短　　報　**Use of the Gas-Phase Oxygen Electrode in the Measurements of Photosynthesis of Tree Leaves***

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I . Introduction

Nutritive and physiological conditions of planted trees must be monitored and maintained to be healthy for successful reforestation. They are sometimes monitored by the photosynthetic properties of leaves which are calculated from photosynthetic rates under various light intensities.

DELIEU and WALKER (1981) have designed a gas-phase oxygen electrode (GOE) to measure photosynthesis in a leaf, and have obtained satisfactory results. In the measurement by GOE, rates of O_2 evolution from a leaf disc of less than 10 cm^2 can be determined with a great accuracy (BJÖRKMAN and DEMMIG, 1987). Besides, the experimental system has the advantage in being assembled at small cost and operated easily. There is a 1 to 1 (mol ratio) relationship between the CO_2 fixed and the O_2 evolved, at least in the photosynthesis described by the general overall equation. Accordingly, it may well be that the GOE measurement will become one of the most desirable methods to make a check on the nutritive condition and physiological activity of trees or seedlings.

Photosynthetic rates of leaves usually are measured by an infrared gas analyzer (IRGA). Therefore, for establishing the GOE measurment as a reliable method for determining the photosynthetic rates of leaves, comparisons of the GOE measurements with the IRGA measurements would be indispensable. In this paper, we compared photosynthetic rates of *Fagus crenata* BL. and *Quercus serrata* THUNB. leaves measured by GOE with those measured by IRGA at various light intensities, and discussed practical merits and problems of GOE.

II. Materials and Methods

Experimental materials were collected in August to September of 1991 from two trees with heights of 5 to 6 m. One a *F. crenata* and the other a *Q. serrata*, were grown at an experimental nursery on the Ueda campus of Iwate University. Three branches with length of 80 to 100 cm were cut from both the upper part (sampling for sun leaves) and the lower part (sampling for shade leaves) of the crown of the *F. crenata*. For the *Q. serrata*, three branches of the same size were cut from the upper part of the crown (sampling for sun leaves).

Oxygen evolution or consumption by a leaf disc of 10 cm^2 size was measured by a GOE unit (LD2/2 ; Hansatech Co., King's Lynn, England) according to the manufacturer's instructions, where air enriched with CO_2 to 5% was used. The leaf disc was preilluminated about 300 μmol quanta m^{-2} s^{-1} for ten to twenty minutes until the rate of O_2 evolution became constant. Thereafter, the O_2 evolution from the leaf disc was measured under various light intensities ; the O_2 consumption by dark respiration was measured last. Light was supplied by a halogen lamp (EFR A1/232 ; Philips, Germany). Light intensity on the leaf disc was changed by inserting black Saran shading cloth screens between the apparatus and the light source. The light intensity was measured by a photon flux sensor (IKS-25 ; Koito, Tokyo). The leaf disc temperature was kept at 25°C during the measurements.

In the IRGA(VIA-300 ; Horiba, Kyoto)measurement a leaf attached to a branch was put in an assimilation chamber equipped with a temperature control-unit (MC-A3-W ; Koito, Tokyo). Light was supplied by an electric discharge lamp (HGI-TS ; Mitsubishi, Tokyo). The CO_2 concentration in the chamber was kept

within a range of 340 to 360 $\mu l\, l^{-1}$ during the measurements. The photosynthetic rates of leaves also were measured in various CO_2 concentrations under the light of about 600 μmol quanta $m^{-2}\,s^{-1}$. CO_2 concentrations of air passed through the chamber were controlled by mixing air with a small CO_2 concentration with 5% CO_2 (SHIRAHATA and HASHIMOTO, 1992).

III. Results

The photosynthetic rates at various light intensities for the sun leaves of *F. crenata* are shown in Figs. 1 and 2. There was no large difference between the GOE and the IRGA measurements in the initial slope of the photosynthetic response to light intensity curve (a); the means were 0.097 $(\mu mol\ O_2)\,(\mu mol\ quanta)^{-1}$ in the former and 0.095 $(\mu mol\ CO_2)\,(\mu mol\ quanta)^{-1}$ in the latter (Table 1). The GOE measurements gave significantly large rates of dark respiration. Rates of net photosynthesis at high levels of light intensities (p_h) were about 50% larger in the GOE measurements than in the IRGA measurements; 16 to 21 $(\mu mol\ O_2\ m^{-2}\,s^{-1})$ and 11 to 13 $(\mu mol\ CO_2\ m^{-2}\,s^{-1})$, respectively.

The GOE and IRGA measurements provided almost the same initial slopes of photosynthetic response curves to light intensities (a) for the shade leaves of *F. crenata*, as were seen for the sun leaves; the means were 0.082 $(\mu mol\ O_2)\,(\mu mol\ quanta)^{-1}$ in the former and 0.083 $(\mu mol\ CO_2)\,(\mu mol\ quanta)^{-1}$ in the latter (Table 1, Fig. 3). Rates of net photosynthesis at high levels of light intensities (p_h) were 2.5 to 5.0 $(\mu mol\ O_2\ m^{-2}\,s^{-1})$ in the GOE measurements and 3.9 to 4.2 $(\mu mol\ CO_2\ m^{-2}\,s^{-1})$ in the IRGA measurements (Fig. 4). The means of p_h were not so different between the two kinds of measurements, but rates of dark respiration were much greater in the GOE measurements.

The photosynthetic response curves to CO_2 concentrations for the sun and shade leaves of *F. crenata* are shown in Fig. 5. The photosynthesis rapidly increased at first with increasing CO_2 concentrations, then they reached a plateau at about 1,000 $\mu l\, l^{-1}$ for the sun leaves and at about 500 $\mu l\, l^{-1}$ for the shade leaves. The maximal rates of net photosynthesis were in the range of 19 to 24 $(\mu mol\ CO_2\ m^{-2}\,s^{-1})$ for the sun leaves and 5 to 8 $(\mu mol\ CO_2\ m^{-2}\,s^{-1})$ for the shade leaves. Accordingly, the maximal photosynthetic levels in the above CO_2 response curves seemed to agree with the rates of light-saturated net photosynthesis obtained from the GOE measurements for the sun leaves (Figs. 2 and 5), but such agreement was not shown for the shade leaves (Figs. 4 and 5).

The photosynthetic response curves to light intensities and CO_2 concentrations for the *Q. serrata* leaves (sun leaves) are shown in Fig. 6. Rates of net photosynthesis measured by GOE were 22 to 24 $(\mu mol\ O_2\ m^{-2}$
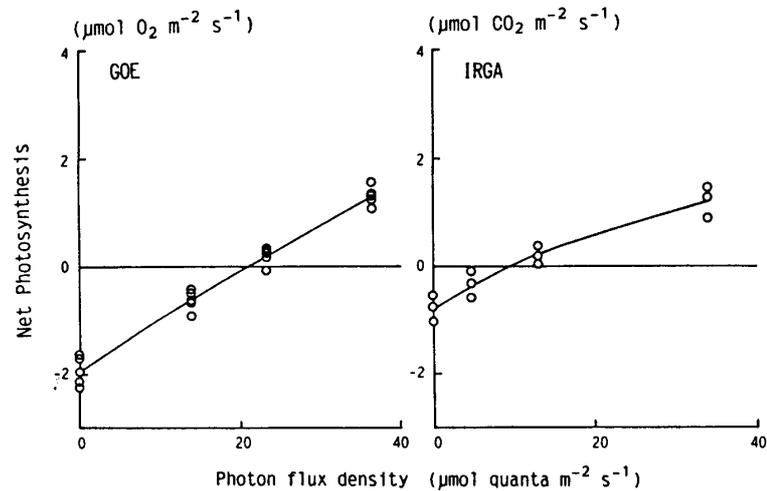

Fig. 1. Photosynthetic response curves to photon flux density (at low levels of light intensities) for sun leaves of *F. crenata*

GOE means the measurement by a gas oxygen electrode, and IRGA means the measurement by an infrared gas analyzer.
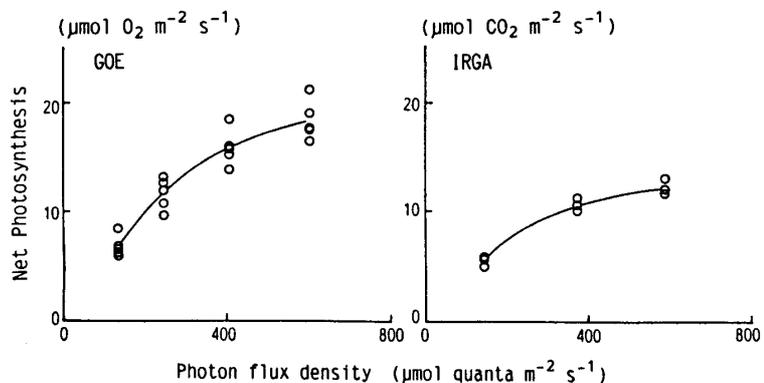

Fig. 2. Photosynthetic response curves to photon flux density (at high levels of light intensities) for sun leaves of *F. crenata*

Table 1. Initial slopes (a) of photosynthetic response curves to photon flux density and net photosynthesis rates (p_h) at high levels of light intensities for sun and shade leaves of *F. crenata*

	GOE			IRGA		
	Sample sizes (no.)	a (μmol CO_2) (μmol quanta)$^{-1}$	p_h (μmol CO_2 m^{-2} s^{-1})	Sample sizes (no.)	a (μmol CO_2) (μmol quanta)$^{-1}$	p_h (μmol CO_2 m^{-2} s^{-1})
Sun leaves	5	0.097(0.008)	18.4 (1.6)	3	0.095(0.005)	12.2 (0.5)
Shade leaves	4	0.082(0.006)	3.38(0.98)	3	0.083(0.013)	4.11(0.14)

Numerals are means and standard deviations.
Initial slope (a) of photosynthetic response curve to light intensity for each leaf disc is derived from its regression equation (HASHIMOTO, 1991).

s^{-1}) at high levels of light intensities. With increasing CO_2 concentrations the photosynthetic rate rapidly increased at first, then reached a plateau at about 1,000 μl l^{-1}, as was the case of the sun leaves of *F. crenata*. The CO_2-saturated rates of net photosynthesis were in the range of 22 to 26 (μmol CO_2 m^{-2} s^{-1}). Thus, estimates of the light-saturated net photosynthesis obtained from the GOE measurements agreed approximately with the maximal rates in the photosynthetic response curve to CO_2 concentrations.

IV. Discussion

The initial slope of the photosynthetic response curve to light intensity is linked directly with the quantum efficiency of photosynthesis, which is one of the key factors in understanding the photosynthetic properties of the leaves of plants living under low levels of light intensities such as in lower shaded parts of tree crowns and on forest floors (BOARDMAN, 1977 ; KOZLOWSKI et al., 1991). The initial slopes ((μmol O_2) (μmol quanta)$^{-1}$) obtained from the GOE measurements almost agreed with those ((μmol CO_2)(μmol quanta)$^{-1}$) from the IRGA measurements, not only for sun leaves but also for shade leaves (Table 1,
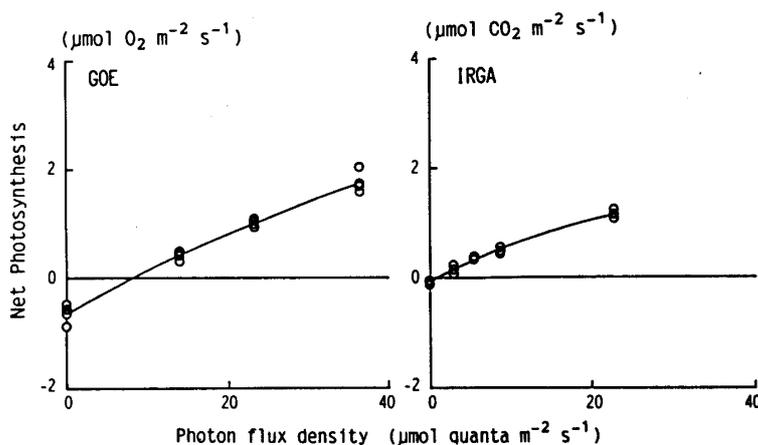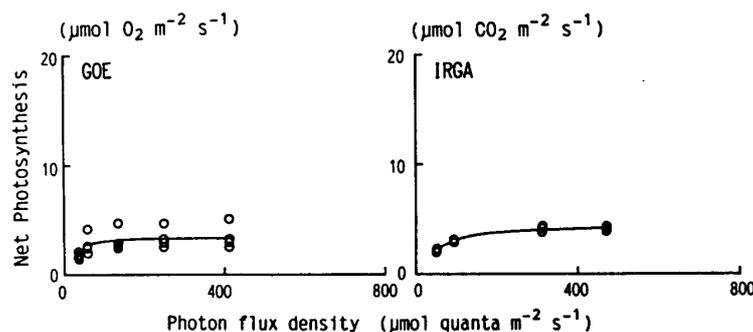

Fig. 3. Photosynthetic response curves to photon flux density (at low levels of light intensities) for shade leaves of *F. crenata*

Fig. 4. Photosynthetic response curves to photon flux density (at high levels of light intensities) for shade leaves of *F. crenata*
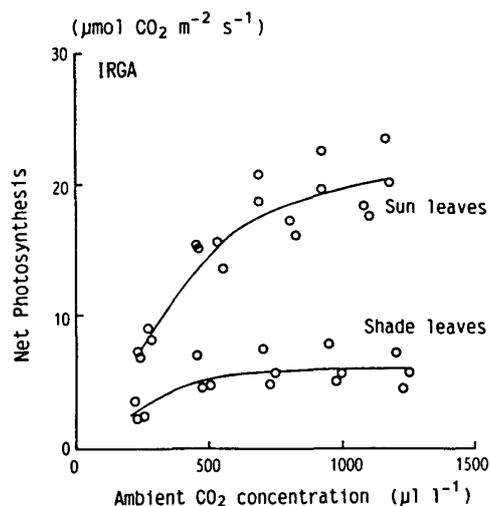
Figs. 1 and 3). These results imply that the difference of the ambient CO_2 concentrations between the two kinds of measurements does not affect greatly the quantum efficiency under low levels of light intensities like around the light-compensation points. Considering that the experimental system has the advantage in being assembled with small cost and guarantees great accuracy, the GOE measurements will be most useful in investigating the quantum efficiency of photosynthesis.

In the GOE measurements, leaf discs are cut from leaf blades, so it might be considered that the abnormal respiration occurs in cells adjacent to cut cells. In a preliminary test, however, such effect on the dark

Fig. 5. Photosynthetic response curve to CO_2 concentrations at about 600 μmol quanta $m^{-2} s^{-1}$ (from the IRGA measurements) for sun and shade leaves of *F. crenata*
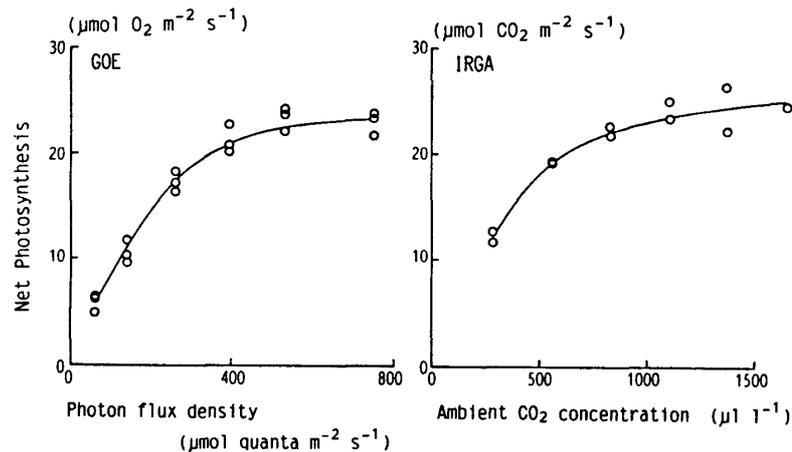

Fig. 6. Photosynthetic response curve to photon flux density (from the GOE measurement) and that to CO_2 concentration at about 600 μmol quanta $m^{-2} s^{-1}$ (from the IRGA measurement) for sun leaves of *Q. serrata*

respiration rate was not seen probably because the proportion of cut cells to intact cells was negligible. The significantly large rates of dark respiration which were observed in the GOE measurements, accordingly is due to other factors ; One possibility is, for instance, to assume that the substrate of dark respiration accumulates during the photosynthesis measurements because of the cuts of vascular tissues.

The photosynthetic rates of tree leaves linearly increase at first, as well as in the other C_3 herbaceous plants, with increasing ambient CO_2 concentrations, and then shows slow increases to reach a constantly level CO_2-saturation at about 1,000 or 2,000 μl l^{-1} (KOZLOWSKI *et al.*, 1991 ; IMAI, 1988). Under such large concentrations of CO_2, it is likely that the RuBP oxygenase reaction is suppressed, and so the photorespiration declines to an extremely low level (BERRY and BJÖRKMAN, 1980). The GOE has been considered from these stand-points, if the other experimental conditions, such as light intensity and temperature, are adequate to allow reasonable estimates of maximal rates of photosynthesis to be derived (WALKER, 1988). It follows that the measurement data by GOE provide potential capacities of photosynthesis of leaves whereas those by IRGA (determined under usual concentrations of CO_2) are actual capacities.

As is shown in Figs. 2 and 5, and in Fig. 6, estimates of the light-saturated net photosynthesis (μmol O_2 $m^{-2} s^{-1}$) obtained from the GOE measurements approximately agree with the CO_2-saturated photosynthetic rates (μmol CO_2 $m^{-2} s^{-1}$) measured by IRGA for the sun leaves of *F. crenata* and *Q. serrata*. However, the light-saturated photosynthetic rates measured by GOE are small compared with the CO_2-saturated photosynthetic rates measured by IRGA for the shade leaves of *F. crenata* (Figs. 4 and 5). Accordingly, the measurement data by GOE always does not show the maximal estimates of net photosynthetic rates. The present results imply that the photosynthetic response of leaves to large concentrations of CO_2 would differ due to the environmental conditions under which plants or leaves grow ; large intercellular concentrations of CO_2 would adversely affect some photosynthetic processes for shade leaves.

In this paper the GOE measurements were made in 5% CO_2 for all of the leaf discs, according to WALKER (1988). This level of CO_2 concentration would have been too much for the shade leaves of *F. crenata* to exert maximal rates of photosynthesis. Taking the responses of each material to various CO_2 concentrations into account, the GOE measurements will become more reliable ways to examine the nutritive conditions and the physiological activities of trees.

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