A MODELLING APPROACH OF ACETYLENE REDUCING ACTIVITY OF PLANT-RHIZOSPHERE DIAZOTROPH SYSTEMS

J.P. BALANDREAU[1], C.R. MILLIER[2], PIERETTE WEINHARD[1], P. DUCERF[1] and Y.R. DOMMERGUES[1]

INTRODUCTION

Acetylene reducing activity (ARA) of plant-diazotroph systems whether obligatory or associative is known to be affected by environmental factors, by plant factors and by specific characteristics of associated diazotrophs. Marked and rapid variation in ARA occur due to the number of fluctuating environmental factors acting to limit ARA.

Diurnal ARA variations were reported in the case of symbiotic systems such as legume-*Rhizobium* (Hardy *et al.*, 1968; Magué and Burris, 1972) and non-legume-actinomycetes like organisms (Wheeler, 1971; Bond *et al.*, 1975). In the case of grass-rhizosphere diazotroph systems (Balandreau *et al.*, 1974), variation in the ARA was related to variation in the photosynthetic activity of the plant, rate of translocation of photosynthates to the roots and rate of exudation of compounds from the roots. These processes were all sensitive to light intensity, temperature and other environmental parameters. Variations in ARA from day to day are also known to occur, which result from daily variations in environmental parameters (e.g. Balandreau, 1975). Moreover variation in ARA throughout the plant growth cycle can also be expected (e.g. Hardy *et al.*, 1973; Balandreau and Dommergues, 1973).

Variation in ARA may obscure the interpretation of field experiments where estimations of N_2 fixation are made by extrapolating over long periods of time. Precise estimation of N_2 fixation would necessitate a large number of ARA measurements - at least 8 per day with 5 replicates (Balandreau *et al.*, 1974) for the duration of the experiment.

[1] Centre de Pedologie du C.N.R.S., B.P. 5, 54500 VANDOEUVRE-LES-NANCY, France.

[2] Centre National de Recherches Forestieres, Champenoux, 54280 SEICHAMPS, France.

The object of the present investigation is to attempt to correlate observed ARA with soil and climatic factors and with plant growth in order to build a preliminary model (1) allowing the interpolation of a limited number of ARA determinations over periods of time extending from one day to a whole plant growth cycle, (2) predicting variation in ARA from environmental data or simple plant growth parameters.

MATERIAL AND METHODS

ARA was assayed *in situ* using a device designed not to disturb the plant and consisting of two components: 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 and a polyethylene bag which was fitted on to the upper rim of the cylinder. Water was placed in the rim of the cylinder to seal the volume containing the plant and enclosed by the bag and the cylinder. Acetylene (10% in volume) was injected into the bag together with propane (tracer gas). After 1 h incubation ethylene production was determined by gas chromatography according to the procedure described elsewhere (Balandreau and Dommergues, 1973). ARA was expressed as nmoles of ethylene evolved/h/700 cm^2 cylinder area). The plant used for these investigations was maize grown in a typical eutrochrept soil located at La Bouzule experimental farm, 20 km north of Nancy, France. A preliminary series of estimations of ARA made in 1971 showed that N_2 fixation by the maize native diazotroph system, was 1-3 kg N_2/ha. growth cycle (Balandreau, 1975).

A second series of ARA measurements, used to build the model of diurnal variation, were taken every 3 h without interruption during 5 days at the beginning of July 1973. Simultaneously the following parameters were monitored:

AT: air temperature measured under shelter.

AH: air humidity measured under shelter.

LE: amount of light energy received by the plant measured with a thermolinear pyranometer, expressed as J cm^{-2}.

UST: temperature of the upper soil horizon (5 cm in depth).

IST: temperature of the intermediate soil horizon (18 cm in depth).

LST: temperature of the lower soil horizon (30 cm in depth).

SW: soil water content expressed on a wet weight basis .

LW: dry leaf weight expressed in g per plant.

A third series of ARA estimations were made to build a model of variation in ARA throughout the whole growth cycle of maize. ARA was estimated every 2 h, one day per week, during the maize growth cycle of 1975.

Non planted controls showed no significant ARA, thus indicating that ARA originated from the native diazotrophs associated to the maize rhizosphere (Fig. 1).

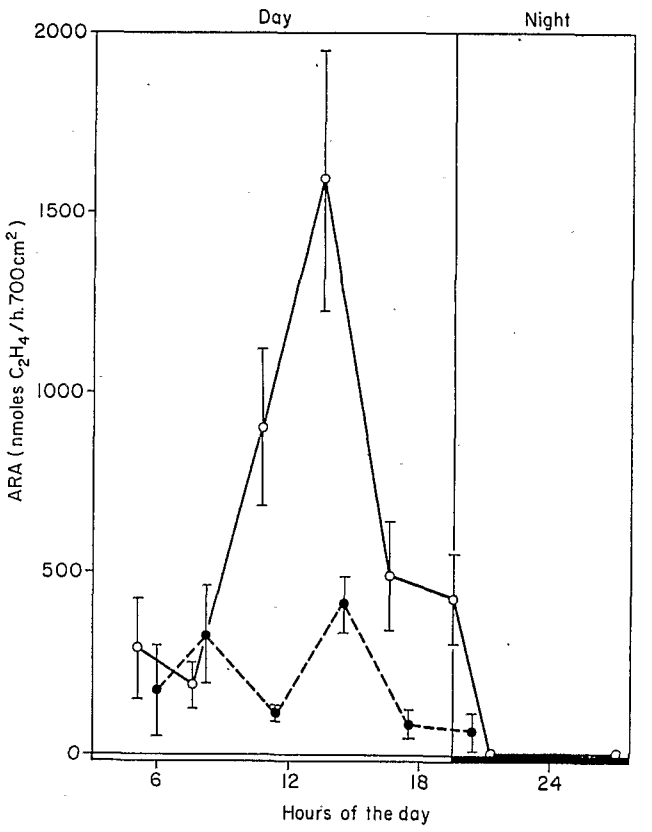

Fig. 1. *Diurnal variations in acetylene-reducing activity (ARA) in the rhizosphere of maize (full line) and in non planted soil (dotted line); measurement performed at flowering stage. Limits show standard error of means.*

MODELLING OF DIURNAL VARIATIONS OF ARA

Fig. 1 is an example of nycthemeral pattern of ARA as observed in the maize field. It shows one mid-day peak at 15 h, very similar to that already reported in the case of rice (Balandreau *et al.*, 1976). A midnight peak also occurred, but only when night temperature was below 18 to 20°C. The occurrence of a midnight peak of ARA was reported by Döbereiner and Day (1974) when temperatures were high enough (20°C) to allow hydrolysis of starch reserves and further translocation of solubilized compounds (West, 1971) towards the sites of utilization by diazotrophs.

Diurnal variations in ARA were significantly and positively correlated (at the .05 probability level) with 4 out of the 7

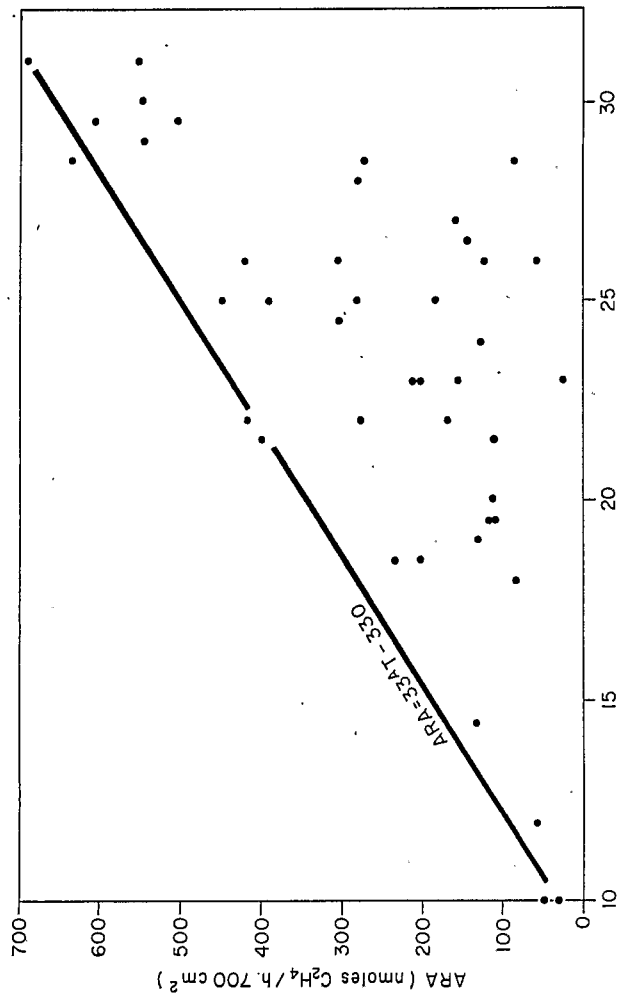

Fig. 2. *Acetylene reducing activity (ARA) plotted against air temperature (AT) measured in July 1973 (investigations on nycthemeral variations).*

environmental parameters monitored, i.e. AT, LE, UST, IST.

When ARA was plotted against one of the environmental parameters, corresponding points appeared to be located between the x axis and a straight line, considered the envelope of the points (Fig. 2). This straight line was obtained either graphically or by calculation. The graph was then used to calculate the equation of the straight line:

$$ARA = aAT + b$$

Points on the straight line were related to situations when the parameter studied - e.g. AT in Fig. 2 - was the limiting factor; points below the straight line were related to situations when other parameters were limiting. When IST was lower than 20°C, ARA was nil.

A set of 4 equations was thus obtained:

(I) ARA = 33 AT - 330
(II) ARA = 40 UST - 694
(III) ARA = 1,95 LE + 140
(IV) ARA = 0, if IST 20.

Resolving equations I, II, III, IV, gave 4 ARA values; the lowest value had to be retained since ARA actually depended on the "most" limiting factor.

This model appeared to describe 70% of ARA diurnal variations, which is not perfect but is an encouraging result.

MODELLING OF VARIATION IN ARA THROUGHOUT THE PLANT GROWTH CYCLE

Calculations by computer are not yet completed. However, preliminary interpretations showed that soil water content (SW) played a major role. (1) When SW was higher than 18%, ARA was maximum if other environmental parameters were not limiting; such values were designed as potential maximum ARA (PMARA). PMARA could be related to LW by a simple equation:

$$PMARA = k.LW$$

k being a constant. (2) When SW was lower than 18%, SW appeared to be a limiting factor. ARA measured at the mid-day peak (MDARA) appeared to be related to SW by the following equation:

$$MDARA = PMARA\ (mSW - p)$$

m and p being constants.

MDARA/PMARA was correlated to SW at the .01 probability level. Actual values of constant k, m and p will be published later.

DISCUSSION

ARA of the maize-rhizosphere diazotroph system was shown to depend primarily upon the stage of growth of the plant. Midday ARA (MDARA) was proportional to the leaf weight (LW), in the absence of limitations imposed by other environmental factors.

The influence of environmental parameters on ARA was also significant, but more difficult to elucidate because of interactions. Light energy (LE) was a major factor which was responsible for rapid ARA variations, together with air temperature (AT) and soil temperature (UST, IST). Such variations often resulted in large diurnal variations of ARA. Soil water content varied more slowly so that its effect could be shown clearly only when measurements were made over periods of time greater than 24 h.

Abrantes *et al.* (1975) found that the main factors limiting ARA were air temperature and NH_4^+ content of the soil solution, but that soil water content (SW) was limiting only when the wilting point was reached. Our results confirm the effects of temperature and soil water content reported by Abrantes *et al.*; like these authors, we detected a threshold under which soil water content was limiting.

Surprisingly, in preliminary experiments, no effect of soil combined nitrogen could be detected, possibly because of the spacial heterogeneity of this soil parameter.

The two models of ARA variations related to two time scales (daily and the whole growth cycle) proposed are obviously still in the preliminary stage and require improvements. We are currently trying to develop a unique model integrating the results of the separate preliminary models presented here. Generalisation of such an integrated model to other types of environments and other plant-diazotroph systems should increase the accuracy and precision of evaluations of ARA *in situ*.

Bowen and Rovira (1973) advocated the use of the modelling approach in rhizosphere studies. The preliminary results presented here support the value of such an approach which is a prerequisite for the development of current investigations (e.g. Bülow and Döbereiner, 1975) aimed at the improvement of the efficiency of plant-rhizosphere diazotroph systems.

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