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Fate of Vinclozolin in Creeping Bentgrass Turf Under Two Application Frequencies

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The frequent use of fungicides to suppress diseases is a common practice in the management of golf course putting greens. As indicated by Walker et al. (1986), little data is available on the fate of fungicides in soil. The extent to which multiple applications of fungicides, or any chemical, impact their fate is unclear. Most studies have only examined single applications of a fungicide even though multiple applications throughout the growing season are commonly used. Projects that have dealt with multiple applications studied a series of plots over several years when fungicides were applied once each year. When multiple applications have been studied, the phenomenon of enhanced biodegradation has often been observed (Chapman et al., 1986, Martin et al., 1991, Slade et al., 1992, and Walker et al., 1986). This is a process in which microorganisms become enzymatically adapted or the population increases and pesticides are degraded more rapidly with each subsequent application. For example, the time for 50% degradation of iprodione in soil declined from 30 to 12 days after a second application and to 4 days after a third application (Walker, 1987). Microbial adaptation can increase the rate of degradation, yet it is not often studied in the turf environment where multiple applications of the same fungicide are common. As a result, the patterns of fungicide degradation expected with multiple applications across a single season are unknown.

Few pesticide fate studies determined residue concentrations after multiple applications of fungicide in all three matrices of the turf environment: soil, thatch, and leaf tissue (Sears and Chapman, 1979). Many have examined soil (Chapman et al., 1986, Walker et al., 1986, Walker, 1987), and others, just soil and thatch (Niemczyk et al., 1988, Niemczyk and Krueger, 1987, Niemczyk and Chapman, 1987). Stahnke et al. (1991) conducted a thorough experiment of the environmental fate of the herbicide pendimethalin in Kentucky bluegrass. Leaf tissue, thatch, and three increments of soil to a depth of 10 cm were sampled. The herbicide was retained in the leaf tissue and thatch, while little pendimethalin moved into the soil. Frederick, et al. (1994) conducted a laboratory study of the degradation of three fungicides, including vinclozolin, in Kentucky bluegrass clippings, thatch and soil. Vinclozolin was completely degraded after 21 days in soil. Slower degradation of the fungicide in thatch and grass clippings was observed. Vinclozolin concentration in thatch samples was 13% of applied at the end of the experiment (56 days).

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The objectives of this field study were to determine the fate of the fungicide vinclozolin ((RS)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione) in soil, thatch, and grass clippings of creeping bentgrass turf (*Agrostis palustris*) and to determine the depth of movement of the fungicide into the soil profile after multiple applications.

MATERIALS AND METHODS

This experiment was conducted across one turfgrass growing season, from 17 May to 4 October, 1993, at the Purdue Agronomy Research Center, West Lafayette, Indiana on ‘Pennlinks’ creeping bentgrass turf plots. The plots had been previously established on Chalmers silty clay loam soil (fine-silty, mixed, mesic Typic Halaquoll) with 4.7% organic matter, 26% clay, a pH of 7.1, and no history of vinclozolin use. Plots were 4 by 1.5 m and separated on all sides by 0.5 m untreated border. Treatments were replicated four times and randomized within each replication. Thatch was approximately 1 cm thick but varied across the plot area. Irrigation was applied as needed throughout the experiment to prevent moisture stress. Plot areas were mowed to a height of 1.25 cm every two days and each day that a sample set was taken. The turf was fertilized on 27 May, 17 June, and 17 September using The Andersons Tee Time 25-5-15 fertilizer at a rate based on 24.4 kg N ha^{-1} (0.5 lb N per 1000 ft^2).

A water dispersible, granule powder formulation of vinclozolin (Curalan DF®, BASF Corporation, Research Triangle Park, NC) was applied at the highest recommended curative application rate (12.3 kg $ha-^1$ or 6.2 kg a.i. $ha-^1$) to the plots. This rate was used to mimic an extreme, but normal, rate of field application. Treatments were applied with a pressurized CO_2 backpack sprayer at 221 kPa (32 psi) equipped with Tee-Jet 8003 nozzles that delivered 0.16 L per m^2 (4 gal per 1000 ft^2). The treated turf was allowed to completely dry before any water was applied. Application frequencies were: no application (control), every two weeks, and every four weeks for a period of 16 weeks. A total of 0 (control), 8 (every two weeks), and 4 (every four weeks) applications of vinclozolin were made during this period.. Plots were treated in the mornings on scheduled treatment days, and fungicide solutions were mixed just prior to being sprayed.

Soil, thatch, and grass clipping samples were taken 1, 3, and 7 days following application, and then weekly until the next application. When samples were taken, grass clippings were collected from each plot in the basket of a lawn mower. Three cores were taken with a soil probe to a depth of 4 cm from each plot. The grass was cut off the top of the core and discarded. Thatch and soil were separated, and the three subsamples from each plot were thoroughly mixed. Three subsamples were taken from each plot with a soil probe to a depth of 61 cm (2 ft) at the beginning, middle, and end of the summer to determine the depth of vinclozolin movement into the soil. Cores were separated into 7.5 cm increments, and the subsamples from each plot were well mixed. Samples were only taken from control plots every four weeks.

Samples of soil, thatch, and grass clippings (5 g, 1 g, and 1 g) respectively, were weighed into glass vials. Fungicide was extracted from each sample by shaking with 5 mL of isooctane for one hour (Frederick et al., 1994). Approximately 1 mL of solution was filtered through 25 mm, 0.45 µm nylon Titan® HPLC syringe filters (SRI, Somerset, NJ) directly into 2 mL gas chromatography vials. The samples were stored at -20°C unless immediately analyzed. A Hewlett Packard model 5890 GC equipped with an electron capture detector (300°C) and a split/splitless capillary injector (280°C) was used for analysis of the extracts. Vinclozolin was separated with a PTEM-5 fused silica capillary column (0.25 µm x 30 m, Supelco Inc., Bellefonte, PA) using helium carrier gas. The oven temperature was programmed from 90°C to 250°C at 10°C mim^{-1}, and held for 5 min. Data were acquired with a Hewlett Packard 3396 integrator, and fungicide concentrations were quantified against external standards. Method blanks were tested at the beginning of the experiment and showed no interfering peaks at the retention time for vinclozolin. Two injections were made for each extract.

Fungicide concentration was plotted against time where Day 0 was the first application date (17 May 1993). The error bars were calculated from the standard deviation of the replications (Samuels, 1989).

RESULTS AND DISCUSSION

Loss of vinclozolin was clearly seen in grass clippings and thatch of the creeping bentgrass turf plots under both application frequencies (Figure 1 and 2). In each case, vinclozolin concentrations declined between applications, and there was no vinclozolin detected at the termination of the project. The trends of vinclozolin recovery for the plots treated every two weeks were similar to those treated every four weeks. First order decay rate constants, k (day^{-1}), were calculated from:

$$X = Ae^{-kt}$$

where X was the concentration (µg g^{-1}) of fungicide at time, t (days), and A was the initial concentration recovered (µg g^{-1}) at time 0 (Table 1 and 2). These data show that the rate constants generally increased following each fungicide application. This trend was especially evident in the thatch samples.

Table 1. Decay rate constants, k, in grass clippings and thatch after each of four applications of vinclozolin.

Application #	1	2	3	4
Grass Clippings	0.14	0.29	0.19	0.38
Thatch	0.015	0.67	NR[1]	NR

[1] NR: Vinclozolin was not recovered in these samples.

Table 2. Decay rate constants, k, after each of eight applications of vinclozolin.

Application #	1	2	3	4	5	6	7	8
Grass Clippings	0.20	0.22	0.42	0.31	0.21	0.14	0.52	0.56
Thatch	0.065	0.011	0.35	2.8	NR[1]	NR	NR	NR

[1] NR: Vinclozolin was not recovered in these samples.

The average concentration of vinclozolin detected in grass clippings one day after the initial application was approximately 700 µg g^{-1} (Figure 1). The vinclozolin concentration declined between each application and over the course of the summer. The amount of vinclozolin detected one day after application became progressively lower. This trend was seen when applications were made every two or four weeks. However, there was more variability under more frequent application pattern, possibly reflecting differences in weather conditions.

Concentrations of the detectable vinclozolin residues in thatch (Figure 2) were approximately ten times lower than those detected in grass clippings. Approximately 17 $\mu g\ g^{-1}$ of vinclozolin was detected after the initial application for both 2 and 4 week application schedules. The fungicide concentration in thatch samples taken the day following treatment increased after the first two applications when the frequency was every four weeks and increased after the first four applications when the frequency was every two weeks. The maximum concentrations detected were approximately 30 $\mu g\ g^{-1}$. However, no vinclozolin was detected after the first two applications when the frequency was every four weeks and after the first four application when the frequency was every two weeks.

Concentrations of vinclozolin recovered from soil were approximately 100 times lower than those obtained in grass clippings and ten times lower than those in thatch. When applications were made at four week intervals, the concentration in soil after the initial application was less than 1 $\mu g\ g^{-1}$. After the second and third applications, the average concentrations reached approximately 4 $\mu g\ g^{-1}$ and 2 $\mu g\ g^{-1}$, respectively. Concentrations never exceeded 1 $\mu g\ g^{-1}$ when vinclozolin applications were made every two weeks. Under both application frequencies, no vinclozolin was detected after 85 days.

Vinclozolin (less that 200 $\mu g\ g^{-1}$) was detected in the grass clippings from the control plots following an application and was most likely due to drift of chemical when applications were made to target plots. Much lower concentrations (less that 5 $\mu g\ g^{-1}$) of vinclozolin were detected in the thatch from the control plots. No vinclozolin was recovered from the upper 4 cm of soil from control plots. No vinclozolin was recovered from subsurface soil samples from 4 to 61 cm from any plot.

Several factors contribute to the loss of pesticides when they are applied in the field: runoff, leaching, photodegradation, volatilization, sorption, chemical conversion, and microbial degradation. The plot area was flat and completely covered with turf; thus, losses due to runoff were minimal. Since the vinclozolin concentrations in the soil were very small and none detected passed 4 cm depth, we concluded that the fungicide did not leach through the soil profile. Air temperature data were obtained for the duration of the experiment and there was no correlation between air temperature and the dissipation rate of vinclozolin. Losses from photodegradation were also estimated to be minimal. The losses due to volatilization were estimated to be approximately 0.35% of the total amount applied. This estimation was determined by the behavior assessment model for trace organics in soil introduced by Jury et al. (1983). The default parameters of the model were used with the exception of the following: organic carbon partition coefficient, for thatch, 1.39 $m^3\,kg^{-1}$ (Dell et al., 1994); Henry's Law constant for vinclozolin, 1.17×10^{-9}; and half-life of vinclozolin, 7.7 days (Frederick et al., 1994). Because runoff, leaching, photodegradation, and volatilization were not

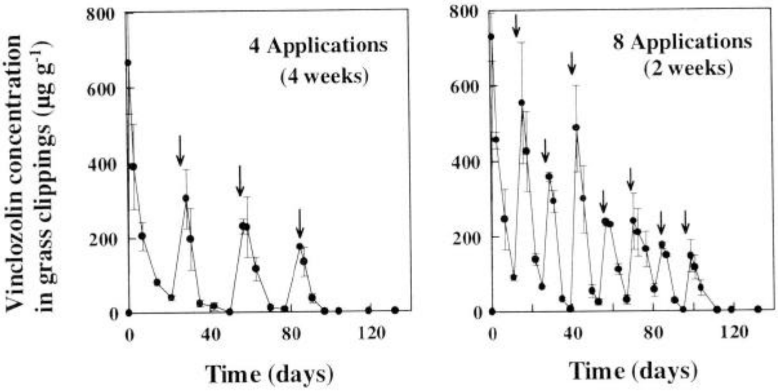

Figure 1. Vinclozolin concentrations in grass clippings removed from creeping bentgrass plots. Arrows indicate application dates. Clippings were recovered 1, 3, and 7 days following application, and then weekly until the next application. Error bars represent the standard deviation of the sample replications.

significant factors in the degradation of vinclozolin, we concluded that microbial degradation was the primary cause of loss of chemical.

The high concentration of vinclozolin detected in the grass clippings was attributed to the vinclozolin being directly applied onto the leaf tissue and the clippings recovered shortly thereafter. The rapid decline in concentration as compared to the initial application can be explained by enhanced biodegradation of vinclozolin by an adapted population of phyllosphere residents. This is a process in which microorganisms became adapted in degrading vinclozolin and are able to use the chemical more readily with each successive application. Enhanced biodegradation of vinclozolin has been shown to occur in soil (Slade et al., 1992 and Walker et al., 1986) and we feel it also occurs in the phyllosphere of turfgrass. Walker (1987) showed that the time for 50% degradation of vinclozolin in soil was 30, 22, and 7 days after the first, second, and third applications.. Our data suggests that the adapted population became fully established after 40 days. Work by Niemczyk (1987) showed development of an enhanced population in turfgrass thatch. Our data show similar trends. The increase in rate constants after repeated application of vinclozolin (Table 1 and 2) was especially evident in the thatch samples. The extension of the population to the leaf surfaces is possible given the short mowing height of turfgrass and the intimate contact between the soil and turfgrass. Foot traffic, irrigation and rainfall may have allowed the transfer of the population to the leaf surfaces. Since most of the vinclozolin stays on the leaf tissue of the turf, precautions should be taken when handling these clippings, especially for a few days after application.

While most of the fungicide was retained on the leaf tissue of the plants, a small amount of vinclozolin was detected in the thatch layer of the turf plots. This indicates movement of vinclozolin through the grass canopy and into the thatch. The same pattern of enhanced degradation of vinclozolin seen in the grass clippings was also

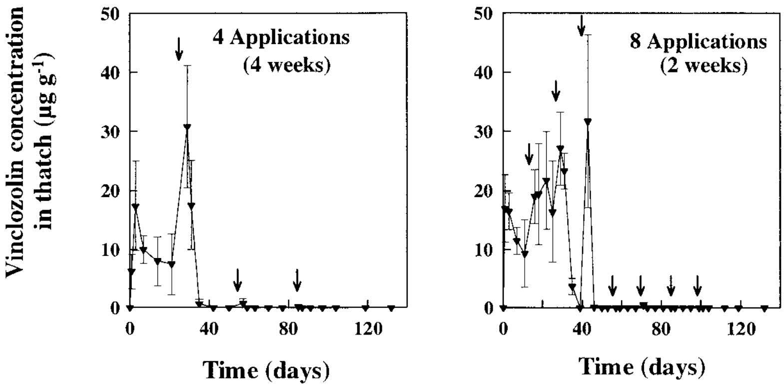

Figure 2. Vinclozolin concentrations in thatch removed from creeping bentgrass plots. Arrows indicate application dates. Thatch was recovered 1, 3, and 7 days following application, and then weekly until the next application. Error bars represent the standard deviation of the sample replications.

observed in thatch. As peak concentrations of vinclozolin decreased in clippings, concentrations also decreased in the thatch.

The average vinclozolin concentrations in soil were low, indicating the fungicide is degraded or becomes bound to the lignin and organic material in the leaf tissue and thatch, allowing little leaching of the fungicide into soil. This is further supported by the lack of vinclozolin detected in the subsurface samples taken during the study period. The fungicide did not move beyond a 4 cm soil depth for either application schedule. This supports the work of Dell et al. (1994) who showed that thatch has a high capacity to adsorb fungicides, thus reducing the probability that these chemicals will move into the soil profile or offsite. The movement of insecticides through the soil profile has also been shown to be minimal (Sears and Chapman, 1979; Niemczyk and Krueger, 1987; Niemczyk, 1987).

In conclusion, vinclozolin, when applied at recommended application rates to these creeping bentgrass plots, did not move down the soil profile and was not a likely threat to water table contamination. This experiment determined the patterns of degradation for only one fungicide. Many pesticides, even other fungicides, may behave otherwise and results may differ under different field conditions. But, by learning the routes of fungicide loss, informed use-management decisions can be made.

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