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Effect of Acetylene on Root Respiration and Acetylene Reducing Activity in Nodulated Soya Bean

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ABSTRACT

Acetylene decreased root and nodule respiration, as measured by CO_2 evolution of nodulated or non-nodulated *Glycine max*. An inhibition of 25 to 35% in 15 to 30 minutes occurred when 13% C_2H_2 was introduced in the gas flux which aerated the root nutrient solution. When the light intensity was doubled to 800 microeinsteins per square meter per second, the inhibition increased to 50% and nodule acetylene reduction activity was inhibited 50%.

ARA[1] is a frequently used method for determining the nitrogenase activity in N_2 fixing plants. The method has been extensively discussed (4), but as I began to use it in my experimentation, I noticed that acetylene had effects on root respiration, about which little information is available. Only a few authors have measured RR simultaneously with ARA. Berlier and Lespinat (2) observed no decrease of CO_2 evolution rate by pea and soybean nodulated roots after C_2H_2 introduction. Minchin *et al.* (7) found a considerable decrease (50%) of ARA and RR in *Trifolium* and other species occurring within 10 to 30 min of contact with C_2H_2. This decrease was dependent on plant cultivar, age, and light intensity, and has not always been confirmed (3). Drevon (personal communication) found no inhibition of RR by C_2H_2. Winship and Tjepkema (10) found a 10% constant and reversible decrease in the respiration of isolated *Frankia* nodules, possibly due to lowered O_2 partial pressure during the measurements. McDowall and Kristjansson (6) observed a 30% increase in CO_2 evolution in a long-term experiment, while after 90 min the effect varied from a 2% stimulation to a 19% inhibition.

In my experiments, I studied the short-term (1 h) effects of contact with C_2H_2 on the ARA and respiration of nodulated roots of soybean and on the photosynthesis of the shoot.

MATERIAL AND METHODS

Plant Material

Seeds of *Glycine max* (L.) Merr. cv Kingsoy were sterilized and germinated in perlite. Plantlets were inoculated with *Bradyrhizobium japonicum* strain PJ 17 and grown in aerated nutrient solution (5). The solution was supplemented with 1 mM urea during the first 20 d of cultivation. Plants were grown at first in a greenhouse, then in a controlled climate chamber at 25°C, 70 to 80% RH, and a continuous light intensity of about 400 $\mu E \cdot m^{-2} s^{-1}$.

Continuous light eliminated daily rhythms of root respiration and ARA, which facilitates the interpretation of results. The plants grew normally in these conditions and their respiratory capacity, when put in the dark, was the same as that of control plants.

Measurement of Root Respiration and ARA

Prior to measurements, the plants were transferred to a measuring chamber illustrated in Figure 1. Roots were aerated by compressed air containing about 380 ppm CO_2 at a flow rate of usually 20 L h^{-1}. Root respiration was calculated from

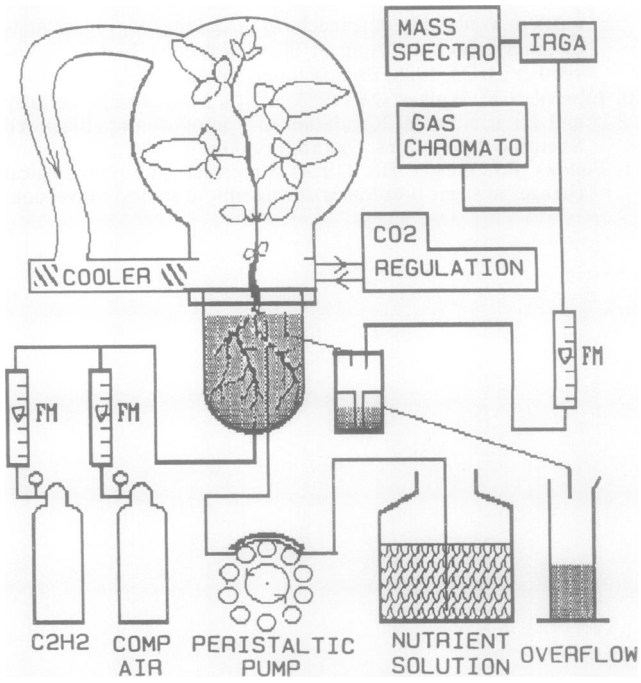

Figure 1. System for measuring root respiration. FM, flowmeter; IRGA, infrared CO_2 analyzer.

[1] Abbreviations: ARA, acetylene reducing activity; RR, root respiration expressed in ml CO_2 per hour per plant.

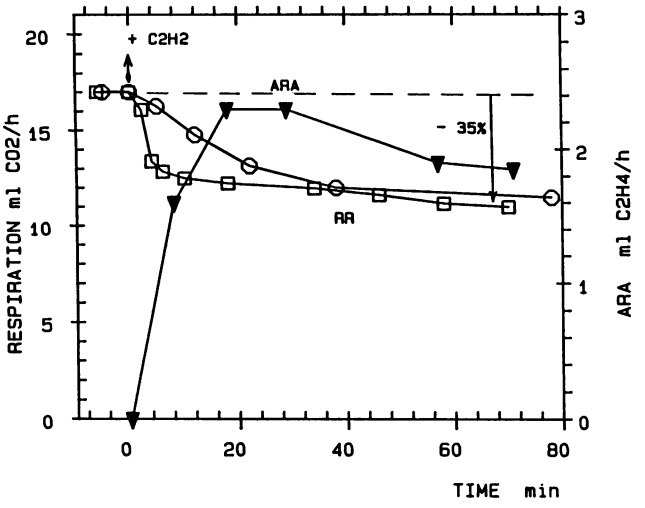

Figure 2. C_2H_2 effect on the respiration of nodulated (□) or nonnodulated (○) soybean roots. C_2H_2 (13%) was introduced in the gas flow aerating the roots at the time shown by the arrow. The respiration rates of the nonnodulated plant were divided by 1.63 for a better readability of the figure. C_2H_4 evolution (▼) was measured simultaneously in the nodulated plant.

the CO_2 enrichment of outlet air measured with a Maihak Finor CO_2 analyser.

For ARA measurements, 3 L h^{-1} of C_2H_2 were added to the compressed air flux, giving a concentration of around 13% C_2H_2. The roots remained submerged in the nutrient solution. Gas samples were taken at the root outlet with a syringe or introduced directly in the gas chromatograph (Delsi DI 200) by a pneumatic 6-way valve. Acetylene used (grade N26 from Air Liquide France) contained around 6 ppm ethylene. The gas was washed in water before entering the root chamber.

Measurement of Net Photosynthesis and Shoot Respiration

The shoot was enclosed in an air-tight glass vessel (Fig. 1). CO_2 concentration was kept at 340 ppm by pulsed injections monitored by a computer (1). Net photosynthesis was calculated from the record of CO_2 injection pulses. For the occasional measurement of dark respiration the CO_2 regulation was stopped; the rate of respiration was calculated from the rate of increase of CO_2 concentration in the chamber. This method ensured a good evaluation of transitory rates.

RESULTS

The kinetics of root respiration (including the CO_2 evolution by nodules) was followed before and after C_2H_2 introduction in the root compartment (Fig. 2). C_2H_4 evolution was sampled at intervals. RR decreased shortly after C_2H_2 introduction and stabilized by 20 min. The level of inhibition, 35% here, ranged from 15 to 35% in other experiments. C_2H_4 evolution increased to a plateau in about 15 min. A small decrease followed in the case presented but was not always present. There was no effect on photosynthesis (data not shown).

The same experiment was done with soybean plants that had not been inoculated and were cultivated on the same nutrient solution supplemented with 4 mM sodium nitrate (Fig. 2). The response was very similar, although slower, to that of nodulated plants.

The responses of RR and ARA to increasing C_2H_2 show a similar concentration dependence (Fig. 3). The responses saturate near 5% C_2H_2. This implies that although most of the experiments were done at 13% C_2H_2, the results may probably be extended to C_2H_2 concentrations between 5 and 10%, which are used in most ARA assays.

Another question was whether the inhibition of RR observed was due to the dilution of O_2 following C_2H_2 addition in the gas stream. I measured the RR and ARA at various O_2

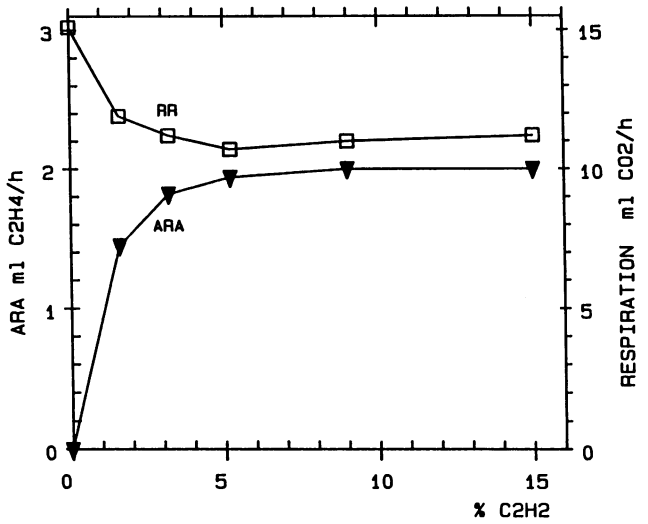

Figure 3. C_2H_2 concentration effect on ARA and respiration of nodulated roots.

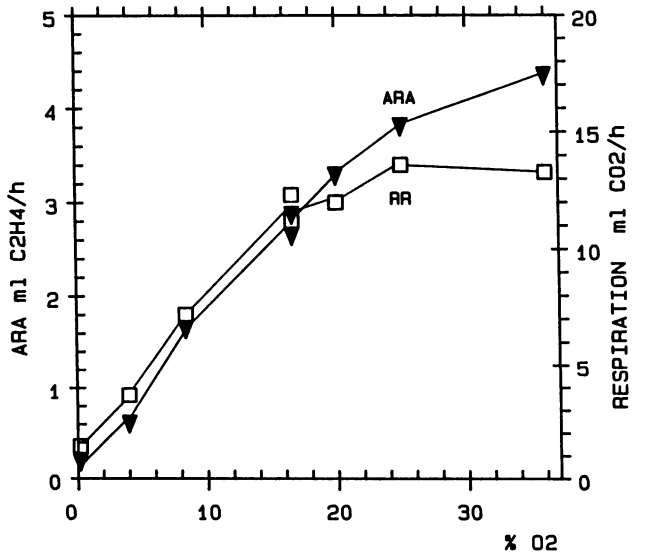

Figure 4. Oxygen concentration effect on ARA and respiration of nodulated roots (measured at 13% C_2H_2).

concentrations from 0.2 to 36% (Fig. 4). RR and ARA were similarly dependent on O_2, except above 21% O_2 where RR saturated faster than ARA. The introduction of 13% C_2H_2 would lower the O_2 concentration to 17.9%. The decrease of RR due to this dilution would be around 7%; hence, the dilution of O_2 during the ARA test can explain only a small part of the observed inhibition of RR. The corresponding decrease of ARA is more (15%) because it saturates only at higher O_2 concentration.

The influence of light intensity was also considered. In a first type of experiment, I doubled the light intensity after having applied C_2H_2 for some time, until the level of RR was stabilized. The doubling of the light intensity increased from 16% to 47% the inhibition due to the presence of C_2H_2 (Fig. 5). Inhibitions of RR at 800 $\mu E \cdot m^{-2}\ s^{-1}$ were consistently between 40 and 50%; that is, in the mean, twice the inhibition at 400 $\mu E \cdot m^{-2}\ s^{-1}$.

In a second type of experiment, C_2H_2 was introduced a few hours after the doubling of the light intensity (Fig. 6). In this case the 41% inhibition of RR was accompanied by a 52% inhibition of ARA after 90 min. The maximal value of ARA was reached between 15 and 30 min, as at normal light.

For comparison, C_2H_2 effect on leaf respiration in the dark was also tested. A stimulation of about 40% of leaf respiration was observed a few minutes after C_2H_2 introduction (Fig. 7).

DISCUSSION

Inhibition of RR and ARA by C_2H_2 was first reported by Minchin *et al.* (7); however, only in conjunction with a simultaneous inhibition of ARA. They mentioned a large variability in the degree of inhibition observed. My results show the importance of the light intensity used, as the inhibition of ARA is totally suppressed, and that of RR reduced by 50%, by halving the light intensity. This agrees with unpublished data of Drevon reporting inhibition of ARA at high light intensity. Also, contrarily to Minchin *et al.* (7), I found that the inhibition also occurs in nonnodulated soybean plants. This needs confirmation; it suggests that the response of roots to acetylene differs slightly from the response of nodules. The response of nodulated roots probably reflects mainly the nodule response, as the respiration of nodules is known to be very active, and there was a large mass of nodules in the experimental plants. The variability of the effect with species, age, light, and other conditions might be the cause of the discrepancy of results between the various authors.

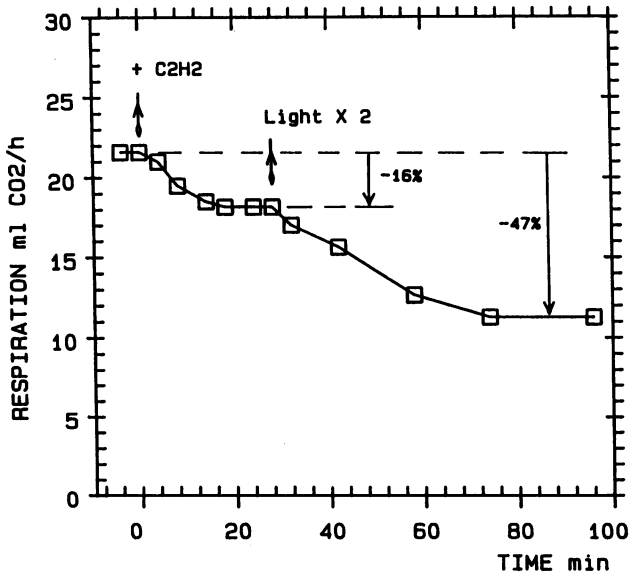

Figure 5. Interaction between C_2H_2 and high light effects on the respiration of nodulated roots. C_2H_2 was introduced to the roots at time 0. When root respiration reached a steady state in about 30 min, light intensity in the shoot compartment was doubled (arrow) from 400 to 800 $\mu E \cdot m^{-2}\ s^{-1}$, bringing a new steady state with a higher inhibition of the respiration rate.

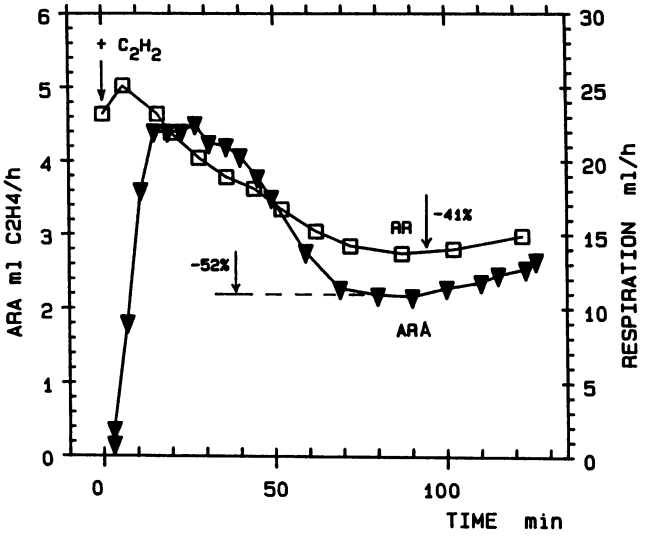

Figure 6. C_2H_2 effect on ARA and respiration of nodulated roots at 800 $\mu E \cdot m^{-2}\ s^{-1}$ light intensity. Other conditions as in Figure 2.

Practically, I would suggest that the precautionary steps to be taken in measuring nitrogenase activity with C_2H_2 should not necessarily be as drastic as recommended by Minchin *et al.* (7), as the inhibition of ARA by C_2H_2 occurs mainly at high light intensity, and even then the ARA level remained stable for more than 15 min after the maximum was reached. Long periods of stable ARA measurements have, in fact, been observed in many cases (3, 6, 10). The stability of ARA after the first 30 min should be checked if longer measurements are to be made. Care should also be taken of controlling C_2H_2 and O_2 concentrations: in the usual test conditions (10% C_2H_2 and no O_2 control), ARA measurements should be corrected by about 10%. On the other hand, all effects were fully reversible when C_2H_2 was applied for no more than a few hours.

There was no effect on photosynthesis in the short-term, but some inhibitory effect was observed after 3 d of repeated contact with C_2H_2 (data not shown). The repeated loss of N_2 fixation may cause a deficiency of nitrogen, that would be expected to alter the photosynthetic rate, but the inhibition of root respiration could as well have brought a deficiency in the nutrition of other elements.

Although it is still too soon to propose explanations for the

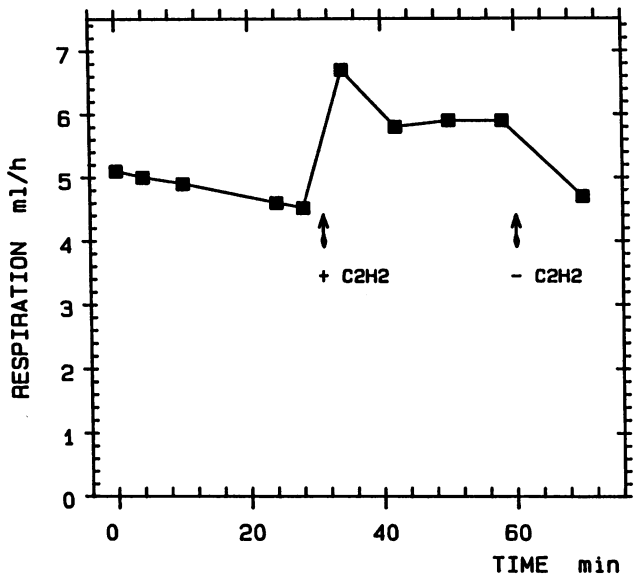

Figure 7. C_2H_2 effect on foliar respiration in soybean. C_2H_2 (15%) was introduced in the shoot compartment 30 min after the onset of darkness (time 0).

phenomena observed, I envisioned that it might be due to ethylene that is contained as an impurity in the acetylene (around 6 ppm C_2H_4). This hypothesis was, however, dismissed by the fact that 50 ppm ethylene in air without acetylene had no action on root respiration (data not shown).

The assay of C_2H_2 action on leaf respiration, intended as a test, showed a reverse action of that on roots: the effect was a 40% stimulation of leaf respiration, similar to the effect usually seen with ethylene (8). Although the effect of acetylene on roots is unexplained, it shows a considerable difference between shoot and root respiratory metabolism.

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