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Short Communication

A Simple Technique for the Rapid Determination of Plant CO_2 Compensation Points

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This technique was developed to screen large numbers of plants for individual ones with low CO_2 compensation points. It was thought that low compensation points might be correlated with high photosynthetic efficiency, and that plants possessing them might provide useful material for a breeding program. The technique was designed originally for use on excised leaf segments from tobacco, but has since proved adaptable for use with detached leaves and leaf segments from a variety of plants. It calls for the use of one infra-red CO_2 analyzer and will produce results at rates of up to one a minute.

Table I. *Compensation Point Values in CO_2 by the Mylar Bag Technique*

Illumination was at 1000 ft-c and the temperature was 25 C.

Initial Atmosphere	Replicate number	*Nicotiana tabacum*	*Zea mays*	*Pelargonium*	*Typha latifolia*	*Lycopersicon esculentum*
Air containing 300 μl/liter CO_2	1	50	<2	62	45	33
	2	51	<2	60	46	34
	3	51	<2	66	47	32
	4	49	<2	62	49	32
	5	50	<2	64	47	34
CO_2-free air	1	49	<2	60	47	34
	2	51	<2	61	47	34
	3	50	<2	62	49	32
	4	49	<2	64	49	34
	5	50	<2	61	47	33
Mean		50.0	<2	62.2	47.3	33.2
Values determined by other workers at 25 C		48	2.7	55, 80	...	...
Reference		5	4	1, 2	...	...

The principle underlying the method is extremely simple. The plant material is allowed to photosynthesize in a CO_2-impermeable Mylar bag. When it reaches its compensation point, the gas in the bag is squeezed out into an infra-red analyzer, and the CO_2 concentration is measured.

MATERIALS AND METHODS

Mylar is the registered trademark for a polyester film made by E. I. du Pont de Nemours & Co., Inc. Mylar bags used for this work were obtained from Vac Pac, Inc., Box 6339, Baltimore, Maryland 21203. The CO_2 analyzer was a Beckman 15A, and the growth chamber was an E.G.C. type M.3. fitted with a polyethylene curtain across the entrance to minimize temperature changes when opened.

Two replicate portions of leaf material, each of approximately 60 cm^2 (the exact size is not critical), were used for each determination. In tobacco they took the form of 8- × 8-cm squares of leaf tissue cut from either side of the midrib of the same leaf, but whole detached leaves of other plants were used with equal effect. The leaf material for each replicate was floated, undersurface uppermost, on 15 ml of distilled water at the desired temperature in an open 10-cm square plastic Petri dish. Each dish was slid horizontally to the far end of a 16- × 45-cm Mylar bag. One of the replicate bags was pumped up with atmospheric air and closed halfway along its length by twisting tightly and sealing the twist with a burette pinchcock. The remaining bag was flushed by filling it three times with CO_2-free air and expelling the contents. It was then filled with CO_2-free air, twisted halfway down, and sealed with a pinchcock. Both bags were laid horizontally in a growth chamber under defined conditions of temperature and illumination for a period of 1 hr. The gas atmosphere in the bag

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was then sampled while still under illumination and was analyzed for CO_2 concentration.

The technique for sampling the gas atmosphere in the bags was as follows. A sampling probe was made from a 5-ml graduated pipette, the oral end of which was connected via a small magnesium perchlorate drying column to an infra-red CO_2 analyzer. The volume of the whole system, including the analyzer, was less than 200 ml. The tip of the pipette was inserted into the mouth of the bag down as far as the pinchcock. The neck of the bag was then twisted tightly around the body of the pipette *in the opposite direction to the twist originally employed to seal the bag*. The neck of the bag was gripped firmly, the pinchcock removed, and the body of the bag squeezed. The complementary nature of the two twists allowed the bag to open in the position vacated by the pinchcock and the gas atmosphere within was expelled by manual pressure into the CO_2 analyzer.

RESULTS

Table 1 shows the compensation point values determined by this method for a number of species together with some values observed by workers using conventional techniques. Agreement between replicates is good and there is also good agreement with the findings of other workers. It is perhaps worth noting that the high compensation point of *Typha latifolia* is surprising in view of the claim by McNaughton and Fullem (3) that it has only a low rate of photorespiration.

DISCUSSION

This technique has a built in check that sufficient time has been allowed for incubation since each of the two replicates start from either side of the compensation point, and can give identical results only if the compensation point has been reached. The method has the advantage over the conventional closed circuit gas flow system in that the CO_2 analyzer is not tied to one sample throughout the experiment, and so permits a larger number of determinations to be made with the instrument. Also, subatmospheric pressures cannot be developed within Mylar bags as they are at points within closed circuit flow systems. This, coupled with the very low permeability of Mylar film to CO_2, makes the method inherently free of errors due to leaks. To check on this, Mylar bags were filled with CO_2-free air but no plant material and incubated as for compensation point determination. After 1 hr, the CO_2 concentration within the bags due to leakage from the atmosphere was in the region of 2 to 3 μl/liter. The error in the measured compensation point due to leakage will be less than this because a proportion of the CO_2 leaking in will be fixed by the leaf. Polyethylene bags are not recommended as a substitute for Mylar bags because of the very high permeability of polyethylene to CO_2. Samples of polyethylene bags tested for leakage, as above, proved to be 20 to 30 times more permeable than their Mylar equivalents.

Temperature control within the Mylar bags is good. When under illumination at 1000 ft-c in the growth chamber at 24 C, the temperature of the water in which the leaf was floating was less than one degree above the temperature of the chamber after 1 hr of incubation.

With this method, it is the rate at which bags can be filled and subsequently analyzed which limits the throughput of the system. For large scale use, it is suggested that the incubation period be carried out in a growth room at the periphery of a large turntable rotating at one revolution per hour. One person could fill the bags and load them onto the turntable at a fixed point, and a second person could analyze them when they passed him after 1 hr of incubation. By this means it should be possible to obtain results at a steady rate of one a minute.

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