oculated with the mutants were less than those of plants inoculated with the parental strains (Table 2). The ratios of nodule mass to dry weight for plants nodulated with the $H_2$-uptake negative mutants were greater than comparable ratios for plants inoculated with the $H_2$-uptake positive parental strains (Table 2). The increase in dry weight and nitrogen contents of plants nodulated by the $H_2$-uptake positive strains cannot be accounted for by differences in nodule weight.

In agreement with previous reports (*3–5, 11*) all $H_2$-uptake positive strains produced nodule bacteroids with hydrogenase activity (Table 2), but no activity was detected in the nodule cytosol (soluble nonbacteroid part of the nodule). In contrast, no measurable hydrogenase activity could be detected in bacteroids from nodules that evolved $H_2$, and these observations agree with the other reports (*6, 12*). The acetylene reduction rates of nodules formed by the $H_2$-uptake negative mutant strains were somewhat less than acetylene reduction rates of nodules formed by the $H_2$-uptake positive parental strains. In comparison with the plants inoculated with the $H_2$-uptake negative mutants, the percentage increases from inoculation with the $H_2$-uptake positive parental strains were: dry weight, 32; nitrogen, 13; and total nitrogen in plants, 49 (Table 2). Although all three of the $H_2$-uptake negative mutants behaved similarly in previous studies (*13*) in this experiment, the mean total nitrogen content of plants inoculated with SR3 was less than that of plants inoculated with mutants of SR1 or SR2. The mutants were selected on the basis of their inability to take up $H_2$, and further characterization of them is required and is in progress.

In both experiments a regression analysis revealed highly significant positive correlations between relative efficiency values and total nitrogen contents of plants ($r^2 = .92$ and $r^2 = .90$ for data in Tables 1 and 2, respectively). Also, in both experiments, analyses of variance revealed that dry weights, percentage nitrogen, and total nitrogen in plants inoculated with the $H_2$-uptake positive strains were significantly higher than comparable values for plants inoculated with the $H_2$-uptake negative strains (Tables 1 and 2). The data from the two different types of experiments are consistent, showing in both cases that plants with nodules containing the hydrogenase system fixed more $N_2$ and produced greater dry weights than plants that were nodulated with the $H_2$-uptake negative strains.

Any process that would decrease $H_2$ evolution by nitrogenase or contribute toward recycling of the evolved $H_2$ should increase nodule relative efficiency, as defined by Schubert and Evans (*4*), and should lead to increased $N_2$ fixation and plant growth, provided that $N_2$ fixation is limited by the energy provided by carbon substrates. Hardy and Havelka (*14*) have suggested that the supply of carbon substrates from photosynthesis is a major factor limiting $N_2$ fixation by legumes. The diversion of energy from nitrogenase by $H_2$ evolution would be expected to have a deleterious effect on $N_2$ fixation and plant growth. The maximum benefits of the hydrogenase system to $N_2$ fixation in the legume-*Rhizobium* symbiosis would be predicted when the energy supply for support of $N_2$ fixation is limiting.

The results of these greenhouse experiments are consistent with those obtained in a field trial (*11*). From the combined evidence we conclude that incorporation of the hydrogenase system into the gene pool of existing rhizobial inocula is desirable and may contribute toward increased productivity of soybeans. Beneficial effects of the hydrogenase system in a strain of cowpea *Rhizobium* also has been observed (*15*). It seems reasonable that use of $H_2$-uptake positive strains of *Rhizobium* species for the inoculation of other legumes may contribute to increased efficiency of the $N_2$-fixing process.

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## Ammonia Volatilization from Senescing Leaves of Maize

Abstract. *Ammonia release by plants growing in normal air is reported. Contrary to observations made at high ambient ammonia concentrations, corn plants did not absorb ammonia in normal air but released it as they senesced, even while photosynthesizing actively.*

Studies of the global ammonia cycle have either ignored living plants (*1*) or treated them solely as sinks (*2, 3*). Several workers have demonstrated that plants assimilate gaseous ammonia (*2, 4*), but they imposed external partial pressures of ammonia (30 to 20,000 nbar) that were much higher than those found in unpolluted areas (1 to 8 nbar) (*5*). Their results have been extrapolated to field conditions (*2*) on the assumption that the rate of absorption of ammonia is the product of the stomatal conductance to diffusion of ammonia and the concentration of ammonia in the surrounding air, the concentration in the leaf being assumed zero. The assumption that the internal concentration is negligible appeared to be borne out in experiments in which high external concentrations were used (*2, 4*), but these imposed concentrations were so high as to obscure the presence of a small internal partial pressure of a few nanobars. Meyer (*6*) showed that plants released ammonia into air from which the ammonia had been re-


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Table 1. Rates of evolution of ammonia from senescing and nonsenescing leaves of *Zea mays*.

| Plant nitrogen status | Half of plant | Assimilation of $CO_2$ ($\mu$mole/m$^2$-sec) | Senescing leaves present | Evolution of $NH_3$ (nmole/m$^2$-sec) | Standard error | Mean $NH_3$ partial pressure (nbar) |
|---|---|---|---|---|---|---|
| High N | Top | 8.9 | No | 0.00 | ±0.07 (6)* | 5 |
| High N | Bottom | 7.6 | No | 0.05 | ±0.05 (7) | 3 |
| Low N | Top | 6.3 | No | 0.07 | ±0.06 (6) | 3 |
| Low N | Bottom | 3.7 | Yes | 0.57 | ±0.07 (12) | 4 |

*The number of measurements is given in parentheses.

moved and that, of the species tested, corn released the least. He concluded that absorption generally predominated under normal conditions. We report that there is no net uptake by corn plants of the small amounts of ammonia normally found in the atmosphere; corn plants release ammonia in normal air as they senesce, even while still showing positive net assimilation of carbon dioxide. To our knowledge, these are the first observations of ammonia release by plants growing in normal air.

We exposed plants to ambient air having realistically low partial pressures of ammonia (5 ± 3 nbar). Corn (*Zea mays* L. var. NES 1002) plants were grown in pots in a glasshouse, using Hoagland nutrient solution with two nitrate nitrogen concentrations, 3.4 and 35 meq/liter. Between 78 and 116 days after germination a plant was taken and the aerial portion was enclosed in an acrylic chamber measuring 13 by 42 by 155 cm. The chamber was sealed at the base of the stem, using Terostat (*7*) to exclude the soil as a source of or sink for ammonia. The chamber was surrounded by a bank of vertical fluorescent lights, and both the chamber and lights were air-cooled with a 30-cm high-speed fan. The air temperature in the chamber was 25° ± 3°C. The lights were surrounded by a reflective aluminum cylinder, and the irradiance on one side of a vertical plane in the chamber was 500 $\mu$E/m$^2$-sec. Air was drawn from outside the building through the chamber and into ammonia collectors, which were similar to those of Denmead *et al.* (*8*). The air then passed through a flow meter to a pump. Air was also drawn through a similar system in parallel but with no plant chamber interposed. In both cases the flow rate was 30 liter/min.

Rates of $CO_2$ assimilation were determined with a Maihak Unor 2 infrared gas analyzer. After 90 minutes the ammonia collectors were eluted with distilled water and the ammonia concentration was determined by using both an Orion specific ion electrode and a Technicon autoanalyzer employing an indophenol method. Both of the latter techniques detect amines as well as ammonia, and the possible contribution of amines to the measured rates was unknown. Both techniques gave consistently similar results, and tests of recovery in which known realistic quantities of ammonia were released in the plant chamber showed that there were no significant leaks or absorbance of ammonia before the air reached the ammonia collectors. Once a day a blank run was made with the plant chamber empty. The flux of ammonia from the plant was determined from the differing amounts of ammonia in the collection tubes and then corrected for any apparent evolution or assimilation from the blank run.

A series of measurements was made on the younger (top) and older (bottom) leaves of high- and low-nitrogen plants. Rates of photosynthetic assimilation of $CO_2$ (Table 1) were lower in the older leaves of the high-nitrogen plants than in the younger leaves, and lower again in the younger leaves of the low-nitrogen plants. The rates were markedly depressed in plants having some senescing leaves. The plants were healthy and in separate experiments the young leaves of the high-nitrogen plants, at a $CO_2$ partial pressure of 320 $\mu$bar and an irradiance of 2 mE/m$^2$-sec, had a $CO_2$ assimilation rate of 30 $\mu$mole/m$^2$-sec. The lower rates for the same leaves shown in Table 1 are due to the lower irradiance and significant depletion of $CO_2$ in the chamber.

The results (Table 1) demonstrate that ammonia is not taken up by nonsenescing leaves of corn in atmospheres containing the low concentrations of ammonia typically found in unpolluted areas. From separate experiments at the appropriate irradiance, temperature, and partial pressure of $CO_2$, we estimated stomatal conductance to the diffusion of both $NH_3$ and water vapor of the high-nitrogen plants as 0.2 mole/m$^2$-sec, equal to 0.5 cm/sec in the older measure of conductance (*9*). With ambient partial pressures of ammonia ranging from 2 to 8 nbar we would expect to find rates of uptake of ammonia between 0.40 and 1.60 nmole/m$^2$-sec if the internal concentrations of ammonia were zero. As it was, no uptake occurred.

The most interesting feature of the results was the volatilization of ammonia from senescing leaves. The rate at which this occurred, equivalent to 7 g of N per hectare per day with a leaf area index of 1, is not high enough to account for observations of declining total nitrogen in grain crops after flowering (*10–12*) but may be significant in terms of the turnover of ammonia in the troposphere. The release of ammonia over Britain has been estimated (*1*) as 100 kilotons per year, equivalent to 0.8 nmole/m$^2$-sec or 9 g of N per hectare per day. In this estimate it was assumed that living, senescing, and rotting vegetation contribute negligible amounts. Although we do not exclude the possibility of foliar absorption of ammonia by other means (*7, 13*), our results indicate that studies of the global ammonia cycle that have either ignored living plants as possible sources (*1*) or treated them solely as sinks (*2, 4*) are likely to be in error.

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