Influence of Light Intensity at Different Temperatures on Rate of Respiration of Douglas-Fir Seedlings

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Abstract. The rate of photorespiration of Douglas-fir seedlings was measured under different light intensities by: (1) extrapolating the curve for CO$_2$ uptake in relation to atmospheric CO$_2$ content to zero CO$_2$ content, and (2) measuring CO$_2$ evolution of the plants into a CO$_2$-free airstream. Different results, obtained from these techniques, were believed to be caused by a severe restriction of the photosynthetic activity when the latter was used. With the first method, CO$_2$ evolution was lower than the dark respiration rate at low light intensity. For all temperatures studied (6°, 20°, 28°) a further increase in light intensity raised the CO$_2$ evolution above dark respiration before it leveled off. The rate of CO$_2$ evolution was stimulated by increase in temperature at all light intensities. With the CO$_2$-free air method, CO$_2$ evolution in the light was less than dark respiration at all light intensities.

In recent years considerable evidence has been produced concerning the influence of light on plant respiration. The magnitude of photorespiration varies between plant species. In some plants the CO$_2$ evolved in photorespiration is a major proportion of the CO$_2$ fixed in photosynthesis; in others, such as maize, photorespiration appears to be negligible or non-existent (5).

In explaining differences in rate of apparent photosynthesis, photorespiration should be related to plant type and environment. It has been suggested that the high rate of apparent photosynthesis in maize is caused by its low rate of photorespiration (5, 17).

In a study of environmental influences on rate of apparent photosynthesis of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings, effects of light intensity and temperature on photorespiration were examined.

Materials and Methods

The rate of CO$_2$ exchange between intact shoots of Douglas-fir seedlings and the surrounding atmosphere was measured at different external CO$_2$ concentrations between 600 ppm and the CO$_2$ compensation point, where there is no net uptake of CO$_2$. The CO$_2$ concentration was measured with a Beckman Model 15A CO$_2$ analyzer in a continuous airstream of a closed system containing shoots of 3 Douglas-fir seedlings grown in a 17.5 cm pot. The seedlings were 2 months old, about 8 cm tall and had numerous fully developed leaves. The 3 shoots were enclosed in a double-walled chamber with a plexiglass top and sealed airtight from the roots during measurement. Air temperature in the chamber was controlled to ±0.5° by regulating a flow of cooled water between the walls of the plant chamber. The leaf surface temperature, measured with a thermocouple, was about 1° higher than the air temperature, when a light intensity of 2500 ft-c was used. In experiments at this light intensity and at 3300 ft-c, the air temperature was kept 1° lower than the desired leaf temperature. Adjustment was not made at lower light intensities. Light was filtered through a 5 cm layer of water from a 750 w G.E. reflector spot lamp. The intensity was regulated by adjusting the distance to the light source.

The laboratory air was enriched with CO$_2$ to a concentration of about 600 ppm in the closed system, and the rate of CO$_2$ uptake in light was measured with decreasing concentration down to the CO$_2$ compensation point. The system was then left for about 15 minutes to ensure a steady state. This procedure was repeated for different light intensities and temperatures. After studying CO$_2$ uptake in light, the chamber was darkened and the rate of CO$_2$ evolution measured after a 5-minute interval. The rate of CO$_2$ uptake by the plants in light was plotted against the external CO$_2$ concentration, and the resulting graphs were extrapolated to zero CO$_2$ concentration to obtain the CO$_2$ evolution in light. This method was followed by Decker (3) who interpreted the rate at zero CO$_2$ concentration as photorespiration. Tregunna et al. (14) used essentially the same principle to calculate photorespiration at the CO$_2$ compensation point. They multiplied the CO$_2$ concentration at the compensation point by the slope of the curve for rate of CO$_2$ uptake plotted against CO$_2$ concentration.

Moss (10), El-Sharkawy et al. (4), and Goldsworthy (6) used another method of measuring
photorespiration. With a stream of CO₂-free air passing into a chamber containing leaves, they measured the rate of CO₂ evolution by the leaves in light and in darkness in the outgoing air. This method was applied in the present study as a comparison for the first-mentioned technique. The airflow must be rapid to minimize CO₂ build-up in the chamber and refixation of CO₂ in the light. The upper limit of the airflow rate is set by the accuracy of measuring CO₂ content of the outgoing air. The airflow used was 1.42 l/min and the chamber volume 1.34 l. In no instance did the CO₂ concentration of the outgoing air exceed 12 ppm. A closed air system was also used for this method and CO₂ was absorbed in a column of “Acarite” (Arthur H. Thomas Company, Philadelphia, Pennsylvania) before entering the plant chamber. Laboratory air was periodically circulated through the chamber to avoid abnormal O₂ concentrations. A light period of at least one-half hour preceded measurements in the light. Dark respiration rates were subsequently measured.

The study was performed with 3 groups of 3 seedlings. Each group was used for all the experiments and measurements were replicated 4 to 6 times as a total for the 3 groups.

Results

In figure 1, the rate of CO₂ uptake by Douglas-fir seedlings is plotted for different external CO₂ concentrations and light intensities with a temperature of 20°C. The resulting curves are extrapolated to zero CO₂ concentration to give a measure of photorespiration at different light intensities. All rates of CO₂ exchange are expressed as mg CO₂ per hour per dm² leaf surface area (both sides). The figure shows that CO₂ concentration limited the rate of CO₂ uptake at all light intensities. The slope of the lines was affected by light intensity, probably because of a light-CO₂ interaction on photosynthesis, but the extent to which CO₂ concentration affected photorespiration under different light conditions is not known. The implication of this is discussed later on.

To arrive at photorespiration at 6°C and at 28°C experiments, similar to that illustrated in figure 1, were made at these temperatures, using the same light intensities and CO₂ concentrations. The rates of CO₂ evolution derived from extrapolation to zero CO₂ concentration for the temperatures of 6°C, 20°C, and 28°C are plotted in figure 2 against light intensity. The rates in darkness were determined directly. The figure shows that the rate of CO₂ evolution decreased initially at the 2 higher temperatures when light intensity was increased from the dark. With a further increase in light intensity, the rate of CO₂ evolution rose to a steady rate above that in the dark at all temperatures.

The rate of CO₂ evolution increased with in-

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Fig. 1. The rate of carbon dioxide exchange by Douglas-fir seedlings in relation to external CO₂ concentration at different light intensities.

Fig. 2. The effect of light intensity at different temperatures on rate of CO₂ evolution by Douglas-fir seedlings; results obtained by extrapolating CO₂ exchange curves to zero external CO₂ content.
crease in temperature from 6° to 28° (fig 2). This increase in rate was evident in darkness as well as in light of various intensities. The temperature coefficient ($Q_{10}$) for the rate of CO$_2$ evolution was 1.9 both in darkness and in light of 2500 ft-c in the temperature range of 20° to 28°. For the temperature interval 6° to 20° the $Q_{10}$ was 3.0 in darkness and only 1.4 at 2500 ft-c. More extensive experiments are required to establish the significance of this feature.

The influence of light intensity and temperature on the CO$_2$ compensation point is shown in Table I.

Table I. Carbon Dioxide Compensation Point (ppm CO$_2$) of Douglas-Fir Seedlings in Relation to Light Intensity and Temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>6°</th>
<th>20°</th>
<th>28°</th>
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<tbody>
<tr>
<td><strong>Light intensity</strong></td>
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<td>ft-c</td>
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<td></td>
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<tr>
<td>200</td>
<td>33</td>
<td>165</td>
<td>380</td>
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<td>400</td>
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<td>1000</td>
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<td>54</td>
<td>81</td>
</tr>
<tr>
<td>2500</td>
<td>33</td>
<td>54</td>
<td>65</td>
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<tr>
<td>3300</td>
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<td>55</td>
<td>65</td>
</tr>
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The CO$_2$ compensation point is the CO$_2$ concentration at which the rates of photosynthesis and respiration are equal. A change in the compensation point results from a change in the rate of one or both of these processes. Light intensity had a considerable effect on the compensation point at 20° and 28° but not at 6°. Temperature influenced it at all light intensities. The increase in compensation point with increase in temperature indicates that the faster increased respiration more than it did photosynthesis at low CO$_2$ concentration. The possibility that photosynthesis decreased with the increase in temperature is unlikely. The table also shows that temperature had more effect on the compensation point at low than at high light intensity. This shows that low light was more restrictive for photosynthesis than for respiration. A similar conclusion can be made from the fact that the compensation point decreased with increase in light intensity at 20° and at 28°. Only at low temperature (6°) did light influence photosynthesis and respiration to the same extent.

The rate of CO$_2$ evolution by Douglas-fir seedlings into a CO$_2$-free airstream at 20° is shown in figure 3, in relation to light intensity. Evolution of CO$_2$ decreased steadily with increase in light intensity up to 1000 ft-c. With further increase in light there was no consistent change in rate. Because the time response can be significant (10), the plants were kept at different light intensities for 1.5 hours.

**Discussion**

The basis of the extrapolation and the CO$_2$-free air method is that photosynthesis is zero at zero CO$_2$ concentration, and CO$_2$ evolution at this point should therefore be respiration. What is not considered with either method is that part of the CO$_2$ evolved in respiration may not be released to the outside atmosphere but refixed in photosynthesis. As the 2 methods do not measure the same CO$_2$ evolution for zero CO$_2$ concentration, the straight or nearly straight line relationship between CO$_2$ exchange and CO$_2$ concentration, which exists above the compensation point (fig 1), does not continue as a straight line with decreasing CO$_2$ below this point. Evolution of CO$_2$ measured by extrapolation to zero CO$_2$ therefore estimates the evolution which occurs above, but not below, the compensation point.

In the CO$_2$-free air method only the ingoing air has a zero concentration and the outgoing air a concentration up to 12 ppm, but this does not explain the difference in results between the methods. This can be seen from figure 1 by extrapolating to 12 rather than to zero ppm CO$_2$. At 12 ppm the evolution of CO$_2$ still increases with increase in light of high intensity and reaches a value higher than dark respiration. However, it is evident from this figure that serious errors could result with higher concentrations of the outgoing air than used in this study because more of the CO$_2$ evolved will be refixed in photosynthesis.

Another source of difference between the 2 methods could be that stomatal apertures change at low CO$_2$ concentration, thereby changing the rate of gas exchange. If change occurs it would be expected that stomates are more widely open at zero than at higher CO$_2$ concentrations (7). This would mean that more of the CO$_2$ produced would escape to the outside atmosphere, thus increasing
rather than decreasing the apparent photorespiration with the CO₂-free air method.

An obvious difference in the physiological conditions under which the 2 methods are studied is that with the CO₂-free air method photosynthesis is restricted to the amount of CO₂ released in respiration while the extrapolation method allows for a more normal rate of photosynthesis. If photosynthesis influences photorespiration directly or indirectly we can expect different results. This seems probable from the work by Zelitch (15,16,17). He has shown that a product of photosynthesis, glycolic acid, is a likely substrate of photorespiration. It may not be the only substrate used in light since Moss (10) and Goldsworthy (6) found a higher rate of photorespiration than dark respiration using the CO₂-free air method, and this is only possible if photorespiration utilizes an endogenous source of carbon compound. El-Sharkawy et al., using the same method, found a lower rate of CO₂ evolution in the light than in the dark for the plant species they studied (4).

Further evidence of at least partial dependence of photorespiration on photosynthetic activity is given by Tregunna et al. (13) for soybean leaves and pepperia shoots. A close relationship between the 2 processes is indicated by Poskuta et al. (12) from work with metabolic inhibitors. In the present study, the increase in CO₂ evolution with increase in light (fig 2) followed the light curve for photosynthesis of Douglas-fir seedlings (unpublished results): the rate of photosynthesis is also saturated at 2500 ft-c with a temperature of 20°. Results of the method measuring CO₂ evolution into a CO₂-free airstream for different light intensities (fig 3) is therefore not considered an estimate of photorespiration which accompanies normal photosynthetic activity. The figure does indicate that a considerable amount of the CO₂ released in respiration can escape to the atmosphere without being recycled in the process of photosynthesis.

Hoch et al. (8) found a decreased rate of O₂ uptake at low light intensity compared to the rate in darkness for a blue-green alga. They attributed the decreased O₂ uptake to a light inhibition of dark respiration. Further evidence of such an inhibition was obtained by Forrester et al. (5): Ozbun et al. (11), working with bean leaves, suggested that 1 of 2 dark-respiration pathways is inhibited in the light. Similar to the work by Ozbun et al., Brown and Weis (1) found for the green alga Ankistrodesmus braunii that light induced an inhibition of CO₂ evolution and an enhanced O₂ consumption at high intensities. They stated that this indicates an interaction between a photosynthetic reductant and the respiratory mechanism. For Scenedesmus, Marsh et al. (9) found no detectable effect of light on the turnover of the citric acid cycle. Although there is much evidence for a light inhibition of dark respiration in different plants, it seems likely that at least part of the decreased rate of CO₂ evolution found in the present study at low light intensity, compared to the rate in darkness (fig 2), is caused by a recycling of respiratory CO₂ in light (4,10). The amount of CO₂ refixed will depend on the CO₂ concentration gradient between the sites of respiration and photosynthesis as well as on the resistance to CO₂ diffusion between these points and to the outside atmosphere. The rates of photorespiration given in figure 2 are therefore the apparent minimum and to arrive at the actual rate the recycled CO₂ should be added.

An additional factor that could affect estimation of photorespiration, by the extrapolation method, is an effect of CO₂ concentration above the compensation point on photorespiration. When using this method, it is generally assumed that there is no such effect (2,3). However, if photorespiration increases with increasing photosynthesis above the CO₂ compensation point, results obtained from extrapolation will underestimate photorespiration occurring at normal atmospheric CO₂ concentration.

The apparent rate of photorespiration, as given in figure 2, comprises a considerable part of the net CO₂ exchange by Douglas-fir seedlings. A further appraisal of the importance of photorespiration in Douglas-fir productivity will be made in a study of light and temperature effects on the rate of photosynthesis of this plant.

Literature Cited


