Rates of Photosynthesis in Attached and Detached Bean Leaves, and the Effect of Spraying with Indoleacetic Acid Solution 1, 2

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Introduction

The rate of photosynthesis in a given leaf is generally considered to be limited either by external or environmental factors such as light, CO₂ supply, or temperature, or by internal factors such as pigment concentration, enzyme concentration and turnover time, or some other factors associated with the nutritional status of the plant. However there are reports that rates of photosynthesis may be influenced by the presence or absence or developmental stage of roots (8,11) or by stimulation of roots (1). It has also been shown that hormone treatment of soybean plants increases their rate of translocation (9), and it has been suggested that the demand for photosynthetic in roots may influence translocation (11,16) and hence photosynthesis. It thus seems possible that the rate of photosynthesis may also be subject to internal control resulting from stimuli from other parts of the plant. Since many photosynthesis experiments are done on detached leaves, the work reported here was undertaken to compare photosynthetic rates of attached and detached leaves, and to search for evidence of possible internal control mechanisms of photosynthesis.

Materials and Methods

CO₂ Uptake. Measuring the net CO₂ exchange of a leaf is a well-established procedure for short-term determination of the rate of photosynthesis. Gross rates can be calculated by making an allowance for respiration, but this requires the assumption that respiration continues at the same rate in light as in darkness and this is a matter of controversy (2,13). In order to avoid this controversy as far as possible, a technique has been devised which consists essentially of supplying CO₂ containing C¹⁴O₂ of known specific activity to a leaf sealed in a chamber containing a Geiger tube. The rate of disappearance of C¹⁴ is initially proportional to the rate of gross CO₂ uptake, until the C¹⁴O₂ begins to be diluted by respired C¹²O₂. A measurement is completed in 5 to 10 minutes with a minimum disturbance of the leaf.

The leaf to be studied is put into a glass chamber with its petiole sealed into a split stopper with a 1:1 beeswax-vaseline mixture. The leaf should be left with the chamber open for a few hours to equilibrate. A detached leaf can be supported in the chamber in a small beaker of water. A C¹¹-sodium carbonate solution containing about 10 μc of C¹⁴ is added to a test tube equipped with a side arm having a stopcock, and the tube is stoppered with a serum bottle stopper. The tube is evacuated via the side arm, the stopcock is closed, and 3 ml of lactic acid are introduced into the tube with a syringe. The tube is heated to boiling for 1 to 2 seconds on a cool flame to release the C¹⁴O₂. The leaf chamber is then evacuated to 300 mm Hg and the CO₂-generating tube, with the syringe still in place, is connected to it. The syringe barrel is unscrewed from the needle, and air flushes the C¹¹O₂ into the chamber, after which it is closed. The whole process of C¹⁴O₂ addition takes about 15 seconds and the leaf is subjected to partial vacuum for less than 10 seconds. (No effect of evacuating the leaf, even for periods up to several minutes, could be found on subsequently measured rates of photosynthesis.) The disappearance of the C¹⁴O₂ from the chamber is measured on a recording Geiger counter with an Anton Model 222 geiger tube sealed into the lid of the chamber. A small vibrating diaphragm pump circulates the atmosphere in the chamber, thus ensuring rapid mixing of C¹⁴O₂ and C¹²O₂. The measured rate of photosynthesis is not affected by varying the pumping rate from 20 to 1500 cc minute. An infrared CO₂ analyser is used to measure the CO₂ content of air entering the apparatus. The rate of gross CO₂ uptake is calculated from the disappearance of radioactivity from the atmosphere which is measured on the chart record of the geiger counter. This decrease is linear during the first 5 to 10 minutes, during which time about one quarter of the supplied CO₂ is taken up by the leaf under normal experimental conditions. During this time the specific activity of C¹⁴O₂ in the chamber is not measurably diluted by respired C¹²O₂. Hence the rate of gross CO₂ uptake is proportional to the initial rate of decrease in radioactivity, and may be calculated from it since the starting CO₂ concentration in the apparatus is known.

1 Received October 27, 1964.
2 This work was supported by grants from the National Research Council of Canada and is partly based on experiments reported in the M.A. Thesis of Wendy B. Turner.
**Light.** Two banks of fluorescent lights (24 bulbs) were used in the experiments. The lamps were Gro-lux, warm white and high intensity white in the proportions 1:1:2. These gave an overall illumination of 1500 ft-c. For higher intensities, incandescent lamps with water screens were added. To be sure we were operating within light saturation, light curves such as the one shown in figure 1 were plotted and it can be seen that 1500 ft-c is well within the light saturation range. The temperature was kept within the range of 24 to 27°C, and did not vary more than 1° in any series of measurements.

**Plant Material.** The plants used were *Phaseolus vulgaris* (var. Pencil Pod Black Wax) which were grown in pots of soil in the greenhouse. They were well watered each morning so that water stress is unlikely to have occurred. Experiments were done on primary and trifoliate leaves.

**Stomatal Measurements.** The Darwin-Pertz parameter cup method (6) was used to measure relative stomatal opening. Further details of this procedure are described by Laidlaw and Knight (12).

**Results**

**Studies on Attached and Detached Leaves.** It is possible that a control mechanism operating from distant parts of a plant may affect the rate of photosynthesis in a leaf. If so, differences observed between attached and detached leaves will be important. For this reason, measurements of the rates of photosynthesis of comparable attached and detached leaves were made, with the results shown in figure 2. It can be seen from this figure that, although there was considerable variation in the rates of photosynthesis with time, no great differences occurred between attached and detached leaves either old or young, up to 2 days after detachment. There was, however, a clear-cut 10 minute lag phase following illumination in the attached leaf which the detached leaf did not exhibit, either immediately after excision, or up to 19 hours later. The lag phase in the attached leaf always lasted for 10 minutes and occurred after dark intervals of only 15 or 20 minutes. No lag phase was found if the leaf was only darkened for 10 minutes. Illuminating or darkening the rest of the plant had no effect on the lag phase. The data illustrating these points are shown in table I.

It seemed probable, from earlier work (3,4,10), that the lag phase observed in attached leaves was the result of stomatal behaviour. Hence measurements of the comparative opening of stomata in

**Table I. Effect of Preillumination on the Lag Phase of Photosynthesis of Attached or Detached Bean Leaves**

<table>
<thead>
<tr>
<th>Attached or detached (D) (hr detached)</th>
<th>Pretreatment of leaf</th>
<th>Rest of plant</th>
<th>Lag phase, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Light 60</td>
<td>Light</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>Dark 60</td>
<td>Dark</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>60</td>
<td>Light</td>
<td>10</td>
</tr>
<tr>
<td>A</td>
<td>60</td>
<td>Dark</td>
<td>10</td>
</tr>
<tr>
<td>A</td>
<td>60</td>
<td>Light</td>
<td>10</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>Light</td>
<td>5</td>
</tr>
<tr>
<td>A</td>
<td>30 10</td>
<td>Light</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>30 15</td>
<td>Light</td>
<td>10</td>
</tr>
<tr>
<td>A</td>
<td>30 30</td>
<td>Light</td>
<td>10</td>
</tr>
<tr>
<td>D (0)</td>
<td>Light 60</td>
<td>Light</td>
<td>0</td>
</tr>
<tr>
<td>D (2)</td>
<td>Light 60</td>
<td>Light</td>
<td>0</td>
</tr>
<tr>
<td>D (20)</td>
<td>Light 60</td>
<td>Light</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. Light intensity curve for photosynthesis of an attached bean leaf in incandescent light.

![Fig. 1](https://www.plantphysiol.org)

**Fig. 2.** Rates of photosynthesis in attached and detached primary bean leaves.
attached and detached leaves were made during the initial 10 minutes of illumination. Rates of photosynthesis were determined at the same time on comparable leaves. Typical results of these experiments are shown in figure 3. It may be seen that the stomata of both the attached and detached leaves began to open immediately upon illumination and continued to open for at least 20 minutes. CO₂ uptake, however, began abruptly at almost full velocity either immediately in detached leaves or after 10 minutes in attached leaves. It may also be seen that the stomata of the attached leaves, which had a lag phase, appeared to open a little more quickly than those of the detached leaves which had no such lag phase. Therefore, there seems to be no connection between the lag phase in photosynthesis and stomatal behaviour.

Effects of External Stimuli on the Rate of Photosynthesis. A series of experiments was done to determine if it was possible to affect the rate of photosynthesis in a leaf by applying various stimuli to distant parts of the plant. In the first of these, 1 leaf of a plant was placed under normal conditions in the photosynthesis chamber and the rest of the plant was heated with an infrared lamp until the plant was eventually cooked. The rate of photosynthesis of the leaf under measurement, protected from direct heat by aluminum foil, did not change during 3 days of such treatment. Several experiments were conducted in which the rest of the plant was illuminated at different levels to see if variations in the overall supply of photosynthetic in the rest of the plant could affect the rate of photosynthesis in the leaf under study, which was held under standard conditions. There was no such effect. Defoliating the rest of the plant, or dipping the roots in ice water or sucrose solution, as done by Belikov (11), also had no effect. The results of all these experiments are shown in table II. It may be concluded that none of the treatments to the rest of the plant could elicit any response from the leaf under observation, hence no evidence of a controlling mechanism originating in other parts of the plant could be found.

Photosynthesis in a Leaf during Development of the Plant. The course of development of a plant is such that the requirement for assimilation in any leaf may be changed from time to time. In order to see if any change in requirement might be correlated with a change in rate, a series of experiments was done to study the rate of photosynthesis of a leaf during the development of the plant. The leaf chosen was nearly expanded at the beginning of the experiment, and completed its growth during the

Table II. Effect of External Stimulation Applied to Distant Parts of the Plant on the Rate of Photosynthesis of 1 Leaf.

Leaves of different size and age were used in various experiments, hence the initial rates of photosynthesis cannot be compared. The illumination was 1500 ft-c and the temperature of the leaf was 25°C.

<table>
<thead>
<tr>
<th>Treatment of rest of plant</th>
<th>Rate of photosynthesis in experimental leaf, µg CO₂/min</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots in 2 M sucrose (30 min)</td>
<td>59</td>
<td>6</td>
</tr>
<tr>
<td>Roots in ice water (30 min)</td>
<td>61</td>
<td>59</td>
</tr>
<tr>
<td>Dim light (200 ft-c for 3 hr)</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Darkness (3 hr)</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Light (1500 ft-c) and fan draft</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Defoliated</td>
<td>16.5</td>
<td>18</td>
</tr>
<tr>
<td>Heat (15 lamp, up to 3 days)</td>
<td>12.5</td>
<td>13</td>
</tr>
<tr>
<td>Leaves wilted from heat</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>
experiment. Readings were taken at the same times of day for up to 10 successive days. There was a tendency to a gradual increase in photosynthesis as the leaf grew, but this was minor compared to a series of irregular fluctuations which appeared to be correlated with the appearance of buds. Whenever an axial bud appeared, the rate of photosynthesis in the leaf rose 20 to 110% for about a day and then fell back to the original rate. A plant stripped of its new leaves and its buds as they appeared exhibited a rise in rate just before a bud was removed, but the response was less pronounced. Results of 2 typical experiments are shown in figure 4. In 1 such experiment on a defoliated plant (fig 4, upper curve), a bud grew unnoticed through the beeswax-vaseline and the response was observed before the bud was discovered and removed. It may be concluded that there is a response in the rate of photosynthesis of a leaf when an axial bud breaks dormancy and begins to grow.

The Effect of Auxin on the Rate of Photosynthesis. The response of a leaf to the breaking of an axial bud suggested a possible auxin effect, since buds produce auxin in large quantities and this could be transported to the leaves (7). Consequently bean leaves, both young and mature, attached and detached, were sprayed with a 15 mg/liter solution of indole-3-acetic acid (IAA) containing 5 mg/liter ethanol and 0.05% Tween 80. The leaf was first placed in the chamber and several readings were taken to establish its normal rate of CO2 uptake. Then the IAA solution was sprayed on the upper surface of the leaf. Although there are no stomata on the upper surface of the bean leaf, auxin is known to penetrate bean leaves very quickly through the epidermis (15). Readings were taken as close together as possible for the next 1 to 2 hours. Some typical results are shown in figure 5. It can be seen that within 30 minutes after spraying, the rate of photosynthesis always increased rapidly to a value nearly double that before spraying, and then fell back to the original rate. The response could be obtained repeatedly in the same leaf. Primary or trifoliate leaves, old or young, attached or detached, all gave the same type of response. The response was slightly less pronounced in old or mature leaves than in young ones. Control leaves, sprayed with the same solution but without IAA showed no such increase in rate, and sometimes even showed a decrease. Leaves sprayed with tryptophane solution of 20 mg/liter showed only a small increase in photosynthetic rate.

A primary bean leaf was sprayed with various concentrations of IAA from 0.1 mg/liter to 100 mg/liter and its increase in CO2 uptake rate over the control was measured, with the results shown in figure 6. The curve shows a broad maximum over

![Fig. 4. Rates of photosynthesis in bean leaves attached to plants which were either allowed to develop normally (solid) or which had all buds and new leaves removed as they appeared (dotted).](image)

![Fig. 5. The effect of sprays on the rate of photosynthesis in bean leaves. A) The effect of IAA (15 mg/liter); B) the effect of water, tryptophan (20 mg/liter) and IAA (20 mg/liter); C) the effect of repeated spraying with IAA (15 mg/liter). All sprays contained ethanol (5 mg/liter) and Tween 80 (0.05%). S marks the time of spraying.](image)

![Fig. 6. Percent increase in rate of photosynthesis of a bean leaf at the time of maximum response (approx 30 min) following spraying with IAA solutions of different concentrations.](image)
the range 10 to 50 mg liter IAA, with lower responses at greater or lesser concentrations. It was thought that this response might be due to an effect of auxin on the stomata, so experiments were done using the Darwin-Pertz porometer cup. Measurements of relative stomatal opening were taken before and after spraying with water or auxin. The auxin did not cause the stomata to open wider, but as can be seen in figure 7, it had quite the opposite effect.

It can thus be concluded that an effect similar to the bud break response though of shorter duration, is obtained when leaves are sprayed with IAA, and this is not an effect of the auxin on the stomata but a more direct effect on the rate of CO₂ assimilation.

**Site of Action of the Auxin Effect.** It would thus appear that auxin has an effect on some mechanism which controls the rate of photosynthesis. The question must now be asked: at what point does auxin affect the rate of photosynthesis? It might affect the rate of CO₂ diffusion. If the rate of photosynthesis is normally limited by CO₂ diffusion, and the action of auxin is to increase it, then any other factor which increases CO₂ diffusion should have a similar effect on photosynthesis. Increasing CO₂ concentration would have this effect. Experiments were done in which the rate of photosynthesis of a bean leaf was measured at CO₂ concentrations from 0.01 to 0.22%. The results are shown in figure 8. It can be seen that from 0.02 to 0.22% CO₂ there was no change in the rate of photosynthesis. Therefore, it may be concluded that the action of auxin is not on CO₂ diffusion, but must be on some part of the mechanism of photosynthesis or the rates of reaction or diffusion of intermediates in the carbon cycle.

If auxin affects the rate of the photochemical

![Graph showing the effect of spraying water or IAA solution on stomatal aperture](image)

**Fig. 7.** The effect of spraying water or IAA solution (15 mg/liter) on the relative stomatal aperture of an attached bean leaf. Relative stomatal aperture derived from porometer readings (see caption for fig 3).

![Graph showing CO₂ concentration vs. rate of photosynthesis](image)

**Fig. 8.** The effect of CO₂ concentration on the rate of photosynthesis in a bean leaf at 1500 ft-c illumination. The arrow designates the CO₂ concentration at which previous experiments were done.

or light reaction, then its effect should be maximal at light intensities where light is limiting, but small or nonexistent at light saturation, where some other reaction system is limiting. If, on the other hand, auxin affects some aspect of the dark reactions, then its effect should be greatest at light saturation and least at lower light intensities where light is limiting. A series of experiments were performed in which a leaf was placed in the chamber at various light intensities ranging from 200 to 4000 ft-c, and determinations of the rate of CO₂ uptake were made to establish the normal rate at each light intensity. The leaf was then sprayed with auxin and readings were taken until the peak response was obtained and until the rate returned to normal. All this was done at each light intensity. The results of a typical experiment are shown in figure 9, where the normal rate and peak responses are plotted for various light intensities. It may be seen that auxin caused only a slight enhancement of the rate of CO₂ assimilation at lower light intensities, but caused a

![Graph showing CO₂ concentration vs. rate of photosynthesis](image)

**Fig. 9.** The effect of auxin spray (10 mg/liter) on the light intensity curve for bean leaf photosynthesis. Readings were taken at each light intensity on unsprayed leaves (■—■) and at the time of peak response following spraying with 1AA solution (○—○).
marked increase at light intensities above 1000 ft-c, the point of light saturation. It may thus be concluded that auxin stimulates the rate of CO₂ assimilation by affecting some mechanism which normally limits the rate of dark reactions in photosynthesis.

Discussion

From these results, it appears that the rate of photosynthesis is an attribute of a particular leaf and is not normally affected by stimuli applied to other parts of the plant. However, it can be affected by stimuli occurring at a distant site, namely, by the breaking of dormancy of an axial bud. A similar response can be obtained when a leaf is sprayed with IAA, i.e., a stimulation of the rate of photosynthesis occurs. Although only IAA was used in these experiments, Coulombe and Paquin (5) have found a similar stimulation of photosynthesis rate, as well as respiration and translocation, by gibberellic acid. It is interesting to note that a similar sort of reaction has been recently reported by Livne (14), namely that photosynthetic carbon fixation of healthy bean leaves may be greatly increased by infection of distant leaves with the rust Uromyces phaseoli. The stimulation of photosynthesis was proportional to the intensity of infection. These various observations suggest that photosynthesis, like so many other physiological processes, may be influenced by hormones. It is thus possible that the increased demand for photosynthate created by a newly developing bud may be met by an increased rate of photosynthesis, as well as translocation (5,9), in nearby mature leaves, caused by the increased export of auxin from the bud itself.

Summary

The gross rate of photosynthesis of a bean leaf (*Phaseolus vulgaris* var. Pencil Pod Black Wax), measured as C₁⁴O₂ uptake, is an attribute of the leaf and is not normally affected by external stimuli applied to other parts of the plant. Attached and detached leaves behave in the same manner except that attached leaves exhibit a well-defined 10-minute lag phase.

The rate of photosynthesis of a leaf rises to a sharp peak for a period of several hours concurrently with the breaking of dormancy of an axial bud. Similar peaks but of about an hour's duration, were caused by spraying the leaf with solutions of indoleacetic acid. This effect was not found in controls sprayed with water nor was it a stomatal effect. The action of auxin in promoting CO₂ uptake appears to be associated with the dark reactions of photosynthesis, not the photochemical reactions or the diffusion of CO₂.

The possibility is suggested that the bud break effect is due to the action of auxin produced in the growing bud.

Literature Cited