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UREA HYDROLYSIS IN SOILS: FACTORS INFLUENCING THE EFFECTIVENESS OF PHENYLPHOSPHORODIAMIDATE AS A RETARDANT

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Summary—Studies to determine the factors influencing the effectiveness of phenylphosphorodiamidate (PPD) to retard urea hydrolysis in soils showed that the inhibitory effect of PPD on hydrolysis of urea by soil urease increased markedly with the amount of PPD added and decreased markedly with time and with increase in temperature from 10 to 40°C. They also showed that the ability of PPD to retard urea hydrolysis in 15 surface soils selected to obtain a wide range in properties was significantly correlated with organic C content ($r = -0.68^{**}$), total N content ($r = -0.74^{**}$), cation-exchange capacity ($r = -0.65^{**}$), sand content ($r = 0.66^{**}$), clay content ($r = -0.64^{**}$) and surface area ($r = -0.60^{*}$), but was not significantly correlated with pH, silt content, urease activity or $CaCO_3$ equivalent. Multiple-regression analyses indicated that the effectiveness of PPD to retard urea hydrolysis in soils tends to increase with decrease in soil organic-matter content.

INTRODUCTION

The use of urea as a nitrogen fertilizer has increased dramatically during the past 20 years, and all indications are that urea will become the most important solid fertilizer in world agriculture (Tomlinson, 1970; Engelstad and Hauck, 1974; Beaton, 1978). This has stimulated research to find methods of reducing the problems encountered in use of urea as a fertilizer. These problems result largely from the rapid hydrolysis of fertilizer urea to ammonium carbonate through soil urease activity and the concomitant rise in pH and accumulation of ammonium. They include damage to germinating seedlings and young plants, nitrite or ammonia toxicity or both, and gaseous loss of urea N as ammonia (Gasser, 1964; Tomlinson, 1970; Engelstad and Hauck, 1974).

One approach to reducing the problems associated with the use of urea as a fertilizer is to find compounds that will inhibit urease activity and thereby retard urea hydrolysis when applied to soils in conjunction with urea fertilizer. This approach has received considerable attention during the past decade, and many compounds have been patented as inhibitors of urea hydrolysis in soils (Mulvaney and Bremner, 1981). We have shown (Martens and Bremner, 1982) that phenylphosphorodiamidate (PPD) was the most effective of 12 phosphoroamides evaluated for retardation of urea hydrolysis in soils and that it was considerably more effective than five compounds known to be among the most effective of the compounds thus far proposed for inhibition of urease activity in soils (hydroquinone, catechol, 1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone and phenylmercuric acetate). PPD was among a group of phosphordiamides patented by East German researchers (Held *et al.*, 1976) for reducing gaseous loss of urea fertilizer N as ammonia, and it has given promising results in greenhouse and field research (Matzel *et al.*, 1978a, b, 1979; Heber *et al.*, 1979; Vlek *et al.*, 1980; Byrnes *et al.*, 1983; Kämpfe *et al.*, 1983). Our purpose was to identify the factors influencing the effectiveness of PPD to retard urea hydrolysis in soils.

MATERIALS AND METHODS

The soils used (Table 1) were surface (0–15 cm) samples of soils selected to obtain a wide range in pH (4.6–8.0), texture (5–57% sand, 11–55% clay) and organic-matter content (0.30–6.73% organic C). Unless otherwise specified, each sample was air-dried and crushed (<2 mm). In the analyses reported in Table 1, pH, $CaCO_3$ equivalent, organic C, texture and urease activity were determined as described by Zantua and Bremner (1975). Total N was determined by a semimicroKjeldahl procedure (Bremner, 1960), cation-exchange capacity was determined as described by Keeney and Bremner (1969), and surface area was determined by the method of Heilman *et al.* (1965) modified as described by Cihacek and Bremner (1979). The organic C, $CaCO_3$ equivalent, total N and cation-exchange capacity analyses were performed on <0.14 mm soil. The other analyses reported were performed on <2 mm soil.

PPD was obtained from K & K Labs Division, ICN Pharmaceuticals, Plainview, New York Other chemicals used were obtained from Fisher Scientific Co., Itasca, Illinois.

Unless otherwise specified, the following procedure was used to study the effect of PPD on urea hydrolysis in soils. Five-gram samples of air-dried soil were placed in 65 ml glass bottles and treated with 2 ml water containing 10 mg urea or with 2 ml water containing 10 mg urea and 1–125 (usually 25) μg PPD. The bottles were stoppered and placed in an incubator maintained at 20°C. After various times (1–21 days), triplicate bottles were removed from the incubator, and urea in the incubated soil samples was extracted with 2 M KCl containing 5 μg phenyl-

Table 1. Analyses of soils

Soil	pH	Organic C (%)	Total N (%)	Sand (%)	Silt (%)	Clay (%)	$CaCO_3$ equivalent (%)	CEC[a]	Surface area ($m^2\ g^{-1}$ soil)	Urease activity[b]
Storden	8.0	0.30	0.059	51	31	18	20.8	9.5	49	14.2
Rosebud	8.0	0.59	0.071	57	24	19	1.7	14.7	67	14.2
Ida	7.9	0.88	0.110	5	71	24	14.2	15.2	92	18.9
Canyon	7.9	0.89	0.107	56	22	22	15.5	14.4	57	23.6
Lindley	5.0	1.68	0.143	40	42	18	0	11.5	52	18.9
Dickinson	6.3	1.90	0.160	53	36	11	0	14.0	32	21.1
Muscatine	6.0	2.25	0.215	5	67	28	0	23.1	109	28.3
Indian Head	7.6	2.25	0.367	14	31	55	2.8	36.8	221	37.8
Moody	6.2	2.65	0.254	5	67	28	0	21.0	127	23.6
Nicollet	6.4	3.08	0.259	46	32	22	0	21.7	81	66.1
Hayden	6.9	3.21	0.227	53	34	13	0.2	16.3	40	80.2
Harps	7.9	3.21	0.335	27	41	32	12.9	31.7	125	47.2
Clyde	5.1	4.23	0.351	15	57	28	0	18.2	103	33.0
Okoboji	6.3	5.86	0.544	19	40	41	0	40.2	180	84.9
Okojobi	4.6	6.73	0.598	12	50	38	0	38.0	127	56.6

[a]Cation-exchange capacity (m-equiv 100 g^{-1} soil).
[b]Determined by nonbuffer method of Zantua and Bremner (1975). Expressed as μg urea hydrolyzed $h^{-1}\ g^{-1}$ soil (37°C).

Table 2. Effect of air-drying soil on effectiveness of PPD to retard urea hydrolysis[a]

Soil	% Inhibition of urea hydrolysis[b] FM	AD
Indian Head	90	91
Nicollet	94	93
Harps	85	87
Clyde	89	89
Okoboji (pH 4.6)	65	66

[a]Samples of field-moist and air-dried soils (5 g dry material) were incubated (20°C; 2 ml water) for 24 h after treatment with 10 mg urea and 25 μg PPD.
[b]FM, field-moist soil; AD, air-dried soil.

mercuric acetate ml^{-1} (Douglas and Bremner, 1970) and determined by the colorimetric method of Mulvaney and Bremner (1979). Percentage inhibition of urea hydrolysis by PPD was calculated from $(C - T)/C \times 100$, where T = amount of urea hydrolyzed in the soil sample treated with PPD, and C = amount of urea hydrolyzed in the control (no PPD added).

All analyses and experiments reported were performed in duplicate or triplicate. Correlation and multiple-regression analyses were performed using the Statistical Analysis System (SAS) on an IBM 360 computer.

RESULTS AND DISCUSSION

Evaluation of experimental technique

To study the effect of any compound on urea hydrolysis in soil, it is first necessary to check that it does not interfere with the method used to measure urea hydrolysis. Tests showed that PPD did not interfere with measurement of urea hydrolysis in soil by the procedure described even when it was applied at the rate of 200 $\mu g\ g^{-1}$ soil.

Tests showed that the results obtained in studies of the effectiveness of PPD to retard urea hydrolysis in soils were not affected if the soils were air-dried before use (Table 2) or if the PPD was added to soils immediately before, with or immediately after addition of urea (Table 3).

Aqueous solutions of PPD were prepared immediately before use because tests showed that storage of aqueous solutions of PPD decreased their effectiveness to retard urea hydrolysis in soils (Table 4).

Effects of soil properties

The results obtained in a study of the effects of PPD (5 $\mu g\ g^{-1}$ soil) on urea hydrolysis in 15 diverse surface soils incubated at 20°C for 7 days after treatment with urea (Table 5) were statistically anal-

Table 3. Effects on urea hydrolysis of adding PPD to soils before, with or after urea[a]

Soil	Time of hydrolysis (days)	% Inhibition of urea hydrolysis by PPD[b] A	B	C
Rosebud	3	94	93	93
	7	86	85	84
Dickinson	3	96	95	95
	7	93	92	93
Indian Head	3	92	90	91
	7	63	62	61
Nicollet	3	94	92	92
	7	86	85	86
Harps	3	83	84	83
	7	50	49	48

[a]5 g Samples of soil were incubated (20°C; 2 ml water) for 3 or 7 days after treatment with 10 mg urea and 25 μg PPD.
[b]A, PPD added immediately before urea; B, PPD added with urea; C, PPD added immediately after urea.

Table 4. Effect of storing an aqueous solution of PPD on its effectiveness to retard urea hydrolysis in soils[a]

Soil	Storage temperature (°C)	Time of storage (days)				
		0	7	14	21	28
		% retardation of urea hydrolysis				
Moody	20	95	90	88	85	81
	30		89	84	80	75
Lindley	20	97	92	89	86	82
	30		91	85	82	79

[a] 5 g samples of soil were incubated (20°C) for 2 days after treatment with 1.5 ml water containing 10 mg urea and 0.5 ml of an aqueous solution of PPD (50 $\mu g\ ml^{-1}$) that had been stored at 20° or 30°C for various times.

Table 5. Inhibitory effect of PPD (5 ng^{-1} soil) on urea hydrolysis in different soils[a]

Soil	% Inhibition of urea hydrolysis by PPD
Storden	81
Rosebud	86
Ida	64
Canyon	90
Lindley	90
Dickinson	93
Muscatine	75
Indian Head	63
Moody	80
Nicollet	86
Hayden	84
Harps	50
Clyde	39
Okoboji (pH 6.3)	61
Okoboji (pH 4.6)	37

[a] 5 g samples of soil were incubated (20°C; 2 ml water) for 7 days after treatment with 10 mg urea and 0 or 25 μg PPD.

Table 6. Correlations between soil properties and inhibitory effect of PPD on urea hydrolysis (15 soils)

Soil property	Correlation coefficient (r)
Organic C content	−0.68**
Total N content	−0.74**
Urease activity	−0.29
Cation-exchange capacity	−0.65**
Sand content	0.66**
Silt content	−0.46
Clay content	−0.64**
Surface area	−0.60*
pH	0.29
$CaCO_3$ equivalent	0.03

*Significant at 5% level.
**Significant at 1% level.

ysed to determine the relationships between these effects and the soil properties listed in Table 1. Simple correlation analyses showed that percent inhibition of urea hydrolysis by PPD was negatively correlated with organic C content ($r = -0.68$**), total N content ($r = -0.74$**), cation-exchange capacity ($r = -0.65$**), clay content ($r = -0.64$**) and surface area ($r = -0.60$*) and was positively correlated with sand content ($r = 0.66$**), but was not significantly correlated with pH, silt content, urease activity or $CaCO_3$ equivalent (Table 6).

Correlation analyses also showed that organic C content was highly correlated with total N content ($r = 0.95$**), and that cation-exchange capacity was highly correlated with both organic C content ($r = 0.76$***) and total N content (0.90***). Because organic C content and total N content are indexes of organic-matter content and because cation-exchange capacity is closely related to organic-matter content, the highly-significant simple correlations between percent inhibition of urea hydrolysis by PPD and organic C content, total N content and cation-exchange capacity (Table 6) suggest that organic-matter content accounts for most of the observed variation in the effectiveness of PPD to retard urea hydrolysis in different soils.

Multiple-regression analyses of the data obtained with the 15 soils studied showed that the variation in percent inhibition of urea hydrolysis by PPD (5 $\mu g\ g^{-1}$) was best accounted for ($R^2 = 88\%$) by the following equation (a):

$$PI = 206.67 - 17.67\ (\text{organic C content}) + 0.35\ (\text{urease activity}) - 12.17\ (\text{pH}) - 2.65\ (\text{clay content}) + 0.37\ (\text{surface area}) - 0.42\ (\text{silt content}) + 1.49\ (\text{cation-exchange capacity}) \quad (a)$$

where PI = percent inhibition of urea hydrolysis. The numerical coefficients (b-values) of the soil properties in this equation and their significance are reported in Table 7.

It is evident from Table 7 that most of the variation in percent inhibition by PPD could be accounted for by organic C content, urease activity, pH, cation-exchange capacity, clay content and surface area. It is noteworthy that the soil property with the most significant numerical coefficient in equation (a) was organic C content (Table 7). This finding is in harmony with the simple correlation analyses reported in Table 6 and supports the conclusion that the effectiveness of PPD to retard urea hydrolysis in soils tends to increase with decrease in soil organic-matter content.

Effects of amount of inhibitor, time or temperature

A study of the effects of different amounts of PPD on urea hydrolysis in 15 soils showed that percent inhibition of urea hydrolysis by PPD increased with the amount of PPD applied and that the effect of

Table 7. Numerical coefficients (b-values) of soil properties in equation and significance of these coefficients

Source	b-value
Intercept	206.67
Organic C content	−17.67**
Surface area	0.37
Urease activity	0.35
pH	−12.17*
Clay content	−2.65*
Silt content	−0.42
Cation-exchange capacity	1.49

*Significant at 5% level.
**Significant at 1% level.

Table 8. Effects of different amounts of PPD on urea hydrolysis in soils[a]

Soil	Amount of PPD added ($\mu g\ g^{-1}$ soil)													
	0.2		0.5		1.0		3.0		5.0		10.0		25.0	
	2 d	10 d	2 d	10 d	2 d	10 d	2 d	10 d	2 d	10 d	2 d	10 d	2 d	10 d
	% retardation of urea hydrolysis													
Storden	63	19	74	22	78	31	83	44	93	46	94	69	99	78
Rosebud	54	49	63	55	73	56	84	62	95	73	96	79	99	86
Ida	62	23	72	24	87	31	87	37	96	43	96	73	98	81
Canyon	64	43	74	54	91	56	94	57	96	67	97	78	98	86
Lindley	59	48	61	58	69	68	85	71	97	80	98	90	99	91
Dickinson	88	1	90	18	91	27	92	53	97	61	98	77	98	86
Muscatine	81	2	87	14	88	25	90	27	95	38	95	51	99	67
Indian Head	64	0	67	0	75	9	81	17	91	32	93	44	98	53
Moody	49	42	65	53	70	54	88	61	95	67	98	78	98	83
Nicollet	66	36	77	48	85	58	91	65	93	67	95	76	98	85
Hayden	84	0	91	18	94	28	94	32	95	43	99	59	99	79
Harps	41	0	47	0	60	0	73	0	85	5	91	12	95	70
Clyde	48	0	61	0	67	0	77	0	88	0	90	13	94	44
Okoboji (pH 6.3)	54	0	65	0	72	6	77	13	86	36	89	44	95	69
Okoboji (pH 4.6)	19	0	31	0	37	1	55	6	56	18	59	25	70	30
Average	60	18	68	24	76	30	84	36	91	45	93	58	96	71

[a] 5 g samples of soil were incubated (20°C; 2 ml water) for 2 or 10 days (d) after treatment with 10 mg urea and different amounts of PPD.

Table 9. Effect of time on effectiveness of PPD to retard urea hydrolysis in soils[a]

Soil	Time (days)					
	2	3	7	10	14	21
	% retardation of urea hydrolysis by PPD					
Storden	93	90	81	46	33[b]	0[b]
Rosebud	95	94	86	73[b]	57[b]	38[b]
Ida	96	96	64	43[b]	17[b]	0[b]
Canyon	96	92	90	66[b]	51[b]	27[b]
Lindley	97	96	90	80	58[b]	40[b]
Dickinson	97	96	93[b]	61[b]	8[b]	0[b]
Muscatine	95	95	75[b]	38[b]	9[b]	0[b]
Indian Head	91	92	63[b]	32[b]	0[b]	0[b]
Moody	95	86	80	67	54[b]	39[b]
Nicollet	93	94	86[b]	67[b]	50[b]	15[b]
Hayden	95	95[b]	84[b]	43[b]	11[b]	0[b]
Harps	85	83[b]	50[b]	5[b]	0[b]	0[b]
Clyde	88[b]	82[b]	39[b]	0[b]	0[b]	0[b]
Okoboji (pH 6.3)	86	86	61[b]	36[b]	0[b]	0[b]
Okoboji (pH 4.6)	56	54	37[b]	18[b]	0[b]	0[b]
Average	91	89	72	45	23	11

[a] 5 g samples of soil were incubated (20°C; 2 ml water) for various times after treatment with 10 mg urea and 0 or 25 μg PPD.

[b] Complete hydrolysis of urea was observed in the absence of PPD.

Table 10. Effect of temperature on effectiveness of PPD to retard urea hydrolysis in soils[a]

Soil	Time of hydrolysis (days)	Temperature (°C)			
		10	20	30	40
		% inhibition of urea hydrolysis			
Rosebud	3	92	94	82	63
	7	87	86	66	1
Dickinson	3	97	96	70	14
	7	96	93	0	0
Indian Head	3	90	92	41	18
	7	84	63	0	0
Moody	3	93	86	72	59
	7	82	80	51	0
Nicollet	3	96	94	75	58
	7	94	86	51	4
Harps	3	82	83	12	0
	7	64	50	0	0
Average	3	91	90	59	35
	7	85	76	28	1

[a] 5 g samples of soil treated with 10 mg urea and 25 μg PPD were incubated (2 ml water) at temperature specified for 3 or 7 days.

increasing the amount of PPD applied was considerably more marked after 10 days than after 2 days (Table 8). It is noteworthy that as little as 0.2 μg PPD g^{-1} soil markedly inhibited urea hydrolysis in 2 days in the 15 soils studied and substantially retarded urea hydrolysis in 10 days in seven of these soils.

The results obtained in a study of the effect of PPD (5 $\mu g\ g^{-1}$ soil) on urea hydrolysis in 15 soils incubated for various times after treatment with urea (Table 9) show that the inhibitory effect of PPD on urea hydrolysis decreased markedly with time and that, on the average, the percent inhibition of urea hydrolysis by PPD observed after 10 days was only about half of that observed after 2 days.

A study of the effect of PPD (5 $\mu g\ g^{-1}$ soil) on urea hydrolysis in six soils incubated at various temperatures for 3 and 7 days showed that the inhibitory effect of PPD on urea hydrolysis decreased markedly with increase in temperature from 10 to 40°C and that the effect of temperature on inhibition of urea hydrolysis by PPD was considerably greater after 7 days than after 3 days (Table 10).

To summarize, our work has shown that the effect of PPD on urea hydrolysis in soils increases with the amount of PPD applied, decreases with time and with increase in temperature, and tends to increase with decrease in soil organic-matter content.

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