Photoacoustic Measurements of C_2H_4 Production and Entrapment in Plants: A Comparison with Gas-Chromatographic Results

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Introduction

We compare the superior performance of the photoacoustic detection method to gaschromatography in measuring the C_2H_4 production of and entrapment in *Rumex* plants during submergence.

C_2H_4 is a well-known plant hormone. Already in 1935 [1] it was shown that many plant materials produce ethylene. C_2H_4 can e.g. stimulate the ripening of fruit, the wilting of flowers or the abscission of leaves.

In our experiment we measured the C_2H_4 production and C_2H_4 release of *Rumex palustris* plants. These plants grow in a zone near rivers, where they are flooded at irregular intervals when the water level in the river rises. They respond to this inundation by an enhanced growth of the petioles, a process triggered by an increased C_2H_4 production [2].

We have investigated plants under waterlogged and submerged conditions. The amount of entrapped C_2H_4 inside the plant tissue that had been submerged for 24 hours has been measured using the photoacoustic method. A large discrepancy was found with measurements using the gaschromatographic method.

Experimental set-up

As radiation source for our photoacoustic measurements we use an infrared CO_2 waveguide laser (9-11 μm wavelength). A small resonant acoustic cell is placed inside the laser cavity between the discharge tube and the output mirror [2]. Due to the small overall volume (35 cm^3) it can be used in low flow regimes (1 l/hour) and still have a fast response time. The total set-up has an absorption sensitivity of $1.8 \cdot 10^{-10}$ cm^{-1}. For the strongest vibrational absorption of C_2H_4 on the 10P14 CO_2 laser line (949.479 cm^{-1}) we can reach a detection limit of 6 ppt (1 ppt=1:10^{12}).

To measure the C_2H_4 production of plants the photoacoustic cell is connected to a flow-through system. Air from the plant cuvettes is stripped of CO_2 by a KOH-based CO_2 scrubber to prevent interference effects of CO_2 absorption on the C_2H_4 signal.

Biological applications

In figure 1 the C_2H_4 production of a single *R. palustris* plant is compared to that of a control plant. After an acclimatization period of 18 hours (With both production levels on 3-4 nanoliter per gram dry weight per hour (nl./g DW. h.) the plant is completely inundated (W) for a 24 hours period. In this period first a decrease

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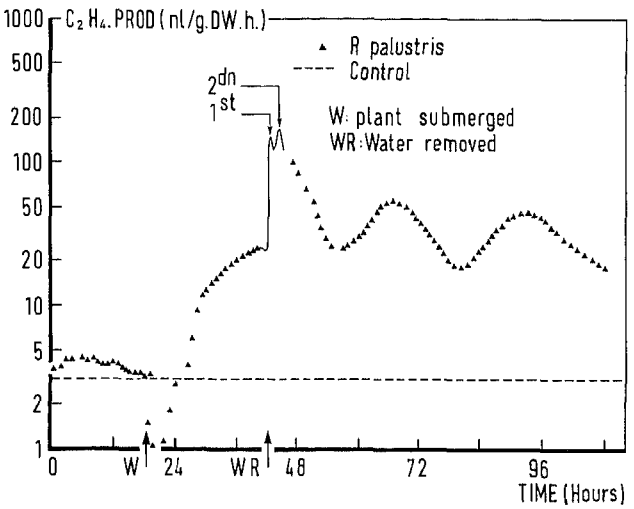

Figure 1: The C_2H_4 production of a *Rumex palustris* plant (26-30 days old) is measured during submergence (from W) and waterlogging (from WR) compared to that of a control plant. Clearly one sees a first C_2H_4 peak from the entrapped C_2H_4 and a second from the conversion from ACC. (Data are so close together that a line is drawn).

is measured of the C_2H_4 concentration in the air above the water level caused by the slow diffusion rate of C_2H_4 in water (10^{-4} x slower than in air). Afterwards the measured C_2H_4 level rises again due to the stress induced production of the plant.

When the waterlevel is lowered from submergence to waterlogging a fast first peak of C_2H_4 occurred within 1 hour, followed by a second, gradual one with a maximum after 3-4 hours. The first peak is attributed to the entrapped C_2H_4 inside the plant tissue during submergence. Ethylene is released immediately after lowering of the water level. The width of this peak is given by the response time of the system (a combination of flow velocity through the cuvettes and the volume of the cuvettes, tube, photoacoustic cell etc.). The second peak is connected to the onset of ACC conversion (1-aminocyclopropane-1-carboxylic acid), yielding C_2H_4. During the inundation period ACC probably accumulates inside the root tissue, but cannot be converted into C_2H_4 since this conversion needs oxygen . After this second peak the C_2H_4 production remained higher for several days. Inspite of the continuous light and temperature regime a diurnal rhythm in the ethylene production is observed.

From the integrated first peak we calculate the total amount of C_2H_4 entrapped: for this specific plant 12 ppm. or 12 nanoliter per milliliter internal air volume. (The average internal air volume amounts to 0.35 ml.) This concentration is in contradiction to the results of gaschromatographic measurements which yield an average concentration of 0.5 ppm C_2H_4 inside the internal air volume of the plant[3]. Because concentrations of exogenous C_2H_4 become effective on the growth of petioles at a level of 5ppm [3] the gaschromatographic result appear to be doubtful.

The discrepancy between the two measurements can be explained by the difference in plant treatment. Before the C_2H_4 gas sample is injected into the gaschro-

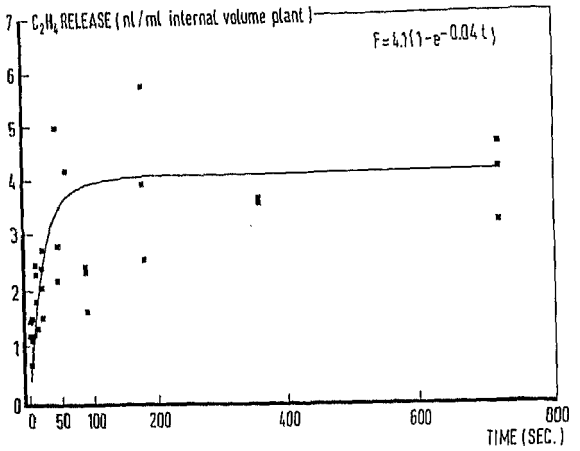

Figure 2: The C_2H_4 release of *R. palustris* plants is plotted against the exposure time to the atmosphere after a 24 hour period of submergence. The amount of C_2H_4 is normalized for its production rate and its internal volume. C_2H_4 release = $4.1(1\text{-}e^{-0.04t})$ nanoliter/plant.

matograph the plant is removed from the water, cut into pieces and put into an ammonium sulphate solution from where the internal air volume of the plant is collected by vacuum extraction[3]. During the treatment (which can take a few minutes) a large amount of C_2H_4 can escape from the plant.

In a separate photoacoustic experiment we have investigated the C_2H_4 release rate, lifting the plant above the water surface during a limited period of time. (fig 2.) and afterwards submerging it again. Just before the lifting of the plant the ethylene production rate (nl / plant h.) was determined. The ratio of the mean C_2H_4 production rate (n=36) divided by each individual production rate was used to correct the ethylene release after de-submergence. High ethylene production rates will lead to high internal ethylene concentrations during submergence and thus to high release peaks after de-submergence. The ratio corrects for inter plant variation in ethylene production under water. The air exposure time varied from 2 till 360 seconds. From the concentrations in the air flow we calculated the total amount of C_2H_4 that was released during this period; corrected for the production ratio it is plotted in figure 2. From this figure we obtained a mean internal concentration of 4.1 ± 0.3 ppm C_2H_4 in the plant tissue just before de-submergence. The $^1/_e$ release time is 25 seconds. This demonstrates that, unless the plants are handled very carefully the photoacoustic method is far more suitable for reliable measurements of C_2H_4 production and release than the gaschromatographic method.

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[3] L.A.C.J. Voesenek and C.W.P.M. Blom, Plant, Cell and Environment 12 (1989) 433-439