

Potential phytotoxicity associated with the use of soil urease inhibitors

[phenylphosphorodiamidate/*N*-(*n*-butyl)thiophosphoric triamide]

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ABSTRACT Recent work in our laboratory showed that the adverse effect of urea fertilizer on seed germination and seedling growth in soil is due to ammonia produced through hydrolysis of urea by soil urease ($\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2$) and can be eliminated by amending the fertilizer with a small amount of a urease inhibitor such as *N*-(*n*-butyl)thiophosphoric triamide or phenylphosphorodiamidate. Continuation of this work showed that these inhibitors can induce leaf-tip necrosis in plants. Research to account for this phytotoxicity indicated that it resulted from an accumulation of toxic amounts of urea in plants through inhibition of urease activity by *N*-(*n*-butyl)thiophosphoric triamide and phenylphosphorodiamidate. Support for this conclusion was provided by experiments showing that these urease inhibitors increased both leaf-tip necrosis and urea concentrations in wheat (*Triticum aestivum* L.) and sorghum [*Sorghum bicolor* (L.) Moench] plants grown in soils treated with urea and that the necrotic areas of such plants had a much higher concentration of urea than did the nonnecrotic areas. The potential of urease inhibitors for inducing phytotoxicity should not preclude their use to eliminate the adverse effects of urea fertilizers on seed germination and seedling growth in soil because the ammonia produced through hydrolysis of urea fertilizer by urease is much more detrimental to plant growth than is the urea accumulation induced by urease inhibitors.

Although urea has significant advantages as a nitrogen fertilizer and is now the most extensively used fertilizer in world agriculture (1–3), there are problems associated with its use (4, 5). One of these problems is that urea has adverse effects on seed germination and seedling growth in soil (6). Recent work in our laboratory showed that these adverse effects are due to ammonia produced through hydrolysis of urea fertilizer by soil urease and can be eliminated by amending the fertilizer with a small amount of a soil urease inhibitor such as *N*-(*n*-butyl)thiophosphoric triamide (NBPT) or phenylphosphorodiamidate (PPD) (7).

We report here the results of studies to investigate the possibility that amendment of urea fertilizers with urease inhibitors such as NBPT or PPD might induce leaf-tip necrosis in plants. This possibility was investigated because Eskew *et al.* (8) observed in recent work with soybeans that a reduction in leaflet urease activity induced by a deficiency of nickel (an essential component of urease) can lead to leaflet-tip necrosis and suggested that this necrosis resulted from an accumulation of toxic amounts of urea in the leaflets.

MATERIALS AND METHODS

The studies reported were performed with seeds of wheat (*Triticum aestivum* L.) and sorghum [*Sorghum bicolor* (L.)

Table 1. Properties of soils used

Soil	pH	Organic carbon, %	Sand, %	Clay, %	CCE, %	Urease activity
Buckney	8.3	0.7	80	6	30	26
Sparta	5.5	0.8	75	9	0	20

CCE, CaCO_3 equivalent. Urease activity is expressed as μg of urea hydrolyzed per hr per g of soil at 37°C.

Moench]. The seeds used were obtained from the Iowa State University Seed Laboratory.

The soils used (Table 1) were surface (0–15 cm) samples of Entic Hapludolls of the Buckney and Sparta series. Before use, each sample was air-dried and crushed to pass through a 2-mm screen. The soil analyses reported in Table 1 were performed as described by Zantua and Bremner (9).

NBPT was obtained from Allied (Solvay, NY). PPD was obtained from K & K Lab Division, ICN. All other chemicals used were obtained from Fisher (Itasca, IL).

The following procedure was used to study the effects of PPD and NBPT on growth, leaf-tip necrosis, and urea content of sorghum and wheat plants grown in soils treated with urea. Fifteen seeds of the plant under study were placed 2 cm below the surface of soil in a plastic pot (75 × 120 mm). These pots contained 500 g of air-dried soil that had been moistened with 25 ml of water containing 20 mg of K_2SO_4 and 20 mg of NaH_2PO_4 . After placement of the seeds, the pots were treated with 50 ml of water containing 0.5 g of urea (control) or with 50 ml of water containing 0.5 g of urea plus 0.05, 0.50, or 5.0 mg of PPD or NBPT. The pots were then placed in a growth chamber that was maintained at 22°C and illuminated 12 hr/day. The pots were watered daily to replace water lost by evaporation, as determined by weighing the pots. After 21 days, the plants were removed from the pots, rinsed with water to remove soil particles, dried for 3 days at 65°C, and weighed. Leaf-tip necrosis was assessed by separating the necrotic portions of the plant shoots from the nonnecrotic portions, weighing both portions, and calculating the percentage of leaf-tip necrosis as (weight of necrotic portions/weight of necrotic plus nonnecrotic portions) × 100. Urea in the plant shoots was determined by grinding the necrotic and nonnecrotic portions of the shoots with 2 M KCl containing 5 μg of phenylmercuric acetate per ml (40 ml of KCl solution per g of plant tissue) in a mortar, centrifuging the extract at 18,000 × *g* for 30 min, and analyzing the extract for urea as described by Mulvaney and Bremner (10).

The procedure described was modified to compare the effects of PPD and NBPT on leaf-tip necrosis and urea content of wheat plants grown in Buckney soil and autoclaved Buckney soil, the modification being that the pots containing 500 g of air-dried soil moistened with 25 ml of

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Abbreviations: PPD, phenylphosphorodiamidate; NBPT, *N*-(*n*-butyl)thiophosphoric triamide.

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Table 2. Effects of different amounts of PPD and NBPT on growth of wheat plants in soils treated with urea and on leaf-tip necrosis and urea content of these plants

Urease inhibitor	Inhibitor added, mg per 100 mg of urea	Growth in 21 days, g (dry wt) per pot	Leaf-tip necrosis, % dry wt	Urea content, % dry wt
Buckney soil				
None	—	1.1	0	<0.01
PPD	0.01	1.4	0	<0.01
	0.10	3.2	0	<0.01
	1.00	4.7	2.1	0.03
NBPT	0.01	5.7	2.9	0.04
	0.10	6.5	6.9	0.35
	1.00	3.4	24.5	1.46
Sparta soil				
None	—	1.3	0	<0.01
PPD	0.01	2.0	0	<0.01
	0.10	3.3	0.7	0.02
	1.00	4.4	4.1	0.05
NBPT	0.01	4.7	2.8	0.04
	0.10	5.3	6.1	0.31
	1.00	6.8	9.8	0.45

The soil concentration of urease inhibitor was equivalent to 0.10, 1.0, or 10 μg per g of soil. The soil concentration of urea was equivalent to 1 mg per g of soil.

water containing 20 mg of K_2SO_4 and 20 mg of NaH_2PO_4 were treated with 50 ml of water containing 0–0.5 g of urea or with 50 ml of water containing 0–0.5 g of urea and 2.5 mg of PPD or NBPT. The autoclaved soils used were obtained by heating air-dried soils at 120°C for 2 hr. They exhibited no urease activity when analyzed by the nonbuffer method of assaying soil urease activity described by Zantua and Bremner (9).

The procedure used to study the effects of PPD and NBPT on urease activity in wheat and sorghum plants grown in soils was similar to that described for study of the effects of these compounds on growth, leaf-tip necrosis, and urea content of wheat and sorghum plants grown in soils treated with urea, but the soils used were not treated with urea, and the shoots of the plants were removed from the pots after 21 days and

Table 3. Effects of different amounts of PPD and NBPT on growth of sorghum plants in soils treated with urea and on leaf-tip necrosis and urea content of these plants

Urease inhibitor	Inhibitor added, mg per 100 mg of urea	Growth in 21 days, g (dry wt) per pot	Leaf-tip necrosis, % dry wt	Urea content, % dry wt
Buckney soil				
None	—	0.5	0	<0.01
PPD	0.01	0.9	0	<0.01
	0.10	3.3	0	<0.01
	1.00	6.8	2.9	0.04
NBPT	0.01	5.4	1.0	0.02
	0.10	6.3	6.8	0.30
	1.00	4.1	22.4	1.40
Sparta soil				
None	—	0.8	0	<0.01
PPD	0.01	3.5	0	<0.01
	0.10	3.9	0	<0.01
	1.00	4.5	4.2	0.04
NBPT	0.01	5.0	1.5	0.03
	0.10	6.0	3.7	0.09
	1.00	6.5	8.2	0.32

The soil concentration of urease inhibitor was equivalent to 0.10, 1.0, or 10 μg per g of soil. The soil concentration of urea was equivalent to 1 mg per g of soil.

Table 4. Urea content of necrotic and nonnecrotic portions of wheat and sorghum plants grown in soils treated with urea or with urea plus PPD or NBPT

Plant	Urease inhibitor	Urea content, % dry wt	
		Necrotic portion of plants	Nonnecrotic portion of plants
Buckney soil			
Sorghum	None	—	<0.01
	PPD	1.4	<0.01
	NBPT	6.2	0.02
Wheat	None	—	<0.01
	PPD	0.5	<0.01
	NBPT	5.9	0.02
Sparta soil			
Sorghum	None	—	<0.01
	PPD	0.5	<0.01
	NBPT	3.9	<0.01
Wheat	None	—	<0.01
	PPD	0.6	<0.01
	NBPT	4.6	<0.01

The soil concentration of urease inhibitor was equivalent to 10 μg per g of soil. The soil concentration of urea was equivalent to 1 mg per g of soil.

immediately weighed and analyzed for urease activity as described by Hogan *et al.* (11).

All experiments were performed in quadruplicate.

RESULTS AND DISCUSSION

Table 2 shows the results obtained in a study of the effects of different amounts of PPD and NBPT on growth, leaf-tip necrosis, and urea content of wheat plants grown in soils treated with urea, and Table 3 shows the results of a similar study with sorghum plants. The data reported show that NBPT and PPD increased both leaf-tip necrosis and urea content of wheat and sorghum plants grown in soils treated with urea. Statistical analysis of the data showed that the leaf-tip necrosis induced by PPD and NBPT was highly correlated ($r = 0.99$) with the urea accumulation induced by these compounds, which indicates that the necrosis observed resulted from accumulation of toxic amounts of urea through inhibition of urease activity by PPD and NBPT. NBPT was

Table 5. Effects of PPD and NBPT on leaf-tip necrosis and urea content of wheat plants grown in Buckney soil and autoclaved Buckney soil treated with different amounts of urea

Urease inhibitor	Urea added, μg per g of soil	Leaf-tip necrosis, % dry wt		Urea content, % dry wt	
		BS	ABS	BS	ABS
None	0	0	0	<0.01	<0.01
	125	0	0	<0.01	0.01
	250	0	0	<0.01	0.02
	500	0	0.5	<0.01	0.05
PPD	1000	0	13.0	<0.01	1.24
	0	0	0	<0.01	<0.01
	125	0	0	<0.01	<0.01
	250	0	0.1	<0.01	0.03
NBPT	500	0	1.0	0.01	0.10
	1000	1.3	19.0	0.04	2.79
	0	0	0	<0.01	<0.01
	125	0	2.0	0.01	0.09
NBPT	250	0.6	7.0	0.05	0.74
	500	10.0	20.0	1.37	3.27
	1000	27.0	56.0	2.60	6.67

The soil concentration of urease inhibitor was equivalent to 5 μg per g of soil. BS, Buckney soil; ABS, autoclaved Buckney soil.

superior to PPD for reducing the adverse effect of urea fertilizer on early plant growth in soil when these inhibitors were applied at the rate of 0.01 or 0.10 mg per 100 mg of urea to the Buckney or Sparta soil and at the rate of 1.00 mg per 100 mg of urea to the Sparta soil. However, the growth of wheat and sorghum plants observed when NBPT was applied at the rate of 1.00 mg per 100 mg of urea to the Buckney soil was less than that observed with the same rate of PPD, presumably because NBPT caused much more extensive leaf-tip necrosis than did PPD applied at the same rate (Tables 2 and 3).

A study of the effects of PPD and NBPT on the urea content of necrotic and nonnecrotic areas of wheat and sorghum plants grown in soils treated with urea showed that the urea detected in these plants was confined almost exclusively to the necrotic areas (Table 4). This is further evidence that the necrosis induced by these urease inhibitors resulted from accumulation of toxic amounts of urea through inhibition of urease activity.

Table 5 shows that autoclaving of soil to destroy its urease activity induced leaf-tip necrosis and urea accumulation in wheat plants grown in soil treated with urea and also markedly increased leaf-tip necrosis and urea accumulation in these plants when the soil was treated with urea and PPD

Table 6. Effects of different amounts of PPD and NBPT on urease activity in shoots of wheat and sorghum plants grown in soils for 21 days

Urease inhibitor	Inhibitor added, μg per g of soil	Urease activity in plant shoot	
		Sorghum	Wheat
Buckney soil			
None	—	2.18	2.44
PPD	0.1	2.22	2.49
	1.0	2.19	2.41
	10.0	2.03	2.24
NBPT	0.1	2.06	2.24
	1.0	1.94	1.97
	10.0	1.83	1.56
Sparta soil			
None	—	2.32	2.63
PPD	0.1	2.33	2.60
	1.0	2.28	2.55
	10.0	2.09	2.36
NBPT	0.1	2.04	2.42
	1.0	1.95	2.39
	10.1	1.90	2.29

Urease activity is expressed as μmol of $\text{NH}_3\text{-N}$ produced per hr per g of plant tissue through hydrolysis of urea by plant urease (30°C).

or NBPT. This indicates that the leaf-tip necrosis observed in plants grown in soils treated with urea and PPD or NBPT resulted primarily from inhibition of soil urease activity by PPD and NBPT. This conclusion is supported by the experiments reported in Table 6, which showed that PPD and NBPT did not significantly decrease urease activity in wheat or sorghum plants grown in soils for 21 days. It also is supported by the finding that the leaf-tip necrosis induced by NBPT exceeded that induced by the same rate of PPD because NBPT is more effective than PPD for inhibition of urease activity in soil (12).

In summary, the work reported shows that PPD and NBPT can induce leaf-tip necrosis in plants grown in soils treated with urea and indicates that this necrosis is due to accumulation of toxic amounts of urea in such plants through inhibition of urease activity. However, extensive leaf-tip necrosis was observed only when the soil concentrations of PPD and NBPT markedly exceeded those likely to exist when these compounds are used as fertilizer amendments to reduce problems encountered in use of urea as a fertilizer. We conclude that the potential of urease inhibitors for inducing phytotoxicity should not preclude their use to eliminate the adverse effects of urea fertilizers on seed germination and seedling growth in soil because it is evident from Tables 2 and 3 that the ammonia produced through hydrolysis of urea fertilizer by urease is much more detrimental to plant growth than is the urea accumulation induced by urease inhibitors.

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