UPTAKE AND EVOLUTION OF CARBON DIOXIDE DURING PHOTOSYNTHESIS: A SURVEY OF PLANT DIVISIONS

by

JUDITH MARGARET HORGAN

B. Sc. Melbourne University, 1963

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

MARCH 1968

EXAMINING COMMITTEE APPROVAL

C. D. NELSON
Senior supervisor

F. J. F. FISHER
Supervising committee

W. E. VIDAVER
Supervising committee

L. D. DRUEHL
Chairman of examining committee

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ABSTRACT

The exchange of CO_2 in light was studied in plants of six divisions. The rates of CO_2 uptake in light, of CO_2 evolution in darkness, and the concentration of CO_2 at CO_2 compensation point were measured in a closed system with an infrared gas analyser. The CO_2 evolution rate in light was then calculated from these measurements. Rates of CO_2 exchange and CO_2-compensation points were similarly measured at several concentrations of oxygen between 2% and 100%.

The CO_2 concentration at CO_2 compensation point was a linear function of O_2 concentration in eleven of the twelve species studied. The CO_2 compensation point of corn was not affected by O_2. At 60% O_2, the inhibition of rates of CO_2 uptake was of the same magnitude in all species studied.

The minimum rate of CO_2 evolution in light was found to be at least twice the rate of CO_2 evolution in the dark in ten of eleven species.

ACKNOWLEDGEMENT

I wish especially to thank Dr. C. D. Nelson for his help and encouragement during this project, and for suggesting the topic. I am grateful to Dr. F. J. F. Fisher for his stimulating discussions, and to Dr. W. E. Vidaver, Dr. R. C. Brooke, and Dr. G. R. Lister for their help in the preparation of this thesis. I want to express my appreciation to Bob Thompson, Mary Jo Duncan, Ross Bulley, Stan Maleszewski and other members of the Biological Sciences Department for discussions of ideas and technical problems. My thanks are due to Miss Shirley Vander Molen and the other office staff for the typing of this thesis.

TABLE OF CONTENTS

	Page
Examining Committee Approval	i
Abstract	ii
Acknowledgement	iii
Table of Contents	iv
List of Tables	v
List of Figures	vi
Introduction	1
Materials and Methods	
1. Measurement of CO_2 exchange in a closed system	10
2. Apparatus for CO_2 measurement	10
3. Illumination and temperature	18
4. Accuracy and variation of CO_2 measurements	20
5. Plant sources and growth conditions	23
6. Experimental procedure	25
Results	
1. The effect of oxygen concentration on carbon dioxide compensation points	27
2. Comparison of rates of CO_2 exchange	27
3. The effect of oxygen concentration on CO_2 exchange	38
Discussion	40
Summary and Conclusions	53
Bibliography	54

LIST OF TABLES

		Page
Table 1.	Plants used in experiments	24
Table 2.	CO_2 concentrations at CO_2 compensation point at various concentrations of O_2	31
Tables 3,4.	Rates of CO_2 exchange in 21% O_2	33,34.
Table 5.	Variations in rates of CO_2 exchange in 21% O_2	36
Table 6.	Effect of 60% O_2 on rate of CO_2 uptake	39

LIST OF FIGURES

		Page
Fig. 1.	Apparatus for CO_2 measurement	11
Fig. 2.	Flow diagram of apparatus for CO_2 measurement	12
Fig. 3.	Emission spectra of light used in experiments	19
Figs. 4-6	The effect of O_2 concentration on carbon dioxide compensation point	28-30
Fig. 7.	The relationship of measurable data to calculated CO_2 evolution in light	45
Fig. 8.	Effects of departures from linearity of APS/ $[CO_2]$ curves on various methods of estimation of CO_2 evolution in light	46

INTRODUCTION

The relationship between photosynthesis and respiration in the light has commonly been studied by measurement of CO_2 and O_2 exchange by green plants. In early work it was assumed that CO_2 is evolved at the same rates in light and dark (Rabinowitch, 1951), and the true rate of photosynthesis has been considered to be the measurable rate of CO_2 uptake plus the rate of CO_2 evolution in darkness. However recent experiments indicate that the rates of evolution of CO_2 are not the same in darkness and light. The effects of variations in oxygen, light and temperature on CO_2 evolution are radically different in light compared to darkness, suggesting two different processes. Such differences have been demonstrated by many workers using diverse methods and organisms. Isotopic CO_2 and O_2 exchange were studied by Semenenko (1964), Ozbun, Volk and Jackson (1964) and Goldsworthy (1966). Rates of uptake and exchange transients of CO_2 have been analysed by Decker (1957, 1959), Tregunna (1963) and Tregunna, Krotkov and Nelson (1966). Holmgren and Jarvis (1967) studied CO_2 exchange at extremely low CO_2 concentrations.

Poskuta, Krotkov and Nelson (1967) and Zelitch (1966) studied CO_2 exchange with several metabolic inhibitors. These papers represent only a part of the recent evidence showing that different processes of CO_2 evolution operate in light and dark.

This project was designed to investigate the CO_2 evolution in the light, in a broad range of species from six Plant Divisions by measuring CO_2 exchange at several O_2 concentrations with an Infrared Gas Analyzer.

The measurable rate of CO_2 uptake in light or apparent rate of photosynthesis (APS) is considered to represent the net difference between the rates of CO_2 uptake and simultaneous CO_2 evolution (Tregunna, Krotkov and Nelson, 1966). The rate of CO_2 evolution in light may be calculated from the measurable APS rate (when CO_2 concentration is limiting CO_2 uptake) if the CO_2 concentration at CO_2 compensation point ($[CO_2]_{CCP}$) is known.

$$\text{The rate of } CO_2 \text{ evolution in light} = \frac{\text{APS} \times [CO_2]_{CCP}}{[CO_2] - [CO_2]_{CCP}}$$

where $[CO_2]$ is the concentration of CO_2 at which the rate of APS is measured. This equation was derived by Tregunna (1963) from work with detached tobacco leaves. Zelawski (1967) cites evidence that the values calculated by this method correspond to those from measurements of

CO_2 evolution in a CO_2-free system, but details of this work are not available at present. If any of the CO_2 evolved is reassimilated, this equation will be imprecise, although it may usefully indicate minimum values.

At a given light intensity and temperature, the rate of CO_2 uptake varies directly with the ambient concentration of CO_2 (below CO_2 saturation) (Gaffron, 1960; Decker, 1957). The relationship between CO_2 concentration and the rate of CO_2 evolution in light is unknown, but independence is assumed until a suitable test is devised (Brown and Tregunna, 1967). If the rate of CO_2 evolution in light does vary with CO_2 concentration (either directly, or indirectly by dependence on photosynthesis), the above method of calculation would have to be modified.

The rate of CO_2 uptake in light is a function of light intensity, when other environmental factors are constant (Gaffron, 1960). High light intensities saturate CO_2 uptake in many plants but not in others such as corn (Lemon, 1963; Gaastra, 1963) and Pinus sylvestris (Zelawski and Kinelska, 1967). The saturating light intensity is significantly lower for CO_2 evolution than for CO_2 uptake (Poskuta, Nelson and Krotkov, 1966). Holmgren and Jarvis (1967) found that a light intensity of 5×10^4 ergs cm^{-2}

sec^{-1} (at 20.2° C) saturated CO_2 efflux into nearly CO_2-free air for Rumex acetosa. At the light compensation point, generally about 50 ft-c (Rabinowitch, 1951), net CO_2 uptake ceases, and CO_2 uptake and evolution are equal in rate (Zelawski, 1967).

The rate of CO_2 uptake in light is reversibly reduced by increased O_2 concentration over the range of 1% to 100%. This decrease of net CO_2 uptake reflects a change in the ratio of CO_2 uptake and evolution rates due to a light and O_2- stimulated increase in CO_2 evolution, and a direct inhibition of CO_2 uptake by O_2 (Tregunna, Krotkov and Nelson, 1966). Turner and Brittain (1962) have extensively reviewed the effects of O_2 on photosynthesis, especially in algae. Björkman (1966) found the inhibition of CO_2 uptake by O_2 to be independent of light over a wide intensity range. However he does not consider the extent of CO_2 evolution in the light, nor the possibility of O_2 affecting CO_2 uptake in two ways.

There is considerable disagreement about the source of CO_2 evolved in light. Some CO_2 is derived from the oxidation of glycolate recently produced by photosynthesis (Goldsworthy 1966; Zelitch 1966). However evidence is equivocal about whether some CO_2 is evolved in light by the respiratory mechanism that operates in darkness. Tregunna, Krotkov and

Nelson (1966) conclude, partly because of the extrapolation of CO_2 evolution in the light to zero at zero O_2 concentration, that dark respiration is inhibited in light, and is replaced, in some plants by a light-stimulated process (see also Brown and Tregunna, 1967). As it is not known how much evolved CO_2 is reassimilated, the calculated values of CO_2 evolved may be minimum values, and dark respiratory CO_2 production may be masked even at low O_2 concentrations. Zelawski (1967), Semenenko (1964) and Hoch, Owens and Kok (1963) present results consistent with decreasing rates of "dark" CO_2 evolution with increasing light intensities from darkness up to the light compensation intensity. Thus in experiments using higher than compensating light intensities, it could be assumed that all CO_2 is evolved by light-stimulated processes. Zelitch (1964, 1966) suggested that even at high light intensities, CO_2 evolved in light is derived both from oxidation of glycolate, and from the same respiratory mechanism which is operative in darkness. He distinguished the two processes by their different temperature sensitivities.

The methods used in the experiments in this thesis did not identify the source of the CO_2 evolved in the light, calculated values therefore refer to total evolution

of CO_2 in light irrespective of its origin.

Many photosynthetic plants exhibit a CO_2 compensation point (CCP) (Gabrielson 1948, 1949). This is the concentration of ambient CO_2 at which the plant shows no net exchange of CO_2 and represents the equilibrium between uptake and evolution of CO_2 (Lister, Krotkov and Nelson, 1961). Plants with a CCP of about 50 ppm CO_2 or more (in 21% O_2) have been termed high CPP plants (Downton and Tregunna, 1968) as distinguished from low CCP plants, which can reduce the CO_2 concentration in a closed system to 5 ppm or less. Low CPP plants include tropical grasses such as corn and sugarcane (Moss, 1962), some, but not all, species of the families Amaranthaceae, Chenopodiaceae and Portulacaceae (Tregunna and Downton, 1967) and the algae Chlorella, Scenedesmus, Gonium, Enteromorpha and Fucus (Brown and Tregunna, 1967). Downton and Tregunna (1968) showed that plants found by Hatch, Slack and Johnson (1967) to possess the C4 photosynthetic carboxylation pathway (Hatch and Slack, 1966), were invariably low CCP plants. None of the high CCP plants tested showed appreciable fixation of CO_2 into C4 compounds (Downton and Tregunna, 1968). Grasses of the low compensation chloridoid-eragrostoid or panicoid lines of evolution (Downton and Tregunna, 1968) were found by Downes and Hesketh (1968) to have the same rates of CO_2

uptake at both 21% and low concentration of oxygen. These authors showed that in temperate, high CCP grasses found by Hatch et al. (1957) to have the Calvin and not the C4 pathway, photosynthetic rates were considerably enhanced at low concentrations of O_2 as compared to rates at 21% O_2.

Tregunna et al. (1966) found that in tobacco, a high CCP plant, the concentration at CO_2 at CCP was a linear function of O_2 concentration. In soybean, this relationship is directly proportional from 1% to 100% O_2 and approaches zero at low O_2 concentrations (Forrester, Krotkov and Nelson, 1966). The effects of O_2 on CCP's have been attributed by these authors to the stimulation of CO_2 evolution in light , since they found in soybean that CO_2 evolution in darkness was not increased by O_2 concentrations above 2%. Ducet and Rosenberg (1962) list many species and types of tissue for which CO_2 evolution in darkness is saturated by low concentrations of O_2.

Forrester et al. (1966) concluded that in corn, a low CCP plant, dark respiration is inhibited by light without being replaced by the CO_2-evolving process which operates in high CCP plants. El-Sharkawy, Loomis and Williams (1967) considered that both high and low CCP plants produce CO_2 in light, whether by dark respiration or photo-respiration. They suggested that low CCP plants reassimilate

all the CO_2 produced, while high CCP plants are less efficient at refixation of respiratory CO_2, and hence evolve some of this to the atmosphere. Such a difference may be due to morphology (Lake, 1967; Downes and Hesketh, 1968) rather than to a biochemical effect.

The experiments presented in this thesis deal with the relationship between carbon dioxide compensation points and O_2 concentration for species of several major plant groups. Some data are included of the minimum rates of CO_2 evolution in light as calculated using the method of Tregunna, Krotkov and Nelson (1966). However a reliable quantitative study by this method is outside the scope of a survey of plants, since such a study would require individual consideration of the light saturation curve of APS for each species, and replicate CO_2-exchange determinations of several samples at various O_2 concentrations. Considerable variation is found in CO_2-exchange rates. This might be expected, due to the known sensitivity of the systems involved to such factors as physiological age, time of season and light pretreatment. By contrast, the determination of CO_2 compensation points may yield reproducible values. Moss (1962), for example, commented on the striking constancy of the CCP's of varieties of

corn and tomato. He found variation of less than 5 ppm CO_2 between measurements taken over a period of six months at various light intensities above 2000 ft-c.

MATERIALS AND METHODS

1. Measurement of CO_2 exchange in a closed system

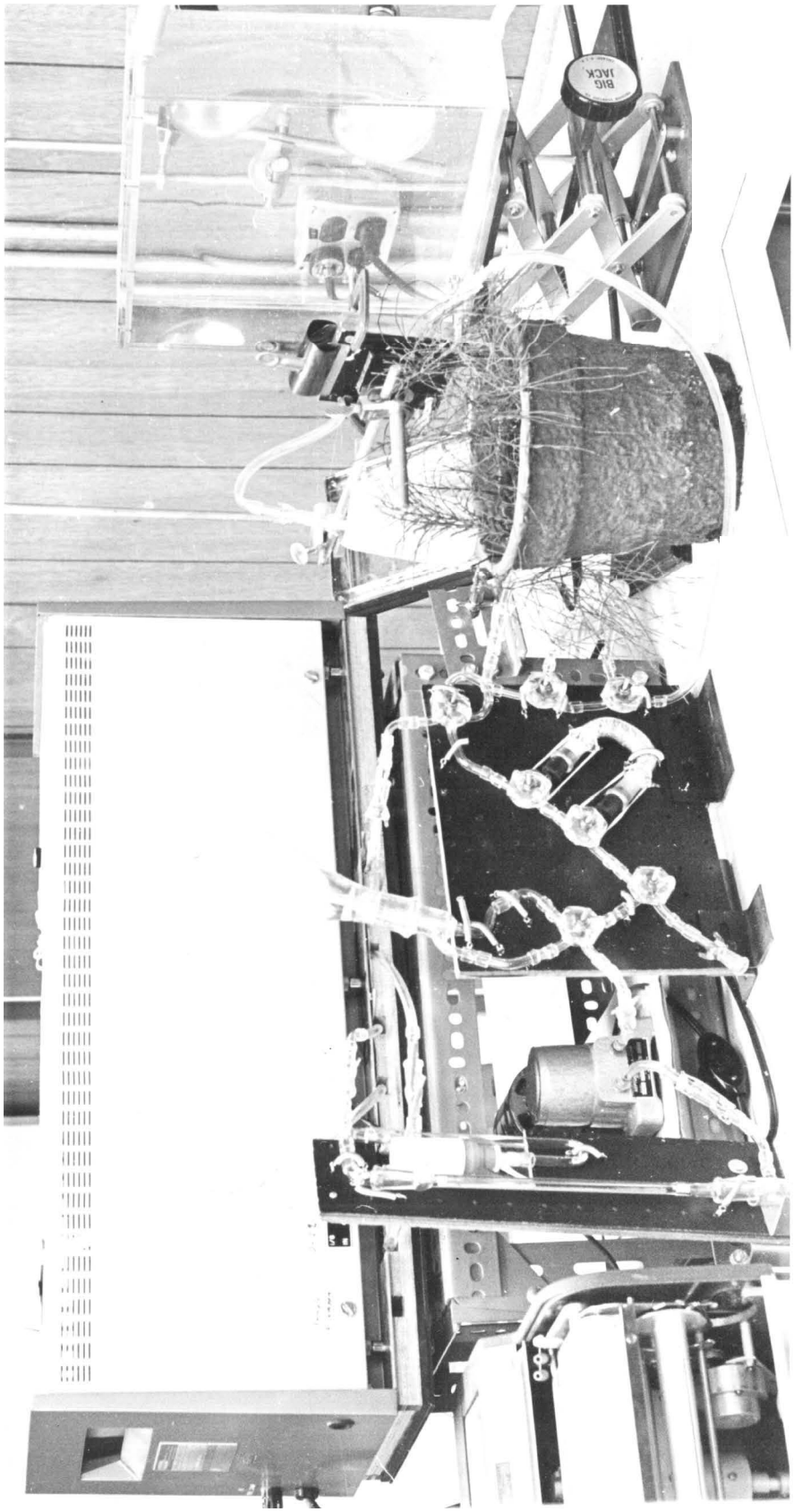
Rates of carbon dioxide by an attached leaf were calculated from changes of CO_2 concentration measured with a Beckman model 215 Infrared Gas Analyser (IRGA) in a closed system. The apparatus, described in detail below, was adapted after that described by Lister, Krotkov and Nelson (1961). Since the concentration of CO_2 was measured in a closed system of known volume, and at constant temperature and pressure, the amount of CO_2 exchanged by the leaf per unit time could be calculated.

2. Apparatus for CO_2 measurement

A continuous record of the IRGA output was obtained by means of a trace on a rectilinear grid by a Texas Instruments "Rectiriter" recorder.

Between the leaf chamber and the IRGA, gas passed through a circuit of glass and "Tygon" tubing, as shown in Figs. 1 and 2. Four-way stopcocks, which permitted the selection of either of two gas flow paths, diverted the flow from the basic CO_2-measurement circuit to accessory circuits for removal of CO_2, or the addition and mixing of gases.

Fig. 1. -- Apparatus for measurement of CO_2 exchange.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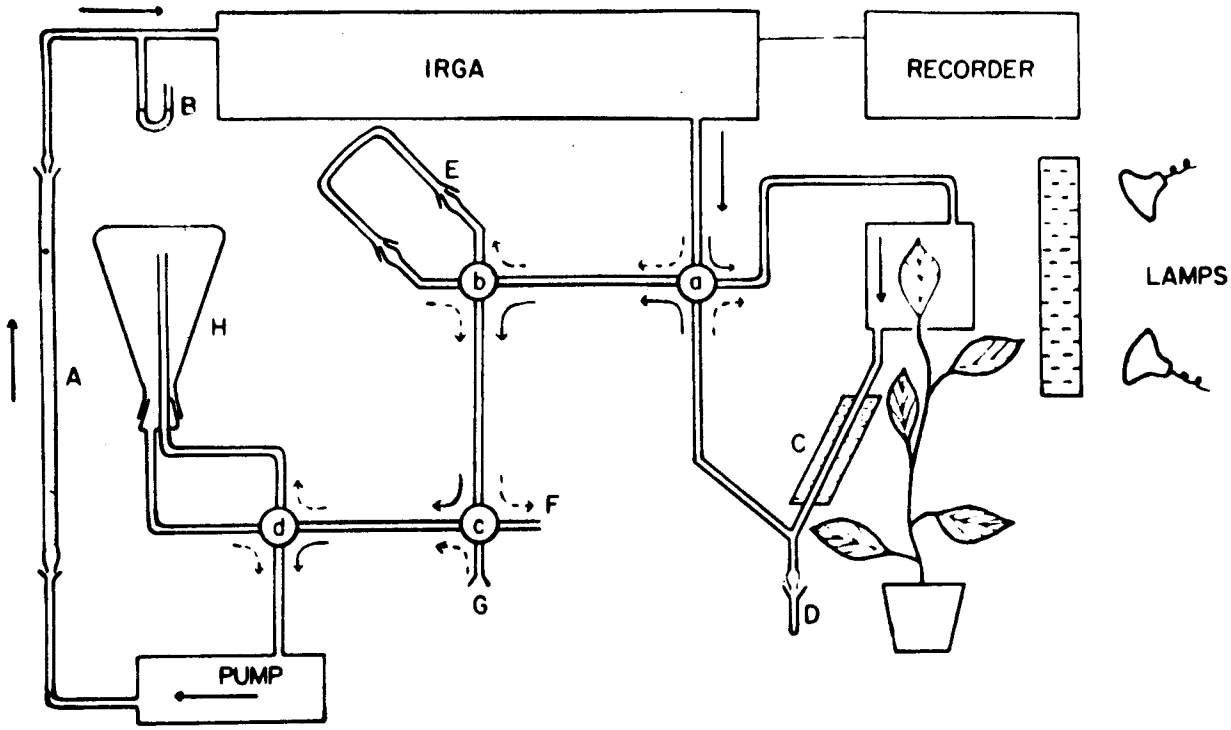


A Flowmeter
B Mercury manometer
C Ice-water jacket
D Removable flask for condensed water
E CO_2 absorbant
F Outlet during gas addition
G Inlet for adding gases
H Ballast flask for gas mixing
Four way stopcock
Path of flow during $[CO_2]$ measurement
Path of flow during
a bypass of leaf chamber
b removal of CO_2
c addition of gas
d mixing of gas

Fig. 2. Flow diagram of apparatus for measurement of CO_2 exchange.

A diaphragm pump ("Neptune Dyna-Pump" - model 2, Universal Electric Co., U. S. A.) circulated the gases through the closed circuit at a rate of 0.9 litres per minute. A glass flowmeter A, Fig. 2 (Matheson Co. No. 303, tube size R-2-15-B) was located on the output side of the pump in the circuit. A mercury manometer (B, Fig. 2) was incorporated into the flow circuit to ensure that the system operated at constant pressure. This was especially important when using the ballast flask (H, Fig. 2) for gas mixing and volume measurement.

Water vapour control

The gas stream passed through a cooling condenser immediately after the leaf chamber (C, Fig. 2). The condenser was introduced to remove excess water vapour transpired by the leaf, as this otherwise condensed in the circuit as droplets which interfered with gas flow. The condenser consisted of a glass Y-tube cooled by a jacket of ice water. The cold tube walls effectively removed some water from the gas stream without appreciably changing the temperature of the gas. As the gas stream passed through the upper part of the Y-tube, the waste liquid flowed down into the stem of the Y (D, Fig. 2) and was periodically removed. Water vapour remaining

in the gas stream interfered slightly with CO_2 determination, and is discussed below.

Leaf chambers

Two chambers of different sizes were made from sheets of 6 mm thick plexiglass, and each was fitted with a reflecting back of aluminum foil. The larger chamber, suitable for fern fronds and *Ricinus* leaves was 12 x 24 x 0.6 cm (volume 195 ml), and the smaller chamber, used for all other plants, was 7 x 8 x 0.6 cm (volume 50 ml). The plant stem fitted into a small gap in the chamber wall, which was made airtight with plasticine around the stem. The plexiglass lid was sealed to the chamber with petroleum jelly, clamped, and a strip of plasticine applied around the joint as a final precaution against leaks.

Calibration of the IRGA

The zero reference gas for all CO_2 measurements was dry nitrogen which was trickled continuously through the reference cell as a precaution against any slow leakage of atmospheric CO_2 into the cell. Calibration of the IRGA was difficult due to the non-linearity of its response. A gas mixture, certified by the Matheson Co. (Canada) to be 350 ppm CO_2 in air, was used as the upscale calibration

standard. The zero point was established with dry nitrogen. Intermediate CO_2 concentrations were interpolated according to the calibration curve supplied with the IRGA. The final calibration curve was converted to a numerical table which was used in the calculation of all results. During calibration, standardization and experiments, the CO_2 concentration was always measured with the pump operating and the system at a known pressure.

A CO_2 absorbant (Ascarite) was used when necessary to remove CO_2 from gas mixtures. Ascarite scrubbing was also used to check the zero point of the IRGA during experiments, since the reading thus obtained corresponded to the zero point established with dry nitrogen gas.

Volume Measurements

The flow system for CO_2 determination may be conveniently considered in two parts: --

i) The leaf chambers, condenser and leads attached to stopcock a, in Fig. 2. Variously sized chambers may be substituted. The volumes of these components, and of the ballast flasks, were determined by measuring the volume of water they could contain.

ii) The basic circuit. This includes the IRGA sample tube, and the entire tubing circuit, bypassing

the leaf chamber and other components which may be switched into the circuit for special purposes. The volume of the basic circuit was determined in the following two ways.

Method 1. The volume of water contained by the circuit external to the IRGA was measured. This figure was added to the volume of the IRGA sample tube as specified by the Beckman manual. The basic circuit volume by this method is 146 ml.

Method 2. The technique of gas mixing is outlined in the following section. If, by use of the ballast flask, gas of known volume and CO_2 concentration is mixed with gas in the basic circuit at a different but known concentration and the same pressure, the volume of the basic circuit may be calculated.

For example: --

Using the ballast flask, 275 ml of gas containing 100 ppm CO_2 was mixed with gas in the basic circuit containing 350 ppm CO_2. After mixing, the final concentration of CO_2 was 187 ppm. The volume, x, of the basic circuit may thus be calculated: --

$$100\ (275) + 350\ (x) = 187\ (275 + x)$$

The basic circuit volume (x) determined by this method

was 147 ml.

The total volume of the CO_2-measuring circuit (225 ml or 373 ml) was the smallest possible, using the available components. These small volumes enabled low rates of CO_2 exchange to be measured in a short time.

Gas Mixing Technique

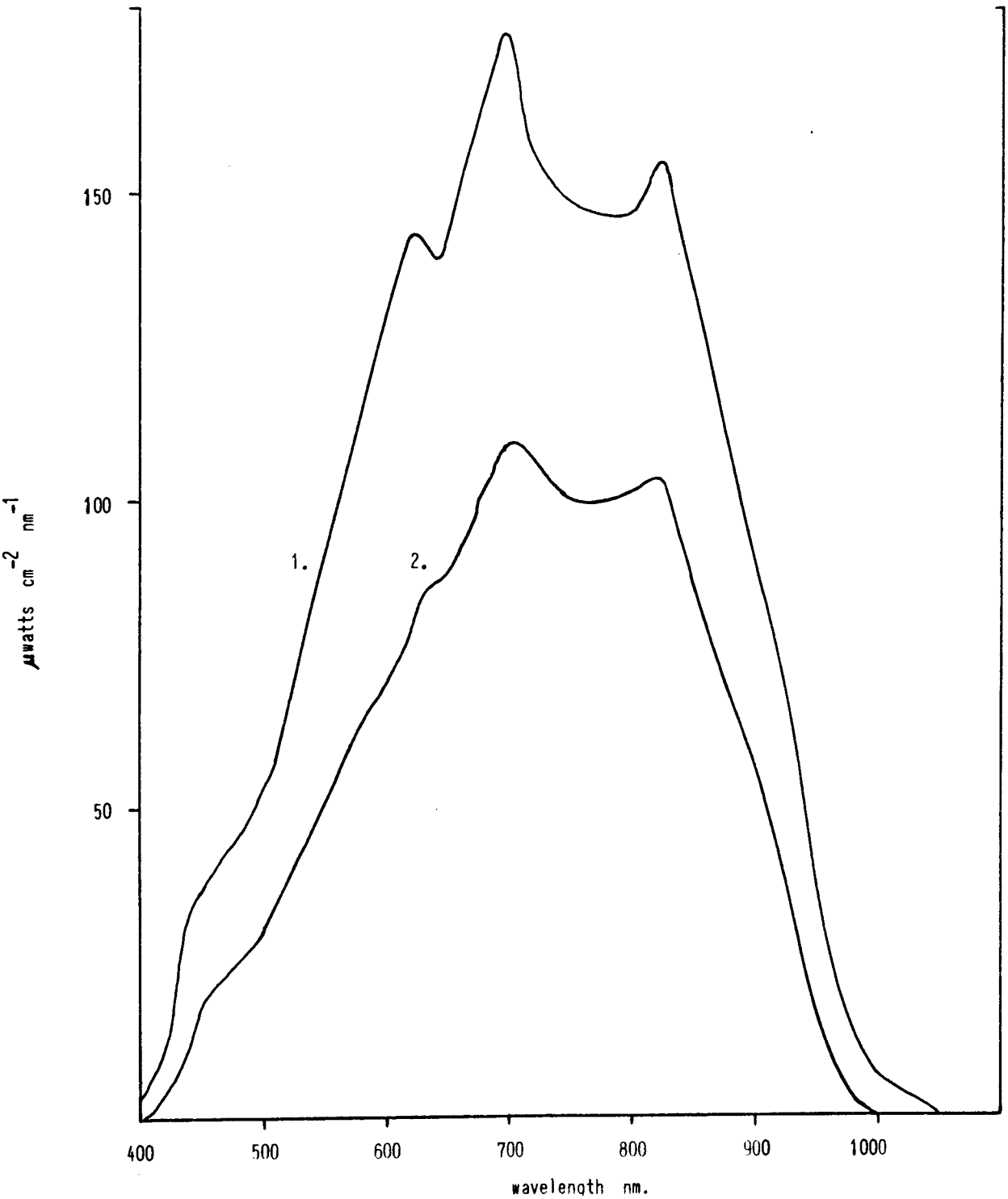
A system of interchangeable ballast flasks (H, Fig 2) permitted mixing of nitrogen, oxygen and air to give several different concentrations of O_2 and CO_2. A four-way stopcock (d, Fig. 2) attached by tubing and "Quick-fit" joints to flasks of known volume, could be switched so that the gas stream flowed through, or bypassed the ballast flasks. To obtain the same percentage of O_2 with different sized leaf chambers, both chambers were incorporated in the system during gas mixing. When the desired gas mixture was achieved, the unoccupied chamber and the ballast flask were switched out of the system.

For example: -- To obtain 71% O_2, both leaf chambers were filled with nitrogen (containing 100 ppm CO_2) and switched out of circulation. The basic circuit and a 525 ml ballast flask were filled with O_2 and closed. These two components were then mixed, and circulated until the IRGA indicated uniform CO_2 throughout the

system. Thus 672 ml O_2 were added to 273 ml N_2, making a total volume of 945 ml, of which 672 ml is 71%. The CO_2 in the N_2 was used as a marker to indicate that mixing of the gases was complete.

3. Illumination and temperature

Incandescent light was provided by two Sylvania EBR 375 watt movie lights. These lights have internal reflectors giving a beam angle of 25°. The normal lamp life of four hours was extended by running lamps at 90 volts instead of the rated voltage of 115 - 120 v. This lower voltage produces an emission spectrum corresponding to a colour temperature lower than the rated 3,400°K. The emission spectrum was therefore measured (Fig. 3) using an ISCO model SR spectroradiometer (Instrument Specialties Co. U.S.A.), after excessive infrared radiation had been absorbed from the light beam by passage through 10 cm of water in a tank made from 0.6 cm thick plexiglass. Cold water was continuously circulated through the filter. The distance between the plant chamber and lamps was 30 cm for most experiments, but was greater where lower light intensities were required. Light intensity was measured in foot candles (ft-c) with a "Sekonic Studio-deluxe" photographic exposure meter with a flat white diffusing disc over the detector. The light intensities used in


150
100
50
μwatts cm^{-2} nm^{-1}
1.
2.
400
500
600
700
800
900
1000
wavelength nm.

experiments, 1800 ft-c and 4200 ft-c, corresponded to 1.3×10^5 ergs $cm^{-2} sec^{-1}$ and 2.7×10^5 ergs cm^{-2} sec^{-1} of total light energy reaching the leaf.

Temperature measurement

The air temperature in the laboratory did not vary more than 2°C during any one experiment. The temperature of the gas stream in the closed circuit was measured with a small thermometer inside the plant chamber. Temperatures and variations are stated in the experimental results.

4. Accuracy and variation of CO_2 measurements

a. Calibration curve and accuracy of the IRGA

The response of the IRGA to CO_2 concentration is nonlinear, thus the instrument accuracy and the smallest unit of CO_2 concentration that can be precisely read from the recorder trace (*) depend on the concentration of CO_2 measured. The values given in the following table incorporate the effects of the width of the recorder pen trace, and the readability of the calibration curve of the IRGA. The accuracy of the IRGA is specified by the manufacturer to be ± 1%, and the sensitivity to be 0.5% of full scale.

Approximate range ppm CO_2	Smallest readable unit CO_2 ppm. (*)	Accuracy of CO_2 measurement ± ppm CO_2
0 - 25	1.5	3
25 - 100	2.0	4
100 - 200	3.0	6
200 - 270	3.5	7
270 - 400	4.0	8
400 - 600	5.0	10

b. Water vapour

Water vapour in the gas stream interferes with CO_2 determination. According to the manufacturer's specifications, 3.5% water vapour gives a reading equivalent to 5 ppm. CO_2 with the detector of this particular instrument. The percentage by weight of water vapour in saturated air at various temperatures is: --

21° C	-	1.6% water vapour
24° C	-	1.9%
27° C	-	2.3%
31° C	-	2.9%

Thus at the temperatures of the experiments (20° C - 30° C), the reading due to water vapour is equivalent to less than 4 ppm CO_2, which is within the limits of accuracy of the IRGA. When Ascarite was used to absorb CO_2 during standardization of the IRGA, the zero point obtained with moist gas compensated for the reading due to water vapour.

c. CO_2 leakage

A standard rate of leakage at atmospheric CO_2 into

the initially CO_2-free closed system was determined over a period of four hours. Before and after each experiment, the CO_2 leak rate was measured over a period of fifteen minutes to check that it did not exceed the standard rate. Thus the rate of CO_2 leakage with a given CO_2 concentration in the system could be estimated using the standard rate. The contribution of CO_2 leakage was calculated for individual results of several plants. For example, a leak rate giving an error of 2 ppm of the compensation CO_2 concentration, leads to a rate of CO_2 evolution in light 3% higher than the rate corrected for CO_2 leakage. These errors are insignificant compared to the differences between replicate determinations, therefore the results presented have not been corrected for CO_2 leak rate, which is a constant factor at a given CO_2 concentration.

d. Oxygen leakage

A standard rate of O_2 leakage for the closed circuit was measured with a Beckman Oxygen Analyzer at the same time as the CO_2 leak rate. During experiments, the rate of O_2 leakage was estimated by comparing the measured CO_2 leak rate with the two corresponding standard rates. At very high or low O_2 concentrations, where O_2

leakage was appreciable, the concentrations of O_2 in the results have been corrected for leakage. For example, zero O_2 was corrected to 2% O_2, and 100% O_2 to 97% O_2.

e. Precision of rate measurements

The CO_2-concentration readings used for calculation of APS rates at 300 ppm CO_2 are liable to errors due to the rapid rate of CO_2 uptake by the plant in the light at 300 ppm CO_2, which leads to difficulty in precise reading at the recorder chart. Furthermore the calibration curve and instrument errors are relatively high at 300 ppm CO_2. A difference of 3 ppm $CO_2 min^{-1}$ would lead to a 4% error in the calculated value for CO_2 evolution in light. Although the actual errors varied for each individual result, they were of the above order of magnitude and were thus insignificant compared with those of plant variation.

5. Plant sources and growth conditions

Table 1 lists the plants used in the experiments and their sources. The plants are grown in the laboratory under a battery of 40-watt fluorescent lights (Westinghouse cool white) which provided 1300 footcandles light at average plant height. A cycle of 16 hours of light and 8 hours darkness was used. The plants were open to room

TABLE 1. PLANTS USED IN EXPERIMENTS

DIVISION	SPECIES	COMMON NAME	SOURCE
BRYOPHYTA	Marchantia polymorpha L.	Liverwort	*
	Hylocomium splendens (Hedw.) Bry. Eur.	Moss	*
LYCOPODOPHYTA	Lycopodium obscurum L.	Clubmoss	*
	Selaginella wallacei Hieron.	Clubmoss	*
ARTHROPHYTA	Equisetum arvense L.	Horsetail	*
PTERIDOPHYTA	Polystichum munitum (Kaulf.) Presl.	Sword fern	*
CONIFEROPHYTA	Tsuga heterophylla (Raf.) Sarg.	Western hemlock	*
	Thuja plicata D. Don.	Redcedar	*
	Pseudotsuga menziesii (Mirb.) Franco.	Douglas-fir	***
ANTHOPHYTA	Alnus rubra Bong.	Alder	*
	Glycine max L. var. Comet.	Soybean	**
	Lycopersium esculentum Mill. var. Ace.	Tomato	**
	Ricinus communis L. var. Sanguineus	Castor bean	**
	Rubus ursinus Cham. and Schlecht.	Bramble	*
	Zea mays L.	Corn	**

Source

* Transplanted from the field to pots.

** Grown in the laboratory from certified seed five weeks before experiments.

*** Three year old potted nursery stock.

air, at a temperature of 23°C ± 2°C. They were grown in soil, with monthly supplements of fertilizer (100 ml of 0.2% solution of Hisol 20-20-20). Those plants grown from seed were under these conditions from the time of germination. The older plants spent at least two months in this growth regime after transfer from the field.

6. Experimental procedure

One hour after the beginning of the daily light cycle, a leaf, attached to the plant, was sealed into the leaf chamber, and room air (about 0.03% CO_2) circulated past the leaf for one hour under the high intensity of incandescent light used in the experiments. This period of high intensity illumination was necessary to ensure a steady rate of photosynthesis. After standardization of the IRGA, the circuit was filled with room air (about 21% O_2, 0.03% CO_2) and closed. The leaf was illuminated until the CO_2 concentration in the circuit had decreased to a constant value, the CCP. The leaf chamber was then covered with an opaque black plastic sheet and the rate of CO_2 evolution in darkness was determined. The transients of CO_2 evolution due to the transfer of the leaf from light to darkness (Tregunna,

1963) were found to disappear with 20 minutes of darkness, and the steady rates measured after this time are given in results.

To determine the CCP and the rates of APS and CO_2 evolution in darkness at different O_2 concentrations, the O_2 content of the circuit was adjusted as outlined in the Methods, page 17. When the leaf had produced sufficient CO_2 in the dark (400 ppm) it was illuminated for determination of the new APS rate. The temperature of the gas stream passing through the leaf chamber was recorded at 15 minute intervals throughout the experiment.

Measurements of APS rate were made at the average atmospheric concentration of CO_2 (300 ppm) so that experimental results would have some relevance to field conditions, and also for comparison with published results (e.g. Brown and Tregunna, 1957). The change in CO_2 concentration within the range 315 - 285 ppm CO_2 was used as an approximation of the instantaneous rate of APS at 300 ppm CO_2.

RESULTS

1. The effect of oxygen concentration on carbon dioxide compensation points.

The relationship between O_2 concentration and the CO_2 concentration at CCP is shown for twelve species in Figs. 4, 5 and 6. The basic data of the graphs are included, with temperatures and light intensities, in Table 2.

In eleven of the twelve species tested, the CCP is a linear function of O_2 concentration. These species are high CCP plants, as distinguished from low CCP plants such as corn, in which O_2 concentration does not affect CCP (Fig. 6). At 21% O_2 or lower, the CCP's of red-cedar and tomato are similar to those of the other high CCP plants measured. However at 60% O_2, the CCP is nearly the same as in 21% O_2. This effect is further considered in Results, section 3.

2. Comparison of rates of CO_2 exchange.

The results presented in Tables 3, 4 and 5 are derived from measurements as follows:

a. The rate of CO_2 uptake (APS) in light at a mean concentration of 300 ppm CO_2

b. The steady rate of CO_2 evolution in darkness, measured

Figs. 4, 5 and 6

The effect of oxygen concentration on carbon dioxide compensation point.

•	=	Single CCP determination.
•(3)	=	3 CCP determinations with the same result.
]	=	Range of CCP's determined.

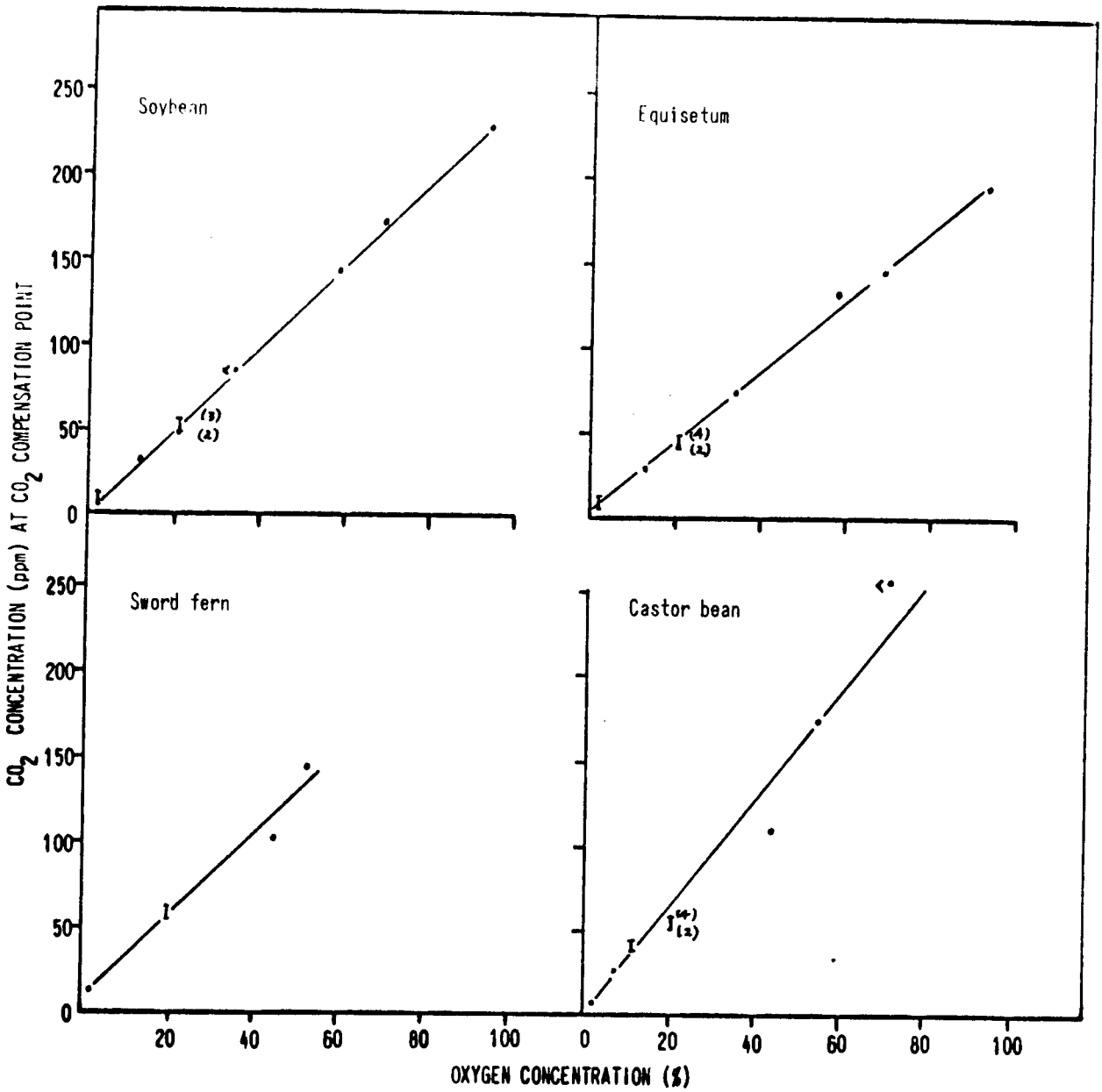

Soybean
Equisetum
Sword fern
Castor bean
CO2 CONCENTRATION (ppm) AT CO2 COMPENSATION POINT
OXYGEN CONCENTRATION (%)
0
50
100
150
200
250
20
40
60
80
100

Fig. 4.

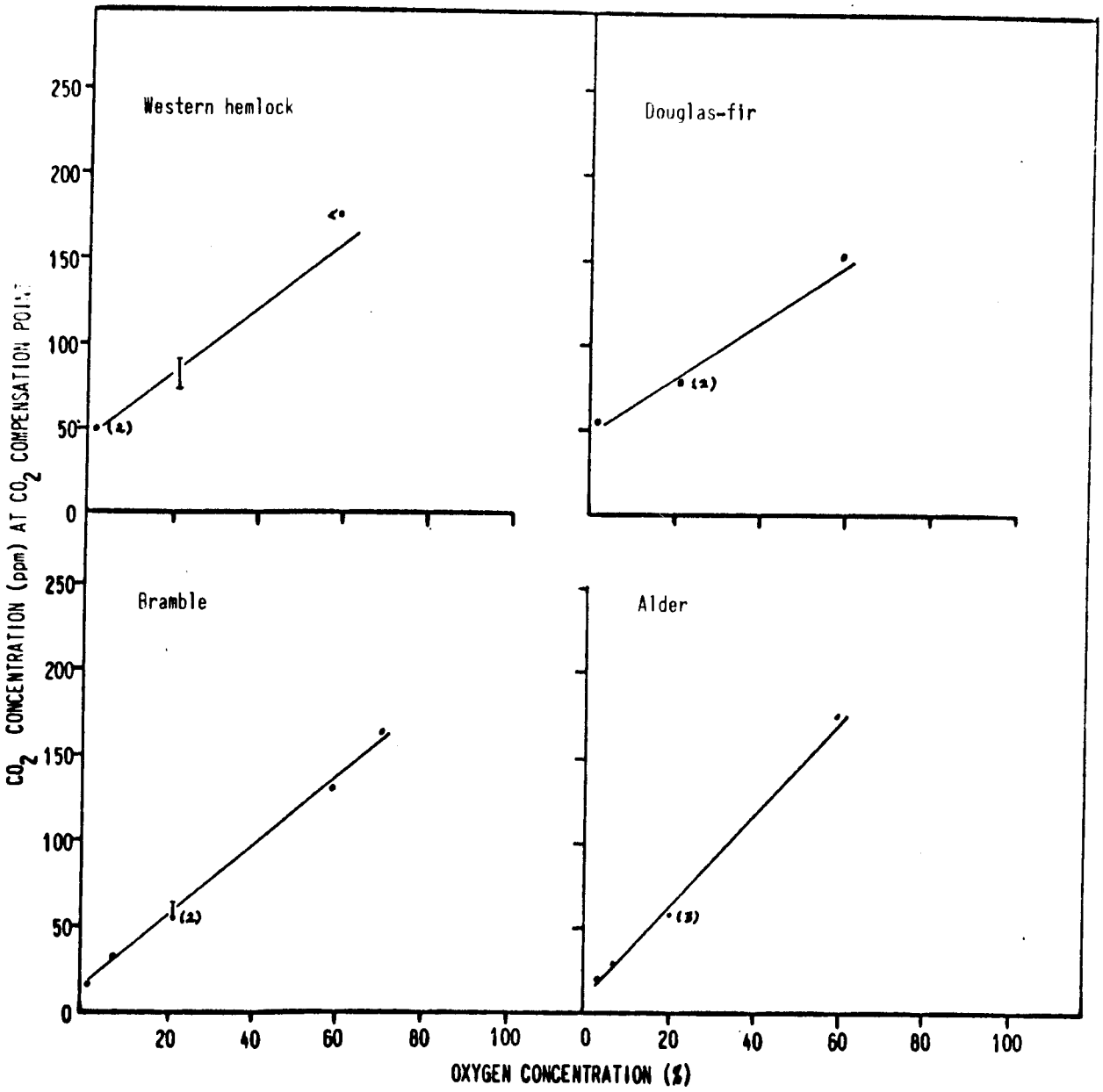

Western hemlock
Douglas-fir
Bramble
Alder
250
200
150
100
50
0
20
40
60
80
100
CO_2 CONCENTRATION (ppm) AT CO_2 COMPENSATION POINT
OXYGEN CONCENTRATION (%)

Fig. 5.

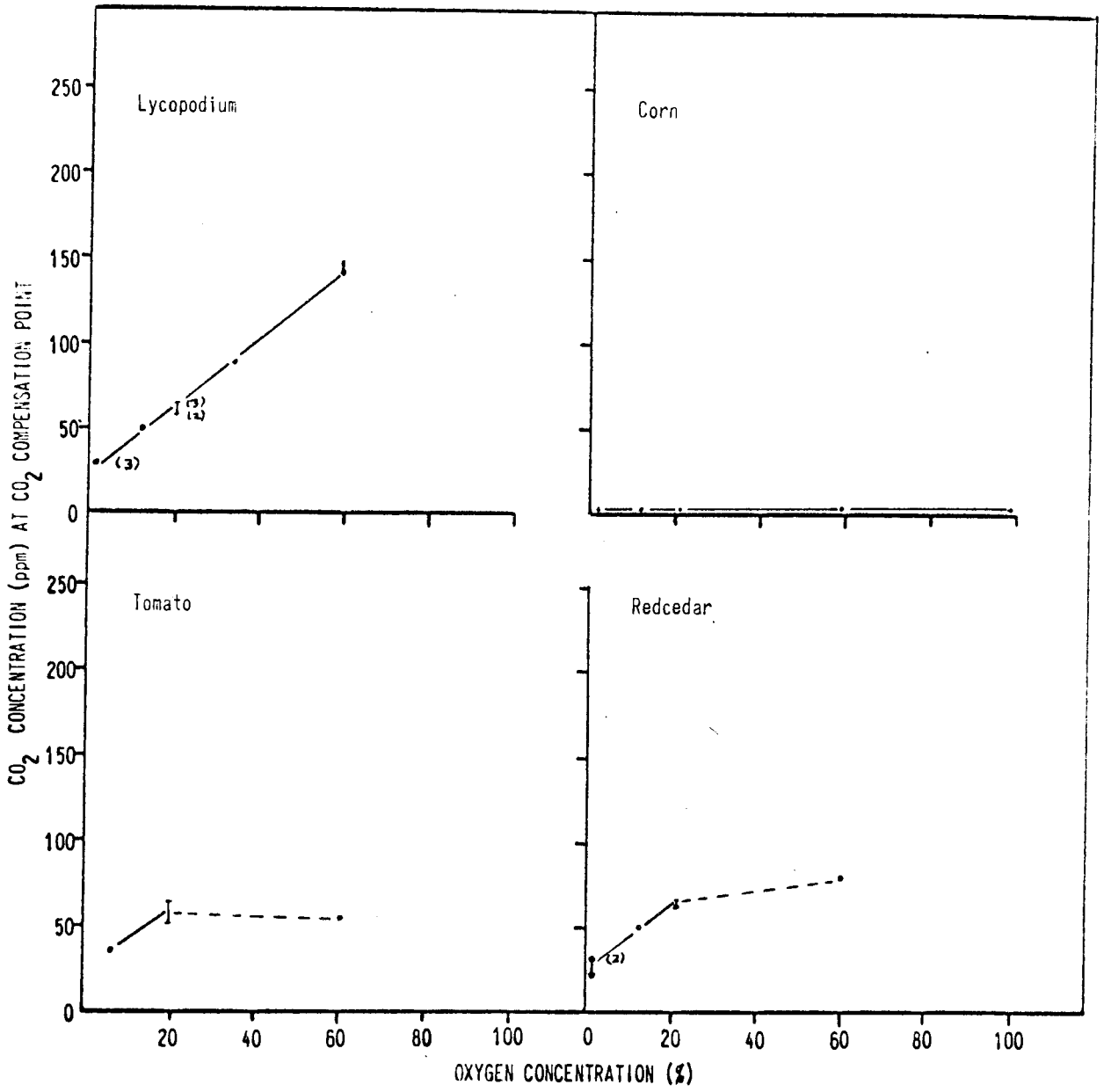

Lycopodium
Corn
Tomato
Redcedar
CO_2 CONCENTRATION (ppm) AT CO_2 COMPENSATION POINT
OXYGEN CONCENTRATION (%)
0
50
100
150
200
250
20
40
60
80
100
(1)
(2)
(3)

Fig. 6.

Table 2. CO_2 concentrations (ppm) at CO_2 compensation point for various concentrations of O_2

Plant	Temp ±1°C	Light intensity (ft—c)	Concentration of oxygen 2%	7%	12%	21%	35%	45%	59%	71%	97%
Hylocomium	26	600				120					
Lycopodium 2	22	1800	30(3)		50	58(2),62(3)	90		145.152		
Selaginella	27	4200				110					
Equisetum 2	22	1800	6,12(2)		32	42(2),50(4)	75		135	149	202
Sword fern	26	4200	13			56,63		104	149		
Western hemlock	26	1800	48(2)			73,81,88			<180		
Redcedar	23	1800	24,34(2)		50	64,66			80		
Douglas—fir	26	1800	57			81(2)			157		
Alder	25	4200	20		32	56,59(2)			178		
Soybean	27	4200	7,10(2)			49(3),55(3)	< 88		152	168	235
Tomato	22			37		51,57,65			54		
Castor bean	29	4200	4	30	40,45	54(2),60(4)		113	178		<265
Bramble	20	4200	19	35		56,60,63			136	170	
Corn	22	4200	3		3	3			4		5

Plants are arranged in taxonomic order since there was no significant difference between the two light intensities.
Figures in parentheses show the number of duplicate results.

immediately before or after a.

c. The concentration of CO_2 (ppm) at CO_2 compensation point.

d. The rate of CO_2 evolution in light at 300 ppm CO_2 as calculated: --

$$\text{Rate of } CO_2 \text{ evolution in light} = \frac{\text{APS} \times [CO_2]\ CCP}{[CO_2] - [CO_2]\ CCP} \quad \text{Equation 1}$$

This rate should be considered as a minimum, as the extent of reassimilation of CO_2 is not known for these plants. The units are $\mu gCO_2 min^{-1} g^{-1}$ fr wt for rates, and concentrations, $\mu gCO_2/100ml$ for CO_2 compensation points.

Tables 3 and 4 show that (under the conditions of these experiments) the minimum rate of CO_2 evolution in light at 300 ppm CO_2 and 21% O_2 is greater than the rate of CO_2 evolution in darkness (in most plants by a factor of more than two) in all plants tested except corn and Lycopodium. Several factors make quantitative comparisons of these rates inappropriate between different plants. Taxonomically, physiologically and morphologically, the tested plants are extremely diverse. The temperatures and light intensities optimal for growth and photosynthesis are unknown for most of these species. The light intensities used were found to be above light saturation of APS for swordfern, redcedar, alder, Lycopodium and bramble,

Table 3.
Rates of CO_2-exchange ($\mu g\ CO_2\ min^{-1} g^{-1}$ fr wt) in air (21% O_2, 0.03% CO_2) at 1800 ft-c light intensity.

Plant	Temp. °C.	APS_{300} A	CO_2 evolution dark B	CO_2 evolution light C	CO_2 CCP (ppm)	A/B	A/C	C/B
Marchantia	27	(2.4)	(1.7)	—	—	1.4	—	—
Lycopodium 2.	22	23.1	8.6	6.0	62	2.7	0.7	3.9
Equisetum 2.	22	57.2	4.5	9.6	43	12.7	2.1	5.9
Hemlock	26	25.5	5.3	n.c.	81	4.8	n.c.	n.c.
Redcedar	24	24.1	5.7	6.8	66	4.2	1.2	3.5
Douglas-fir	26	27.8	6.4	n.c.	81	4.3	n.c.	n.c.

Rates and compensation points are individual measurements of one plant. The instrumental error is ±0.2 $\mu g\ CO_2\ min^{-1} g^{-1}$ fr wt for rates, and ±1.5 ppm for CO_2 compensation points.

APS_{300} = rate of CO_2 uptake at 300 ppm CO_2

CO_2 light evolution = minimum rate of CO_2 evolution in light at 300 ppm CO_2 (calculated with equation 1.

n.c. = not calculated (explanation in text)

( ) = rate per leaf as ppm CO_2/min.

— = not determined

A/B = ratio of APS_{300}/CO_2 dark evolution

A/C = ratio of APS_{300}/CO_2 light evolution

C/B = ratio of CO_2 light evolution/CO_2 dark evolution

Table 4.
Rates of CO_2 exchange ($\mu g\ CO_2\ min^{-1}g^{-1}$ fr wt) in air (21% O_2, 0.03% CO_2) at 4200 ft-c light intensity.

Plant	Temp. °C.	APS_{300} A	CO_2 evolution dark B	CO_2 evolution light C	CO_2 CCP (ppm)	A/B	A/C	C/B
Lycopodium 1.	27	(19)	(8.6)	(5.8)	70	2.2	3.2	0.7
Lycopodium 2.	22	14.7	13.2	5.7	84	1.1	2.6	0.4
Selaginella	27	(4.7)	(0.75)	(2.7)	110	6.3	1.7	3.6
Equisetum 1.	28	85.5	12.2	27.2	73	7.0	3.1	2.3
Sword fern	26	(66)	(8.2)	(15.2)	56	8.0	4.3	1.9
Alder	25	200.8	17.0	49.1	59	11.8	4.1	2.9
Soybean	27	183.1	14.4	34.9	48	12.7	5.2	2.4
Tomato	22	118.9	8.2	32.9	65	14.5	3.6	4.0
Tomato	22	80.8	4.9	16.5	51	16.5	4.9	3.4
Castor bean	29	126.1	11.1	27.7	54	11.4	4.5	2.5
Bramble	20	113.1	10.0	25.9	56	11.3	4.4	2.6
Corn 1.	29	(58)	(5.6)	(0.9)	3	10.4	6.4	0.2
Corn 2.	22	102.2	7.8	1.0	3	13.1	1.2	0.1

Symbols are explained below table 3.

however the remaining plants were not tested.

Rates of CO_2 exchange differed considerably when measured several times during an experiment. Such variations may be intrinsic to the plant (e.g.endogenous rhythm), or may be influenced by the timing and nature of intervening light, dark, and oxygen treatments. Variation in rates may also reflect a slow adaptation to the experimental conditions. At any one O_2 concentration, CCP's were consistently lower after several hours of experiments, which may reflect decreasing induction effects. Table 5 shows the range of variation in rates and CCP for two species. The experimental leaves were measured on several successive days, and left in a stream of room air in darkness for about ten hours between periods of measurement. The highest CCP, highest dark respiration rate and lowest APS rate were those measured early on the first day. Variations on subsequent days were smaller. Table 5 also includes results for different plants of the same species. Lycopodium 1 was a mature fruiting plant and was measured six months earlier than Lycopodium 2 which was a young growing shoot from the same rhizome. Similarly there was a six month interval between measurements on Equisetum 1 and 2. Although

Table 5.
Rates of CO_2-exchange ($\mu g\ CO_2\ min^{-1}g^{-1}$ fr wt) in air (21% O_2, 0.03% CO_2)

	Plant.	Temp. °C.	Light Intensity ft-c	APS_{300} A	CO_2 evolution dark B	CO_2 evolution light C	CO_2 CCP (ppm)	A/B	A/C	C/B
	Equisetum									
*	1	28	4200	85.5	12.2	27.5	73	7.0	3.1	2.3
1	2	21.5	1800	42.7	8.5	8.1	48	5.0	5.3	0.9
2	2	22	1800	66.6	8.1	13.3	50	8.2	5.0	1.6
2	2	23	1800	42.7	4.5	6.8	41	9.5	6.3	1.5
2	2	23	1800	57.2	4.5	9.6	43	12.7	6.0	2.1
	Lycopodium									
	1	27	4200	(9.7)	(4.3)	(2.9)	70	2.2	3.3	0.7
4	2	22	4200	14.7	13.1	5.7	84	1.1	2.6	0.4
1	2	21.5	1800	15.2	13.2	7.1	96	1.2	2.1	0.5
2	2	21.5	1800	23.0	8.6	6.0	62	2.7	3.8	0.7
3	2	20	1800	21.0	7.5	5.0	58	2.8	4.2	0.7
4	2	20	1800	19.1	8.4	5.0	62	2.3	3.8	0.6

( ) = Rate per branch as μgCO_2/min. Other symbols are explained beneath Table 3.

* = day of measurement

rates differ quantitatively, the patterns are consistent within both species.

Table 3 does not include calculated values of CO_2 evolution in light for Douglas-fir or western hemlock because of the relatively large quantity of non-photosynthetic tissue in the twigs measured. The CCP/$[O_2]$ relationship for these two species is linear, as shown in Fig. 4, and of the same general slope as other high CCP plants, but the extrapolated value of CO_2 evolution at zero $[O_2]$ is conspicuously high, and probably represents respiratory CO_2 of non-photosynthetic tissue.

The meaning and usefuless of the calculated values of CO_2 evolution in light are considered further in the Discussion. However it must be stressed that the equation is a form of expression of the extrapolation of the curve of APS rate vs $[CO_2]$ to its intercept of the APS rate axis at zero $[CO_2]$ (Tregunna, 1963). These relationships are shown graphically in Fig. 7. The calculated value of CO_2 evolution in light thus represents a theoretical rate at zero $[CO_2]$ and the assumptions mentioned in the introduc-duction should be considered in the interpretation of these calculated values.

3. The effect of O_2 concentration on CO_2 exchange

The rates of CO_2 uptake at 300 ppm CO_2 are compared at 21% O_2 and 60% O_2 in Table 6. Rates of CO_2 uptake range between 23 and 210 $\mu g CO_2 min^{-1} g^{-1}$ fr wt at 21% O_2, and between 5 and 42 μg $CO_2 min^{-1} g^{-1}$ fr wt at 60% O_2. In all cases 60% O_2 strongly inhibited the uptake of CO_2. This inhibition was consistent among the plants examined, and was of the same order of magnitude (63 to 87% inhibition) in all plants despite the wide differences in rates of CO_2 uptake at the same O_2 concentration.

The degree of inhibition of APS by 60% O_2 in redcedar and tomato was within the range of other plants tested. By contrast, 60% O_2 did not appear to affect CCP in these species to the same extent as in other high CCP plants, as described in Results, section 1. However further experiments are necessary to confirm these effects.

Table 6. Effect of oxygen concentration on the rate of APS_{300} ($\mu g\ CO_2\ min^{-1}\ g^{-1}$ fr wt)

	APS_{300}		Inhibition of APS_{300}
	21% O_2	60% O_2	
Alder	208.8	27.9	87%
Bramble	113.1	42.0	63%
Castor bean	90.4	17.6	81%
Douglas-fir	27.8	6.0	78%
Equisetum 2.	57.2	7.3	87%
Lycopodium 2.	23.1	6.3	73%
	23.1	7.3	68%
Redcedar	24.1	6.2	74%
Soybean	183.1	31.6	83%
Tomato	118.9	29.0	76%
	80.8	29.0	64%
Western hemlock	25.5	5.3	79%

$$\text{Inhibition of } APS_{300} = \frac{APS_{300} \text{ at 21\% } O_2 - APS_{300} \text{ at 60\% } O_2}{APS_{300} \text{ at 21\% } O_2} \times 100$$

DISCUSSION

The relationship between O_2 concentration and the CO_2 compensation point (CCP) as described in section 1 of Results, is linear in the species tested from the Lycopodophyta, Arthrophyta, Pteridophyta, Coniferophyta and Anthophyta. These results agree with those of Tregunna, Krotkov and Nelson (1966) for tobacco, and Forrester et al. (1966) for soybean. The lowering of CCP from that in 21% O_2 to near zero in 2% O_2 in species of the Chlorophyta and Phaeophyta suggests that a linear relationship holds in at least some algae (Brown and Tregunna, 1967).

The contrasting lack of effect of O_2 on CCP in corn agrees with published results (Forrester, et al., 1966). Such a lack of effect has also been shown for certain grasses (Downes and Hesketh, 1968), algae (Brown and Tregunna, 1967) and species of the Chenopodiaceae, Portulacaceae and Amaranthaceae (Tregunna and Downton, 1967), although no evidence of the effects of O_2 concentrations higher than 21% was presented in these three papers.

Tregunna, et al., (1966) and Forrester, et al., (1966) found, in tobacco and soybean, that the CCP is zero when

extrapolated to zero O_2. Within the instrumental error, my results for soybean, Equisetum, castor-oil plant, and probably sword fern, do show this extrapolation to zero. The extrapolation of CCP to CO_2 concentrations as high as 50 ppm at zero O_2 in other species examined, could reasonably be attributed to several causes. The most likely, especially in Douglas-fir and western hemlock, is the presence of relatively large quantities of non-photosynthetic tissue. Whether CO_2 evolution by non-green tissues are stimulated, unaffected or inhibited by light (e.g. Tregunna, 1963; Weintraub, 1944), the effect of light is not the same as in chlorophyllous tissue (Voskresenskaya, 1961; Tregunna, 1963). Thus the same relationships between CO_2 evolution in light, and other environmental factors cannot be expected to hold for mixed tissues as in purely chlorophyllous tissues. The plants tested were not examined microscopically for the amount of non-green tissue, although this was obvious in the twigs of hemlock and Douglas-fir. Thus no estimate of the magnitude of this effect was possible in these experiments. It is also possible that the failure of the CCP/$[O_2]$ relationship to extrapolate to zero at zero O_2 represents a true evolution of CO_2 by

some process other than the suggested glycolate metabolism. An evolution of CO_2 in these conditions in some species and not in others has been suggested to indicate differences in reassimilation of respiratory CO_2 (El-Sharkawy and Hesketh, 1967). Moss (1962) found a CCP of 9 ppm for corn, and 7 ppm for sugar cane (also a low CCP plant) in air, but as he does not give the experimental (or instrumental) error, it is not possible to decide whether there is a real, though small, evolution of CO_2 in these species. Thus the relatively high CCP's at low O_2 found in tomato, Lycopodium, western hemlock, redcedar, alder, bramble and Douglas-fir, may represent a real evolution of CO_2 in light, an inefficiency of reassimilation, or evolution of CO_2 by non-photosynthethic tissue. The available data are insufficient to favour any particular one of these explanations.

The deviations of CCP's found at high O_2 concentrations in redcedar and tomato, from a linear relationship with O_2, have interesting implications. However further experiments at other O_2 concentrations are necessary to confirm these effects. These low CCP's in redcedar and tomato followed low rates of photosynthesis over two to three hours. The O_2 concentration in the system at the end of this period

was not measured, but since the CO_2 leak rate was measured during this experiment, and the O_2 leak rate was previously found to correlate with the CO_2 leak rate, the expected loss of O_2 from the system could be calculated, and was found to be quite insufficient to account for the CCP's being nearly the same as in 21% O_2.

In Figures 4, 5 and 6 the graphs of CCP vs $[O_2]$ differ in slope between species. This difference has not been expressed quantitatively, since there were insufficient replicate data available for most of the plants examined. A more comprehensive study might show a relationship between the slope of this line and rates of CO_2 exchange in light, and thus enable prediction of rates at other O_2 concentrations. My data show no marked differences in the effect of O_2 on CCP's (except for corn) between species from the various divisions.

The ratio between CO_2 fixation and loss (expressed in Tables 3 - 5 as APS_{300}/CO_2 dark evolution) may be taken broadly to indicate dry matter production in normal air. Although metabolic activities other than the rates of CO_2 exchange at the time of the experiment cannot be considered in this ratio, the highest values are found for relatively fast growing Anthophyta, and the lowest values in relatively slow growing plants such as Lycopods

and Bryophytes. Equisetum and sword fern show intermediate values which may correlate in natural conditions with high growth rates over a limited period of time. The effects of these ratios will be modified by the relative lengths of time in darkness and light, and other environmental factors such as temperature.

Rates of CO_2 evolution in light were calculated using equation 1. The relationships of the values of this equation are shown graphically in Figure 7. This calculation gives a theoretical rate of CO_2 evolution at zero CO_2. If it is assumed (Brown and Tregunna, 1967) that CO_2 evolution does not vary with the concentration of CO_2 or with the rate of CO_2 uptake, this calculated rate (in the absence of reassimilation) may be added to the rate of APS, at a given CO_2 concentration, to give the gross rate of photosynthesis. El-Sharkawy, Loomis and Williams (1967) measured CO_2 evolution in light and darkness in CO_2-free air, and concluded that reassimilation of CO_2 produced in light (whether by dark respiration, or other processes) can account for the differences between high and low CCP plants. Experiments combining some of their methods with those of Tregunna et al. (1966), (and considering the different assumptions in these two approaches) may clarify the actual rates of

Equation 1 (Irogunna,1963)

$$\text{Rate of } CO_2 \text{ evolution in light} = \frac{APS \times [CO_2]_{CCP}}{[CO_2] - [CO_2]_{CCP}}$$

Where $[CO_2]$ = the concentration of CO_2 at which the rate of APS was measured.

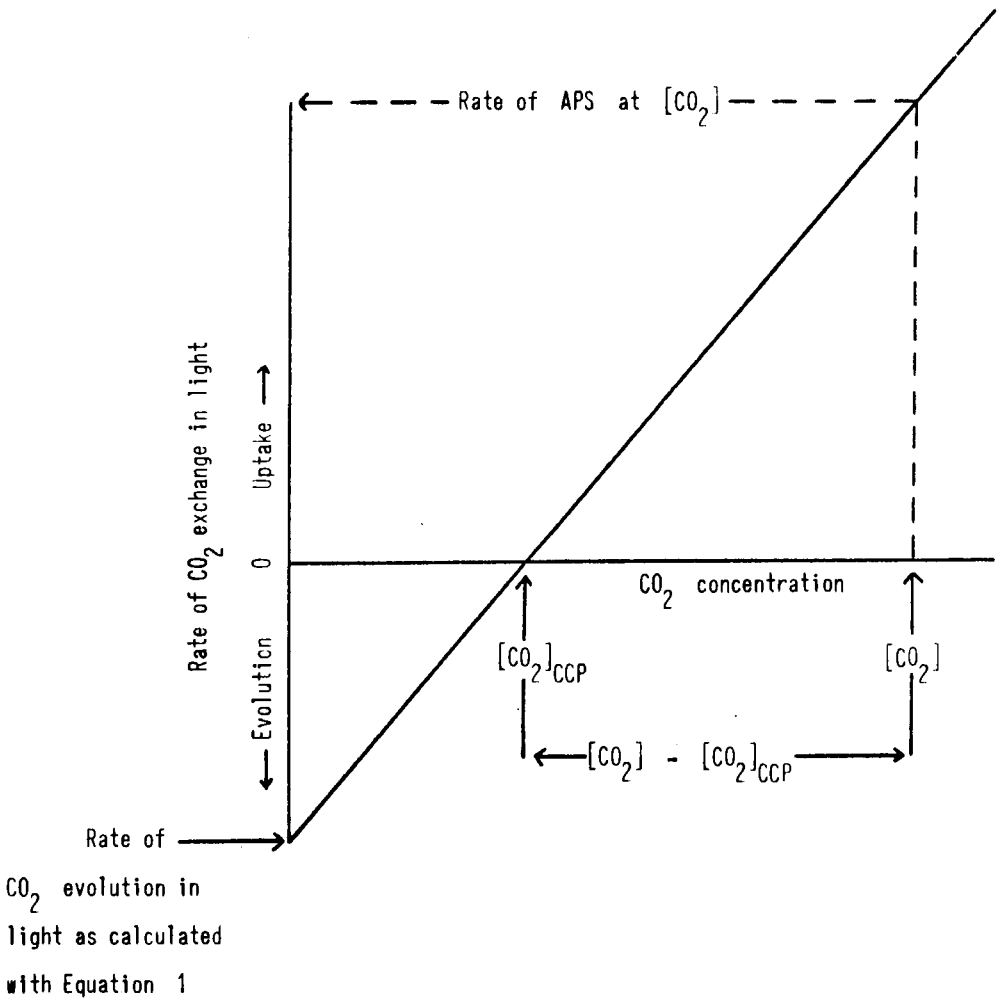


Fig. 7 - The relationship of measurable data to calculated CO_2 evolution in light.

Fig. 8a.

•———• Rate of APS of tobacco leaves at 2500 ft-c and 25° - 26°C. Points are the means of 45 measurements. The CCP is the mean of 15 measurements. (Data from Decker, 1957).

- - - - Extrapolation of the lower segment of the APS/ $[CO_2]$ curve to give A, the minimum rate of CO_2 evolution in the light by Decker's method.

——— The relationships of Equation 1 (Tregunna, 1963) which gives B, as the minimum rate of CO_2 in the light.

Fig. 8b.

Data of Holmgren and Jarvis (1967) showing departures of the CO_2 exchange rate from linearity above and below CCP. The data below CCP were obtained from the efflux of CO_2 into CO_2-free air.

Rumex acetosa at 20.6°C and 8 x 10^4 ergs cm^{-2} sec^{-1}, which is above saturation for APS and for CO_2 efflux.

- - - - Extrapolation of the linear part of the APS/$[CO_2]$ curve to give the minimum rate of CO_2 evolution in the light by Decker's method.

Fig. 8. EFFECTS OF DEPARTURES FROM LINEARITY OF THE APS/$[CO_2]$ CURVE ON VARIOUS ESTIMATES OF CO_2 EVOLUTION IN THE LIGHT.

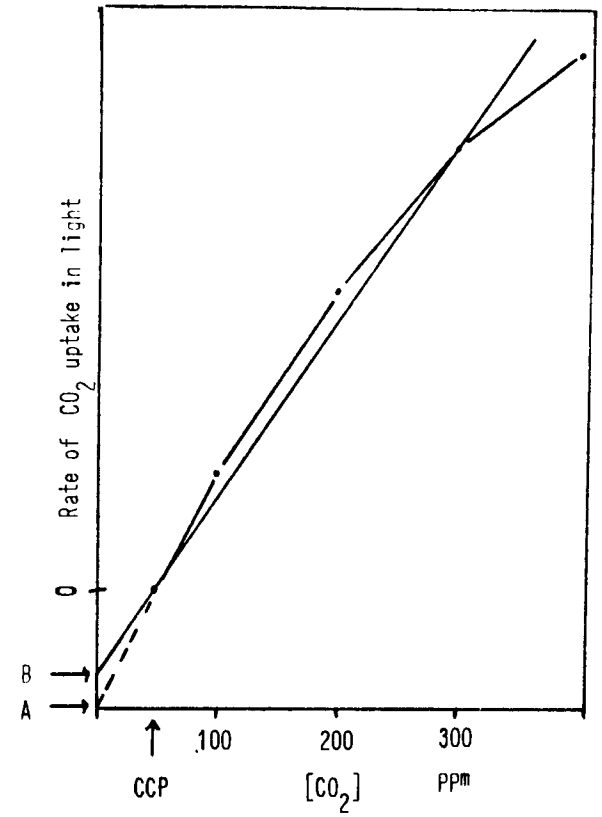

Rate of CO_2 uptake in light
0
B
A
100
200
300
CCP
$[CO_2]$
PPm

Fig. 8a.

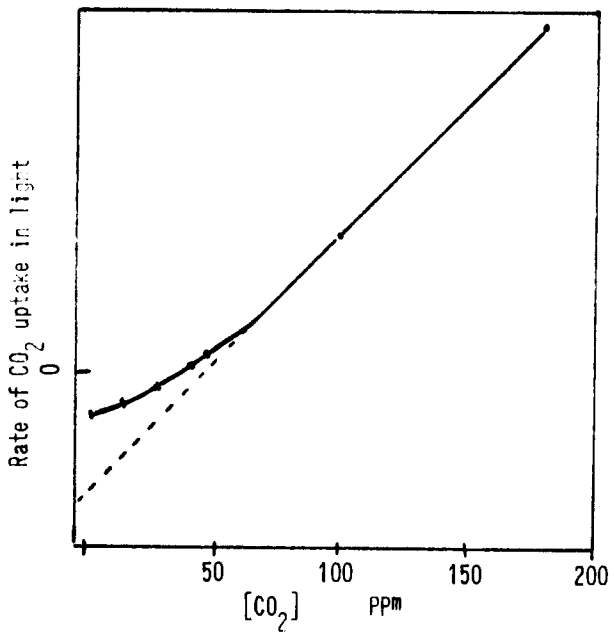

Rate of CO_2 uptake in light
0
50
100
150
200
$[CO_2]$
PPm

Fig. 8b.

CO_2 evolution. In one of their experiments, El-Sharkawy et al. (1967) used different flow rates of CO_2-free air, which would provide an alternative sink for otherwise reassimilated CO_2. Different flow rates at various concentrations of O_2, together with analysis of the resistances to CO_2 movement in leaves (El-Sharkawy and Hesketh, 1965; Lake, 1967) may lead to a more quantitative comparison between CO_2 actually produced and that evolved to the atmosphere.

The rates of CO_2 exchange in Tables 3 - 5 show that the minimum rate of CO_2 evolution in light, as calculated by Equation 1, is greater than that in darkness, in most cases by a factor of 2 or more. Although direct quantitative comparisons are inappropriate between such different species, and plant variation is considerable (Table 5), the general relationships between CO_2 uptake, light and dark CO_2 evolution rates and CCP are clear. Lycopodium was the only plant for which the CO_2 evolution in the dark was not smaller than the calculated minimum in light. However, relative to CO_2 uptake, dark respiration was particularly high in Lycopodium, while the calculatedCO_2 light evolution bore much the same relationship to CO_2-uptake rate as in other species. Without more knowledge of the optimal conditions for growth and photosynthesis of Lycopodium,

speculation on the importance of these differences in CO_2 exchange are hardly justified.

Rates of CO_2 evolution in light measured by other workers vary considerably with experimental methods and conditions, as well as species. Krotkov, Runeckles and Thimann (1958), measuring total ^{14}C uptake or dilution found that the CO_2 output in wheat was doubled in light compared to that in darkness, but was 75% less in light than darkness in pea leaves. These experiments used 5% CO_2. Lister et al., (1961) using ^{14}C tracer with an IRGA (closed system), found CO_2 evolution at CCP in the light to be only about 20% of that in the dark in red pine. Decker (1957) found consistently higher minimum rates in light compared with dark, in various plants including gingko and a fern. He used 0.03% CO_2 with an IRGA (closed system) and rates were calculated by extrapolation of APS rates to zero CO_2. Hoch, Owens and Kok (1963) using a mass spectrometer, found that O_2 uptake was inhibited by low intensity light, and accelerated at medium and high light intensities in two species of unicellular algae, Scenedesmus and Anacystis. These effects had different wavelength dependences. Ozbun, Volk and Jackson (1964) measured O_2 and CO_2 exchange simultaneously (at timed intervals) with a mass spectro-

meter and found, in bean leaves at about 1% O_2 and 1% CO_2, that light depressed CO_2 evolution and accelerated O_2 consumption relative to these rates measured in darkness. Evolution of CO_2 and uptake of O_2 in darkness decreased as leaves matured, but the evolution of CO_2 in light remained constant when the O_2 uptake decreased with maturity. They consider that reassimilation did not account for the low rate of CO_2 evolution in light. The rate of evolution of O_2 during photosynthesis was considerably greater than the rate of CO_2 uptake in a mature leaf at 1500 ft-c. These differences in results may be partly due to methods and conditions, however it appears that gas exchange relations may be very complicated, quite apart from the transient phenomena associated with changing conditions (e.g. from light to darkness) as outlined by Tregunna (1963) and Semenenko (1964).

If some uncontrolled variable is affecting CO_2 uptake, it may very well be inappropriate to use Equation 1. In my experiments, light intensity and quality, relative humidity, O_2 and CO_2 concentrations and temperature were either controlled or known. The most obvious uncontrolled variables were the length of time in light and darkness at various O_2 concentrations. Tregunna, Krotkov and Nelson (1966) used a uniform cycle of short light and

dark periods during CO_2-exchange measurements of tobacco leaves, but this procedure was not practical for slowly metabolising plants, such as Lycopods and Bryophytes, especially at high O_2 concentrations (see Table 2). Another example of an uncontrolled variable is the "sleep" reaction of soybean leaves. In 21% O_2, the CO_2-uptake rate of soybean at 300 ppm CO_2 was 183 μg $CO_2/min^{-1}g^{-1}$ fr wt (table 4). When the folding of leaves indicated the "sleep" reaction, the CO_2 uptake rate fell to 22.6 μ g $CO_2/min^{-1}g^{-1}$ fr wt (at 300 ppm CO_2). However these widely different CO_2 uptake rates were both associated with CCP's of 55 ppm (Table 2).

The effects of time in light and dark would be expected to differ with species. The induction phenomena of CO_2 uptake are complicated. Mokronosov and Nekrasova (1966) found in potato that,

> "The magnitude of the induction losses of photosynthesis reaches 60-90% of the stationary state in a two-to-four hour exposure in darkness. The most substantial changes in the photosynthetic intensity occur during the first 2-5 min. of illumination, when more than half of the induction losses are eliminated....

..However....even two hours after the beginning of illumination, changes are still observed in the intensity of the fixation of CO_2 and in the ratio of the products of photosynthesis."

In my experiments the period of high intensity illumination was one hour (after about one hour low intensity illumination). Since the first measurements of CO_2 exchange after this period were always lower than those made later, they doubtless reflected continued induction effects, and were not used in the results. A further factor which may affect calculations of CO_2 evolution is departure from linearity of the APS/ $[CO_2]$ curve. The present data are not amenable to analysis in this respect but a brief outline of some examples in published data is given in Fig. 5. The APS/ $[CO_2]$ curves commonly differ slightly from linearity (e.g. Decker, 1957). Equation 1 uses the instantaneous rate or slope of the APS/ $[CO_2]$ curve at a particular concentration, thus the interrelationships of the values of Equation 1 will differ slightly at different CO_2 concentrations with the curvature in APS/ $[CO_2]$. This gives results rather higher than does Decker's method (Fig. 7) of extrapolation of the APS curve. Holmgren and Jarvis (1967) found that the CCP determined directly (by adjusting the ambient CO_2 concentra-

tion until there was no net CO_2 flux) was lower than that obtained by extrapolating the straight line part of the APS/ $[CO_2]$ curve to the APS axis.

These differences are assumed to be unimportant, especially since the extent of reassimilation is unknown, in gross estimates of CO_2 evolution in light. The comparability of data obtained by CO_2-free air methods, and those from APS rates is not known. Decker (1957) found that keeping a leaf at CCP for a period ranging from a few seconds to one hour had no effect on subsequent rates of CO_2 uptake, however the effects of CO_2-free air may well be different. Thus Equation 1 and other current methods of estimation of the rate of CO_2 evolution in light are unlikely to be quantitatively precise. Lake (1967) has restated Equation 1 so as to include factors relating to the resistances to CO_2 fluxes within the leaf. Further experimentation on reassimilation of CO_2 may clarify the actual rate. However, despite the limitations on the quantitative interpretations of calculations of CO_2-evolution rates, Equation 1 and similar approximations provide a readily determinable parameter for the comparison of CO_2 exchange in different species and environmental conditions.

SUMMARY AND CONCLUSIONS

1. The CO_2 concentration at the CO_2 compensation point is linearly related to O_2 concentration in eleven of twelve plant species studied. These results agree with published data for tobacco and soybean, showing that this relationship holds in species of six plant divisions. The lack of effect of O_2 concentration on the CO_2 compensation point in corn was confirmed.

2. The calculated rate of CO_2 evolution in the light is greater than the rate of CO_2 evolution in darkness by a factor of two or more in ten of eleven species studied. (The exception, Lycopodium, is discussed.)

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