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EFFECT OF FOLIAR APPLICATION OF UREA AND AMMONIUM NITRATE ON THE DRY WEIGHT AND

PROTEIN CONTENT OF MAIZE PLANTS

by

Alvaro Fiallos

A thesis submitted in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved :

Major Professor

Committee Member

Committee Member

Dean of Graduate Studies

UTAH STATE UNIVERSITY Logan, Utah

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Alvaro Fiallos

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ABS TRACT

Effect of Foliar Application of Urea and Ammonium Nitrate on the Dry Weight and Protein Content of Maize Plants

by

Alvaro Fiallos, Master of Science Utah State University, 1969

Major Professor: Dr. Frank B. Salisbury Department: Plant Science

Urea and ammonium nitrate were applied to leaves of maize plants growing in growth chambers on nutrient solutions containing three different concentrations of ammonium nitrate. Dry weights, and the soluble protein contents of leaves, stems and roots were measured.

Both urea and ammonium nitrate did increase the dry weights of leaves and stems when ammonium nitrate was used in the nutrient solutions (0.5 and 2.5 mM/liter). When nitrogen was not used in the nutrient solutions, no increments of dry weight occurred. The protein contents of leaves were increased for plants in the same nutrient solutions that produced increases in the dry weights, except that urea did not increase protein contents of stems.

The dry weights of roots were increased by foliar applied urea when ammonium nitrate was used in the nutrient solutions but not when the nutrient solutions were without nitrogen. Ammonium nitrate applied to the foliage did not increase the dry weights of roots. The protein contents of roots were not increased by urea or ammonium nitrate applied to the leaves.

The increments in the dry weights and protein contents given by foliar applied urea were superior to those of foliar applied ammonium nitrate, with the exception of the protein content of stems.

(38 pages)

INTRODUCTION

The application of nitrogen to the leaves of crops has become increasingly important, especially in those regions where high rainfall rapidly leaches out or hinders their application to the soil.

Of all conventional compounds used in fertilization, urea, because of its non polar organic properties, seems to be the most rapidly absorbed by the leaves (Hinsvark, Wittwer, and Tukey, 1953). In addition to this, urea also increases the permeability of the cuticular membrane, stimulating in this way the uptake of nutrients that are simultaneously applied (Yamada, Wittwer, and Bukovac, 1965; Yamada et al., 1965). These advantages of urea resulted in a complete concentration of interest of many workers on urea to such an extent that the other nitrogenous compounds have been largely neglected as foliar applicants.

Some nitrogen containing compounds such as ammonium nitrate have proven to be as effective or superior to urea when they are applied to the roots of crops (Templeman, 1961; Low and Piper, 1961; Court, Stephen, and Waid, 1962, 1964; Court et al., 1963; Devine and Holmes, 1963a, 1963b; Stephen and Waid, 1963a, 1963b; Pyl'neva and Mosolov, 1964). Furthermore, although foliar applications of ammonium nitrate gave no significant increases of dry weight of maize according to Thomas (1954), increases were reported by Ivanov (1959). In other crops, such as sugar beets, Thorne (1954, 1955, 1957) and Thorne and Watson (1956) have reported promotive effects of ammonium nitrate on the yield and quality of the crop.

The objectives of this experiment were to evaluate the effects of foliar applied ammonium nitrate compared with those of urea, when maize plants were grown in nutrient solutions containing three different concentrations of ammonium nitrate.

REVIEW OF LITERATURE

Since there are many reviews on the foliar nutrition of plants (Boynton, 1954; Wittwer and Teubner, 1959; Dimond, 1962; Wittwer, 1964; Franke, 1966; Mogilner and Orioli, 1967; Orioli, 1967), we will not present an exhaustive review here.

The nutrition of plants through the leaves and through the roots is clearly a similar process. Foliar application of urea causes a redistribution of nutrients flowing from the root system, their movement into lateral branches being stimulated, while movement in the main stem is suppressed (Shereverya, 1959). The effect of foliar applied nitrogen on other minerals is not yet clear. Some workers claim that it reduces the uptake of phosphate and potassium as well as nitrogen by maize roots (Pavlov, 1960; Pavlov and Ivanov, 1960; Barat and Das, 1962), while Dorogi (1967) reported an increased uptake of those elements. Later it was found that this effect was related to plant age and to the concentration of the elements available to the roots. Pavlov, Ivanov and Razuvaeva (1961) found that when maize plants were vegetative, foliar applied urea resulted in a delay in the absorption of nitrogen through the roots, but applications during the flowering period resulted in an enhancement. Grechukhina and Timofaeva (1961) reported that when the plants are growing in nutrient solutions high in nitrogen, foliar application of urea decreased the absorbtion of this nitrogen and slightly increased that of phosphate and potassium. Earlier, Thorne (1954) concluded

that when the element applied to the leaves is high in the soil, its own root absorption will be decreased.

The protein content and the dry weight of plants have been reported to increase when nitrogen compounds are applied to the leaves. Thorne and Watson (1956), working with sugar beets, found that leaf sprays with ammonium nitrate and urea increase the protein yield and the weight of the leaves. They also calculated that 20 to 30 per cent of the nitrogen in the leaf sprays was converted into protein in the leaf laminae. Increases of protein in spring wheat were produced when urea and ammonium nitrate were applied to the leaves (Shereverya, 1959). Pavlov (1960) reported that in maize, protein content in the leaves increased by 16-32 per cent, in the stem by 25-41 per cent, and in the grain by 8-12 per cent, compared with controls not receiving foliar nitrogen. He also found that alanine and glutamic acid in the root sap markedly decreased, and threonine, tyrosine, and phenylalanine also decreased, but in less proportion. (Pleshkov, Snmyreva, and Ivanko (1959) and Bekmukhamedova (1961) reported those amino acids as the most abundant and the first found in maize plants grown on normal concentrations of nitrogen.) Pavlov explained that when nitrogen is applied to the leaves, the newly formed sugars are utilized as acceptors of the sprayed nitrogen (keto acids), decreasing in this way the quantity of sugar translocated to the roots, even though net photosynthesis increases, and, as a consequence, decreases the quantity of the acceptor of \mathtt{NH}^+_Δ in the roots.

The superiority of root nutrition over foliar nutrition has

been reported (Thomas, 1954; Tueva, 1960; Forshey, 1963). Mogilner and Orioli (1967) concluded that nitrogen nutrition only through the leaves cannot produce a normal nitrogen metabolism of the whole plant, unless the leaves insure the translocation of adequate nitrogen to the roots, satisfying in this way their metabolic requirements. Some reports claim superiority (Rajat and Singh, 1963) and others similarity (Jones and Lancaster, 1967) of foliar nutrition to root nutrition.

MATERIALS AND METHODS

The maize variety Golden Bantam Cross was used, and the experiment was conducted under growth chamber conditions with 13 hours of light, 26 C ($\frac{+}{-}$ 1 C) day temperature, 20 C ($\frac{+}{-}$ 1 C) night temperature, and 70 per cent ($\frac{+}{-}$ 5 per cent) relative humidity.

Seeds were germinated on moist vermiculite in a plastic tray. After all the seedlings had emerged, modified Hoagland's Solution Number 1 (Hoagland and Arnon, 1950) containing no nitrogen was applied. (This solution will be referred to as Nutrient Solution.)

When the plants reached a length of 10 cm, they were transplanted to polyethylene pots (10x10x15 cm) with vermiculite. Three pots, containing 2 plants per pot, were set on plastic trays, forming a unit. No nitrogen was used at this time, because the effectiveness of foliar applied nitrogen has been found to be higher in this way than when nitrogen is used as a "starter" (Thomas, 1954). When all the plants recovered from transplanting, the first part of the experiment was conducted.

In order to find out the optimum concentration of nitrogen required under the present conditions, five concentrations (10, 6, 4, 2, and 0 mM/liter) of urea or ammonium nitrate were applied to the plants of each unit. The addition of one liter of nutrient solution with nitrogen was repeated every three days without further addition of water. Every six days the remaining solution was thrown away, and the trays were washed with distilled water. The plants grew for 30

days, after which they were harvested and analyzed (see below). The results are shown in Figures 1 through 4. Because of the lower yields of urea, it was decided to use only ammonium nitrate in the nutrient solution.

After the optimum nitrogen concentration was found, the main section of the experiment was conducted. The plants were grown as described in three different concentrations of ammonium nitrate: zero, one-tenth, and one-half of the optimum concentration. Three different concentrations of ammonium nitrate or urea were applied to the leaves of these plants: zero, 50, and 100 mM/liter. The latter is the maximum concentration that can be applied to the leaves of corn without causing damage (Thomas, 1954).

Foliar solutions were applied with a manual sprayer that produces a fine mist. In order to insure against contaminating the roots by the foliar solution, the pots were covered with plastic, and the stems were sealed with paraffin. The foliar solutions were applied every six days, making a total of four applications, applying about 40 ml of solution each time. Before and after each foliar application, one pot (two plants) was weighted in order to determine the quantity of solution that remains on the leaves of the plants. This quantity varied from 1 to 80 per cent of the applied solution as the plant growth increased.

Observations and measurements

Daily observations were conducted and the apparent differences of the plants noted. A sample of one unit consisting of 3 pots (6 plants) was taken at the time of the first application (zero time and



Figure 1. Effects of various concentrations of ammonium nitrate in nutrient solution on the dry weights of maize plants. Note optimum concentration at about 5 mM/liter.



Figure 2. Effects of various concentrations of ammonium nitrate in nutrient solution on the soluble protein content of maize plants. Note optimum concentration at about 5 mM/liter.



Figure 3. Effects of various concentrations of urea in nutrient solution on the dry weights of maize plants.



Figure 4. Effects of various concentrations of urea in nutrient solution on the soluble protein content of maize plants.

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zero growth). After 30 days the last sample was taken, consisting of all the remaining units. The plants were divided into leaves, stems, and roots, and the fresh weight was immediately measured after harvesting. Five plants, of each unit, were dried in an oven at 80 C for 10 hours and the dry weight determined. The remaining plants were ground in a high speed electric blender with distilled water. The mixture was then filtered with gentle suction in a Buchner funnel with filter paper No. 2. The filtered mixture was diluted with distilled water in a proportion of 1:10, and the Folin-Phenol Test (Lowry et al., 1951) was conducted in order to determine the soluble protein content of each part of the plant.

RESULTS

The plants growing without nitrogen showed a yellowish-green color that persisted during the entire time of the experiment; in the older leaves this color turned to complete yellow, and the tips dried out. The stems did not grow much, remaining very thin and giving to the plants a spindly look. All of the plants growing without nitrogen in the nutrient solution showed these same symptoms to some extent, even though they received foliar applied nitrogen. Plants growing in 0.5 mM/liter of nitrogen in the nutrient solution exhibited these deficiency symptoms slightly during the first two weeks of the experiments, after which they appeared healthy. Plants growing in 2.5 mM/liter nitrogen remained healthy and green. All of the plants growing in the same nitrogen concentration appeared identical.

Leaves

Figure 5 shows the dry weights of the leaves. The third treatment (100 urea-2.5) gave the largest increments above controls, and neither urea nor ammonium nitrate was effective when no nitrogen was given to the roots. The protein contents of the leaves as influenced by foliar applied urea and ammonium nitrate to plants growing in three levels of ammonium nitrate in the nutrient solutions are presented in Figure 6. In this case, the combination that resulted in the largest increment in protein content was 50 mM/l urea-2.5 mM/l $\rm NH_{4}NO_{3}$, followed by 100 mM/l urea-2.5 mM/l $\rm NH_{4}NO_{3}$. Protein contents





Figure 5. Dry weights of leaves as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.





Figure 6. Soluble protein content of leaves as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.

were increased markedly by the foliar solutions when no nitrogen was used in the root solutions.

The dry weights of the leaves were not significantly affected by the foliar solutions, variations being due to the nutrient solutions alone, but the protein contents were affected by both the foliar and the root solutions, producing a highly significant interaction (Table 1).

Source of	Degree of	Mean squ	ıare	F ratio		
variance	freedom	Dry weight	Protein	Dry weight	Protein	
Foliar solution	4	1.32	2.11	1.51	5.75*	
Root solution	2	96.94	22.44	111.04*	60.11*	
Interaction	8	0.77	3.57	0.88	9.73*	
Error	30	0.87	0.37			
Total	44					

Table 1. Analysis of variance of dry weight and protein content of leaves

*Significant difference at 0.05 per cent.

Stems

In Figures 7 and 8, the dry weights and protein contents of the stems as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions are presented. Apparently dry weights are increased more by the foliar application of urea



* mM of $\rm NH_4NO_3$ per liter of nutritive solution (root solution) ** mM per liter (foliar solution)

Figure 7. Dry weights of stems as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.





Figure 8. Soluble protein content of stems as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.

combined with 2.5 mM/l $\rm NH_4NO_3$ in the nutrient solution, but the protein content is higher with ammonium nitrate applied to the leaves and 2.5 mM/l $\rm NH_2NO_3$ in the nutrient solution.

Table 2 shows that the foliar and root solutions did have significant effects on the dry weight and protein contents of the stems, but they did not interact.

Source of	Degree of	Mean squ	lare	F ratio		
variance	freedom	Dry weight	Protein	Dry weight	Protein	
Foliar solution	4	3.17	2.05	3.57*	4.54*	
Root solution	2	48.89	21.26	48.19*	47.00*	
Interaction	8	1.78	0.84	2.00	1.86	
Error	30	0.89	0.45			
Total	44					

Table 2. Analysis of variance of dry weight and protein content of stems

*Significant difference at 0.05 per cent.

Roots

Figure 9 shows that 100 mM/l urea with 2.5 mM/l $\rm NH_4 NO_3$ root solution gave larger increments of dry weight, while the other treatments to the leaves were not significantly different. They can be grouped together by the concentrations of the nutrient solutions and independently of the compounds and the concentrations.





Figure 9. Dry weight of roots as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.

Figure 10 indicates that the protein contents of the leaves were affected only by the nutrient solutions and not by the foliar solutions.

Dry weights of the roots were significantly affected by foliar and root solutions, producing a significant interaction, but the protein contents were affected only by the root solutions (Table 3).

Source of	Degree of	Mean squ	uare	F ratio		
variance	freedom	Dry weight	Protein	Dry weight	Protein	
Foliar solution	4	1.16	0.05	4.11*	0.18	
Root solution	2	23.23	17.15	82.46*	57.62*	
Interaction	8	1.07	0.22	3.80*	0.73	
Error	30	0.28	0.30			
Total	44					

Table 3. Analysis of variance of dry weight and protein content of roots

*Significant difference at 0.05 per cent.





Figure 10. Soluble protein content of roots as a function of nitrogen concentrations applied as foliar sprays and supplied in nutrient solutions.

DISCUSSION AND CONCLUSIONS

In general, urea seems to give larger increments of dry weight and protein content than ammonium nitrate, even though the differences were not significant in some cases. This superiority of urea may be based upon its properties as reported by Hinsvark, Wittwer, and Tukey (1953), Yamada, Wittwer, and Bokovac (1965), and Yamada et al. (1965). It could also be a consequence of the use of ammonium nitrate in the nutrient solutions, affecting the foliar absorption of the same compound in the same way that one element may affect its own absorption (Thorne, 1954). This is supported by the report of Ivanov (1959), who found a reduction of the foliar surface area when ammonium nitrate was foliar-applied to maize growing in high nitrogen levels in the soil.

Ammonium nitrate, as compared with the controls (Table 4), did increase the dry weights and protein contents of leaves and stems; decreasing the dry weights of roots.

The protein contents of roots were not affected by either foliar applied urea or ammonium nitrate. This might be because of a low translocation of the nutrient to the roots from the leaves. Probably because of the age of the plants, the foliar-applied nitrogen was utilized entirely by the leaves. This was reported to be true for young tissues (Thorne, 1957).

The effects of urea and ammonium nitrate applied to the leaves was found to be independent of the concentration of the compound,

Leave	s	Stem	IS	Root	
Dry weight %	Protein %	Dry weight %	Protein %	Dry weight %	Protein %
138.74	111.28	171.01	66.67	117.80	98.78
118.08	101.22	131.36	127.27	82.63	101.83
120.66	134.45	188.76	88.89	111.44	110.97
126.94	100.00	132.54	117.68	87.29	100.00
100.00	100.00	100.00	100.00	100.00	100.00
	Leave Dry weight % 138.74 118.08 120.66 126.94 100.00	Leaves Dry weight Protein % % 138.74 111.28 118.08 101.22 120.66 134.45 126.94 100.00 100.00 100.00	Leaves Stem Dry weight Protein Dry weight % % % 138.74 111.28 171.01 118.08 101.22 131.36 120.66 134.45 188.76 126.94 100.00 132.54 100.00 100.00 100.00	Leaves Stems Dry weight Protein Dry weight Protein $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ 138.74 111.28 171.01 66.67 118.08 101.22 131.36 127.27 120.66 134.45 188.76 88.89 126.94 100.00 132.54 117.68 100.00 100.00 100.00 100.00	Leaves Stems Root Dry weight Protein Dry weight Protein Dry weight Protein Dry weight Dry weight Not weight Dry weight Not weight Dry weight Not weight Dry weight Dry weight Not weight </td

Table 4. Dry weight and protein content of maize plants as affected by foliar solutions as the source of variation

^amM per liter.

and always related to the higher concentration of the ammonium nitrate (2.5 mM/liter) in the root solution. To obtain the greatest increases in dry weights and protein contents of the plants, nitrogen had to be applied simultaneously by both methods.

The application of nitrogen to the leaves of young maize plants under field conditions might provide some needed nitrogen through the leaves, and the fraction that falls on the soil will be used as a supplement of the non-leached soil fertilizer. In this way the need of both applications will be partially fulfilled.

The application of 30 liters/Ha of a 1.25 M solution (most popular application of urea in the corn fields of Nicaragua) will provide almost the same quantity per unit plant as the sprayed solutions in our experiment (0.4 to 32 and 0.5 to 43 mg/plant of urea and ammonium nitrate respectively, taking 1 and 80 per cent of the total applied solution as the fraction that remains on the leaves).

With the use of the "Low Volume" system of aerial spray, we might be able to apply saturated solutions of urea or ammonium nitrate without much injury to the leaves, providing in this way more nitrogen to the leaves and to the soil. Further experiments are needed to make suitable field recommendations.

SUMMARY

Foliar applied ammonium nitrate increased dry weights of leaves and stems of maize plants but decreased dry weights of roots. Ammonium nitrate as a foliar applicant did not affect protein contents of leaves or roots, but increased protein contents of stems.

Foliar sprays of urea gave larger dry weight increments than ammonium nitrate for the different parts of the plant, but these differences were not significant for leaves. The protein contents of leaves were increased by foliar applied urea, being significantly different from protein contents due to ammonium nitrate. The protein contents of stems and roots were decreased by foliar applied urea.

The increments of dry weight and protein content were independent of the concentrations of the foliar-applied solutions. The interactions between foliar-applied and root-applied solutions were found to be significant for the dry weights of roots and the protein contents of leaves. Even though the interactions in the others were not significant, urea applied to leaves, combined with higher concentrations of ammonium nitrate in the nutrient solutions, gave better results in most cases. When ammonium nitrate was not used in the nutrient solutions, the foliar applicants increased neither dry weights nor protein contents.

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VITA

Alvaro Fiallos

Candidate for the Degree of

Master of Science

Thesis: Effect of Foliar Application of Urea and Ammonium Nitrate on the Dry Weight and Protein Content of Maize Plants

Major Field: Plant Science

Biographical Information:

- Personal Data: Born at Leon, Nicaragua, March 8, 1942, son of Mariano Fiallos and Soledad Oyanguren de Fiallos; married Maria Antonieta Gutierrez, October 24, 1964; three children--Alvaro, Ana Victoria, and Maria Antonieta.
- Education: Attended elementary school in Leon, Nicaragua; graduated from Instituto Nacional de Occidente (High School) in 1960; received the Bachelor of Science degree from Escuela Nacional de Agricultura y Ganaderia, Managua, with a major in agronomy, 1964. Completed requirements for the Master of Science degree, specializing in plant nutrition, at Utah State University in 1969.
- Professional Experience: 1965 to present, assistant professor at Biology Department, Universidad Nacional de Nicaragua, Leon.