EFFECTS OF NO_3^-, NH_4^+, AND UREA ON EACH OTHER'S UPTAKE AND INCORPORATION

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The purpose of these studies is to determine the optimal use by wheat plants of the N sources expected from processing biological waste products, NO_3^-, NO_2^-, NH_4^+, and urea. Our approach is to determine the uptake and metabolic products of each N source (from single and multiple component solutions), inhibitory effects of each, feedback inhibition, and overall in vivo regulation of the rates of assimilation of each by wheat plants. Previously, we have determined the interactions of NO_3^-, NO_2^-, and NH_4^+ on each other's uptake and incorporation. This report deals with urea assimilation and some of its effects on NO_3^- and NH_4^+ assimilation which have been completed to date.

MATERIALS AND METHODS

Plant Material. Wheat (Triticum aestivum cv. Yecora Rojo) seedlings were grown hydroponically. Seeds were surface sterilized in sodium hypochlorite (5% v/v) for 15 min, rinsed with distilled water, and germinated at 25°C in aerated deionized water in the dark. After 24 h, the

germinated seeds were spread on a layer of cheesecloth supported on a stainless steel screen suspended about 1 cm above the surface of 1 l of aerated 0.2 mM $CaSO_4$ solution and placed in the dark at 25°C. After 7 days, the seedlings were transferred to aerated one-quarter-strength Hoagland solution lacking N (3) and placed in a controlled environmental growth chamber. Seedlings were grown under conditions of continuous light for 3 d at 25°C or for 3 weeks under a 16-h photoperiod at 25°/15°C light/dark temperature. Photon flux density at the seedling canopy was 400 $\mu Em^{-2}s^{-1}$, and RH was maintained at 60-65%. In some of the continuous light experiments, the seedlings were transferred after 2 days to nutrient solutions containing 1 mM ^{14}C-urea, NO_3^-, or NH_4^+ (preinduced seedlings).

$\underline{NH_4^+, NO_3^-, \text{ and Urea Uptake}}$. Uptake of NH_4^+, NO_3^-, and urea were determined by following their disappearance from the uptake solution with time as described previously in studies of NO_3^- uptake (1).

$\underline{\text{In Vivo Assimilation of Absorbed } NH_4^+, NO_3^-, \text{ and Urea}}$. $\underline{\text{In vivo}}$ assimilation of absorbed NH_4^+, NO_3^-, or urea were determined simultaneously along with uptake. The difference between the total amount of NH_4^+, NO_3^-, or urea absorbed and

that accumulated in the seedling, root, or shoot was considered to be assimilated *in vivo*.

NH_4^+, NO_3^-, and Urea Analysis. The tissue was ground with a chilled mortar and pestle in 5 volumes of deionized water and centrifuged at 30,000 x g for 15 min. NH_4^+ in plant extracts was determined by fluorimetric detection, following separation by HPLC (20 mM KH_2PO_4, pH 6.2) on a Whatman Partisil-10-SCX cation exchange column by post column derivatization with o-phthaldialdehyde (OPA) (5) determined spectrophotometrically at 210 nm following separation b HPLC on a Whatman Partisil-10-SAX anion exchange column (6). Urea was determined both spectrophotometrically at 189 nm and radiometrically. Urea was determined in plant extracts by counting plant extracts both before and after addition of excess urease. Free urea was determined by difference.

RESULTS

Analyses. This year we developed an automated HPLC assay for the uptake of urea along with NO_3^-, NO_2^-, and NH_4^+ by wheat seedlings from a full component nutrient solution utilizing a microcomputer-based system (Fig. 1). Except for the metabolic studies where a tracer is required, this

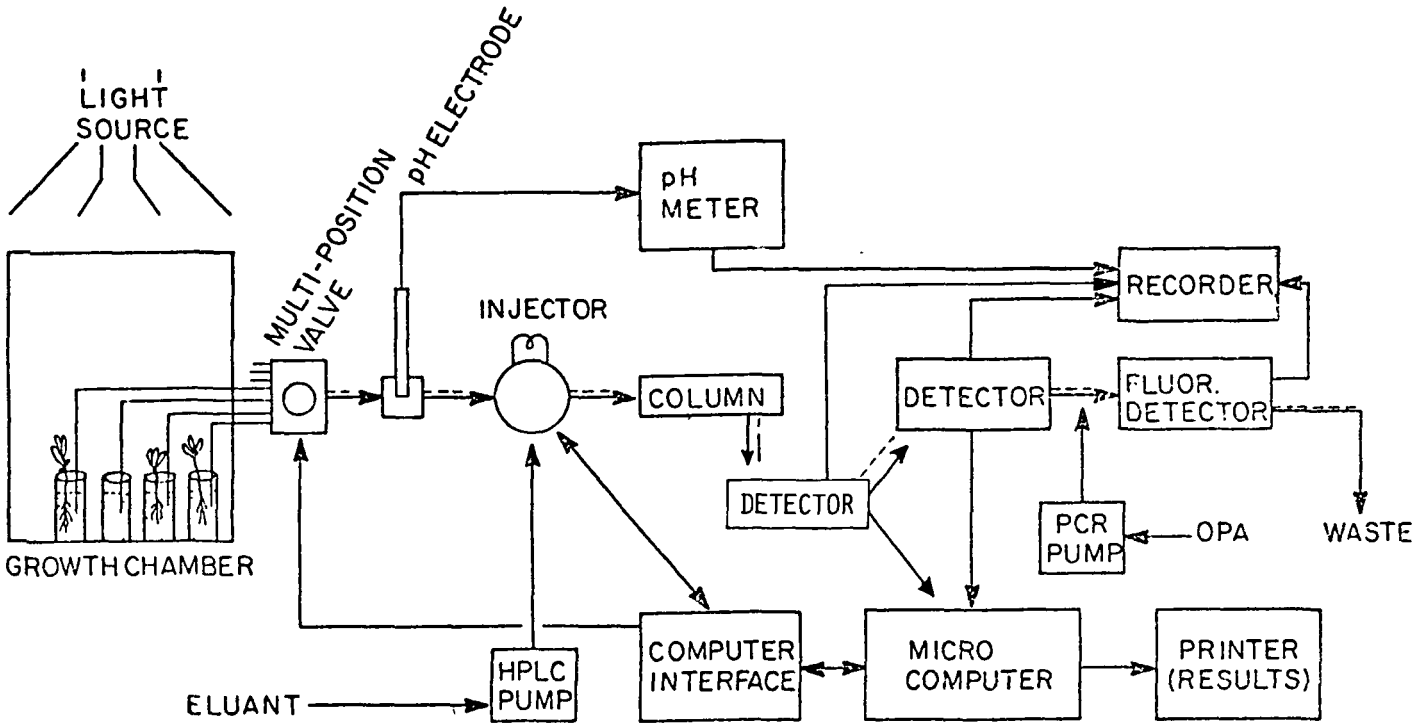

Figure 1. Flow diagram for determining uptake kinetics of N species by wheat seedlings. Detector 1 is a UV absorbance detector set at 189 nm for determination of urea. Detector 2 is a UV detector set at 210 nm for determination of NO_3^- and NO_2^-. Detector 3 is a fluorescence detector used for NH_4^+ determination.

removed the need to use ^{14}C-urea with its problems of contamination. This system was previously described in the analysis of NO_3^- and NO_2^-. The NH_4^+ assay has been improved by forming the OPA derivative in a flow system with detection by spectrofluorometry.

Uptake. Urea uptake was very slow in comparison to NO_3^- and NH_4^+ (Fig. 2) and showed a long lag before uptake began. The wheat seedlings were put in the presence of each of the above N compounds for 24 h to induce their transporters before the uptake studies were begun. The results shown are double the real rate of urea uptake since each urea molecule contains two N atoms. Of the three N compounds tested thus far, NH_4^+ uptake was the most rapid.

Assimilation. Figure 3 shows the concentration of urea, NH_4^+ and NO_3^- inside the plants. These concentrations are a function of uptake minus the amount further assimilated. Almost all of the urea absorbed by either roots or leaves is assimilated (Table I, Fig. 4).

Urea on NO_3^- and NH_4^+ Uptake. Urea facilitated the uptake of NO_3^- by wheat seedlings (Fig. 5), primarily because the induction of the NO_3^- transporter was much faster and a

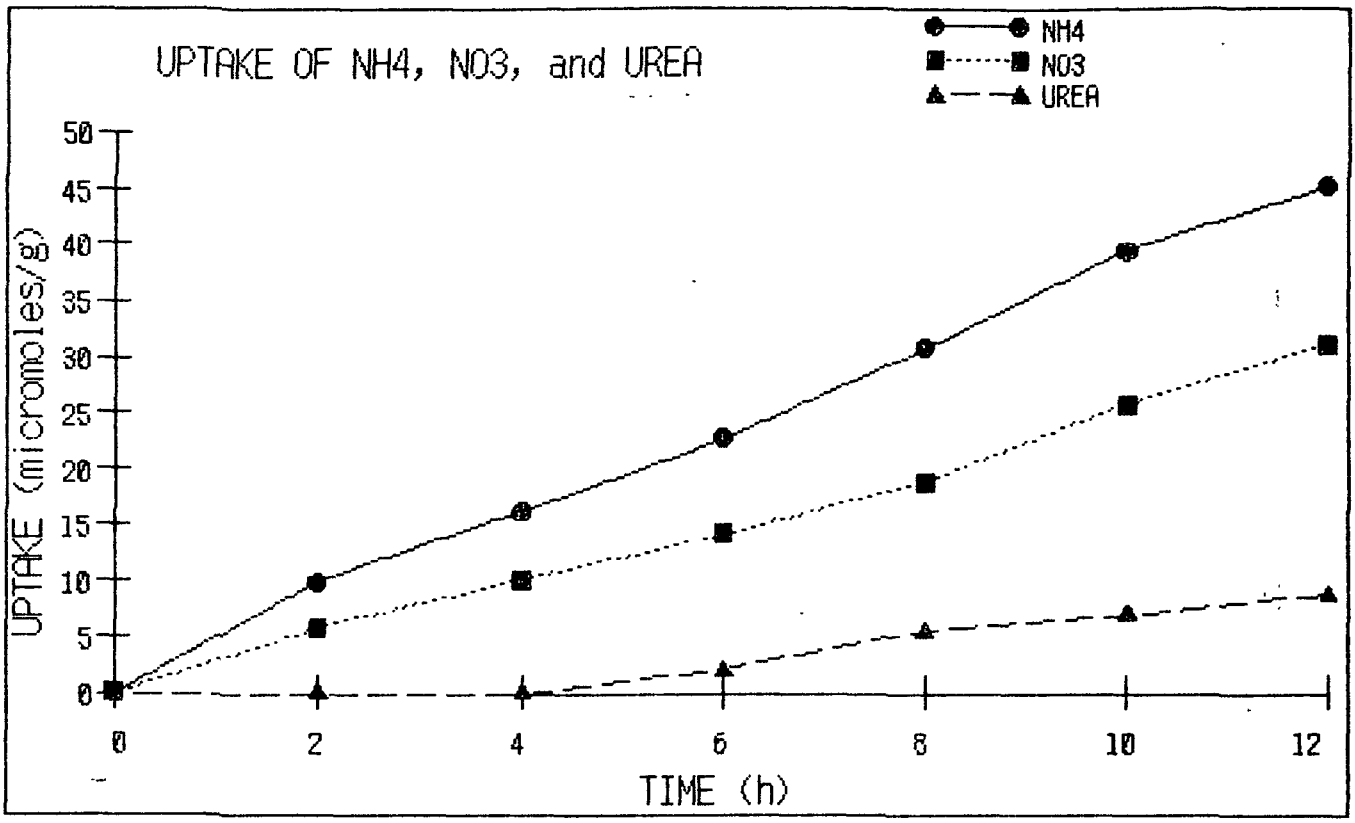

Figure 2. Comparison of urea, NO_3^-, and NH_4^+ uptake by pre-induced wheat seedlings. Seedlings were grown hydroponically in N-free solutions for 7 d in darkness followed by 2 d in continuous light. Groups of eight seedlings were transferred to pretreatment solutions containing 1 mM ^{14}C-urea, NO_3^-, or NH_4^+ in one-quarter strength Hoagland solution lacking N. After 24 h, the seedlings were transferred to 140 ml of fresh uptake solutions. Uptake of urea, NH_4^+, and NO_3^- were determined by sampling solutions every 2 h. Data is presented on a μmole N/g fresh weight basis.

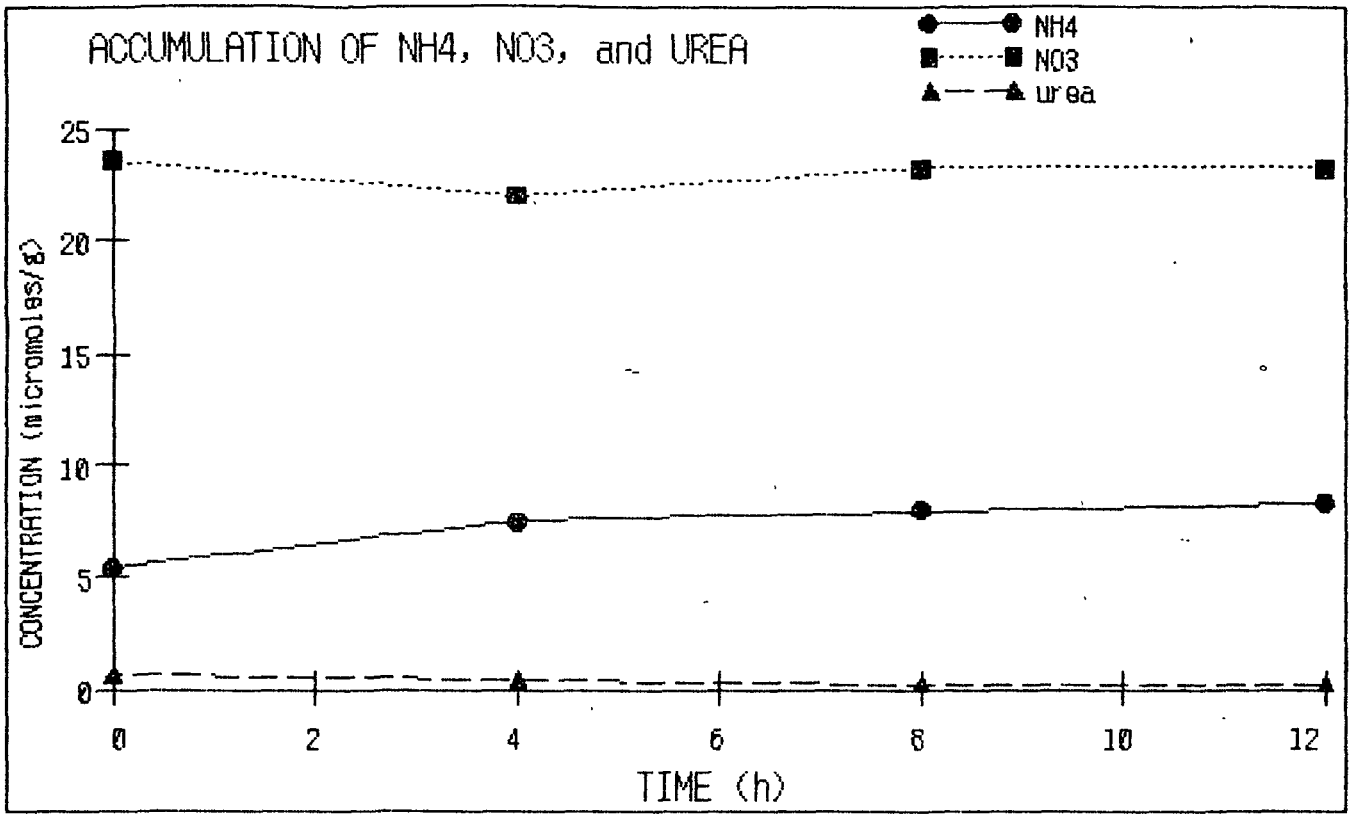

Figure 3. Accumulation of urea, NO_3^-, and NH_4^+ in preinduced wheat seedlings. Seedlings were grown as described in Fig. 2. NO_3^-, NH_4^+, and urea tissue levels were determined every 4 h as described in "Materials and Methods." Data is presented on a μmol/N g fresh weight basis.

Table I. Urea assimilation in wheat roots, leaves, and whole seedlings. Wheat seedlings were grown hydroponically for 3 d in continuous light as described in Fig. 2. Groups of 8 seedlings were separated into roots and shoots or used as whole seedlings. Roots and whole seedlings were placed in 40 ml of aerated uptake solutions containing 1 mM ^{14}C-urea. Shoots were placed base down in vials containing 10 mls of 5 mM ^{14}C-urea. Uptake and assimilation of urea were determined as described in "Materials and Methods" for one 6-h time point.

	Uptake	Assimilation	% Assimilation
	µmole N/gfw x 6 h		
Root	12	11.8	98
Shoot	10	9.9	99
Whole Seedling	11	10.8	98

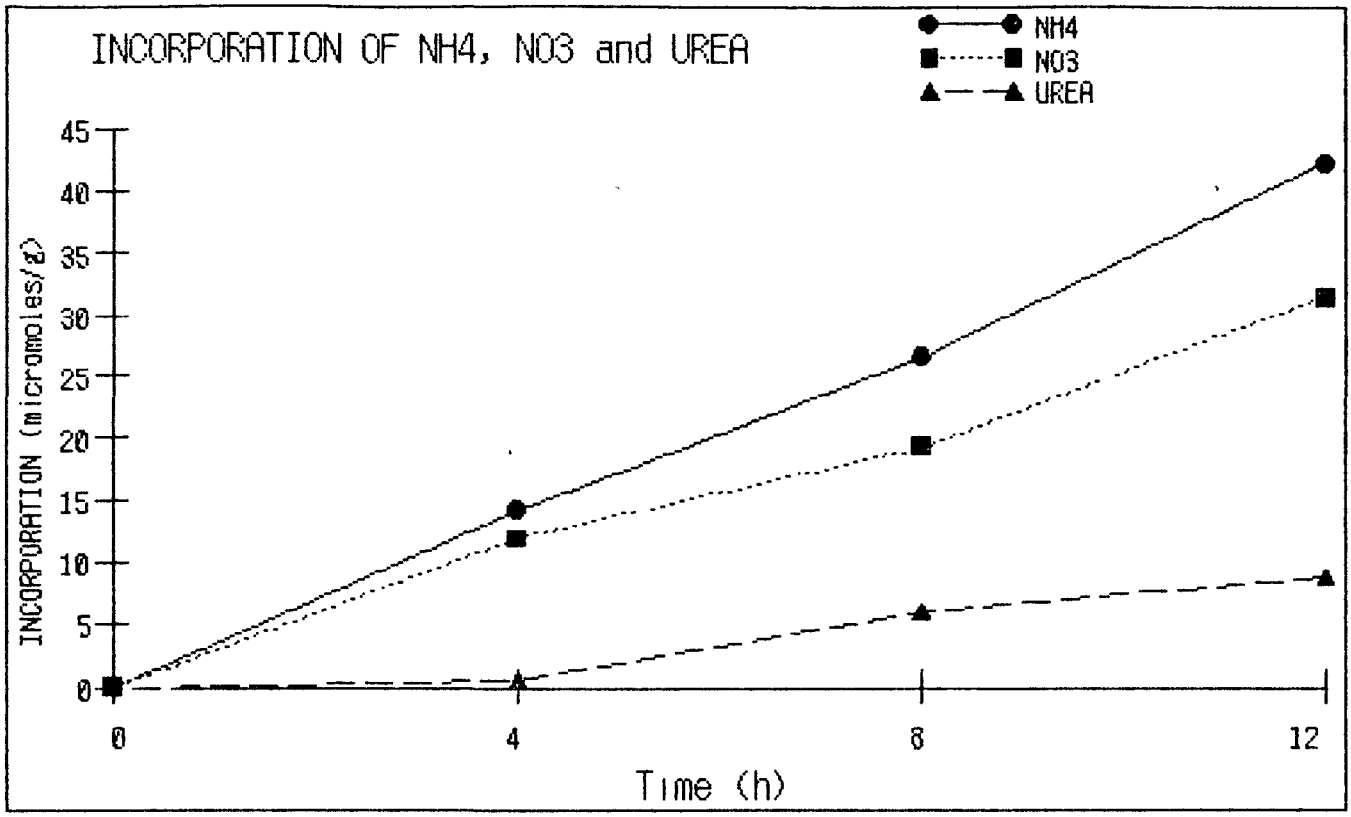

Figure 4. Assimilation of urea, NO_3^-, and NH_4^+ by preinduced wheat seedlings. Seedlings were grown as described in Fig. 2. N assimilation was determined as described in "Materials and Methods."

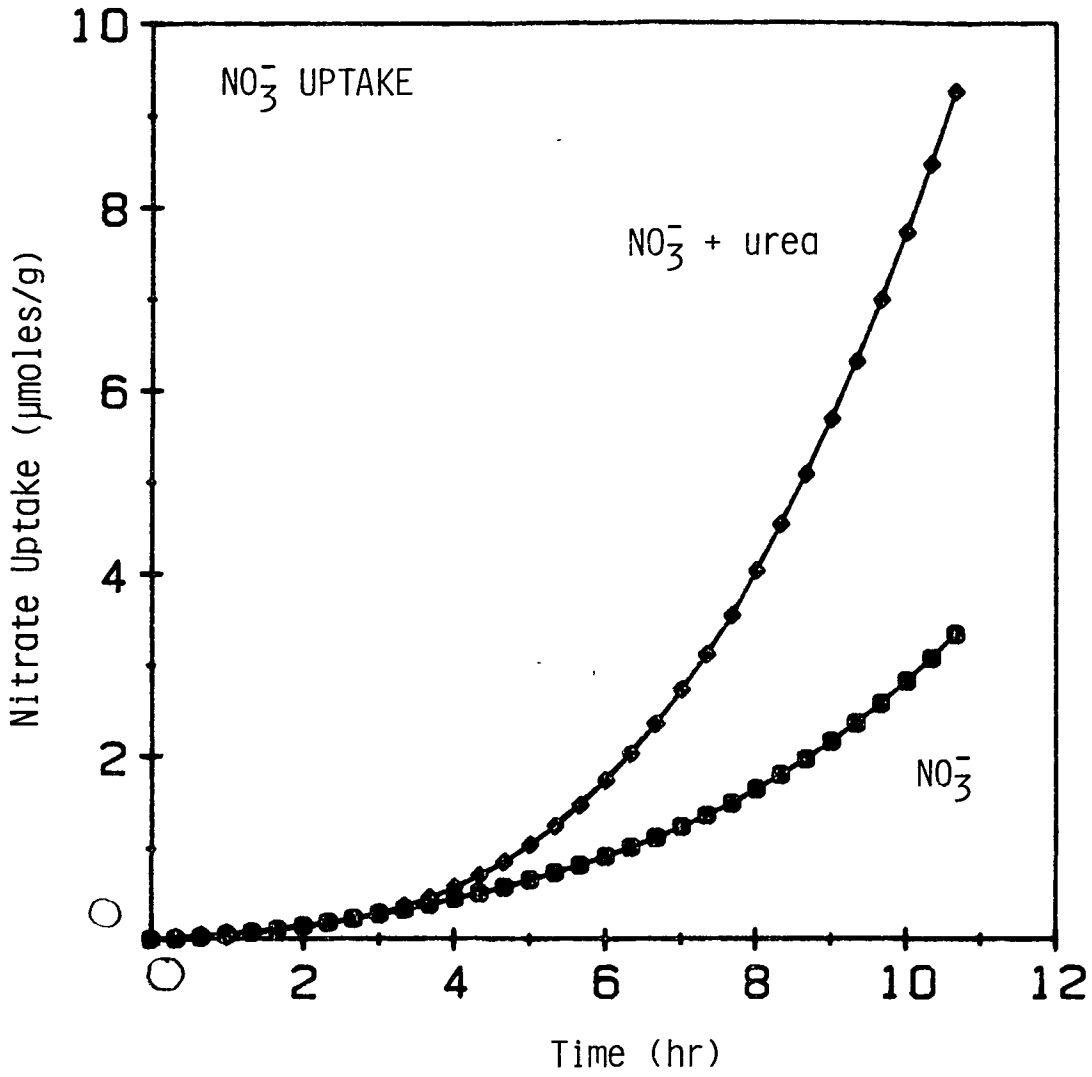

Figure 5. Effect of urea on NO_3^- uptake. Wheat seedlings were grown for 3 d in continuous light. Groups of 8 seedlings were transferred to 40 ml of uptake solutions containing 1 mM KNO_3 with and without 1 mM urea. Depletion of NO_3^- was monitored by sampling solutions every 20 min using an HPLC autosampling system.

greater induction occurred in the presence of urea. Urea had little effect on NH_4^+ uptake (Fig. 6).

Urea and NH_4^+ on NO_3^- Reduction in Wheat Leaves. NH_4^+ facilitated NO_3^- reduction while urea decreased NO_3^- reduction (Table II). Since this experiment was done on an equimolar basis, the urea supplied twice as much NH_4^+ as did the NH_4^+ treatment. We are at the stage now of supplying urea at half the concentration of NH_4^+ for a comparison on the basis of NH_4^+ concentration after assimilation of urea.

Growth of Wheat Plants in Urea. Wheat plants grew the least in urea compared to NO_3^- and NH_4^+ (Table III). As expected, the plants grew slightly better in NO_3^- than NH_4^+.

DISCUSSION

The automated analytical system described allows a precise estimate of the induction and activity of the transporters of the N compounds involved in the study. The importance of studying the inducibility of the transporters is to determine their stability throughout the growing season. Indications are that they decrease in both stability and activity as the plant roots age. If the transporters are under constant induction or turnover, as the

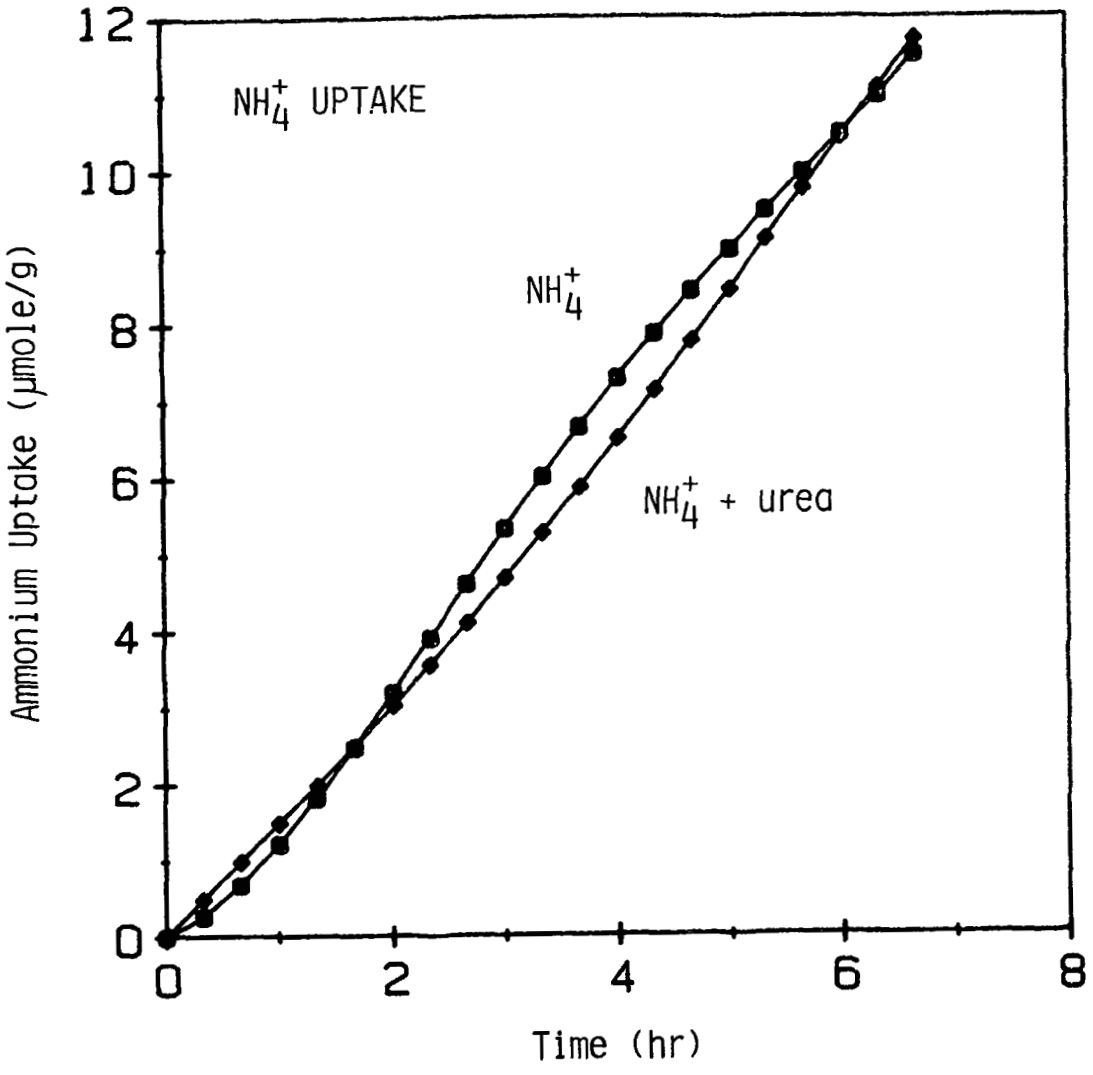

Figure 6. Effect of urea on NH_4^+ uptake. Wheat seedlings were grown for 3 d in continuous light. Groups of 8 seedlings were transferred to 40 ml of uptake solutions containing 1 mM NH_4^+ as $(NH_4)_2SO_4$ with and without 1 mM urea. Depletion of NH_4^+ was monitored by sampling solutions every 20 min using an HPLC autosampling system.

Table II. Effect of NH_4^+ and urea on NO_3^- reduction in wheat leaves in light. Wheat seedlings were grown for 3 d in continuous light. Leaves from groups of 8 seedlings were excised and placed base down in vials containing 10 ml of 5 mM NO_3^-, 5 mM NO_3^- + 5 mM NH_4^+ or 5 mM NO_3^- + 5 mM urea. Uptake and reduction were determined for a 24-h absorption period as described in "Materials and Methods."

Treatment	Uptake	Reduction	% Reduction
	µmol/gfw x 24 h		
NO_3^-	90	65.7	73
NO_3^- + urea	92	48.8	53
NO_3^- + NH_4^+	91	79.2	87

Table III. Effect of urea, NH_4^+, and NO_3^- on the growth of wheat plants. Wheat seedlings were grown hydroponically in N-free solutions for 7 d in continuous darkness, followed by 2 d under a 16/8-h light/dark regimen. Groups of 5 seedlings were then transferred to 5 l of nutrient solutions containing one-quarter strength Hoagland solution lacking N. Nitrogen was supplied as 1 mM KNO_3, 1 mM NH_4HCO_3 or 1 mM urea. Solutions were changed daily. Root and shoot weights were determined at the end of the 3-week experimental period.

N Source	Root	Shoot	Whole Plant
		weight (g)	
NO_3^-	7.2	12.4	19.6
NH_4^+	6.7	11.4	18.1
Urea	4.3	8.0	12.3

plant ages, it may lose or develop decreased ability to maintain their presence in the plasmalemma. Do the transporters disappear when the inducing N compounds are depleted from the nutrient solution? Can they be reinduced when the inducing compound is again added to the nutrient solution? Are they stabilized when the inducing compound is always present?

It has been shown previously that the NO_3^- and NO_2^- transporters are induced by the presence of their substrates (2, 4). Indications are that the urea transporter is induced and that the induction requires quite a long time (over 2 days) (Fig. 2). Work is continuing to verify this observation.

Uptake of urea is extremely slow when compared to NO_3^- or NH_4^+ (Fig. 2), which seems to result in decreased growth (Table III) with urea as the only source of N. Once urea is taken up, it is very efficiently assimilated; therefore, uptake seems to be the rate-limiting step to its utilization by wheat seedlings. When the four N species used in our studies are fed to roots, NH_4^+, urea, and NO_2^- are assimilated almost totally in the roots while NO_3^- is assimilated primarily in wheat leaves. Thus, NO_3^- seems to be the only one of the four whose reduction may be facilitated more directly by

photosynthetically derived electrons. Urea decreased NO_3^- reduction in leaves (Table III), however, since urea is mainly assimilated in roots, it will likely have little effect on NO_3^- assimilation on a whole-plant basis. This remains to be determined.

At equimolar concentrations, urea facilitated NO_3^- uptake (Fig. 5). The increased uptake was largely due to decreasing the time required for induction of the NO_3^- transporter and increasing the amount of transporter induced. Urea had little effect on NH_4^+ uptake (Fig. 6).

Future studies:

1. Determine if presence of urea induces a urea transporter or if the transporter is constitutive.
2. Determine the stability of the transporters as wheat ages.
3. Complete the studies showing the interactions of urea, NH_4^+, NO_3^-, and NO_2^- on each other's induction, uptake, and further assimilation in roots and intact seedlings.
4. Complete growth studies as a function of the assimilation of the four mixed N sources at differing concentrations.

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