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DIURNAL CHANGES IN ACETYLENE REDUCTION IN FIELD-GROWN COWPEAS AND SOYBEANS

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Summary—The effects of five environmental factors on variation in the rate of C_2H_2 reduction in two cowpea (*Vigna unguiculata* L. Walp.) and two soybean (*Glycine max* L. Merrill) cultivars were examined at two stages of growth in the field. Diurnal changes in C_2H_2 reduction, estimated as μmoles C_2H_4 produced·g^{-1} nodules·h^{-1}, over a 30 h period were compared with changes in soil, canopy and air temperatures, global radiation, and vapour pressure deficit. Although the environmental factors showed one maximum and one minimum during a diurnal cycle, the C_2H_2 reduction rate in nodulated cowpea roots showed two peaks: one between 0600–1200 and another between 1800–2400 and two minima: one between 1200–1600 and another between 2400–0600. Variation in C_2H_2 reduction in soybean nodules did not show any definite pattern. Vapour pressure deficit appeared the most likely factor influencing the decline in C_2H_2 reduction between 1200–1600.

The rate of C_2H_2 reduction in cowpeas was greater at the pre-flower than at the early pod-fill period; the rate in soybeans was not greatly different at early flower or early pod-fill.

INTRODUCTION

The facility and sensitivity of the C_2H_2–C_2H_4 assay as a measure of nitrogenase activity make it a useful tool for studying daily changes in N_2 fixation in legumes. Bergersen (1970) cautioned against using the method to estimate N_2 fixation unless the conversion ratio for the particular legume-*Rhizobium* system is established. Thus, the method may be employed in conjunction with ^{15}N methods to measure N_2 fixation or it may be used alone to measure C_2H_2 reduction. Several workers (Bergersen, 1970; Hardy *et al.*, 1968; Mague and Burris, 1972) have used the method to show that the daily changes in nodule function depend on environmental factors such as solar radiation, air temperature and relative humidity.

Over the past 3 yr it has been observed (Ayanaba, unpublished) at the International Institute of Tropical Agriculture (IITA) that the rate of C_2H_2 reduction by rhizobia in field-grown cowpeas and soybeans shows one maximum and one minimum during the light period and again increases towards the early evening. In nodulated roots, C_2H_2 reduction during one diurnal cycle is reported to show a maximum during the light period and a minimum during the dark period whether the nodules are of plants grown in the field (Hardy *et al.*, 1968; Mague and Burris, 1972) or in cabinets (Bergersen, 1970; Minchin and Pate, 1974) or even of non-legumes (Wheeler, 1971). Recently, however, Balandreau, Millier and Dommergues (1974) reported that C_2H_2 reduction in field-grown peanuts showed two peaks during one daily cycle.

Because of these considerations the effects of certain environmental variables on C_2H_2 reduction were examined in field-grown legumes over a 30 h period. Two cowpea and two soybean cultivars were used to permit comparison of cultivars as well as species.

MATERIALS AND METHODS

Two cowpea cultivars, TVu 37 (Pale Green) and TVu 1190, and two soybean cultivars, TGm 80 (Bossier) and TGm 280-3, were sown in the field in a randomized complete block with four replications. Plots were 3 × 3 m. Cowpeas were sown without inoculation at a spacing of 15 × 50 cm. Cowpea is indigenous to this area and rhizobia are numerous in the soil; inoculation is unnecessary (Ayanaba, unpublished). Soybean seeds were inoculated with Nitragin "S" inoculant (Nitragin Co., Milwaukee, WI, USA) and sown at a spacing of 5 × 50 cm. Soil was fertilized with 20 kg·ha^{-1} of P as single superphosphate 5 days after planting (DAP). Because it was the dry season, plants were watered twice weekly by sprinkler irrigation. Weeds were removed by hoeing. Plants were protected from pests by spraying with monocrotophos (*O,O*-dimethyl-*O*-[2-methylcarbamoyl-1-methyl-vinyl]-phosphate).

In a second experiment, TVu 37, TGm 80 and TGm 280–3 were sown during the rainy season, following the procedures given above.

Environmental variables were regularly monitored during each of three sampling periods. Global and net radiation were measured with a Kipp solarimeter and a Fritschen type net radiometer respectively. The former was located on the roof of one of the main laboratory buildings about 300 m from the experimental site; the latter, at the IITA Weather Station, about 75 m away. Vapour pressure deficit (the difference between actual and saturation vapour pressure of the air) was deduced from air temperature and relative humidity measured with a hygrothermograph in a standard screen located at the Weather Station. Soil temperature was monitored by thermistor probes buried 5 cm in the soil. Similar probes fixed about 3/4 up the plant canopy, inside wooden, well-aerated

double-roofed screens, were used to measure temperature in the canopy. The top of the screens, which were almost level with the top of plant canopies, were covered by a sheet of aluminium foil to reduce radiation load to a minimum.

Plants were harvested to measure C_2H_2 reduction at different physiological stages of growth: (a) cowpeas at 34–35 DAP (pre-flower); (b) soybeans at 41–42 DAP (early flower), and (c) both crops at 55–56 (pod fill). At each sampling, three plants from each replication were dug up one after the other, the nodulated roots were cut and each root put in a 250 ml bottle. Bottles were stoppered with No. 77 Suba-Seal stoppers (Suba-Seal Works, Barnesley, U.K.), 25 ml of air was withdrawn with a syringe and replaced with 25 ml welding grade C_2H_2. The bottles were placed in a 26°C incubator within 30 min after the plants were dug up. After 30 min incubation, 3 ml of gas was removed from each bottle with a syringe and stored in pre-evacuated tubes. The C_2H_2 produced was determined by modifying the procedure of Dobereiner, Day and Dart (1972) and using a Beckman GC-45 gas chromatograph fitted with a 2 × 6 mm stainless steel column packed with 80–100 mesh Porapak N (Waters Associates, Waltham, MA, USA). The operating temperatures were respectively 150, 80, 110 and 150°C for injector, oven, detector line and dectector. Fresh weight of nodules/plant was determined after the gas samples were removed; C_2H_4 produced·g^{-1} fresh nodules was then calculated.

At the second sampling (55–56 DAP), five additional plants each of TVu 37 and TGm 80 were removed at 0900, 1500, 2100, 2400 (day 1) and 0500 and 0900 (day 2) and incubated at ambient field temperature within 5 min after being dug up. This was to test the differences in C_2H_2 reduction between field-incubated samples and those incubated at 26°C.

In the second experiment, ten plants each of the three cultivars were dug up at 2 h intervals between 0800 and 1600. Nodulated roots were incubated at ambient field temperature and C_2H_4 production was estimated as described above.

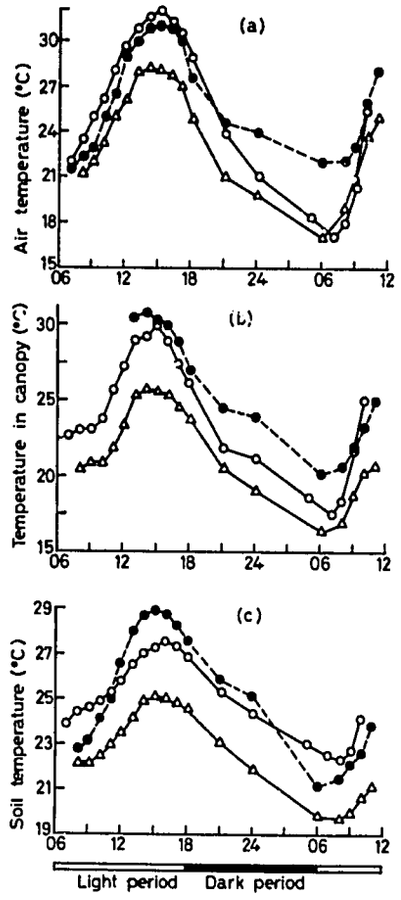

Fig. 1. Diurnal changes in air temperature (a), temperature in the canopy (b) and soil temperature (c). The first observations were made 34–35 days after planting of cowpeas (●), the second were made 41–42 days after planting of soybeans (△), and the third were made 55–56 days after planting of both legumes (○). Temperature in the canopy during the first sampling period was not monitored before 1300.

RESULTS AND DISCUSSION

Air and soil temperatures and temperature in the canopy were distinctly lower during the second sampling; the widest range in values occurred during the third and first sampling periods (Fig. 1(a–c)). The higher amount of global radiation (Fig. 2(b)) during the third sampling period suggests high rates of photosynthesis. Furthermore the higher radiation and net radiation (not shown), coupled with the high vapour pressure deficit values (Fig. 2(a)) (low relative humidity values) during this period also suggest higher rates of evapotranspiration (Penman, 1961; Tanner and Gardner, personal communication). This, according to results obtained by DeWit (1958) and more recently by Tanner and Gardner (personal communication) implies greater dry matter accumulation.

Rates of C_2H_2 reduction, as a measure of N_2ase activity, are shown in Figs. 3 and 4. The standard error was large, possibly, because of variability in soil factors and nodule yield. Because cowpeas were not inoculated, variation in the *Rhizobium* strains that nodulated the cowpeas could have contributed to the observed variation in mean C_2H_2 reduction values.

Rates of C_2H_2 reduction, especially in cowpeas were highest at night as well as during the day. In some instances (Fig. 3, TVu 37), there was no sharp peak during the light period. In others (Fig. 3, TVu 37, TVu 1190), the daytime peak was lower than the night-time peak. The general pattern of C_2H_2 reduction was as follows: (a) 0600–1200, increasing rate of C_2H_2 reduction and intensity of the measured environmental factors; (b) 1200–1600, drop in the rate of C_2H_2 reduction, but environmental factors had attained their maxima for the day and were falling; (c) 1800–2400, a second increase in rate of C_2H_2 reduction coincident with a drop in the intensity of the environmental factors to their morning levels and (d) 2400–0600 of the second day, a decline in C_2H_2 reduction rate and attainment of the absolute minimum intensity of the environmental factors. The results of the second experiment (Table 1), conducted for part of the day, confirm the observations summarized in (a) and (b) above.

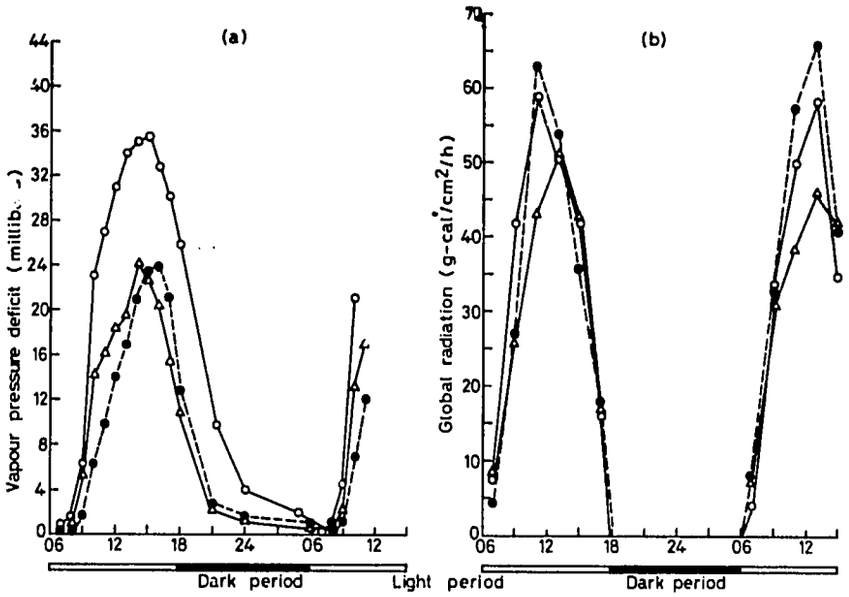

Fig. 2. Diurnal changes in the vapour pressure deficit (a) and global radiation (b) for the three sampling periods. See Fig. 1 for explanation of symbols.

The environmental variables we monitored show one cycle during each day. Consequently, it is reasonable to relate variation in N_2 fixation (C_2H_2 reduction), showing one maximum and one minimum during a given diurnal period, to this cycle in environmental variables. Thus, a maximum between noon and early evening and a minimum at night have been established for nodules of legumes (Bergersen, 1970; Hardy *et al.*, 1968; Mague and Burris, 1972) and non-legumes (Wheeler, 1971). These changes were related to changes in solar radiation. Mague and Burris (1972) also showed that C_2H_2 reduction in soybean was related to changes in air but not soil temperature.

Recently, however, Balandreau *et al.* (1974) reported that C_2H_2 reduction in nodules of peanuts grown in the field in the Ivory Coast were maximal at about 0600 and again between 1500–1800. Minchin and Pate (1974) found that pea plants grown in cabinets fixed more N_2 during the night than during the day, but they observed only one peak in fixation during a 24 h period. The present study reports two peaks in rates of C_2H_2 reduction in a 24 h period. The actual times when the peaks in this study occurred differ from those reported by Balandreau *et al.* (1974) possibly because of differences in legume species and environmental conditions.

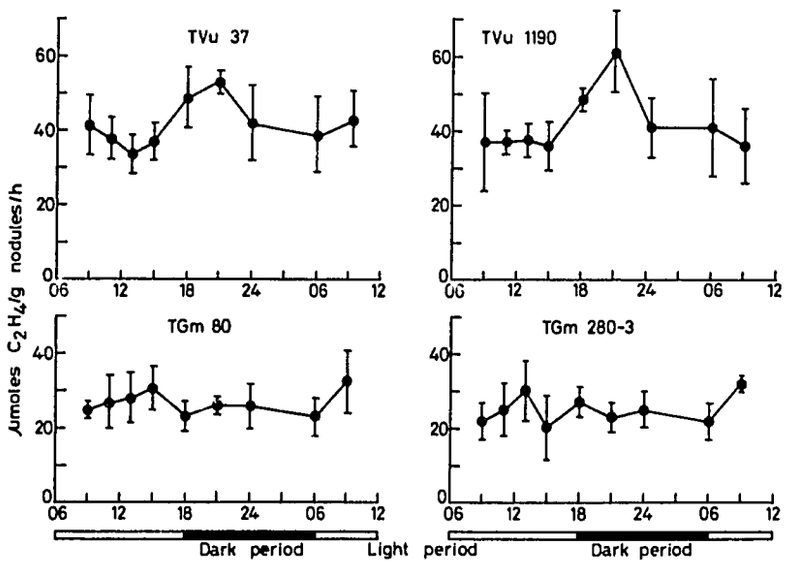

Fig. 3. Diurnal changes in C_2H_2 reduction in nodulated roots of cowpeas (TVu 37 and TVu 1190) harvested 34–35 days after planting and soybeans (TGm 80 and TGm 280-3) harvested 41–42 days after planting. Vertical lines are standard errors of means of replicates.

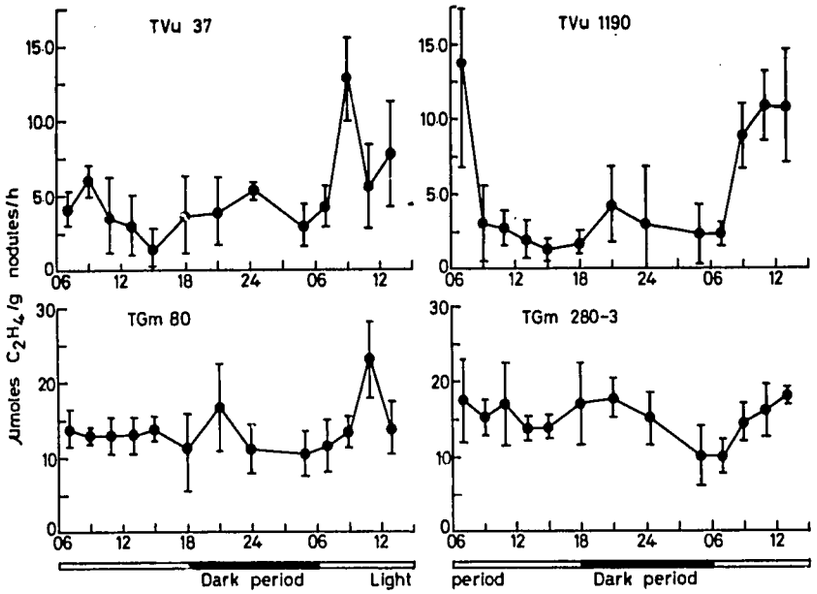

Fig. 4. Diurnal changes in C_2H_2 reduction in nodulated roots of cowpeas (TVu 37 and TVu 1190) and soybeans (TGm 80 and TGm 280-3) harvested 55–56 days after planting. Vertical lines are standard errors of means of replicates.

Once carbohydrates become available and N_2 fixation commences, fixation should continue until the carbohydrate supply is exhausted. Why then, did the rate of C_2H_2 reduction decline between 1200 and 1600? We hypothesize that environmental stress or stresses caused the decline. Balandreau *et al.* (1974) suggested that decreased atmospheric humidity during the afternoon decreased C_2H_2 reduction in peanuts. Minchin and Pate (1974) reported that when the relative humidity in growth cabinets was decreased, C_2H_2 reduction in pea plants also decreased because of increased transpiration. The decrease in C_2H_2 reduction between 1200–1600 in the present study was, therefore, most probably due to the large vapour pressure deficit (low relative humidity). A sample comparison of C_2H_2 reduction rates in the two cowpea cultivars confirms this hypothesis. Radiation values measured at the pre-flower and early pod-fill growth periods were similar, but vapour pressure deficit was greater during early pod-fill and the values were considerably greater during the afternoon. Correspondingly, the duration of the afternoon minimum in C_2H_2 reduction rate was longer during the early pod-fill than during the pre-flower period.

A study with soybeans by Huang, Boyer and Vanderhoef (1975a) showed a positive correlation between transpiration and C_2H_2 reduction, a result which is different from what we report. Huang *et al.* were dealing with moisture-stressed plants over a four day period. When soil moisture was not limiting the correlation was negative, as we and others (Balandreau *et al.*, 1974; Minchin and Pate, 1974) report. Huang, Boyer and Vanderhoef (1975b) indeed concluded from a subsequent study that the positive correlation observed between transpiration and C_2H_2 reduction during desiccation was fortuitous and that the observed decline was rather the result of inhibition of shoot photosynthesis.

Because N_2 fixation is dependent on carbohydrate supply (Minchin and Pate, 1974; Wheeler, 1971) it will be of interest to determine in subsequent experiments if sugar concentrations in nodules also increase at night. This would explain whether translocation was decreased as suggested by Minchin and Pate (1974) or whether sugar was continuously being translocated, but N_2 fixation interrupted, as a result of relative extremes in environmental factors, e.g. soil temperature.

Table 1. Variation in global radiation and C_2H_4 formation in nodulated roots of cowpea (TVu 37) and soybeans (TGm 80 and TGm 280-3) incubated under field conditions. C_2H_4 values are means ± standard error

Time	Global radiation ($g\text{-}cal\cdot cm^{-2}$)*	TVu 37	TGm 80	TGm 280-3
		$\mu moles\ C_2H_4\cdot g^{-1}\ nodules\cdot h^{-1}$		
0800	ND	31.9 ± 9.7	29.2 ± 7.7	29.2 ± 8.7
1000	10.2	14.0 ± 4.5	43.9 ± 6.1	41.5 ± 5.9
1200	30.4	6.9 ± 3.4	32.1 ± 6.2	[illegible] ± 7.9
1400	60.9	3.2 ± 1.9	22.1 ± 8.7	29.1 ± 8.3
1600	50.7	10.8 ± 3.3	28.3 ± 5.2	19.0 ± 6.7

* Values are integrated over 2 h intervals, i.e. the first value (barely detectable) corresponds to radiation received between 0600–0800, the second between 0800–1000, etc.

Table 2. The effects of field incubation and incubation in the laboratory at 26°C on C_2H_4 formation in nodulated roots of cowpea (TVu 37) and soybean (TGm 80) over a 24 h period. C_2H_4 values are mean ± standard error

			TVu 37		TGm 80	
			μmoles $C_2H_4 \cdot g^{-1}$ nodules$\cdot h^{-1}$ when incubated under conditions shown			
Day	Time	Air temperature (°C)	Field	26°C	Field	26°C
1	0900	25.0	8.9 ± 4.4	6.0 ± 1.2	17.2 ± 4.7	12.7 ± 1.0
	1500	32.0	5.4 ± 2.9	1.5 ± 1.3	15.1 ± 4.7	14.0 ± 1.6
	2100	23.7	8.4 ± 1.3	3.9 ± 2.3	4.7 ± 1.4	17.0 ± 5.7
	2400	21.1	11.6 ± 2.6	5.4 ± 0.7	6.2 ± 1.5	1.3 ± 3.0
2	0500	18.4	2.8 ± 2.1	3.1 ± 1.4	10.2 ± 5.0	10.8 ± 3.0
	0900	20.4	15.0 ± 3.4	12.9 ± 2.8	26.5 ± 3.3	13.5 ± 2.0

Because soil and air temperatures at night are usually lower than during the day, it is possible that increased C_2H_2 reduction rates at night are due to incubation at the elevated temperature of 26°C. To test this possibility, nodulated roots of one cowpea cultivar (TVu 37) and one of soybean (TGm 80) were incubated in the presence of C_2H_2 in the field at selected periods. The results (Table 2) show that the double peaks and double minima in rates of C_2H_2 reduction occurred whether incubation was at ambient field temperature or 26°C. Incubation of cowpeas at the lower, ambient temperature actually increased C_2H_2 reduction rates at 2100 and 2400. The temperatures encountered in the field (except at 1500 and 0500) and that used in the laboratory were within the optimum range of 20–30°C for C_2H_2 reduction in cowpeas (Ayanaba, unpublished) and soybeans (Hardy *et al.*, 1968).

In addition to the more pronounced night-time peak in C_2H_2 reduction, both cowpea cultivars also reduced less C_2H_2 during early pod-fill than during pre-flower (e.g. for TVu 1190, maximum rates at these stages were respectively 14.0 and 61.5 μmoles C_2H_4 produced$\cdot g^{-1}$ nodules$\cdot h^{-1}$). By contrast, soybeans maintained quite similar reduction rates at early flower and early pod-fill. Mague and Burris (1972) observed that early maturing soybeans attained maximum rates of C_2H_2 reduction sooner and that reduction also declined sooner than late maturing cultivars. The days-to-maturity ratings of the cowpeas used in these experiments are 70–80 days; of the soybeans, 90–105 days (International Institute of Tropical Agriculture, 1974). It was, therefore, reasonable to observe no differences in C_2H_2 reduction rates between cultivars of the same species. However, the decline in maximum rates of C_2H_2 reduction occurred sooner in cowpeas than soybeans.

In view of the results reported here and because soybeans yield more seed than cowpeas (IITA, 1974), it is of practical interest to compare many more legume species and cultivars from more ecological zones to determine whether these patterns of C_2H_2 reduction (N_2 fixation) are characteristic of certain legumes only and whether there is any affinity with protein content and grain yield.

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