PATTERN S OF PHOTOSYNTHESIS UNDER NATURAL ENVIRONMENTAL CONDITIONS

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Abstract. Net photosynthesis in six different conifers was studied under various natural environmental conditions. Changes in the pattern of photosynthesis on clear days, especially the midday decrease, are apparently primarily controlled by changes in leaf water potential. In noble fir and Scots pine, water potential probably acts mainly through its influence on stomatal movement. In grand fir, Douglas-fir, hemlock, and Sitka spruce, however, some other mechanism, probably mesophyll resistance to CO₂ diffusion, seems to play a more important role. Both mechanisms probably operate concurrently in all species. Daily variations in leaf water potential seem to occur primarily in response to changes in atmospheric moisture, or, more precisely, vapor pressure gradient from leaf to atmosphere. Variation in carbohydrate content, through its influence on solute concentration, may also influence leaf water potential.

INTRODUCTION

The pattern of assimilation in forest trees differs markedly on days with different local weather conditions and, most often, the highest rates of assimilation occur on cloudy or overcast days (Polster 1950, Pisek and Tranquillini 1954, Gentle 1963, Helms 1963). On overcast days, net photosynthesis normally reaches a peak about midday and then gradually decreases throughout the remainder of the day. On clear days, there is normally a morning peak followed by a marked depression in photosynthesis and a second, lower peak (Rabinowitch 1945, Polster 1950, Stocker 1960). The objective of this research was to study intensively the pattern of net photosynthesis in conifer seedlings of the Pacific Northwest and, if possible, to explain these patterns by measurement of various plant and environmental factors. The research was part of a larger investigation which was also designed to study and compare photosynthetic rates and efficiency of seedlings of west coast conifers under a wide range of natural environmental conditions and to use the information in explaining ecological differences between species. Results of the measurements of photosynthetic rates and efficiency will be presented in a subsequent paper.

MATERIALS AND METHODS

Plant material and environment

Seedlings (2-0 stock) of six species—Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), grand fir (Abies grandis (Lindley)), western hemlock (Tsuga heterophylla (Rafinesque) Sargent), Sitka spruce (Picea sitchensis (Bongard)), noble fir (Abies procera), and Scots pine (Pinus sylvestris (L.))—were out-planted in plots along north-south transects extending from deep within a 35- to 40-year-old Douglas-fir stand into an adjacent open area cleared of all vegetation including herbaceous plants. The four most interesting environmental situations were those designated as open (full exposure), outside stand border, inside stand border, and deep inside stand (deep shade). The location designated as “outside stand border” was immediately adjacent to the outer margin of the Douglas-fir stand, while the one designated as “inside stand border” was
approximately 20 ft inside the stand. Thus the environment at both locations was influenced by the Douglas-fir stand, but the influence of the stand was much greater at the location just inside the stand border. The location deep inside the stand was approximately 125 ft from the margin on the stand.

Light intensity was often greater than 11,000 ft-c in the open on clear summer days. Intensity at the stand border (outside) was normally in the range of 1,000–2,000 ft-c except in late afternoon when the area received direct sunlight for a short period. Inside the stand border the plants received little direct sunlight and intensity was normally less than 1,000 ft-c. Light intensity deep inside the stand was normally less than 100 ft-c and seldom over 200 ft-c except during occasional "sun flecks." Environmental factors other than light were also modified by the Douglas-fir stand. The effect of the stand on air temperature, as compared to the open area, was to reduce the maximum temperatures and to slightly increase the minimum temperatures. At locations influenced by the stand, relative humidity, especially on clear days, was higher than in the open. A more detailed description of the environment at the different locations will be presented in a subsequent paper dealing with ecological differences between the six species.

Seedlings were also planted at locations intermediate in position between the open area and the stand border (outside), and between the location inside the stand border and deep shade. These seedlings were not studied intensively, however, since their environment and rates and patterns of net photosynthesis were almost identical to seedlings in the open area or in deep shade.

Experimental plantings were made in January 1962 and January 1963. Two transects of plots were established in 1962 and one transect in 1963. The 1962 plots contained six seedlings of each species, while those established in 1963 contained 20 seedlings of each species. Thus 32 seedlings of each species were planted at each experimental location. The study area was located on Charles Lathrop Pack Demonstration Forest which is owned by the College of Forestry of the University of Washington and located near LaGrande, Washington.

Measurement of plant factors and processes

An infrared gas analyzer was used for the measurement of net photosynthesis during the daylight hours and of respiration at night. Gas samples were collected from a small plastic (polypropylene) cuvette which surrounded the foliage sample. The total needle mass sampled was kept at about 0.5 g, oven-dry weight. For all samples, a single branch from the uppermost whorl of branches which developed during the second growing season in the field was used. Thus, seedlings used from June 1963 to about June 1964 were planted in January 1962 and those used from June 1964 until the end of the study were planted in January 1963. In all cases, the needles were fully developed and, depending on the species, 2–4 inches of the branch tip were placed in the cuvette.

An automatic switching mechanism at the CO₂ analyzer permitted cyclic sampling at six separate points. Since at least one point (line) was always used to monitor ambient CO₂ concentration, up to five seedlings could be sampled at any time. The sampling procedure was varied so that at times all sample lines were placed in one location in order to compare photosynthesis in different species under the same environment. At other times the sample lines were placed along the environmental transect, and rates and patterns of assimilation were compared for the same species over a range of environments. Net photosynthesis was normally determined for a period of 3 days on each sample seedling after which the foliage sample was removed and needle dry weight determined. Seedlings were normally used only once except at the location deep inside the stand where survival was poor. The investigation extended over a 2-year period beginning in June 1963. Thus measurements were made in all seasons of the year. A total of 390 seedlings was used in the investigation.

Plant factors which were measured in the spring, summer, and fall of 1964, included leaf water potential, sugar content of the foliage, leaf temperature, and relative stomatal aperture. Leaf water potential and sugar concentrations were determined from foliage samples of the same seedling taken at periodic intervals throughout the day. An electric psychrometer, similar to the one described by Spanner (1951), was used for water potential measurements. Sugars, reducing and non-reducing, were determined colorimetrically by a modification of the method given by Bernfield (1955). Leaf temperature was measured continuously by a thermocouple attached to the under surface of the needle. On two occasions, field measurements of relative stomatal aperture were made throughout the day and into the night. In addition, stomatal measurements were made in the

Leaf water potential is the difference in free energy or chemical potential between pure water and the water contained in the leaf cells. The potential of pure water is zero. Therefore, the potential of water in leaf cells is less than zero (a negative number).
laboratory under controlled conditions of light, temperature, and humidity. All stomatal measurements were made by an infiltration technique described by Fry (1965). Measurements listed above as well as the measurement of environmental factors were made concomitantly with measurements of CO₂ assimilation.

Measurement of environmental factors

Continuous measurements of light intensity, air temperature, and relative humidity were made for the duration of the study. Light intensity was measured by means of selenium photocells and/or radiometers. Temperature and humidity were measured with wet-dry bulb thermocouples and with hygrothermographs. All light intensity and thermocouple temperature measurements were recorded on a 24-point millivolt recorder. Soil moisture was determined by gravimetric sampling.

Results and Discussion

Patterns of net photosynthesis

Under fully exposed conditions, the typical pattern of photosynthesis on overcast or cloudy days was one in which net photosynthetic rates increased to a maximum about noon, then either decreased or remained constant for an hour or two and finally decreased (Fig. 1). On bright sunny days in the growing season, assimilation normally increased rapidly, reaching a peak at about 9–12 AM, then decreased until late afternoon when it again increased and reached a second but much lower peak (Fig. 2). Patterns were basically the same at all seasons of the year, but in the colder months the period of photosynthesis was shorter, rates of net photosynthesis were lower, and the depression on sunny days was less than in the warmer months.

The patterns described above for exposed conditions were typical for all six species. With Scots pine, however, the midday decrease on clear days was often not so marked as for the other species (Fig. 2), and rates were much more variable. Also with Scots pine, the average daily rate of photosynthesis increased after several days of sunny weather (Table 1), but with the other species a progression of clear days resulted in a progressive decrease in total photosynthate production. Furthermore, there was a noticeable interaction between weather conditions and species in terms of assimilation rates. Species showing the highest rate on clear days (Scots pine and noble fir) did not necessarily show the highest
rate on overcast days (compare noble fir and grand fir, Fig. 1 and 2). In general, net photosynthesis in Scots pine, and to a lesser extent noble fir, appeared less adversely affected by sunny conditions than in the other species.

At the locations near the stand border (outside and inside the stand) the basic patterns of net photosynthesis were the same as for the open area. However, there were differences in rate of photosynthesis, timing of the peak rate of photosynthesis, and in the magnitude of the depression on clear days. For all species except Scots pine and noble fir it was quite common for peak rates of photosynthesis and total photosynthate production to be higher outside the border than in the open area even on overcast days. Also, the depression on clear days was usually not so great as in the open. Inside the border peak rates of photosynthesis occurred later in the day and the depression on clear days, although still very apparent, was much less than in the open. Daily photosynthate production by the seedlings was lower inside the stand border than in the open on overcast days but, except for Scots pine and noble fir seedlings, was higher on bright sunny days. Short-term fluctuations in rates of photosynthesis were much more common at the stand border, especially inside the border, than in the open due to greater short-term variation in light intensity. As with the open area, daily weather conditions seemed to control the basic pattern of net photosynthesis so that no great seasonal differences were apparent. In the colder months, however, rates of net photosynthesis were lower, the photosynthesizing period was shorter, and midday depression was less on clear days.

Under deep shade the pattern of photosynthesis was controlled by variations in light intensity. Peaks of photosynthesis as well as rate were determined by the occasional sun flecks which penetrated the dense canopy.

The discussion which follows is concerned with the general pattern of photosynthesis which was common to all locations, except deep shade. As will be seen, however, comparisons of rates and pattern of photosynthesis under the different en-

<table>
<thead>
<tr>
<th>No. days with sun</th>
<th>Average temperature (°C)</th>
<th>Average V.P.D. (mm Hg)</th>
<th>Average daily rate of assimilation (µg CO₂/m²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dusky fir</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>12.80</td>
<td>1.44</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>15.88</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
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<td>20.13</td>
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</tr>
<tr>
<td>4</td>
<td>26</td>
<td>15.03</td>
<td>0.52</td>
</tr>
</tbody>
</table>

**Explanations of patterns**

Much has been written concerning daily patterns of photosynthesis and especially the midday decrease in photosynthesis. The literature suggests at least nine possibilities that might account for the depression, as follows: (a) a daily decrease in the CO₂ content of the air; (b) bleaching of chlorophyll (Bohning 1949); (c) increased chloroplasm temperature (Kozlowski 1957); (d) photooxidative deactivation of enzymes (Rabinowitch 1945); (e) increased respiration (Kozlowski 1957); (f) accumulation of photosynthetic end products (Thomas and Hill 1949); (g) stomatal closure (Maskell 1928, Stäfelf 1935, Nutman 1937); (h) increased mesophyll resistance to CO₂ diffusion (Gaastara 1959, Shimshi 1963, Bierhuizen and Slatyer 1964); and (i) water deficit in the leaves (Pisek and Tranquillini 1954).

In the present investigation, the first five of these possibilities could not account for the differences in pattern of photosynthesis on overcast and clear days. As for possibility (a), the CO₂ content of the atmosphere was remarkably constant and there was often a marked decrease in net photosynthesis even though there was no change in CO₂ concentration. The data for photosynthesis under various environmental conditions (along the environmental transect extending from within the stand to the open field) indicate that suggestions (b), (c), (d), and (e) can also be eliminated as major causes of the midday decrease. The decrease was common at the location just inside the stand border on clear days and sometimes occurred deep inside the stand. This would strongly suggest that bleaching of chlorophyll and photooxidative deactivation of enzymes are not factors in the depression because light at both locations was from a diffuse source, and intensity was less than 1,000 ft-c near the border and normally less than 200 ft-c deep inside the stand.

When the data are considered in relationship to daily variation in temperature, there is little likelihood that high chloroplast temperature and increased rates of respiration (resulting from increased temperature) could account for the observed decrease in assimilation. On August 17, 1963, for example, at the location just inside the stand, there was a 59% reduction in net assimilation of Sitka spruce from 9:30 AM (maximum rate) until 3:30 PM (minimum rate), after which the rate again increased. During this period the air temperature increased only 3.0°C (15.0° to
18.0°). Leaf temperature was never more than 0.5° higher than air temperature. Under more exposed conditions, increased temperature did result in some decrease in net photosynthesis due to an increase in respiration. On several occasions, however, calculations were made of the reduction in net photosynthesis which could be expected from the increase in temperature. Even under the most extreme conditions (hot summer days) respiration could not account for all the reduction, and on cooler days increased respiration commonly accounted for less than 15% of the midday reduction in assimilation.

The possibility that reductions in the rate of photosynthesis result from a clogging of the photosynthetic mechanism due to an accumulation of photosynthetic end products cannot be substantiated by the findings of this study. Sugar concentration in the foliage did increase during the period when rate of photosynthesis was decreasing; but normally, sugar content continued to increase even after net photosynthesis began to increase in late afternoon (Fig. 3). Also, the rate of sugar accumulation was as high and sometimes higher on overcast mornings than on clear mornings. It is not implied, however, that sugar accumulation has no influence on patterns of photosynthesis. As will be shown later, sugar concentrations may affect photosynthesis through an influence on cell water relations.

Leaf water status and photosynthesis

Of the possible explanations for the diurnal decrease in photosynthesis, (f), (g), and (h) are all related in some way to the water status of the plant. They may either directly affect the status of the water in the plant or be affected by it. The last named possibility, water deficit, is a "catch all" in that the possible influence of the water status is recognized but no explanation is given as to how the water deficit acts to bring about a reduction in photosynthesis.

Since these possibilities are related to the status of the water in the plant, it was decided to first determine whether or not photosynthetic rates are related to water regime in the foliage. Water status was determined by periodic measurement of leaf water potential with an electric psychrometer.

In agreement with the work of Boyer (1964), photosynthesis was found to be well correlated with changes in leaf water potential. In Douglas-fir, grand fir, hemlock, and Sitka spruce correlation coefficients were 0.96, 0.95, 0.90, and 0.95 respectively and were statistically significant in all cases. On sunny days, photosynthesis tended to decrease as leaf water potential decreased and to increase when it increased (Fig. 4). The afternoon increase in water potential apparently began about an hour before the increase in net photo-

![Fig. 3. Photosynthesis and reducing-sugar content of the foliage in single seedlings of Douglas-fir and grand fir on a selected representative day in July 1964. Pattern is basically the same for all species except that in Scots pine the change in sugar concentration appeared to be much less than for the other species.](image)

![Fig. 4. Leaf water potential and CO₂ assimilation in a Douglas-fir seedling on a clear summer day (1964). Relationship is basically the same for grand fir, hemlock, and Sitka spruce.](image)
synthesis, but this could not be definitely established because of the limitations of the sampling procedure.

The relationship of leaf water potential to photosynthesis was not so obvious in noble fir and Scots pine as in the other species. Morning photosynthesis in noble fir decreased as water potential decreased (Fig. 5), after which there appeared to be an almost inverse relationship between the two. With Scots pine there was normally a decrease in photosynthesis during midday, and there appeared to be a tendency for a slight diurnal decrease in water potential. A preliminary examination, however, indicated no direct relationship between the two.

From these preliminary observations it might be concluded that plant water status, as evidenced by leaf water potential, is not an important factor in controlling the daily pattern of photosynthesis in all species tested. However, this does not seem probable. More likely, water potential is important in the control of photosynthesis in all species tested, and the apparent difference between species is due to a difference in the mechanism by which water potential acts to control photosynthesis.

**Stomatal and mesophyll resistance to CO₂ diffusion**

Leaf water potential could conceivably influence patterns of photosynthesis through its influence on stomatal closure and/or mesophyll resistance to CO₂ diffusion. Although the exact mechanism of stomatal movement has not been definitely established, there is ample evidence that opening and closing ultimately depend on increasing and decreasing the supply of water to the guard cells (Zelitch 1963). In the liquid phase of CO₂ diffusion, control of photosynthesis could be exerted through resistance to gaseous movements into or within the mesophyll cells. Changes in the water regime of the mesophyll cells could have a marked influence on CO₂ solubility and diffusion. Rawlings (1963) concluded that mesophyll resistance was not constant, but was a function of water potential and could vary with transpiration.

To determine if either or both of these possible resistances are involved in the midday reduction of photosynthesis, measurements of relative stomatal aperture were made in the field and in the laboratory in a controlled environment chamber. Measurements were made in the summer and fall of 1964 on foliage of the current year. In the laboratory relative stomatal aperture was determined under conditions of decreasing relative humidity and increasing temperature. A marked difference in stomatal movement was found between species. In one experiment the stomata of grand fir were less responsive than those of noble fir (Table 2). Stomata of grand fir and Douglas-fir tended to close gradually while those of noble

**Table 2.** Relative stomatal aperture in four species as determined by infiltration and expressed in pounds of pressure at various temperatures and levels of humidity (A single seedling of each species was used throughout the test.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Relative humidity (%)</th>
<th>Temperature (°F)</th>
<th>Infiltration pressure (psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grand fir</td>
<td>Noble fir</td>
</tr>
<tr>
<td>9:45</td>
<td>92</td>
<td>70</td>
<td>5.5</td>
</tr>
<tr>
<td>11:00</td>
<td>60</td>
<td>75</td>
<td>6.0</td>
</tr>
<tr>
<td>13:00</td>
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<td>14:30</td>
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<td>15:30</td>
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<td>17.0</td>
</tr>
<tr>
<td>16:30</td>
<td>15</td>
<td>98</td>
<td>38.0</td>
</tr>
</tbody>
</table>

*Humidity and temperature were changed to next level immediately after the time shown.  
*A reading of 40-40 psi indicates that the stomata are effectively closed.*
fir closed very rapidly once closure had begun. Bannister (1964) has also noted that all species do not react in the same manner to internal water deficits in the leaves, stomatal closure occurring much sooner in some than in others.

A second laboratory experiment on stomatal movement confirmed the findings of the first. In addition, grand fir showed a marked reduction in photosynthesis before there was any appreciable change in stomatal aperture. In noble fir, however, the pattern of photosynthesis appeared to be more strongly related to stomatal movements (Fig. 6).

![Graph](image)

**Fig. 6.** CO₂ assimilation and relative stomatal aperture as determined by infiltration in noble fir and grand fir. A reading of 30-40 psi indicates that the stomata are effectively closed. Results are for a controlled laboratory test conducted in September 1964. Current-year needles from a single seedling of each species were used throughout.

Laboratory measurements of stomatal aperture in Scots pine indicated that, under conditions of decreasing relative humidity, the stomata may close and later open again. This possibly occurs many times in the course of a day. Other researchers (Ehrler, Nakayama, and van Bavel 1965) have recently reported opening and closing cycles of less than 30 min. Hemlock and Sitka spruce seedlings were not used in the laboratory experiments. Based on field observations, however, it appears that stomatal movements in these species more closely resemble those of grand fir and Douglas-fir than those of noble fir or Scots pine.

Field tests of stomatal movement confirm the laboratory observations. In Douglas-fir and grand fir there was a reduction in photosynthesis with very little change in stomatal aperture (Fig. 7). Concomitant measurements of photosynthesis and stomatal aperture were not made for the other species, but the stomata of noble fir and Scots pine were much more responsive to closure than were those of the other species. At night the stomata of Scots pine and noble fir showed complete closure, but stomatal closure was not observed in grand fir. Douglas-fir was intermediate between the two extremes. Hemlock and Sitka spruce stomata showed some closure but not so much as Douglas-fir.

These findings are consistent with the observed patterns of change in both water potential and photosynthesis. In species where stomatal closure is of primary importance, it would be expected that there would first be a decline in water potential until the stomata closed. After closure, water loss by the plant would then be reduced so that water potential would again increase. Later, the stomata might open, followed by a second decline in water potential due to increased transpiration. Fig. 5 shows that this is consistent with the pattern of change of water potential in noble fir. In Scots pine there is evidence that the same pattern is followed, but that the stomata open and close much more rapidly than in noble fir. This would account for the more rapid short-term fluctuation in photosynthesis in Scots pine as compared to the other species. It might also account for the net diurnal decrease in water potential being less
than in other species, and for the rate of photosynthesis being less affected by a progression of warm, clear days. During periods of stomatal closure transpiration would be reduced and partial recharge might occur. To thoroughly investigate the relationship between water potential and photosynthesis in this species would require short term (5–10 min) sampling of both water potential and stomatal aperture throughout the photosynthesizing period.

In this investigation, the reduction in net photosynthesis not accounted for by stomatal closure has been attributed to mesophyll resistance. No quantitative measurements were made, but of the many possibilities considered, this phenomenon is the only one that will fully account for the observed reductions in all cases. There is ample evidence in the literature of the existence of such a phenomenon, and in several cases it has been reported to be of more importance than stomatal resistance in reducing photosynthesis (Gaastra 1959, Rawlings 1963, Bierhuizen and Slatyer 1964, Fry 1965).

The two phenomena, stomatal closure and mesophyll resistance, do not operate to the mutual exclusion of each other. Stomatal closure occurs in all species, and under conditions such as low soil moisture and low humidity, it is of primary importance in regulating photosynthesis. There was also an indication that in noble fir a certain amount of reduction in photosynthesis occurred before stomatal closure became important.

Environmental factors and leaf water potential

Daily variations in leaf water potential were most closely related to variations in the vapor pressure deficit (VPD) of the atmosphere. With a decrease in moisture content of the air, the vapor pressure gradient between air and foliage increases, resulting in more rapid water loss from the plant. This in turn results in water stress conditions within the plant and possible reduction in photosynthesis because of hydroactive stomatal closure and/or mesophyll resistance. This agrees with the later work of Philpot (1965) who reported that diurnal fluctuation in moisture content of ponderosa pine and whiteleaf manzanita leaves was correlated with changes in relative humidity.

Soil moisture also influences leaf water potential. As soil moisture is lowered, leaf water potential decreases daily and the effect of low atmospheric moisture is intensified.

Sugar accumulation and patterns of photosynthesis

Another factor which has some influence on daily variation in photosynthesis is sugar accumulation in the leaves (Fig. 8). Sugar accumulation may affect photosynthesis by directly influencing stomatal movements as suggested by Yemm and Willis (1954), or it may affect osmotic pressure of the cells and membrane permeability as suggested by Shreve (1931) and Boon-Long (1941). Sugar accumulation, however, seems to be of less importance than vapor pressure deficit. Even on overcast days there is an increase in sugar, with relatively little decrease in photosynthesis or leaf water potential as compared to sunny days. The daily increase in sugar is in the reducing form, which results in part from an accumulation of the end product of photosynthesis (glucose). It appears, however, that there is also a conversion from the non-reducing to the reducing form since reducing sugars increased at a faster rate than total sugars (Fig. 8).

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Literature Cited


