EFFECTS OF LIGHT QUALITY AND INTENSITY ON PHOTOSYNTHESIS
AND PHOTORESPIRATION IN ATTACHED LEAVES

by

NORMAN ROSS BULLEY

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EXAMINING COMMITTEE APPROVAL

Dr. G. H. Geen
Chairman
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Dr. O. Björkman
External Examiner
CARNEGIE INSTITUTION OF WASHINGTON

Dr. C. L. Kemp
SIMON FRASER UNIVERSITY

Dr. G. R. Lister
SIMON FRASER UNIVERSITY

Dr. E. B. Tregunna
UNIVERSITY OF BRITISH COLUMBIA

Dr. J. F. Turner
Visiting Professor
SIMON FRASER UNIVERSITY

Dr. W. E. Vidaver
SIMON FRASER UNIVERSITY
ABSTRACT

The object of this investigation was to study the effects of light quality on photosynthesis and photorespiration.

The effects of light quality on plants which have photorespiration (radish leaves) was measured and compared to the effects of light quality on plants which do not have photorespiration (corn). Action spectra were determined in the region where apparent photosynthesis is proportional to light intensity. The action spectra for radish leaves were measured at constant incident energy and constant incident quanta at 2 and 21% O₂ and 300 μl/l CO₂. The percent inhibition of apparent photosynthesis due to 21% O₂ at each wavelength was found to be constant. The CO₂ compensation points at 21% O₂ for light intensities greater than the light compensation point were found to be the same for the wavelength regions used.

A reduction of the light compensation point at 665 nm and low CO₂ concentrations was found at 21% O₂. The compensation point at 21% O₂ was found to be reduced in 4% O₂ by the same amount for wavelengths from 435 to 665 nm. The action spectrum for corn at 21% O₂ and 300 μl/l CO₂ was found to be unaffected by a reduction in the O₂ concentration to 2% O₂.
The amino acid fraction of the carbon-14 labelled products of photosynthesis in soybean leaves using narrow wave bands of the visible spectrum in the blue to yellow region, was found to be unaffected by light quality. A stimulation of the accumulation of sucrose was found in the blue light (450 nm) when compared to the green or yellow wavelengths or white light.

The size of the post-illumination CO$_2$ burst from soybean leaves was directly proportional to the rate of photosynthesis before the onset of darkness and was found to be unaffected by the wavelength of light in the preceding light period. The peak of the burst at 21% O$_2$ was found to occur within 8 sec after the onset of darkness and lasted for about 5 sec before beginning to decrease. No CO$_2$ burst was found for leaves at 2% O$_2$ but the kinetics of the decay of photosynthesis and the continuation of or induction of dark respiration at 2 and 21% O$_2$ appeared to be linked. The size of the burst was found to be about 8% larger than the decrease in photosynthesis produced by 21% O$_2$ when compared to the rate at 2% O$_2$. Sudden decreases in the light intensity, from 38 to 100% of the original intensity, were found to produce an apparent CO$_2$ burst even when there was still a high rate of photosynthesis after the intensity change.
High specific activity $^{14}$CO$_2$ feedings at four CO$_2$ compensation points produced by four different O$_2$ concentrations indicated that (1) the rates of CO$_2$ exchange at the CO$_2$ compensation points increased with light intensity even though the compensation points did not change, (2) the rates of CO$_2$ exchange at the compensation point and 10% O$_2$ were comparable to the rate of CO$_2$ uptake at 2% O$_2$ and the same CO$_2$ concentration and (3) these rates of CO$_2$ exchange increased at higher compensation points but tended to saturate above 40% O$_2$.

These results would indicate that photorespiration is not a blue light stimulated process with special pigments but that it is very closely linked to photosynthesis and has a rate that is much higher than the rate of respiration in the dark.
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"Photorespiration ..... a direct photochemical acceleration of normal respiration which disappears in the dark as instantaneously as does photosynthesis. The possibility of such an effect is a nightmare oppressing all those who are concerned with exact measurement of true photosynthesis."

E. I. Rabinowitch 1951

To measure what might be called the true rate of photosynthesis by a leaf, it would be necessary to measure the actual number of CO$_2$ molecules reduced to some organic compound per unit time. This would include CO$_2$ from the outside of the leaf and CO$_2$ which was being released by respiratory processes within the leaf. Some of this CO$_2$ released inside the leaf would also escape to the outside of the leaf. The measurements of rates of photosynthesis using an infrared CO$_2$ gas analyser indicate only the net rate of CO$_2$ uptake in the light which is the difference between the CO$_2$ that is entering the leaf and the CO$_2$ that is leaving the leaf. This net CO$_2$ uptake is normally referred to as apparent photosynthesis (APS). It is very difficult to measure either of the two actual rates of CO$_2$ exchange at the surface of the leaf.
What allowance to make for respiration in the light has been a subject of controversy for studies of quantum efficiency of photosynthesis, primary productivity, genetic selection of plants for crops with the highest photosynthetic capacity, intermediary metabolism, and other areas of research. Until recently most workers when correcting measurements for respiration have assumed that dark respiration continued on at the same rate in the light and used this as their value. Recently this concept has been questioned and different lines of research have given support to the theory that dark respiration is replaced or supplemented in the light by a different process called photorespiration.

If a leaf is kept at 2% O₂ rather than 21% O₂, it apparently does not evolve any CO₂. The resulting rate of CO₂ uptake has been called true photosynthesis by some authors but it does not include any CO₂ from respiration in the leaf which might be refixed before it can escape to the outside of the leaf. Dark respiration is unaffected by O₂ concentrations greater than 2%. The effect of O₂ and light on CO₂ release during photosynthesis is interpreted as evidence that photorespiration is stimulated by O₂ concentrations from 2 to 100% O₂ (6*) and increases with light intensity (21).

* Prologue references begin on page 134
It has also been found that when a photosynthesizing leaf which has photorespiration is switched from light to dark, it has a very high rate of respiration for about the first minute after it is placed in the dark (a post-illumination burst, PIB) before this high rate decreases to the normal dark rate of respiration. The size of the PIB has been shown to increase with increasing light intensities below light saturation of photosynthesis used in the preceding light period (20). Plants which have photorespiration but are at 2% O₂ do not show any signs of this PIB.

Blue light of low intensity has been reported to stimulate respiration in algae (12) and higher plants (22) and to stimulate photorespiration in some other higher plants (17, 18). A blue light stimulation of the production of amino acids (1, 23) and also of glycolic acid in algae grown in blue light (19) has been reported. Since glycolic acid has been proposed as a possible substrate for photorespiration (24, 25, 26, 27) it may be that the effects of blue light on respiration and glycolate metabolism are connected.

If photorespiration is a truly light-activated process
then it might be expected to respond to different wavelengths of light in a manner that is not the same as the action spectrum of photosynthesis. It has been found that some plants are unable to remove all of the $\text{CO}_2$ from a closed chamber. The $\text{CO}_2$ concentrations at which the equilibrium between $\text{CO}_2$ uptake and $\text{CO}_2$ evolution by the leaf are equal is called the $\text{CO}_2$ compensation point. If any particular wavelength of light were to stimulate $\text{CO}_2$ evolution, then at this wavelength there should be an increase in the compensation point relative to the value at any other wavelength of light.

Thus there was evidence that the rate of $\text{CO}_2$ evolution from leaves increases in blue light, that the $\text{CO}_2$ evolved in the light may come from the glycolate pathway in the leaf and that blue light stimulates the production of glycolate. A series of experiments was set up to determine whether photorespiration was really a process stimulated by blue light and a different process from dark respiration.

The first series of experiments (Chapter 1) were designed to measure the action spectrum of photosynthesis over the visible part of the spectrum at 21% $O_2$ for leaves that have photorespiration (radish) and for leaves that are
reported to have no photorespiration (corn). These action spectra were then compared with the action spectra for the same leaves at 2% O₂ where it has been reported that photorespiration appears to be greatly reduced. This was carried out at both normal CO₂ concentrations (300 µl/l) and at low CO₂ concentrations (< 60 µl/l).

In previous unpublished attempts to measure an action spectrum for photosynthesis and photorespiration, it was found that if narrow band-pass filters were used there was insufficient energy available for accurate measurement of the resulting rates of net CO₂ fixation. The 5,000 watt xenon arc lamp was found to have sufficient energy to carry out these measurements. The filters used for the control of light quality and intensity are described in Appendix A. To facilitate measurements of the total energy and spectral distribution of the light available from the xenon lamp and rates of net CO₂ exchange for the leaves, computer programs which would assist with each of these calculations were set up and are described in Appendices B and C.

The second series of experiments (Chapter 2) was carried out to try to determine whether blue or green light had an effect on the size of the post-illumination burst from leaves.
A kinetic analysis of the PIB was carried out over the first 16 sec after the onset of darkness in an attempt to obtain information which would help to determine whether the PIB was connected in some way to the rate of respiration which was going on in the previous light period or whether it was an oxidation process which commenced with the onset of darkness.

Since photorespiration has been shown to depend on recent products of photosynthesis and a blue light stimulation of the oxidation of this photosynthate might possibly be masked by the gas exchange techniques being used in the other experiments, Chapter 3 describes a preliminary study of the 14C-products of photosynthesis using light from the blue to the yellow regions of the spectrum isolated with narrow band-pass filters.

During the experiments in Chapter 2, the problem arose as to what the CO₂ compensation point really represented. What are the actual rates of CO₂ exchange at the compensation point and are they affected by light intensity and O₂ concentration? To answer this question, experiments using high specific activity 14CO₂ for feedings at the CO₂ compensation point were carried out and are described in
Chapter 4. Appendix D describes experiments carried out to determine the sensitivity of the infrared CO\textsubscript{2} gas analyser to $^{14}$CO\textsubscript{2}. This information was required before the experiments of Chapter 4 could be attempted.

Photorespiration as defined by Rabinowitch is only measured by the isotope techniques in Chapter 4. Chapters 1 and 2 depend on there being a high O\textsubscript{2} requirement for photorespiration. Chapter 2 also requires a continuation of a constant rate of photorespiration for a few seconds after a change in the rate of photosynthesis due to a reduction in the light intensity.
Chapter 1

Photosynthesis: Action Spectra for Leaves in Normal and Low Oxygen
INTRODUCTION

There is much evidence available (22) to support the theory that many plants give off CO$_2$ in the light by a process that is not the same as the one responsible for the evolution of CO$_2$ in the dark. Fixation of CO$_2$ by photosynthesis makes the direct measurement of CO$_2$ evolution in the light (photorespiration) difficult. A comparison of the major methods for estimating the rate of photorespiration has been made by Hew (9). He found that the methods of Decker (5), Tregunna et al. (19), Bidwell (1) and the direct measurement of CO$_2$ evolution into CO$_2$-free air gave similar values.

Whether photorespiration is a true light stimulated process with its own pigments is not known. There is evidence that blue light of low intensity stimulates respiration in *Chlorella* (13). Voskresenskaya (21) has shown that short wave radiation (400 to 580 m$\mu$) of low intensity stimulates oxygen uptake in tobacco and broadbean leaves. Poskuta has reported a three fold increase in photorespiration in blue light relative to the rate in red light for spruce (17), wheat, soybean, oleander, and swiss chard (16). On the other hand, plants unable to carry out photosynthesis are also
unable to carry out photorespiration (6, 10).

If the action spectra of photosynthesis and photorespiration are different, then the action spectrum of photosynthesis measured at 21% \( O_2 \) is really a composite of the action spectra of photosynthesis and photorespiration. It has been reported that photorespiration is greatly reduced by lowering the oxygen concentration from 21% \( O_2 \) to 2% \( O_2 \) (7, 19). Thus the difference between the action spectra of net \( CO_2 \) uptake at 2% \( O_2 \) and 21% \( O_2 \) may be used to represent the action spectrum of photorespiration. Björkman (2) has reported that the percent inhibition of net \( CO_2 \) uptake due to 21% \( O_2 \) is greater in far red light (40% at 704 \( \mu m \)) than in red light (31% at 654 \( \mu m \)) for \textit{Plantago} at low light intensities (less than 5\text{nW/cm}^2\text{sec}^{-1} \text{absorbed}). He also reported that the percent inhibition of net \( CO_2 \) uptake caused by 21% \( O_2 \) when compared to the rate at 0.2% \( O_2 \) is the same for wavelengths of light from 440 to 700 \( \mu m \) for \textit{Mimulus cardinalis} (3). The following report describes experiments which compare the action spectra of apparent photosynthesis at 21% and 2% oxygen for radish, a plant which has photorespiration, and for corn, a plant which is reported to have no photorespiration (8). The experiments were carried out at both 300
\( \mu l/l \) CO\(_2\) and at the CO\(_2\) compensation point.

**MATERIALS AND METHODS**

Radish and corn plants were grown from seed in pots of garden soil and kept in a greenhouse at 19\(^\circ\) to 24\(^\circ\). The day length was held at 16 hr by the use of fluorescent lighting. Twenty day old radish plants and fifty day old corn plants were moved to a growth chamber which was maintained at a day/night temperature of 25\(^\circ\)/17\(^\circ\) with a 16 hr photoperiod of 1000 ft-c supplied by Sylvania grow lux fluorescent tubes supplemented with incandescent lamps. The plants were kept in the growth chamber for at least two days before being used.

The radish leaves to be used were not fully expanded and were selected for a cross sectional area of about 9 sq. cm. on one side. An individual attached blade was sealed in a plexiglass chamber which was blackened on the outside except for a 9.6 cm\(^2\) circular area on the front to permit the passage of light. Leaf temperature was continuously recorded by a copper-constantan thermocouple located in the leaf chamber on the darkened side of the leaf.

For the corn leaf, two circular chambers were clamped
together, one on either side of the leaf, enclosing 9.6 cm$^2$ of leaf tissue. The front of the chamber was clear, the back and sides of the chamber were black. The air stream passing through the chamber was split to pass over both sides of the leaf and rejoined outside the chamber.

Both an open and a closed system were used to measure rates of net CO$_2$ uptake (apparent photosynthesis, APS) in different wavelength regions of the spectrum and at different oxygen concentrations.

In the closed system, air was continuously circulated through the leaf chamber, flow meter, and infrared CO$_2$ gas analyser (Beckman, Model 215) at 2.5 l/min. The gas analyser (IRGA) was standardized at the start and finish of each day's experiments using standard gas mixtures of 350 and 100 µl/l CO$_2$ from Matheson of Canada Ltd. The volumes of the systems including the chamber were 216±6 ml for the corn. Knowing the volume of the system and the rate of disappearance of CO$_2$ from this volume, the rate of apparent photosynthesis for a given CO$_2$ concentration can be calculated. The O$_2$ concentration was measured with a Beckman oxygen electrode (Model 777).

An attached leaf was placed in the plexiglass chamber in
white light of 2,500 ft-c, the CO₂ concentration was held at 325 μl/l. After a constant rate of apparent photosynthesis had been reached (in about 30 min) the system was closed and the CO₂ concentration in the system recorded with time down to the CO₂ compensation point. The system was then flushed with the standard gas mixture (350 μl/l CO₂ in air), closed, and the CO₂ concentration recorded to the compensation point. The light intensity or quality was then changed and the cycle repeated. The rate of apparent photosynthesis for different CO₂ concentrations was determined first at 21% O₂ and then at 2.0±0.5% O₂ for a given light quality and intensity. It was found that the subsequent rate of apparent photosynthesis at 21% O₂ was the same as before the low O₂ treatment. The rate of dark respiration was measured at the end of each day's experiment.

In the open system, gas of 340 μl/l CO₂ and 2% O₂ or 350 μl/l CO₂ and 21% O₂ was passed over the leaf at 400 ml/min. The change in CO₂ concentration was measured with a Beckman infra-red CO₂ analyser and the flow rate with a Matheson flowmeter (model 302). The rate of net CO₂ uptake was calculated from the change in CO₂ concentration times the flow rate. In both the closed and open systems, after any
change in light intensity or quality a four minute adaptation period at 350 μl/l CO₂ was found to be sufficient for the leaves to reach a constant rate of apparent photosynthesis. The rates of photosynthesis at 450 mμ and 665 mμ were measured at the start and end of each day's experiments to be sure that the rates remained constant during the day and to use as a standard in comparing the results recorded on different days. Changes in gas flow rates above 350 ml/min in the open system were found to have no significant effect on the rate of apparent photosynthesis.

During the experiments, the leaf was illuminated by a 5,000 watt xenon lamp. The light was focused with a quartz lens and passed through a 15 cm water filter and a heat filter (Dicrolite). The interference filters used for the isolation of the wavelength regions were Balzer Filtraflex B-40 (HW 11±1) and Schott Depal (HW 17±3). The combination of filters used reduced the second-order spectrum to less than 1% transmission. The light intensity was controlled with Balzer neutral density filters and recorded with a Yellow Springs Instrument Co. radiometer (Model 65). The sensing probe was housed in a brass heat sink to reduce base line drift due to external temperature fluctuation. The
light intensity at the edge of the leaf was found to be 60% of that at the center. Values reported here are the intensities at the center of the leaf.

An ISCO spectroradiometer was used to determine the effects of high light intensity on the spectral distribution of the interference filters. The temperature on the back side of the filter increased from 24° to 29° ± 1° after 1/2 hour exposures to the high intensity xenon lamp but the 1/2 band width of the filters showed no measurable change.

EXPERIMENTAL AND RESULTS

A series of experiments was carried out to find the relationship between apparent photosynthesis and light intensity at each wavelength since saturating light intensities could not be obtained at all wavelengths. Fig 1 illustrates the results found for two wavelengths on one day for a radish leaf. The rate of apparent photosynthesis was proportional to the light intensity over the range of intensities studied and was found to be so for all wavelength regions studied at both 21% O₂ and 2% O₂ for radish and at 21% O₂ for corn. Similar results were found using both open
Figure 1  Effect of light intensity on net CO₂ fixation rates (APS) in attached radish leaves at 300 μl/l CO₂ and 21% O₂ for narrow wavelength regions 619 mμ and 450 mμ.
and closed systems. A minimum light intensity of about
0.75 \times 10^4 \text{ ergs cm}^{-2} \text{ sec}^{-1} was required for net CO$_2$ fixation
at 300 \mu l/l CO$_2$. Results from three or more days' experiments
were used to make graphs of apparent photosynthesis vs
incident light intensity for each wavelength region. No
fewer than twelve points for radish and eight points for
corn between the values of 3 \times 10^4 and 7 \times 10^4 \text{ ergs cm}^{-2} \text{ sec}^{-1}
were used for any one wavelength. From these results, values
of apparent photosynthesis for a given light intensity at a
given wavelength were found by interpolation. The estimate of
variance of the predicted value of the photosynthetic rate
(Y in (4)) for the selected light intensity of 4.0 \times 10^4 \text{ ergs cm}^{-2} \text{ sec}^{-1}
varied from \pm 2 to \pm 5\% depending on the wavelength.

Radish

Fig 2 represents the action spectrum of net CO$_2$ uptake
by radish leaves at 300 \mu l/l CO$_2$ and 21\% O$_2$. The rate of
apparent photosynthesis is relatively constant with wavelength
for a constant incident energy of 4.0 \times 10^4 \text{ ergs cm}^{-2} \text{ sec}^{-1}
from 435 m\mu to 550 m\mu with a 20\% dip at 520 m\mu. The rate of
apparent photosynthesis then increases with increasing wave-
length to a peak around 665 m\mu and then drops rapidly for wave-
lengths above 680 m\mu. The ratio of the peak rates at equal
Figure 2  Action spectra of net CO₂ fixation rates in attached radish leaves for constant incident energy and for constant incident quanta at 300 μl/l CO₂ and 21% O₂.
The energies of red and blue light was red:blue equals 1.5:1.0. To obtain an action spectrum at constant incident quanta, the number of quanta equal to $4.0 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ at 547 m$\mu$ was calculated and then the energy equal to this number of quanta calculated for each wavelength. Using this value, the rate of apparent photosynthesis for this energy could be found from the graphs of apparent photosynthesis vs intensity by interpolation as before. Plotted in this manner, the action spectrum in Fig 2 indicates a greater rate of apparent photosynthesis in the blue part of the spectrum than for an equivalent number of quanta in the red with the same dip as before in the green and the same sharp drop after 680 m$\mu$.

These action spectra represent net CO$_2$ uptake where both photosynthesis and photorespiration occur. It has been shown that photorespiration has a high O$_2$ requirement (7, 19). Therefore any effect of photorespiration on the action spectrum can be eliminated by measuring the action spectrum for photosynthesis at a low O$_2$ concentration. Fig 3 indicates the action spectrum of apparent photosynthesis at 300 µl/l CO$_2$, constant incident energy and 21% O$_2$, or 2% O$_2$. The general shapes of the two curves are similar, but there is a greater difference between the two curves at the longer wavelengths.
Figure 3  Action spectra of net CO₂ fixation rates in attached radish leaves at 2% O₂ and 21% O₂ for a constant incident energy of 4.0 x 10⁴ ergs cm⁻² sec⁻¹ and 300 μmol CO₂·l⁻¹.
than at the shorter. The rates of apparent photosynthesis at both \( O_2 \) concentrations were also higher at the longer wavelengths than at the shorter. Therefore comparison of the absolute rates of photosynthesis do not indicate clearly whether lowering the \( O_2 \) concentration alters the shape of the action spectrum of photosynthesis. The comparison of shape was done by looking at the proportional effect of \( O_2 \) on rates at different wavelengths.

A standard value of apparent photosynthesis of 5.5 mg \( \text{CO}_2 \ \text{dm}^{-2} \ \text{hr}^{-1} \) at 21% \( O_2 \) and 300 \( \mu \text{l/l} \ \text{CO}_2 \) was chosen. The intensity of light needed to give this rate at each wavelength was found from the graphs of apparent photosynthesis vs intensity. Using this intensity, the rate of apparent photosynthesis at 300 \( \mu \text{l/l} \ \text{CO}_2 \) and 2% \( O_2 \) was found for each wavelength, and these values were plotted as shown in Fig. 4. The value of dark respiration (DR) is included to indicate its size relative to the difference between the rates of apparent photosynthesis at 21% and 2% \( O_2 \). The experimental error found for any one wavelength can account for the differences in rates of apparent photosynthesis for the different wavelengths at 2% \( O_2 \). Thus at 300 \( \mu \text{l/l} \ \text{CO}_2 \) the lowering of the \( O_2 \) concentration has the same effect on
Figure 4  Effect of lowering the oxygen concentration from 21% to 2% O₂ for constant rates of net CO₂ fixation at 21% O₂ and different wavelengths of light for attached radish leaves. DR is the rate of respiration after 30 minutes in the dark.
apparent photosynthesis across the visible part of the spectrum from 402 to 700 m\(\mu\).

The action spectra described above were measured at 300 \(\mu\)l/l \(\text{CO}_2\) where the rate of \(\text{CO}_2\) assimilation is much greater than the rate of \(\text{CO}_2\) production. Any effect of light quality on photorespiration would be more apparent where these processes have equal rates. To see if the direct relationship between photosynthesis and photorespiration exists at low \(\text{CO}_2\) concentrations, the interactions among light intensity, oxygen concentration, and \(\text{CO}_2\) concentration, were studied at the \(\text{CO}_2\) compensation point. Typical results for the wavelengths studied (450, 501, 547, 601, 665 m\(\mu\)) at both 21% and 4% oxygen are shown in Fig 5. The values of the \(\text{CO}_2\) compensation point at different light intensities for 450, 501, 547, 601 m\(\mu\) are combined in curve (a). Curve (b) is for 665 m\(\mu\). The two curves are not significantly different for intensities greater than \(0.7 \times 10^{-8}\) ein cm\(^{-2}\) sec\(^{-1}\). For light intensities less than this, the \(\text{CO}_2\) compensation point tends to be lower for the longer wavelength (665 m\(\mu\)) than the other shorter wavelengths even though the rates of apparent photosynthesis at 300 \(\mu\)l/l \(\text{CO}_2\) and constant quanta in the red are slightly lower than those in the blue.
Figure 5  Effect of light intensity on the CO$_2$ compensation point for attached radish leaves at 21% and 4% O$_2$ for wavelengths: (a) 601, 547, 501, 450 m$\mu$ and (b) 665 m$\mu$. 
region of the spectrum. For the five wavelengths studied, the 
CO₂ compensation point was constant for light intensities 
above 1.0 x 10⁻⁸ ein cm⁻² sec⁻¹ (light compensation point at 
60 μ1/l CO₂ and 21% O₂) and was independent of wavelength.
Under these conditions either photosynthesis and photorespira-
tion were light saturated (do not increase with increasing 
light intensity) or if photosynthesis increases with increasing 
light intensity then photorespiration must increase by an equal 
amount. Thus photosynthesis and photorespiration at light 
intensities above light compensation point are directly related 
at 21% O₂ and low CO₂ concentrations as well as at higher CO₂ 
concentrations. Fig 5 also shows the effect of lowering the 
oxygen concentration to 4 percent on the CO₂ compensation 
point. When light was saturating (1.0 x 10⁻⁸ ein cm⁻² sec⁻¹) 
the CO₂ compensation point was lowered in proportion to the 
oxygen concentration; at lower light intensities the curve 
changes more sharply from a horizontal line to an almost 
vertical line. Lowering the oxygen concentration did not 
cause a proportional reduction in the minimum light intensity 
required to achieve compensation. Dark respiration was 
probably contributing CO₂ under these conditions.
Corn

The action spectrum of net CO$_2$ uptake for corn at 300 μl/l CO$_2$, 21% O$_2$ and constant incident energy is shown in Fig 6. The spectrum is much like that for radish except for a broader dip in the green portion of the spectrum. The reduction of the O$_2$ concentration from 21% to 2% had no significant effect on the rates of net CO$_2$ fixation at 300 μl/l CO$_2$ for the wavelength regions tested from 435 μm to 665 μm. It was also found that for a given light intensity, the curve of apparent photosynthesis vs CO$_2$ from 300 μl/l CO$_2$ to less than 9 μl/l CO$_2$ was unchanged by lowering the O$_2$ concentration from 21% to 2%.

Since corn is reported to have a very low CO$_2$ compensation point and therefore no photorespiration, the effect of light intensity on this low compensation point was studied to compare with a similar curve for radish measured at 4% O$_2$ where photorespiration is considered to be greatly reduced. Fig 7 shows that the compensation point remained very low for light intensities greater than 0.3 x 10$^{-8}$ ein cm$^{-2}$ sec$^{-1}$ and was found to be constant and less than 9 μl/l CO$_2$. No effect of wavelength on the CO$_2$ compensation point at low light intensities was found.
Figure 6 Action spectrum of net CO$_2$ fixation rates in attached corn leaves for constant incident energy of $4.0 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ at 300 $\mu$l/l CO$_2$ and 21% O$_2$. 
Figure 7 Effect of light intensity on the CO$_2$ compensation point for attached corn leaves at 21% O$_2$. 
DISCUSSION

The rates of net CO$_2$ uptake by radish leaves at 300 μl/l CO$_2$ were measured for light intensities below saturation but above those intensities required for light compensation. At these intensities net CO$_2$ uptake was found to be proportional to incident light intensity for all of the wavelength regions studied. From the graphs of apparent photosynthesis vs light intensity at the different wavelength regions of the visible part of the spectrum, rates of apparent photosynthesis at constant energy or constant quanta can be selected to plot an action spectrum. These action spectra, measured where both photosynthesis and photorespiration occur, can then be compared with action spectra obtained under conditions where photorespiration is considered to be greatly reduced.

As indicated by Fig 2, if the action spectrum is based on constant incident energy, the red portion of the spectrum is more effective at carrying out photosynthesis than either the blue or green regions. If the comparison is made on the basis of a constant number of quanta in each region of the spectrum, then the blue region of the spectrum becomes as effective or
even slightly more effective than the red portion of the spectrum. Considering that on a normal sunny day, the light from the sun has an approximately equal incident energy distribution across the visible part of the spectrum, the constant energy action spectrum may be the more meaningful in indicating the relative contribution of each part of the visible spectrum to net CO₂ fixation. To see the effect of CO₂ evolution in the light on the action spectrum of photosynthesis, Fig 3 compares the rates of net CO₂ fixation at 21% O₂ and 2% O₂ and constant incident energy. The comparison indicates that the CO₂ evolution due to the increase in O₂ concentration is present in all parts of the visible part of the spectrum. The greatest difference between the rates of net CO₂ fixation at 2% O₂ and 21% O₂ occurs at 665 mμ. If constant rates of photosynthesis are obtained at the different wavelengths and 21% O₂, then lowering of the O₂ concentration to 2% to reduce photorespiration results in the same increase in net CO₂ fixation. The above results indicate that at a constant CO₂ concentration and increasing light intensity, the inhibitory effect due to O₂ increases with increasing apparent photosynthesis but that the percentage inhibition of photosynthesis is relatively constant.
These results confirm those found by Björkman (3) for *Solidago viraeura* and *Mimulus cardinalis* in which he found a constant percentage inhibition of CO\textsubscript{2} fixation for the wavelength regions studies at 300 \textmu l/l CO\textsubscript{2} when the oxygen concentration was increased from 0.2\% to 21\% O\textsubscript{2}. Such effects have also been reported previously for studies with white light (2, 20). The percentage inhibition of photosynthesis due to O\textsubscript{2} was not found to be greater at the longer wavelengths as has been reported earlier (2). The rate of apparent photosynthesis for radish at 709 m\textmu was very low being about 11\% of the rate at 654 m\textmu for equal incident quanta of $1.0 \times 10^{-8}$ ein cm\textsuperscript{-2} sec\textsuperscript{-1}. The lowering of the oxygen concentration at 709 m\textmu did increase the rate of apparent photosynthesis at this wavelength but accurate reproducible results were difficult to obtain due to the very low rates. Since in his earlier paper (2) Björkman reported rates of net CO\textsubscript{2} fixation at 704 m\textmu equal to about 38\% of those at 654 m\textmu for equal incident quanta, of $0.5 \times 10^{-8}$ ein cm\textsuperscript{-2} sec\textsuperscript{-1} his experimental conditions were apparently quite different from ours.

The increase in net CO\textsubscript{2} fixation due to the lowering of the O\textsubscript{2} concentration was not observed in corn. This lack of response of photosynthesis to O\textsubscript{2} concentrations from 2\% O\textsubscript{2} to
21% O₂ in corn was found for all wavelengths tested and for CO₂ concentrations from 300 µl/l to the CO₂ compensation point of less than 9 µl/l CO₂.

The action spectra for corn and radish indicated that rates of apparent photosynthesis in 21% O₂ are higher in the green part of the spectrum than might be expected from absorption spectra of isolated chloroplasts (12). The ratio of the rate in red light to the minimum in green light was 1.7:1.0 for radish, 1.9:1.0 for wheat (11) and 1.6:1.0 for Euphorbia milli (14). The ratio of the rates at equal energies of red and blue light was red:blue equals 1.5:1.0 for radish as compared to 1.3:1.0 for wheat (11) and 1.1:1.0 for Euphorbia milli (14). Thus whole leaves appear to be very effective in their ability to use wavelengths of light in the green and blue portions of the spectrum. The action spectra for radish and corn are quite different in shape from that published earlier by Hoover for wheat but have similar peak rates around 440 and 660 mµ and a minimum at about 520 mµ.

The effects of O₂ on photosynthesis are not clearly understood. Several explanations are possible for the inhibitory effects of oxygen on net CO₂ fixation (20). Since there is no measurable inhibitory effect of oxygen from 2% to 21% on corn
and other low compensation point plants, it seems unlikely that the large changes in net CO$_2$ fixation that we observe are primarily due to the effects of O$_2$ on the photochemical steps of photosynthesis.

The evidence given here that photorespiration and photosynthesis are closely linked at all wavelengths in the visible part of the spectrum and that an increase in photosynthesis is accompanied by a proportional increase in photorespiration would support the findings of Hew and Krotkov (10) and Downtown and Tregunna (6). Hew and Krotkov (10) found that plants having their normal photosynthetic processes either inhibited or blocked by a mutation, also lacked photorespiration. Downtown and Tregunna (6) found that in wheat leaves which had their photosynthetic capacity blocked by 3-(3,4-dichlorophenyl)-1,1 dimethyl urea (DCMU), the CO$_2$ that was evolved in the light was not due to photorespiration since it was insensitive to high O$_2$ concentration and thus more likely due to dark respiration. Thus anything that appears to interfere with photosynthesis also seems to affect photorespiration.

The above conclusions were also valid at the CO$_2$ compensation point where CO$_2$ uptake and evolution are equal. There was no effect of changing wavelength on the CO$_2$
compensation point for intensities greater than $1.0 \times 10^{-8}$ ein cm$^{-2}$ sec$^{-1}$. This more critical test of the effect of wavelength on the interaction between photosynthesis and photorespiration did indicate a differential effect of 665 μ light versus the effect of shorter wavelengths when energies less than $0.7 \times 10^{-8}$ ein cm$^{-2}$ sec$^{-1}$ were used.

The differential effects of wavelength on the CO$_2$ compensation point of radish leaves at low light intensities may be related to reports by Kowallik and Gaffron (12) and Voskresenskaya (21) of effects of blue light on respiration. These effects are probably not related to photorespiration. A stimulation of photorespiration by blue light at the intensities reported by Poskuta et al. (16, 17) was not found in radish or soybean (Builey, unpublished).
LITERATURE CITED


Chapter 2

The Post-illumination CO$_2$ Burst and Its Possible Relationship to Photorespiration
INTRODUCTION

It has been reported that the rate of respiration for some green leaves in the light (photorespiration) is higher than in the dark (16). It has also been reported that photorespiration increases with increasing light intensity and high \(O_2\) concentration and is thus not the same process as dark respiration which is unaffected by \(O_2\) concentrations greater than 2% \(O_2\) (5, 16).

When a green leaf in air (350 \(\mu\)l/l \(CO_2\), 21% \(O_2\), balance \(N_2\)) is switched from light to dark, there appears to be a high initial rate of \(CO_2\) evolution. It has been proposed that this post-illumination \(CO_2\) burst (PIB) is a continuation of a high rate of respiration which was proceeding in the light (3, 12). This interpretation has been challenged (10) and an alternative has been proposed (2).

If the PIB is the extension of photorespiration into the dark period, it could be useful as a quantitative measurement. Other approaches to the measurement of photorespiration suffer from the problem of fixation of \(CO_2\) before it can leave the leaf. Samish and Koller attempt to correct for this, but there appears to be an error in their equation 6 (13) which would invalidate the calculations.
The rate of photosynthesis of some leaves is not affected by high O\textsubscript{2} concentrations (6). These leaves are reported to have no photorespiration, no PIB (15) and a metabolic pathway for the fixation of CO\textsubscript{2} which is not the same as the Calvin cycle (8, 9).

The purpose of the following experiments was: (1) to study the effect of O\textsubscript{2} on the kinetics of the PIB in a plant which has photorespiration; (2) to study the effect of light intensity and quality in the preceding light period on the magnitude of the PIB; (3) to see if there was any relationship between the size of the PIB and the inhibitory effect of 21% O\textsubscript{2} on the rate of net CO\textsubscript{2} fixation in the light in 2% O\textsubscript{2}; and (4) to study the effect of small changes in light intensity to determine whether the PIB can be observed without darkening the leaf.

MATERIALS AND METHODS

Soybean plants (Glycine max L. var. Comet) were grown from seed in pots of garden soil and kept in a growth chamber which was maintained at a day/night temperature of 25°/17° with a 16 hr photoperiod of 1,000 ft-c supplied by
fluorescent and incandescent lamps.

A single attached soybean leaflet on the second trifoliate leaf was sealed in a plexiglass chamber and illuminated with white light of 2,500 ft-c from a 5,000 watt xenon lamp. The control of the light intensity and quality of the system is described in Appendix A. The chamber is darkened on the sides and back.

The inside of the chamber is cylindrical (radius = 1.8 cm, depth = 0.6 cm) with four fine copper wires set in the plexiglass to hold the leaf in the center of the chamber, ensuring the flow of air over both sides of the leaf. The total volume of the chamber is 6.4 ml and with the flow rate of 6.66 ml/sec, the gas in the chamber was changed about once every second. An open ended gas flow system was used. Standard air (350 µl/l CO₂, 21 or 2% O₂, balance N₂) was passed through one of the cells of the infrared gas analyser (Beckman Model 215), over the leaf, back through the other cell of the analyser, through a flow meter and out to atmosphere. The analyser was calibrated for a full scale deflection of 100 µl/l CO₂ (265 - 365 µl/l CO₂) and has a scale adjustment switch which expands 1/6 of the scale to full scale, giving a difference of 16 2/3
µl/l CO₂ as full scale deflection on a 10 cm null point recorder (Riken Denshi Model SP-J2). A one hour adaptation period in white light and standard air was carried out at the start of each experiment. The light intensity and/or quality was then adjusted to the desired experimental conditions and 15 min light, 5 min dark cycles were given.

Using the standard air and a flow rate of 400 ml/min the time response of the system was found by injecting small amount of CO₂ (0.1 ml of CO₂ in nitrogen in <1 sec) into the empty leaf chamber. The time for any change in the CO₂ concentration in the leaf chamber to be seen in the IRGA was 2.5 ± 0.5 sec and the time taken for the CO₂ entering the IRGA to reach the exit and begin leaving was 12 ± 0.5 sec. Since the volume of the IRGA measuring cell is 100 ml and the flow rate is 400 ml/min, if no mixing occurs it should have required 15 sec for the CO₂ to travel from one end of the IRGA to the other. Thus, some mixing does occur but, based on the conditions present, the Reynolds number for the gas flow in the IRGA measuring cell is greater than the critical point which should ensure turbulent rather than laminar flow.
RESULTS AND DISCUSSION

Kinetics of the Post-illumination Burst

Fig 1 is a reproduction of the gas exchange data produced when a soybean leaf in white light (17 x 10^4 ergs cm^-2 sec^-1 between 400 and 700 nm) is placed in the dark. The gas entering the leaf chamber was 350 µl/l CO₂, 21% O₂, balance N₂. As the CO₂ concentration begins to rise after the onset of darkness, the scale expansion switch is thrown as the CO₂ concentration passes 350 µl/l making the CO₂ concentration readings during respiration more pronounced. The 2.5 sec time delay of the system between changes in the CO₂ concentration in the chamber and the time for these changes to be recorded by the IRGA is shown in Fig 1 and subtracted from all other reported readings. It can be seen that there is an abrupt increase in the CO₂ concentration as measured by the IRGA which reaches a constant rate of change after about 7 sec and this constant slope lasts for about 6 sec. The IRGA reading becomes greater than 350 µl/l CO₂ after 13 sec. This reading reaches a peak after about 22 sec, falls to a slight dip and then tends to level off after about 2 min.

Fig 2 (...) shows the IRGA recording of a similar graph (without the scale expansion) compared to a simplified
Figure 1  Net CO$_2$ uptake and evolution rates for a soybean leaflet at 21% O$_2$ during the dark period following a 15 min light period.
Figure 2 Net CO$_2$ uptake and evolution rates for a soybean leaflet at 21% O$_2$ during the dark period following a 15 min light period. (-----) is the actual IRGA recording, (——) is the CO$_2$ concentration entering the IRGA based on the changing slope of the recorded CO$_2$ concentration in the IRGA, (▼▼▼) is the theoretical IRGA reading based on the calculated CO$_2$ concentration entering the IRGA.
IRGA recording
- CO₂ entering IRGA
△ predicted IRGA value based on CO₂ entering

15 min light

Time (sec)

0 8 16 24 32 40 48

μM CO₂

365
360
355
350
345
340
335
330

mg CO₂ H₂O dm⁻²

+10 +8 +6 +4 +2 0 -2 -4 -6

-45-
graph of the CO₂ concentration which must have been entering the IRGA to produce the IRGA recording (Fig 2 ———). Since the concentration of the gas in the IRGA is known at the onset of darkness and it is the same throughout the length of the measuring cell, the true CO₂ concentration of the gas entering the IRGA can be calculated from the recorded average CO₂ concentration in the cell and the known CO₂ concentration leaving the cell. After 12 sec the CO₂ concentration leaving the IRGA is no longer constant and will begin to increase slowly. After 15 sec the CO₂ that entered the IRGA at the onset of darkness will be leaving and due to mixing, its exact concentration is very difficult to determine.

It can be seen from the calculated values of the CO₂ concentration entering the IRGA (Fig 2 ———) that there is a net CO₂ evolution from the leaf within 5 sec after darkness and that this rate of CO₂ evolution appears to reach a plateau at about 7 sec. This high constant rate of CO₂ evolution continues for about 6 sec and then drops off sharply. It then reaches a slowly increasing rate of respiration after about 1 min. Based on the simplified values for the CO₂ concentrations that were entering the IRGA during the first 15 sec, the theoretical readings of the IRGA can be calculated and compared
to the actual reading. Fig 2 indicates that the recorded readings (.....) and the theoretical readings (vvvv) based on the calculated CO₂ concentrations entering the IRGA (-----) are in close agreement.

The data for a soybean leaf under the same conditions as in Fig 2 except at 2% O₂ is given in Fig 3. Curve (······) is the actual recording, curve (-----) is a simplified calculation of the CO₂ concentration that must have been entering the IRGA to give curve (······) over the first 14 sec and curve (vvvvv) is the theoretically predicted IRGA readings based on curve (-----). It can be seen that the CO₂ concentration rises sharply at the onset of darkness to a net rate of CO₂ evolution after about 7 sec and continues to rise at a slower rate up to the 14 sec mark. There was no evidence of any PIB. The IRGA reading reached 350 μl/l CO₂ after 13 sec of darkness in 21% O₂, and after 19 sec in 2% O₂. Preceding photosynthetic rates of 5 to 15 mg CO₂ hr⁻¹ dm⁻² did not affect these times. The values of respiration after 60 sec at 21 and 2% O₂ are not significantly different and both rates were slowly rising to a second peak which occurred after about 2.5 min before both curves declined to a more constant rate of dark respiration after about 15 minutes.
Figure 3 Net CO$_2$ uptake and evolution rates for a soybean leaflet at 2% O$_2$ during the dark period following a 15 min light period. (-----) is the actual IRGA recording, (- - - -) is the CO$_2$ concentration entering the IRGA based on the changing slope of the recorded CO$_2$ concentration in the IRGA, (vvvv) is the theoretical IRGA reading based on the calculated CO$_2$ concentration entering the IRGA.
It should be noted that at 21% O₂ the rate of CO₂ evolution reaches a maximum at the same time that a net CO₂ evolution rate begins at 2% O₂. Thus at 2% O₂, 7 sec are required for the rate of dark respiration and the decreasing rate of photosynthesis to become equal. If this same balance were to occur at 21% O₂ and superimposed on this was a continuing rate of photorespiration, the peak rate of CO₂ evolution would be expected to occur after 7 sec. Since the dark rate of respiration is still increasing after 7 sec at 2% O₂ and at 21% O₂ there is a plateau, this would indicate that the rate of photorespiration must be dropping. The actual CO₂ concentrations entering the IRGA cannot be determined accurately between the 16 and 50 sec after darkness. But since the two recordings of the CO₂ concentration entering the IRGA at 2 and 21% O₂ come together after about 50 sec, photorespiration is apparently reduced to zero between 16 and 50 sec after darkness.

The difference between the two curves at 2 and 21% O₂ could be accounted for if, at the low O₂ concentration, CO₂ fixation was prolonged in the dark. This could effectively remove any indication of the PIB at 2% O₂. If, as has been recently reported in isolated chloroplasts (7),
O₂ does not affect the light reactions of photosynthesis, then the reducing power generated for a given light intensity at 2 and 21% O₂ should be the same. Since the rate of CO₂ fixation is either higher at 2% O₂ than at 21% O₂ (if O₂ inhibits photosynthesis) or is similar to the rate at 21% O₂ (if O₂ stimulates respiration and does not affect photosynthesis), then the size of the remaining reducing pool at 2% O₂ would be the same as, or smaller than, the pool at 21% O₂ at any one time. At the onset of darkness, this remaining pool at 2% O₂ then should be used up at the same time or earlier than at 21% O₂. From this simplified view, it does not appear likely that photosynthesis in the dark would be prolonged at 2% O₂ when compared to 21% O₂. Similarly, if the size of the reducing pool were to affect the continuation of photosynthesis in the dark, then the timing of the peak PIB should have changed with large changes in the preceding light intensity. This was not found. The peak always occurred at the same time, independent of the previous light intensity.

Based on the study of the kinetics of the PIB in soybean leaves at 350 μl/l CO₂, the PIB reaches a peak within 7 sec after the onset of darkness. The timing and shape of the
burst as seen by direct examination of the IRGA recording is an artifact of the system. The slow (between 30 and 60 sec) times reported previously for the PIB to reach its maximum (2, 3, 14, 15) may be due to the same type of error. The reports of the PIB occurring between one and two minutes after the onset of darkness (4, 10, 12) are related more to the second peak which was observed in these experiments after about 2 min. This second peak did appear to be affected by light intensity but not by $O_2$ concentrations between 2 and 21% $O_2$. Most of these reported bursts at longer times probably include both the initial burst and this slower, longer burst.

Heath and Orchard (10) have reported that the PIB at 12° increased two fold for changes in light intensity from 100 to 2700 ft-c but that the compensation point was unaffected by light intensity. They felt that since the compensation point appears to reflect the rate of respiration in the light and that the PIB and the compensation point respond differently to light, then the PIB can not be related to the light respiration rate. Their assumption that the rates of $CO_2$ exchange at the compensation point do not change with light intensity is not supported by the results for soy-
bean (Chapter 4). The increase in the rate of CO₂ fixation with light intensity at the compensation point could explain their increases in the CO₂ burst with light intensity.

It has been shown that *Amaranthus edulis*, a low compensation point plant, does have a PIB at 21% O₂ (2). This would indicate that the PIB is not related to photorespiration. But, since the PIB is normally removed by low O₂ concentrations, and the PIB of *A. edulis* was only slightly reduced by using 2% O₂, it is possible that the PIB in *A. edulis* is a different process and unrelated to the PIB that we observe here. Thus for leaves at 2% O₂, the curve of the CO₂ concentration entering the IRGA versus time (Fig 2 ′′′) appears to be the resultant of two processes, the decay of CO₂ fixation and the restoration or continuation of the rate of dark respiration. At 21% O₂ (Fig 1 ′′′) the equivalent curve would appear to be the resultant of the same two processes plus a third process which was proceeding in the light but which does not begin to decay until at least 7 sec into the dark period.
Effect of Wavelength on the PIB Size and its Relationship to the Inhibitory Effect of 21% O$_2$ on Photosynthesis

If the rate of CO$_2$ evolution which occurs 8 sec after a leaf is placed in the dark is used to represent the size of the PIB, then these values of the PIB can be compared in relation to various pretreatments in the preceding light period. For this study, the PIB was measured at 21% O$_2$ using five different wavelength regions of the spectrum (peak transmission of 470, 520, 568, 613 and 670 nm) in the preceding light period and the rate of CO$_2$ evolution after 8 sec of darkness was compared to the previous rate of photosynthesis (Fig 4). For the comparison, the PIB has been added to the rate of photosynthesis in the preceding light period to give an indication of the size of the total rate of CO$_2$ uptake from the atmosphere if the PIB really does represent the rate of respiration in the light.

The equation of the line in Fig 4 using a linear regression analysis is

$$y = 1.72x + 0.05$$

The points on the figure are a composite of the previously listed wavelengths. The PIB was found to be affected by all
Figure 4 Effect of light intensity and quality on the magnitude of the post-illumination CO$_2$ burst (PIB) from soybean leaves. The PIB has been added to the rate of CO$_2$ uptake in the light to give an indication of the magnitude of true photosynthesis if the PIB represents the rate of respiration in the light.
of the five wavelengths of light used in the preceding light period in the same way as photosynthesis, including an increase in the PIB with light intensity as has been reported earlier (3, 15). The seven points which appear to be below the line are for all five wavelength regions on two leaves on different days. If the PIB represents the rate of CO₂ release to the outside of the leaf in the light it would have the effect of reducing the rate of CO₂ uptake at 350 μl/l CO₂ by 41.9%.

Fig 5 illustrates the effect of 21% O₂ on the rate of photosynthesis at 2% O₂ and 350 μl/l CO₂. The data were obtained using the closed system as described in Chapter 1. The inhibitory effect of 21% O₂ was proportional to the rate of photosynthesis as reported previously (Chapter 1) (1,11) and at 350 μl/l CO₂ represented about a 34% inhibition of the rate of photosynthesis at 2% O₂. This value was about 9% smaller than the decrease that would be experienced if the rate of respiration measured by the PIB was the rate of photorespiration in the light. Since some of the CO₂ respired in the light would be expected to be refixed in photosynthesis, it would be expected that if these two measurements are of the same process, then the one measured in the light should be smaller
Figure 5 Effect of 21% $O_2$ on the rate of net $CO_2$ uptake at 2% $O_2$ and 350 $\mu l/l CO_2$ in attached soybean leaves.
due to this refixation.

**Effect of Changes in Light Intensity on the Rate of CO$_2$ Evolution in the Light**

If the PIB is just the result of an imbalance between the rates of CO$_2$ uptake and evolution due to a disturbance in the CO$_2$ uptake rate, then sudden changes in the light intensity from one set intensity to different lower intensities (darkness being the extreme lower value) might show a particular type of effect. At steady-state in the first light intensity, CO$_2$ uptake ($P_1$) minus CO$_2$ evolution ($R_1$) is equal to apparent photosynthesis (APS)

$$P_1 - R_1 = APS_1$$

and at the lower light intensity, $P_2 - R_2$ would be equal to $APS_2$. The initial effect of reducing the light intensity, if $P_1$ decreases faster than $R_1$, might give equation 2.

$$P_2 - R_1 = APS_3$$

At steady-state, $R$ is a constant proportion of $P$ (Figs 4 and 5), and so $APS_3$ would be lower than $APS_2$ by an amount equal to $R_1 - R_2$. If the PIB is not a continuation of light respiration but is a true CO$_2$ burst beginning at the onset of darkness, it should not occur if the light intensity is only lowered to a level which is still sufficient for a
Fig 6 shows four sample IRGA recordings for the same leaf as it was switched from one light intensity to other lower light intensities. It can be seen that the slope of the initial increase in CO$_2$ concentration after the intensity change, increases with increasing percentage change in the intensity. An apparent CO$_2$ burst was always present. As before, since the slope of the changing CO$_2$ concentration is a straight line for a short time, the CO$_2$ concentration entering the IRGA was constant and could be calculated (Table I). A constant rate of apparent photosynthesis at the new light intensity was achieved after about three minutes. If the new light intensity was not sufficiently greater than the light compensation point, an increasing rate of photosynthesis resulted for a longer period of time (curve b). If the magnitude of the PIB 8 sec after a light intensity change is assumed to be the rate of respiration in the light, then both $R_1$ and $R_2$ can be calculated from Fig 4 for the measured rates of APS$_1$ and APS$_2$. APS$_2$ was measured at 5 min after the change in light intensity. From these values, APS$_3$ can be calculated (equation 2). The same calculations were made using the effect of oxygen on photosynthesis.
Figure 6 Net CO$_2$ uptake and evolution rates for a soybean leaflet at 21% O$_2$ in response to sudden changes in the light intensity.
15min light

% decrease in intensity
(a) 100
(b) 90
(c) 75
(d) 51
Table I. The measured rates of photosynthesis during responses to sudden changes in the light intensity compared to the theoretically predicted rates of photosynthesis.

<table>
<thead>
<tr>
<th>Row</th>
<th>% reduction in intensity*</th>
<th>100</th>
<th>90</th>
<th>84</th>
<th>76</th>
<th>69</th>
<th>61</th>
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<td>APS before change in intensity**</td>
<td>8.55</td>
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<td>9.0</td>
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<td>9.22</td>
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<td>3.15</td>
<td>5.18</td>
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<td>respiration rate after intensity change ($R_2$)</td>
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<td>11.3</td>
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<tr>
<td>8</td>
<td>APS$_3$ measured (8 sec) after intensity change</td>
<td>-5.85</td>
<td>-1.15</td>
<td>-0.9</td>
<td>+0.45</td>
<td>+2.7</td>
<td>+5.4</td>
<td>+4.5</td>
<td>+5.8</td>
</tr>
<tr>
<td>9</td>
<td>APS$_3$ calculated (8 sec) after intensity change based on PIB</td>
<td>-5.85</td>
<td>-5.85</td>
<td>-3.15</td>
<td>-0.9</td>
<td>+1.35</td>
<td>+4.95</td>
<td>+4.4</td>
<td>+5.85</td>
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<td>APS$_3$ calculated (8 sec) after intensity change based on $O_2$ effect</td>
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<td>-1.72</td>
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<td>+2.5</td>
<td>+5.52</td>
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</tr>
</tbody>
</table>

*Light intensity before change: $17 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$

**All rates are in mg CO$_2$ hr$^{-1}$ dm$^{-2}$
(Fig 5) as a measure of $R_1$. These calculated rates of $APS_3$ (Table I Row 9) can then be compared to the actual rate of $APS_3$ (Table I Row 8) measured by the IRGA 8 sec after darkness (Table I). The respiration rates in Table I are based on the PIB. Similar calculations of $APS_3$ were made based on the inhibitory effect of oxygen in place of the PIB (Table I Row 10). The rate of photosynthesis after 5 min at the lower intensity is also included.

The correlation between the actual rate of $APS_3$ after 8 sec and the value calculated from the PIB is best at the lower percentage changes in the light intensity. As the percentage change increases, the role of dark respiration may become more significant and the ratio of the PIB to the preceding rate of photosynthesis may change.

A better agreement is found between the pairs of values of measured $APS_3$ and the calculated $APS_3$ based on the $O_2$ effect. This would indicate the $CO_2$ concentration entering the gas analyser 8 sec after the light intensity change is the resultant of the new rate of photosynthesis ($P_2$) and the old rate of light respiration ($R_1$) based on the effect of oxygen on $APS$. Of course, neither of these rates allow for refixation of $CO_2$. Because the correction of photosynthesis
for respiration in the light if applied to the transient
gives rates which are in agreement with those actually mea-
sured, it is concluded that the transient expresses the con-
tinuation of a process occurring in the previous steady-
state. The PIB data are consistent with the hypothesis that
the PIB represents the total rate of CO₂ production during
photosynthesis, but the data do not prove the hypothesis.

Thus it appears that the PIB does not depend on the
oxidation of some photosynthetic pool which commences at
the onset of darkness but is the resultant of two processes
which change at different rates when subjected to changes
in the light intensity. This would lend support to the
theory that the PIB which is sensitive to high O₂ concen-
trations is due to a continuation of a high rate of photo-
respiration in the light that is also sensitive to O₂ con-
centrations greater than 2% O₂.
LITERATURE CITED


Chapter 3

Effects of Light Quality on the Distribution of Carbon-14 among the Products of Photosynthesis
INTRODUCTION

It has previously been reported that the distribution of $^{14}$C among the early products of photosynthesis was unaffected by the wavelength of light used during the $^{14}$CO$_2$ feeding experiments (2, 9). It has also been reported that red light stimulates the production of carbohydrates (11) and blue light stimulates the production of amino acids (1, 6, 11). Most of these experiments have been carried out, with the exception of those by Cayle et al (2), with broad band filters which transmit mainly in the red or blue regions of the spectrum but still transmit considerable amounts of energy in both the blue and red regions of the spectrum.

In view of the recent reports of a blue light stimulation of photorespiration (7, 8) and other forms of respiration (5, 10) and that photorespiration may be connected with the early metabolism of glycolic acid (12), a re-examination of the effects of blue to yellow light on the early products of photosynthesis was made using narrow wave band regions of the visible part of the spectrum (transmission peaks 450, 501, 547, 601 nm H. W. = 18 nm).
MATERIALS AND METHODS

Soybean plants were grown from seed in pots of garden soil in a greenhouse and moved to a growth chamber when they were twenty days old. The growth chamber was maintained at a day/night temperature of 25º/17º with a 16 hr photoperiod of 1,000 ft-c. The plants were kept in the growth chamber for at least two days before being used.

The middle attached leaflet of the second trifoliate leaf was sealed in a plexiglass chamber in a closed system. The system contained an infrared CO₂ gas analyser (Beckman Model 215), a flow meter (Matheson Model 302), a circulating pump and a CO₂ generating flask. The leaf chamber could be dialed out of the closed system and into an open system connected to a standard gas supply (250 µl/l CO₂, 21% O₂, balance nitrogen). The leaflet was pre-treated in white light (17 x 10⁴ ergs cm⁻² sec⁻¹ between 400 and 700 mµ) and 300 µl/l CO₂, 21% O₂, balance nitrogen at a flow rate of 2.5 l/min until a constant rate of net CO₂ uptake had been reached (usually about 30 min). The light quality and intensity were then changed to the conditions to be used during the ¹⁴CO₂ feeding and the leaflet was kept at
these conditions for 20 min. A new constant rate of photosynthesis was achieved after about 10 min. The leaflet was then dialed out of the system and continually flushed with the standard gas. $^{14}$C-Na$_2$CO$_3$ was added to the CO$_2$ generating flask, the system closed and 2N H$_2$SO$_4$ added. The CO$_2$ generating flask was dialed out of the system when the gas analyzer gave a reading of 350 $\mu$1/l CO$_2$ and the leaf chamber dialed back in. When the CO$_2$ concentration in the closed system had been reduced to 250 $\mu$1/l CO$_2$, the leaflet was dialed out of the system, flushed with standard gas for one min to remove any free $^{14}$CO$_2$ before opening the chamber and then killed in boiling alcohol (80% ethanol).

The subsequent treatments are shown on the flow chart (Fig 1). The total activity in the leaflet was calculated as the sum of activities in the ethanol soluble fraction, the digested starch fraction, and the products of wet combustion (4). The autoradiograms from the paper chromatograms of the total ethanol soluble fraction did not indicate any obvious changes in the distribution of carbon-14 among the radioactive compounds. One spot which appeared to contain more than one compound was suspected of containing sucrose and possibly serine and glycine (spot A). The
Figure 1 Flow diagram for the extraction and determination of the carbon-14 products of photosynthesis.

Activity measurements.
L. S. C.- Liquid scintillation counter
A. G. C.- Automatic Geiger counter

1. Boiling 80% ethanol insoluble fraction less starch.
2. Starch digest.
3. Boiling 80% ethanol soluble fraction.
4. Spot A.
5. Eluate of spot A.
6. Amino acid fraction.
7. Organic acid fraction.
8. Sugar fraction.

Solvent systems for paper chromatography.

A. 1. phenol:water:ammonium hydroxide (267:37:1)
2. propanol:water:ethyl acetate (7:2:1)
B. 1. phenol:water:ammonium hydroxide (300:75:1)
2. butanol:water:acetone:diethylamine (10:5:10:2)
C. 1. ethanol:water:ammonium hydroxide (35:13:2)
2. ethyl acetate:acetic acid:water:sodium acetate 
   * (20:11:10:48)

* Sodium acetate proportion in mg, all other proportions in ml.
amylase in boiling 80% K phosphate ethanol buffer

wet corn combustion

evaporate to dryness

wired leaf solvents A extract chromatography autoradiography

NH_4OH eluate

I I for~ic acid flash evaporator

Rexin 201 lorganic acids I flash evaporator...

solvents B solvents C solvents A chromatography autoradiography

solvents A leaf extract chromatography

dried leaf extract to dryness evaporator

extract leaf L5°C extract leaf L5°C

wet K phosphate amylose in boiling 90%
twelve other major areas of activity on the chromatogram were cut out and their activity determined (Table II). Spot A was cut out and the compounds eluted and separated into amino acids, organic acids and sugars on ion exchange resin columns. Their $^{14}$C content was determined quantitatively in each fraction by automatic Geiger counting. Corrections were made for background and for counting efficiency. The three types of compounds were then resolved to their individual components by means of two dimensional paper chromatography and the compounds containing $^{14}$C were located by autoradiography.

RESULTS

Although the experiment was designed to fix the same amount of $^{14}$CO$_2$ in the same length of time, Table I indicates that this was not the case. In experiment 8, the leaf appeared to wilt during the experiment and in experiments 6 and 10 the rate of CO$_2$ uptake decreased during the feeding for no observable reason. As can be seen from Table I, no data consistently showed an effect of wavelength on the distribution of $^{14}$C among the three main fractions of the
Table I  Percentage distribution of carbon-14 among the three main fractions of photosynthetic products from a soybean leaflet fed $^{14}\text{CO}_2$ at different wavelengths of light.

<table>
<thead>
<tr>
<th>Wavelength peak (nm)</th>
<th>Expt.</th>
<th>Light Intensity $\times 10^{-2}$ ergs cm$^{-2}$ sec$^{-1}$</th>
<th>Feeding time (min)</th>
<th>Total activity (cpm $\times 10^{-7}$)</th>
<th>Ethanol soluble (%)</th>
<th>Ethanol insoluble less starch (%)</th>
<th>Starch (%)</th>
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<tr>
<td>White (400-700nm)</td>
<td>1</td>
<td>17.5</td>
<td>6</td>
<td>7.42</td>
<td>69.8</td>
<td>19.1</td>
<td>11.1</td>
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<tr>
<td></td>
<td>2</td>
<td>33.0</td>
<td>6</td>
<td>7.65</td>
<td>77.2</td>
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<td>11.9</td>
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<td>5.19</td>
<td>79.8</td>
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</table>
products of photosynthesis. Although there appears to be an increasing percentage in the starch fraction with increasing wavelength for comparable feeding times, a high percentage was not found in the starch fraction in white light. The lowest percentage in the ethanol insoluble fraction, less starch, was found at 450 nm and the highest at 501 nm but in the second pair of feedings at 450 and 501 nm the percentages were about the same. The percentage in the ethanol insoluble fraction, less starch, decreased with longer feeding times in all but one case (450 nm). Table II shows the distribution of 14C among the 14 main spots from the chromatogram of the 80% ethanol soluble fraction (Fig 2). Spot A included spots 9 and 10. The values in Table II for spots 9 and 10 are lower than they should be since the values given are based on the separation of spot A into amino acids, organic acids, and sugars which had about an 80% recovery. Spot 9 was mainly serine with small amounts of glycine being found in feedings 8 and 10. The proportions of 14C in the organic acid spots 7 and 8 appeared to change with wavelength of illumination. These spots have not been identified chemically. The main results of Table II are summarized in Table III along with the major carbon-14 products of
Table II Percentage distribution of carbon-14 among the 14 major products of the 80% ethanol soluble fraction from a soybean leaflet fed $^{14}\text{CO}_2$ at different wavelengths of light.

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* minimal values; uncorrected for percent recovery from ion exchange columns.
Figure 2  Location of 14 major radioactive spots on the paper chromatogram of the 80% ethanol soluble fraction from a soybean leaflet. The $^{14}\text{CO}_2$ feeding was for 6 min in white light (17 x $10^4$ ergs cm$^{-2}$ sec$^{-1}$) at 325 $\mu$l/l CO$_2$, 21% O$_2$. 
propanol:water:ethyl acetate (7:2:1)

phenol:water:ammonium hydroxide (267:37:1)
Table III  Percentage distribution of carbon-14 in the major products of photosynthesis from a soybean leaflet fed $^{14}$CO$_2$ at different wavelengths of light.

<table>
<thead>
<tr>
<th>Wavelength peak (nm)</th>
<th>Expt. no.</th>
<th>Feeding time (min)</th>
<th>Serine*</th>
<th>Amino acids</th>
<th>Sucrose*</th>
<th>Insolubles less starch</th>
<th>Starch</th>
<th>Total carbohydrate</th>
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<td>22.0</td>
<td>7.4</td>
<td>12.8</td>
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</tbody>
</table>

* minimal values; uncorrected for % recovery from ion exchange columns.
photosynthesis. The main amino acids recovered (aspartate, glutamate, serine, glutamine, alanine, \(\beta\) amino butyric acid and proline) have been combined. Both the combined total and the value for serine alone might have been lower in 450 nm light but were definitely not higher than at any of the other wavelengths studied. In sucrose there appeared to be a greater accumulation of \(^{14}\text{C}\) at the 450 nm feedings, relative to the other feedings. There may be some protein in the insoluble fraction but a previous report for kidney beans (6) indicated that of the total \(^{14}\text{C}\) fixed, not greater than 3% was in protein. Consequently, radioactivity in the insoluble fraction is assumed to be in carbohydrates. The distribution of \(^{14}\text{C}\) among the carbohydrates at the different wavelengths seemed to vary considerably but the total percentage was about the same.

DISCUSSION

The distribution of \(^{14}\text{C}\) from \(^{14}\text{CO}_2\) among the ethanol soluble, ethanol insoluble excluding starch, and starch products of photosynthesis was essentially unaffected by any of the four wavelengths of light used from 450 to 601 nm when com-
pared to white light. At longer feeding times and thus reduced fixation rates there appeared to be an increase in the percentage of $^{14}\text{C}$ in the ethanol soluble fraction. There was no evidence that blue light as compared to green, yellow or white light stimulated the accumulation of amino acids as has been reported earlier (1, 6, 11). This would support other reports in which no stimulation of $^{14}\text{C}$ accumulation in amino acids by blue light was found in \textit{Chlorella} (2) or in tobacco leaves (9). It is quite possible that over longer times, a change in the intermediary metabolism pattern could result in the accumulation of amino acids in blue light. This type of alteration in the accumulation pattern of soybean leaves was not observed during these 6-15 min feedings. The amount of carbon accumulated in serine and glycine was not stimulated by blue light alone as has been reported (1,11) and might even have been reduced in blue light relative to the white light or the other wavelengths studied.

There was evidence that the blue light (450 nm) did stimulate the accumulation of sucrose in the leaf. This may have been at the expense of carbohydrates of the ethanol insoluble fraction and amino acids but this was not certain. A build up of sucrose could result if blue light had an
inhibiting effect on translocation. At the present time, there is no evidence for or against such a proposal. If the results of further study supported the evidence that blue light does cause an accumulation of sucrose and a reduction in the ethanol insoluble carbohydrates (cellulose, hemicellulose) this could result in an accumulation of amino acids not due to a change in the primary products of photosynthesis but as a change in the pattern of subsequent metabolism. Reduction in the availability of one organic acid, or an amino acid such as serine, could also cause an accumulation of radioactivity in others.

In contrast to the gas exchange data in Chapter 1, where the action spectra of photosynthesis and photorespiration are the same at normal CO₂ concentrations, the wavelength of light did alter the distribution of carbon-14 among the products of photosynthesis.

Whatever the effect of wavelength on metabolism, it does not appear to be on photorespiration. Lowering the oxygen concentration to 1% has been reported to reduce the \(^{14}\text{C}\) incorporated into glycine in soybean leaves and increase the incorporation into sucrose (3). These effects are accompanied by a great reduction in the CO₂ compensation point.
and an increase in the rate of photosynthesis both of which are related to photorespiration (Chapter 1). Such a correlation was not found here.
LITERATURE CITED


Chapter 4

Effect of O₂ on the Rates of CO₂ Exchange at the Compensation Point of Soybean Leaves
INTRODUCTION

The inhibitory effects of oxygen on the rates of photosynthesis has been a subject of controversy since it was first reported in 1920 by Warburg for *Chlorella* (19) and by McAlister and Myers for higher plants (10). Turner andBrittain (16) have summarized much of the experimental evidence used in support of the various hypotheses that have been suggested to explain this "Warburg effect". In recent years one of the theories, the concept of a light stimulated respiration which is sensitive to oxygen concentration (photorespiration) has received considerable study. Attempts have been made on higher plants to measure rates of $O_2$ uptake and evolution in the light using mass spectroscopy (12) and $CO_2$ exchange rates using the infrared $CO_2$ gas analyser (1, 2, 4, 5, 6, 9, 11, 14, 17). Since both $CO_2$ and $O_2$ uptake and evolution are proceeding at the same time and internal recycling can occur, the accurate measurement of the exchange rates for either gas is very difficult.

If photorespiration is measured as the rate of $CO_2$ evolution into $CO_2$ free air (2, 4, 6, 11), then this method will maximize the problem of a partial refixation by photo-
synthesis of the CO₂ released by the respiratory process. If the technique of extrapolating the graph of apparent photosynthesis vs CO₂ concentration to 'zero' CO₂ concentration is used (1, 3, 15), this implies that the proportionality between apparent photosynthesis (APS) and CO₂ concentration which exists for CO₂ concentrations greater than the CO₂ compensation point will still hold for CO₂ concentrations less than the compensation point. This possibility has been challenged by several workers (5, 17). If the decrease in net CO₂ uptake due to an increase in O₂ concentration is used as the rate of photorespiration, this method assumes that the decrease in net CO₂ uptake due to an increase in O₂ concentration is due to a stimulation of respiration and not to a decrease in photosynthesis. This interpretation has been recently challenged by Samish and Koller (13) in which they argued that the decrease in photosynthesis due to increased O₂ concentrations was due to a direct inhibition of photosynthesis and not a stimulation of respiration.

The only CO₂ concentration at which the CO₂ uptake and evolution rates are the same is at the CO₂ compensation point. At this concentration there is no further net gain of CO₂ to the plant but it has been shown that CO₂ exchange
is still proceeding (8). This CO₂ compensation point has been shown to be proportional to the oxygen concentration surrounding the leaf (3, 15). Above some minimum light intensity (light compensation point) when the light intensity increases, the CO₂ compensation point does not increase but remains constant (18, 20). This was also found to be true at different wavelengths of light in Chapter 1. Whether the rates of CO₂ uptake and evolution are increasing at the same rate with increasing light intensity is not known.

Also, for a given light intensity, it is not known whether the rates of CO₂ fixation and evolution at the CO₂ compensation point and 21% O₂ differ from the rates of fixation and evolution at 40% O₂ and its higher CO₂ compensation point.

If O₂ inhibits photosynthesis and does not affect respiration, then when the O₂ concentration increases, to maintain the balance between photosynthesis and respiration the compensation point must also increase. This increase in the CO₂ concentration would increase the rate of photosynthesis just balancing the constant rate of respiration. If this is true, the rates of CO₂ uptake and evolution at the CO₂ compensation point would be expected to be the same at
40% and 21% O₂ for a given light intensity.

The higher CO₂ compensation point at the higher oxygen concentrations could also be due to a stimulation of respiration. This would cause a shift to a higher CO₂ compensation point where the increased photosynthetic rate due to the higher CO₂ concentration would be able to balance the increased rate of respiration. Thus the rates of CO₂ uptake and evolution at the CO₂ compensation point would be expected to be higher at 40% O₂ than at 21% O₂ for a given light intensity.

The following experiments were carried out to try to lend support to either of these hypotheses or some intermediate in which rates of both photosynthesis and photorespiration are affected by oxygen.

MATERIALS AND METHODS

A closed system was used containing a diaphragm pump, flow meter, infrared CO₂ gas analyser (Beckman Model 215), oxygen electrode (Beckman Model 777), Geiger tube (Anton 222), CO₂ generating flask, and a leaf chamber. The leaf chamber and CO₂ generating flask could be switched out of the system
without opening the system.

A single attached soybean leaf was sealed in the plexiglass chamber and illuminated with white light (about $10 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ from 400 to 720 nm). Air containing 350 μl/l CO$_2$ and 21% O$_2$ was passed over the leaf at 2.5 l/min for about one hour until a constant rate of photosynthesis was reached. The system was closed and the CO$_2$ concentration in the system reduced to the compensation point by the leaf. The system was opened, recharged with 350 μl/l CO$_2$ and 21% O$_2$ in air, closed and the CO$_2$ concentration again reduced to the compensation point. The leaf chamber with its leaf was then dialed out of the system, a 50 μl sample of radioactive sodium carbonate solution containing 76.6% Na$_2^{14}$CO$_3$ added to the CO$_2$-generating flask, and the gaseous CO$_2$ removed from all of the system except the leaf chamber by a CO$_2$ scrubber. Twenty drops of 18 N H$_2$SO$_4$ were added to the sodium carbonate through a serum cap and when the total CO$_2$ concentration ($\text{CO}_2^T$ in equation 4) in the system reached the CO$_2$ compensation point the generating flask was dialed out. By manipulating the CO$_2$-generating flask and the CO$_2$ scrubber any CO$_2$ concentration could be attained in the closed system. This process usually took between 2 and 3 minutes.
the CO₂ concentration was at the compensation point, the leaf chamber was dialed back in and the changes in $^{12}\text{C}O_2$ concentration measured by the infrared gas analyser and the changes in $^{14}\text{C}O_2$ concentration measured by the Geiger tube. The effect of the high $^{14}\text{C}O_2$ concentrations on the infrared gas analyser is described in Appendix D and is included in the calculations.

After 15 minutes, the CO₂ in the system was removed by the scrubber and the system opened to a gas stream of 350 μl/l CO₂ and 21% O₂ in air. If the system was closed again, very little $^{14}\text{C}O_2$ was found to be released by the leaf in either the dark or the light. The plant was normally left in the light for about one hour. The process was then repeated on the same leaf but at either a new O₂ concentration or a new light intensity after a suitable adaptation period at the new conditions. At the end of the first experiment, the rates of apparent photosynthesis at 2% O₂ were determined for the light intensity or intensities that were to be used on that leaf and again checked at the end of the second feeding. Holding the leaf in the small chamber for four minutes at its compensation point was found to have no apparent effect on the CO₂ concentration of the compensa-
tion point or on subsequent rates of net CO\textsubscript{2} fixation.

CALCULATIONS

If CO\textsubscript{2} of specific activity 76.6 atoms percent \(^{14}\text{C}\) is generated in a closed system, the proportion of the response on an infrared gas analyser which is due to the \(^{14}\text{CO}_2\) can be calculated. It was found that the Beckman IR 215 is 4.8 ± 2.2% as sensitive to \(^{14}\text{CO}_2\) as it is to \(^{12}\text{CO}_2\) (Appendix D). Thus for any reading on the analyser in which 76.6% of the CO\textsubscript{2} is \(^{14}\text{CO}_2\),

\[
\frac{76.6 \times \frac{4.8}{100}}{76.6 \times \frac{4.8}{100} + 23.4} \times 100 = 13.6\%
\] (1)

13.6% of the reading will be due to the \(^{14}\text{CO}_2\) present (equation 1), and 86.4% of the reading will be due to the \(^{12}\text{CO}_2\) present. If \(^{12}\text{CO}_2\_S\) \(\mu l/l\) carbon dioxide are present in the system at the time of the \(^{14}\text{CO}_2 + ^{12}\text{CO}_2\) generation, and the analyser indicates an increase of \(^{12}\text{CO}_2\_G\) \(\mu l/l\) carbon dioxide, then the total \(^{12}\text{CO}_2\_T\) \((^{12}\text{CO}_2\_T\) in the system is found from equation 2.
The amount of \(^{14}\text{CO}_2\) present may be calculated from equation 3.

\[
^{14}\text{CO}_2 = \frac{86.4}{100} \times \text{CO}_2 + \frac{76.6}{234} \times \text{CO}_2 = 2.83 \text{CO}_2
\]  

and the total amount of \(^{12}\text{CO}_2\) (\(^{12}\text{CO}_2\)) in the system can be found from the sum of equations 2 and 3.

\[
^{12}\text{CO}_2 = \text{CO}_2 + 3.69 \text{CO}_2
\]

The initial rate of \(^{12}\text{CO}_2\) evolution was calculated from the initial slope of the \(^{12}\text{CO}_2\) vs time graph (equation 5) at time zero when the leaf chamber was dialed back into the system.

\[
\text{Net}^{12}\text{CO}_2\text{ evolution rate} = \frac{d^{12}\text{CO}_2}{dt}
\]

The initial rate of \(^{14}\text{CO}_2\) evolution was calculated from the slope of the \(^{14}\text{CO}_2\) activity curve vs time where cpm (time = 0) was the total activity in the system at time zero.
\[ \text{14}_\text{CO}_2 \text{ uptake rate} = \frac{2.83 \times \text{CO}_2}{\text{G}} \times \frac{d^{14}\text{CO}_2}{dt} \quad (6) \]

The total rate of uptake was calculated from the initial rate of \( ^{14}\text{CO}_2 \) uptake times the ratio of the total \( \text{CO}_2 \) atoms present to the \( ^{14}\text{CO}_2 \) atoms present.

\[ \text{Rate of total } ^{2} \text{CO}_2 \text{ uptake} = \frac{2.83 \times \text{CO}_2}{\text{G}} \times \frac{d^{14}\text{CO}_2}{dt} \times \frac{^{12}\text{CO}_2 + 3.69\text{CO}_2}{2.83 \times \text{CO}_2 \text{G}} \quad (7) \]

The net \( ^{12}\text{CO}_2 \) evolution rate (equation 5) and the \( ^{14}\text{CO}_2 \) uptake rate (equation 6) should be equal.

RESULTS

The measurements of the rates of \( ^{14}\text{CO}_2 \) fixation and net \( ^{12}\text{CO}_2 \) evolution at the compensation point were made independently. As Table I shows, the rates were equal within the limits of the experiment. This internal comparison indicates that the technique used gave valid measurements of the rates of \( \text{CO}_2 \) exchange at the compensation point.

The rate of \( ^{14}\text{CO}_2 \) uptake measured at the compensation
Table 1  Effect of O₂ concentration and light intensity on rates of CO₂ uptake and evolution at the compensation point.
Rates of CO₂ uptake at 2% O₂ and the CO₂ compensation point concentrations are given for comparison.

<table>
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<tr>
<th>[O₂]</th>
<th>No. of leaves</th>
<th>Compensation point u1/1 CO₂</th>
<th>Light intensity (x 10⁻⁴ ergs cm⁻² sec⁻¹)</th>
<th>°Rate of net ¹²CO₂ evolution</th>
<th>°Rate of ¹⁴CO₂ uptake</th>
<th>°Rate of total CO₂ uptake</th>
<th>**Rate of net CO₂ uptake at 2% O₂ (mg CO₂/hr/dm²)</th>
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<td>10</td>
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<td>30.3</td>
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<td>1.76</td>
<td>1.76</td>
<td>2.38 ± 20%</td>
<td>2.88 ± 22%</td>
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<td>5</td>
<td>6.5</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.98 ± 23%</td>
<td>2.08 ± 25%</td>
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<td>8</td>
<td>52</td>
<td>28</td>
<td>3.68</td>
<td>3.35</td>
<td>4.48 ± 17%</td>
<td>5.57 ± 12%</td>
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<td>3.48 ± 14%</td>
<td>4.31 ± 11%</td>
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<td>110</td>
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<td>6.24</td>
<td>9.1 ± 15%</td>
<td>12.2 ± 24%</td>
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<td>3.51</td>
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<td>5.75 ± 22%</td>
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<td>4.15</td>
<td>4.64</td>
<td>5.4 ± 28%</td>
<td>10.3 ± 17%</td>
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</table>

°Rates of CO₂ exchange at the compensation point.
**Rates of net CO₂ uptake at 2% O₂ and the CO₂ concentration of the compensation point for other O₂ concentrations.
***Standard deviation expressed as a percentage of the mean.
point could be a rate of photosynthetic $^{14}\text{C}_2\text{O}_2$ fixation or it could be due to an equilibrium reaction between the $^{14}\text{C}_2\text{O}_2$ outside the leaf and the $^{12}\text{C}_2\text{O}_2$ pool inside the leaf. It has been shown that $\text{CO}_2$ outside the leaf can enter the leaf and be fixed within 2 sec and that this $\text{CO}_2$ can be respired and begin to reappear outside the leaf in 15 to 45 sec or possibly less (9). It has also been found that the $\text{CO}_2$ pool size in the leaf is very small (7). Therefore the measurement of the rate of $^{14}\text{CO}_2$ uptake is more likely a measure of its rate of photosynthetic fixation than a measure of its equilibration rate with an internal $^{12}\text{CO}_2$ pool.

A Fisher t test for the comparison of paired samples showed that the rates of $\text{CO}_2$ uptake at the compensation point were always greater at the high light intensity than at low light intensity. Both rates of $\text{CO}_2$ exchange (photosynthesis and respiration) at the compensation point were found to increase with light intensity (Fig 1A) even though the $\text{CO}_2$ compensation point remained the same.

The $\text{CO}_2$ compensation point was found to be proportional to the $\text{O}_2$ concentration (Fig 2) as has been reported previously (3, 15). The rates of $\text{CO}_2$ uptake and evolution at the compensation point also increased as the increasing
Figure 1 (A) Effect of $O_2$ concentration on the rates of $CO_2$ uptake and evolution at the $CO_2$ compensation point at high and low light intensities.

- $28 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ (400 - 700 nm)

- $6.5 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ (400 - 700 nm)

Figure 1 (B) Rates of $CO_2$ uptake at 2% $O_2$ and the $CO_2$ concentrations of the compensation points in Fig A at high and low light intensity.

- $28 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ (400 - 700 nm)

- $6.5 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ (400 - 700 nm)
Figure 2  Effect of O₂ on the CO₂ compensation point in attached soybean leaves.
O₂ concentration resulted in an increased CO₂ compensation point (Table 1). These increased rates of CO₂ exchange were not proportional to the CO₂ compensation point but tended to saturate for O₂ concentration above 40% O₂ (Fig 1A).

For each of the CO₂ concentrations at the compensation points, the rate of CO₂ fixation at that CO₂ concentration and 2% O₂ was determined (Fig 1B). This rate of CO₂ fixation at the compensation point relative to the rate at 2% O₂ gives a percentage inhibition of the maximum rate of fixation due to the increasing O₂ concentration (Fig 3). At low light intensity, the rate of CO₂ fixation at 10% O₂ was not significantly different from the rate at 2% O₂ and the same CO₂ concentration (based on the Fisher t test for the comparison of paired samples). The rate of CO₂ fixation for all of the other compensation points was always less than the rate of CO₂ fixation at 2% O₂ and the same CO₂ concentration (Table 1). This percentage inhibition of the rate of net CO₂ fixation at 2% O₂ was found to increase with increasing O₂ concentration (Fig 3). The value on Fig 3 for low light intensity and 80% O₂ is the only value where the CO₂ concentration is no longer a limiting factor.
Figure 3  The rate of CO₂ fixation at the CO₂ compensation point expressed as a percentage of the rate of CO₂ fixation at 2% O₂ and the same CO₂ concentration.
% inhibition of APS2%O₂

% oxygen

- 28x10⁴ ergs cm⁻² sec⁻¹
- 6.5x10⁴ ergs cm⁻² sec⁻¹
DISCUSSION

Since the CO₂ compensation point is proportional to the oxygen concentration, it might be expected that it would give an indication of the relative rates of photosynthesis and respiration at different compensation points. However, it has been shown that the compensation point is not affected by light intensities above the light compensation point (18, 20). The CO₂ compensation point fails to show that the rate of photosynthesis at the compensation point increases with light intensity and that this increase is exactly matched by an increase in the rate of photorespiration (Table I). This increase in the rate of respiration would appear to be a true light stimulation of respiration. If it were the result of an increase in the carbon substrate for photorespiration due to an increased rate of photosynthesis, then small increases in CO₂ concentration would have the same result and lead to a higher compensation point which is not found. The increase in light intensity could also reduce the stomatal resistance to CO₂ diffusion. A decrease in resistance could result in a greater rate of CO₂ evolution and uptake at the compensation point but would not necessarily change the
$CO_2$ compensation point.

The compensation point also exaggerates the effect that $O_2$ concentration has on the rates of $CO_2$ uptake and evolution at the compensation point. Where it would appear that the rates of $CO_2$ exchange at the compensation point should increase with $O_2$ concentration from 2 to 80% $O_2$, the compensation point gives no indication of the saturating effect on these rates for $O_2$ concentrations above 40%.

There are at least two basically different hypotheses which could be proposed to explain these results. Oxygen could either inhibit photosynthesis or stimulate photorespiration or some combination of both. If the increasing $O_2$ concentration inhibits photosynthesis, then the higher the $O_2$ concentration, the greater the internal resistance to $CO_2$ fixation and the greater the percentage of any respired $CO_2$ that would appear outside the leaf. This higher $CO_2$ evolution rate to the outside of the leaf would result in an increase in the compensation point outside the leaf. The external $CO_2$ concentration would increase until the diffusion gradient for $CO_2$ into the leaf was high enough to result in a rate of $CO_2$ fixation equal to the increased rate of $CO_2$ evolution to the outside of the leaf. If there were
no change in the stomatal resistance with increasing O₂ concentration (no evidence is available to the contrary) it would be expected that the rates of CO₂ influx would decrease with increasing oxygen and constant CO₂ concentration (Fig 1A vs Fig 1B). As the compensation point increases with O₂ concentration, the rates of CO₂ exchange would still increase because of the greater percentage of respired CO₂ which appears outside the leaf. Based on this theory the saturation of the rates of CO₂ exchange at high O₂ concentrations would not be expected.

Samish and Koller (13) have shown that based on their calculation, most of the inhibition of photosynthesis due to oxygen observed by different researchers (3, 15) can be accounted for by the researcher's incorrect use of the external CO₂ concentration for the actual internal CO₂ concentration in calculating rates of photosynthesis. Their calculations show that at higher O₂ concentrations an increase in the mesophyll resistance to CO₂ fixation could account for the decreased rates of net photosynthesis. Their calculations are based on the equation: \( \phi = P - L \) where \( \phi \) is the magnitude of net photosynthesis, \( P \) is the influx of CO₂ into the leaf and \( L \) is the efflux of CO₂ from the leaf.
From this equation they develop the relationship that:

\[ P = \frac{[\text{CO}_2]_{\text{ext}}}{R_{\text{CO}_2}} = \frac{[\text{CO}_2]_{\text{int}}}{r_m} \]

where \([\text{CO}_2]_{\text{ext}}\) is the \text{CO}_2 concentration outside the leaf, \(R_{\text{CO}_2}\) represents the overall resistance to the \text{CO}_2 uptake from the bulk atmosphere, and \(r_m\) is the residual intercellular resistances to \text{CO}_2 uptake from the intercellular atmosphere in the mesophyll \([\text{CO}_2]_{\text{int}}\) into the photosynthetic sites.

Their second equation can be rearranged to:

\[ R_{\text{CO}_2}/r_m = \frac{[\text{CO}_2]_{\text{ext}}}{[\text{CO}_2]_{\text{int}}} \]

Below \text{CO}_2 compensation, where \([\text{CO}_2]_{\text{int}} > [\text{CO}_2]_{\text{ext}}, r_m > R_{\text{CO}_2}\), but \(R_{\text{CO}_2}\) is the total resistance, and \(r_m\) is only part of it. If \([\text{CO}_2]_{\text{int}}\) is redefined as the intercellular \text{CO}_2 contributed from outside the leaf, then their algebraic development, \(r_m = [\text{CO}_2]_{\text{int}}/L\) at compensation, is not valid since \([\text{CO}_2]_{\text{int}}\) is not equal to the external \text{CO}_2 concentration. Thus even though their basic concept may be valid, that the inhibition of photosynthesis due to \text{O}_2 can be explained by an increase in the mesophyll resistance and not due to a stimulation of photorespiration, it is impossible to test this theory based on their equations.

In the second hypothesis, if \text{O}_2 does not inhibit photosynthesis but stimulates respiration, the increase in the compensation point would also be expected to be proportional to
the O₂ concentration; the increased rate of respiration being balanced by the increased rate of CO₂ fixation at the higher compensation point. Again for O₂ concentrations above 40% O₂, it would have to be proposed that this stimulatory effect of O₂ becomes saturated. But if this were so then the compensation point should also level off in the same manner which was not found (Fig 2). Thus neither hypothesis appears able to explain fully the experimental results.

The results reported here could be explained by a stimulation of photorespiration by increasing O₂ concentration accompanied by an increase in the stomatal diffusion resistance. An increase in the stomatal resistance to CO₂ diffusion would not alter the CO₂ compensation point but it would lower the rates of CO₂ exchange at the compensation point. This could also explain earlier reports (3, 15) in which the inhibitory effect of O₂ on rates of APS was attributed to two affects: (1) the stimulation of photorespiration which could be seen by the increase in the CO₂ compensation point with O₂ and (2) the direct inhibition of photosynthesis which resulted in the decreasing carboxylation efficiency which was observed with increasing O₂ concentration. This direct affect of O₂ on photosynthesis could be explained by an increase in stomatal resistance with O₂ concentration and would not require a direct inhibition of CO₂ fixation.
LITERATURE CITED


7. Jolliffe, P. Personal communication.


At the time that this research was begun, there were three tropical plants (corn, sorghum and sugarcane) which were known to have different gas exchange kinetics from all other plants tested. These plants had rates of CO$_2$ fixation reported to be almost twice that of other plants (5, *), these rates were unaffected by O$_2$ concentrations from 2 to 21% O$_2$ (7, 21), they had a very low CO$_2$ compensation point (7, 14) and these plants had no post-illumination burst. All other plants that had been studied at that time had generally lower rates of apparent photosynthesis: these rates were inhibited by oxygen concentrations greater than 2% O$_2$, they had high CO$_2$ compensation points (~50 μl/l CO$_2$) at 21% O$_2$, the compensation points were proportional to oxygen concentration and they had a significant post-illumination burst at 21% O$_2$. It was thought that these characteristics were related and due to a stimulation of the rate of CO$_2$ evolution in the light called photorespiration which was not the same process as dark respiration. The primary purpose of my research was then to try to determine the action spectrum of photorespiration.

*Epilogue references begin on page 134
Since that time many more similarities and differences between these two types of plants have been found. Of the four main evolution lines in the Gramineae (Festucoideae, Bambusoideae, Eupanicoideae, and Chloridoideae), all plants properly classified in the Chloridoideae-grastoid and Panicoid lines have characteristics similar to the tropical grasses mentioned earlier (3). These tropical grasses and some dicotyledon species (13) have their primary products of photosynthesis mainly in C-4 dicarboxylic acids (8, 13), low compensation points (3), no effect of O₂ on rates of photosynthesis, retained 12-15% of assimilated carbon-14 products in the fed area after 24 hr (11), and are anatomically and cytologically quite different from temperate grasses (2, 3, 13). Temperate grasses and the other tropical grasses on the other hand have the primary carbon-14 products of photosynthesis normally associated with the Calvin cycle, high CO₂ compensation points, higher rates of photosynthesis at 2% O₂ than at 21% O₂, 30-50% of assimilated carbon-14 in the fed area after 24 hr (11), and different anatomical and cytological features (2, 3).

Thus photorespiration is only one aspect of a much greater area of research involved in distinguishing between these two evolutionary types of plants. With an understanding
of the basic causes of these differences it might be possible by genetic selection or use of inhibitors to select the more desirable characteristics for producing plants with higher rates of primary productivity. The recent report that the two physiological and phenotypically different types of plants have been found within the same genus may permit work along the genetic selection lines to begin (4).

From the work reported in this thesis there are several lines of research that should be continued. The action spectra at 2 and 21% O₂ on a wider variety of plants should be carried out (especially blue spruce because of Poskuta's work) to be sure that the effects reported here are not confined to particular families of plants but are a more general phenomenon. These investigations should also be carried out on leaves of different ages to see whether the close relationship between the action spectra of photosynthesis and photorespiration continues on in older leaves.

The simultaneous measurements of photosynthesis and photorespiration at the compensation point were carried out at the compensation point due to technical problems that arise in trying to carry these measurements out at any other CO₂ concentration. With modifications in the techniques, it might be possible to measure simultaneous rates
of CO₂ exchange at CO₂ concentrations normally experienced by plants. This could add very useful information in helping to distinguish between the two main current interpretations of the O₂ effect on photosynthesis in whole leaves. The effect of O₂ concentrations on stomatal movement is also needed in this area of research. With the system used in Chapter 2 and the use of an analogue computer it should be possible to do a careful analysis of the PIB during the entire first 60 sec after the onset of darkness. This would give added information as to the decay of this high rate of respiration in the light and its possible relationship to the continuation of or beginning of dark respiration.

Since it is now possible to achieve high rates of photosynthesis using narrow band-pass filters on whole attached leaves, further experiments on the effects of light quality on the carbon-14 products of photosynthesis should be carried out. These should include pulse feeding experiments to study the effects of wavelength of light on the subsequent distribution patterns of carbon-14 in the leaf and the possible effects that these patterns might have on translocation.
Appendices
APPENDIX A

Control of Light Quality and Intensity

The xenon lamp when operating at its rated power of 5,000 watts produces large amounts of energy in the visible far red and infra-red regions of the spectrum. Figure 1 shows the spectrum of the light as recorded by the ISCO spectroradiometer 28 inches from the lamp:

(a) without any filters
(b) with a 15 cm water filter
(c) with 1 Dicrolite heat filter
(d) with 1 Dicrolite heat filter plus a 15 cm water filter.

Since neither the water filter nor the Dicrolite filter is capable of removing all of the radiation for wavelengths longer than 700 nm, the water and Dicrolite filters together were used during all experiments. The interference filters in combination with the water and Dicrolite filters have less than 1% transmission for wavelengths from 700 to 2,000 nm (Cary Spectrophotometer Model 14). With the water and Dicrolite filters in place, the intensity of the xenon lamp was about 100,000 ft-c (Sekonic Studio Deluxe ft-c Meter
Figure 1  Spectral distribution of light from a xenon lamp with Dicrolite and water filters.
Xenon Lamp and:

(a) No filters
(b) 15 cm H₂O filter
(c) 1 dicrolite filter
(d) 15 cm H₂O + dicrolite filters

Light Intensity $\mu$watts cm⁻² nm⁻¹ vs. Wavelength (nm)
Model L-28C) or $4 \times 10^6$ ergs cm$^{-2}$ sec$^{-1}$ (Yellow Springs Instrument Company Radiometer Model 65).

**Light Intensity**

The light intensity was controlled with Balzer neutral density filters. The percent transmission of the filters was found to vary slightly from the stated percent on the filter when the filters were used in pairs. Thus intensities were measured each day with the radiometer for each combination of filters used.

**Light Quality**

**Narrow Band-Pass Filters:**

Table I is a list of interference filters used for the isolation of narrow wavelength-regions of the spectrum. The transmission curves of the Balzer and Schott filters were checked in the Perkin Elmer spectrophotometer (Model 450) (three examples of each brand of filter are in Fig 2A and 2B). The two types of filters have very different transmission curves. The Balzer filters (Fig 2B) have a narrow half band width but their transmission curves flair near the bottom to over 70 nm at 0.56% transmission. It was found that the percent of the transmitted energy outside one full band width was $19 \pm 3\%$ outside 30 nm was $9 \pm 3\%$. For the Schott filters
Table 1  Make and $\frac{1}{2}$ band widths of interference filters.

<table>
<thead>
<tr>
<th>Wavelength Peak (nm)</th>
<th>$\frac{1}{2}$ band Width (nm)</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>402</td>
<td>16</td>
<td>S</td>
</tr>
<tr>
<td>435</td>
<td>18</td>
<td>S</td>
</tr>
<tr>
<td>450</td>
<td>16</td>
<td>S</td>
</tr>
<tr>
<td>466</td>
<td>15</td>
<td>S</td>
</tr>
<tr>
<td>480</td>
<td>12</td>
<td>S</td>
</tr>
<tr>
<td>501</td>
<td>18</td>
<td>S</td>
</tr>
<tr>
<td>520</td>
<td>10</td>
<td>B</td>
</tr>
<tr>
<td>547</td>
<td>14</td>
<td>S</td>
</tr>
<tr>
<td>580</td>
<td>17</td>
<td>S</td>
</tr>
<tr>
<td>601</td>
<td>22</td>
<td>S</td>
</tr>
<tr>
<td>619</td>
<td>10</td>
<td>B</td>
</tr>
<tr>
<td>630</td>
<td>12</td>
<td>B</td>
</tr>
<tr>
<td>654</td>
<td>13</td>
<td>B</td>
</tr>
<tr>
<td>665</td>
<td>12</td>
<td>B</td>
</tr>
<tr>
<td>680</td>
<td>10</td>
<td>B</td>
</tr>
<tr>
<td>694</td>
<td>9</td>
<td>B-20</td>
</tr>
<tr>
<td>709</td>
<td>17</td>
<td>S</td>
</tr>
</tbody>
</table>

S - Schott Depal

B - Balzer Filtraflex B-40
Figure 2 (A) Transmission curves of Schott Depal filters.

Figure 2 (B) Transmission curves of Balzer filtraflex B-40 filters.
(Fig 2A), a wider half band width is listed but their transmission curves do not flair as much at the base (to 50-60 nm). Only 1±0.5% of the total energy transmitted was outside one full band width. Thus even though the curves are similar at the base, the half-band widths of the Schott filters give a clearer picture of their spectral properties.

**Broad Band-Pass Filters:**

Pairs of variable band pass interference filters (set 60) from Optics Technology Inc. were used to measure light quality effects where higher intensities were required than were available with the narrow band-pass filters. The pairs of filters combined with a far red cut off filter (700B) were capable of transmitting at least $15 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ from the xenon lamp over a 10 cm$^2$ area. The percent transmissions of the pairs of filters that were used are shown in Fig 3 and 4. The 700B-450B combination (Fig 4) does not give a true picture of the light used in the experiments since the xenon lamp produces very little energy below 410 nm and the water and Dicrolite filters remove most of the energy that would be transmitted by the second peak around 720 nm. The heavy dotted line on Fig 4 indicates the spectral distribution of the light from the xenon lamp which passes through the
Figure 3  Transmission curves of paired Optics Technology blocking filters with a far red blocking filter (700B).
Figure 4  Transmission curves of paired Optics Technology blocking filters with a far red blocking filter (700B). The heavy line (----) is the spectral distribution of light from the xenon lamp through the 700B and 450B filters (adjusted to a peak height at 470 nm equal to the % transmittance of the filters at that wavelength).
700B-450B filters as recorded by the ISCO spectroradiometer (the peak height at 470 nm as measured by the spectroradiometer has been adjusted to that of the spectrophotometer for comparison).
APPENDIX B

Computer Program for Calculating the Energy Distribution of the Light Recorded on the ISCO Spectroradiometer.

The Instrumentation Specialties Company (ISCO) spectroradiometer comes equipped with an automatic recorder. Each point on the chart of intensity vs wavelength must be corrected for the machine’s response to different wavelengths. A simple computer program has been set up to carry out the correction of the chart paper values for machine response based on a calibration curve made for the spectroradiometer using a standard lamp (No. 173) from ISCO. The pen response of the recorder for the radiometer is not rapid enough to keep pace with a sharp peak in a spectrum and results should be evaluated with this in mind. The spectroradiometer scanner can be operated by hand and thus eliminate pen response time but a separate calibration curve is required if it is to be used in this manner.

The results given by the program are:

(a) total incident energy in $\mu$ watts cm$^{-2}$ between the wavelengths selected (program calculates the area under the curve of intensity vs wavelength
by use of the trapezoidal rule).

(b) total energy in \( \mu \) watts \( \text{cm}^{-2} \) in each of seven regions of the spectrum (these wavelength regions may be changed to suit individual needs).

\[
\begin{array}{cccccccc}
V & B & G & Y & O & R & FR \\
400-420 & 420-490 & 490-580 & 580-590 & 590-650 & 650-700 & 700- *
\end{array}
\]

(c) wavelength in nm at the midpoint of the energy distribution.

(d) average intensity in \( \mu \) watts \( \text{cm}^{-2} \text{ nm}^{-1} \) over the spectrum.

(e) plot of wavelength vs energy at 10 nm intervals.

The spectroradiometer has scales ranging from full scale 0.3 \( \mu \) watts \( \text{cm}^{-2} \) to 1,000 \( \mu \) watts \( \text{cm}^{-2} \). The chart paper values are taken at 10 nm intervals from 400 nm to any value above 710 nm up to a maximum of 1,050 nm. Each of these values must be corrected for (a) the response of the machine to different wavelengths and (b) the full scale range chosen. The correction factors for the response of this machine to different wavelengths from 400 to 1,050 nm are included in the program and were measured on February 21, 1968. These values must be changed after each new calibration of the radiometer. All readings from the print out of the program are based on 30 or
100 μ watts cm\(^{-2}\) as full scale. When the radiometer is on full scale 30, all chart values must be multiplied by 0.3. This scale factor and its use are discussed below. Similarly, when on scales other than the 30 or 100 μ watt cm\(^{-2}\) scales, all values from the print out of the program must be multiplied by the appropriate correction factors listed below.

If on full scale 0.3, multiply all program readings by 0.01

<table>
<thead>
<tr>
<th>Value (μ watt cm(^{-2}))</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>3.0</td>
<td>0.1</td>
</tr>
<tr>
<td>10.0</td>
<td>0.1</td>
</tr>
<tr>
<td>30.0</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>1,000</td>
<td>10</td>
</tr>
</tbody>
</table>
Data Cards

Card 1.  (a) punch in any information desired in first 70 columns, i.e. date, experimental conditions, etc.

(b) the scale factor of 0.3 is placed in columns 77 and 78 of card 1 if the machine is on full scale, 0.3, 3, 30, or 300. If the machine is on scale 1, 10, 100, or 1,000, the scale factor is 1 and columns 77 and 78 are left blank.

Card 2.  In the first three columns put the number of points from the chart to be used, right justified.

i.e., if 66 readings from 400 nm to 1,050 nm are used, the 66 is placed in columns 2 and 3.

Card 3.  Starting with the chart reading from 400 nm, punch data, one chart paper value for every 10 spaces starting in columns 1, 11, 21, etc. (8 numbers to a card.

The data cards are added to the program (Fig 1) for use in the computer.
Figure 1 Computer program for calculating the energy distribution of the light recorded on the ISCO Spectroradiometer.
DIMENSION W(66),F(66),R(66),CI(66),H(19),A(8),CHART(100)
DATA F/1,2,3,4,5,6,7,8,9,0,995,990,980,970,950,940,
745,730,720,710,700,690,680,670,660,650,640,630,
620,610,600,590,580,570,560,550,540,530,520,510,
500,490,480,470,460,450,440,430,420,410,400,390,
380,370,360,350,340,330,320,310,300,290,280,270,
260,250,240,230,220,210,200,190,180,170,160,150,
140,130,120,110,100,90,80,70,60,50,40,30,20,10,0/}
DATA ECT,STAR,BLANK/4,4,4,4,4/}
DC I =1,166
1 M(I+40)+I(I-1)+10
19 READ(I,2,END=100)HED,SCALE
2 FCMATR(194,F4.0)
3 FCMATR(13/(R(I),I=1,4))
4 IF(SCALE.EQ.0.) GO TO 4
5 DC S=1,N
7 M(I)=I-1,N
8 IF(I+1)=I+1,N
10 DC 7 =1,7
12 IF(I=1)=SUM-SUM
13 SUM=SUM+W(I)
14 SUM=SUM/(MIN-400.)
15 S=SUM/2.
17 WRITE(6,10)EC
19 FCMATR(1,20X,1944///)
21 WRITE(6,14)
23 FCMATR('NM RAW F CI///')
25 DC I =1,N
27 CHART(I)=GET
29 I=CI(I)+.5
31 IF (I-I+1) =1
33 IF (1,GT,100) =1,100
35 CHART(I)=STAR
37 WRITE(6,13)W(J),R(J),F(J),CI(J),CHART
39 CHART(I)=BLANK
41 FCMATR('F6.0,F7.1,F8.3,F7.1,2X,100A1)
43 WRITE(6,15)W(J)
45 FCMATR(2X,'ENERGY PER REGION///',7X,'Y',14X,'B',14X,'G',14X,'V',
47 14X,'C',14X,'X',13X,'FR///',4X,'400-420',8X,'420-430',8X,'430-450',
49 8X,'450-460',8X,'460-480',8X,'480-500',8X,'500-520',8X,'520-540',
51 8X,'540-560',8X,'560-580',8X,'580-600',8X,'600-620',8X,'620-640',
53 8X,'640-660',8X,'660-680',8X,'680-700',8X,'700-720',F4.0///)
55 WRITE(6,16)A(W(I)),I=1,7
57 FCMATR(112D6F15.2///)
59 DO R =1,150
61 T = BCH(15.5+I)
63 IF(I=9)+1,9,9
65 T = T
67 WRITE(6,17)
69 FORMAT('***DID NOT FIND HALF-AREA///')
71 D=TI
73 CI=SI
75 X=10.*CI/G
77 WHX=I+15+X
79 WRITE(6,18)SUM,WH,SUM
81 FORMAT('TOTAL ENERGY IS',F12.2,'X','WAVELENGTH AT .5 IS',F6.1,
83 'X','INTENSITY IS',F10.2///)
85 GO TO 19
100 STOP
END
FUNCTION BORY(Y,N)
DIMENSION Y(11)
SUM=(Y(I)+Y(IN)/2.
M=2
D=I+1,N
SUM=SUM+Y(I)
800=SUM+10.
RETURN
END
APPENDIX C

Computer Program for Calculating Rates from Curves of CO$_2$ Concentration Versus Time

To measure the rate of apparent photosynthesis (APS) at a particular CO$_2$ concentration in a closed system, the slope of the graph of CO$_2$ concentration vs time must be read at the desired CO$_2$ concentrations. To observe the effect of CO$_2$ concentration on the rate of apparent photosynthesis, slopes from the graph of CO$_2$ concentration vs time are taken at several different CO$_2$ concentrations and these values are plotted in a graph of APS vs CO$_2$ concentration. A computer program has been set up to carry out these calculations. The computer accepts values of CO$_2$ concentration vs time and calculates a third order polynomial equation for the curve using the method of least squares (all points given equal weighting). It then differentiates this equation and prints out values for the rate of apparent photosynthesis and the CO$_2$ concentration at each time interval. Since the rate of apparent photosynthesis at the compensation point is zero, the longer the curve of CO$_2$ vs time remains at the compensation point, the greater will be the distortion of the third order
equation of $\text{CO}_2$ vs time and the subsequent differential of this equation used to give APS vs time. Therefore, when carrying out the curve fitting of APS vs time, the computer uses only numerical values of APS greater than 3, in any units, all lower values are excluded. This value of APS was found to occur at $\text{CO}_2$ concentrations about 10 $\mu$l/l above the $\text{CO}_2$ compensation point, and the removal of these values was found to have very little effect on either the calculated rates of APS or on the values of APS found when the curve of APS vs $\text{CO}_2$ was extrapolated to zero $\text{CO}_2$ concentration. The program takes the values of APS greater than 3, with their respective $\text{CO}_2$ concentration, at the given time intervals, and fits them to a second order polynomial using the method of least squares to give an equation of APS vs $\text{CO}_2$ concentration. From this equation, values of APS at pre-selected values of $\text{CO}_2$ concentration are printed out (0, 25, 50, ...... 375 $\mu$l/l). All values of APS at $\text{CO}_2$ concentrations lower than the $\text{CO}_2$ compensation point are calculated by extrapolation of the equation of APS vs $\text{CO}_2$ concentration. The program also gives the root mean square value for each equation to give an indication of how well the data fit the prescribed equation.
Data Cards

Card 1. Punch in any information desired in first 70 columns, i.e. date, experimental conditions, etc.

Card 2. In the first three columns put the number of points from the graph to be used, right justified.

Card 3. Values of CO₂ versus time are punched one pair of values to a card. The time is placed in the first 10 columns and its corresponding CO₂ concentration in columns 11 to 20.

The data cards are added to the program (Fig 1) for use in the computer.

Computer Print Out

Coefficients are: Coefficients for third order equation of CO₂ vs time. X = time, Y = CO₂, Calc = calculated [CO₂] based on third order equation, Diff = Y-Calc, Y Prime = first differential of third order equation.

Coefficients are: Coefficients for second order equation of Y Prime vs CO₂, CO₂ = Y, D CO₂/ DT = Y Prime, Calc and Diff as above. Selected values of D CO₂/ DT (APS) are then listed for different CO₂ concentrations.
Figure 1: Computer program for calculating rates from curves of CO$_2$ concentration versus time.
-127-

(a)
-127-  
(b)
SIMULTANEOUS EQUATIONS BY ELIMINATION WITH ROW INTERCHANGES

DIMENSION A(2),R(2)
INTEGER ROW,COL,VAR
REAL R,MAX,VAR

IF (VAR.GT.0.0) GO TO 5

CONTINUE

IF (VAR.GT.0.0) GO TO 99

RETURN

-127-
(c)
APPENDIX D

Sensitivity of the Infrared CO₂ Gas Analyser to ¹⁴CO₂

Introduction

Radioactive carbon dioxide (¹⁴CO₂) is used in many biological experiments related to photosynthesis, photorespiration, translocation and the products of photosynthesis. Rates of photosynthesis are normally measured by the rate of ¹⁴CO₂ uptake as measured by a Geiger tube or the rate of ¹²CO₂ as measured by an infrared gas analyser. To the author's knowledge, the sensitivity of the infrared gas analyser to ¹⁴CO₂ concentrations is not known. Most experiments use very low concentrations of ¹⁴CO₂ and any effects that the ¹⁴CO₂ might have on the ¹²CO₂ readings are not discussed. The following experiments were carried out to determine the effect of ¹⁴CO₂ on a Beckman infrared gas analyser (Model 215).

Materials and Methods

A closed system was used containing a diaphragm pump, flow meter, infrared CO₂ gas analyser (IRGA), and a CO₂ generating flask. The IRGA was calibrated each day with
standard gases from Matheson of Canada Limited.

A 100 μl sample of a known concentration of \( ^{12}C \)-sodium carbonate in 0.12 ml NaOH was pipetted into the CO$_2$ generating flask and the system flushed with nitrogen and closed. To the sodium carbonate, 0.2 ml of 18 N H$_2$SO$_4$ was added through a serum cap, the pump turned on, and the system allowed to equilibrate. Normally about two minutes with continuous agitation of the generating flask was required before the CO$_2$ concentration remained constant. Knowing the number of μ moles of \( ^{12}C \)-sodium carbonate added (\( m_{12} \)) and the final reading of the IRGA in μl/l CO$_2$ (\( IR_{12} \)), the sensitivity (\( S_{12} \)) of the IRGA to a known amount of \( ^{12}C \)CO$_2$ for that system could be calculated (equation 1).

\[
S_{12} = \frac{IR_{12}}{m_{12}}
\]  

This standardization was carried out with two standard sodium carbonate solutions. The system was flushed with nitrogen after each experiment and the generating flask removed and washed and dried. A sample of a known concentration of \( ^{14}C \)-sodium carbonate in 0.12 N NaOH containing a known ration of \( ^{14}C/^{12}C \) was added to the generating flask and the experiment repeated. The sensitivity of the IRGA to \( ^{14}C \)CO$_2$ (\( S_{14} \)) when generated from a mixture of \( ^{14}C \)CO$_2$ and
\(^{12}\text{CO}_2\) of known specific activity was calculated from equation 2.

\[
S_{14} = \frac{\text{IR}_t - C \times mc \times t_\frac{1}{2} \times ^{12}\text{C}/^{14}\text{C} \times S_{12}}{M_{14}}
\] (2)

\(\text{IR}_t\) is the IRGA reading in \(\mu\text{l/l CO}_2\) after the acidification of the \(^{14}\text{C}\)-sodium carbonate in the system, \(C\) is a constant (16), \(mc\) is the number of millicuries per mole of \(^{14}\text{CO}_2\), \(t_\frac{1}{2}\) is the half life of \(^{14}\text{CO}_2\), \(M_{14}\) is the number of \(\mu\) moles of \(^{14}\text{CO}_2\) generated, \(M_{12}\) is the number of \(\mu\) moles of \(^{12}\text{CO}_2\) generated, \(^{12}\text{C}/^{14}\text{C}\) is the ratio of carbon atoms in the sodium carbonate.

The sensitivity of the IRGA to \(^{14}\text{CO}_2\) relative to \(^{12}\text{CO}_2\) (\(S_{rel}\)) was calculated by dividing equation 2 by equation 1 and the result expressed as a percent (equation 3).

\[
S_{rel} = \frac{S_{14}}{S_{12}} \times 100
\] (3)

The radioactive sodium carbonate was purchased from:

1. Atomic Energy of Canada S. A. 76.6 \(^{14}\text{C}\) to total C present.
2. International Chemical and Nuclear Corporation S. A. 52.7 \(^{14}\text{C}\) to total C present.

The activities of the samples (mc/mole) being used were measured in a Packard Tri-carb scintillation spectrometer (series 3000).
Results and Discussion

Two different $^{14}\text{C}/^{12}\text{C}$ ratios were tested in the IRGA. The total CO$_2$ concentrations generated in the closed system varied from 600 μl/l CO$_2$ (October 22, 1968) to 40 μl/l CO$_2$ (December 3, 1968).

The results in table I indicate that the IRGA has a sensitivity to $^{14}$CO$_2$ which is about 5% of that for the detection of $^{12}$CO$_2$. No correction has been made for the possibility that high $^{14}$CO$_2$ concentration might alter the sensitivity of the gas analyser to $^{12}$CO$_2$. No significant difference in the sensitivity of the analyser to $^{14}$CO$_2$ was found between the two $^{14}$CO$_2$/$^{12}$CO$_2$ ratios or for the different CO$_2$ concentrations.

The low sensitivity of the infrared gas analyser to $^{14}$CO$_2$ is not completely unexpected. The Beckman analyser does not use the whole absorption spectrum of CO$_2$ in its measurements. The detector cell of the IRGA contains $^{12}$CO$_2$ at a reduced pressure. This gas absorbs the infrared radiation which passes through the sample cell and compares it to a known amount of infrared radiation passing down the reference cell. Any gas in the sample cell which absorbs infrared radiation
Table 1. Sensitivity of the Infrared Gas Analyser to $^{14}\text{CO}_2$ Relative to $^{12}\text{CO}_2$.

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of Samples</th>
<th>$^{14}\text{C}/^{12}\text{C} \times 100$</th>
<th>M$_{12}$</th>
<th>M$_{14}$</th>
<th>S$_{12}$</th>
<th>S$_{14}$</th>
<th>S$_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 22, 1969</td>
<td>3</td>
<td>76.6</td>
<td>0.88</td>
<td>2.88</td>
<td>140</td>
<td>7.26</td>
<td>5.19</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>76.6</td>
<td>0.44</td>
<td>1.44</td>
<td>140</td>
<td>11.5</td>
<td>8.2</td>
</tr>
<tr>
<td>December 3, 1968</td>
<td>4</td>
<td>A. 76.6</td>
<td>0.44</td>
<td>1.44</td>
<td>132</td>
<td>3.25</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>B. 52.7</td>
<td>1.46</td>
<td>1.625</td>
<td>132</td>
<td>6.57</td>
<td>4.98</td>
</tr>
<tr>
<td>December 4, 1969</td>
<td>5</td>
<td>76.6</td>
<td>0.455</td>
<td>1.515</td>
<td>132</td>
<td>7.53</td>
<td>5.73</td>
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</tbody>
</table>

Arithmetic mean and standard deviation of $S_{rel}$ is $4.82 \pm 2.2$
in the region of peak absorption of $^{12}\text{CO}_2$ (1239.4± 50 cm$^{-1}$) will interfere with the detection of this gas. Theoretically, the replacement of $^{14}\text{C}$ for $^{12}\text{C}$ in CO$_2$ should shift the absorption curve by the square root of the reduced masses. The actual shift in the absorption peak of $^{12}\text{CO}_2$ due to the substitution of $^{14}\text{C}$ for $^{12}\text{C}$ has been measured by Nielson et al. (15). They found that the band center at 2349.4 cm$^{-1}$ for $^{12}\text{CO}_2$ has been reduced to 2225.85 cm$^{-1}$ for $^{14}\text{CO}_2$ which was in agreement with the theoretically predicted shift. This means that the infrared radiation absorbed by $^{14}\text{CO}_2$ in the sample cell is outside the main absorption region of $^{12}\text{CO}_2$ and as a result should not be detected by the IRGA and should have very little effect on the detection of $^{12}\text{CO}_2$ by the infrared gas analyser. A small amount of overlap of the two absorption curves could account for the low measurable sensitivity of the IRGA to $^{14}\text{CO}_2$.

Thus for low concentrations of $^{14}\text{CO}_2$ (<600 μl/l), the infrared CO$_2$ gas analyser is only 5% as sensitive to $^{14}\text{CO}_2$ as it is to $^{12}\text{CO}_2$. 
LITERATURE CITED


Curriculum Vitae

Name: Norman Ross Bulley

Place and year of birth: Toronto, Ontario 1938

Education:
- University of Toronto
  Chemical Engineering
  B.A.Sc. 1961
- Ontario College of Education
  Summer School 1961, 1962
- Queen's University
  Summer School 1963, 1964
- University of Western Ontario
  Summer School 1965
- Simon Fraser University
  Graduate Studies 1966-1969

Experience:
- Secondary School Teacher
  Etobicoke, Ontario 1961-1965
- Neuchâtel Junior College
  Neuchâtel, Switzerland 1965-1966
- Teaching Associate
  Department of Biological Sciences
  Simon Fraser University
  Burnaby, B. C. 1966-1969

Awards:
- International Nickel Company
  Award for summer study 1964, 1965
- N. R. C. Studentship 1968-1969