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Stem-nodulating legumes as green manure for rice in West Africa

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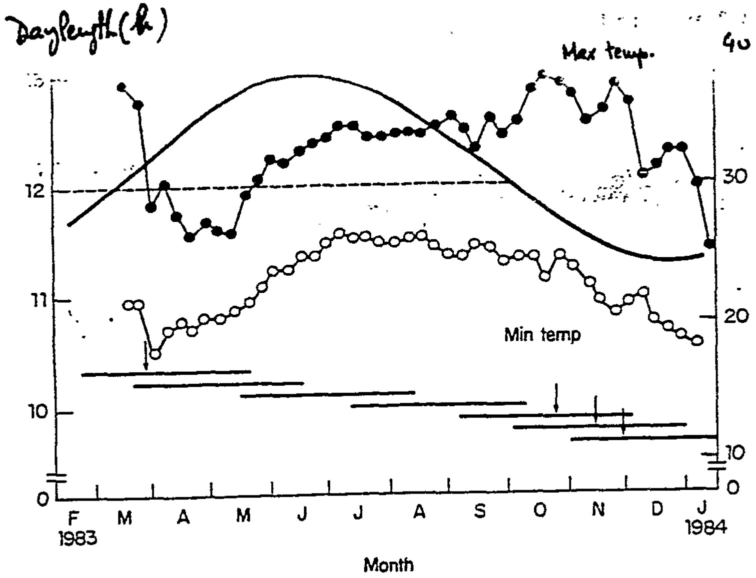
(pp. 97-109)

In West Africa, the stem-nodulating legumes *Sesbania rostrata* and *Aeschynomene afraspera* generally behave as wild annual plants in periodically flooded soils. They are particularly sensitive to photoperiod and temperature; at the latitude of Senegal (15 °N), they grow well during the rainy season (Jun-Sep). *S. rostrata* and *A. afraspera* are fast-growing and fix N_2 more actively than most root-nodulating legumes. Stem nodules are less affected than root nodules by the inhibitive action of flooding and combined N. Stem nodules result from the infection of predetermined sites with specific strains of *Rhizobium*. In nature, when soils already harbor native stem strains, nodules appear on the lower parts of the stems; however, their distribution is often irregular. Stem inoculation is generally recommended to optimize N_2 fixation. When used as green manure at the beginning of the rainy season, *S. rostrata* and *A. afraspera* can provide more than 100 kg N/ha to a rice crop, resulting in significant yield increases. *S. rostrata* also acts as a plant trap for the pathogenic nematodes *Hirschmanniella oryzae* and *H. spinicaudata*, the prevalent species in flooded ricefields in West Africa.

Nitrogen is one of the most important factors governing plant productivity: to produce 100 kg of grain, rice requires 1.8-2.0 kg N (Patnaik and Rao 1979). The plant absorbs N from three major sources: soil N, fertilizers, and biological N_2 fixation. In West Africa, soil N reserves are limited, and N fertilizers are often too expensive for African farmers. Biological N_2 fixation presents an appealing alternative to fertilizers that has often been underutilized. Roger and Watanabe (1984) reviewed the potential for practical utilization of biological N_2 fixation technologies in rice.

It is well known that long-term productivity may decline unless efforts are taken to maintain soil fertility. The utility of green manure for increasing soil productivity has been recognized from early times in some rice-growing areas, particularly China, India, and Northeast Asia (Jiao Bin 1983, Singh 1984, Watanabe 1984, Wen Qi-Xiao 1984). The potential benefits are many. Green manure can increase soil N content, concentrate P and significantly increase the available phosphate content in the soil, maintain and renew soil organic matter, and improve soil structure and physical characteristics (Jiao Bin 1983). N_2-fixing plants that have been used as green



1. Flowering behavior of *Sesbania rostrata* in response to daylength and mean weekly temperatures. Plants were sown in 30-liter PVC pots with a diameter of 30 cm and filled with 20 kg of Bel-Air soil (4 plants/pot). After 3 wk growth, the soil was kept waterlogged. Plants were inoculated by spraying the stems with a culture of *Rhizobium* ORS 571 3 wk after seeding, then at 2-wk intervals. Growth cycle = 13 wk. Arrows indicate beginning of flowering.

Potential N_2 fixation

We estimated N_2 fixation by *S. rostrata* during the 1985 rainy season using the ^{15}N isotope dilution method and the difference method. There was good agreement between the methods. The results are as follows:

1. About 30 g N/m^2 could be fixed in 53 d by *S. rostrata*, which confirms its high N_2-fixing potential (Table 2).

Table 1. Effect of *Sesbania rostrata* planting date on growth parameters and N_2-fixing activity (acetylene reduction activity [ARA]) at 5, 7, and 9 wk after seeding.[a] ORSTOM Dakar, Senegal, 1983.

Planting date	Plant height (m)			N content (g/plant)			Stem ARA (µmol C_2H_4/plant per h)		
	5	7	9	5	7	9	5	7	9
22 Feb	0.19	0.38	0.85	0.01	0.08	0.37	8	46	71
22 Mar	0.18	0.39	0.84	0.02	0.09	0.33	2	25	93
17 May	0.31	0.72	1.34	0.04	0.24	0.73	21	156	164
12 Jul	0.76	1.54	2.13	0.14	0.80	1.62	65	172	171
6 Sep	0.72	1.49	1.80	0.19	0.77	1.08	62	169	144
4 Oct	0.28	0.52	0.77	0.02	0.09	0.22	6	31	40
1 Nov	0.23	0.29	0.35	0.01	0.03	0.09	3	9	18

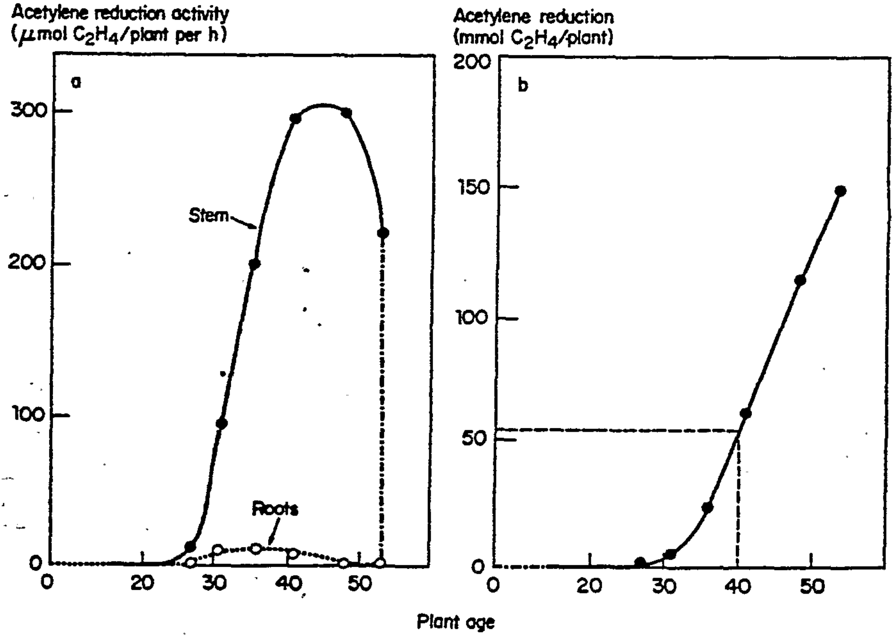
[a] Mean of 4 replications. WAS = weeks after seeding.

2. Because of stem nodulation, *S. rostrata* can actively fix N_2 even in waterlogged conditions (Saint Macary et al 1985). In [illegible] experiments with plants grown in waterlogged soil, root nodulation was poor. N_2-fixing activity was due mainly to stem nodules (about 96% of total ARA) (Fig. 2).
3. Because acetylene reduction is an indirect method of estimating N_2-fixing activity, integrating it into the curve that represents stem nodule activity provides a cumulative curve that allows a qualitative estimate of N_2 fixed at different plant ages (Fig. 2). We found that a) N_2 fixation in *S. rostrata* became significant 35 d after seeding (DAS), 14 d after inoculation; b) the N_2 fixed accumulated linearly

Table 2. N_2 fixation by 53-d-old *S. rostrata* as estimated by 2 different methods (Rinaudo and Moudiongui 1987).

Method	N_2 fixation expressed as		
	N (%) derived from N_2 fixation	g N_2/pot[a]	g N_2/m^2[a]
Direct isotope dilution	38.5	2.09 ± 0.52	30.3 ± 7.3
Difference	43.5	2.38 ± 0.20	33.5 ± 2.8

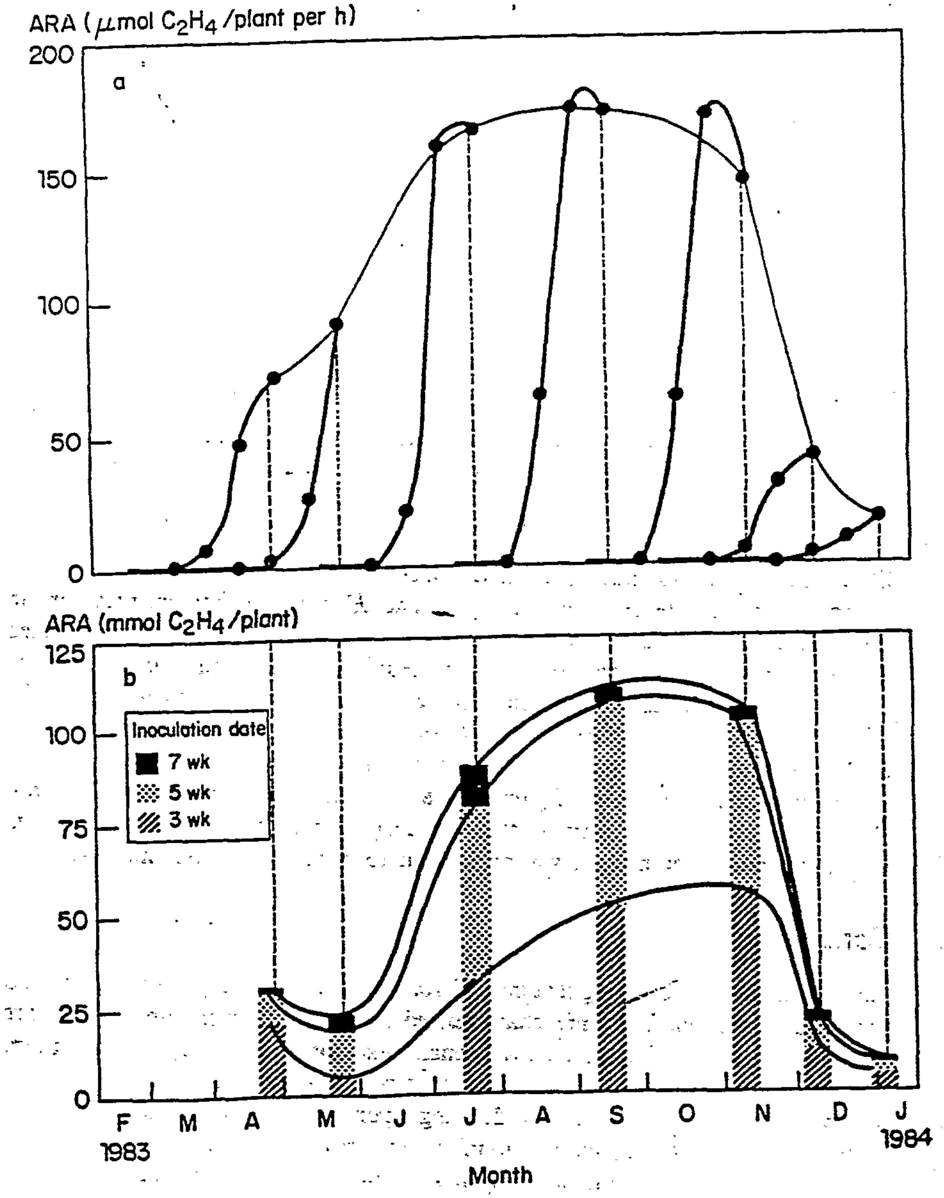
[a] Mean value ± confidence interval (P=0.05). Containers were 30-liter PVC pots filled with Bel-Air soil, each with 4 plants inoculated with *Rhizobium* ORS 571 at 21 and 31 d after seeding.



2. N_2-fixing activity (acetylene reduction) of *S. rostrata* as a function of plant age. a) acetylene reduction activity, b) cumulated values.

[illegible] alginate polymer (Jung et al 1982). Seed [illegible] inoculation [illegible]

Satisfactory stem nodulation is obtained by spraying the shoots with a suspension containing about 10^8 bacteria/ml, using the following bacterial suspensions: liquid culture of *Rhizobium*, colloidal suspension obtained by mixing entrapped rhizobia (alginate polymer) in a phosphate buffer (0.06 M, pH 6.8), or [illegible] ile..



3. N_2-fixing activity (ARA) of *S. rostrata* as a function of planting date. a) ARA as a function of plant age, b) contribution of stem portions inoculated 3, 5, and 7 WAS to total activity of 9-wk-old plants.

Inoculation is generally recommended to optimize N_2 fixation by stem-nodulating legumes. The problem is choosing a suitable inoculation date.

Figure 3 shows the N_2-fixing activity (acetylene reduction) of *S. rostrata* as a function of plant age and planting date. Plants were inoculated at 3, 5, and 7 WAS. The N_2-fixing activity of 9-wk-old plants was the sum of activities of stem portions inoculated successively at 3, 5, and 7 wk.

The activity of each stem portion has been integrated to calculate the contribution of stem nodules—initiated at different inoculation dates—to total activity during growth.

Results show that the activity was mainly due to nodules initiated 3 and 5 WAS. If we attribute the nodules initiated by 3-wk-old plants to spontaneous nodulation (which affects the lower parts of the stems), then the most suitable date for inoculation is 5 wk. The corresponding stem nodules contributed about half the total N_2-fixing activity.

Using stem-nodulating legumes as green manure

Microplot experiments

In microplot experiments during several rainy seasons, use of stem-nodulating legumes *S. rostrata, A. afraspera*, and *A. nilotica* as green manure significantly increased rice grain and straw yields and the N content of both grain and straw (Alazard and Becker 1987; Rinaudo et al 1982, 1983; Rinaudo and Moudiongui 1987).

Experiments with *S. rostrata* were performed with two soils: Bel-Air soil, a typical sandy soil of Senegal (common name: Dior), and Tilene soil, an alluvial soil of the Senegal valley. In both cases, rice grain yields, N uptake by the plant, and number of productive tillers were markedly influenced by green manure (grain yields more than doubled). Significant responses to residual N were obtained with the second rice crop (Table 6, 7).

Similar results were obtained with *A. afraspera* and *A. nilotica* (both species form the second cross-inoculation group of the genus *Aeschynomene* and develop profuse stem nodulation) (Table 8). Furthermore, it could be expected that the N remaining in the soil after a rice crop would benefit a second crop, as was observed with a *S. rostrata* crop. Only 25-35% of the total N accumulated by *Aeschynomene* was transferred to the rice crop (Alazard and Becker 1987).

Experiments on 25-m^2 plots

We collaborated with the Senegalese Institute of Agricultural Research (ISRA) and the West Africa Rice Development Association (WARDA) to perform larger scale irrigated experiments.

At the ISRA station at Djibelor (Casamance, south of Senegal), *S. rostrata* green manure had a more marked effect on rice yields than did organic matter (Table 9). It doubled grain yield (Diack 1986).

and *A. afraspera* as green manure for rice at the beginning of the rainy season. Total dry matter yields of about 10 t/ha have been obtained from both species at 8-9 wk growth. That represents an accumulation of more than 200 kg N/ha.

Assuming that about 50% of the N accumulated in legumes originates from biological N_2 fixation, it appears that green manuring with stem-nodulating legumes could provide about 100 kg N/ha to a rice crop. Results obtained at IRRI (1985, 1986) are consistent: *S. rostrata* was more effective than other *Sesbania* species as a premonsoon green manure for lowland rice.

References cited

Alazard D (1985) Stem and root nodulation in *Aeschynomene* spp. Appl. Environ. Microbiol. 50:732-734.

Alazard D, Becker M (1987) *Aeschynomene* as green manure for rice. Plant Soil 101(1):141-143.

Alazard D, Duhoux E (1987) Nitrogen-fixing stem nodules on *Aeschynomene afraspera*. Biol. Fert. Soils 4(1/2):61-66.

Becker M, Alazard D, Ottow J C G (1986) Mineral nitrogen effect on nodulation and nitrogen fixation of the stem-nodulating legume *Aeschynomene afraspera*. Z. Pflanzenernaehr. Bodenk. 149:485-491.

Camara I, Diara H (1986) Evaluation de la valeur agronomique de *Sesbania rostrata* comme source d'azote en riziculture irriguée dans la vallée due fleuve Senegal. Paper presented at the IFS-CRDI-ORSTOM Workshop on Biological Improvement of Soil Fertility, 15-19 Mar 1986, Dakar.

Carrol B J, Gresshoff P M (1983) Nitrate inhibition of nodulation and nitrogen fixation in white clover. Z. Pflanzenphysiol. 110:77-88.

Dazzo F B, Brill W J (1978) Regulation by fixed nitrogen of host symbiont recognition in the *Rhizobium* clover symbiosis. Plant Physiol. 62:18-21.

Diack M (1986) Multiplication de *Sesbania rostrata* et son effet sur la production du riz. Paper presented at the IFS-CRDI-ORSTOM Workshop on Biological Improvement of Soil Fertility, 15-19 Mar 1986, Dakar.

Dreyfus B, Dommergues Y R (1980) Non inhibition de la fixation d'azote par l'azote combiné, chez une legumineuse à nodules caulinaires, *Sesbania rostrata*. C.R. Acad. Sci. Ser. D, 291:767-770.

Dreyfus B, Dommergues Y R (1981) Nitrogen-fixing nodules induced by *Rhizobium* on the stem of the tropical legume *Sesbania rostrata*. FEMS Microbiol. Lett. 10:313-317.

Dreyfus B, Gillis M, Van Montagu M, Garcia J L, De Ley J (1987) Characterization of *Azorhizobium stiponodans*, genus novus, a stem-nodulating bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacteriol. (in press)

Duhoux E (1984) Ontogenese des nodules caulinaires du *Sesbania rostrata* (legumineuse). Can. J. Bot. 62:982-994.

Eaglesham A R J, Szalay A A (1983) Aerial stem nodules on *Aeschynomene* spp. Plant Sci. Lett. 29:265-272.

Germani G, Reversat G, Luc M (1983) Effect of *Sesbania rostrata* on *Hirschmanniella oryzae* in flooded rice. J. Nematol. 15:269-271.

Hardy R W F, Holstein R D, Jackson E K, Burns R C (1968) The acetylene ethylene assay for N_2 fixation. Plant Physiol. 43:1185-1207.

Houward F (1980) Influence of ammonium and nitrate nitrogen on nitrogenase activity of pea plants as affected by light intensity and sugar additions. Plant Soil 54:271-282.

International Rice Research Institute (1985) Annual report for 1984. P.O. Box 933, Manila, Philippines. 548 p.

International Rice Research Institute (1986) Annual report for 1985. P.O. Box 933, Manila, Philippines. 555 p.

Jiao Bin (1983) Utilization of green manure for raising soil fertility in China. Soil Sci. 135:65-69.

Jordan D C (1984) Family III. *Rhizobiaceae*. Pages 234-257 *in* Bergey's manual of systematic bacteriology. 9th ed. Vol. I. N. R. Krieg and J. G. Hold, eds., The Williams & Wilkins Co., Baltimore.

Jung G, Mugnier J, Diem H G, Dommergues Y R (1982) Polymer-entrapped *Rhizobium* as an inoculant for legumes. Plant Soil 65:219-231.

Moudiongui A, Rinaudo G (1987) Effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction) of the tropical legume *Sesbania rostrata*. Mircen J. (in press)

Pariselle A, Rinaudo G (1987) Study of interrelationships between *Sesbania rostrata*, *Hirschmanniella oryzae* and rice yields. Rev. Nematol. (in press)

Patnaik S, Rao M V (1979) Sources of nitrogen for rice production. Pages 25-43 *in* Nitrogen and rice. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Rinaudo G, Dreyfus B, Dommergues Y (1982) *Sesbania rostrata* as a green manure for rice in West Africa. Pages 441-445 *in* B.N.F. Technology for tropical agriculture. P. H. Graham and S. C. Harris, eds., Centro Internacional de Agricultura Tropical, Cali, Colombia.

Rinaudo G, Dreyfus B, Dommergues Y (1983) *Sesbania rostrata* green manure and the nitrogen content of rice crop and soil. Soil Biol. Biochem. 15:111-113.

Rinaudo G, Moudiongui A (1987) Fixation d'azote par *Sesbania rostrata*: son utilisation comme engrais vert. Bulletin de Recherches Agronomiques Gembloux. (in press)

Roger P A, Watanabe I (1986) Technologies for utilizing nitrogen fixation in wetland rice: potentialities, current usage, and limiting factors. Fert. Res. 9:39-77.

Saint Macary H, Marqueses E A, Torres R O, Morris R A (1985) Effect of flooding on growth and nitrogen fixation of two *Sesbania* species. Philipp. J. Crop Sci. 10:17-20.

Schaede R (1940) Die Knollchen der adventiven Wasserwurzeln von *Neptunia oloracea* und ihre Bakteriensymbiose. Planta 31:1-21.

Singh N T (1984) Green manures as sources of nutrients in rice production. Pages 217-228 *in* Organic matter and rice. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Tsien H C, Dreyfus B, Schmidt E L (1983) Initial stages in the morphogenesis of the nitrogen-fixing stem nodules of *Sesbania rostrata*. J. Bacteriol. 156:888-897.

Watanabe I (1984) Use of green manures in Northeast Asia. Pages 229-234 *in* Organic matter and rice. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Wen Qixiao (1984) Utilization of organic materials in rice production in China. Pages 45-56 *in* Organic matter and rice. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Wong P P (1980) Nitrate and carbohydrate effects of nodulation and nitrogen fixation (acetylene reduction) activity of lentil (*Lens esculenta* Moench). Plant Physiol. 66:78-81.

Notes

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