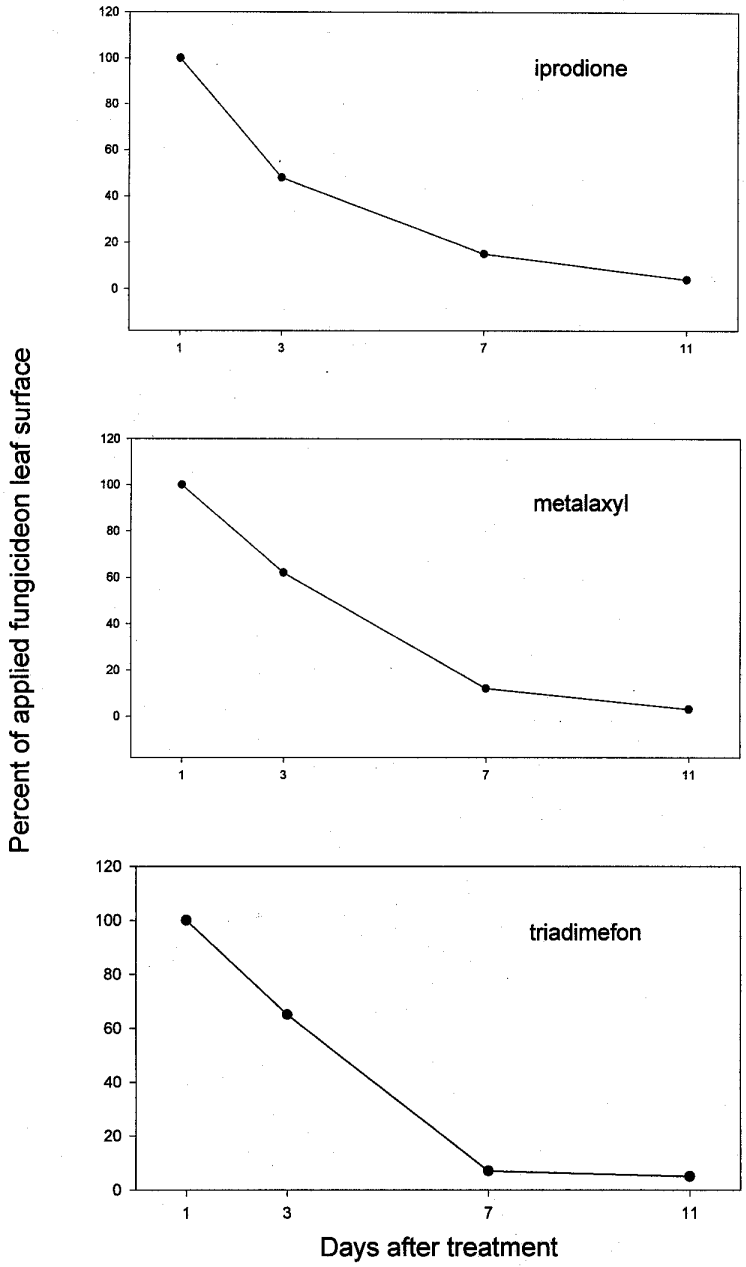

Fig.1. Averaged dissipation of iprodione, metalaxyl, and triadimefon over 8 application cycles.

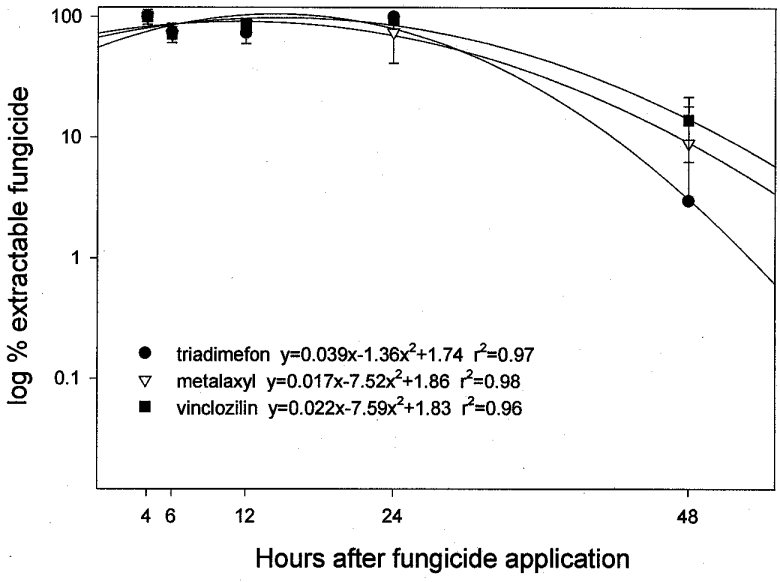

Fig.2. Percent of extractable fungicide at 4, 6, 12, 24, and 48 hours after application.

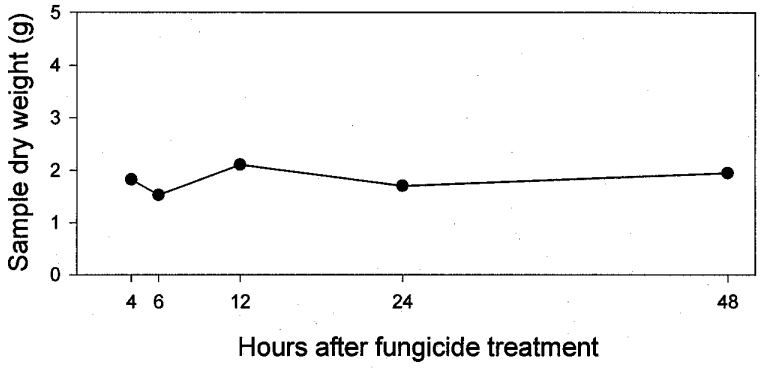

Fig. 3. Average dry weight of harvested clippings at 4, 6, 12, 24, and 48 hours after fungicide application

Table 1. Sampling cycle 5 recovery of ^{14}C-fungicide from turfgrass clippings.

Fungicide	Pre-extraction§	Post-extraction (Non-extractable)	Extractable	Mineralized
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Triadimefon	100	56	42	2
Metalaxyl	100	67	30	3
Iprodione	100	62	33	5

§ Data normalized to reflect pre-extraction as 100% of total fungicide amount.