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Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease

(biuret/phenylphosphorodiamidate)

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ABSTRACT **Studies using seeds of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), and corn (*Zea mays* L.) indicated that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease and is not due to urea itself, to urea fertilizer impurities such as biuret, or to nitrite formed by nitrification of urea nitrogen. Support for this conclusion was obtained from (*i*) comparison of the effects on seed germination in soil of purified urea, urea fertilizers, urea fertilizer impurities, and compounds formed by enzymatic and microbial transformations of urea in soil; (*ii*) studies showing that ammonia volatilized from soils treated with urea completely inhibited germination of seeds close to, but not in contact with, these soils; and (*iii*) experiments showing that the adverse effect of urea fertilizer on seed germination in soil was completely eliminated when the soil was autoclaved to destroy urease or was treated with phenylphosphorodiamidate to inhibit soil urease activity before treatment with urea fertilizer.**

Although urea has significant advantages as a nitrogen (N) fertilizer and is now used extensively in world agriculture, there are problems associated with its use (1–3). One of these problems is that urea fertilizers can have adverse effects on seed germination, seedling growth, and early plant growth in soil (4–6). Studies to account for these effects have given divergent results, and several explanations have been advanced for each effect (6–11). For example, studies to account for the adverse effect of urea fertilizer on seed germination have suggested that it is due to urea fertilizer impurities such as biuret (7), to cyanate formed by isomerization of urea in aqueous solution (8), to the high pH or the high concentration of ammonium ions resulting from hydrolysis of urea fertilizer by soil urease (9–11), to the ammonia produced by urea hydrolysis (10, 11), or to nitrite produced through nitrification of urea N by soil microorganisms (10, 11).

We report here evidence that the adverse effect of urea fertilizer on seed germination in soil is due to the ammonia formed through hydrolysis of urea by soil urease ($NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$).

MATERIALS AND METHODS

The seed germination studies reported were performed with seeds of barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), rye (*Secale cereale* L.), and wheat (*Triticum aestivum* L.). Wheat seeds were obtained from Merschman Seed and Fertilizer (West Point, IA), and corn seeds were obtained from Mike Brayton Seeds (Ames, IA). The other seeds used were obtained from May Seed and Nursery (Shenandoah, IA).

The soils used (Table 1) were surface (0–15 cm) samples of Entic Hapludolls of the Buckney and Sparta series and Typic Hapludolls of the Dickinson and Clarion series. Before use, each sample was air-dried and crushed to pass through a 2-mm screen. The analyses reported in Table 1 were performed as described by Zantua and Bremner (12).

Table 1. Properties of soils used

Soil		pH	Organic carbon, %	Sand, %	Silt, %	Clay, %	CCE,† %
Series	Subgroup*						
Buckney	EH	8.3	0.7	80	14	6	30
Sparta	EH	5.5	0.8	75	16	9	0
Dickinson	TH	6.3	1.9	45	42	13	0
Clarion	TH	6.2	2.2	51	31	18	0

*EH, Entic Hapludoll; TH, Typic Hapludoll.
†CCE, $CaCO_3$ equivalent.

The following procedure, based on the rules for seed testing published by the Association of Official Seed Analysts (13), was used to study the effects of different compounds on germination of seeds on germination paper and soil. Two 85-mm discs of germination paper (Steel Blue Anchor seed germination blotter, Anchor Paper, St. Paul, MN) or 40 g of air-dried soil were placed in a 15 mm × 100 mm Petri dish (Fisher Scientific) and moistened with 10 ml of water (control) or with 10 ml of water containing the compound(s) under study. Seeds (25 with corn, 100 with others) were placed between the discs of germination paper or on the soil, and the Petri dish was covered with a lid and kept in the dark for 7 days in an incubator maintained at 20°C. The number of germinated seeds was then counted and calculated as a percentage of the number of seeds sown. The criterion for germination was the emergence of a radicle and a coleoptile that were longer than the seed.

To determine the effect of ammonia volatilized from soils treated with urea on germination of seeds close to, but not in contact with, these soils, air-dried soil (10 g) was placed in the outer compartment of a modified Conway microdiffusion unit (14) and treated with 4 ml of water (control) or with 4 ml of water containing 4 mg of urea and 0 or 40 μg of phenylphosphorodiamidate (PPD). Twenty seeds of barley, corn, rye, or wheat were placed on moist germination paper in the inner compartment of the unit. The unit was then covered with a lid and placed in an incubator at 20°C for 7 days. The number of germinated seeds was then counted. All germination tests reported were performed in quadruplicate.

Urea fertilizers were obtained from C. F. Industries (Long Grove, IL), Cominco American (Spokane, WA), and Farmland Industries (Kansas City, MO). Purified urea was obtained from Fisher Scientific. PPD was obtained from ICN, and jack bean urease (urea amidohydrolase, EC 3.5.1.5) was

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Abbreviation: PPD, phenylphosphorodiamidate.
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Table 2. Effects of different amounts of purified urea, urea fertilizers, and urea fertilizer impurities on germination of seeds in Dickinson soil

Substance added to soil	Amount added, mg/g of soil	% germination of seeds in 7 days			
		Barley	Corn	Rye	Wheat
None (control)	—	94	86	95	92
Purified urea	0.5	95	85	95	94
(Fisher Scientific)	1.0	96	84	93	92
	1.5	77	74	63	38
	2.0	10	36	0	0
	2.5	0	0	0	0
Urea fertilizer	0.5	93	85	93	92
(Cominco American)	1.0	96	87	93	94
	1.5	78	73	66	42
	2.0	9	36	0	0
	2.5	0	0	0	0
Urea fertilizer	0.5	93	84	96	93
(C. F. Industries)	1.0	95	84	92	95
	1.5	80	69	63	45
	2.0	9	42	0	0
	2.5	0	0	0	0
Urea fertilizer	0.5	95	87	93	95
(Farmland Industries)	1.0	97	86	95	92
	1.5	76	74	59	46
	2.0	11	33	0	0
	2.5	0	0	0	0
Biuret	0.5	96	82	95	92
	1.0	93	85	93	94
	1.5	92	86	95	75
	2.0	95	83	92	47
	2.5	95	74	93	36
Cyanuric acid	0.5	95	86	96	95
	1.0	93	85	92	93
	1.5	92	87	93	91
	2.0	93	83	95	94
	2.5	97	86	94	92
		LSD* (0.05) 9.7			

*Least significant difference.

obtained from Sigma. All other chemicals used were obtained from Fisher Scientific.

RESULTS AND DISCUSSION

Comparison of the effects of different amounts of purified urea and urea fertilizers on seed germination in Dickinson soil showed that the adverse effects of different amounts of urea fertilizers were not significantly different from those of the corresponding amounts of purified urea (Table 2). Similar results were obtained with Buckney soil. The deduction from this observation that the adverse effect of urea fertilizers on seed germination is not due to fertilizer impurities was supported by our finding that biuret and cyanuric acid, compounds formed during manufacture of urea fertilizers, had little, if any, effect on seed germination in soil when compared with urea (Table 2). Urea fertilizers usually contain only small amounts of biuret and other impurities, and the concentrations of biuret and cyanuric acid after application of urea fertilizers to soil are much smaller than those tested in the work reported in Table 2.

Table 3. Effects of different forms of N on seed germination in Dickinson soil

Form of N added to soil	Amount of N added, mg/g of soil	% germination of seeds in 7 days			
		Barley	Corn	Rye	Wheat
None (control)	—	96	85	94	93
Urea	0.25	95	85	96	91
	0.50	85	83	79	63
	1.00	0	0	0	0
Ammonium	0.25	93	82	94	92
as $(NH_4)_2SO_4$	0.50	96	86	92	94
	1.00	94	84	96	91
Ammonium	0.25	95	83	95	91
as $(NH_4)_2CO_3$	0.50	93	85	94	93
	1.00	27	75	6	10
Ammonium	0.25	95	86	93	91
as NH_4OH	0.50	26	85	94	92
	1.00	0	0	0	0
Nitrite	0.10	95	85	93	92
as KNO_2	0.25	67	72	54	36
	0.50	16	3	17	11
	1.00	0	0	0	0
Nitrate	0.25	95	87	95	94
as KNO_3	0.50	93	83	95	93
	1.00	94	85	92	94
			LSD* (0.05) 11.7		

*Least significant difference.

Rotini (8) hypothesized that the phytotoxic effects of urea are due to ammonium cyanate formed by isomerization of urea in aqueous solution, but Low and Piper (15) calculated that only trace amounts of ammonium cyanate can occur in soils treated with urea, and Court *et al.* (11) could not detect cyanate in such soils. We found that cyanate as KCNO did not affect seed germination in soil even when it was applied at the rate of 1.0 mg/g of soil.

Table 4. Comparison of effects of urea on germination of seeds on germination paper, soils, autoclaved soils, and autoclaved soils treated with jack bean urease

Seeds	Urea conc., mg/ml of water	% germination of seeds in 7 days											
		Germination paper	Soils*				Autoclaved soils*†				Autoclaved soils treated with urease*†‡			
			D	C	B	S	D	C	B	S	D	C	B	S
Barley	0	95	95	93	94	96	95	94	96	94	94	96	97	93
	10	94	0	0	0	0	94	92	95	96	0	0	0	0
Corn	0	86	87	86	85	84	88	84	85	83	84	86	84	84
	10	84	0	0	0	0	86	84	82	85	0	0	0	0
Rye	0	94	95	94	96	93	95	96	93	94	93	96	93	95
	10	92	0	0	0	0	94	94	95	93	0	0	0	0
Wheat	0	94	93	92	94	95	93	93	92	94	92	94	91	92
	10	91	0	0	0	0	92	93	92	93	0	0	0	0

*D, Dickinson; C, Clarion; B, Buckney; S, Sparta.
†Soils were autoclaved at 120°C for 2 hr.
‡Autoclaved soils were treated with jack bean urease (15 international units/g of soil) immediately before addition of water containing urea.

Table 5. Effect of PPD on germination of seeds in soils treated with purified and fertilizer urea

Soil*	Soil treatment	% germination of seeds in 7 days			
		Barley	Corn	Rye	Wheat
D	None (control)	96	84	95	92
	Purified urea	0	0	0	0
	Purified urea + PPD	93	85	92	94
	Fertilizer urea	0	0	0	0
	Fertilizer urea + PPD	96	87	93	95
C	None (control)	93	82	93	93
	Purified urea	0	0	0	0
	Purified urea + PPD	97	84	95	91
	Fertilizer urea	0	0	0	0
	Fertilizer urea + PPD	96	85	93	94
B	None (control)	94	85	92	92
	Purified urea	0	0	0	0
	Purified urea + PPD	95	86	95	93
	Fertilizer urea	0	0	0	0
	Fertilizer urea + PPD	93	86	94	92
S	None (control)	95	83	95	93
	Purified urea	0	0	0	0
	Purified urea + PPD	93	86	96	91
	Fertilizer urea	0	0	0	0
	Fertilizer urea + PPD	94	85	92	94

Air-dried soil (40 g) in a Petri dish was moistened with 10 ml of water (control) or with 10 ml of water containing 100 mg of purified urea (Fisher Scientific) or fertilizer urea (Cominco American) and 0 or 1 mg of PPD. Seeds (25 with corn, 100 with others) were then placed on the moist soil, and the dish was covered with a lid and placed in an incubator at 20°C for 7 days.
*D, Dickinson; C, Clarion; B, Buckney; S, Sparta.

When urea is added to soil, it is hydrolyzed by soil urease with formation of ammonia and carbon dioxide, and the ammonium retained by the soil is subsequently oxidized to nitrite and nitrate by the nitrifying soil microorganisms. Table 3 shows the results of a comparison of the effects of different amounts of N as urea, ammonium, nitrite, and nitrate on seed germination in Dickinson soil (similar results were obtained with Sparta soil). The data reported show that, when applied at the rate of 1.00 mg/g of soil, N in the form of urea, ammonium hydroxide, or nitrite completely inhibited seed germination, N in the form of ammonium carbonate markedly reduced seed germination, and N in the form of ammonium sulfate or potassium nitrate had no effect on seed germination. Because tests showed that 0.1 M potassium carbonate (pH 10.8) had no effect on seed germination in soil whereas 0.1 M ammonium carbonate (pH 7.9) markedly inhibited seed germination, the data in Table 3 indicate that the differences in the results obtained with solutions of ammonium hydroxide, ammonium carbonate, and ammonium sulfate were related to the ammonia content rather than to the pH of these solutions. Our finding that N in the form of nitrite was more toxic to seeds than N in the form of urea merits attention because nitrite is produced during nitrification of urea N in soil, and nitrite accumulation has been observed in soils treated with urea. However, the nitrite N content of soils treated with urea rarely exceeds 0.05 mg/g of soil, and Table 3 shows that nitrite N added at the rate of 0.10 mg/g of soil had no effect on seed germination. Also, analyses for nitrite N by a colorimetric procedure (16) showed that the maximal concentration of nitrite N over 7 days after treatment of the Dickinson or Sparta soil with urea N at the rate of 1.00 mg/g of soil was 0.04 mg/g of soil, which is considerably below the concentration of nitrite N found to affect seed germination in these soils.

The conclusion from Table 3 that the adverse effect of urea on seed germination is due to ammonia formed through hydrolysis of urea by soil urease is supported by Table 4, which shows the results of a comparison of the effects of urea on germination of seeds on germination paper, soils, autoclaved soils, and autoclaved soils treated with jack bean urease. The data in Table 4 show that, whereas urea at the concentration tested completely inhibited seed germination in soils that had not been autoclaved to destroy urease activity or in soils that had been autoclaved and treated with jack bean urease, it had no effect on seed germination on germination paper or in soils that had been autoclaved at 120°C for 2 hr [destruction of soil urease activity by this treatment was confirmed by the method of Zantua and Bremner (12)].

Table 6. Inhibition of seed germination by ammonia volatilized from soils treated with urea

Soil*	Soil treatment	% germination of seeds in 7 days			
		Barley	Corn	Rye	Wheat
D	None (control)	95	86	95	93
	Urea	0	0	0	0
	Urea + PPD	94	85	95	92
C	None (control)	95	87	95	92
	Urea	0	0	0	0
	Urea + PPD	96	84	94	94
B	None (control)	94	85	94	94
	Urea	0	0	0	0
	Urea + PPD	96	86	96	92
S	None (control)	93	84	93	93
	Urea	0	0	0	0
	Urea + PPD	93	86	96	91

Air-dried soil (10 g) in the outer compartment of a microdiffusion unit was treated with 4 ml of water (control) or with 4 ml of water containing 4 mg of urea and 0 or 40 μg of PPD, and 20 seeds of the plant specified were placed on moist germination paper in the inner compartment of the unit. The unit was then covered with a lid and placed in an incubator at 20°C for 7 days.
*D, Dickinson; C, Clarion; B, Buckney; S, Sparta.

Further evidence that the adverse effect of urea on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease was provided by the experiments reported in Tables 5 and 6. Previous work in our laboratory (17) showed that the adverse effects of purified urea on germination of barley, oat, sorghum, and wheat seeds in soil could be eliminated by treating the urea with a small amount of PPD, a potent inhibitor of urease activity. Table 5 shows that the adverse effects of both purified and fertilizer urea on germination of barley, corn, rye, or wheat seeds in soil were completely eliminated when PPD was added to the urea. Table 6 shows that ammonia volatilized from soils treated with urea completely inhibited germination of seeds close to, but not in contact with, these soils and that no inhibition of seed germination by urea was observed when the soils were treated with PPD to prevent formation of ammonia through hydrolysis of urea by soil urease.

To summarize, the work reported shows that the adverse effect of urea on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease and is not due to urea itself, to urea fertilizer impurities such as biuret, or to nitrite formed through nitrification of urea N by soil microorganisms.

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