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Evaluation of Phenoxyaminocyclotriphosphazatrienes as Sustained-Action Soil Urease Inhibitors

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Laboratory incubation experiments were conducted over a period of 36 days to evaluate soil urease inhibition with time at 30 °C by the phenoxy derivatives of the phosphazene compound 2,2,4,4,6,6-hexaaminocyclotriphosphazatriene. The derivatives were (1) 2-phenoxy-2,4,4,6,6-pentaaminocyclotriphosphazatriene, (2) 2,4-diphenoxy-2,4,6,6-tetraaminocyclotriphosphazatriene, and (3) 2,4,6-triphenoxy-2,4,6-triaminocyclotriphosphazatriene. After the first 16-h incubation (immediate inhibition), the monophenoxyphosphazene exhibited a 95% inhibition, whereas the triphenoxyphosphazene only inhibited 22%; the compound phenyl phosphorodiamidate, by comparison, inhibited 100%. The immediate inhibition by the phosphazene compounds decreased with an increase in the number of phenoxy substitutions. The sustained inhibition (more than 4 days), which ranged from 40% to 95%, tended to increase with an increase in the number of phenoxy substitutions but tended to decrease with an increase in incubation temperatures from 20 to 40 °C.

Ammonia volatilization losses from broadcast urea on unsaturated soils can be serious (Terman, 1979) and can result in significantly decreased nitrogen use efficiency. One approach to increasing N use efficiency is to use urea amended with a chemical compound to retard its rapid hydrolysis (Sahrawat, 1980; Mulvaney and Bremner, 1981; Hauck, 1984). This approach has received much attention in the last 10-12 years, and several organic, inorganic, synthetic, and natural chemical compounds have been tested (Sahrawat, 1980; Mulvaney and Bremner, 1981; Liao and Raines, 1982; Martens and Bremner, 1984; Bremner and Chai, 1986). Of the compounds tested, phenyl phosphorodiamidate (PPDA) (Held et al., 1976; Martens and Bremner, 1984) and *N*-(*n*-butyl)thiophosphoric triamide (Bremner and Chai, 1986) have been reported to exhibit high soil urease inhibition.

In an attempt to identify effective sustained-action urease inhibitors, Peters et al. (1988) recently synthesized and characterized three phosphazene compounds: (1) 2-phenoxy-2,4,4,6,6-pentaaminocyclotriphosphazatriene (2) 2,4-diphenoxy-2,4,6,6-tetraaminocyclotriphosphazatriene, and (3) 2,4,6-triphenoxy-2,4,6-triaminocyclotriphosphazatriene.

These compounds were in fact developed essentially for retarding hydrolysis of broadcast urea in floodwater and at the floodwater-soil interface of submerged rice soils. We

therefore first conducted preliminary investigations on soil urease inhibition properties of these compounds using a Crowley soil incubated under unsaturated conditions. In this paper, we reported these preliminary data on the temporal changes in soil urease inhibition by three phosphazene compounds. In separate studies conducted using several different submerged soils incubated in the greenhouse (without rice plants), we have also studied the inhibitory effects of these compounds on urea hydrolysis in floodwater and at the floodwater-soil interface, and the relevant data will be reported elsewhere.

MATERIALS

The three phosphazene compounds synthesized and characterized by Peters et al. (1988) were used (Table I). For comparison purposes, phenyl phosphorodiamidate supplied by ICN Pharmaceuticals (Plainview, NY) and recrystallized twice from ethanol; 2,2,4,4,6,6-hexaaminocyclotriphosphazatriene, prepared according to the procedure described by Sowerby and Audrieth (1961); and phosphoryl triamide prepared according to the procedure described by Klement and Nielsen (1960) were also included in this study (Table I).

An air-dried surface soil sample (0-15 cm, <2 mm) of Crowley silt loam (Typic Albaqualf, fine montmorillonitic, thermic) was used: pH (H₂O), 6.3; organic matter, 1.7%; cation-exchange capacity (CEC), 16.2 mequiv/100 g; urease activity, \approx 18 μ g of urea hydrolyzed/g per h (near field capacity and at 30 °C).

EXPERIMENTAL PROCEDURE

In order to study the temporal changes in soil urease inhibition by a chemical compound or a mixture of compounds in a well-mixed soil system, the following procedure

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Table I. Compounds Evaluated as Soil Urease Inhibitors

no.	compound	formula	cis/trans ratio	solubility (H ₂ O, g/L, at 25 °C)
1	2-phenoxy-2,4,4,6,6-pentaaminocyclotriphosphazatriene	N ₃ P ₃ (NH ₂) ₆ (OC ₆ H ₅)		86.40
2	2,4-diphenoxy-2,4,4,6,6-tetraaminocyclotriphosphazatriene	N ₃ P ₃ (NH ₂) ₄ (OC ₆ H ₅) ₂	2.60	0.11
3	2,4,6-triphenoxy-2,4,6-triaminocyclotriphosphazatriene	N ₃ P ₃ (NH ₂) ₃ (OC ₆ H ₅) ₃	0.55	0.02
4	phenyl phosphorodiamidate	(NH ₂) ₂ PO(OC ₆ H ₅)		6.643
5	2,2,4,4,6,6-hexaaminocyclotriphosphazatriene	N ₃ P ₃ (NH ₂) ₆		a
6	phosphoryl triamide	(NH ₂) ₃ PO		a

^aVery soluble.

of successive incubations was used:

a. Preincubation. Unsaturated Crowley soil (≈16% water, w/w) was prepared by spraying with water and repeated mixing. The moist soil sample was preincubated in a closed plastic bucket for 24–48 h at room temperature.

b. Incubation. One milliliter of each of the freshly prepared aqueous solutions or well-stirred suspensions containing 7 μmol of each compound or mixtures of the compounds was evenly distributed over a 120-g sample of the unsaturated Crowley soil (from the preincubated soil), which was spread out on a plastic sheet, and mixed well. The treated soil sample was transferred to a PVC pipe (12 cm in length by 4 cm in internal diameter) with one end closed. The average bulk density of the soil column was about 1.1 g/cm³. The open end of each pipe containing soil was covered with Parafilm (with a pinhole). A sufficient number of the treated and untreated (check) soil columns to provide triplicates were incubated for 0, 3, 7, 14, 21, and 35 days in a vertical position at >70% relative humidity and at 20, 30, or 40 °C. The water content of the incubated soil was maintained by dropwise addition of the required amount of water onto the top surface of the soil column and monitoring the weight of each soil column system every week.

c. Reincubation. After the desired incubation period, the soil from each PVC pipe (in triplicate) was removed from the cylinder and spread out separately on a plastic sheet. One milliliter of urea solution (50 mg of urea/mL) was distributed on the soil layer and mixed well. The soil was then returned to the PVC pipe, and the soil column was packed by hand to give an average bulk density of about 1.1 g/cm³. The open end of the pipe was covered with Parafilm (with a pinhole), and the soil column was reincubated as above. At the end of the 16-h reincubation, at 30 °C and ≥70% relative humidity, the cylinders containing the soil samples were removed from the incubator and the urea remaining unhydrolyzed in the soil was extracted with freshly prepared 1 N KCl containing 10 ppm PPDA as a urease inhibitor and determined colorimetrically on a Technicon AutoAnalyzer (Technicon, 1974). Preliminary observations indicated that 10 ppm of PPDA completely inhibited urea hydrolysis during KCl extraction and did not interfere in the colorimetric determination of urea. The amount of urea hydrolyzed in the 16-h reincubation period was calculated as the difference between urea added and urea remaining unhydrolyzed. Soil urease activity was calculated as micrograms of urea hydrolyzed/gram per hour at 30 °C, and the percentage of apparent inhibition of soil urease was calculated as suggested by Douglas and Bremner (1971).

RESULTS AND DISCUSSION

Figure 1 shows the changes, with time, in apparent soil urease inhibition by the six chemical compounds well-mixed in soils and incubated at 30 °C and >70% relative humidity.

The inhibition after the first 16-h incubation (hereafter called immediate inhibition) varied from 22% (compound

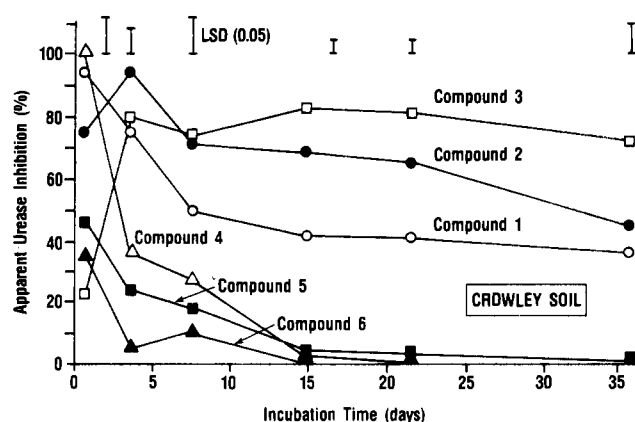


Figure 1. Changes in apparent inhibition of soil urease by the compounds in well-mixed unsaturated soil systems at 30 °C with time.

3) to 100% (compound 4). With increasing incubation time, the inhibition by compounds 4–6 rapidly decreased and essentially disappeared at 14 days. These temporal changes in inhibition may be due to their degradation in soil with time. Martens and Bremner (1983) suggest that compound 4 decomposes in soil with time. In previous work, the pH-dependent hydrolysis products of compound 4 have been identified as phenyl phosphoramidate and NH₃ in acid medium and phosphorodiamidic acid and phenol in alkaline medium (Austin et al., 1984). These degradation products when tested separately exhibited little or no soil urease inhibition (Savant et al., 1983). Compound 5 also degrades in soil (Dick and Tabatabai, 1978), which may explain the decreased urease inhibition by this compound in the well-mixed soil system.

The 95% immediate inhibition shown by compound 1 gradually decreased to about 35% at the end of the 36-day test period. For compound 2, inhibition increased from 75% (after 16 h) to 95% (after 4 days) and thereafter very gradually decreased to 45% (after 36 days). With compound 3, the inhibition increased markedly from 22% after 16 h to 80% at 4 days of incubation. It then tended to level off for the remainder of the test period.

The above results suggest that the inhibition properties of the compounds were influenced by the number of phenoxy substitutions on compound 5. In general, an increase in the number of phenoxy substitutions decreased the water solubility (Table I; Mader et al., 1965) and probably increased the stability of compounds 1–3 as compared with that of compound 5. The data presented in Figure 1 indicate that the immediate inhibition followed the order of their solubilities in water (Table I; Figure 1). However, the sustained inhibition seemed to be dependent on the number of phenoxy substitutions and therefore presumably on their stabilities in soil.

The data given in Figure 2 indicate the marked influence of incubation temperatures on the inhibition by compounds 1–4. In general, an increase in temperature increased the immediate inhibition except for compound 3

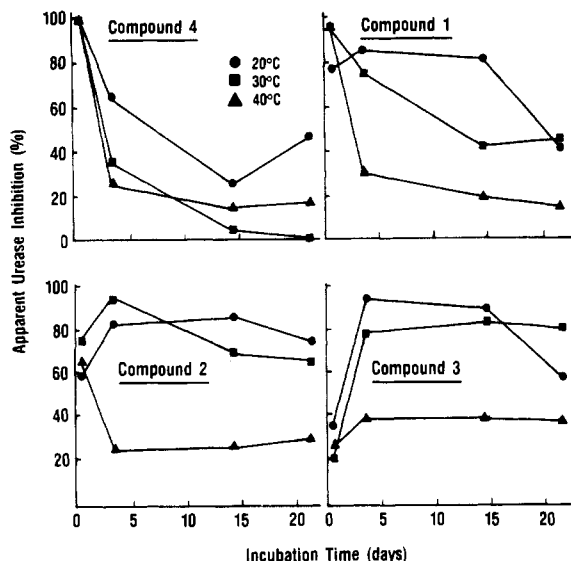


Figure 2. Effect of incubation temperatures on apparent urease inhibition by the compounds in well-mixed unsaturated Crowley soil systems.

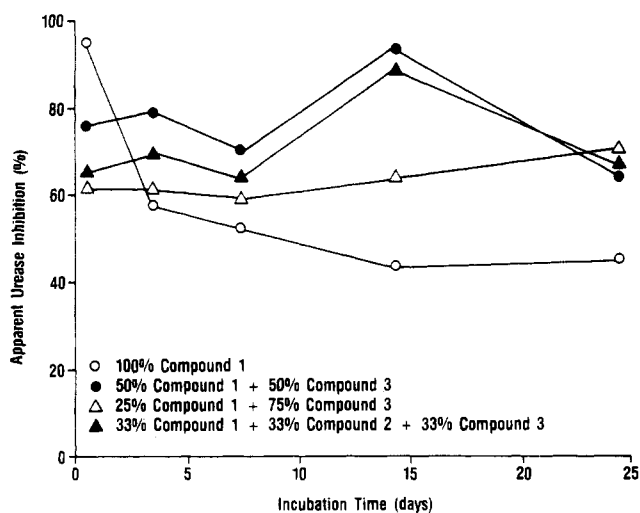


Figure 3. Changes in apparent soil urease inhibition by the mixture of the compounds in well-mixed soil systems at 30 °C with time.

but decreased the sustained inhibition.

Medina and Sullivan (1987) proposed the use of a few binary or tertiary mixtures of compounds 1–3 to obtain nearly linear sustained urease inhibition at levels of 60% or more for the first 3 weeks. They inferred this by using (a) the inhibition data collected for the individual compounds 1–3 (Figure 1) and (b) the Monte Carlo statistical simulation model for predicting the inhibition by mixtures of them. A separate experiment was conducted to verify this prediction, and the results obtained for three mixtures are shown in Figure 3. The immediate inhibition varied systematically with the percentage of compound 1 in the mixture, which suggests that the immediate inhibition depended on its presence in a given mixture. Thereafter, the inhibition was nearly linear and sustained in the range

of 60–90% and was attributed to the presence of compounds 2 and 3 in the mixtures.

Finally, these data on the observed soil urease inhibition for the phosphazenes and the mixtures of phosphazenes in well-mixed soil systems should be interpreted with great caution because the urease inhibition property of a given chemical compound present in surface-applied urea granules is likely to be influenced by soil bulk density, water solubility, and diffusion of the compound in relation to that of urea in unsaturated soil (Savant et al., 1988). Before their agronomic evaluation for dryland crops, therefore, there is an apparent need to study rate and extent of inhibition of hydrolysis of surface-applied urea granules containing one or more of these phosphazenes in unsaturated soils.

Registry No. 1, 98814-82-5; 2, 98816-27-4; 3, 2785-43-5; 4, 7450-69-3; 5, 13597-92-7; 6, 13597-72-3; urease, 9002-13-5.

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