Proc. Indian natn. Sci. Acad. **B61** No.1 pp. 57-62 (1995)

Predicting Nitrogen Fixation and N Accumulation in Field Grown Annual *Sesbania* spp.

D L N RAO and S K GHAI

Central Soil Salinity Research Institute, Karnal 132 001

(Received on 22 December 1993; after revision 8 July 1994;
Accepted on 25 July 1994)

Field studies on two annual legumes *Sesbania cannabina* and *Sesbania* PL Se-17, used for green manuring, showed that nodulation was effective, nodule number and biomass increased upto 45 d - as explained by a linear (r^2=0.83 and 0.82 for the two species) and log-linear (r^2=0.81 and 0.78) functions. N_2ase and % shoot N showed a curvilinear response and were explained by cubic (r^2=0.75 and 0.34) and quadratic (r^2=0.46 and 0.81) functions. Peak N_2ase for 'cannabina' and 'Se-17'was at 33 and 28 d resulting in peak shoot N content at 40 and 41 d. Total shoot N content showed a log- linear response (0.92 and 0.93). N_2ase activity showed a poor correlation with shoot N content when the growth upto 60 d or even 45 d was considered, but was significant (r^2=0.69 and 0.54) upto 40 d. Seasonal integration of nitrogen fixation and its relationship to shoot N content over the 15-45 d period showed that fixation could meet most of the plant demand. When the whole growth period of 15-60 d was considered it was seen that fixation was not sufficient and uptake from soil N would be significant.

Key Words: Green manuring, Modelling, Nitrogenase, Nitrogen fixation, Nodulation, *Sesbania*

Introduction

Annual legumes, particularly *Sesbania* spp. have proven very popular for green manuring , because of their vigorous growth and high N fixing ability (Ghai et al. 1988, IRRI 1988, Rao & Gill 1993). Currently, in comparative trials for evaluating suitability of plant species for green manuring purposes, biomass production, N content and N accumulation in plant tops (indirect indices of N fixing ability) are the most widely used indices (Ghai et al. 1985, Rao 1986, IRRI 1988). While such indirect indices as N accumulation are useful agronomic parameters, it is important to assess the nitrogenase activity of *Sesbania* spp. and understand its relationship with N accumulation in plant tops with progressive growth. Knowledge of the peak stage of N fixation (nitrogenase activity) would be another factor in addition to biomass production in helping to decide the right age for incorporation of green manures in the soil, so as to conserve soil N and increase the efficiency of green manuring. The use of mathematical functions and models to describe soil microbial events is a recent development (Smith 1982) and can lead to better insights into the descriptive and predictive aspects of diverse biological processes in soil, e.g., nitrogen fixation (Guofan & Deng 1991). In this paper we have derived various functional relations for nodulation, nitrogenase activity and N accumulation for prediction and optimization of N fixation.

Materials and Methods

In a study on green manuring with two annual *Sesbania* spp. (Ghai et al. 1988) an area of high alkalinity (30 x 5 m) was used to study nitrogenase activity of root nodules and N accumulation in plant tops with time. The soil was a typic natrustalf, sandy loam, pH_2 (1:2 soil-water suspension) 9.5, electrical conductivity, EC_2 1.3 dS (deci-siemens) m^{-1}, Org.C 0.3% , total N 0.06% and $KMnO_4$ oxidizable N 44mg kg^{-1}. Fertilizer nitrogen or potash were not applied but P @ 17.5kg ha^{-1} as single superphosphate was applied when preparing the field. Seeds of *S.cannabina* (Retz.) Pers. (syn.*S.aculeata* L. Pers.) pre-soaked in water for 18hr, and of *Sesbania* sp. PL Se-17 scarified in concentrated sulfuric acid for 15min and then soaked in water, were broadcast @ 60kg ha^{-1} (100 seed wt = 1.9 and 0.9g respectively) on well prepared soil, and flooded with water. From 15 to 60 days after sowing (DAS), three plants of each species were carefully uprooted daily and the roots were washed in water over a nest of 3 sieves, removed and patted dry on filter papers. The nodulated roots were assayed for nitrogenase activity by acetylene reduction (Bergerson 1980). For this the intact root systems were carefully placed in 15ml test tubes (for plants upto 30d age) or 300 ml glass bottles fitted with suba-seals for older plants. After a 30 min incubation in an atmosphere of 10% acetylene at 30°C, a sample of the gas phase was analyzed for C_2H_2 on a Nucon 5700 gas chromatograph fitted with a flame ionization detector and a poropak-T column. After the assay, the nodules were removed, counted and their fresh and dry weights determined. The root and shoot biomass was dried at 70°C in an oven and weighed and N content determined by Kjeldahl method using a Kjeltec-2 N analyzer. Calculation of potential dinitrogen fixation was as per Hansen and Pate (1987). Linear and quadratic regressions were used to describe the shoot biomass; nodule number, fresh weight and nitrogenase activity; % N and N uptake in shoot trends with time (figure 1). For all the above indices an attempt was made to arrive at the best equations (table 2) which could predict the parameter as function of time (days of growth). For the purpose of estimating N fixation from nitrogenase activity (and see its relation to N accumulation in plant tops, table 3), the daily measurements of C_2H_2 reduction, being discrete values, they were converted to N fixed/day and summed up over 5-6 days to obtain cumulative values for the given time interval. Since N uptake is a continuous process , cumulative uptake in the corresponding time interval was calculated as the difference in N uptake/plant between the j^{th} day and the i^{th} day; but this ran into difficulties because of considerable daily variations in N uptake due to spatial variability of soil properties of the experimental site. So the values of estimated N uptake between two time intervals were arrived from the linear regression equation of N uptake versus time. The nitrogenase activity and nitrogen accumulation over the whole growing season was numerically integrated from the corresponding daily TARA (Total acetylene reducing activity, µmol plant hr^{-1}) and daily increments of N uptake respectively by using the Simpson's rule which is based on piece-wise, quadratic approximation of a function between two finite limits (Jeffrey Johnson 1987). Mathematically,

$$I = \int_a^b f(x)\, d(x) \sim h/3 \; [f_o + 4 \sum_{i=1,3}^{2n-1} f(x_i) + 2 \sum_{i=2.4}^{2n-2} f(x_i) + f_{2n}]$$

where $h = b - a / 2n$, and $f_i = f(x_i)$; $i = 0,1,2,3....2n$.

Again because of the day to day variation of N uptake in plants, net N uptake day^{-1} values at discrete intervals of time required for integration

were predicted from the linear regression equation.

Results and Discussion

Nodulation began early, nodules were concentrated mainly on the crown root and were effective with a high nodule score of 4 on the 5 point scale of Corbin et al. (1977). Both nodule number and mass increased upto 45 d (table 1) and were well explained by a linear and log-linear function respectively (figure 1b,c and table 2). Similarly there was a steady accumulation of biomass in shoot and could be explained by a log-linear function (figure 1a, table 2).

Nitrogenase activity of root nodules at all the stages was higher for PL Se - 17 as compared to 'cannabina'. Similarly N content in the shoots of PL-Se 17 was higher at most stages of growth (table 1). The nitrogenase activity in root nodules and % N in shoot showed similar curvilinear increase with time (figure 1d & e) and were explained by cubic and quadratic functions (table 2). Nitrogenase activity peaked at 33 and 28 days, whereas the peak shoot N content was on day 40 and 41 for 'cannabina' and 'Se-17' respectively. Similarity of curves for nitrogenase activity and concentration of N in shoot against time, sequential increase and a gap of 10 days

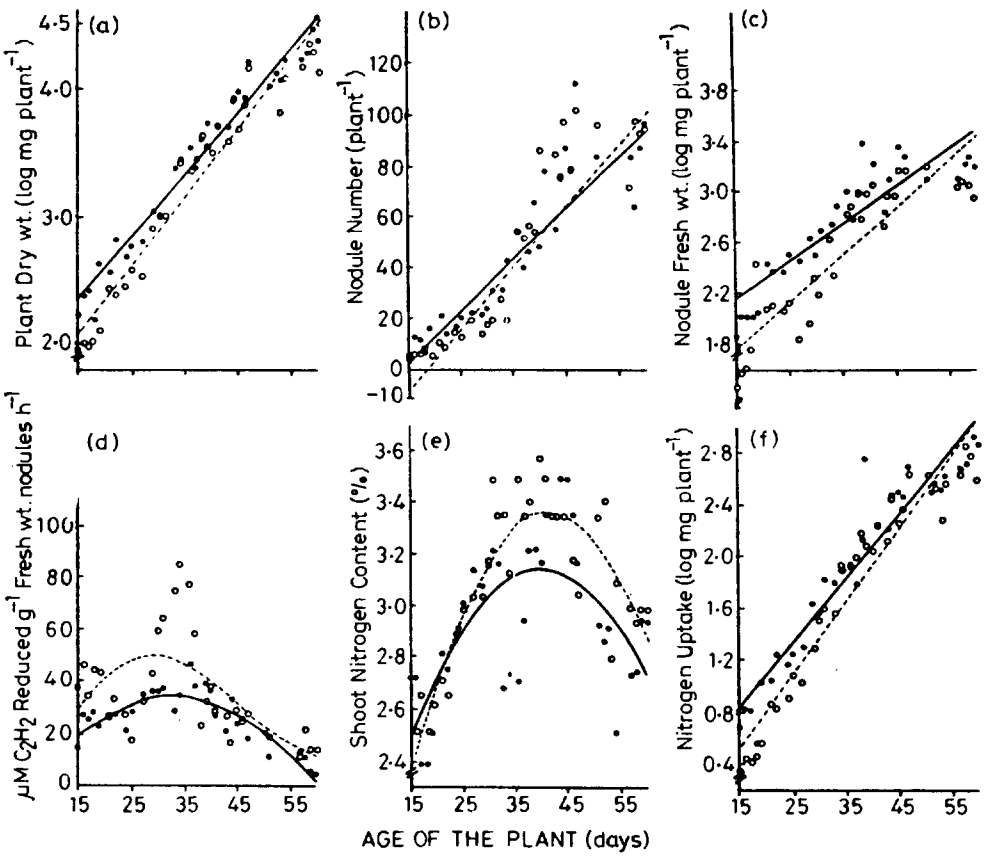

Figure 1 Dry matter accumulation (a) nodule number (b) and fresh wt.(c) nitrogenase activity (d) N concentration (e) and uptake (f) in shoots of two annuals, *Sesbania cannabina* (●—●) and *Sesbania* sp. PL Se-17 (O—O) at different growth stages in an alkaline soil

between the two peaks reflects the time taken for fixed products in root nodules to be translocated to the shoots. Total N accumulation also increased with time and was well explained by a log-linear function (figure 1f, table 2). Although the shape of curves of N_2 ase in nodules and % shoot N regressed against time was similar and both peaked in a sequential manner, yet over the whole study period of 15-60 days, the correlation between them was very poor and nonsignificant for both the species. The coefficient of determination of % N in shoot over nitrogenase activity of root nodules (independent variable) remained ≤ 0.2 for linear, and ≤ 0.3 for quadratic, cubic or log functions and the F-test in all cases was non-significant. The correlations were non-significant even when the analysis was done only for the 15-45 day period. Only when analysis was restricted upto the peak stage, i.e.,when observations of only 15-40 days were taken into account, the correlation (0.69 and 0.54) was significant. Rao and Gill (1993) have reported a poor correlation between acetylene reduction of root nodules of several accessions of annual *Sesbania* spp. and their shoot N concentration or N uptake when sampled at a fixed stage of crop growth (50 DAS). So, for comparative evaluation of legumes, traditional indexes of N fixation like % N and N uptake can only be relied when sampling is done at their respective peak stages of shoot N concentration or nodule nitrogenase activity and not at any randomly chosen growth stage.

Cumulative N fixation peaked at 36-41 d and declined thereafter (table 3) and was very low at 57-60 d at which stage plant N content was high. So at these later stages as biomass production increases rapidly, demand for N is high but N fixation has gone down, the plants will predominantly derive N from soil pool. Seasonal integration of nitrogenase activity showed that 'cannabina' and 'Se-17' showed similar N_2 ase activity of 885 and 871 μ mol C_2H_2 red. plant^{-1} hr^{-1} and corresponded to N fixation of 198 and 195 mg N fixed plant^{-1} respectively in 45 days

Table 1 *Nodulation, nitrogenase activity and N uptake by* ***Sesbania cannabina*** *and* ***Sesbania*** *PL Se-17 at various growth stages (average of daily values, plant^{-1})*

Growth stage* (days)	Nodule no.	Nodule mass (mg fr.wt.)	Nitrogenase activity#	Shoot biomass (g DW)	N (%)	N uptake (mg)
15-21	13.7	148	24.0	0.27	2.59	7.1
(6)	7.1	95	38.5	0.13	2.58	3.4
22-27	19.1	272	28.0	0.60	2.97	17.6
(4)	13.8	118	27.2	0.31	2.89	9.0
29-34	30.4	520	34.7	1.80	3.00	53.2
(6)	19.4	228	65.6	1.34	3.25	42.2
36-41	55.9	1287	36.3	3.84	3.09	120.7
(6)	68.4	861	42.8	3.57	3.44	122.8
43-47	81.6	1459	23.9	9.73	3.32	322.6
(5)	88.4	1112	24.4	7.65	3.25	244.8
51-54	84.0	1378	11.2	13.21	2.80	366.7
(4)@	87.2	1733	19.5	10.19	3.15	324.7
57-60	83.3	1609	8.0	22.97	2.83	655.0
(4)	89.9	910	15.6	17.32	2.97	513.2

*figures in parentheses represent no. of sampling days in the study interval. Figures in ordinary and bold face are of 'cannabina' and Se-17 respectively.

#μ moles of acetylene reduced g^{-1} fr.wt. nodule hr^{-1}.

@nodulation and N_2ase are for only day 51.

Table 2 *Equations to predict various parameters of nitrogen fixation as a function of time (d) for* ***Sesbania*** *spp. grown for green manure in an alkaline soil*

Parameter	*S.cannabina*	*Sesbania* PL Se - 17
Dry wt plant^{-1} (mg)	$y = 41.69 . 10^{0.049d}$	$y = 18.20.10^{0.054d}$
	$R^2 = 0.98$	$R^2 = 0.94$
Nodule no.	$y = - 33.80 + 2.54d$	$y = - 63.87 + 3.95d$
plant^{-1}	$R^2 = 0.83$	$R^2 = 0.82$
Nodule wt.	$y = 54.95. 10^{0.029d}$	$y = 17.78 . 10^{0.037d}$
plant^{-1}(mg)	$R^2 = 0.81$	$R^2 = 0.78$
Nitrogenase activity*	$y = - 12.56 + 2.83d - 0.043d^2$	$y = 63.95 + 9.35d - 0.24d^2 + 0.002d^3$
	$R^2 = 0.75$	$R^2 = 0.34$
%·shoot N	$y = 1.47 + 0.084d - 0.001d^2$	$y = 0.94 + 0.118d - 0.001d^2$
	$R^2 = 0.46$	$R^2 = 0.81$
N uptake	$y = 1.17 . 10^{0.050d}$	$y = -2.08 . 10^{0.056d}$
plant^{-1}(mg)	$R^2 = 0.92$	$R^2 = 0.93$

*µmoles C_2H_2 reduced g^{-1} nodule fr.wt. hr^{-1}.

Table 3 *Nitrogen fixation (C_2H_2 reduction) in* ***Sesbania cannabina*** *(SC) and* ***Sesbania*** *sp. PL Se-17 (Se-17) in relation to shoot N accumulation in various growth stages*

Growth stage (days)	Cumulative C_2H_2 reduced (µ mol plant^{-1}hr^{-1})		Estimated N_2 fixation (mg N plant^{-1})		Total N accumulated (mg N plant^{-1})	
	SC	Se-17	SC	Se-17	SC	Se-17
15-21	25.6	14.5	5.7	3.2	7.3	4.3
22-27	56.8	18.8	12.7	4.2	13.1	8.4
29-34	107.0	95.9	24.0	21.5	29.3	20.6
36-41	276.7	211.4	62.0	47.3	65.5	50.6
43-47	174.0	135.2	39.0	30.3	114.7	96.8
57-60	50.1	51.6	11.2	11.6	432.6	437.4

(15-60 DAS). But in the same period N uptake (estimated from linear regression line, figure 1c) was 442 and 283 mg N, which means that N fixation could meet only 45 and 69 % of the demand for nitrogen by 'cannabina' and 'Se-17' respectively. N fixation thus appears to have met only half to two-thirds of the requirement; however these appear to be underestimates, since N uptake at later stages was exaggerated due to the equations used for predicting N uptake. Better fits could not be obtained and this illustrates the limitations of using equations to describe or model biological phenomena which are more complex than physical and chemical processes. When the calculations were restricted to earlier growth stages i.e., 15-45 day period it was seen that 78 and 100 % of the N requirement of the two species was met from fixation. The differ-

ence between the species was because of higher nitrogenase activity of PL Se-17 coupled with its lower N demand due to lesser biomass production.

Results presented here show that peak nitrogenase activity in *Sesbania* spp. occurs at 30 d resulting in peak nitrogen accumulation in the shoots at 40 d and that growing them beyond 45 days is not beneficial for conserving the soil N resource because as biomass production increases rapidly, following this period, the plants would start drawing nitrogen from the soil reserves. In low external input sustainable agricultural systems (LEISA) which depend on minimum depletion of the soil stocks of important nutrients like nitrogen such information is useful for practicing green manuring on a more scientific basis.

Acknowledgments

The authors are grateful to Drs N T Singh and I P Abrol, present and former Director, C S S R I, Karnal for providing the facilities for this investigation and to Mr H S Tomar for the excellent technical assistance in the field sampling and laboratory analysis.

References

Bergerson F J 1980 *Methods For Evaluating Biological Nitrogen Fixation* (Chichester: John Wiley)

Corbin E J , Brockwell J and Gault R R 1977 Nodulation studies in chickpea (*Cicer arietinum*); *Aust.J.Exp.Agric.Anim.husb.* **17** 126-134

Ghai S K , Rao D L N and Batra L 1985 Comparative study of the potential of sesbanias for green manuring; *Trop.Agric.* (Trinidad) **62** 52-56.

——, —— and ——1988 Nitrogen contribution to wetland rice by green manuring with *Sesbania* spp. in alkaline soil; *Biol. Fert. Soils* **6** 22-25

Guofan L and Deng T 1991 Mathematical model of the relationship between nitrogen fixation by black locust and soil conditions; *Soil Biol. Biochem.* **23** 1-7

Hansen A P and Pate J S 1987 Comparative growth and symbiotic performance of seedlings of *Acacia* spp. in defined pot culture or as natural understorey components of a eucalypt forest plantation in S.W.Australia; *J. Expt. Bot.* **186** 13-25 .

International Rice Research Institute 1988 *Sustainable Agriculture.Green Manure in Rice Farming* (Los Banos: IRRI) 379 pp

Jeffrey Johnson K 1987 *Numerical Methods in Chemistry* (New York: Marcel Dekker) 503 pp.

Rao D L N 1986 *Sesbania for Green Manuring. Better Farming in Salt Affected Soils,* No **6** (Karnal: CSSRI)

—— and Gill H S 1993 Nitrogen fixation, biomass production and nutrient uptake by annual *Sesbania* species in an alkaline soil; *Biol. Fert. Soils* **15** 73-78

Smith O L 1982 *Soil Microbiology - A Model of Decomposition and Nutrient Cycling* (Cleveland: C R C Press)