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AN IMPROVED CLOSED SYSTEM FOR CONTINUOUS MEASUREMENT OF PHOTOSYNTHESIS, RESPIRATION AND TRANSPIRATION

(with a summary in English)

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INTRODUCTION

In a previous paper (VAN HOLSTEIJN 1979) a prototype closed system for CO_2-exchange measurements on whole plants was described. Compared to an open system (JARVIS and CATSKÝ 1971) a closed system has the advantage that the same experimental plant can be exposed to a large series of external CO_2-concentrations. An additional advantage is the relatively easy determination of the CO_2-compensation concentration (JACKSON and VOLK 1970) and the carboxylation efficiency (AUGUSTINE et al. 1976). The closed system mentioned above has been used to study the influence of irradiance, external CO_2-concentration, temperature and cultivation conditions on net photosynthesis of whole sweet pepper (NILWIK 1980a, 1980b) and lettuce plants (VAN HOLSTEIJN 1981). In this paper an extension of the closed system is described, which enables measurement of transpiration simultaneously with measurement of the CO_2-exchange. The transpiration data will facilitate the estimation of plant resistances to water vapour and carbon dioxide (e.g. JARVIS 1971). In addition some other improvements of the system are described.

METHODS AND DESCRIPTION

Measurement of transpiration

In the original design of the closed system measurement of transpiration was planned by determining the difference in water vapour content between the in- and outgoing air of the plant chamber. During preliminary experiments two Vaisala humidity sensors (SUATOLA and ANTSON 1973) and an infrared gas analyser (URAS-2, Hartmann and Braun) were used for this purpose. However, due to the high wind speed in the plant chamber (in centre 0.8 m s^{-1}), the differences in water vapour content are too small (< 100 mg m^{-3}) to obtain accurate readings with both methods. For this reason it was decided to obtain transpiration measurements by a direct assessment of the loss in weight of the plant.

The actual system consists of an electronic precision balance (Sartorius type 1364 MP) connected to a 30-point recorder (Leeds and Northrup, Speedomax 250) via an interface (Fray Data FD 1019).

The electronic balance is positioned in the plant chamber with the experimental plant placed on top. The root part of the plant is sealed in an airtight perspex cylinder. The measuring range of the balance is 0–4000 g, while the read-out of the weight is available as a digital signal and on a display with an accuracy of 0.01 gram.

Using the 30-point recorder all relevant measurements (e.g. from the infrared gas analyser, humidity sensor, thermocouples, photocells etc.) are periodically printed on a chart. The time interval between two print-outs of the signal from the interface will be defined as one 'weighing-cycle'. The length of a 'weighing-cycle' depends on the total number of channels connected and on the number of replicate channels used for each incoming signal. In the experimental set-up described in this paper the length of a 'weighing-cycle' amounted to 24 seconds.

Apart from converting the digital signal of the electronic balance to an analog signal suitable for the 30-point recorder, the interface acts as a 'digital filter'. Due to vibrations originating from the temperature control system and due to movements of the leaves of the experimental plant as a result of the turbulence of the air in the plant chamber the measured weight of the plant will be continuously fluctuating. However, it can be expected that, during an adequately chosen time interval in which temperature, irradiance and humidity conditions do not vary, the real weight of the plant will show a linear decrease with time. Furthermore, it can be assumed that the differences between the real and the measured weight follow a normal distribution (except for a possible constant deviation between real and measured values which will not influence the result since only a rate is determined). These remarks suggest the following 'filtering' procedure: the 'filtered' weight at time t should be calculated from a linear regression equation obtained via the 'least squares method' (DRAPER and SMITH 1966) using all the readings of the weight which were collected during a chosen time interval Δt preceeding time t.

With the interface this is actually carried out as follows: readings of the weight on the electronic balance are collected every 0.5 seconds. At the same time that the recorder-channel connected to the interface is printed on the chart, a request for 'revision of output' is sent from the recorder to the interface. At this request the interface calculates the required 'filtered' weight by linear regression as outlined above using all the data collected from the balance during the last k 'weighing-cycles'. This value is used for the next recorder print-out (implying that the printed result always has a time-lag of one 'weighing-cycle'). The number k of 'weighing-cycles' to be incorporated in the calculation of the 'filtered' weight can be manually set between $k = 1$ and $k = 10$ and changed during operation. Thus, in the experimental set-up described in this paper, the time interval from which readings of the weight are incorporated in the calculation of the print-out can be varied stepwise from 24 to 240 seconds corresponding to a regression in which 48 to 480 data points are used respectively. All calculations are carried out with the absolute values of the measured weights on the balance. Thus, the transpiration will be visible as an increase in the signal printed on the recorder chart. The calculated values, however, are transmitted from the interface to the recorder either modulo 1 or modulo 10 grams. In this way the full scale of the recorder chart can be chosen to represent either 1 or 10 grams of water transpired, while this also results in an automatic reset to zero when the maximum scale deflection has been attained.

Automatic control of CO_2-level

When in the closed system the photosynthetic rate at a certain ambient CO_2-concentration needs to be determined, the period of time required for a small decline in the CO_2-concentration at that ambient CO_2-concentration is measured. To obtain accurate data this decline in CO_2-concentration should be taken sufficiently small because of the non-linear response of net photosynthesis to external carbon dioxide concentration (e.g. BIERHUIZEN and SLATYER 1964). For a new reading a small amount of CO_2 should be re-injected in the system to obtain the desired ambient CO_2-concentration again. Manual injection of pure CO_2 with a syringe was used in the initial experiments carried out in the system, but accurate injection proved to be troublesome. Furthermore, the continuous attendance is a major drawback when monitoring the photosynthetic rate for long periods. For this reason automatic control of the CO_2-level seemed desirable.

The injection of CO_2 is carried out with the use of a peristaltic pump (Meredos type SP-TI) connected to a magnetic valve (Asco) which is attached to the plant chamber. When the pump is operating a choice between continuous or intermittent injection is available. In the latter case the time interval between injections can be independently set from 1–200 seconds. This allows a range of injection rates between 0.1 and 20 ml per minute.

An electronic control unit (TFDL type 923404) operates the injection system described above. By means of two potentiometers two set-points S_{min} and S_{max} are selected from the range at which the infrared gas analyser has been calibrated. When the CO_2-concentration in the closed system drops below S_{min} the peristaltic pump is switched on and the magnetic valve opened to allow injection of CO_2. Both are switched off again when the CO_2-concentration surpasses S_{max}. In this way monitoring of the photosynthetic rate during long periods is simplified.

In an analogous way respiration rates can be continuously measured. In this case the control unit is switched to 'flushing' mode and operates two magnetic valves, both attached to the plant chamber.

The first valve is connected to a cylinder supplying CO_2-free air, the second valve acts as overflow when the first is open. Again two set-points S_{min} and S_{max} are selected, but now both magnetic valves are opened when the CO_2-concentration in the closed system increases above S_{max} while closure occurs when the CO_2-concentration drops below S_{min}.

Miscellaneous additions

The minimum volume of the closed circuit (i.e. the plant chamber plus the channel containing cooling and heating units) was determined at 180 dm^3 (VAN HOLSTEIJN 1979). This volume is too large to obtain accurate data from CO_2-exchange measurements with very small amounts of plant material. For the latter purpose an additional cylindrical perspex plant chamber was constructed by Wientjes, Roden, The Netherlands. The internal diameter of this chamber amounts to 280 mm, the internal height of bottom and top part to 70 and 110 mm

respectively. Bottom and top part are airtight connected with O-rings and metal clips. This small chamber is connected directly to the infrared gas analyser, resulting in a closed circuit with a volume of 18 dm^3. Adequate mixing of the air in the small plant chamber is obtained by using two small fans (Micronel type V361M). The air temperature can be kept constant with an accuracy of $\pm$ 0.3 °C by placing the small chamber in the existing plant chamber and using the temperature control system of the latter. The temperature inside the small chamber is measured by manganine-constantan thermocouples (0.2 mm^2) and the level of irradiance by selenium photocells (Megatron). The system described above was used to measure respiration rates of cut Gerbera flowers (VAN MEETEREN, unpublished data) and the photosynthesis of shoot initials of Douglas fir grown in vitro (EVERS 1981).

During initial experiments the maximum level of irradiance available in the plant chamber (220 W m^{-2}) was still too low to obtain light-saturated values of net photosynthesis when applying high external CO_2-concentrations to relatively large plants. Furthermore, a comparison of net photosynthetic rates under a level of irradiance attained in late spring and summer was not possible. In order to increase the maximum level of irradiance available, the existing five 400 W HPLR-lamps were replaced by five 400 W SON/T-lamps (TEMPLING and VERBRUGGEN 1977). The latter are placed in a specially designed trapezoidal reflector (Philips' Lightning Design and Engineering Centre). In this way a level of irradiance of 360 W m^{-2} can now be obtained at plant level.

Another advantage of a closed system is the fact that the CO_2-exchange rates can with relative ease also be determined at external oxygen concentrations other than 20 %. At the start of the measurement the desired O_2-concentration is obtained by injecting O_2 or flushing with N_2.

The O_2-concentration is continuously measured using an oxygen monitor (Beckman OM-15) with a range from 0–100 % O_2 and an accuracy $< \pm$ 1 %. During short-term measurements changes in the O_2-concentration in the plant chamber resulting from the O_2-exchange of the experimental plant can be neglected. In the future this set-up will be used for comparison between photosynthetic rates at 20 and 1 % ambient oxygen, which will provide estimates of the photorespiration (JACKSON and VOLK 1970).

To facilitate an easier calibration of the infrared gas analyser the Wösthoff gas mixing pumps type SA 27/2a and /3a were replaced by two Wösthoff pumps type IM 301/A-F connected in series. With the latter a finer range of mixtures can be obtained while furthermore no change of gears is necessary when another mixture is desired.

SOME PRELIMINARY RESULTS

The calculation of photosynthesis and respiration in the closed system has been described in the previous paper (VAN HOLSTEIJN 1979). The transpiration rate can be calculated directly from the recorder chart. It should be mentioned

that, although the humidity in the plant chamber can not be controlled, a constant value for the humidity is attained ($\pm$ 0.5%) in the range between 60 and 75% as soon as the temperature is sufficiently constant ($\pm$ 0.5°C). This justifies the assumption made in the construction of the 'digital filter' that the weight of the plant on the balance shows a linear change with time. To obtain accurate readings the number of 'weighing-cycles' necessary for the calculations of the interface was varied between 5 and 10 depending on the magnitude of the transpiration rate.

For the calculation of various plant resistances to water vapour and carbon dioxide the following equations based on FICK's diffusion law are used (compare JARVIS 1971). The transpiration rate (T) is represented as

$$T = (V_1 - V_2)/R_{H_2O} \quad (1)$$

where V_1 is the water vapour content of saturated air at plant temperature and V_2 the water vapour content of the ambient air. The plant temperature can be determined using thin manganine-constantan thermocouples (0.2 mm^2) touching the adaxial side of several leaves without influencing the registration of the plant weight. V_2 is determined from the readings of the humidity sensor and shaded thermocouples in the ambient air. It should be remarked, however, that as a result of the high wind speed in the plant chamber the differences between plant and air temperature of a freely transpiring plant are always smaller than 0.3°C. For the same reason no ambiguity can occur in determining V_2 due to a positional gradient in the plant chamber.

Equation (1) is used to calculate R_{H_2O}, the total plant resistance to water vapour (i.e. including boundary layer, cuticular and stomatal resistance). The photosynthetic rate (P) is represented as

$$P = (C_{ex} - C_{chl})/RT_{CO_2} \quad (2)$$

where C_{ex} is the ambient CO_2-concentration and C_{chl} the CO_2-concentration at the chloroplasts. By means of equation (2) RT_{CO_2}, the total plant resistance for CO_2, can be calculated when assuming $C_{chl} = 0$. RT_{CO_2} can be further separated according to

$$RT_{CO_2} = RG_{CO_2} + R_{res} \quad (3)$$

where RG_{CO_2} is the plant resistance for gaseous diffusion of CO_2 and R_{res} a residual resistance, called the mesophyll resistance (GAASTRA 1959). R_{res} is calculated using equation (3), and remarking that $RG_{CO_2} = 1.371\ R_{H_2O}$ (JARVIS 1971, pp. 567–569). Finally, expressing the photosynthetic rate (P) as

$$P = (C_{ex} - C_{int})/RG_{CO_2} \quad (4)$$

the CO_2-concentration in the substomatal cavities, C_{int} can be calculated using equation (4). To facilitate handling of all data a program was made for a portable desk calculator HP 9815A. All relevant readings are entered directly from the recorder chart into the program which carries out all necessary calculations.

Some preliminary results with lettuce, cv. 'Deciminor' are shown in Figures 1 and 2. The plants were sown on June 25 and cultivated in a glasshouse during July-August 1980 at average day/night temperatures of 26/19°C.

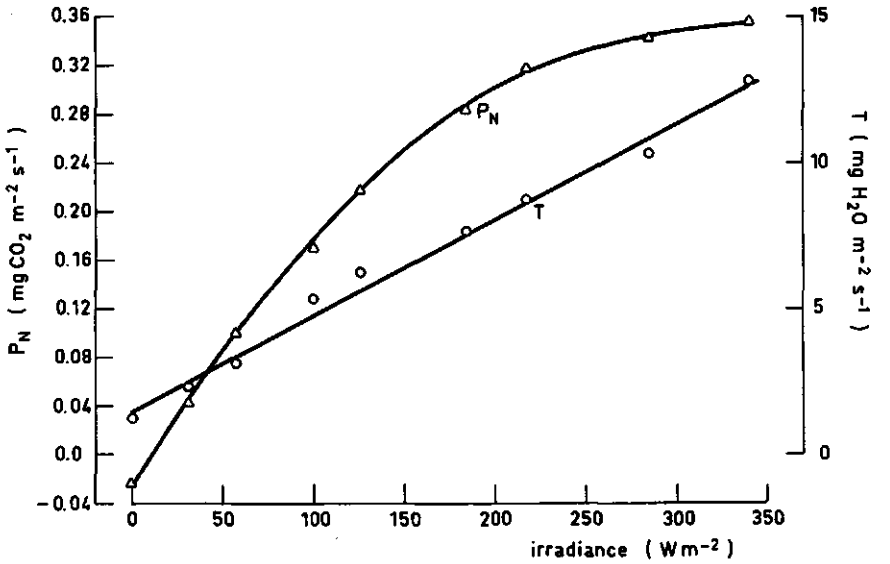

Fig. 1-A. Response of net photosynthesis (P_n) and transpiration (T) to irradiance for lettuce cv. 'Deciminor'. The measurements were carried out at 21.0°C and an external CO_2-concentration of 1540 mg m^{-3}.

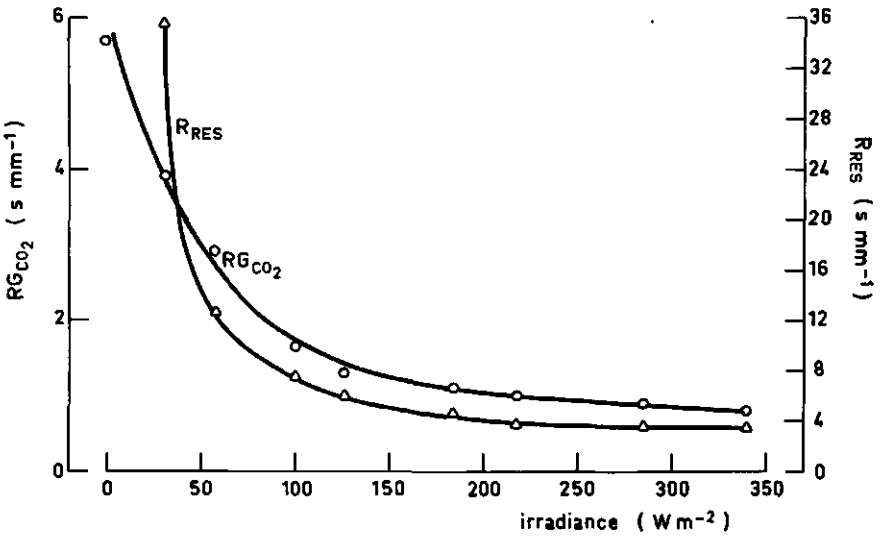

Fig. 1-B. Calculated values for plant resistance to gaseous diffusion of CO_2 (RG_{CO_2}) and residual resistance (R_{res}) plotted against irradiance.

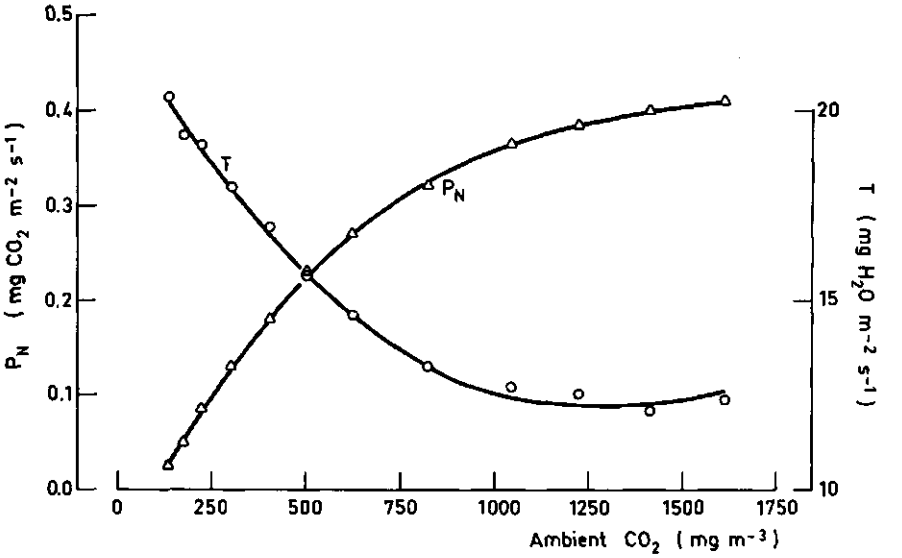

Fig. 2-A. Response of net photosynthesis (P_n) and transpiration (T) to external CO_2-concentration for lettuce cv. 'Deciminor'. The measurements were carried out at 21.5°C and 350 W m^{-2}.

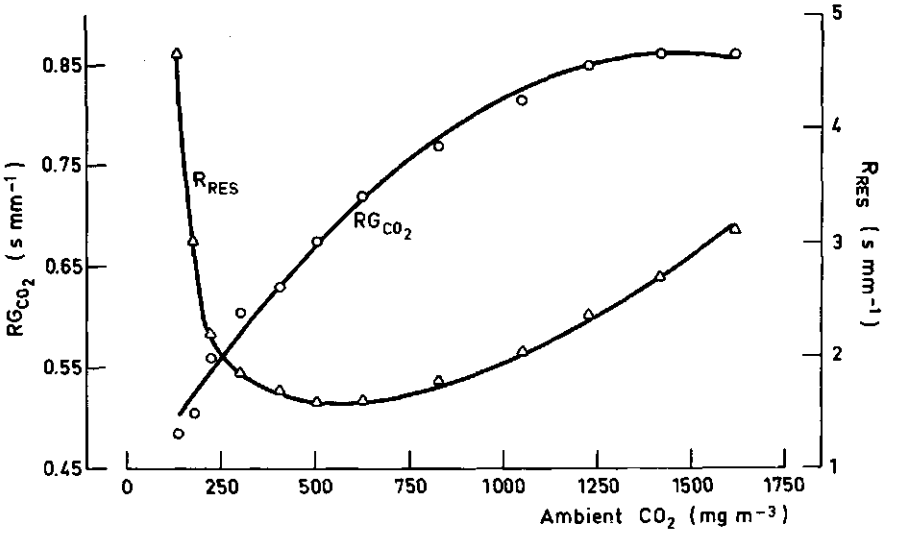

Fig. 2-B. Calculated values for plant resistance to gaseous diffusion of CO_2 (R_{CO_2}) and residual resistance (R_{res}) plotted against the external CO_2-concentration.

Fig. 1-A shows the response of net photosynthesis (P_n) and transpiration (T) to the level of irradiance at 21.0 °C and 1540 mg m^{-3} ambient CO_2. The leaf area of the experimental plant was 0.148 m^2. The procedure followed in measuring a 'light-series' was outlined by NILWIK (1980a). For P_n the usual saturation curve was obtained, while T showed a linear increase with increasing level of irradiance. The plant resistance for gaseous diffusion of CO_2 (RG_{CO_2}) and the residual resistance (R_{res}) are shown in Fig. 1-B. Both strongly decrease with increasing level of irradiance. The difference in the order of magnitude of RG_{CO_2} and R_{res} should be noted.

Fig. 2-A shows the response of P_n and T to ambient CO_2-concentration at 21.5 °C and 350 W m^{-2}. The leaf area of the experimental plant was 0.184 m^2. The procedure for measuring a 'CO_2-series' was described previously (NILWIK 1980b). A saturation curve was obtained for P_n, while T strongly increased at ambient CO_2-concentrations below 900 mg m^{-3}. Values for RG_{CO_2} and R_{res} are shown in Fig. 2-B. RG_{CO_2} decreased with decreasing ambient CO_2-concentration. R_{res} also exhibited an initial gradual decrease with decreasing CO_2-concentration but strongly increased in the range between 300 mg m^{-3} and the carbon dioxide compensation concentration (about 120 mg m^{-3}). Again the difference in the order of magnitude of RG_{CO_2} and R_{res} should be noted.

SUMMARY

A system for the measurement of transpiration has been added to an existing closed system for the measurement of the CO_2-exchange of whole plants. The transpiration rate is determined through continuous assessment of the plant weight using an electronic precision balance with an accuracy of 0.01 gram. Fluctuations in the readings of the weight are suppressed by means of a 'digital filter'. This procedure is discussed. The 'filtered' weight is printed on a recorder chart. The calculation of various plant resistances to water vapour and carbon dioxide are outlined.

A system for the automatic control of the CO_2-level in the closed system is also described. This facilitates monitoring of photosynthetic and respiratory rates during long periods. In addition some other improvements of the closed system are described.

Preliminary results showing the influence of the level of irradiance and ambient CO_2-concentration on photosynthesis, transpiration and plant resistances for CO_2 are presented for lettuce cv. 'Deciminor'.

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