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Diurnal Variations of Nitrogenase Activity in the Field

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Nitrogenase activity was determined in situ by repeated assays at regular intervals to compare the patterns of diurnal variations occurring in a rice field, a grassland, and a peanut field. In the rice field only one peak occurred (mid-day), whereas the other systems exhibited typical two-peak patterns, which were suspected to be induced by climatic conditions or possibly by a specific rhythm of exudation. Observed data on diurnal variations in nitrogenase activity were fitted to different models of diurnal variation comprised of computed periodic curves.

Under natural conditions the nitrogenase activity (NA) of symbiotic systems has been shown to fluctuate diurnally; such fluctuations have been noted in the case of *Alnus glutinosa* and *Myrica gale* (8) and *Glycine max* (5, 6). Thus far, diurnal variations have not been investigated for nonsymbiotic systems. This work was initiated to study the fluctuations occurring in certain asymbiotic nitrogen fixation systems and, in particular, to examine whether such fluctuations were synchronized with daily changes in the physical environment.

The following systems, all located in the Ivory Coast, were studied: (i) an irrigated rice field, at Lamto; (ii) different types of grassland, including an artificial grassland (*Panicum maximum*) at O.R.S.T.O.M. research station, Adiopodoume, and three natural grasslands (*Hyparrhenia* sp. and *Andropogon* sp., *Loudetia simplex*) at the Lamto IBP station; and (iii) a peanut field (*Arachis hypogea*) at Lamto, which was used as a control.

MATERIALS AND METHODS

NA was assayed in situ by the acetylene reduction technique using a nondisturbing device consisting of two parts: an open metal cylinder 700 cm^2 in area and 15 cm in height, which was pushed into the soil to surround an individual plant or a cluster of plants, and a bell jar which could be adjusted on the upper rim of the cylinder with a water seal. Acetylene was generated in the bell jar, and propane was injected as a tracer gas. After 0.5-h and 1-h incubations, ethylene production in the bell jar was determined by gas chromatography according to procedures described elsewhere (1). NA was estimated 8 to 11 times per 24-h period, and the estimations were performed at regular or almost regular intervals. Each estimation was the mean of 4 to 5 individual measurements (samples); each sample represented a different plant-soil system. NA was expressed as micromoles of C_2H_4 per hour per 700 cm^2 (cylinder area). Integrated daily nitrogenase activity (INA) was determined graphically and expressed as micromoles of C_2H_4 per day per 700 cm^2. Because each cylinder was placed around a cluster of plants and the number of clusters varied according to the plant, the results could not be expressed on a per hectare basis. Ethylene is known to be produced in small quantities by plants, fungi, and bacteria. However, because laboratory experiments conducted on similar soil types had shown only negligible production of non-nitrogenase-formed ethylene, no corrections were made for this possible ethylene background.

To check the diurnal character of the observed variations of NA, agreement of the corresponding data (samples and means) with different periodic models was tested by an analysis of variance (3). This agreement was expressed in terms of percentage and as a comparison of residual variance (total variance minus variance attributed to the model) and standard error. The models were computed periodic curves: symmetrical sine curves and sine curves extended by adding high-order harmonics. A stepwise procedure selected the significant terms of the regression. The basic model was: $Y_t = A_0 + A_1 \cos 2\pi(t/T) + B_1 \sin 2\pi(t/T) + A_2 \cos 4\pi(t/T) + B_2 \sin 4\pi(t/T) + \ldots + A_n \cos 2n\pi(t/T) + B_n \sin 2n\pi(t/T) + \epsilon_t$. Y_t is the measure of the variable at time t; t is time; T is the fundamental period (T = 1 day = 24 h); ϵ_t is a normal variate with mean 0 and variance σ^2; A_0 is the constant coefficient of the regression equation; A_1, A_2, etc. are the regression coefficients of Y_t versus cos $2\pi(t/T)$, cos $4\pi(t/T)$, etc.; and B_1, B_2, etc. are the regression coefficients of Y_t versus sin $2\pi(t/T)$, sin $4\pi(t/T)$, etc.

The phase of the first harmonic, ψ_1, is expressed in the relationship: $tg\ \psi_1 = -B_1/A_1$. The amplitude of the first harmonic is: $A_1^2 + B_1^2$. Terms of the other harmonics can be described similarly.

RESULTS

Rice field. Figure 1 shows that one mid-day peak occurred at 15 h. The best agreement of

data with a periodic curve was obtained for the following function: NA = 9.765 − 10.210 sin 15 (t + 5 h 06) + 4.750 sin 30 (t − 1 h 07) − 3.510 sin 45 (t − 3 h 07).

This function accounted for 97.0% of the variation between means and 63.9% of the variation between samples. Furthermore, $F_{6.4}$ = 22.44 and $F_{6.4}$ 1% = 15.21 ($F_{6.4}$ 1%:F value read on a Fisher and Snedecor F variable table with 6 and 4 degrees of freedom at .01 risk, $F_{6.4}$:observed F value) (Table 1). The INA activity computed according to the above function was: INA = 246.6 μmol of C_2H_4 per day per 700 cm^2.

Grasslands. NA in grasslands was always much lower than in the rice field (compare curves a and c, Fig. 2). Variations were relatively important and erratic. No significant agreement between data concerning grasslands and computed periodic curves could be demonstrated. Yet the following function concerning *P. maximum* grassland is worth mentioning because $F_{1.8}$ = 5.12 and $F_{1.8}$ 5% = 5.32 (Table 1):

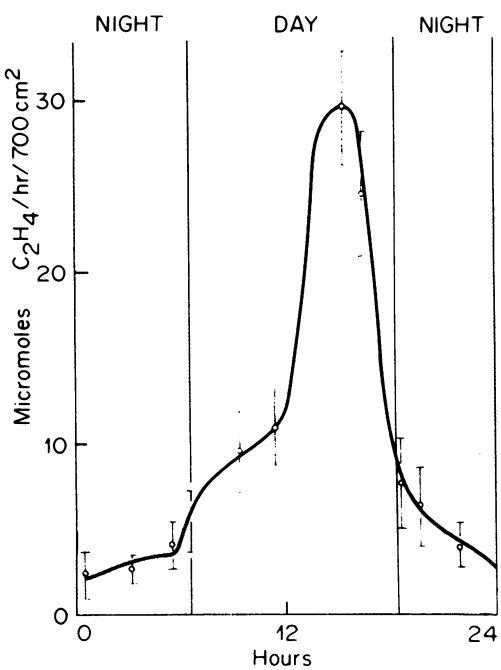

FIG. 1. *Diurnal variations of NA in a rice field as assayed by acetylene reduction; calculated curve, observed means, and standard errors (shown by limits).*

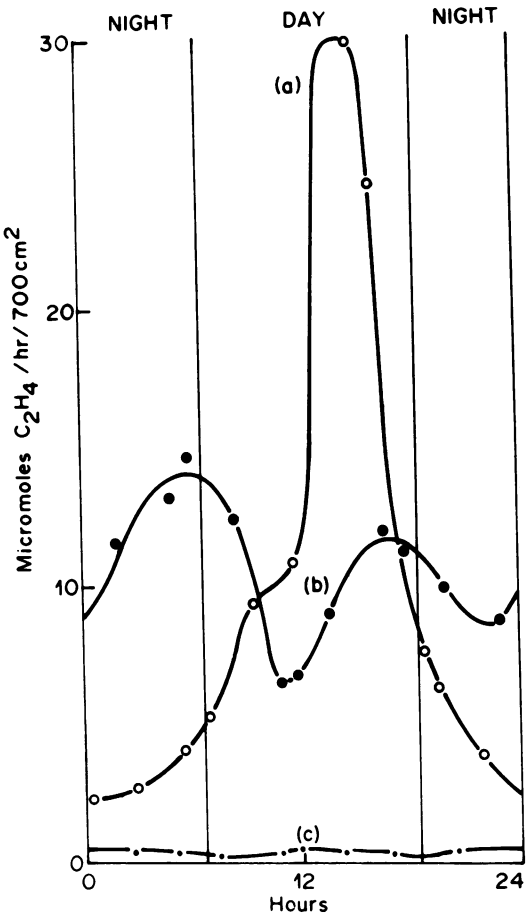

FIG. 2. *Diurnal variations of NA in (a) a rice field, (b) a peanut field, and (c) a P. maximum grassland, measured by acetylene reduction assay. Standard errors related to rice and peanut fields are given in Fig. 1 and 3, respectively. Adopted scale did not allow the drawing of standard errors for P. maximum.*

TABLE 1. *Agreement of data with computed periodic curves*

Type of field	Degree of agreement of data (%)[a]		Analysis of variance test	
	Means	Samples	Observed F[b]	Tabular F with associated level of significance P
Rice	97.0	63.9	22.44	15.21 (P = 0.01)
Panicum maximum	38.9	9.7	5.12	5.32 (P = 0.05)
Peanut	89.9	32.1	13.44	9.15 (P = 0.01)

[a] Means are the average of 4 to 15 individual measurements (samples).
[b] F is the classic Fisher-Snedecor variable.

NA = .3776 + .0618 cos 30 t(t hours, 30 t degrees).

The corresponding curve is a two-peak curve, but, due to the scale adopted, the peaks could not be represented on Fig. 2.

The INA computed according to this periodic curve was: INA = 7.421 μmol of C_2H_4 per day per 700 cm², which is very low when compared to the rice field figure (246.6 μmol).

Peanut field. Variations in acetylene reduction showed a double-peak pattern (Fig. 3) as in the case of *P. maximum*, but differed significantly from the latter in having a much higher mean level. The two-peak pattern obtained here for peanuts is in contrast to the one-peak pattern of variations published (6) for another legume, soybeans. The best agreement of data for the peanut field with a periodic curve was obtained for the following function: NA = 10.531 + 1.615 sin 15 (t + 2 h 04) + 2.605 sin 30 (t − 2 h 24).

This function explained 89.9% of the variations between means and 32.1% of the variation between samples. Furthermore, $F_{4,6}$ = 13.44 and $F_{4,6}$ 1% = 9.15.

The INA computed according to this periodic curve was: INA = 264.150 μmol of C_2H_4 per day per 700 cm².

DISCUSSION

A comparison of NAs for the three crop systems is given in Fig. 2. The mean level of NA in the rice system was of the same order of magnitude as that of symbiotic systems and is in agreement with the results of several previous investigations (7, 9). In comparison with the rice and peanut systems, grassland appeared to be much less active, although N_2 fixation was not negligible.

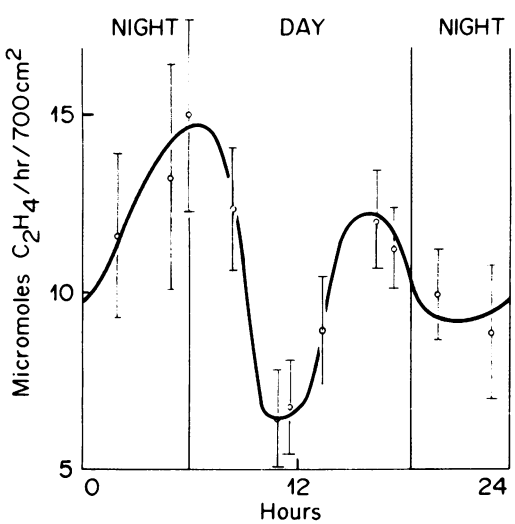

FIG. 3. *Diurnal variations of NA in a peanut field as assayed by acetylene reduction.*

TABLE 2. *Calculation of daily NA*[a]

Type of field	Integration of means[b]	Extrapolation of a unique mean corresponding to:	
		Maximum level of NA	Minimum level of NA
Rice	246.600	556.8	56.0
Panicum maximum	7.421	12.0	6.0
Peanut	261.150	350.4	153.6

[a] NA is expressed as micromoles of C_2H_4 per day per 700 cm².

[b] The 8 to 11 means were obtained throughout a 24-h period.

Interpretation of the first harmonic terms of the models is relatively simple when related to the underlying diurnal phenomenon. As to the higher harmonic terms, their interpretation is not so evident. The patterns give an implication of biological rhythms with 12-h periods for the second harmonic, 8 h for the third, and so on. Thus, the proposed models must be considered as having only general descriptive value in their present stage of refinement.

Because N_2 fixation in the rhizosphere presumably depends primarily on exudation (4), the two main types of N_2 fixation patterns shown here, the single- and the double-peak patterns, were considered to be influenced not only by environmental parameters acting on exudation, but also by the particular pattern of exudation of each plant species. As far as environmental parameters are concerned, light was obviously prominent, but other factors such as air or soil moisture content, or both, were thought to play a significant role. Thus, the decrease of N_2 fixation occurring in the afternoon could be attributed to a reduction of atmospheric humidity. On the other hand, some grasses, namely *P. maximum*, were suspected of having a mid-night flush of exudation which did not exist in other plants such as rice. This night peak presumably followed the hydrolysis of carbon storage products accumulated during the day and their subsequent translocation and exudation in the rhizosphere (2).

The findings reported in this study have implications relative to the use of the acetylene reduction assay as an estimate of nitrogen fixation in the field. Because NA varied according to the time of measurement, only integrated estimations based on data obtained throughout a 24-h period could be considered valid. Extrapolation of data obtained at a given time of the

day may give way either to overestimations or to underestimations. The extent to which such extrapolations can lead to erroneous estimates of NA is illustrated in Table 2 with data from the three sites investigated.

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