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

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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	Relative Amt.
	—%—	mg m^{-2} d^{-1}	g ha $^-d^{-1}$	—%—
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.