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Nitrogen-15 Dioxide Uptake and Incorporation by *Phaseolus vulgaris* (L.)

Hugo H. Rogers, Janet C. Campbell, and Richard J. Volk

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Abstract. *The sorption rate and metabolic fate of nitrogen dioxide, a major air pollutant, have been determined for* Phaseolus vulgaris *(L.). Sorption was determined kinetically by chemiluminescent monitoring of $^{15}NO_2$ removal from the test atmosphere and directly by mass spectrometric analysis of nitrogen derived from the plant tissue. Sorptive processes were first order with respect to $^{15}NO_2$ concentration. Virtually all of the $^{15}NO_2$ taken up was metabolized.*

Vegetation is recognized as a sink for atmospheric pollutants, and efforts have been made to characterize the interactions between plants and pollutants. Studies of pollutant sorption by plants furnish information that can help in assessing the efficacy of atmospheric scrubbing and in understanding the fate of pollutant molecules within plants. Some of these molecules can be phytotoxic and some can serve as plant nutrients (for example, S and N pollutants). Nitrogen dioxide (NO_2), a major air pollutant, might conceivably serve in either role, depending on dosage. A detailed review of its biological activity has been published (*1*).

Only two investigations of $^{15}NO_2$ uptake by plants have been reported (*2*, *3*), and both were conducted with concentrations of NO_2 that were higher than concentrations in the normal atmosphere, which range from about 0.0002 to 0.5 parts per million (ppm). Durmishidze and Nutsubidge (*2*) used $^{15}NO_2$ at 5400 ppm to demonstrate the incorporation of ^{15}N into amino acids for a variety of plant species. They also observed ^{15}N translocation between shoots and roots. Yoneyama *et al.* (*3*), using $^{15}NO_2$ concentrations of 0.5 to 4.0 ppm, demonstrated that ^{15}N accumulation by three plant species was accompanied by increases in both nitrite and nitrite reductase levels. The interpretation of these results, however, is limited by the static exposure conditions used, which would have limited CO_2 fixation and produced maximum stomatal opening.

The purpose of the present study was to estimate foliar sorption of $^{15}NO_2$ at normal atmospheric concentrations and to measure the extent of ^{15}N incorporation into the major nitrogenous fractions. For comparison, NO_2 uptake was also measured with a sensitive kinetic method that depended on chemiluminescent monitoring of NO_2 removal from the test atmosphere.

Phaseolus vulgaris (L.) 'Bush Blue Lake 290' (snap bean) was seeded three to a 177-ml Styrofoam cup containing a substrate of one part of peat moss–vermiculite mixture (Redi-Earth) and two parts of gravel. Plants were grown in a controlled environment room of the North Carolina State University Phytotron (*4*) with a day length of 9 hours and day and night temperatures of 26° and 22°C, respectively. The quantum flux density was 660 microeinsteins per square meter per second (400 to 700 nm), and relative humidity ranged from 55 to 65 percent during the day and 75 to 85 percent at night. Cups were watered lightly with deionized water until seedling emergence, after which they received a nutrient solution (*4*) twice daily. Plants were

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thinned to one per cup at 7 days from seeding and exposed to $^{15}NO_2$ at 14 days.

Six plants per experimental run were exposed to $^{15}NO_2$ in each of two 200-liter chambers of the continuous stirred tank reactor design (*5*). The chamber system was housed in a controlled environment room. All internal parts of the system were made of Teflon or glass in order to minimize surface reaction. The chambers were cylindrical with three vertical baffles and an impeller to provide stirring (120 rev/min). During exposure, the cup (containing soil and root) of each plant was sealed into a glass container with a split plate-glass lid with fluorocarbon grease. This permitted only the plant tops to take up the $^{15}NO_2$ and excluded evaporation from the soil surface, allowing computation of total leaf diffusion resistance (aerodynamic plus stomatal). Airflow through each of the two chambers was continuous at 10 liters per minute. There was a common inlet and separate outlets. Sampling steps lasted 2 minutes; each outlet was sampled twice as often as the inlet. The $^{15}NO_2$, dew point, and temperature were measured at the common inlet and at both outlets. The monitors used were: for NO_2, Monitor Labs 8440 NO_x analyzer; for dew point, EG&G 880 hygrometer; and temperature, Yellow Springs 44203 thermistors. Leaf temperature was estimated with No. 36-gauge type T thermocouples, and leaf area was determined with a Lambda 3100 area meter.

The sorption of the $^{15}NO_2$ was determined by two techniques: kinetically by assessing removal of NO_2 from the exposure chamber airstream, and directly by measuring the ^{15}N sorbed by the plant tissue. The $^{15}NO_2$ was supplied to the system airstream through a stainless steel capillary (for flow control) from a tank containing 99 percent enriched $^{15}NO_2$ in nitrogen (Prochem) at a concentration of 102 ppm. Plants were exposed for 3 hours to $^{15}NO_2$ at concentrations approximately 10, 15, and 32 parts per hundred million (pphm), after which they were harvested and freeze-dried. Four replications were run at each NO_2 concentration except the highest, where four additional exposures were made to provide enough plant material for fractionation. Plant tops and roots were prepared for mass spectrometric determination of ^{15}N abundance by a modified Dumas procedure (*6*). The plant material was mixed with oxidizing agents (CuO and $KClO_4$), elemental Cu to reduce nitrite and nitrate to N_2, and CaO to absorb all generated gases (for example, CO_2 and H_2O) except N_2. The mixture was sealed in a glass ampule and heated at 560°C for 10 hours. The resultant N_2 was introduced into a mass spectrometer (Consolidated Electrodynamics 21-620) through an ethanol–dry ice trap for ^{15}N analysis. To obtain an absolute measure of ^{15}N tissue content, we determined total N by a Kjeldahl procedure (*7*).

Fractionation was carried out on four pooled sets of six plants each: the plants had been exposed to about 32 pphm $^{15}NO_2$ for 3 hours. The distribution of ^{15}N was examined among four categories of compounds, which were separated by tissue extraction (*8*) in a mixture of methanol, chloroform, and water (13:4:3, by volume): alcohol-insoluble compounds (primarily proteins and nucleic acids), alcohol-soluble compounds (predominantly amino acids and amides), chloroform-soluble compounds (primarily lipids and chlorophyll), and nitrate.

Results are presented in Table 1. An average of 65 percent of the NO_2 determined to have been removed from the air was accounted for in the plant tissue. Losses most probably occurred during plant tissue preparation. Destruction and reaction of the NO_2 could also have taken place in the chamber atmosphere and on plant and chamber surfaces.

Table 1. Nitrogen-15 content of snap bean tops and roots after exposure to $^{15}NO_2$ for 3 hours. Exposure concentrations, leaf area, total diffusion resistance (R_{total}), and the kinetic value for NO_2 sorbed are given. All values (mean ± standard error) are for four relications of experiments with six plants each.

Concentration of $^{15}NO_2$ (pphm)	Leaf area (dm^2)	R_{total} (sec/cm)	Amount of ^{15}N sorbed (μmole/g dry weight)				Direct total/kinetic* (%)
			Kinetic	Direct			
				Total	Tops	Roots	
32.5 ± 1.6	5.23 ± 0.21	1.73 ± 0.11	15.64 ± 0.55	10.72 ± 0.51	10.36 ± 0.51	0.36 ± 0.09	69
15.2 ± 1.0	4.85 ± 0.21	1.70 ± 0.03	7.26 ± 0.15	4.69 ± 0.28	4.49 ± 0.28	0.20 ± 0.02	65
9.7 ± 0.5	4.88 ± 0.16	1.34 ± 0.11	3.45 ± 0.16	2.05 ± 0.13	1.96 ± 0.12	0.09 ± 0.02	60

*(Direct total/kinetic) × 100: determined at each concentration.

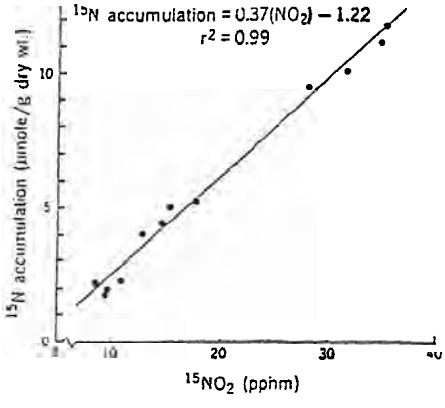

Fig. 1. Regression of ^{15}N accumulation on $^{15}N_2$ exposure concentration. The linear regression equation and the square of the correlation coefficient are shown.

Table 2. Nitrogen-15 content in fractions of tops and roots of snap bean exposed to 32 pphm $^{15}NO_2$ for 3 hours. Each value is the mean ± standard error of duplicated analyses performed on pooled material from 24 plants.

Material	Excess ^{15}N (atom %)	Amount of ^{15}N Sorbed (μmole/g dry weight)	Fraction/total* (%)
Insoluble			
Tops	0.300 ± 0.000	6.74 ± 1.22	
Roots	0.052 ± 0.001	0.76 ± 0.01	
Chloroform-soluble			
Tops	0.395 ± 0.001	0.29 ± 0.03	
Roots	0.075 ± 0.004	0.02 ± 0.01	
Soluble reduced N			
Tops	0.642 ± 0.001	3.40 ± 0.07	32.0
Roots	0.115 ± 0.007	0.65 ± 0.01	
Nitrate			
Tops	0.074 ± 0.007	0.27 ± 0.02	2.5
Roots	0.040 ± 0.001	0.17 ± 0.01	

*[(Tops + roots)/total] × 100: determined for each fraction.

However, appropriate corrections were made from runs made without plants. Although most of the ^{15}N derived from $^{15}NO_2$ was found in the tops, 3 to 5 percent was translocated to the roots. Figure 1 shows a linear correlation between NO_2 concentration and ^{15}N accumulation in snap bean. This demonstrates the first-order nature of the NO_2 sorptive process (that is, the first-order rate constant is independent of concentration). Previous studies in our laboratory (*5*) have shown that under constant environmental conditions the rate of NO_2 uptake by several crop species is independent of concentration (that is, first order) over the range 0 to 58 pphm. Rates were found to increase with increasing light and to be linearly correlated with the reciprocal of total leaf diffusion resistance.

Fractionation results are given in Table 2. Approximately 97 percent of the absorbed $^{15}NO_2$ was incorporated into reduced nitrogen compounds during the 3-hour exposure period. Nitrogen dioxide is known to react in aqueous solution to form nitrate and nitrite ions. The low levels of ^{15}N observed as nitrate, a common storage form, suggested that most of the absorbed NO_2 formed nitrite, which is rapidly assimilated via nitrite reductase. These data show conclusively that N derived from the air pollutant NO_2 is metabolized by snap bean and incorporated into various plant nitrogen fractions. This conclusion is supported by several lines of indirect evidence. For example, Faller (*9*) recorded stimulation of plant biomass when NO_2 was supplied as the sole source of fixed nitrogen. Similarly, Matsushima (*10*), using ^{14}C, showed an increase in amino acid synthesis when citrus was exposed to NO_2 and suggested that the N source was NO_2. Finally, Zeevaart (*11*) reported that nitrate reductase was induced by NO_2 and that levels of nitrate, nitrite, and protein increased in NO_2-exposed plants.

We have demonstrated the sorption of NO_2 by snap bean by a direct technique under usual plant growth conditions and at NO_2 levels well within recorded ambient ranges. We have further shown that this NO_2 is rapidly metabolized and incorporated into organic nitrogen compounds.

HUGO H. ROGERS
JANET C. CAMPBELL
Atmospheric/Vegetation Effects Research, AR-SEA-USDA, and Botany Department, North Carolina State University, Raleigh 27650
RICHARD J. VOLK
Soil Science Department, North Carolina State University

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