# Air Containing Nitrogen-15 Ammonia: Foliar Absorption by Corn Seedlings

Abstract. *Thirty-day-old corn seedlings, grown in the greenhouse with different concentrations of supplemental nitrate nitrogen, were moved to a constant-temperature growth chamber and sealed in a 560-liter tent made of polyvinyl chloride. The plants were exposed to air containing ammonia labeled with nitrogen-15 (1, 10, and 20 parts per million) for 24 hours and then harvested. The nitrogen-15 content of the tops and roots showed that at 1 part per million 43 percent of the ammonia was absorbed, whereas at 10 and 20 parts per million, 30 percent of the ammonia was absorbed. The results demonstrate that growing plants may be a natural sink for atmospheric ammonia.*

In his quest for a cleaner environment, man is becoming increasingly concerned about nitrogen compounds, that is, nitrogen oxides, ammonia, and amines that are volatilized into the air and may be absorbed by water. De Saussure published in 1804 his observations on the presence of $NH_3$ in the atmosphere. Yet we still do not know whether $NH_3$ is an air pollutant and how it participates in the nitrogen cycle. We therefore are seeking information concerning the natural sources and sinks of this constituent of the atmosphere. Our results with $^{15}NH_3$ showed that foliar absorption by plants is a natural sink for atmospheric $NH_3$. The results also indicate that plants may leak small amounts of $NH_3$ or amide N into the atmosphere.

A Platner loam soil from eastern Colorado received an application of 30 ppm of S as $K_2SO_4$, 100 ppm of P as concentrated superphosphate, and 5 ppm of Fe as ferric ethylenediaminedi[*o*-hydroxy]phenylacetic acid. Four samples (2 kg each) of the soil were fertilized with $KNO_3$ (0, 25, 50, and 100 ppm of N) and placed in plastic-coated cardboard cartons lined with polyethylene bags. Each carton was seeded with three seeds of Illinois Foundation corn (hybrid WF9X38-11), and the seedlings were thinned to one plant when they were approximately 5 cm high. Different seeding dates were used for each replication (three replications), which permitted us to have only four plants, one representing each supplemental N concentration, ready for exposure to $^{15}NH_3$ at one time. When the plants were 28 to 30 days old, and about 35 cm high, they were moved from the greenhouse to a growth chamber (*1*), in which they were conditioned for several days at 21°C. The soil-root system was then sealed by securing the top of the polyethylene bag to the cornstalk, and the plants were moved into the polyvinyl chloride tent in the growth chamber. The tent had been tested with a halogen gas and a halogen gas detector, and no leaks were found. Normally, the tent is inflated and self-supporting. However, during the transfer of the plants, the walls of the 60.9 by 60.9 by 152.2 cm (560-liter) tent were supported with wire hoops covered with Tygon tubing. After the plants had been transferred, the tent was sealed and inflated with air. The air within the tent was circulated with a peristaltic pump.

Plastic films are somewhat permeable to small gas molecules like $CO_2$, $O_2$, and $H_2O$ (*2*). Because of the size and bulk of the tent, we were unable to test it for $NH_3$ permeability, but we did test the polyethylene bags used to contain the soil. Hydrochloric acid was sealed in the bags, which were then placed in 10-liter glass vacuum desiccators and exposed to atmospheres containing 10 or 50 ppm of $NH_3$ for 24 hours. The HCl was analyzed for $NH_4^+$ content, and no measurable increases in $NH_4^+$ were found.

Labeled $NH_3$ (79.8 percent $^{15}N$) from a 500-ml glass storage flask was sorbed into a 10-ml, high-pressure cylinder-cryosorption pump (*3*) filled with polyaromatic polymer beads (Porapak Q). Using the pressure generated in the cryosorption pump, we were able to bring small glass transfer bulbs of known volumes to predetermined pressures of $^{15}NH_3$. The labeled $NH_3$ was transferred from the transfer bulb into the plastic tent with the peristaltic pump. We began with the lowest level of $^{15}NH_3$ (1 ppm atmospheric concentration) and proceeded to the next higher concentrations (10 and 20 ppm) after we had exposed three replications at staggered intervals to the 1-ppm concentration.

The corn plants were kept in the $^{15}NH_3$ atmosphere for 24 hours (12 hours of light and 12 hours of dark). The temperature of the growth chamber was 21°C. However, during the light period, the temperature inside the polyvinyl tent rose to 23°C. Since the walls of the tent were maintained at the temperature of the growth chamber (21°C), any water transpired or guttated by the plant condensed on the walls or bottom of the tent. After 24 hours, the tent was opened. No attempt was made to trap any $^{15}NH_3$ remaining in the atmosphere. The plants were removed, and any water that had collected on the walls and bottom of the tent was siphoned up and acidified. The

Table 1. Total $^{15}N$ content in corn tops and roots and the percentage of excess $^{15}N$ in these plant parts; total $^{15}N$ absorbed per plant and the $^{15}N$ absorbed ($\mu g/cm^2$) by the leaf surface in 24 hours.

| $KNO_3$ N prior treatment (ppm N) | $^{15}N$ in tops | | $^{15}N$ in roots | | Total $^{15}N$ per plant ($\mu g$) | $^{15}N$ absorption by leaf surface ($\mu g/cm^2$) |
|---|---|---|---|---|---|---|
| | Excess* (%) | Total ($\mu g$) | Excess* (%) | Total ($\mu g$) | | |
| *Exposure to 1 ppm atmospheric $^{15}NH_3$ (mean of three replications)†* | | | | | | |
| 0 | 0.0600 | 13 | 0 | 0 | 13 | 0.020 |
| 25 | 0.0649 | 58 | 0 | 0 | 58 | 0.068 |
| 50 | 0.0484 | 46 | 0 | 0 | 46 | 0.033 |
| 100 | 0.0552 | 44 | 0 | 0 | 44 | 0.038 |
| *Exposure to 10 ppm atmospheric $^{15}NH_3$ (mean of two replications)†* | | | | | | |
| 0 | 0.4336 | 143 | 0.0963 | 24 | 167 | 0.173 |
| 25 | 0.4317 | 262 | 0.1458 | 40 | 302 | 0.249 |
| 50 | 0.3579 | 293 | 0.1534 | 40 | 333 | 0.256 |
| 100 | 0.3012 | 284 | 0.1347 | 28 | 312 | 0.250 |
| *Exposure to 20 ppm atmospheric $^{15}NH_3$ (mean of three replications)†* | | | | | | |
| 0 | 1.5960 | 506 | 0.4121 | 43 | 549 | 0.669 |
| 25 | 0.7659 | 422 | 0.3202 | 55 | 477 | 0.438 |
| 50 | 0.8300 | 587 | 0.3433 | 61 | 642 | 0.612 |
| 100 | 0.9643 | 623 | 0.3271 | 43 | 666 | 0.655 |

* Two check plants, that is, plants not exposed to $^{15}NH_3$, were carried for each N treatment. These corn plants were separated into tops and roots, and $^{15}N$ determinations were made in order to establish the normal $^{15}N$ abundance of these plants. $n = 16$, $\bar{x} = 0.3618$ percent $^{15}N$, $s^2 = 0.00015$, and $s \pm 0.0123$. † There was a total of 0.3746 mg of $^{15}N$ for the 1 ppm atmospheric $NH_3$, 3.746 mg of $^{15}N$ for 10 ppm, and 7.689 mg of $^{15}N$ for 20 ppm.

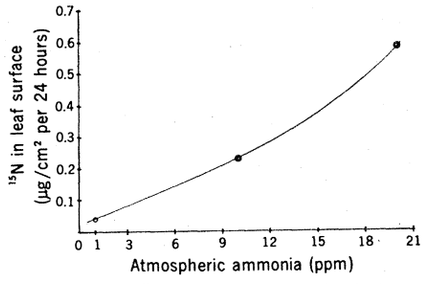


Fig. 1. The effect of the atmospheric $NH_3$ concentration of the tent on the rate of absorption of labeled ammonia nitrogen by corn leaf surfaces.

interior walls and bottom of the tent were wiped with cellulose (Celfibe) towels. The $NH_3$ in the tent water and in the wipes was recovered by MgO distillation, and then any amide N remaining in these solutions was hydrolyzed by the addition of NaOH and distilled as $NH_3$. After determination of the ammonium by titration, the sample was redistilled and saved for $^{15}N$ analysis as described below.

The plants were separated into tops and roots, and the leaf surface area of each plant was estimated by measuring the length and widest width of each leaf and then multiplying the product by 0.7. The constant of 0.7 was determined from the ratio of actual leaf area determined with a planimeter to the product of leaf length times maximum leaf width of a corn plant. We assumed both top and bottom surfaces of the leaf might absorb $NH_3$, and thus we doubled the leaf area.

The tops and roots were dried at 65°C and then ground in a Wiley mill to pass a 60-mesh screen. The total N of each sample was determined by Kjeldahl methods (*4*). The ammonia was redistilled and recovered for $^{15}N$ analysis. Cross contamination of samples by any $NH_4^+$ absorbed on the walls of the glass distillation equipment was avoided by distilling $NH_3$ from an alkaline $(NH_4)_2SO_4$ (normal $^{15}N$ abundance) solution through the still between samples (*5*). Ammonium was converted to $N_2$ gas (*6*), and the abundance of the $^{15}N$ was determined on a quadrupole mass spectrometer (*1*).

Samples fertilized with nitrate N enhanced the absorption of $^{15}NH_3$ (Table 1). However, the addition of 25 ppm of nitrate N was just as effective as additions of larger amounts. The four plants in the chamber at one time representing the various nitrate-N fertilizer concentrations absorbed 0.161, 1.114, and 2.334 mg of $^{15}N$ when the $NH_3$ concentrations were 1, 10, and 20 ppm, respectively. Of the $^{15}NH_3$ introduced into the tent, 43 percent was absorbed when the $NH_3$ was 1 ppm, whereas 30 percent was absorbed when the concentration was 10 and 20 ppm. There is the possibility that $NH_3$ at the higher concentrations was toxic to the seedlings, but we saw no visual symptoms in the 24 hours of exposure that indicated toxicity. Toxicity of $NH_3$ at various concentrations could probably be determined by measuring $CO_2$ uptake. When the concentration of $NH_3$ becomes toxic, it would probably affect photosynthesis and $CO_2$ fixation. Even though we have established that corn seedlings readily absorb atmospheric $^{15}NH_3$, we have not established the rate at which they can absorb $NH_3$. In our study it is entirely possible that most of the $^{15}NH_3$ was absorbed in the first hour. We used a 12-hour light and a 12-hour dark period, and no attempt was made to determine whether absorption proceeds faster in the light than in the dark, although light should affect stomatal opening and thus affect absorption.

The absorption of $^{15}N$ (per square centimeter of leaf surface) is given in Table 1, and the relation between the atmospheric $NH_3$ and the means of $^{15}N$ absorption per square centimeter of leaf surface are shown in Fig. 1. The absorption increases with increasing $NH_3$ levels, with six times more $^{15}N$ being absorbed when the $NH_3$ concentration was 20 ppm as compared to absorption at 1 ppm.

Our study does not explain what mechanisms are involved in the $NH_3$ absorption. Physical absorption, chemical exchange, and metabolic assimilation are all likely to be involved. When the $^{15}NH_3$ concentration in the atmosphere was 1 ppm, the $^{15}N$ in the root tissue was not enriched more than the amount of the normal standard deviation for check samples (Table 1). When the concentration in the atmosphere was increased 10- or 20-fold, excess $^{15}N$ was detected in the root tissues. Of the absorbed $^{15}NH_3$, 7 to 16 percent was translocated to plant roots (Table 1; 10 and 20 ppm $^{15}NH_3$ treatment). The translocation of $^{15}N$ to the corn roots indicated that perhaps some of the absorbed $^{15}NH_3$ was being metabolized by the corn plants. In order to determine whether the absorbed $^{15}NH_3$ was being converted to amino acids and protein, a single corn plant (highest nitrate preliminary treatment and exposure to 20 ppm $^{15}NH_3$ for 24 hours) was extracted with hot 70 percent ethanol. The ethanol soluble N was separated into $NH_4^+$, amide and free amino acids, and ethanol insoluble (protein) as outlined by Stewart and Porter (*7*). The data expressed on a dry weight basis are presented in Table 2. The percentage of excess $^{15}N$ for each fraction is also presented in Table 2. The ammonia N accounts for about 2 percent of the total N in the corn tops and about 1 percent of the total N in the roots. The free amino acid fraction and protein account for more than 90 percent of the N in the corn seedlings. The excess $^{15}N$ is higher for the free amino acid and the protein fractions, and, since these fractions make up most of the N in this plant, it is apparent that the absorbed $^{15}NH_3$ was being rapidly metabolized to amino acids and protein, probably via glutamic acid (*8*) or carbamyl phosphate (*9*). The initial assimilatory compounds have not been identified, and very short time intervals (exposures for minutes or seconds) would be required to identify such compounds.

Table 2. Ethanol (70 percent) fractionation of plant N and absorbed $^{15}N$.

| N fraction | Tops (%) | | Roots (%) | |
|---|---|---|---|---|
| | N | Excess $^{15}N$ | N | Excess $^{15}N$ |
| *Ethanol soluble* | | | | |
| $NH_4^+$ | 0.084 | 0.5582 | 0.016 | 0.1426 |
| Amide | 0.015 | | 0.010 | 0.1346 |
| Free amino acids | 0.763 | 0.6447 | 0.172 | 0.2385 |
| *Ethanol insoluble* | | | | |
| Protein | 3.605 | 0.6734 | 1.339 | 0.2627 |

One-tenth of the $^{15}NH_3$ introduced into the tent was found in the transpired water. The towels contained another 5 or 6 percent of the labeled N. The abundance of $^{15}N$ in the water ranged from 38 to 74 percent. The higher amounts were found where the high concentrations (20 ppm of $NH_3$) were introduced into the tent. In all cases, the introduced $^{15}NH_3$ had been diluted with $^{14}NH_3$, reducing the $^{15}N$ enrichment of the introduced $^{15}NH_3$ from the original 79.8 percent. The $^{14}NH_3$ may have come from several sources: ammonia leakage from plants as suggested by Martin and Ross (*10*); guttation of amides and hydrolysis of these compounds within the tent or during MgO distillation; and finally, there is the possibility that the polyvinyl

chloride tent was permeable to $NH_3$ and that small amounts of $^{14}NH_3$ leaked into the tent. Sodium hydroxide hydrolysis of the MgO-distilled water from the tent did release small amounts of $NH_3$. These data suggest that small amounts of amide N may have leaked or been present in the condensed water from the plants.

Our study illustrates that growing plants are a sink for atmospheric $NH_3$ and can absorb considerable quantities of $NH_3$ from the atmosphere. Green plants, like rain, may cleanse the atmosphere of this possible N pollutant. They may also meet a portion of their N needs by absorbing atmospheric $NH_3$.

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## Dihydroxyphenylalanine in Rat Food Containing Wheat and Oats

Abstract. *Dopa has been identified in rat food by three different fluorimetric assays and paper chromatography. Incubation of the rat food with proteolytic enzymes dramatically increased the measurable free dopa. Analysis of samples of six individual protein-containing constituents of rat food revealed that both wheat and oats contain dopa.*

High concentrations of dopa (dihydroxyphenylalanine) (1 to 2 μg per gram of tissue) in the rat stomach have been observed in this laboratory (*1*). Administration of [$^3$H]tyrosine by stomach tube (10 μc/100 g) to starved rats did not lead to the accumulation of [$^3$H]dopa in the stomach. We therefore suspected that the gastric dopa was of dietary origin. Dopa has not generally been considered to be a dietary constituent, though its glucuronide can be extracted from broad bean, *Vicia faba* (*2*, *3*).

Solid pellets of Big Red laboratory animal chow (Agway, Syracuse, New York) were pulverized with a mortar and pestle and then suspended in water. Portions of the suspension were then analyzed directly for dopa (see Tables 1 and 2) or incubated at 37°C for 16 hours with or without the proteolytic enzyme Pronase [Calbiochem, 0.1 percent (weight to volume)] (*4*). Several drops of ethanol were added to inhibit bacterial growth. After the incubation, one volume of 30 percent trichloroacetic acid was added to four volumes of hydrolyzate, and the resulting mixture was centrifuged at 27,000*g* for 15 minutes. The supernatant was filtered through paper, and the resultant solution was diluted to 10 ml with 7.5 percent trichloroacetic acid (TCA). Portions (0.25 ml) of the diluted supernatant were set aside for the assay of tyrosine (*5*). The dopa was absorbed from the TCA supernatant onto Dowex 50W-4X and then alumina columns as described (*6*). Portions of the final acetic acid eluates from the alumina columns were assayed fluorimetrically for dopa by the trihdroxyindole reaction (*7*). The resulting activation and emission spectra for the dopa extracted from rat food correspond exactly with those of the added dopa standard (Fig. 1a). Identical fluorescence spectra were also obtained when the direct oxidative coupling reaction (*8*) was performed on the alumina eluates (Fig. 1b). When 10.0 g of the rat food was processed, native catechol fluorescence could be seen in the alumina eluate (Fig. 1c). This fluorescence could not have come from dopamine and norepinephrine since, as shown (*6*, *7*), these catecholamines are separated from the dopa by the Dowex chromatography before the alumina absorption. After lyophilization, portions of the alumina eluate were also subjected to ascending paper chromatography with the solvent system butanol, acetic acid, $H_2O$ (4 : 1 : 1). The dried paper chromatogram was sprayed with ethylenediamine, and observed under ultraviolet light. An intense yellow fluorescence was observed with the same $R_F$ (0.16) as that of the added dopa standard.

The free dopa concentration was dramatically increased by the inclusion of Pronase in the incubation mixture (Table 1). Pronase has esterase activity (*9*), and thus could have liberated free dopa from its glucuronide (*3*). Incubation of the rat food with β-glucuronidase H2 (Sigma Chemical) did not, however, increase the measurable dopa. We obtained evidence that the dopa was not simply present as a glucuronide by suspending the food in water, adding equal volumes of pure alcohol, and allowing the mixture to sit in ice for 1 hour to precipitate the protein. The mixture was centrifuged as described, and the supernatant was discarded. The pellet was suspended in water, the suspension was centrifuged, and the supernatant was again discarded. Any dopa-glucuronide would thus have been eliminated. The protein pellet was suspended in water a second time, and then digested with

Table 1. Dopa content of rat food. Data are given as mean ± standard error of the mean (S.E.M.); *N*, number of determinations; TCA, trichloroacetic acid.

| Rat food | L-Dopa (μg/g) | Tyrosine (mg/g) | Dopa/tyrosine |
|---|---|---|---|
| TCA supernatant | 0.087 ± 0.013 ($N = 4$) | | |
| Suspension in $H_2O$ | 0.332 ($N = 2$) | | |
| Incubation of rat-food suspension | | | |
| Without Pronase | 1.29 ± 0.4 ($N = 5$) | 0.175 ± 0.034 ($N = 4$) | $7.4 \times 10^{-3}$ |
| With Pronase | 23.8 ± 3.9 ($N = 9$) | 2.55 ± 0.35 ($N = 6$) | $9.3 \times 10^{-3}$ |
| Incubation of protein precipitate with Pronase | 45 ($N = 3$) | 4.1 ($N = 2$) | $10.9 \times 10^{-3}$ |