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# Symbiotic N Fixation by *Vigna luteloa*, A Legume of Low-Salinity Gulf Coast Marshes

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## Short Papers and Notes:

# SYMBIOTIC N FIXATION BY *Vigna luteloa*, A LEGUME OF LOW-SALINITY GULF COAST MARSHES

Fixation of atmospheric N may be the largest N input to low-salinity marshes of the Gulf Coast region (DeLaune and Patrick, 1990). Although several estimates of N fixation (acetylene reduction) by mats of blue-green algae or by the predominantly non-leguminous marsh vegetation are available (Whitney et al., 1975; Teal et al. 1979; Casselman et al., 1981; DeLaune and Patrick, 1990), little data exists concerning marsh legumes. *Vigna luteola* (Jacq.) Benth. (deerpea) is a perennial, viny legume occuring in fresh to brackish marshes of the Gulf Coast region. *Vigna* may account for 1.5% of total plant abundance throughout the Louisiana coastal marsh zone, and for 3.8% of total plant abundance in the intermediate salinity marshes (Chabreck, 1970).

The objectives of this study were to estimate N fixation (acetylene reduction) rates attributable to both symbiotic sources associated with *Vigna*, and to free-living N fixers in adjacent soils; and to compare landscape-level estimates of N fixation with and without inclusion of *Vigna* patches.

## MATERIALS AND METHODS

On 17 August 1993, three replicate soil cores (14.5 cm diameter x 15 cm deep) were collected from an intermediate marsh at the eastern margin of Four League Bay in Terrebonne Parish, Louisiana. Each core was centered on the base of an individual *Vigna* plant. An abundance of root nodules was visable on the marsh soil surface and to a depth of approximately 1-2 cm. Upon returning to the laboratory, after approximately 24 hours, nodulated root segments were separated from the soil matrix. An $C_2H_2$ reduction assay was used to estimate N fixation rates (Turner and Gibson, 1980). Acetylene was generated from $CaC_2$ and $H_2O$ (Miller, 1965). Samples were placed in 140 ml glass jars, and incubated at room temperature in either air or 90% air: 10% $C_2H_2$. Headspace gas was sampled at 1, 4, and 16 h and concentrations of $C_2H_4$ were determined by gas chromatography (Perkin-Elmer Model 900 GC equipped with a flame ionization detector). Production rates of $C_2H_4$ by nodulated root segments were approximately linear over the first 4 hours of incubation. For soils, 16 hours were required for detectable $C_2H_4$ production.

On 9 September 1993, three different intermediate salinity marsh sites near Raccourci Bay in Terrebonne Parish, Louisiana were sampled. At each site, three replicate pairs of soil cores (14.5 cm diameter x 2 cm deep) were collected from within dense *Vigna* patches and from the adjacent *Spartina patens* stand. Cores were collected from predetermined positions along a transect, with no attempt made to center on a *Vigna* plant base. Immediately after collection, each soil core was cut into four equal wedge-shaped subsamples. Two diagonally opposite subsamples were sealed in a 450 ml glass jar. An air sample was removed through a rubber sampling port, and sufficient $C_2H_2$ was added to achieve a 10% concentration in the jar headspace. A third subsample was pooled with subsamples from the other two cores of the same vegetation type, and sealed in a glass jar to which no $C_2H_2$ was to be added (control). Additional controls were established with $C_2H_2$ incubated in sample jars containing only deionized $H_2O$ and air. Samples were placed in an insulated box to reduce temperature fluctuations. Immediately after, and at 2, 4, 8 and 24 hours after injecting $C_2H_2$; 15 ml headspace gas samples were transferred to vacutainers prior to analysis. Headspace gas was maintained at atmospheric pressure by injecting 15 ml of air after each sample was collected.

N fixation rates were calculated from the moles of $C_2H_4$ produced during the linear phase of incubation (0-2 hours for the field incubations), the sediment surface area represented by the field samples, and a 3:1 conversion ratio for moles of $C_2H_2$ reduced:moles of $N_2$ reduced (Turner and Gibson, 1980). Treatment means were compared using Wilcoxon's signed ranks test (Siegle and Castellan, 1988).

## RESULTS AND DISCUSSION

Both nodulated root segments and nodule-free soil reduced $C_2H_2$ in preliminary assays (Table 1). No detectable $C_2H_4$ was produced by either nodulated roots or soils in the absence of $C_2H_2$. When samples collected on 9 September 1993 were incubated, only one of the six control microcosms produced any detectable $C_2H_4$. Even in this case, $C_2H_4$ production was <1% of that in the $C_2H_2$-treated microcosms.

Rates of N fixation declined in successive field sites (Table 2). This may have been due to the cooler incubation temperatures as the afternoon progressed. Ambient temperatures on this day declined from 32°C at noon, when the first samples were collected, to 28°C at 6 pm, the time at which the samples from the third site had incubated for 2 hours.

N fixation rates across sites were much higher in soils collected from *Vigna* patches (1.90 mg N $m^{-2}d^{-1}$) than from the surrounding *Spartina* stands (0.20 mg N $m^{-2}d^{-1}$, Table 2). Differences between vegetation types were significant at the 0.05 probability level.

Landscape-level N fixation rates for these intermediate marshes can be calculated assuming that *Vigna* occupies 3.8% of the surface area (Chabreck, 1970), and that the remaining marsh surface fixes N at the same rate as did the *Spartina* patches. Relative abundance of *S. patens* in this marsh type was reported to be five times greater than that of any other species, and no other legumes had a relative abundance even one-tenth as great as the relative abundance of *Vigna* (Chabreck, 1970). Fixation estimates are then 1.92 g N $ha^{-1}$ $d^{1}$ without *Vigna* patches, and 2.64 g N $ha^{-1}$ $d^{-1}$ including *Vigna* patches (Table 3). Thus, failing to sample and account for N fixation rates within <5% of the land area could result in a 27% underestimation of N fixation rates.

Ecosystem N budgets for low salinity marshes of this region suggest that N fixation by free-living microbes is a major source of N inputs (60-70 kg N $ha^{-1}$ $yr^{-1}$; DeLaune and Patrick, 1990). Accretion of excess N (20-40 kg N $ha^{-1}$ $yr^{-1}$), was attributed to sediment deposition (DeLaune and Patrick, 1990). The expected relative errors in N fixation estimates based on this study are of similar magnitude as were the excess N accretion rates of the earlier study, and thus may lead to different conclusions

**Table 1.** Rates of $C_2H_4$ production by nodulated *Vigna luteola* roots and underlying soils. Values represent means (CV) of three replicate samples.

| Material | $C_2H_4$ production Rate | |
|---|---|---|
| | $C_2H_2$ treated | Control |
| | —— nmol $g^{-1}$ $d^{-1}$ —— | |
| Nodulated roots † | 33.9 (130) | 0 |
| Soil (2-10 cm depth)§ | 0.15 (63) | 0 |

† $C_2H_4$ production rates were calculated based on the 0-4 hour incubation interval.
§ $C_2H_4$ production rates were calculated based on the 0-16 hour incubation interval.

**Table 2.** N fixation rates of intermediate marsh soils. Values given are the means (CV) of three replicate $C_2H_2$-treated microcosms per site.

| Site | *Spartina patens* | *Vigna luteola* |
|---|---|---|
| | —— mg N $m^{-2}$ $d^{-1}$ —— | |
| 1 | 0.33 (90) | 4.56 (65) |
| 2 | 0.15 (71) | 0.83 (145) |
| 3 | 0.12 (89) | 0.31 (100) |
| Overall mean* | 0.20 (55) | 1.90 (122) |

*Treatment means differed significantly at the 0.05 level using Wilcoxon's signed rank test.

about sediment input rates.

This study demonstrates the potential significance of N fixation by a nodulated legume to low-salinity marsh N budgets, and how estimates of ecosystem function can be influenced by sampling schemes within a heterogeneous landscape.

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**Table 3.** Estimates of landscape-level N fixation rates.

| Vegetation type | Relative Abundance† | N Fixation Rate | Landscape-Level N Fixation Rate Amount | Landscape-Level N Fixation Rate Relative Amt. |
|---|---|---|---|---|
| | —%— | mg $m^{-2}$ $d^{-1}$ | g $ha^{-1}$ $d^{-1}$ | —%— |
| *Vigna* | 3.8 | 1.90 | 0.72 | 27 |
| without *Vigna*§ | 96.2 | 0.20 | 1.92 | 73 |
| Total | 100.0 | | 2.64 | 100 |

† from Chabreck, 1970

§ *Spartina patens* patches were considered representative of all other marsh patch types.