Simultaneous Measurement of Nitrogen Fixation Estimated by Acetylene-Ethylene Assay and Nitrate Absorption by Soybeans

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ABSTRACT

An apparatus was designed for simultaneous measurement of rates of N2 fixation estimated by C2H2-C2H4 assay (N2[C2H2] fixation) and NO3- absorption by roots of intact, nodulated soybeans (Glycine max [L.] Merr.). The principal design features include: (a) a gas-tight mist chamber in which nodulated roots can be exposed simultaneously to C2H2 in the gas phase and to a liquid phase containing NO3- sprayed in a fine mist; and (b) provision for sampling the gas phase for C2H4 determination, and the liquid phase for NO3- determination.

We studied NO3- absorption by soybeans as affected by nodulation, NO3- concentration during assay, and previous N nutrition during growth in nutrient solution culture in controlled environment chambers. It was established that 0.5 mM NO3- nearly saturated the NO3- absorption system of both nodulated and unnodulated soybeans when the concentration dependence of NO3- absorption rate was measured just after flowering began. Nitrate absorption rates were measured after development of N stress in unnodulated plants, and during recovery from N stress in nodulated plants. The results suggested that the lower capacity for NO3- absorption of nodulated plants was a consequence of N stress during the period of nodule growth and development.

Nitrogen [C2H2] fixation rates were compared in intact plants assayed in the mist chamber and in excised roots assayed in both the mist chamber and in glass jars. Excised roots had a lower N2[C2H2] fixation rate than intact plants. The decline observed during the first hour after shoot removal was more pronounced for glass jar-assayed excised roots than for mist chamber-assayed excised roots.

We discuss the advantages of our method for assessing the capability of a nodulated legume to acquire nitrogen through both N2 fixation and absorption and assimilation of NO3-.

Legumes are unique among crop plants in their ability to satisfy their large demand for N either through absorption and assimilation of inorganic N from the soil solution or by symbiotic fixation of atmospheric N2. It has long been recognized (3, 15) that interactions are possible between the processes of: (a) absorption, assimilation, and translocation of NO3-; (b) N2 fixation; and (c) assimilation and translocation of the product NH3.

The concentration-dependent kinetics of NO3- absorption have been reported for only a few species of economic higher plants (10, 13, 18). We are not aware of any similar studies in which NO3- absorption kinetics of soybeans, as influenced by [NO3-], plant age, or the presence or absence of nodules, as measured simultaneously with the reduction of C2H2 by nodulated roots.

Most early applications of C2H2 reduction for investigating legume N2 fixation employed excised nodules, nodulated root segments, or soil cores. Such methods have several potential limitations, however. The adverse effects of O2 depletion (6), water stress (19), ethanol accumulation (20), and decreased carbohydrate supply to nodules (15, 17) have been demonstrated. Extrapolations from measurements on excised tissues might lead to misinterpretation of the nodules' role in integrated whole-plant processes.

A recent consequence has been a trend toward developing techniques and apparatus for in situ C2H2 reduction assays on intact nodulated legumes. Mederski and Streeter (11) devised a method for suspending soybean root systems in a gas-tight enclosure and spraying them with a nutrient mist. Acetylene reduction was then monitored over several days. Although that system was obviously well suited for ion absorption studies, no such investigation was reported. Zobel et al. (24) grew several legume species with well nodulated roots in an "aeroponic" system. Their success suggested that nodules both developed and functioned well in a nutrient mist. We were convinced that a mist or spray would serve equally well for short term studies of absorption kinetics for a single ion (NO3-), and would also facilitate simultaneous measurements of the N2[C2H2]-fixing activity of nodules.

The objectives of the research reported here were to: (a) develop methodology that would facilitate simultaneous measurement on intact nodulated soybean plants of NO3- absorption from a liquid phase, and reduction of C2H2 to C2H4 in a gas phase; (b) evaluate the method by comparison with previously developed techniques; and (c) determine whether nodulated and unnodulated soybeans differ in NO3- absorption capacity, and if so, why.

MATERIALS AND METHODS

ASSAY CHAMBER CONSTRUCTION

Figure 1 diagrams the salient design features of the assay chamber. Each chamber is constructed of 0.63-cm-thick black Plexiglas (Plexiglas G, Rohm & Haas, Philadelphia) and has interior dimensions of 15.0 cm wide by 30.5 cm long by 38.3 cm deep. The total interior volume, excluding nozzles, fittings, and screens, is 17.35 liters. Three stainless steel mist nozzles (Monarch Mfg. Works, Philadelphia) mounted on PVC pipe fittings are positioned to give optimum mist coverage of roots. Two stainless steel screens running the full length of the chamber hold roots in position in the mist pattern and support them above the liquid reservoir.

On the exterior walls of the chamber, flexible silicone-rubber

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1 Abbreviations: PVC: polyvinyl chloride; PPFD: photosynthetic photon flux density; EDDHA: ethylenediamine di-(O-hydroxyphenylacetate); [C2H2]: acetylene concentration; [C2H4]: ethylene concentration; [NO3-]: nitrate concentration; [N2]: N2 fixation. N2 fixation estimated by the C2H2-C2H4 assay.
secondary and tertiary lateral roots. Nodules were first macroscopically visible on the primary root 9 to 10 days after germination was begun. The procedure just described permitted both infection and nodule development to progress in the absence of inorganic N for 14 days while the seedlings' early growth was supported by reserve N stored in the cotyledons.

**Nutrient Solution Schedule.** Full strength formulation of the N-containing nutrient solution was as given by Epstein (4, p. 39), except that Fe-EDDAH was substituted for Fe-EDTA, and 50 μM CoCl₂·6H₂O was added. The full strength N-free nutrient solution contained micronutrients as above, and macronutrients supplied as follows, with final concentrations (mM) in parentheses after each salt: K₂SO₄ (2.0); KH₂PO₄ (1.0); KH₂PO₄ (0.5); MgSO₄·7H₂O (1.0); and CaSO₄·2H₂O (4.0).

In all solutions, excess CaCO₃ (0.5 g/l) was added to buffer against the rapid pH drift commonly experienced when soybeans are grown in unbuffered culture solutions (7, and R. D. Wych, unpublished results). Culture solutions buffered with CaCO₃ remained at pH 7.3 ± 0.2 for up to 1 week.

Seven days after beginning germination, seedlings were transferred from 0.2 mM CaSO₄ to 4-liter polyethylene boxes containing 0.5-strength N-free solution. At 14 days after germination, all seedlings, both inoculated and uninoculated, displayed mild chlorosis and, therefore were transferred to 0.5-strength N-containing solution. Seven days later, inoculated plants were transferred to full strength N-free solution, while uninoculated plants were transferred to full strength N-containing solution. These solution treatments were then imposed for the duration of each experiment. No symptoms of nutrient imbalance were noted on nodulated plants receiving the modified (N-free) solution.

Solution volumes were maintained by adding nutrient solution once a week, midway between complete renewals, and distilled H₂O as needed. Solutions were completely renewed at weekly intervals.

**ASSAYING PROCEDURE.**

The general procedure employed for simultaneous assays was as follows. Permagum sealing compound (Virginia Chemicals, Inc., West Norfolk, Va.) was gently pressed around each plant's stem at the junction with the rubber stopper. The plants were removed from the nutrient solutions, and the roots were rinsed twice in 0.5 mM CaSO₄. The plants were then transferred to a controlled environment chamber where all assays were conducted.

This chamber was also set for a 14-hr photoperiod, 28/22 C day/night temperature cycle, and provided an average PPFD of 450 to 500 μm·m⁻²·s⁻¹ (supplied by both fluorescent and incandescent lamps) at the top of the canopy. Both nodulated and uninoculated plants were subjected to the same change in light conditions between growth and assay environments. Roots were placed for 16 hr in a pretreatment buffer solution (pH 6.0) containing 0.5 mM CaSO₄ and 5.0 mM KH₂PO₄/K₂HPO₄. Assays were begun 4 hr after the day cycle began, allowing time for plant adjustment to the new light conditions during the last portion of the pretreatment period.

To begin an assay, plants and Plexiglas lids were transferred to the mist chamber, which already contained a known volume of NO₃⁻ absorption solution (pH 6.0 buffer, as above, plus KNO₃ of desired [NO₃⁻]). The NO₃⁻ absorption period was initiated by turning on the pump to begin the continuous misting. Temperature inside the root compartment was maintained within ±2 C of the growth chamber air temperature. Assay conditions and [NO₃⁻]'s of the absorption solution specific to a particular experiment are described under "Results."

Acetylene reduction assays were begun by adding 100% C₂H₂ from a compressed gas cylinder at a flow rate of 2.0 liters/min for as long as needed to obtain the desired final [C₂H₂] in the gas phase. When acetylene was captured in a Saran bag and then,
by hand-pumping, mixed thoroughly with the gas mixture inside the
chamber. The gas addition ports were then stoppered, leaving
the interior of the root compartment at atmospheric pressure.

At selected time intervals, the gas phase was sampled for
determination of either [C2H2] or [C2H4]. If a change in gas phase
conditions was desired, the root compartment was flushed with
compressed air. Thirty min was usually sufficient to reduce both
C2H2 and C2H4 to trace amounts. The ethylene and C2H2 concen-
trations of gas samples were determined by flame ionization
detection with a Perkin-Elmer 3920 B gas chromatograph. Nitro-
gen was the carrier gas; the column was packed with Poropak R,
100–120 mesh, and column temperature was 45 C.

The absorption solution was sampled by withdrawing a suffi-
cient volume from the liquid reservoir by syringe. Replacement
of NO3−-depleted absorption solution with fresh solution, or changes
in volume, [NO3 −], or composition of the absorption solution,
were accomplished rapidly. Nitrate concentration of aliquots from
the liquid phase was determined by the salicylic acid method (1).

After each assay, the final volume of absorption solution and the root
volume (by water displacement) were determined. Total
gas phase volume was then calculated by difference. Plants were
separated into component parts, roots were thoroughly rinsed with
distilled H2O, and the tissues were dried at 70 C for 48 hr in a
forced-air oven. After roots were dried and weighed, nodules were
separated from them as time permitted. Nodules were redried at
70 C for 24 hr, and weighed. All C2H2 reduction activities and
NO3− absorption rates were expressed on a unit nodule or root
dry weight basis, respectively. Total N in dried tissue samples was
determined by standard Kjeldahl procedures.

RESULTS

Acetylene Reduction. Figure 2 shows the kinetics of [C2H4] and
C2H2 reduction during a typical assay. Acetylene concentration
declined rapidly during the first 5 to 10 min, after which no
further significant decrease was detected. The initial decline is
attributed to the solution of C2H2 in the liquid phase. The constant
[C2H2] thereafter indicates that equilibration had been attained
between the liquid and gas phases and that the system was
gas-tight.

In this experiment C2H2 reduction was linear throughout the
assay period. Continued production of C2H4 at a constant rate, for
2 hr (Fig. 2) in this experiment and for as long as 3 hr in other
experiments (data not shown), offered indirect evidence that: (a)
the chamber was gas-tight; (b) O2 supply was not limiting; and (c)

![Fig. 2. Kinetics of [C2H4] (Δ) and C2H2 reduction (O) by nodulated soybeans in NO3−-free buffer solution, 40 days after germination. Acety-
lene was added immediately before the first sampling. Values given are
means ± se's of six replicates; se's smaller in value than symbol dimensions
are not shown.](image)

The concentration of C2H2 was saturating for the system and
presumably at the site of reduction (i.e. the nodule interior). In
preliminary experiments on single replicates, analysis of C2H4
samples taken at intervals of 5 to 10 min during the first 30 min
of hr-long assays substantiated that C2H2 reduction proceeded
linearly from the outset (data not shown).

In other preliminary work, we determined experimentally the
response of N2[C2H4] fixation rate to increasing initial [C2H2]s,
from 0 to 0.06 ml/ml. No significant increase in N2[C2H2] fixation
rate was observed at [C2H2]s greater than 0.02 ml/ml (data not
shown). Consequently, all subsequent experiments were con-
ducted at or slightly above 0.02 ml/ml initial [C2H2], unless
specified otherwise.

No detectable amounts of C2H4 were produced by nodulated
plants when C2H2 was not added to the system, nor was any C2H4
produced by unnodulated plants in the presence or absence of
C2H2 (data not shown). Thus, problematical background levels of
endogenous C2H4 are avoided in this system.

We also investigated whether added C2H2 and/or accumulating
C2H4 might alter the kinetics of NO3− absorption. As shown in
Figure 3, the rate of NO3− absorption was not changed by adding
C2H2 midway through the absorption period. The lack of response
to C2H2 permitted flexibility in designing experiments. Acetylene
reduction could be assayed before, after, or during exposure to a
NO3−-containing absorption solution with assurance that the mea-
sured NO3− absorption kinetic data were valid.

Nitrate Absorption. Figure 4 shows the absorption of NO3− as
a function of time for nodulated and unnodulated plants. For
unnodulated plants grown on NO3−, the data suggest a time lag
of, at most, 1 hr before NO3− absorption began at a constant rate.
Nitrate absorption by nodulated roots followed a similar time
course except for a longer time lag. The NO3− absorption rate
from this (0.5 mm) concentration, once it attained a constant value,
was substantially higher for unnodulated plants than for nodulated
plants.

The effect of external [NO3−] on NO3− absorption rate was also
investigated for both unnodulated and nodulated plants. In three
separate experiments, NO3− absorption rate by unnodulated plants
increased as [NO3−] increased (Fig. 5). The data were fitted to a
rectangular hyperbola for illustrative purposes, using the Michaelis-
Menten equation and the double reciprocal method of calcu-
lating Vmax and apparent Km (4, p. 125). This approach to curv-
fitting the data for nodulated plants from two similar experiments
revealed that NO3− absorption rate responded to increasing [NO3−]
with saturation kinetics in only one of the experiments. In the
other experiment the response by nodulated plants to increasing
[NO3−] was better described by a straight line. Despite this uncer-
tainty about the exact kinetic nature of the NO3− absorption

![Fig. 3. Response of NO3− absorption to C2H2. Rate of NO3− absorption
from 0.5 mm KNO3 after addition of C2H2 at about 0.12 ml/ml was not
significantly changed (P = 0.05), as determined by a t test of regression
coefficients for the two intervals. Assays were conducted 34 to 35 days
after germination. Values given are means ± se of six replicates.](image)
process in roots of nodulated plants, it was clear that unnodulated plants had higher NO₃⁻ absorption rates at all [NO₃⁻] than did nodulated plants. The magnitude of the difference changed little above 0.5 mM NO₃⁻.

**Effect of N Stress on Response to NO₃⁻ Concentration.** Despite attempts to equalize the growth rate of nodulated and unnodulated plants nutritionally, by the time flowering began (30 days after germination) unnodulated plants were larger and more vigorous than nodulated plants. On a visual basis, unnodulated plants were greener and had greater total leaf area, more and longer axillary branches, thicker stems, and larger, more extensively branched roots. Differences in dry weight, N percentage, and total N content of component parts of and whole plants supported those observations (e.g. see data for controls in Table II).

Two experiments were performed to determine whether these differences in development might account for the observed differences in NO₃⁻ absorption rates, and to separate N stress effects from the effects of nodulation. The first experiment, after 2 weeks of growth in N-free solution and 1 week in N-containing solution (the sequence routinely given all plants), unnodulated plants were returned to N-free nutrient solution for a 10-day period prior to assay. Nitrate absorption rate was then measured as a function of increasing [NO₃⁻]. These N-stressed unnodulated plants had a $V_{\text{max}}$ of 24.12 μmol of NO₃⁻ absorbed (g root dry weight hr⁻¹) and a $K_m$ of 0.085 mM NO₃⁻. Comparison of these kinetic constants with those presented previously (Fig. 5) shows that the NO₃⁻ absorption systems were similar in roots of both nodulated and N-stressed unnodulated plants.

In the second experiment, nodulated plants were returned to N-containing nutrient solution for 2 and 4 days before the rate of NO₃⁻ absorption from 0.5 mM NO₃⁻ was measured (Table I). Before the return to N, the NO₃⁻ absorption rate of nodulated plants was only 36% of that of unnodulated plants. Two days of exposure to N more than doubled the NO₃⁻ absorption rate. After 4 days the rate had tripled, and capacity for NO₃⁻ absorption was slightly, though not significantly, greater than that of unnodulated plants. In this experiment nodulated plants responded to the return to N with an increase in dry weights, N percentages, and N content of roots, shoots, and whole plants (Table II).

**Effect of Shoot Removal.** Two preliminary experiments sug-

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### Table I. Effect of return to N-containing nutrient solution on rates of NO₃⁻ absorption by nodulated soybeans

<table>
<thead>
<tr>
<th>Days in + N nutrient solution</th>
<th>umoles NO₃⁻ (g root dry wt-hr)⁻¹</th>
<th>Percent of unnodulated control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (control)</td>
<td>16.0 ± 8.8</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>31.8 ± 6.7</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>43.9 ± 6.3</td>
<td>113</td>
</tr>
<tr>
<td>Unnodulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>58.8 ± 11.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean rates followed by the same letter are not significantly different (p = 0.05), as determined by t tests of regression coefficients.

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### Table II. Effect of return to N-containing nutrient solution on dry weight, N percentage, and N content of component parts of nodulated soybeans compared with unnodulated soybeans grown with N continuously

<table>
<thead>
<tr>
<th>Days in + N nutrient solution</th>
<th>shoots</th>
<th>roots</th>
<th>nodules</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.31 ± 1/</td>
<td>3.78 ± 1</td>
<td>0.583 ± 1</td>
<td>10.67 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>7.72 ± 1/</td>
<td>4.43 ± 1</td>
<td>0.484 ± 1</td>
<td>12.67 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>9.71 ± 1/</td>
<td>4.97 ± 1</td>
<td>0.405 ± 1</td>
<td>15.09 ± 1</td>
</tr>
<tr>
<td>Unnodulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>18.53 ± 1</td>
<td>6.91 ± 1</td>
<td>--</td>
<td>25.44 ± 1</td>
</tr>
</tbody>
</table>

Percentage N

<table>
<thead>
<tr>
<th>Days in + N nutrient solution</th>
<th>shoots</th>
<th>roots</th>
<th>nodules</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.47 ± 1/</td>
<td>1.60 ± 1</td>
<td>6.05 ± 1</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>3.44 ± 1/</td>
<td>2.29 ± 1</td>
<td>6.10 ± 1</td>
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</tr>
<tr>
<td>4</td>
<td>3.73 ± 1/</td>
<td>2.63 ± 1</td>
<td>5.93 ± 1</td>
<td>--</td>
</tr>
<tr>
<td>Unnodulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>4.59 ± 1</td>
<td>3.74 ± 1</td>
<td>--</td>
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</tbody>
</table>

N content (g)

<table>
<thead>
<tr>
<th>Days in + N nutrient solution</th>
<th>shoots</th>
<th>roots</th>
<th>nodules</th>
<th>total</th>
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</thead>
<tbody>
<tr>
<td>Modulated</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>0.16 ± 1/</td>
<td>0.06 ± 1</td>
<td>0.033 ± 1</td>
<td>0.252 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>0.27 ± 1/</td>
<td>0.10 ± 1</td>
<td>0.029 ± 1</td>
<td>0.398 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>0.36 ± 1/</td>
<td>0.13 ± 1</td>
<td>0.024 ± 1</td>
<td>0.520 ± 1</td>
</tr>
<tr>
<td>Unnodulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.85 ± 1</td>
<td>0.26 ± 1</td>
<td>--</td>
<td>1.107 ± 1</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter are not significantly different (p = 0.05), as determined by Duncan's multiple range test.
suggested that intact nodulated plants assayed for N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation activity in the mist chamber had substantially higher N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation rates than did excised nodulated root systems assayed in either the mist chamber or in glass Mason jars. Figure 6 shows results of an experiment designed to compare all three of these assay conditions. Nitrogen [C\textsubscript{2}H\textsubscript{2}] fixation rate by intact plants assayed in the mist chamber in NO\textsubscript{3}\textsuperscript{-}-free absorption buffer was constant for 2 hr (Fig. 6). For plants assayed in the mist chamber under similar conditions, but following removal of shoots at time zero, N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation rate was constant for 1 hr. The rate was only 76% of that observed with intact plants, however, and after 1 hr it declined from 77.0 to 21.3 μmol of C\textsubscript{2}H\textsubscript{2} (g nodule dry weight·hr)\textsuperscript{-1}, only 21% of the rate of the intact controls. This lower rate was maintained for at least another 2.5 hr (data not shown).

Excised nodulated roots were incubated in glass jars in an air-C\textsubscript{2}H\textsubscript{2} mixture at an [C\textsubscript{2}H\textsubscript{2}] (0.022 ml/ml) equivalent to that used in the mist chambers. The observed kinetic pattern was similar to that for detopped plants assayed in the mist chamber. The 1st-hr N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation rate was, however, even lower than that observed for excised roots assayed in the mist chamber (36.2 versus 77.0 μmol of C\textsubscript{2}H\textsubscript{2} (g nodule dry weight·hr)\textsuperscript{-1}). Again, 1 hr after excision the rate declined, by 37%. This 2nd-hr rate was slightly, though not significantly, lower than the 2nd-hr rate for excised roots in the mist chamber (Fig. 6).

![Graph](image)

**Fig. 6.** N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation by intact plants (○) and excised roots (△) assayed in the mist chamber, and by excised roots assayed in glass jars (□). Plants were 29 to 30 days old. Assays were conducted in the absence of NO\textsubscript{3}\textsuperscript{-} following 16 hr of pretreatment with NO\textsubscript{3}\textsuperscript{-}-free buffer solution. Assays were conducted concurrently in three mist chambers. The initial [C\textsubscript{2}H\textsubscript{2}]'s exceeded zero because 10 to 15 min elapsed after addition of C\textsubscript{2}H\textsubscript{2} to a given chamber before the first C\textsubscript{2}H\textsubscript{2} sample was taken from that chamber. During that interval, C\textsubscript{2}H\textsubscript{2} addition to the other two mist chambers was sequentially accomplished. Values given are means ± se of three replicates; se's smaller in value than symbol dimensions are not shown.

**DISCUSSION**

As measured in the mist environment of our apparatus, NO\textsubscript{3}\textsuperscript{-} absorption by soybeans was similar in kinetics to NO\textsubscript{3}\textsuperscript{-} absorption reported for other species (2, 9, 13, 18). The 1-hr lag in NO\textsubscript{3}\textsuperscript{-} absorption rate by roots of unnodulated plants already having an induced NO\textsubscript{3}\textsuperscript{-} transport system may represent the time required for ion transport system(s) to adjust from the relatively static environment of standard liquid culture to the well stirred, well aerated mist environment. Once the adjustment has been made, low boundary layer resistance to ion diffusion at the root-solution interface in our apparatus may result in conditions conducive to estimation of maximum potential rates of absorption of NO\textsubscript{3}\textsuperscript{-} (and other ions). Plants are intact during assay, permitting photosynthesis, transpiration, and all metabolic reactions to proceed normally. Absorption from solutions of more than one [NO\textsubscript{3}\textsuperscript{-}] can be measured in sequence with the same plants. Plants need not be sacrificed for assay, so that repeated measurements are possible at several stages of growth.

Huffaker and Rains (8) suggested that NO\textsubscript{3}\textsuperscript{-} absorption capacity in crop plants is closely related to the maximum velocity of NO\textsubscript{3}\textsuperscript{-} absorption measured over the concentration range commonly encountered in agricultural environments. Numerous reports of saturation kinetics for NO\textsubscript{3}\textsuperscript{-} absorption by several species suggest the general applicability of carrier-mediated ion absorption theory to NO\textsubscript{3}\textsuperscript{-} absorption studies (2, 10, 13, 18, 22, 23). The results summarized in this paper suggested that unnodulated soybeans had a V\textsubscript{max}, or capacity for NO\textsubscript{3}\textsuperscript{-} absorption, more than 2.5 times that of the V\textsubscript{max} for nodulated plants. Evidence obtained with N-stressed unnodulated plants, however, suggested that the lower NO\textsubscript{3}\textsuperscript{-} absorption capacity of nodulated soybeans was due as much to N stress as to the state or condition of being nodulated.

Gibson (5) has summarized evidence supporting the observation that legumes normally experience a generalized N stress during the period of nodule growth and nitrogenase synthesis. In the present study, nodulated plants were N-stressed by growth in NO\textsubscript{3}\textsuperscript{-}-free solution, despite an early, brief (7 days) period of N supplementation provided to prevent severe chlorosis and stunting.

Returning N-stressed nodulated plants to NO\textsubscript{3}\textsuperscript{-} resulted in accelerated growth and increases in both per cent N and total N content of both shoots and roots (Table II). Although 4 days of growth in NO\textsubscript{3}\textsuperscript{-} did not result in dry weights, N percentages, or N contents equal to those of unnodulated plants, that length of exposure to NO\textsubscript{3}\textsuperscript{-} was sufficient to effect a full recovery in NO\textsubscript{3}\textsuperscript{-} absorption capacity.

Some of the lower capacity of nodulated plants for NO\textsubscript{3}\textsuperscript{-} absorption might also be ascribed to the absence of a fully operative NO\textsubscript{3}\textsuperscript{-} transport system and to the measurement of absorption rate before induction of such a system had been completed. Admittedly, that parameter was subject to experimental manipulation by choice of pretreatment conditions. Even so, any increase in rate that could have been expected after sufficiently long inducing conditions (Fig. 4) would account for only a fraction of the observed difference in estimated V\textsubscript{max} values. Whether the recovery in NO\textsubscript{3}\textsuperscript{-} absorption capacity (Table I) was due to induction or stimulation of a more efficient NO\textsubscript{3}\textsuperscript{-} absorption system, or to increased photosynthate supply to roots, or both, cannot be ascertained from these data alone. The possibility that some physical basis at the cellular or molecular level exists to explain the lower NO\textsubscript{3}\textsuperscript{-} absorption capacity of nodulated roots was beyond the scope of these investigations.

Our interest in comparing the capabilities of our system with those of widely used acetylene reduction techniques prompted the investigation of the effect of shoot removal. Figure 6 suggests both an immediate and a somewhat delayed effect of shoot removal on N\textsubscript{2}[C\textsubscript{2}H\textsubscript{2}] fixation. The direct comparison of excised roots with control roots, both assayed in the mist chamber, revealed a 24%
Nitrogen [C₂H₄] fixation rate during the 1st hr was substantially lower for roots assayed in glass jars (i.e., by a frequently used technique) than for mist chamber-assayed excised roots (Fig. 6). These data suggest that techniques widely used to measure C₂H₄ reduction preclude estimation of maximum potential N₂ fixation capacity. Too high a nodule-to-volume ratio in the C₂H₄ incubation vessel can reduce N₂[C₂H₄] fixation rates, even during short assays (6, 21), probably through a depletion of available O₂.

Sprent (19) demonstrated the sensitivity of C₂H₄ reduction by detached nodules to either too much or too little water on their surfaces. Sutton and Jepsen (21) concluded from comparisons of nodule slices and detached, whole nodules “that the effect of water-logging (described by Sprent [19]) was to prevent gaseous diffusion through the nodule cortex to the central zone of cells containing nitrogenase.” In our mist system the large gas volume serves as a reservoir for sufficient quantities of O₂, and the liquid that coats the root and nodule surfaces is always saturated with both C₂H₄ and O₂. Consequently, diffusion of these gases across the liquid boundary layer is lessened as a factor limiting rates of C₂H₄ reduction.

Finally, the agitation created as the mist droplets are propelled through the gas space results in a uniformly mixed gas phase. Samples taken for C₂H₄ or C₂H₄ analysis are representative of the entire gas phase, and localized depletion or concentration of the component gases is avoided.

The predominant techniques employed in early investigations of N₂ fixation using the C₂H₄ reduction assay involved incubation of nodulated root segments, excised nodules, or nodule slices in a gas mixture. Nearly all recently reported systems for in situ C₂H₄ reduction assays on intact plants incorporated solid rooting media. Both systems thus lack the capability for studying NO₃⁻ absorption kinetics. Conversely, most conventional ion absorption techniques are not easily adapted for C₂H₄ reduction assay. A gas-tight incubation vessel is required for C₂H₄ reduction, and technical problems of gas exchange between the liquid and gas phases would be more difficult to overcome in a liquid system than in a mist system. We believe that the capabilities of the apparatus we have developed for simultaneous measurement of NO₃⁻ absorption kinetics and N₂[C₂H₄] fixation by intact soybean plants overcome those limitations and afford distinct advantages over previously developed techniques.

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LITERATURE CITED