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Degradation of Chloroneb, Triadimefon, and Vinclozolin in Soil, Thatch, and Grass Clippings

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It is a common practice to use fungicides as a turfgrass management tool on golf courses. Commonly, putting greens, tees, and to a lesser extent, fairways are treated with fungicides to control a wide number of fungal pathogens. Turfgrass management in the golf industry generally also includes daily cutting of putting greens and tri-weekly cutting of fairways resulting in an accumulation of grass clippings. Often these materials are combined with leaves, composted and then used as a planting mulch. A significant portion of the clippings may be spread directly on the roughs. These practices reduce the amount of solid waste sent to landfills, recycle plant nutrients and provide ground cover. However, considerations of the fate of pesticides applied to turf and then transferred to other locations by way of the grass clippings have been limited. It is unclear to what extent pesticides undergo degradation in clippings or during composting. Given that a significant portion of the lawn and turf industry's waste stream is composed of clippings, a clearer understanding of the behavior of chemicals in decaying clippings is needed.

Research has been reported that describes the fate of insecticides and herbicides in soil and thatch (Niemczyk and Chapman 1987; Niemczyk and Krueger. 1987; Weber 1990). As indicated by Walker et al. (1986), little work is available on the general fate of fungicides in soil and turf, and even less work has been reported on fungicide degradation in grass clippings. The objective of our study was to use a laboratory simulation to analyze pesticide residues and establish the patterns of degradation of chloroneb (1,4-dichloro-2,5-dimethoxybenzene), triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butanone), and vinclozolin ((RS)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione) in soil, thatch, and grass clippings.

MATERIALS AND METHODS

Turf and soil samples were collected from a Kentucky bluegrass turf plot grown on Chalmers silty clay loam soil at the Purdue Agronomy Research Center, West

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Lafayette, Indiana. The plot had no history of chloroneb, triadimefon, or vinclozolin use. Twelve soil and thatch samples were collected from the plot area with a cup cutter (11.4 cm diameter). The living grass was cut from the top edge of the thatch layer with scissors and discarded. The soil was separated from the thatch and broken apart by hand, discarding any grass, roots or stones. The thatch was washed with distilled water through a series of sieves (4760 and 177 microns). Both the soil and the thatch were kept moist prior to pesticide application. Grass clippings (2cm in length) were obtained from the baskets on a Toro Greens Master (model GM 3000) lawn mower used to mow the plot.

The fungicides, chloroneb (purity: 99.9%), triadimefon (purity: 99%), and vinclozolin (purity: 99%) (ChemService, West Chester, PA), were applied to the soil, thatch, and grass clippings as individual chemicals and together in a mixture at rates equivalent to those used with the individual chemicals. Rates of 5.7 $\mu g\ g^{-1}$, 1.4 $\mu g\ g^{-1}$, and 2.0 $\mu g\ g^{-1}$ for chloroneb, triadimefon, and vinclozolin were applied to moist soil. The highest recommended field application rate of 8.0 $\mu g\ g^{-1}$ for chloroneb and 2.7 $\mu g\ g^{-1}$ for triadimefon and vinclozolin was used for the thatch and grass clippings. These fungicide application rates are typical of field application levels. The higher rate was used on the thatch and grass clippings to ensure an adequate pesticide recovery and to mimic an extreme field application. To apply the fungicides, soil, thatch, or clipping samples were spread in a thin layer on the bottom of 20.3 cm × 25.4 cm pyrex dishes and the fungicides, dissolved in ethyl acetate, were applied with a disposable pipette. The materials were thoroughly mixed and the ethyl acetate was allowed to evaporate. Water was added to return the samples to a moisture equal to the starting point of 26%, 76%, and 75% for the soil, thatch, and grass clippings, respectively. The soil and thatch samples were incubated in plastic resealable bags while the grass clippings were incubated in 600 mL glass beakers covered with cheesecloth to allow aeration and prevent rapid decomposition. The treatments were replicated three times, incubated at room temperature (25°C) and kept at a constant initial moisture. Moisture levels were evaluated by periodically drying samples and determining the water content on a dry weight basis for the soil and a wet weight basis for the thatch and clippings.

Three subsamples from each of three replication were taken immediately after fungicide application (Time 0). Subsamples were taken from the soil environment (1 g) weekly for seven weeks and from the thatch (1 g) and grass clipping treatments (0.5 g) weekly over the eight week incubation period. The nine replicate values for each matrix were then averaged to give one value for each sampling time. The fungicide was extracted by shaking each matrix with 5 mL of iso-octane for one hour. Approximately 1 mL of solution was filtered directly into 2 mL gas chromatograph (GC) vials using 25 mm nylon Titan® HPLC syringe filters (0.45 µm). The samples were stored at -20°C unless immediately analyzed on the gas chromatograph (GC). A Hewlett Packard 5890 GC equipped with an electron capture detector (ECD) in the splitless mode was used. The injector

temperature was 150°C, and the detector temperature was 300°C. Two µL of the extract containing the chemicals was injected and separated with a PTEM-5 fused silica capillary column (0.25 µm x 30 m) (Supelco Inc., Bellefonte, PA) using helium as a carrier gas. The oven temperature was 70°C for 0.5 min, then increased at 15°C min^{-1} to 175°C, held for 1 min, and then increased at 10°C min^{-1} to 250°C and held for 8 min. Total run time was 24 minutes. Fungicide concentrations were quantified against external standards and data was aquired using a Hewlett Packard 3396 integrator. The lower detection limit was 0.064 µg mL^{-1} for triadimefon and 0.0128 µg mL^{-1} for chloroneb and vinclozolin. Method blanks were tested at the beginning of the experiment and showed no interfering peaks at the retention times for the compounds of interest.

RESULTS AND DISCUSSION

Degradation of the three fungicides was observed in all matrices. Tables 1a and 1b list first-order decay rate constants, k (day^{-1}), for each system. These values were

Table 1a. Decay rate constants, k, for individually applied fungicides

Treatment	Fungicide	Initial recovery (µg g^{-1})	k (day^{-1})	Standard error for k
Soil	Chloroneb	5.53	0.26	0.04
	Triadimefon	2.23	0.16	0.04
	Vinclozolin	1.89	0.86	0.27
Thatch	Chloroneb	7.83*	0.08	0.01
	Triadimefon	5.07	0.08	0.01
	Vinclozolin	5.60*	0.09	0.01
Grass clippings	Chloroneb	6.69	0.01	0.01
	Triadimefon	11.48	0.35	0.10
	Vinclozolin	3.01**	0.09	0.03

Table 1b. Decay rate constants, k, for fungicides applied as mixtures

Treatment	Fungicide	Initial recovery (µg g^{-1})	k (day^{-1})	Standard error for k
Soil	Chloroneb	4.68	0.16	0.03
	Triadimefon	2.09	0.10	0.01
	Vinclozolin	1.63	0.71	0.18
Thatch	Chloroneb	7.61	0.08	0.01
	Triadimefon	5.00	0.06	0.01
	Vinclozolin	4.78	0.05	0.01
Grass clippings	Chloroneb	7.54	0.01	0.001
	Triadimefon	6.68	0.12	0.02
	Vinclozolin	2.94**	0.05	0.01

* Recovery at t = 2 days; ** Recovery at t = 3 days

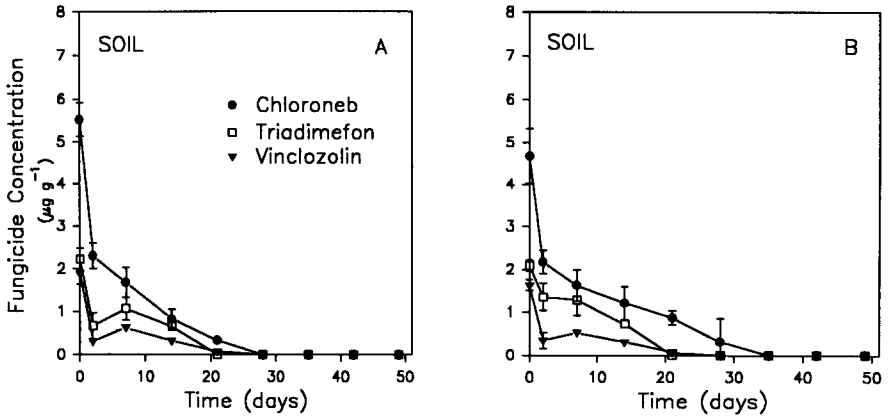

Figure 1. Loss of fungicides from soil after applications made at the average recommended application rate for each chemical when fungicides were added individually (A) and as mixture (B).

determined by the integration of $X = A\exp^{(-kt)}$ where X = concentration (μg g^{-1}) of fungicide at time t, A = initial recovery concentration (μg g^{-1}) for each chemical in each matrix, and t = time (days). Some values were calculated using the concentrations detected on day 2 or 3, as indicated, as the initial recoveries were highly variable. The variation in concentrations during the early incubation may result from uneven application of the fungicides. Repeated sampling aided in mixing and resulted in a better distribution of the fungicides with time.

Limits for detection for triadimefon and vinclozolin in soil were reached after 21 days for both the individual and mixed chemical applications. The three chemicals degraded more rapidly in soil than the other matrices (Fig 1A and B). Degradation of vinclozolin in soil was the greatest among all chemicals and all matrices. Vinclozolin was still detectable in soil on day 21, but was not detected on day 28 regardless of the treatment. When vinclozolin was applied individually the decay rate constant was 0.86 day^{-1}. When the three fungicides were applied as a mixture, the decay rate constant of vinclozolin was 0.71 day^{-1}. When chloroneb was applied individually and as a mixture the decay rate constants were calculated as 0.26 day^{-1} and 0.16 day^{-1}, respectively. The decay rate constants for triadimefon in soil fell below those of chloroneb and vinclozolin under both application treatments. When triadimefon was applied individually the decay rate constant was 0.16 day^{-1} and when it was applied as a mixture the decay rate constant was 0.10 day^{-1}. These results support the findings of Walker (Walker et al. 1986; Walker 1987a;Walker 1987b) who showed iprodione and vinclozolin applied to previously untreated soils had degradation rates reaching 50% loss in 7 to 10 days.

Decay of the fungicides in thatch followed similar degradation trends for both the individual and mixed chemical applications. In thatch, degradation of the fungicides was maximal during the first 21 days (Fig 2A and B). Limits for

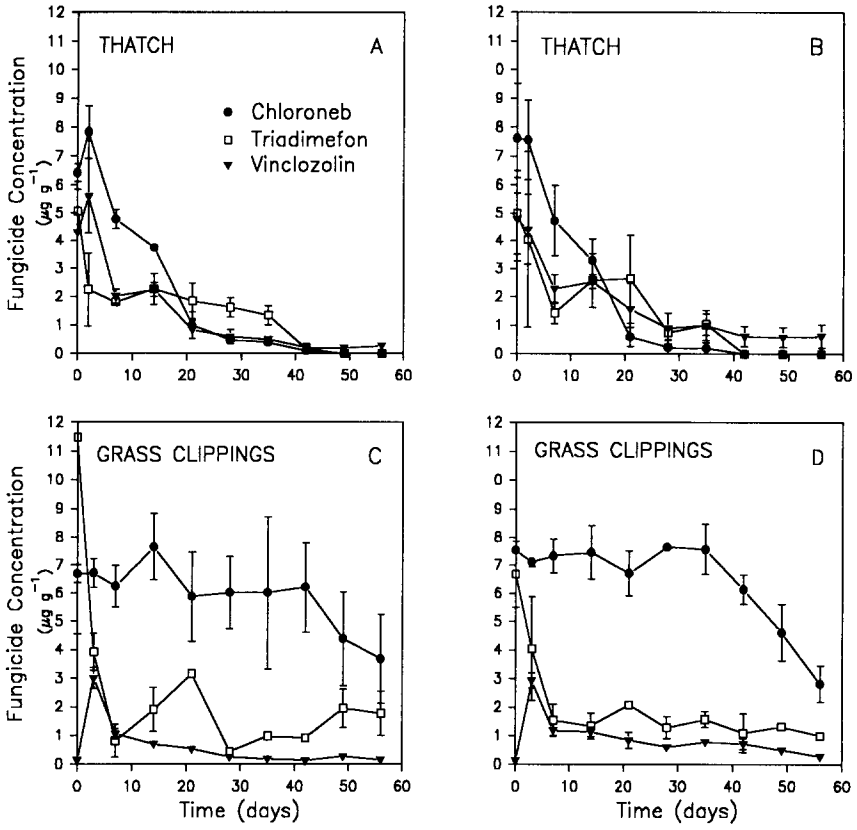

Figure 2. Loss of fungicides from thatch and grass clippings after applications made at the highest recommended application rate for each chemical when fungicides were applied individually (A and C) and as a mixture (B and D).

detection were reached after 42 days for chloroneb and triadimefon when the chemicals were applied individually. However, 0.29 µg g^{-1} of vinclozolin (6.8% of the initial concentration) was detected after 56 days. The decay rate constants calculated for fungicides applied individually in thatch were similar: 0.08 day^{-1}, 0.08 day^{-1}, and 0.09 day^{-1} for chloroneb, triadimefon, and vinclozolin respectively. When they were applied in a mixture, triadimefon and chloroneb were not detected after day 42. Vinclozolin was still detected at a concentration greater than 13% of the initial recovery in the thatch samples at the termination of the experiment. This also followed the pattern set with the individual chemicals. The decay rate constants were 0.08 day^{-1}, 0.06 day^{-1}, and 0.05 day^{-1} for chloroneb, triadimefon, and vinclozolin, respectively, when the fungicides were applied as a mixture. Our laboratory findings compare with the field findings of Niemczyk and Chapman (1987) and Niemczyk and Krueger (1987) who indicate that pesticides degrade much slower in thatch than in soil and that most of the recoverable residues are found in the thatch.

Concentrations of fungicides in the samples of grass clippings remained high over the 56 day study with respect to the soil and thatch samples. Chloroneb, when applied individually, degraded slowly over the first 42 days (Fig 2C). Concentrations began to decrease after 42 days, but greater than 50% of the initial level was recovered when the experiment was terminated. The decay rate constant for chloroneb was 0.01 day^{-1}. The pattern of loss for triadimefon when applied individually was highly variable with a decay rate constant of 0.35 day^{-1}. At the end of 56 days 15.5% of the initial triadimefon was detected. The decay pattern for vinclozolin applied individually was initially variable but after day 7 became less variable. The decay rate constant was 0.09 day^{-1}. When the fungicides were applied as a mixture, little degradation of chloroneb was observed until after 35 days (Fig 2D). Concentrations then decrease steadily to a final concentration of approximately 3 $\mu g\ g^{-1}$ (37.5% of the initial concentration). While not following a true first order decay pattern, the decay constant was again very low at 0.01 day^{-1}. In the clippings, triadimefon degraded rapidly during the first 7 days, but concentrations became nearly constant at approximately 1 $\mu g\ g^{-1}$ (14% of the initial concentration detected) for the duration of the study. Vinclozolin degraded with a rate constant of 0.05 day^{-1} to a final concentration of 0.3 $\mu g\ g^{-1}$. Patterns of loss for the mixed chemicals were nearly identical to that observed with the individual applications.

In soil, vinclozolin degradation has been shown to be biphasic. An initial rapid dissipation rate is displaced with a slower process and 50% of the original chemical remaines after 23 days (Golovleva et al. 1991). Golovleva et al. (1991) concluded that vinclozolin is stabilized by sorption to soil. Though ours was a short-term experiment (56 days), our findings concur with Golovleva et al. (1991) in that vinclozolin in soil tends to have a rapid initial degradation followed by a slower rate. Chloroneb and triadimefon also degraded rapidly over the first 7 days of incubation. However like vinclozolin, after the first week their degradation rates also decreased. These trends tend to support the idea that sorption is stabilizing the chemicals and protecting them from degradation.

Patterns of chemical loss in the clippings are similar to the patterns of loss in the soil and thatch, except for chloroneb. Data presented in figures 2C and D shows that chloroneb is stable in grass clippings under the described conditions. Data from Dell et al. (1993) suggests that sorption potential of the three chemicals is similar. They reported log k_{OW} values of 3.0, 2.3, and 2.7 for vinclozolin, triadimefon, and chloroneb, respectively. Low rates of chloroneb loss in the clippings can be partially explained by a general lack of a significantly sized microbial population able to use the chemical. This conclusion is supported by the fact that degradation of chloroneb in clippings takes over 30 days to initiate (Fig 2C and D). This lag period may represent the time needed for adaption or buildup of the microbial population able to use the chemical.

This study was a laboratory study and did not take into account the affects of

changing incubation parameters such as moisture content, temperature, or pH. These factors may be significant in affecting fungicide degradation and should be explored. Excess moisture may inhibit aerobic metabolism while low moisture content may also impede microbial function and degradation (Fogarty and Tuovinen, 1991). Since degradation generally increases as the temperature increases and this project was incubated at room temperature (25°C), the rates of degradation may be higher if the temperatures were increased. However, little information about thermophilic degradation of pesticides is available.

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