A Simple System for the Rapid Determination of the Anaerobic Compensation Point of Plant Tissue

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Abstract. A method is described for the rapid determination of the anaerobic compensation point (ACP) of plant tissue, i.e., the O_2 concentration at which CO_2 production is minimum. The rate of CO_2 production is measured from tissue exposed to an exponentially declining O_2 concentration produced by a flow of N_2 into a dilution bottle initially containing air. Too rapid a rate of O_2 decline produces abnormal data because of the time required for the tissue to respond to changes in O_2 concentration. The ACP is easily determined from a plot of CO_2 production vs. O_2 concentration. Rates of CO_2 production and ACPs calculated using the exponentially declining system are similar to those calculated from traditional methods of continuously holding tissue under various O_2 concentrations.

Interest in the use of thin plastic films to produce modified atmospheres in packages (Lakin, 1987; Zagory and Kader, 1988) and in the use of low- O_2 atmospheres as quarantine treatments for the control of insects is high (Gaunce et al., 1982; Klag, 1985; Lids-ter et al., 1981; Michell et al., 1984; Soderstrom and Brandl, 1985). Successful application of these developing technologies requires detailed knowledge of the relation between the rate of respiration by the plant tissue and the gaseous atmosphere surrounding it. Of special interest is the anaerobic compensation point (ACP), which is defined as the O_2 concentration at which CO_2 production is minimum (Boersig et al., 1988). The ACP could indicate the lower limit for the O_2 content of storage atmospheres for numerous commodities grown and stored under a wide range of conditions.

The postharvest literature contains various studies in which the ACP has been determined for some commodities (Blackman, 1928; Boersig et al., 1988; Thomas and Fidler, 1933). The traditional method of determining the ACP involves measuring the rate of CO_2 production at several O_2 concentrations. However, this method is time-consuming and often only approximates the true ACP, since it must be calculated from respiration measurements taken at discrete O_2 concentrations. In this paper, we describe a simple, rapid method for determining the ACP in which the O_2 concentrations of the atmosphere surrounding the tissue is reduced from 20.9% to near 0%. This method eliminates the need for syringe-sampling of CO_2 concentrations and for preparing numerous gas mixtures, since continuous readings of

CO_2 production are taken during the exponential decline in O_2 concentration.

The O_2 concentration of the atmosphere flowing past tissue can be reduced exponentially by displacing the air in a large container with a known flow rate of N_2 (Fig. 1). If the dilution container with a volume V is initially filled with air and N_2 enters the con-

tainer at a flow of F , then the O_2 concentration C in the outlet gas stream at any time t is given by the following generalized equation for a one-compartment dilution process [Simon, 1972]: $C = C_0 e^{-(F/V)t} = C_0 e^{-kt}$, where C = concentration at time t ; C_0 = original concentration (i.e., 20.9% O_2); e = base of the natural log (2.71828...); F = flow rate in $ml \cdot min^{-1}$; V = volume (ml); t = time (min); and k = constant (F/V).

This equation assumes that there is complete mixing between the incoming gas and the air in the container. Under this assumption, after one container volume of N_2 has flowed into the container, only 36.8% of the original volume of air will still be in the container. The concentration of O_2 remaining in the container decreases with the volume of N_2 that has flowed through the container, as shown in Table 1. The equation can be used to show that the time (in minutes) required for the removal of 95% of the original concentration of O_2 is three times the volume of the container (in milliliters) divided by the flow rate (in $ml \cdot min^{-1}$).

After five container volumes of N_2 have flowed through the dilution container, the O_2 concentration in the outlet stream would have decreased from 20.9% to 0.14%. Since most plant tissues exhibit the characteristic increase in CO_2 production coincident with the shift from aerobic to anaerobic respiration between 0.5% and 2% O_2 , the system was initially designed to reduce the O_2 concen-

Fig. 1. Schematic of the dilution and analyzing system used to measure rates of CO_2 production from carrot root disks exposed to exponentially declining O_2 concentrations. The solid double lines represent tubes for gas flow, the arrows represent the direction of gas flow, and the dashed lines represent connecting wires used for data transfer. B1 and B2 are barostats to regulate gas pressures of 120 and 60 cm of water to capillaries R1 and R2, respectively.

Table 1. Effect of the flow of N_2 into a dilution bottle of air on the change in O_2 concentration in the outlet gas stream. The flow of N_2 is given in multiples of the volume of the dilution container.

N_2 flow (volumes)	Air removed (%)	Air remaining (%)	O_2 remaining (%)
0	0.0	100.0	20.0
1	63.2	36.8	7.4
2	86.5	13.5	2.7
3	95.0	5.0	1.0
4	98.2	1.8	0.36
5	99.3	0.7	0.14

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Fig. 2. Concentrations of O_2 and rates of CO_2 production by carrot root disks at 20°C during a typical experiment in which the O_2 concentration was reduced from 20.9% to 1% in 730 min.

Fig. 3. Rates of CO_2 production by carrot root disks exposed to distinct O_2 concentrations or to exponentially declining O_2 concentrations. The smooth line was constructed from data from Fig. 2 replotted as CO_2 production vs. O_2 concentration. The symbols represent CO_2 production from two experiments in which rates of CO_2 production were measured in the traditional method during exposure of carrot root disks to the indicated O_2 concentrations for 24 hr.

Fig. 4. Effect of three rates of decline in O_2 concentration on rates of CO_2 production by carrot root disks at 20°C. The time required to reduce the O_2 concentration from 20.9% (air) to 1% O_2 was 260 min (dotted line), 405 min (solid line), or 655 min (dashed line).

tration from 20.9% (air) to 1% in 7 hr. Carbon, dioxide-free air in a 20-liter jar was diluted with pure N_2 at a known flow rate to create the exponentially declining O_2 atmosphere. Minor adjustments to the N_2 flow rate produced 1% O_2 mixtures in 4 to 11 hr. A magnetic stirring bar was used to ensure complete mixing.

A series of experiments were conducted to compare the traditional method of determining the ACP with the exponential dilu-

tion method described above.

Mature carrots were obtained from a local wholesaler and held at 0°C until used. Disks, 5 to 7 mm thick, were cut from the middle sections of the carrots, washed in 200 ppm chlorine solution, rinsed in distilled water, and blotted dry. The disks were randomly sorted into 80-g samples that were placed either in 500-ml glass jars or in glass tubes of a slightly larger diameter than the disks. A flow of $\approx 50 \text{ ml} \cdot \text{min}^{-1}$ of humidified, eth-

ylene-free air was passed through each container for 36 hr to allow dissipation of the wounding response. The glass tubes were used for the exponentially declining O_2 treatments to minimize the void volume and the delay in the change of the O_2 concentration around the tissue. All experiments were run at 20°C.

In one set of treatments, carrot disks were placed in jars ventilated with gas mixtures containing 0%, 0.5%, 1%, 2%, 3%, 4%, or 21% O_2 (balance N_2) for 1 day. Carrots in the other set of treatments were held in an atmosphere in which the O_2 concentration was reduced exponentially to 1.0% in ≈ 260 , 400, or 655 min.

An automated gas sampling system was used to measure the concentration of O_2 and CO_2 in the gas flows (Fig. 1). The apparatus worked as follows. Barostat B1 controlled the pressure of N_2 across a capillary tube, R1, which controlled the rate of flow of N_2 into the dilution bottle. A second barostat, B2, controlled the pressure of the diluted gas mixture across another capillary tube, R2, which controlled the rate of flow to the tissue. The barostats were long tubes partially filled with water. The pressures were 120 and 60 cm of water from barostat B1 and B2, respectively. The gas mixture first passed through the reference cell of the PIR-2000 infrared CO_2 gas analyzer (IRGA) (Horiba Instruments, Irvine, Calif.) before flowing through the glass tube containing the carrot disks. The flow of gas from the chamber holding the carrot disks then passed through the IRGA sample cell before passing through a Model 755 paramagnetic O_2 analyzer (Beckman Instruments, Fullerton, Calif.). The CO_2 and O_2 concentrations were measurable down to $0.001\% \pm 0.0001\%$ and $0.01\% \pm 0.002\%$, respectively. Switching the gas flow between mixtures with dissimilar O_2 and CO_2 concentrations showed that the time required for the O_2 and CO_2 analyzers to respond to the changes and reestablish a stable output was 45 and 5 sec, respectively. Data from each instrument were collected every 5 min with a Hewlett-Packard model 3421A data acquisition unit controlled by a HP71B computer. Data were stored in the HP71B and later downloaded to a PC for processing. Tissue fresh weight, gas flow rates, and measured concentrations of O_2 and CO_2 were used to calculate respiration rates.

When the traditional method to measure the ACP was used, 1-ml gas samples were analyzed for O_2 and CO_2 using an apparatus described by Saltveit and Strike (1989).

The O_2 dilution apparatus produced exponentially declining O_2 concentrations (Fig. 2), as predicted by the equation. The rate of CO_2 production showed a slight increase before declining with the decreasing O_2 concentration. Production of CO_2 started a slow increase when the O_2 concentration declined below 2%, ≈ 10 hr after the start of the experiment.

These changes are more easily seen in a plot of CO_2 production vs. O_2 concentration (Fig. 3). As the O_2 concentration declined exponentially from 21% to 11%, a slight increase was observed in the rate of CO_2 pro-

duction. Below 11%, the respiration rate declined steadily, reaching the ACP at $\approx 1.25\%$ O_2 . The rates of CO_2 production calculated using the traditional method of holding carrot disks continuously under different O_2 concentrations were coincident with the rates calculated using the exponentially declining O_2 system (Fig. 3). However, the ACP determined from the traditional method would have been 1.0% O_2 .

The rate of reduction of O_2 concentration had a significant effect on the ACP (Fig. 4). A rate of dilution that produced 1% O_2 in 260 min did not appear to allow the tissue to adequately respond to the declining O_2 concentrations. The rate of CO_2 production was generally higher than from experiments in which the decline in O_2 took longer, and no increase in CO_2 production was observed at the lower O_2 concentrations. In contrast, rates of dilution that produced 1% O_2 in ≈ 400 to 655 min produced changes in CO_2 production that were coincident.

Previous work has indicated that the ACP shifts to lower O_2 concentrations upon exposure to lower O_2 concentrations (Boersig et al., 1988). While the period of exposure to low O_2 was different between disks held continuously under low O_2 and those held in the exponentially declining O_2 atmosphere, we observed no difference in the ACP (Fig. 3). However, if the reduction in the concentration of O_2 occurred too rapidly (i.e., a reduction from air to 1% O_2 in <300 min), the response of the tissue was affected and

no ACP was observed (Fig. 4). Our studies of the respiratory response of carrot disks to rapid changes in O_2 concentrations indicated that two distinct responses to changes in O_2 concentrations occurred; one at ≈ 10 min and the other at 50 min (unpublished data). The fast response at 10 min occurred only at O_2 concentrations near the ACP, while the slower response at 50 min occurred at O_2 concentrations above the ACP. These results partially explain the CO_2 production data observed during very rapid reductions in O_2 concentration.

The exponentially declining O_2 system can be used to determine the ACP with many fewer samples and without the need for numerous gas mixtures.

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