Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8189–8191, November 1989
Agricultural Sciences

Phytotoxicity of foliar-applied urea

(phenylphosphorodiamidate/soybeans/leaf-tip necrosis)

MICHAEL J. KROGMEIER, GREGORY W. MCCARTY, AND JOHN M. BREMNER*

Department of Agronomy, Iowa State University, Ames, IA 50011

Contributed by John M. Bremner, July 31, 1989

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 ($NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$) and can be eliminated by amending the fertilizer with a small amount of a urease inhibitor such as phenylphosphorodiamidate. Because the leaf-tip necrosis often observed after foliar fertilization of plants with urea is usually attributed to ammonia formed through hydrolysis of urea by plant urease, we studied the possibility that this necrosis could be eliminated or reduced by adding phenylphosphorodiamidate to the urea fertilizer. We found that, although addition of this urease inhibitor to foliar-applied urea increased the urea content and decreased the ammonia content and urease activity of soybean [*Glycine max.* (L.) Merr.] leaves fertilized with urea, it increased the leaf-tip necrosis observed after fertilization. We conclude that this necrosis resulted from accumulation of toxic amounts of urea rather than from formation of toxic amounts of ammonia. This conclusion was supported by our finding that the necrotic areas of soybean leaves treated with urea or with urea and phenylphosphorodiamidate contained much higher concentrations of urea than did the nonnecrotic areas.**

Foliar application of plant nutrients has potential advantages over soil application for fertilization of crops in that it may increase the efficiency of fertilizer use and allow relief of physiological stress (1). Interest in foliar fertilization of soybeans [*Glycine max* (L.) Merr.] was greatly stimulated by work based on the hypothesis that leaf senescence can be delayed and yield increased if nutrients are foliar-applied at seed development because this work indicated that foliar fertilization of soybeans during seed development could lead to substantial increases in the yields of this legume (2). However, most studies of foliar fertilization of soybeans during seed development have given disappointing results (1). For example, Gray (3) reviewed the results of 214 studies and concluded that foliar fertilization of soybeans usually led to a decrease in yield and to some degree of leaf-burn (leaf-tip necrosis).

It is generally believed that leaf-burn is at least partly responsible for the reduced yields observed after foliar fertilization (4) and that it is increased by low humidity and high temperatures and by use of a too concentrated fertilizer solution (2). It is also believed that the burn observed depends upon the form of nitrogen fertilizer used and that urea is less likely to cause foliage burn than other nitrogen fertilizers because it has a lower salt index and is more rapidly absorbed into the leaf (2, 5). However, leaf-burn has often been observed after foliar fertilization of plants with urea, and it has been reported that the leaf-burn observed with urea increases with leaf urease activity (6) and is due to the ammonia produced from urea by this activity (7).

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 ($NH_2\ CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$) and can be eliminated by amending the fertilizer with a small amount of a urease inhibitor such as phenylphosphorodiamidate (PPD) (8). Because the leaf-tip necrosis observed after foliar fertilization of soybeans with urea is usually attributed to ammonia formed through the urea hydrolysis by plant urease (2, 7, 9), we studied the possibility that it could be eliminated or reduced by adding phenylphosphorodiamidate to the foliar-applied urea fertilizer.

MATERIALS AND METHODS

The studies reported were performed with soybean [*Glycine max.* (L) Merr.] plants grown from seeds obtained from the Plant Pathology Department at Iowa State University. The plants were grown in a soil/sand/peat mixture, 2:1:1 (wt/wt).

PPD was obtained from K & K Laboratories, Division of ICN. All other chemicals used were obtained from Fisher.

The soybean plants used to study the effect of PPD on leaf-tip necrosis, urea content, urease activity, and ammonium content of soybean leaves treated with urea were grown from soybean seeds placed 2 cm below the surface of soil/sand/peat mixture in clay pots (400 g of mixture and one seed per pot). The pots were placed in a greenhouse and watered twice daily, and every 2 weeks the soil/sand/peat mixture in each pot was treated with 25 ml of water containing 20 mg of K_2SO_4 and 20 mg of NaH_2PO_4. After 42 days, leaves of the plants were treated with 0.6 ml of water; 0.6 ml of water containing 3, 9, 15, 21, or 27 mg of urea; or 0.6 ml of water containing 3, 9, 15, 21, or 27 mg of urea and 75 μg of PPD. The water used contained 1% Tween as a dispersant. After 7 days, three treated leaves were removed from each plant, and one was rinsed with distilled water, weighed, and analyzed for urease activity as described by Hogan *et al.* (10). The other two leaves were rinsed with distilled water, dried for 3 days at 65°C, and weighed. Leaf-tip necrosis was assessed by separating the necrotic portions of the dried leaves from the nonnecrotic portions, weighing both portions, and calculating the percentage of leaf-tip necrosis as (weight of the necrotic portion/weight of the necrotic plus nonnecrotic portions) × 100. Urea and ammonium were extracted by grinding the leaves in a mortar with 2 M KCl containing 5 μg of phenylmercuric acetate per ml (40 ml of the KCl solution per g of plant tissue) and by centrifuging (18,000 × *g* for 30 min) and filtering the resulting suspension. Urea in the filtered extract was determined by a colorimetric procedure (11), and ammonium was determined with an Orion (Boston) model 95-12 ammonia electrode (12).

All experiments reported were performed in triplicate.

Abbreviation: PPD, phenylphosphorodiamidate.
*To whom reprint requests should be addressed.

Table 1. Effect of PPD on leaf-tip necrosis, urea content, urease activity, and ammonium content of soybean leaves treated with urea

Foliar application		Leaf-tip	Urea		Ammonium
Urea, mg per leaf	PPD, μg per leaf	necrosis, % dry wt	content, % dry wt	Urease activity*	content, % dry wt
0	0	0	<0.01	15.2	0.015
0	75	0	<0.01	6.0	0.012
3	0	0	<0.01	14.0	0.023
3	75	1.2	0.10	8.2	0.009
9	0	0	<0.01	15.3	0.021
9	75	4.3	0.40	3.5	0.013
15	0	1.3	0.10	16.1	0.031
15	75	5.7	0.52	5.8	0.017

*Expressed as μmol of ammoniacal nitrogen produced per hr per g of plant tissue through hydrolysis of urea by plant urease (30°C).

Table 2. Urea content of necrotic and nonnecrotic portions of soybean leaves treated with urea or with urea and PPD

Foliar application		Leaf-tip	Urea content, % dry wt	
Urea, mg per leaf	PPD, μg per leaf	necrosis, % dry wt	Necrotic portion of leaves	Nonnecrotic portion of leaves
3	75	1.2	3.4	0.06
9	75	4.3	4.2	0.23
15	0	1.3	3.9	0.04
15	75	5.7	4.9	0.17
21	0	11.2	4.5	0.02
21	75	20.7	3.7	0.13
27	0	19.4	3.9	0.06
27	75	27.3	3.6	0.21

RESULTS AND DISCUSSION

Table 1 shows the results obtained in a study of the effect of PPD on leaf-tip necrosis, urea content, urease activity, and ammonium content of soybean leaves treated with urea. The data reported show that, although addition of PPD to foliar-applied urea increased the urea content and decreased the ammonium content and urease activity of soybean leaves fertilized with urea, it increased the leaf-tip necrosis observed after fertilization. Statistical analysis showed that the leaf-tip necrosis observed was highly correlated ($r = 0.98$) with urea content.

These observations indicate that the leaf-burn observed after foliar fertilization of soybean plants with urea is not due to formation of toxic amounts of ammonia through urea hydrolysis by plant urease but to an accumulation of toxic amounts of urea in these leaves. This conclusion is in harmony with the recent deduction by Eskew *et al.* (13) that the necrosis they observed with soybeans deficient in nickel (an essential component of urease) was due to an accumulation of toxic amounts of urea resulting from the low urease activity of the nickel-deficient plants.

Determination of the urea contents of necrotic and nonnecrotic portions of soybean leaves treated with urea or with urea and PPD showed that the concentration of urea was much higher in the necrotic than in the nonnecrotic portions of these leaves (Table 2). This is further evidence that the necrosis observed resulted from accumulation of toxic amounts of urea.

The increase in leaf-burn observed when PPD was added to urea applied to soybean leaves is illustrated by Fig. 1, which also shows that no leaf-burn was observed when PPD was applied to soybean leaves in the absence of urea.

In summary, the work reported shows that the leaf-tip necrosis commonly observed after fertilization of soybean leaves with urea is due to accumulation of toxic amounts of urea rather than formation of toxic amounts of ammonia in these leaves and that this necrosis is accordingly increased rather than decreased by addition of a urease inhibitor such as PPD to the urea fertilizer applied.

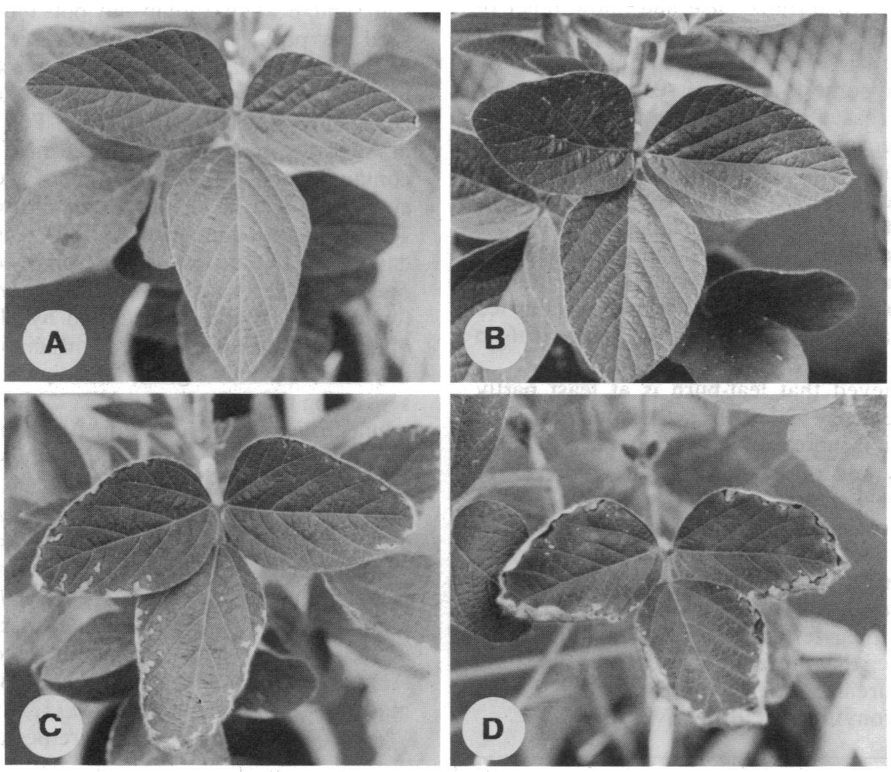

FIG. 1. Soybean leaves 7 days after treatment with 0.6 ml of water (*A*), 0.6 ml of water containing 75 μg of PPD (*B*), 0.6 ml of water containing 15 mg of urea (*C*), or 0.6 ml of water containing 15 mg of urea and 75 μg of PPD (*D*).

This work was supported in part by C.I.L., London, ON, and by the National Fertilizer Development Center, Tennessee Valley Authority, Muscle Shoals, AL. This is journal paper no. J-13637 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Projects 2655 and 2672).

1. Gray, R. C. & Akin, G. W. (1984) in *Nitrogen in Crop Production*, ed. Hauck, R. D. (Am. Soc. Agron., Madison, WI), pp. 579–584.
2. Garcia, R. L. & Hanway, J. J. (1976) *Agron. J.* **68,** 653–657.
3. Gray, R. C. (1977) *Situation 77* (Natl. Fertil. Dev. Ctr., Muscle Shoals, AL), Bull. Y-115.
4. Poole, W. D., Randall, G. W. & Ham, G. E. (1983) *Agron. J.* **75,** 201–203.
5. Rader, L. F., White, L. H. & Whittaker, C. W. (1943) *Soil Sci.* **55,** 201–218.
6. Hinsvark, O. N., Wittwer, S. H. & Tukey, H. B. (1953) *Plant Physiol.* **28,** 70–76.
7. Vasilas, B. L., Legg, J. O. & Wolf, D. C. (1980) *Agron. J.* **72,** 271–275.
8. Bremner, J. M. & Krogmeier, M. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4601–4604.
9. Harper, J. E. (1984) in *Nitrogen in Crop Production*, ed. Hauck, R. D. (Am. Soc. Agron., Madison, WI), pp. 165–182.
10. Hogan, M. E., Swift, I. E. & Done, J. (1983) *Phytochemistry* **22,** 663–667.
11. Mulvaney, R. L. & Bremner, J. M. (1979) *Commun. Soil Sci. Plant Anal.* **10,** 1163–1170.
12. Banwart, W. L., Tabatabai, M. A. & Bremner, J. M. (1972) *Commun. Soil Sci. Plant Anal.* **3,** 449–458.
13. Eskew, D. L., Welch, R. M. & Cary, E. E. (1983) *Science* **222,** 621–623.