All N-containing materials have a $^{15}$N-natural abundance level of 0.3663 atom% (Junk and Svec, 1958). It is therefore possible to use N-salts in $^{15}$N tracer studies, which are depleted instead of enriched in $^{15}$N; however, the maximum difference in atom% $^{15}$N between the $^{15}$N-depleted salt and nonlabeled N material is thus 0.3663. Due to this restricted range in atom% $^{15}$N, $^{15}$N-depleted salts are less suitable for some $^{15}$N experiments. The use of $^{15}$N-depleted material has further been limited not as much by the sensitivity of the mass spectrometer employed as by sample variability (Broadbent and Carlton, 1980). The advantages of using depleted $^{15}$N salts include their low cost and availability, which permit the use of relatively larger experimental plots than are feasible with $^{15}$N-enriched compounds. The $^{15}$N-depleted salts would be especially practical in research assessing the biological N$_2$ fixation of trees that require larger $^{15}$N-labeled subplots than are commonly used in field studies with annual legumes.

This note reports the experimental use of $^{15}$N-depleted (NH$_4$)$_2$SO$_4$ for estimating N derived from N$_2$ fixation and N-allocation in lebbeck [Albizia lebbeck (L.) Benth.] and leucaena [Leucaena leucocephala (Lam.) de Wit], two leguminous trees.

**Materials and Methods**

Seeds of lebbeck and leucaena were scarified and sterilized in concentrated H$_2$SO$_4$ for 15 min, thoroughly rinsed with distilled water, and planted in Leonard jars. The Leonard jar assemblies had a 2-L nutrient solution reservoir containing an N-free nutrient solution (Broughton and Dilworth, 1971). The upper part of the assembly (1 L) was filled with vermiculite. The nutrient solution was supplied to the upper chamber by a cotton wick. Four N treatments were established by weekly applications of 0, 5, 12.5, or 25 mg of $^{15}$N-depleted (NH$_4$)$_2$SO$_4$ added directly to the upper part of the Leonard assembly. After 10 and 18 weeks growth for leucaena and lebbeck respectively, plants were separated into shoots, roots, and nodules for analysis. The atom% $^{15}$N in shoots and roots was linearly correlated to the amount of applied $^{15}$N-depleted (NH$_4$)$_2$SO$_4$. Significant differences in atom% $^{15}$N and percentage N derived from N$_2$ fixation were measured in lebbeck and leucaena when, respectively, more than 8.3 and 2.9% of the total N was derived from (NH$_4$)$_2$SO$_4$. Nodules derived a larger portion of their total N from N$_2$ fixation than did the roots or shoots, indicating that most of the N present in the nodules was derived from N$_2$ fixation. Nodules of both species at the no N treatment showed slightly higher atom% $^{15}$N values than the $^{15}$N-natural abundance level of atmospheric N$_2$.

Additional index words. Leucaena, Leucaena leucocephala (Lam.) de Wit, lebbeck, Albizia lebbeck (L.) Benth, Nitrogen-15 dilution, Rhizobium, Nitrogen-allocation.

Since the heavy, stable isotope of nitrogen, $^{15}$N, has been available (Thade and Urey, 1939), there have been numerous reports of the use of this isotope for the estimation of biological N$_2$ fixation (Hauck and Bystrom, 1970). In field studies, biological N$_2$ fixation (BNF) has been estimated by applying $^{15}$N-enriched salts to the soil and measuring the level of $^{15}$N uptake into both N$_2$-fixing plants and non-N$_2$-fixing reference plants. The $^{15}$N enrichment level of the material applied varies, depending on factors such as the total N applied, the available N in the soil, and the expected total N uptake by the growing crop. Although current ratio mass spectrometers can precisely measure differences as small as 0.0005 atom% $^{15}$N, few field studies have been conducted where materials were applied with an enrichment level of < 1 atom% $^{15}$N.
After seed germination, lebbeck seedlings were inoculated with turbid suspensions of *Rhizobium* strains TAL 1597 and *leucaena* with TAL 1145 (NifTAL Project, Box 0, Paia HI).

Treatments were replicated five times in a completely randomized design. The leucaena and lebbeck seedlings were harvested at 10 and 18 weeks after planting, respectively. Shoots, roots, and nodules were separated, dried for 48 h at 70°C, weighed, and ground in a Cyclone mill.

Plant samples were digested in a salicylic acid-sulfuric acid mixture, using a Se-K₂SO₄/CuSO₄ catalyst (Brenner and Mulvaney, 1982) to include nitrate and nitrite. The distillates were subsampled and total N determined by the indophenol blue method (Keeney and Nelson, 1982). The pH of the remaining distillate was adjusted to a value between 4 and 5 and evaporated to near dryness for mass spectrometer analysis. The ¹⁵N analyses were conducted by Isotope Service, Inc., Los Alamos, NM, and at the laboratory of Dr. R.H. Burris, University of Wisconsin, Madison, WI.

The percentage N derived from N₂ fixation was calculated using the following equations:

1. atom% ¹⁵N-excess sample = atom% ¹⁵N sample - atom% ¹⁵N of comparable part from plants receiving no mineral N.
2. atom% ¹⁵N-excess of mineral N applied = atom% ¹⁵N mineral N applied - 0.3663.

Due to the large seed size, particular for lebbeck, a correction was made for calculating the percent N derived from N₂ of shoots and roots. Total N in seed of lebbeck and *leucaena* was 7.9 and 2.5 mg N, respectively. The partitioning of seed N between shoot and root was assumed to be equal and seed N transported to the initial nodule formation was considered to be insignificant. The difference in atom% ¹⁵N between atmospheric N₂ and seed N was found to be insignificant.

3. Percent N derived from the N₂ fixation and seed N (%NdFAS) =

\[
\left(1 - \frac{\text{atom}\%\ ¹⁵N\ excess\ N₂\ fixing\ plant}{\text{atom}\%\ ¹⁵N\ excess\ mineral\ N\ applied}\right) \times 100
\]

4. Total N derived from atmospheric N₂ and seed N (TNdFAS) = % NdFAS × total N of plant part.

5. Total N derived from atmosphere (TNdFA) in root or shoot = TNdFAS - half of total N present in seed.

6. Percent N derived from atmosphere (%NdFAS) =

\[
\left(\frac{\text{TNdFA}}{\text{total }\ N\ \text{in plant part}}\right) \times 100
\]

For nodules the %NdFAS was calculated as follows:

7. %NdFAS =

\[
\left(1 - \frac{\text{atom}\%\ ¹⁵N\ excess\ in\ nodule}{\text{atom}\%\ ¹⁵N\ excess\ in\ mineral\ N\ applied}\right) \times 100
\]

### Results and Discussion

Total N of the different plant parts of *leucaena* and lebbeck were not significantly different, regardless of the N application. Total mg N + SE of shoots, roots, and nodules were 84.5 + 7.6, 55.2 + 4.1, and 12.0 +

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<th>Table 1. Atom% ¹⁵N and percent N derived from N₂ fixation of shoots, roots, and nodules of lebbeck.</th>
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<td>(NH₄)₂SO₄ applied</td>
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Bayes LSD (0.05) 0.0164 8.3 0.0211 7.5 NS NS

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<th>Table 2. Atom% ¹⁵N and percent N derived from N₂ fixation of shoots, roots, and nodules of <em>leucaena</em>.</th>
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<td>(NH₄)₂SO₄ applied</td>
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Bayes LSD (0.05) 0.0081 2.4 0.0089 2.9 NS NS

1.1 for *leucaena* and 50.9 + 5.4, 75.5 + 8.6, and 19.2 + 3.1 for lebbeck, respectively. Levels of (NH₄)₂SO₄ applied were probably too low to cause detectable responses in dry matter or plant N accumulation.

Tables 1 and 2 show the atom% ¹⁵N and the calculated percent N derived from N₂ fixation (%NdF) for the shoots, roots, and nodules of the *leucaena* and lebbeck. The atom% ¹⁵N in shoots and roots was linearly correlated to the amount of applied ¹⁵N-depleted (NH₄)₂SO₄. The corresponding correlation coefficients for those regression lines for shoots and roots were 0.94 and 0.93 for *leucaena* and 0.96 and 0.93 for lebbeck, respectively. The lowest level of applied N did not generally produce significant differences for atom% ¹⁵N as compared with the no N treatment. Differences occurred as the amount of ¹⁵N-depleted (NH₄)₂SO₄ applied was increased to a level of 12.5 and 25 mg (NH₄)₂SO₄ per week. Data indicate that when more than 3% of total N in *leucaena* and 8.5% in lebbeck is derived from the inorganic N source, significant differences in atom% ¹⁵N can be measured when compared with the seedlings receiving no (NH₄)₂SO₄.

Values for atom% ¹⁵N are lower for lebbeck than for *leucaena* at every level of applied N. This difference is due to the slower growth of lebbeck and the greater amount of isotope it received in comparison to *leucaena*. Lebbeck seedlings had received 0, 40, 100, or 200 mg more ¹⁵N-depleted (NH₄)₂SO₄ than the *leucaena* seedlings at the same treatment level at the time of harvest. The atom% ¹⁵N values of the nodules remained much closer to the value of atmospheric N₂ than did the other plant parts. In the 0 mL N treatment the different plant parts of both species had an atom% ¹⁵N close to the natural ¹⁵N abundance level of atmospheric N₂. Similar higher atom% ¹⁵N values have been reported.
previously for nodules of various annual species (Shearer et al., 1984; Shearer et al., 1982; Steele et al., 1983; Yoneyama et al., 1984). A conclusive explanation for this $^{15}$N enrichment in N$_2$-fixing nodules has not yet been given. The %Ndfa was higher in nodules than in the shoots and roots of plants within a treatment level. This increase suggests that in leucaena and lebbeck, most of the N necessary for nodular tissue formation is derived directly from N$_2$-fixation products and not through reallocation of N from nonnodular tissue. These results agree with those of Ohyama and Kumazawa (1979) and Westermann et al. (1985) who reported that after the incubation of soybean/Glycine max (L.) Merr. nodules with $^{15}$N$_2$ for 24 or 48 h, most of the newly accumulated N in the nodules was derived from N$_2$ fixation. Pate et al. (1979), however, found that only 49% of the N in white lupine (Lupinus albus L.) nodules was derived directly from N$_2$-fixation products and 51% was from the reallocation of N from the shoots, which was cycled back into the nodule. However, those figures were calculated from xylem and phloem sap analyses, and by respiration and transpiration measurements. No information, to our knowledge, has been reported about the origin of N found in nodules of leguminous trees. The allocation of fixed N$_2$ or inorganic (NH$_4$)$_2$SO$_4$ into shoots or roots was similar for both species, independently of the amount of (NH$_4$)$_2$SO$_4$ supplied. Similar results have been found in soybean grown under N$_2$, N$_0$$_3$, or NH$_4$$^+$ (McNeil and LaRue, 1984).

These results demonstrate the usefulness of $^{15}$N-depleted (NH$_4$)$_2$SO$_4$ for assessing BNF and N-allocation by trees in greenhouse studies. The $^{15}$N-depleted N salts may be a useful tool for assessing N$_2$ fixation by trees under natural field conditions.

Acknowledgments

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