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TECHNICAL REPORT

Measurements of photosynthesis in the field: utility of the CO_2 depletion technique

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Abstract. A portable system for the rapid measurement of photosynthetic rates in the field is described and evaluated. The measurement of photosynthesis is based on extraction of volumes of air from a closed system cuvette containing a leaf over a set time interval. CO_2 content of the gas volumes is determined with an infra-red gas analyser, and photosynthetic rate is calculated from the cuvette CO_2 concentration depletion rate. The magnitudes of sources of error with this system were analysed and methods of reducing the errors discussed. Representative examples of data obtainable with this technique are presented.

Introduction

Measurements of photosynthesis are not routinely made in the field because of the complexities and difficulties of operating the equipment and the expense involved in setting up both laboratory and field photosynthetic measurement systems (Hesketh & Musgrave, 1962; Baker & Musgrave, 1964; Mooney *et al.*, 1971; Mooney, 1972; Schulze, 1972; Björkman *et al.*, 1973; Marshall & Biscoe, 1977). Where such measurements have been made interpretation of the results is often difficult because of variability between the limited number of plants that can be sampled. However, measurements of photosynthetic rates under natural field situations are important for many aspects of physiological and ecological studies.

Recently, Atkins & Pate (1977) and Clegg, Sullivan & Eastin (1978) have developed non-steady state methods of infra-red gas analysis that are sufficiently sensitive to get reliable estimates of CO_2 concentrations over the range needed for measuring rates of photosynthesis and dark respiration. The technique involves injecting a sample of CO_2 into a N_2 carrier gas which passes through the sample cell of the infra-red gas analyser (IRGA). The N_2 alone passes through the reference cell of the IRGA. The height of the response peak produced by the IRGA is proportional to the CO_2 concentration of the sample and can be recorded on a recorder. Clegg *et al.* (1978) extracted gas samples from the inlet and outlet ports of a cuvette for measurements of *Sorghum bicolor* dark respiration rates in the laboratory.

We have modified their technique and report the utility of the CO_2 depletion technique for measurements of photosynthesis on plants in the field, far from laboratory facilities. The technique is reliable and easy to operate in the field. A description of the technique, as well as an analysis of the sources of error and examples of the data which can be obtained with it are presented.

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Materials and methods

The gas sample measurement system consisted of a CO_2 gas analyser operated in the absolute mode (Analytical Development Corp., Hoddesdon, England), a millivolt flat-bed recorder (Linear Instruments, Irvine, Calif.), a flowmeter (Matheson Gas Products, Rutherford, N.J.), and a DC pump (W. R. Brown Corp., Chicago, Ill.) (Fig. 1). Electrical power for the IRGA and recorder was furnished by a 400 W generator (Honda Motor Company, Japan). Tygon tubing connected various parts of the system. The flow rate of the carrier gas (CO_2 free air scrubbed by ascarite) was 4 $cm^3\ s^{-1}$. The CO_2 free air necessary for the reference cell was internally generated by the ADC IRGA (air scrubbed by ascarite). Since this IRGA is insensitive to water vapour (not all IRGAs are), no water traps or desiccant which might impede flow rates were necessary. 10 cm^3 gas samples were injected into the carrier gas and the peak obtained on the IRGA as the gas sample passed through the sample cell was recorded. To increase the sensitivity of the recorder, the recorder zero was placed well below the bottom of the scale so that only the upper portion of the peak was seen (Clegg *et al.*, 1978). A sensitivity greater than 1 $cm^3\ m^{-3}$ (upper limit of recorder was then $\sim$350 $cm^3\ m^{-3}$ CO_2 and lower end $\sim$250 $cm^3\ m^{-3}$ CO_2) was then obtained. For convenience in the field, this system was operated on a bench in a tent.

Calibration of the system was achieved by injecting different volumes of a known CO_2 concentration gas (in our case 340 $cm^3\ m^{-3}$ CO_2). A 10 cm^3 injection produced a spike on the recorder equivalent to 340 $cm^3\ m^{-3}$ CO_2, a 9 cm^3 injection a spike equivalent to

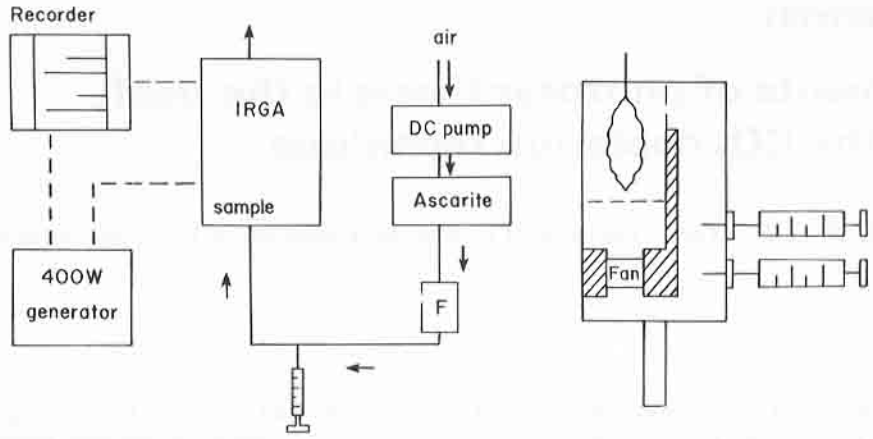

Figure 1. Left. Schematic of gas sampling system for CO_2 depletion method photosynthesis. Solid lines indicate tygon tubing connections and arrows indicate direction of air flow. Dashed lines indicate electrical connections. F = flowmeter, IRGA = infrared gas analyser. Right. Leaf cuvette used in the measurements. A trap door allows rapid sealing of leaf into the cuvette. Hashed marks indicate air flow barriers.

$0{\cdot}9 \times 340$ cm^3 m^{-3} CO_2 or 306 cm^3 m^{-3} CO_2, and so forth. The relationship obtained by this calibration was linear.

The leaf cuvette had an internal volume of approximately 0·3 dm^3 and was made of Plexiglass (Fig. 1). Air circulation was maintained at speeds greater than 1 m s^{-1} using a 6 VDC fan (Micronel, Hudson, Mass.). Two syringes with Luer-Lock type syringe tips were permanently mounted to the sides of the cuvette. The syringes were glass (20 cm^3, B-D multifit) and the plunger was coated with mineral oil. When the syringes were removed from the cuvette, they were stored until processed in a test tube-like rack, with the base of the syringes screwing into Luer-Lock syringe tips embedded into the rack.

For photosynthetic measurements the cuvette was quickly clamped onto a leaf and a gas sample from the cuvette was drawn into one of the syringes. During the measurement period, photosynthetic structures reduced the cuvette CO_2 concentration and after a specified time (usually 20 s), the second syringe was drawn. The concentrations of CO_2 in each of the syringes were then determined.

The rate of photosynthesis is calculated as

$$P = \frac{([CO_2]_b - [CO_2]_e)\,(V)(K)}{(T)(L)} \qquad (1)$$

where P is the photosynthetic rate in μmol CO_2 m^{-2} s^{-1}, $[CO_2]_b$ and $[CO_2]_e$ are the measured CO_2 concentrations in the cuvette at the beginning and then at the end of the measurement period in cm^3 m^{-3}, V is the internal air volume of the cuvette in dm^3, K is a constant that converts CO_2 from mm^3 to μmol (0.0404 μmol mm^{-3} at sea level), T is the time interval of the measurement in seconds, and L is the projected leaf area in m^2.

Accompanying the photosynthetic measurements, additional parameters were measured. Quantum flux density (400–700 nm) was measured with a quantum sensor (model 190-SR, Lambda Instruments, Lincoln, Nebraska). Leaf conductance to water loss was measured with a diffusion porometer (model L1-65, Lambda Instruments). Leaf water potential was measured using a Scholander pressure chamber (PMS Instruments, Corvallis, Oregon).

Results and discussion

An analysis was performed to determine potential sources of variability inherent in the system. In the measurement of CO_2 concentration, two sources of variability exist. The first is user or operator variance, i.e. the variation in repeated analysis of the same sample gas by individual operators. For a 300 cm^3 m^{-3} CO_2 gas sample, this standard deviation equaled 1.04 cm^3 m^{-3} CO_2 ($n = 25$). A second source of variation occurs because not all syringes hold identical volumes at the 10 cm^3 hash mark. For two syringe types (glass = B-D multifit 20 cm^3, plastic = Stylex 20 cm^3), samples of water were drawn to the 10 cm^3 hash mark in fifty syringes, expelled and the water weighed on a balance. The standard deviation in glass syringes was 0.016 cm^3 and in the plastic syringes 0.08 cm^3. For a 300 cm^3 m^{-3} CO_2 gas sample, this standard deviation equals 0.48 cm^3 m^{-3} for glass syringes and 2.4 cm^3 m^{-3} for plastic syringes. Thus for glass syringes, we might expect the standard deviation in CO_2 measurement to be about 1.1 cm^3 m^{-3} CO_2 and for plastic syringes this value would be much greater.

From the time that the gas sample was collected and until it was processed, diffusion of CO_2 from the atmosphere into the syringe was of major concern. The diffusion rate of CO_2 into the syringe was greatly reduced by coating the syringe plunger with mineral oil and by using 20 cm^3 syringes rather than 10 cm^3 syringes (length of plunger barrier was greater). Additionally, during temporary storage the syringe was

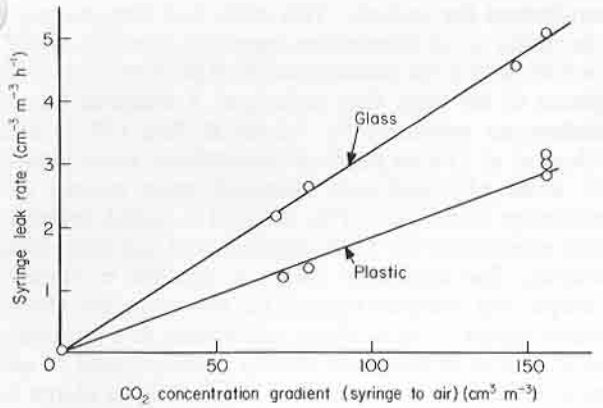

Figure 2. Leak rate of CO_2 from glass syringes and plastic syringes into the air as a function of the CO_2 concentration gradient.

screwed into a locking syringe tip that had been sealed tight with solder. Under these conditions, the CO_2 diffusion rate from the outside air into the syringe was less than 5 cm^3 m^{-3} h^{-1} at a CO_2 concentration gradient of 150 cm^3 m^{-3} CO_2 (Fig. 2). Typical syringe CO_2 gradients are more likely to be approximately 20–50 cm^3 m^{-3} CO_2, which is a CO_2 diffusion rate of less than 1 cm^3 m^{-3} CO_2 h^{-1} for plastic syringes and less than 2 cm^3 m^{-3} CO_2 h^{-1} for glass syringes. In the field, gas samples can easily be processed within 15 min after measurement so that CO_2 diffusion into the syringes is negligible. However, for situations in which the IRGA cannot be taken to the field and gas samples must be transported back to the laboratory over large distances, a correction for CO_2 diffusion rate errors is required. The glass syringes used had slightly greater CO_2 diffusion rates than the plastic syringes (it was still low in both), but the volume variability of plastic syringes made them unreliable for most measurements.

While setting up to make a measurement of photosynthesis using this technique, CO_2 exhaled by the operator is a potential problem and care must be taken not to breathe into the cuvette.

The CO_2 depletion method allows for rapid determinations of CO_2 concentrations and hence rapid measurements of photosynthetic rates. As a consequence measurements can be made on many leaves over a short period. In field situations, this means that photosynthetic measurements can be made on many leaves or individuals of a given species quickly, so that questions of variability between plants or treatments can be answered before environmental conditions change. Our method of measurement is closed system depletion rather than open system depletion as used by Clegg *et al.* (1978). We chose the closed system approach for experiments requiring large sample sizes because it eliminates carrying a bulky air source (compressed air cylinder or pump) and reduces any problems associated with slight fluctuations in incoming CO_2 concentrations.

We have evaluated the utility of this technique for photosynthetic measurements under a variety of field conditions. Three sets of field measurements for which this technique seems appropriate are (1) daily courses of net photosynthesis, (2) determinations of the responses of net photosynthesis to water stress and (3) determinations of the net photosynthetic responses of leaves to changes in irradiance.

Daily courses of photosynthesis were determined using two dominant Sonoran Desert shrubs, *Encelia farinosa* and *E. frutescens*. Measurements were made under late-spring conditions at Shoshone, California where the distributions of both species overlap. Net photosynthesis in both species increased until mid-morning, at which time the net photosynthetic rate plateaued in *E. farinosa* and began to decline in *E. frutescens* (Fig. 3). Closely associated with the changes in net photosynthesis, were concomitant changes in leaf water potential (and presumably also in leaf conductance to water loss). In the late afternoon net photosynthesis declined in both species, and after dusk CO_2 evolution (dark respiration) was measured. The photosynthetic rates measured with the CO_2 depletion technique are very similar to values previously measured for these plants under field conditions using standard gas exchange techniques (Ehleringer & Björkman, 1978).

The response of photosynthesis to changes in leaf conductance was measured on leaves of wild lettuce, *Lactuca serriola* (Fig. 4). These potted plants had been differentially droughted by withholding water for several days. For randomly chosen leaves from different plants both photosynthesis and leaf conductance

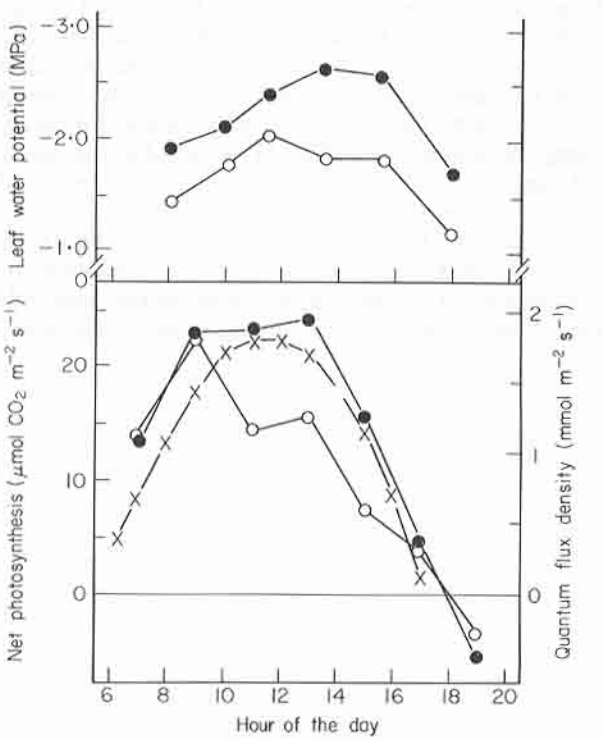

Figure 3. Daily courses of net photosynthesis of *Encelia farinosa* (•) and *E. frutescens* (o), leaf water potentials, and solar quantum flux density (×) (PhAR) through the day on 19 April 1979 at Shoshone, California. Sample size is 4 leaves.

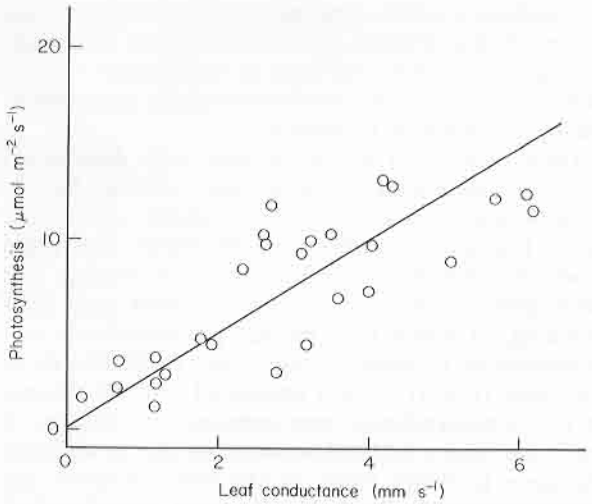

Figure 4. Rates of net photosynthesis in individual leaves of *Lactuca serriola* as a function of leaf conductance to water loss under midday irradiances. Measurements were made on plants in Salt Lake City, Utah.

were measured. Photosynthetic rate declined in response to decreased leaf conductances in a manner similar to that which would be expected from CO_2 diffusion limitations. The utility of the CO_2 depletion technique was that rapid estimates of photosynthetic rate could be obtained on individual leaves at the same rate as leaf conductance estimates.

Photosynthetic-light response curves were determined on leaves of *Quercus agrifolia* (an evergreen tree from Mediterranean-climates of California) under midday irradiances by incremental shading with neutral density screens. Net photosynthesis was measured at each irradiance and was light saturated by 1 mmol m^{-2} s^{-1} (400–700 nm), a pattern typical for many evergreen leaved trees (Fig. 5). At irradiances below 0.5 mmol m^{-2} s^{-1}, the rate of photosynthesis appeared linearly related to irradiance.

Up to now, field measurements of photosynthesis required bulky, relatively immobile systems or radioactive $^{14}CO_2$ techniques. Radioactive methods are by nature not repeatable and require laboratory

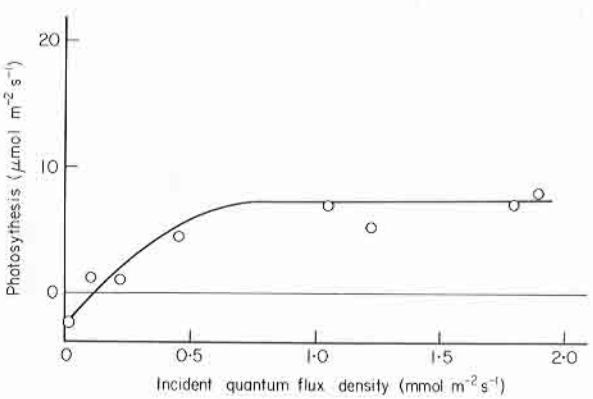

Figure 5. Light dependence of photosynthesis in *Quercus agrifolia*. Measurements were made 19–20 June 1979 on two plants on Jasper Ridge, Stanford, California.

equipment for analysis. This study has demonstrated the utility of an alternative approach, the CO_2 depletion technique for measurements of photosynthesis on plants in the field. Our technique, a modification of techniques presented by Atkins & Pate (1977) and Clegg *et al.* (1978) yields photosynthetic rates similar to those obtained with standard open system gas exchange techniques. This method is useful, reliable, and economical for both physiological and ecological studies. The technique makes it possible to rapidly sample and compare rates of net photosynthesis from many leaves or from many individuals in a relatively brief period of time and also to measure rates of net photosynthesis under natural conditions on plants in remote locations.

Acknowledgments

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References

Atkins, C.A. & Pate, J.S. (1977) An IRGA technique to measure CO_2 content of small volumes of gas from the internal atmospheres of plant organs. *Photosynthetica*, **11**, 214–216.

Baker, D.N. & Musgrave, R.B. (1964) Photosynthesis under field conditions. V. Further plant chamber studies of the effects of light on corn (*Zea mays* L.). *Crop Science*, **4**, 127–131.

Björkman, O., Nobs, M. Berry, J., Mooney, H., Nicolson, F. & Catanzaro, B. (1973) Physiological adaptation to diverse environments: approaches and facilities to study plant responses to contrasting thermal and water regimes. *Carnegie Institution Washington Yearbook*, **72**, 393–403.

Clegg, M.D., Sullivan, C.Y. & Eastin, J.D. (1978) A sensitive technique for the rapid measurement of carbon dioxide concentrations. *Plant Physiology*, **62**, 924–926.

Ehleringer, J. & Björkman, O. (1978) A comparison of photosynthetic characteristics of *Encelia* species possessing glabrous and pubescent leaves. *Plant Physiology*, **62**, 185–190.

Hesketh, J.D. & Musgrave, R.B. (1962) Photosynthesis under field conditions. IV. Light studies with individual corn leaves. *Crop Science*, **2**, 311–315.

Marshall, B. & Biscoe, P.V. (1977) A mobile apparatus for measuring leaf photosynthesis in the field. *Journal of Experimental Botany*, **28**, 1008–1017.

Mooney, H.A. (1972) Carbon dioxide exchange of plants in natural environments. *Botanical Review*, **38**, 455–469.

Mooney, H.A., Dunn, E.L., Harrison, A.T., Morrow, P.W., Bartholomew, B. & Hays, R.L. (1971) A mobile laboratory for gas exchange measurements. *Photosynthetica*, **5**, 128–132.

Schulze, E.-D. (1972) A new type of climatized gas exchange chamber for net photosynthesis and transpiration measurements in the field. *Oecologia*, **10**, 243–251.