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Effects of Commercial Diazinon and Imidacloprid on Microbial Urease Activity in Soil and Sod

C. W. Ingram, M. S. Coyne,* and D. W. Williams

ABSTRACT

Diazinon [*O,O*-diethyl *O*-2-isopropyl-6-methyl(pyrimidine-4-yl) phosphorothioate] and imidacloprid [1-(1-[6-chloro-3-pyridinyl]-methyl)-*N*-nitro-2-imidazolidinimine] are applied to lawns for insect control simultaneously with nitrogenous fertilizers such as urea, but their potential effect on urease activity and nitrogen availability in turfgrass management has not been evaluated. Urease activity in enzyme assays, washed cell assays, and soil slurries was examined as a function of insecticide concentration. Intact cores from field sites were used to assess the effect of insecticide application on urease activity in creeping bentgrass (*Agrostis palustris* Huds.) and bluegrass (*Poa pratensis* L.) sod. Bacterial urease from *Bacillus pasteurii* and plant urease from jack bean [*Canavalia ensiformis* (L.) DC.] were unaffected by the insecticides. Both insecticides inhibited the growth of *Proteus vulgaris*, a urease-producing bacterium, but only diazinon significantly reduced urease activity in washed cells; neither insecticide inhibited urease activity in sonicated cells. Neither diazinon nor imidacloprid inhibited urease activity in Woolper soil (fine, mixed, mesic Typic Argiudoll) slurries, but diazinon slightly inhibited urease activity in Maury soil (fine, mixed, semiactive, mesic Typic Paleudalf) slurries. Imidacloprid had no effect on urease activity in creeping bentgrass or bluegrass sod at up to 10 times the commercial application rate. Diazinon briefly, but significantly, reduced urease activity in bluegrass sod. Co-application of imidacloprid and urea appears to be benign with respect to urease activity in soil and sod. Diazinon, in contrast, appears to have a significant, short-term, inhibitory effect on the microbial urease-producing community, but that effect depends on soil type.

The turfgrass industry has grown steadily since 1945. By 1998 the USDA estimated the sale of turfgrass sod at $835 million nationally (USDA, 1998). Turfgrass acreage in a typical city is comprised of approximately 70% residential lawns and 30% public facilities such as city parks, golf courses, and educational institutes (Cockerham and Gibeault, 1985).

High quality turfgrass for home lawns and golf courses often requires extensive pest control management, and pesticides have become a major component in turfgrass management. Two significant insecticides used by the turfgrass industry are diazinon and imidacloprid (trade name: Merit). Diazinon is a nonsystemic organophosphate insecticide used to control sucking and leaf-eating insects that threaten food crops and urban landscapes (National Pesticide Telecommunications Network, 1998; USEPA, 1988, p. 247–251). Diazinon was restricted from use on golf courses and sod farms in 2000. In 2003 restrictions were extended to lawn, garden, and turf uses. Chemical manufacturers in the United States suspended diazinon production in 2003 with a phase-out from the market by December 2004 (USEPA, 2000). Imidacloprid is a systemic, chloro-nicotinyl insecticide for the control of insects including termites, white grubs, and beetles. Imidacloprid is selectively much more toxic to insects than warm-blooded animals (Buckingham et al., 1997; EXTOXNET, 1996). Imidacloprid is mainly used on golf course fairways and greens, but since 1996 it has been marketed commercially for home lawn care.

The fertility regime is another important component of turfgrass management. Urea is a widely used nitrogenous fertilizer because of its high nitrogen content (46%). Soil ureases hydrolyze urea to plant-available NH_4^+, but under less-than-ideal conditions of elevated soil pH, temperature, and low moisture content, surface-applied urea can volatilize as NH_3–N. Urea N volatilization can be eliminated when urea application is followed by either mechanical irrigation or rainfall (Bovis and Touchton, 1998), and urea application followed by irrigation is routinely used in the turfgrass industry.

Many compounds have been evaluated as urease inhibitors (Bremner and Douglas, 1971), but few meet the requirements for effectiveness at low concentration, nontoxicity, stability, and compatibility with urea application. In particular, few pesticides have been evaluated for their effect on urease inhibition or stimulation. Lethbridge and Burns (1975) observed 40 to 50% urease inhibition 60 d after applying 1000 mg L^{-1} of the organophosphate insecticides malathion, accothion, or thimet to a sandy clay loam. In contrast, Sannino and Gianfreda (2001) observed activation of urease activity in some soils treated with pesticides. Urease inhibition could be beneficial in terms of fertilizer use efficiency and reduced N volatilization, but stimulated urease activity could potentially cause less efficient fertilizer N use and greater runoff and leaching losses of fertilizer N in urban landscapes.

Diazinon and imidacloprid toxicity to the soil metazoan population, their effectiveness in controlling targeted pests, and their losses due to runoff and leaching have been previously studied (Balogh and Anderson, 1992; Eisler, 1986; Vettorazzi, 1976). Diazinon or imidacloprid are typically added to control pests in soil at the same time that urea fertilizer is applied during commercial turfgrass management, but prior studies have not evaluated how commercial use of diazinon and imidacloprid could affect urease activity or urease-

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producing organisms in turfgrass soils. Consequently, this investigation examined the effect of commercial formulations of diazinon and imidacloprid on urease activity in the turfgrass systems.

MATERIALS AND METHODS

Enzyme Assays

Jack bean (23 000 units g^{-1}) and *B. pasteurii* (490 000 units g^{-1}) urease were obtained commercially (Sigma, St. Louis, MO). A unit of activity liberates one μmol NH_3 per minute at 25°C under the assay conditions. Jack bean urease was diluted to 46 units mL^{-1} and *B. pasteurii* urease was diluted to 49 units mL^{-1} by 0.02 *M* sodium-phosphate buffer (pH 7.0) and stored at 4°C in an ice bath until use. Urea stock solution was prepared by dissolving 1.5 g urea and 25 mg bovine serum albumin (Sigma) in 50 mL of 0.75 *M* sodium-phosphate buffer (pH 7.0). A brom cresol green–methyl red indicator solution was prepared by dissolving 60 mg brom cresol green and 40 mg methyl red in 100 mL ethanol.

Diazinon was prepared by diluting an industrial formulation of Hi-Yield diazinon AG500 (Voluntary Purchasing Group, Bonham, TX) (a.i. 48%) in 0.02 *M* sodium-phosphate buffer (pH 7.0). For typical field applications the diazinon concentration is 17 g L^{-1}. Stock solutions at 17, 85, and 170 g diazinon L^{-1} were prepared. Imidacloprid stock solutions were prepared by diluting an industrial formulation of Bayer Merit 75 WP (Bayer Corp. Garden and Professional Care, Kansas City, MO) (a.i. 75%) in 0.02 *M* sodium-phosphate buffer (pH 7.0). For typical field applications the imidacloprid concentration is 1.4 g L^{-1}. Stock solutions at 1.4, 7, and 14 g imidacloprid L^{-1} were prepared.

For enzyme assays, 1.0 mL of diluted urease (4°C), 0.9 mL urea substrate, and 0.1 mL diazinon or imidacloprid were combined in 13- × 100-mm test tubes equilibrated in a 25°C water bath (Gorin et al., 1962). The final concentration of diazinon in each enzyme assay was 0, 0.85, 4.25, or 8.50 g L^{-1}. The final concentration of imidacloprid in each enzyme assay was 0, 0.07, 0.35, or 0.70 g L^{-1}. The control received 0.1 mL of 0.02 *M* buffer at 4°C. To adjust for artifacts (e.g., trace NH_3) from diazinon and imidacloprid being added to the solution, a reagent blank consisting of 1 mL of 0.02 *M* buffer, 0.9 mL urea substrate, and 0.1 mL diazinon or imidacloprid were combined in a test tube for each assay. Each treatment was replicated four times. The enzyme reactions were stopped after exactly 5 min by adding 0.1 *M* HCL. Two drops of brom cresol green–methyl red indicator were added, and the NH_3 concentration was determined by titration with additional 0.1 *M* HCL.

A completely randomized design with a one-way treatment structure was used for statistical analysis by the PROC GLM procedure of SAS (SAS Institute, 1999). An orthogonal polynomial procedure was used to investigate the trends among means.

Growth Studies

A flask containing 50 mL Christensen Urea Broth (per liter: 1.0 g peptone, 1.0 g glucose, 5.0 g NaCl, 2.0 g KH_2PO_4, 12 mg phenol red, 100 mg yeast extract, 2% urea, pH 6.9) was inoculated with *Proteus vulgaris* and incubated at 26°C in a constant temperature incubator–shaker. After 24 h of incubation, 1.0 mL of *P. vulgaris* broth culture was used to inoculate flasks containing 46.5 mL Christensen Urea Broth and 2.5 mL diazinon or imidacloprid stock solution. The final concentration of diazinon in each flask was 0, 0.85, 4.25, or 8.50 g L^{-1}. The final concentration of imidacloprid in each flask was 0, 0.07, 0.35, or 0.70 g L^{-1}. The flasks were incubated at 26°C in a constant temperature incubator–shaker for 96 h. *Proteus vulgaris* was enumerated by dilution plate count every 6 h on plate count agar (Difco, Detroit, MI).

A completely randomized design with repeated measurements using a one-way treatment structure was used to analyze this experiment.

Washed Cell Experiments

Proteus vulgaris was grown for 16 h in 50 mL Christensen Urea Broth, harvested by centrifugation for 15 min at 3500 rpm, and resuspended in 50 mL 0.02 *M* KH_2PO_4 buffer (pH 7.0). The process was repeated three times to remove extraneous growth media and extracellular urease. The washed cells were split into 25-mL aliquots. One aliquot was heat-killed by autoclaving for 15 min at 121°C.

Reaction mixtures were prepared consisting of 41.5 mL of buffer (0.003 *M* KH_2PO_4 and 0.1 *M* $MgSO_4$, pH 7.0), 5.0 mL of 2% urea in distilled H_2O, 2.5 mL of diazinon or imidacloprid stock solution, and 1.0 mL of live or heat-killed cells in 250-mL flasks. A reagent control contained 1.0 mL of buffer instead of cells. The final concentration of diazinon in each flask was 0, 0.85, 4.25, or 8.50 g L^{-1}. The final concentration of imidacloprid in each flask was 0, 0.07, 0.35, or 0.70 g L^{-1}. The flasks were incubated at 26°C in a constant temperature incubator–shaker for 8 h. At 0, 4, and 8 h, a 5.0-mL aliquot was aseptically removed from each flask with a 10-mL syringe, and forced through a sterile 0.45-μm syringe filter into a sterile screw top vial. The samples were stored at 4°C until analysis of NH_3–N by titration.

A completely randomized design using a one-way treatment structure was used for these experiments. There were three replicates of each treatment.

Sonicated Cell Experiments

Proteus vulgaris cells were prepared as described in the washed cell experiments. A 25-mL aliquot of live cells was centrifuged and resuspended in 5.0 mL of buffer. The sample was cooled in an ice-water bath, and the cell suspension was sonicated for 90 s in three separate 30 s periods. The suspension was dispensed into microcentrifuge tubes after sonication, and centrifuged in a microfuge at 10 000 rpm for 2 min. After centrifugation, the supernatant was removed and the broken cells were suspended in 25 mL of buffer. Reaction mixtures were prepared and analyzed as previously described, except that sonicated rather than live cells were used in the assays.

Soil Slurry Assays

Two soils, Maury silt loam and Woolper silty clay loam, were sampled from the surface 0 to 15 cm, sieved through a 2-mm sieve, and stored in field moist conditions (Woolper, 33% gravimetric water content; Maury, 25% gravimetric water content) at 4°C until use. The soils principally differ in organic matter content (2.6% in Maury and 3.3% in Woolper) and pH (5.7 in Maury and 4.8 in Woolper).

The assays were conducted at 26°C in a constant temperature incubator–shaker for 2 h. Five grams of field moist Woolper and Maury soil were weighed into each of 24, 50-mL disposable polypropylene centrifuge tubes and amended with 2.0 mL buffer plus urea (20 g L^{-1} urea, 0.003 *M* KH_2PO_4, 0.1 *M* $MgSO_4$, pH 7.0), 2.5 mL diazinon or imidacloprid stock solution, and 0.5 mL buffer (12 tubes) or toluene (12 tubes). After taking into account the initial moisture content in each soil, and the dilution of the stock insecticide solutions, the

final concentration of diazinon in each tube was approximately 0, 7.0, or 14.0 g L^{-1} and the final concentration of imidacloprid in each flask was 0, 0.6, or 1.2 g L^{-1}.

After the incubation period, 20 mL of 1 *M* KCL solution (acidified with 10 mL of 1 *M* HCl per L) was added to each tube, and the tubes were agitated 30 min. A 2-mL aliquot was removed after agitation and centrifuged 10 min at >5000 rpm. The NH_3–N was then determined by the indophenol method (Ngo et al., 1982).

A completely randomized 2 × 3 factorial design using a one-way treatment structure with three replications per treatment was used for the analysis of these experiments.

Sod Study

Creeping Bentgrass

Creeping bentgrass was maintained at the University of Kentucky Experiment Farm in Lexington on 12 test plots, with dimensions of 1.83 by 3.05 m, which were managed on a sand-based system in accordance with U.S. Golf Association specifications. The plots were constructed of sand with about a 10-mm layer of creeping bentgrass (vegetative and thatch layers). The mixture is 10% organic matter by volume with 90% sand throughout the profile.

The imidacloprid treatments in water were applied at 0 g m^{-2} (control), a field application at 0.066 g m^{-2} (the manufacturer's recommended rate), five times the field application rate (0.33 g m^{-2}), or ten times the field application rate (0.66 g m^{-2}). These application rates were based on the assumption of a typical spray rate of 467 L ha^{-1} and homogenous tank mixing. The application was followed with irrigation (13 mm) to ensure downward movement of the insecticide into the soil. Urea was not added to these plots because under normal management conditions, the plots had already been fertilized with urea before the experiment began (approximately 2.5 g urea per plot).

Before imidacloprid application, a soil core (20 × 70 mm) was removed from each plot to establish the background urease activity. The samples were transported to the lab, subdivided into vegetative, organic, and sand layers, and stored at 4°C in plastic bags until colorimetric ammonia analysis. Once the initial samples were removed, each test plot received an application of imidacloprid with a conventional hand-held pressurized applicator. The test site was irrigated daily (13 mm) at approximately 0500 h. In the experiment this schedule was not altered, and soil samples were collected from the plots following irrigation at approximately 0800 h. One sample from each of the test plots (three replications per treatment) was taken immediately after application (Day 0) and on Days 1, 2, 4, 8, and 16 after application. The samples were transported to the laboratory, separated into a vegetative (0–5 mm), organic (5–10 mm), and sand layers (10–45 mm), then stored at 4°C in plastic bags until urease activity could be assessed.

Urease activity was determined by suspending 2 g of sample (soil or vegetation) in 1.0 mL urea solution (0.48 g/100 mL) and incubating for 2 h at 25°C. After the incubation period, 20 mL of KCl solution was added and the samples were shaken for 30 min. Afterward, a 2-mL aliquot was centrifuged for 10 min at 5000 rpm and NH_3–N measured by the indophenol method (Ngo et al., 1982). Urease activity was defined in terms of the following units: mg NH_3–N kg^{-1} h^{-1}.

Kentucky Bluegrass

The experiment was conducted 24 April to 29 June (for imidacloprid application) and 11 September to 13 November (for diazinon application) at the University of Kentucky Experiment Farm in Lexington on a Maury silt loam soil using nine test plots, with dimensions of 1.83 by 3.05 m each, for each insecticide treatment.

Before insecticide application a core sample was removed from each test plot with a PVC (polyvinylchloride) cylinder (75-mm width by 75-mm depth) to determine baseline urease activity. The interval for subsequent samples was Day 1, 2, 4, 8, 16, 32, and 64 from the initial application. The vegetative (0–5 mm), organic (10–25 mm), and mineral (25–45 mm) layers of the core were separated and the soil was tested for pH, soil water, organic matter content, and urease activity.

Once the initial cores were removed, each test plot received an application of urea fertilizer (58.9 g urea per plot) and irrigation (13 mm). After 24 h, a core sample was removed from each test plot to determine the effect of the urea fertilizer on soil urease activity (Day 0). After these cores were removed in spring, a surface application of imidacloprid was uniformly applied to the test plots using a conventional hand-held pressurized applicator. The test plots were randomly selected to receive no imidacloprid (control), a field application rate at 0.066 g m^{-2} (the manufacturer's recommended rate), or twice the field application rate at 0.132 g m^{-2}. The application was followed with irrigation (13 mm) to ensure downward movement of the insecticide into the soil. This was the only application of imidacloprid applied to the test area.

The test plots for diazinon application to bluegrass were located at the opposite end of the same field as the imidacloprid study on the same Maury silt loam soil. A soil core was removed from each test plot with a PVC cylinder (75-mm width × 75-mm depth) before applying any treatments. The vegetative (0–5 mm), organic (10–25 mm), and mineral (25–45 mm) layers of the core were separated as before, and tested for pH, organic matter content, and urease activity.

After the initial soil cores were removed, each test plot received an application of urea fertilizer (58.9 g urea per plot) and irrigation (13 mm). After 24 h, a core sample was removed from each of the test plots to determine the effect of the urea fertilizer on soil urease activity. After these cores were removed, a surface application of diazinon was applied using a conventional hand-held pressurized applicator. The test plots were randomly selected to receive no diazinon (control), a field application rate of 0.79 g m^{-2} (the manufacturer's recommended rate), or double the manufacturer's application rate (1.59 g m^{-2}). The diazinon application was followed with irrigation (13 mm) to ensure downward movement of the insecticide into the soil. This was the only application of diazinon applied to the test area. A soil core was taken from each test plot for analysis of urease activity. The sampling interval was Days 0, 1, 2, 4, 8, 16, 32, and 64 d from the initial application and urease activity was determined as previously described.

During the course of the field study the maximum daily air temperature rose from 18 to 25°C and there was a total of 178 mm of precipitation in six separate rain events. For both the imidacloprid and diazinon field studies the data were analyzed as a one-way treatment classification in a completely randomized block split plot design using PROC GLM of SAS (SAS Institute, 1999). The block was the plot area for each treatment and split into a vegetative, organic, and mineral layer.

RESULTS

Enzyme Assays

When the treatment effects of diazinon or imidacloprid were corrected for the control there were no signifi-

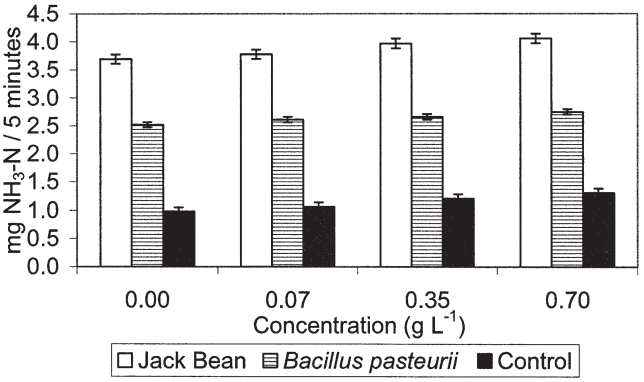

Fig. 1. Effect of imidacloprid on jack bean and *Bacillus pasteurii* urease activity. Error bars represent one standard deviation of the mean.

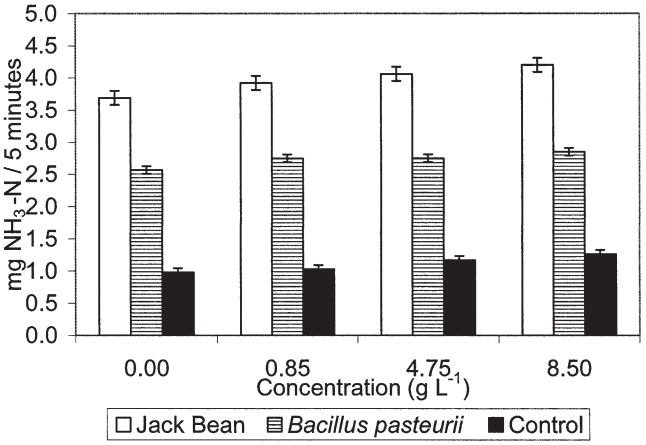

Fig. 2. Effect of diazinon on jack bean and *Bacillus pasteurii* urease activity. Error bars represent one standard deviation of the mean.

cant positive or negative effects ($p > 0.05$) of either insecticide on urease activity at the concentrations employed in this study (Fig. 1 and 2). Jack bean urease routinely produced more NH_3–N than *B. pasteurii* urease in the enzyme assays.

Growth Studies

There was not a significant effect of imidacloprid concentration on *P. vulgaris* growth at 12 or 24 h ($p = 0.559$ and $p = 0.240$, respectively). Increasing diazinon concentration significantly reduced maximum *P. vulgaris* cell density by 24 h ($p = 0.004$). Except for the highest diazinon concentration, specific growth rates (μ) for *P. vulgaris* determined between 12 and 24 h were essentially the same regardless of insecticide treatment, and ranged from 0.11 to 0.13 μ h^{-1}.

Washed Cell and Sonication Experiments

The average NH_3–N produced by heat-treated, whole, and sonicated *P. vulgaris* cells amended with imidacloprid or diazinon is shown in Table 1. Heat-treated cells had no appreciable NH_3–N production during the 8-h incubation. Whole cell NH_3–N production was significantly reduced ($p < 0.05$) at 4 and 8 h as diazinon concentration increased. Imidacloprid had no effect on whole cells. When cells were sonicated there was a slight reduction in NH_3–N production at 4 h as diazinon concentration increased, but by 8 h this was not significant, and the final NH_3–N produced by sonicated cells was quite similar to whole cell controls. Likewise, in sonicated cells exposed to imidacloprid, there was a significant reduction in NH_3–N production at 4 h, but the effect of imidacloprid was not significant at 8 h, and the urease activity in sonicated cells was only slightly reduced compared with whole cells.

Soil Slurry Assays

The average NH_3–N produced during 2 h in each treatment and the accompanying *p* values are shown in Table 2. There were no significant effects of diazinon or imidacloprid in toluene-treated soils ($p > 0.05$). When toluene was eliminated from soil slurries, there was a significant reduction in urease activity in diazinon-treated Maury soil ($p < 0.002$). However, neither diazinon nor imidacloprid had an effect on urease activity in Woolper soil ($p > 0.05$).

Sod Core Study

Urease activity was significantly different ($p < 0.05$) for each layer of the creeping bentgrass cores after imidacloprid application, ranging from 520 ± 50 mg NH_3–N kg^{-1} h^{-1} in the vegetated layer to 100 ± 10 mg NH_3–N kg^{-1} h^{-1} and 20 ± 5 mg NH_3–N kg^{-1} h^{-1} in the organic

Table 1. Production of NH_3–N after 0, 4, and 8 h from heat-treated, whole, and sonicated *Proteus vulgaris* cells exposed to diazinon or imidacloprid.

	Heat-treated			Whole cells			Sonicated cells		
Concentration	0 h	4 h	8 h	0 h	4 h	8 h	0 h	4 h	8 h
g L^{-1}	mg NH_3–N								
Diazinon									
0	4.09	4.21	4.32	0.55	16.26	34.39	2.55	16.29	30.06
0.85	4.03	4.02	4.20	0.22	4.27	5.49	1.59	10.84	29.56
4.25	4.81	4.81	5.12	0.03	1.00	1.05	1.10	11.14	28.93
8.5	6.20	6.09	6.44	0.03	0.06	0.11	0.82	11.33	29.12
p value	0.267	0.293	0.282	0.143	0.004	0.005	0.008	<0.001	0.136
Imidacloprid									
0	3.86	4.16	4.73	0.71	17.08	34.14	1.82	16.87	30.38
0.07	3.35	3.72	3.87	1.06	21.85	34.51	0.54	14.78	30.01
0.35	3.58	3.78	4.02	0.28	13.47	33.26	1.03	13.87	29.70
0.70	3.43	3.66	3.86	0.62	13.65	33.82	0.92	12.43	29.91
p value	0.89	0.90	0.54	0.28	0.55	0.999	0.0564	<0.001	0.411

Table 2. Production of NH_3–N with diazinon or imidacloprid treatment in Maury and Woolper soil slurries.

Insecticide	Concentration	NH_3–N produced†	
		+Toluene	−Toluene
	g L^{-1}	mg kg^{-1}	
		Maury soil	
Diazinon	0	5.13	5.35
	8.5	4.76	3.31
	17.0	4.20	3.38
	p value	0.532	0.002
Imidacloprid	0	5.31	5.24
	0.7	5.32	5.19
	1.4	5.19	4.75
	p value	0.979	0.918
		Woolper soil	
Diazinon	0	7.78	7.63
	8.5	8.67	7.57
	17.0	8.01	7.17
	p value	0.144	0.924
Imidacloprid	0	7.72	7.34
	0.7	7.05	7.18
	1.4	7.60	6.78
	p value	0.200	0.762

† Two-hour incubation.

and sand layers, respectively. There was a significant effect of sample date on urease activity, but when the effects of imidacloprid application were separated for each day of the experiment, and for each layer of the soil core, there was not a significant effect of imidacloprid application rate ($p > 0.05$) regardless of layer or date examined.

In imidacloprid-treated bluegrass cores there was also a significant difference in urease activity between layers immediately after imidacloprid application. Urease activity (all treatments combined) averaged 450 ± 5 mg NH_3–N kg^{-1} h^{-1} in the vegetative layer, 43 ± 2 mg NH_3–N kg^{-1} h^{-1} in the organic layer, and 22 ± 3 mg NH_3–N kg^{-1} h^{-1} in the mineral layer. After partitioning the treatment effects of imidacloprid by layer in bluegrass, there were also no significant effects of imidacloprid application rate ($p > 0.05$) for either the vegetative, organic, or mineral layers (Fig. 3, 4, and 5).

In September and November there continued to be significant differences between urease activity in the different layers of bluegrass sod. Immediately after diazinon application the urease activity was 350 ± 50 mg NH_3–N kg^{-1} h^{-1} in the vegetative layer, 50 ± 5 mg

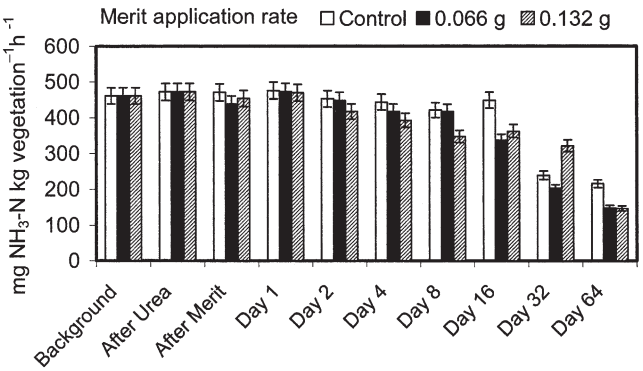

Fig. 3. Effect of imidacloprid on urease activity in the vegetative layer of bluegrass amended with urea (imidacloprid application = g m^{-2}). Error bars represent one standard deviation of the mean.

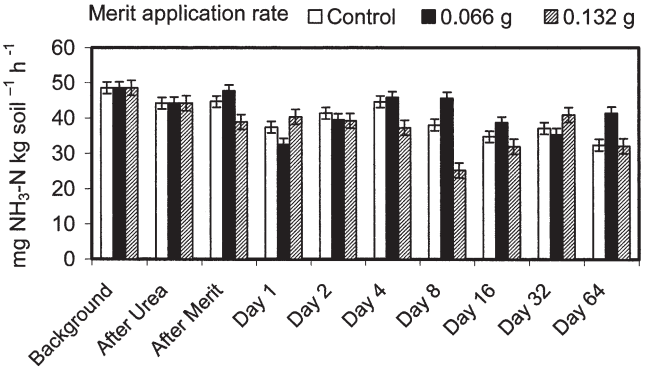

Fig. 4. Effect of imidacloprid on urease activity in the organic layer of bluegrass amended with urea (imidacloprid application = g m^{-2}). Error bars represent one standard deviation of the mean.

NH_3–N kg^{-1} h^{-1} in the organic layer, and 25 ± 5 mg NH_3–N kg^{-1} h^{-1} in the mineral layer. There was no significant effect of diazinon ($p > 0.05$) in vegetative layers of bluegrass, although the decrease in urease activity on Day 2 approached significance ($p = 0.12$) (Fig. 6, 7, and 8). However, immediately after diazinon application (Day 0) until 48 h later, the urease activity in the organic and mineral layers was significantly reduced ($p < 0.001$).

DISCUSSION

The enzyme assays indicated that commercial formulations of neither diazinon nor imidacloprid had a significant effect on pure plant or bacterial ureases. The concentrations of both insecticides ranged from 0 to 0.5 times the recommended field application rates, but considering the potential immobilization of insecticides in soil and dilution in soil solutions, these rates are probably an accurate reflection of the concentration range of insecticides to which soil urease would be initially exposed. Gianfreda et al. (1994) similarly observed that free jack bean urease was relatively unaffected by several pesticides such as glyphosate and paraquat, although its activity was stimulated by carbaryl and diminished by atrazine.

Several studies (e.g., Balogh and Anderson, 1992) have evaluated the fate and persistence of pesticides

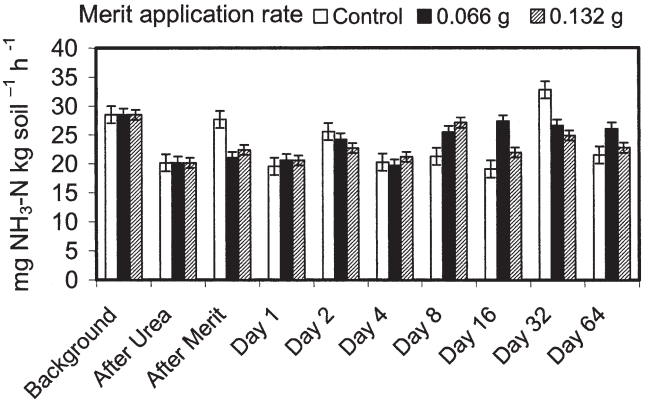

Fig. 5. Effect of imidacloprid on urease activity in the mineral layer of bluegrass amended with urea (imidacloprid application = g m^{-2}). Error bars represent one standard deviation of the mean.

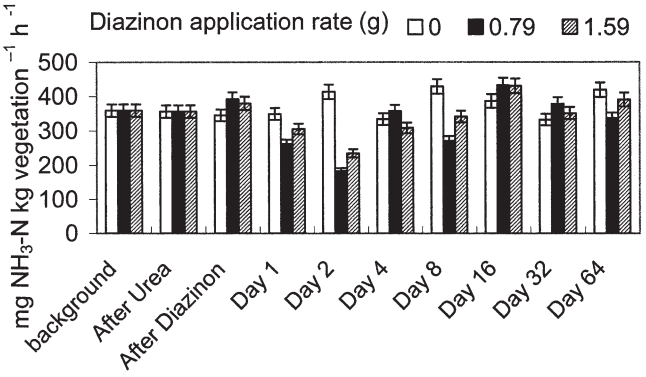

Fig. 6. Effect of diazinon on urease activity in the vegetative layer of bluegrass amended with urea (diazinon application = g m^{-2}). Error bars represent one standard deviation of the mean.

applied to turfgrass systems, and estimates of the pesticide concentration that reaches the soil zone range from 10 to 50% of the applied amount. The implications of this experiment are that the commercial formulations of the insecticides tested would not have a significant effect on extracellular ureases. However, the insecticides could exert an effect on overall soil urease activity by being biocidal to urease-producing organisms or by preventing urea uptake by these organisms.

The growth studies with *P. vulgaris*, a representative heterotrophic bacteria, seemed to indicate that diazinon applications could negatively affect the urease-producing organisms in soil. *Proteus vulgaris* growth after 24 h was inhibited by increasing diazinon and imidacloprid concentrations, although only diazinon caused a significant growth inhibition. The effect could be attributed to an increase in the lag phase of growth, because exponential growth, as indicated by the specific growth rate, was virtually unchanged. Washed cell studies suggested that during this period of diazinon-retarded growth, urease activity was likely reduced due to diminished cell uptake of solution urea. Whole cell urease activity was inhibited while sonicated cells, which exposed intracellular urea, had relatively unaffected urease activity.

If diazinon transiently affected cell growth and urease activity, we would expect to see similar results in soil.

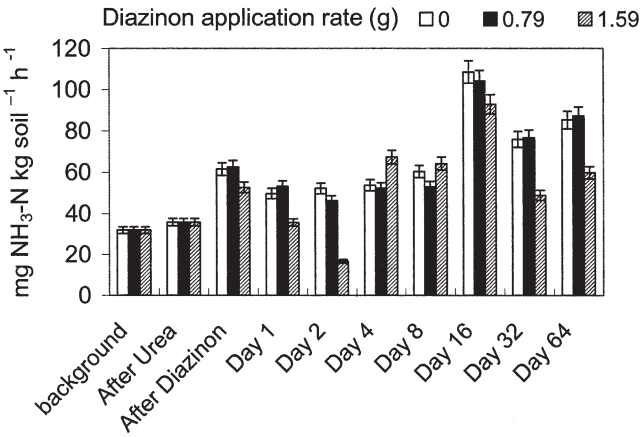

Fig. 7. Effect of diazinon on urease activity in the organic layer of bluegrass amended with urea (diazinon application = g m^{-2}). Error bars represent one standard deviation of the mean.

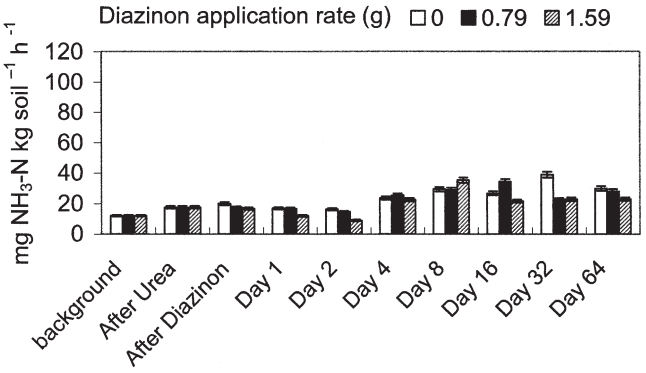

Fig. 8. Effect of diazinon on urease activity in the mineral layer of bluegrass amended with urea (diazinon application = g m^{-2}). Error bars represent one standard deviation of the mean.

This was illustrated by the inhibition of urease activity in Maury soil. When we added toluene, urease activity rates were unaffected by diazinon. Treatments without toluene, in which part of the urease activity was contributed by urease-producing cells, had diminished NH_3–N production, indicating that diazinon was affecting cell activity. In most cases there was a slight but not significant increase in urease activity in the presence of toluene. Nannipieri et al. (2002) report that an artifact of toluene addition is increased permeability of cells to urea. Intracellular urease, which our previous experiments had already demonstrated are unaffected by commercial diazinon formulations, therefore had improved access to substrate. Gianfreda et al. (1994) and Sannino and Gianfreda (2001) noted a similar effect in some soils when methanol was added as a pesticide solvent. They attributed the increased urease activity partly to release of adsorbed urease by the solvent addition as well as to some lysis of cells that released intracellular urease.

Diazinon had no apparent effect in Woolper soil. The variability of soil effects appears to be a common observation from pesticide studies involving urease (Shaffer, 1993). The most likely explanation is that diazinon was adsorbed to soil organic matter in Woolper soil. We cannot discount the possibility that the lower pH in the Woolper soil environment also affected diazinon toxicity or availability. In addition, because we did not look at specific microbial population differences, we cannot eliminate the possibility that the soil urease-producing community in Woolper soil differs sufficiently from that in Maury soil that it resists inhibition by diazinon.

The inhibitory effect of diazinon in Maury soil mirrors similar results obtained by Lethbridge and Burns (1975) for the inhibition of soil urease by various organophosphate pesticides. They observed significant and long-lasting (several weeks) urease inhibition with insecticide concentrations of 1000 mg L^{-1}, about one-third the rate used in the current study. We did not follow the extent of urease inhibition for a longer period, but other results (unpublished) suggest that the inhibitory effects of diazinon in the Maury soil would be short term.

The field studies in creeping bentgrass and bluegrass

sod mirrored the observations we made in laboratory studies with simpler systems. Imidacloprid had virtually no effect on urease activity while diazinon had a transient inhibitory effect. Cores from each sod type were sampled by layer, and in each core urease activity declined as the depth of each layer increased, which has been previously observed (Myers and McGarity, 1968). There were slight differences in urease activity between the vegetative layers of creeping bentgrass and bluegrass, possibly due to diversity in urease producing organisms and different turfgrass management techniques, but these differences quickly disappeared with depth.

The application of both insecticides and urea was immediately followed by irrigation, which would be a standard practice in turfgrass management. The organic layers in creeping bentgrass and bluegrass may not have adequately impeded imidacloprid from being leached from the plots, resulting in a limited interaction of imidacloprid and the soil system. In the Pesticide Information Profile (PIP), imidacloprid had been described as being moderately soluble with moderate binding affinity to organic materials in soils (K_{oc} = 262). It has the potential to move through sensitive soil types including porous, gravelly, or cobbly soils, depending on irrigation practices (EXTOXNET, 1996). Likewise, there was little or no effect of diazinon in the vegetative layer of bluegrass, probably because irrigation leached it into the soil profile before it could manifest an effect on urease-producing microorganisms.

Several studies (Sears and Chapman, 1979; Tashiro, 1982) evaluated the concentration of diazinon that reaches the soil zone, and diazinon residues in each study were found at low concentrations below 13 mm. The top 30 mm of each core (vegetative, 5 mm; organic, 25 mm), may have absorbed much of the diazinon, but at the mineral layer, there could have been sufficient diazinon to inhibit the urease-producing organisms for the initial 48 h of the experiment.

After 96 h (Day 4) of the soil core experiment, the inhibition of urease activity by diazinon had disappeared. Although microbial growth was decreased at just 5% of the recommended field application rate during the initial 24 h of the pure culture studies, the cells eventually recovered. Alternately, diazinon could have been adsorbed by organic matter in the mineral layer in this period.

CONCLUSIONS

Imidacloprid had little if any affect at any level of study. Diazinon did not directly inhibit ureases, but it temporarily reduced the growth and urease activity of a model urease-producing bacterium, *P. vulgaris*, and at realistic field application rates it reduced urease activity in slurries of Maury soil. Because the study employed the commercial formulations available to a typical commercial applicator, it did not eliminate the possibility that the observed effects were strictly due to additives in the commercial formulations rather than the insecticides themselves. The effect of the diazinon formulation appears to be through inhibiting urea uptake, because the inhibition disappeared when intracellular urease was exposed to urea in sonicated cells, or whole cells in soil slurry were made more permeable to urea by adding toluene. Diazinon had the potential to reduce NH_3 loss from the organic and mineral layers of bluegrass sod on a Maury soil, but only at an application rate twice the recommended field application rate, and this resulted in only a short-term (48 h) inhibition of urease activity.

Recommended field application rates of imidacloprid and diazinon followed by common irrigation practices did not have a significant effect on urease in the surface vegetative layer and only limited effects in the organic and mineral layers of turfgrass sod. We conclude that applying commercial imidacloprid and diazinon insecticides along with urea in typical turfgrass management systems has little influence on the subsequent availability of urea N in those systems.

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