Photosynthesis: Action Spectra for Leaves in Normal and Low Oxygen

N. R. Bulley, C. D. Nelson, and E. B. Tregunna

Department of Biological Sciences, Simon Fraser University, Burnaby 2, B. C., Canada and Department of Botany, University of British Columbia, Vancouver 8, B. C., Canada

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Abstract. The action spectrum of apparent photosynthesis for attached radish (Raphanus sativus L. var. Early Scarlet Globe) and corn (Zea mays L. var. Pride V.) leaves was measured at 300 μl/l CO₂ and both 21% and 2% O₂. The spectra were measured at light intensities where apparent photosynthesis was proportional to intensity. For radish, a high compensation point plant, oxygen had an inhibiting effect on photosynthesis at all wavelengths from 402 to 694 μm. If a constant rate of photosynthesis at 21% O₂ for the different wavelengths was chosen, then the percent increase in net CO₂ fixation at 2% O₂ was constant. For corn, a low compensation point plant, no inhibitory effect of oxygen concentration from 2% to 21% O₂ was found over the visible spectrum. The CO₂ compensation point for light intensities greater than the light compensation point was found to be constant and independent of wavelength for both radish and corn leaves. For radish, the lowering of the oxygen concentration from 21% to 2% at these intensities was found to reduce the CO₂ compensation point by the same amount for the wavelengths studied.

There is much evidence available (22) to support the theory that many plants give off CO₂ in the light by a process that is not the same as the one responsible for the evolution of CO₂ in the dark. Fixation of CO₂ by photosynthesis makes the direct measurement of CO₂ evolution in the light (photorespiration) difficult. A comparison of the major methods for estimating the rate of photorespiration has been made by Hews (9). He found that the methods of Decker (5), Tregunna et al. (18), Bidwell (1) and the direct measurement of CO₂ evolution into CO₂ 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 (400-580 μm) of low intensity stimulates oxygen uptake in tobacco and broadbean leaves. Poskuta has reported a 3 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% O₂ 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% O₂ to 2% O₂ (7, 19). Thus the difference between the action spectra of net CO₂ uptake at 2% O₂ and 21% O₂ may be used to represent the action spectrum of photorespiration. Bjorkman (2) has reported that the percent inhibition of net CO₂ uptake due to 21% O₂ is greater in far red light (40% at 704 μm) than in red light (31% at 654 μm) for Plantago at low light intensities. He also reported that the percent inhibition of net CO₂ uptake caused by 21% O₂ when compared to the rate at 0.2% O₂ is the same for wavelengths of light from 440 to 700 μm for Mimulus cardinalis (3). The following report describes experiments which compare the action spectra of apparent photosynthesis at 21% and 2% O₂ 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 μl/l CO₂ and at the CO₂ 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° to 24°. The day length was held at 16 hr by the use of fluorescent lighting. Twenty day old radish plants and 50 day old corn plants were moved...
to a growth chamber which was maintained at a
day/night temperature of 25°/17° 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 2 days before being used.

The radish leaves to be used were not fully ex-
panded and were selected for a cross sectional area
of about 9 sq. cm. on 1 side. An individual attached
blade was sealed in a plexiglass chamber which was
blackened on the outside except for a 9.6 cm² circular
area on the front to permit the passage of light.
Leaf temperature was continuously recorded by a
thermocouple at the front of the chamber.

The radish leaves were reattached and was
maintained at a
blade of about
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During the experiments, the leaf was illuminated
by a 5000 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 were used for isolation of narrow 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 Company
radiometer (Model 65). The sensing probe
was housed in a brass heat sink to reduce base line drift
to external temperature fluctuation. The light inten-
sity 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 deter-
mine the effects of high light intensity on the spectral
distribution of the interference filters. The tem-
perature on the back side of the filter increased from
24° to 29° ± 1° after one-half hr exposures to the
high intensity xenon lamp but the one-half 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 wave-
lengths. Fig. 1 illustrates the results found for 2
wavelengths on 1 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 and closed systems. A minimum light intensity
of about 0.75 × 10⁴ ergs cm⁻² sec⁻¹ was required
for net CO₂ fixation at 300 μl/l CO₂. Results from 3 or more day's experiments were used to make graphs of apparent photosynthesis vs. incident light intensity for each narrow wavelength region. No fewer than 12 points for radish and 8 points for corn between the values of 3 x 10⁴ and 7 x 10⁴ ergs cm⁻² sec⁻¹ were used for any 1 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 x 10⁴ ergs cm⁻² sec⁻¹ varied from ± 2 to ± 5% depending on the wavelength.

**Radish.** Fig. 2 represents the action spectrum of net CO₂ uptake by radish leaves at 300 μl/l CO₂ and 21% O₂. The rate of apparent photosynthesis is relatively constant with wavelength for a constant incident energy of 4.0 x 10⁴ ergs cm⁻² sec⁻¹ from 435 μm to 550 μm with a 20% dip at 520 μm. The rate of apparent photosynthesis then increases with increasing wavelength to a peak around 665 μm and then drops rapidly for wavelengths above 680 μm. The ratio of the peak rates at equal 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 x 10⁴ ergs cm⁻² sec⁻¹ at 547 μm 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 μm.

These action spectra represent net CO₂ uptake where both photosynthesis and photorespiration occur. It has been shown that photorespiration has a high O₂ 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 O₂ concentration. Fig. 3 indicates the action spectrum of apparent photosynthesis at 300 μl/l CO₂, constant incident energy and 21% O₂, or 2% O₂. The general shapes of the 2 curves are similar, but there is a greater difference between the 2 curves at the longer wavelengths than at the shorter. The rates of apparent photosynthesis at both O₂ concentrations were also higher at the longer wavelengths than at the shorter. Therefore comparison of the absolute rates of photosynthesis does not indicate clearly whether lowering the O₂ concentration alters the shape of the action spectrum.

**Fig. 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 μm and 450 μm.

**Fig. 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.

**Fig. 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 μl/l CO₂.
of photosynthesis. The comparison of shape was done by looking at the proportional effect of O₂ on rates at different wavelengths.

A standard value of apparent photosynthesis of 5.5 mg CO₂ dm⁻² hr⁻¹ at 21% O₂ and 300 µl/l CO₂ 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 µl/l CO₂ and 2% O₂ 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% O₂. The experimental error found for any 1 wavelength can account for the differences in rates of apparent photosynthesis for the different wavelengths at 2% O₂. Thus at 300 µl/l CO₂ the lowering of the O₂ concentration has the same effect on apparent photosynthesis across the visible part of the spectrum from 402 to 700 µm.

The action spectra described above were measured at 300 µl/l CO₂ where the rate of CO₂ assimilation is much greater than the rate of CO₂ 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 CO₂ concentrations, the interactions among light intensity, oxygen concentration, and CO₂ concentration were studied at the CO₂ compensation point. Typical results for the wavelengths studied (450, 501, 547, 601, 665 µm) at both 21% and 2% oxygen are shown in Fig. 5. The values of the CO₂ compensation point at different light intensities for 450, 501, 547, 601 µm are combined in curve (a). Curve (b) is for 665 µm. The 2 curves are not significantly different for intensities greater than 0.7 × 10⁻⁸ ein cm⁻² sec⁻¹. For light intensities less than this, the CO₂ compensation point tends to be lower for the longer wavelength (665 µm) than the other shorter wavelengths even though the rates of apparent photosynthesis at 300 µl/l CO₂ and constant quanta in the red are slightly lower than those in the blue region of the spectrum. For the 5 wavelengths studied, the CO₂ compensation point was constant for light intensities above 1.0 × 10⁻⁸ ein cm⁻² sec⁻¹ (light compensation point at 60 µl/l CO₂ and 21% O₂) and was independent of wavelength. Under these conditions either photosynthesis and photorespiration 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% on the CO₂ compensation point. When light was saturating (1.0 × 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.

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**Fig. 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 min in the dark.

**Fig. 5.** Effect of light intensity on the CO₂ compensation point for attached radish leaves at 21% and 4% O₂ for wavelengths: (a) 601, 547, 501, 450 µm and (b) 665 µm.
Corn. The action spectrum of net CO$_2$ uptake for corn at 300 $\mu$g/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 $\mu$g/l CO$_2$ for the wavelength regions tested from 435 m$\mu$ to 665 m$\mu$. It was also found that for a given light intensity, the curve of apparent photosynthesis vs. CO$_2$ from 300 $\mu$g/l CO$_2$ to less than 0 $\mu$g/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 $0.3 \times 10^8$ ein cm$^{-2}$ sec$^{-1}$ and was found to be constant and less than 9 $\mu$g/l CO$_2$. No effect of wavelength on the CO$_2$ compensation point at low light intensities was found.

Discussion

The rates of net CO$_2$ uptake by radish leaves at 300 $\mu$g/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 these graphs of apparent photosynthesis vs. light intensity at the different narrow wavelength regions of the visible part of the spectrum, rates of apparent photosynthesis at constant energy or constant quanta can be selected to plot in 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 first action spectrum may be the more meaningful in indicating the relative contribution of each part of the visible spectrum to net CO$_2$ fixation. To see the effect of CO$_2$ evolution in the light on the action spectrum of photosynthesis, Fig. 3 compares rates of net CO$_2$ fixation at 21% O$_2$ and 2% O$_2$ and constant incident energy. The comparison indicates that the CO$_2$ evolution due to the increase in O$_2$ concentration is present in all parts of the visible part of the spectrum. The greatest difference between the rates of net CO$_2$ fixation at 2% O$_2$ and 21% O$_2$ occurs at 665 m$\mu$. If constant rates of photosynthesis are obtained at the different wavelengths and 21% O$_2$, then the lowering of the O$_2$ concentration to 2% to reduce photorespiration results in the same increase in net CO$_2$ fixation. The above results indicate that at a constant CO$_2$ concentration and increasing light intensity, the inhibitory effect due to O$_2$ increases with increasing
apparent photosynthesis but that the percentage inhibition of photosynthesis is relatively constant.

These results confirm those found by Bjorkman (3) for Solidago virgaurea and Mimulus cardinalis in which he found a constant percentage inhibition of CO$_2$ fixation for the wavelength regions studies at 300 $\mu$L/L CO$_2$ when the oxygen concentration was increased from 0.2% to 21% O$_2$. Such effects have also been reported previously for studies with white light (2, 20). The percentage inhibition of photosynthesis due to O$_2$ 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 $\mu$m was very low being about 11% of the rate at 654 $\mu$m for equal incident quanta of $1.0 \times 10^8$ ein cm$^{-2}$ sec$^{-1}$. The lowering of the oxygen concentration at 709 $\mu$m 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) Bjorkman reported rates of net CO$_2$ fixation at 704 $\mu$m equal to about 38% of those at 654 $\mu$m for equal incident quanta of $0.5 \times 10^8$ ein cm$^{-2}$ sec$^{-1}$, his experimental conditions were apparently quite different from ours.

The increase in net CO$_2$ fixation due to the lowering of the O$_2$ concentration was not observed in corn. This lack of response of photosynthesis to O$_2$ concentrations from 2% O$_2$ to 21% O$_2$ in corn was found for all wavelengths tested and for CO$_2$ concentrations from 300 $\mu$L/L CO$_2$ to the CO$_2$ compensation point of less than 9 $\mu$L/L CO$_2$.

The action spectra for corn and radish indicated that rates of apparent photosynthesis in 21% O$_2$ would be 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 as compared to 1.3:1.0 for wheat (11) and 1.1:1.0 for Euphorbia milli (14). Thus the 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 600 $\mu$m and a minimum of about 520 $\mu$m.

The effects of O$_2$ on photosynthesis are not clearly understood. Several explanations are possible for the inhibitory effects of oxygen on net CO$_2$ 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 directly 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 (10) and Downton (6). Hew (10) found that plants having their normal photosynthetic processes either inhibited or blocked by a mutation, also lacked photorespiration. Downton and Tregunna (6) found that in wheat leaves which had their photosynthetic apparatus 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 but was insensitive to high O$_2$ concentration and thus more likely due to dark respiration than to photorespiration. Thus anything that appears to interfere with photosynthesis also seems to effect 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 $\mu$m light versavis 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 Kowalik 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 (unpublished).

**Literature Cited**


