

THE EFFECT OF LEAF AGE ON THE RATE OF PHOTOSYNTHESIS IN DETACHED LEAVES OF TREE SEEDLINGS

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INTRODUCTION

That the maximum rate of photosynthesis varies with age of the plant material is an assumption which has found its way into many text books of plant physiology (see e.g. 1,2). The experimental evidence offered in its support, however, is surprisingly meagre, and, in particular, data relating to tree seedlings are conspicuously deficient. Experiments recording a reduction in maximum rate of photosynthesis with age of cultures of unicellular algae have been reported by WASSINK and KATZ (1939) and PRATT (1943). Although it is often difficult to be certain that the effects observed with algal cultures are not due merely to "ageing" of the growth medium, PRATT (1940, 1943) has provided unassailable evidence of an ageing effect within the plant cells themselves by extracting an inhibitor of photosynthesis from aged *Chlorella* cells. For trees, evidence has been recorded by WILLSTÄTTER and STOLL (1918), who found a 100 % increase in maximum assimilation level between "young" and "old" leaves of both *Acer pseudoplatanus* and *Tilia cordata*. Expressed per unit of chlorophyll, however, the rate of assimilation showed a 60 % decrease from "young" to "old" leaves and the authors infer from this that a protoplasmic factor other than chlorophyll limits photosynthesis in old leaves. BRIGGS (1920) came to the same conclusion following experiments with etiolated seedlings, while DASTUR (1924, 1925) recorded similar effects in herbaceous plants, but found also that differences in photosynthetic activity run parallel with differences in water content of the leaves. He concluded that the limiting "internal factor" was water.

The present paper describes experiments in which the effect of leaf age on photosynthesis in various species of *Acer* and *Quercus* was studied. In an attempt to distinguish actual and physiological age, data were also obtained for plants grown under different daylength conditions.

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EXPERIMENTAL MATERIAL AND METHODS

Experiments were made with detached leaves of first year seedlings of the following species: — *Acer pseudoplatanus* L., *A. platanoides* L., *A. saccharinum* L., *Quercus robur* L., and *Q. borealis maxima* (MARSH) ASHE. With the exception of the daylength series, the plants were raised in gravel cultures (for details, see 10) under constant conditions of light intensity (5000 lux for 16 hours per daily cycle) and temperature ($20^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$). The daylength series comprised seedlings of *Acer saccharinum* which had been raised under different photoperiods. Plants from long-day (L.D.) conditions (24 hours of light per day) and having 9 leaf pairs, were compared with plants from a short-day (S.D. 10 hours of light per day) and having only 4 leaf pairs.

Carbon dioxide assimilation in relation to light intensity was measured by means of the diaferometer technique developed for botanical purposes by AUFDEMGARTEN (1939) and VAN DER VEEN (1949).

This technique involves the determination of changes in the composition of gas mixtures by measuring the corresponding changes in their thermal conductivity. Since the conductivity of CO_2 is less than that of air and that of O_2 is approximately the same as that of air, a change in the CO_2 content of air will change its heat conductivity; these changes can be measured with the diaferometer. The apparatus used in the present work has been described in detail by SPIERINGS *et al.* (1952). Briefly, a thin platinum wire is heated by a uniform electric current from a 6-volt battery, regulated by a rheostat. The wire is surrounded by the gas under investigation, to which it loses heat. Any change in the thermal conductivity of the gas causes a change in the rate of heat loss from the wire and, consequently, in its temperature. Changes in temperature of the wire effect commensurate changes in its resistance which can be measured by means of a sensitive galvanometer. The sensitivity of the apparatus was such that a deflection on the galvanometer scale of 10 cms represented an assimilation rate of $1.44\text{ cm}^3\text{ CO}_2/\text{hour}$.

Leaves were placed in a glass assimilation chamber kept at constant temperature in a water bath and illuminated either by a 500 watt incandescent lamp or, in a few experiments, by a high pressure mercury vapour lamp (Type, Philips H.O. 2000). A gas flow of constant velocity passed over the leaf and through the diaferometer, where its heat conductivity was measured against that of gas from the same source, but which had by-passed the leaf and, thus, had not changed in CO_2 content. The gas mixture used was air containing 4.65 % CO_2 and was saturated with water before passing the leaf. The light intensity was varied by interposing combinations of Schott neutral glass filters between the lamp and the assimilation chamber and was measured by means of a selenium barrier-layer photocell held flush to the leaf surface. The temperature of the water bath was regulated by a small 20-watt heating coil or by adding ice.

Since these measurements were carried out on detached leaves, it was desirable to find out, at the outset, for how long the normal rate of photosynthesis was maintained, before the effects of removal from the plant were felt in the leaf. Starting 15 minutes after detaching leaves of *Acer saccharinum* from first year seedlings, measurements of CO_2 assimilation at a light intensity of 20 000 lux and a temperature of 24°C were made for periods of 6 hours continuously. The results of 4 such determinations are shown in Fig. 1. Carbon dioxide uptake has been expressed per unit area per unit time and plotted against time.

The figure demonstrates that for the first three quarters of an hour there is an apparent increase in photosynthesis rate, followed by a steady decline for the next five hours.

For all experiments, therefore, leaves were placed in the assimilation chamber immediately after having been detached from the plant and

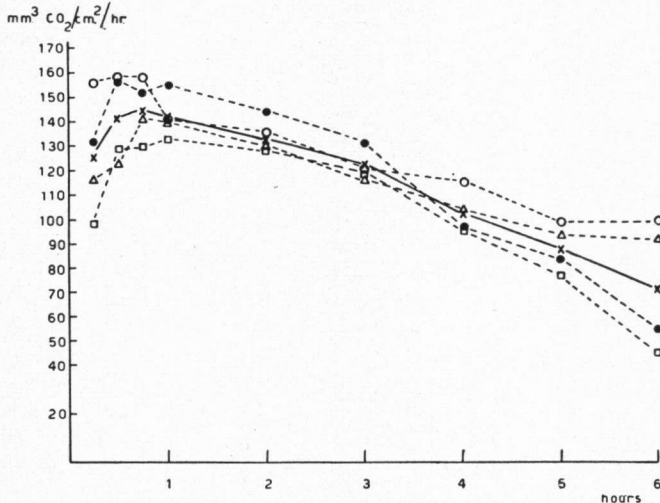


Fig. 1. *Acer saccharinum*. Uptake of carbon dioxide ($\text{mm}^3 \text{CO}_2/\text{cm}^2/\text{hr}$) by excised leaves plotted against time after detachment from first-year seedlings. The solid line represents the mean of 4 leaves shown by dotted lines.

measurements were started after 45 minutes. All experiments were completed within 2 hours of detaching the leaves. Thus, changes in photosynthetic rate over the period of the experiment and due to uncontrollable factors were probably not great. Measurements of the light intensity dependence of photosynthesis were made at about 24°C ; they began with the lowest intensity and worked upwards to the highest. Thus, measurements at the highest light intensities were made when the leaves had been detached from the plant between $1\frac{1}{2}$ and 2 hours. In view of the decline in photosynthesis rate with time, it follows that such measurements when compared with those at lower light intensities, are too low. The error introduced, however, was not regarded as serious.

In all experiments, CO_2 output in the dark was taken as a measure of respiration and it was assumed that light has no influence on respiration. That this is a correct assumption has been questioned by several workers; indeed, according to JAMES (1953), "possible interactions with respiration have been the bugbear of photosynthetic measurements since their beginning". Among others, FOCKLER (1938), McALISTER and MYERS (1940), and MYERS and BURR (1940) have demonstrated an apparent increase in respiration as a consequence of illumination. KOK (1950), on the basis of experiments at low light

intensities, concluded that light reduces or even totally suppresses respiration. On the other hand, recent work with labelled oxygen (O^{18}) has failed to demonstrate any marked effect of light on oxygen uptake by higher plants including several tree species (1951). In the absence of a method of measuring O_2 exchange, therefore, the above assumption was made. Since the output of CO_2 due to respiration relative to the uptake due to photosynthesis is generally very small, serious errors were probably not introduced. Measurements of dark respiration were made immediately before and after the photosynthesis measurements.

EXPERIMENTAL RESULTS

1. *The effect of physiological age of leaf on photosynthesis*

In Figs. 2—6, graphs of CO_2 assimilation against light intensity, for leaves of different physiological ages, have been plotted. Each point represents the mean of 4 determinations. The data from which these figures were obtained are summarised in Table I. The saturation and compensation light intensities were read from the completed figures.

Photosynthesis has been expressed as $mm^3 CO_2$ assimilated per cm^2 leaf area per hour, for each of the leaf ages used. Leaf areas were determined by tracing on to squared paper at the end of each measurement.

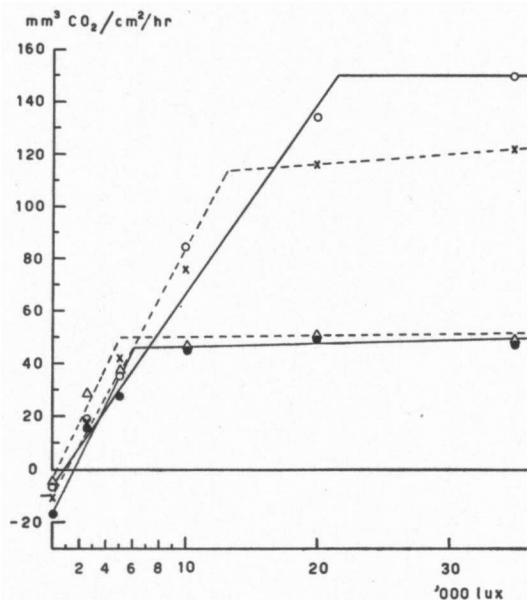


Fig. 2. *Acer pseudoplatanus*. The effect of leaf development stage on the rate of photosynthesis. ●—● young leaf, ×---× half-expanded leaf, ○—○ fully expanded leaf, △---△ old leaf. Each point average of 4 leaves.

TABLE I
Photosynthesis data for detached leaves of different physiological age; means of 4 determinations

Species	Physiological age	Maximum recorded CO ₂ uptake (mm ³ /cm ² /hr)	Mean dark respiration (mm ³ /cm ² /hr)	Saturation intensity (lux)	Compensation point (lux)	Expt. temperature °C.
<i>Acer pseudoplatanus</i>	Young	50.4	17.36	5950	1618	25.4
	Half-expanded	122.4	10.41	14438	1178	25.4
	Fully-expanded	150.6	6.55	21719	893	25.6
<i>Acer platanoides</i>	Old	51.5	4.96	5050	485	25.4
	Young	18.4	11.70	4888	2300	25.1
	Half-expanded	96.1	5.34	10262	583	25.5
<i>Acer saccharinum</i>	Fully-expanded	116.3	8.26	19675	1300	25.6
	Old	49.2	4.84	6975	765	25.9
	Young	48.0	8.62	3988	653	25.1
<i>Quercus robur</i>	Half-expanded	99.1	6.20	13050	845	25.4
	Fully-expanded	138.0	6.86	21100	1025	25.8
	Old	53.2	5.81	5100	565	25.5
<i>Quercus borealis</i>	Young	66.4	6.94	7850	838	25.0
	Half-expanded	94.5	5.42	11763	643	25.6
	Fully-expanded	116.2	3.67	13462	450	25.4
<i>Quercus borealis</i>	Old	70.4	4.17	7550	476	25.4
	Young	56.5	15.72	6700	1518	25.3
	Half-expanded	41.0	6.98	5438	738	25.6
<i>Quercus borealis</i>	Fully-expanded	73.3	7.33	12750	1033	25.6
	Old	23.2	3.98	7313	803	25.3

The physiological ages are described as "young", "half-expanded", "fully-expanded" and "old". "Young" leaves are leaves just formed and growing rapidly. They were light green in colour or, in the case

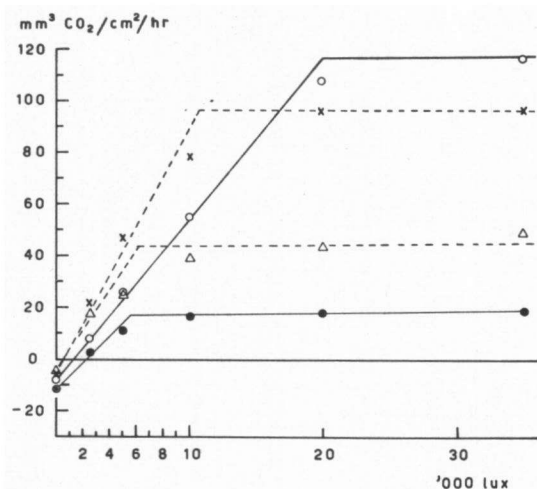


Fig. 3. *Acer platanoides*. The effect of leaf development stage on the rate of photosynthesis. ●—● young leaf, ×—× half-expanded leaf, ○—○ fully expanded leaf, △—△ old leaf. Each point average of 4 leaves.

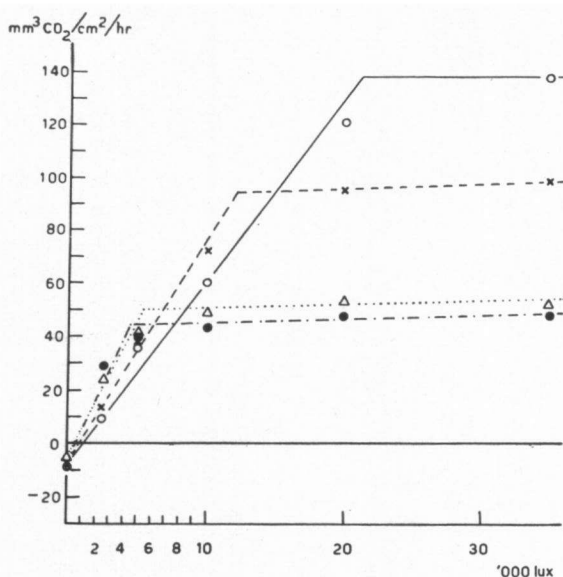


Fig. 4. *Acer saccharinum*. The effect of leaf development stage on the rate of photosynthesis. ●—● young leaf, ×—× half-expanded leaf, ○—○ fully expanded leaf, △—△ old leaf. Each point average of 4 leaves.

of *Acer*, were sometimes pale yellow. "Fully-expanded" refers to the youngest fully-expanded leaf on the plant, while "half-expanded" is intermediate between young and fully-expanded. By "old" is meant the oldest fully-expanded leaf present. The actual ages (approximate values only) were as follows:

Species	Young leaves (days)	Half-expanded leaves (days)	Fully-expanded leaves (days)	Old leaves (days)
<i>Acer</i> spp. and <i>Quercus</i>				
<i>robur</i>	4-6	9-12	14-18	35-50
<i>Quercus borealis</i>	2-3	5-6	10-17	ca. 60

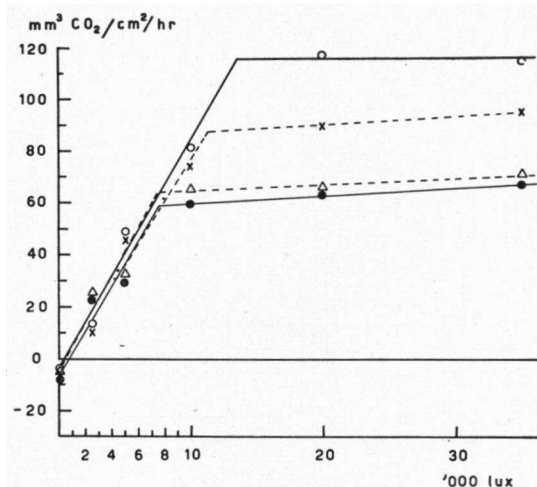


Fig. 5. *Quercus robur*. The effect of leaf development stage on the rate of photosynthesis. ●—● young leaf, ×—× half-expanded leaf, ○—○ fully expanded leaf, △—△ old leaf. Each point average of 4 leaves.

It will be noted that, in the figures, the pre- and post-light-saturation parts of the curves have been prolonged to meet at a point, while in many cases a smooth curve would better fit the plotted values. This has been done in order to obtain a more objective value for the saturation intensity than would otherwise be possible.

It can be seen from the figures and table that, in the case of the *Acer* spp., the maximum level of CO_2 assimilation in very young leaves is relatively low; it is somewhat higher in half-expanded leaves; and highest when the leaves are fully-expanded; the rate in old leaves is about $\frac{1}{2}$ to $\frac{1}{3}$ of that in fully-expanded leaves. Similarly, the light saturation intensity increases up to full expansion, and then falls when the leaves become old. The compensation point values, however, show no definite trend.

In *Quercus robur* (Fig. 5), the pattern is similar but the maximum levels of photosynthesis and saturation intensities are markedly lower

than for any of the *Acer* spp. For *Quercus borealis* (Fig. 6), however, the maximum assimilation level is higher in young than in half-expanded leaves though, again, it is highest in fully-expanded leaves and falls off during senescence. Differences in saturation intensity are less marked and there is little difference between young, half-expanded and old leaves. The highest saturation intensity, however, is again found in fully-expanded leaves.

With regard to absolute values, it is clear that, under the conditions of these experiments, maximum CO_2 assimilation per unit leaf area is relatively greater in the case of *Acer* than in that of *Quercus*.

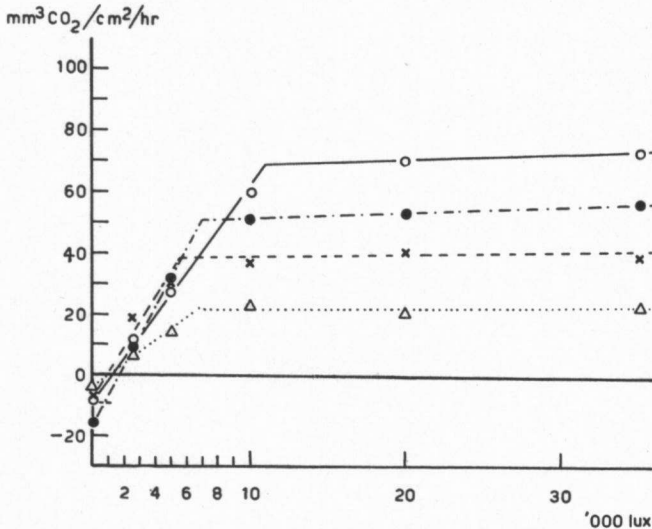


Fig. 6. *Quercus borealis*. The effect of leaf development stage on the rate of photosynthesis. ●—● young leaf, ×---× half-expanded leaf, ○—○ fully expanded leaf, △---△ old leaf. Each point average of 4 leaves.

The values for respiration show the same trends for all species, young leaves showing the highest rate and old leaves the lowest. There is little difference between half-expanded and fully-expanded leaves, and little difference in absolute values between species at these leaf ages. With young leaves, however, the rates of CO_2 output by *Quercus borealis* and *Acer pseudoplatanus* are almost double that of the other species.

2. The effect of actual age of leaf on photosynthesis

Having found big differences in CO_2 assimilation between leaves of different physiological age, it seemed of interest to see whether differences in *actual* age have any marked effect. As has already been said, seedlings of *Acer saccharinum* which had grown under extremes of daylength were used. Plants of the types used are illustrated in Fig. 7. The leaf pair numbered 2 in the S.D. plant has been present for the

same length of time as that on the LD plant. Due to the more rapid growth of the latter, however, there is a big difference in physiological age between the two leaf pairs; whereas in the short day the 2nd leaf pair is only just fully expanded, in the long day, the corresponding leaf pair is approaching physiological old age. In physiological age,

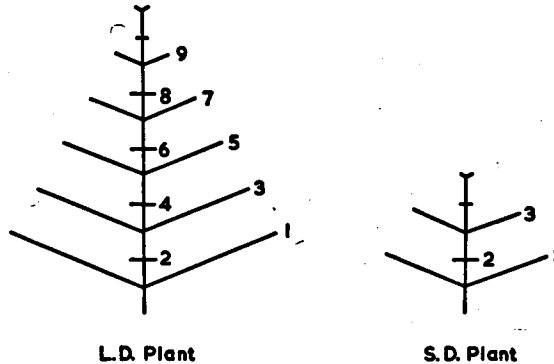


Fig. 7. *Acer saccharinum*. Diagrammatic representation of seedlings grown under extremes of daylength. L.D. = 24 hours light per day; S.D. = 10 hours light per day.

the 2nd leaf pair of the S.D. plant compares with the 7th leaf pair of the L.D. seedling.

By measuring CO_2 assimilation in leaves from the 2nd (L.D. and S.D.) and the 7th (L.D.) leaf pairs, therefore, it should be possible to see how differences in actual age compare with differences in physiological age.

The results of such a comparison are given in Fig. 8. Each point in Fig. 8 is the mean of 5 measurements. The close resemblance between the curves for the 2nd S.D. leaf and that for the 7th L.D. pair, together with the entirely different curve for the 2nd L.D. leaf, demonstrates that the relation between maximum assimilation level and physiological age does not extend to actual leaf age.

DISCUSSION

The increase in the rate of photosynthesis during the first 15 minutes in the experiments illustrated in fig. 1 is unlikely to be due to induction phenomena of the kind described by VAN DER VEEN (1949), though BRIGGS (1933) has shown that induction phases may be of relatively long duration. It may well be due to changes in the rate of gas exchange between the leaf and the surrounding air as a result of an increase in the number of stomata open or in their width. Perhaps an increase in leaf temperature due to illumination is a contributory factor, though this, again, would not be expected to go on for so long.

In general, in these data maximum assimilation level and saturation intensity increase with physiological age up to full leaf expansion as expected. In *Quercus borealis*, however, the maximum assimilation

level was higher in young than in half-expanded leaves. There can be little doubt that the observed effect is a real one. An explanation may lie in the relation between internal factors in photosynthesis and leaf expansion. In addition to its dependence upon chlorophyll concentration (which increases steadily with leaf age), the rate of photo-

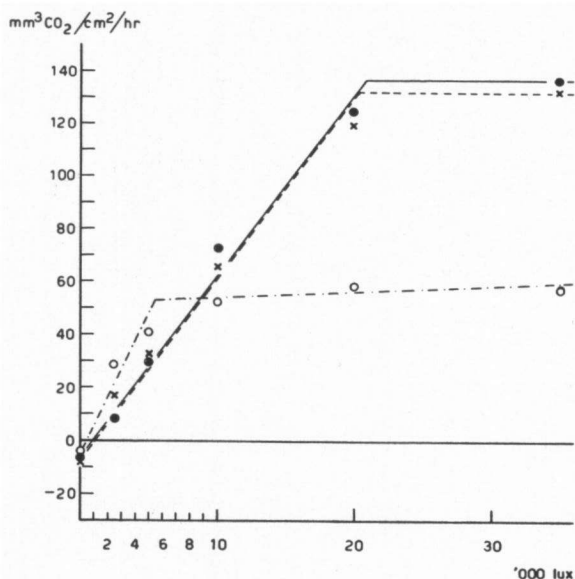


Fig. 8. *Acer saccharinum*. Rate of photosynthesis at $\pm 25^{\circ}$ C. plotted against light intensity for leaves of the same actual age but of different physiological ages (cf. ●—● = 2nd leaf from S.D. plant; and ○—○ = 2nd leaf from L.D. plant) and for leaves of the same physiological age but of different actual ages (cf. ●—● = 2nd leaf from S.D. plant; and ×—× = 7th leaf from L.D. plant). Each point represents the mean of 4 determinations.

synthesis may be determined by other internal factors (e.g. leaf protein) and the absolute amount of such factors may well increase as the leaf grows older. Leaf expansion in *Quercus borealis* is extremely rapid; only 4 days separate "young" and "half-expanded" leaves. Leaf expansion therefore, may outstrip the formation of internal factors, so that, when expressed per unit leaf area, these factors initially decrease with leaf age. Thus, if they limit photosynthesis, this process will show an initial decrease on a leaf area basis. Changes in the level of a single "internal factor" may account for all observed changes in the maximum assimilation rate per unit leaf area. Although the initial rise in rate may be due to an increase in the concentration of chlorophyll with age, the drop in the assimilation rate during leaf senescence cannot be linked with changes in chlorophyll concentration since the latter is reported to increase with age (STALFELT, 1924; RABINOWITCH, 1952). In our material, also,

there was no decrease in chlorophyll concentration with age as judged by eye.

In addition to the work of WILLSTÄTTER and STOLL (1918) and BRIGGS (1920), most more recent schematic representations of photosynthesis contain several enzyme systems (see RABINOWITCH, 1952; WARBURG, 1919; WASSINK *et al.*, 1938). Moreover, the reduction of CO_2 and the development of O_2 involve a number of enzymatic reactions (FRANCK and LOOMIS, 1949). There is some evidence that protein metabolism is intimately bound up with photosynthesis and that the rate of protein synthesis can limit photosynthesis (BURSTROM, 1945; SMITH, 1944).

It is thus clear that the changes in the maximum level of CO_2 assimilation per unit leaf area reported here need not to be ascribed to any single factor. During leaf expansion possible factors limiting photosynthesis are: chlorophyll concentration, moisture content, or the capacity of any enzyme systems involved. During senescence the limiting factor is almost certainly not chlorophyll but it may be either moisture content or an enzyme.

The assumption that CO_2 output during darkness is an accurate measure of respiration during illumination, may not be correct. Also, the experimental technique was not sufficiently exact for precise measurements on this point. With the galvanometer sensitivity used for these measurements, a deflection of 1 cm. represented a CO_2 output of 144 mm^3/hr . Leaves of about 25 cm^2 area were used, and most of the measurements of CO_2 output are based on scale deflections of less than 15 mm. Since it was possible to read only to the nearest 1 mm, the absolute values are subject to relatively large errors. In all species, the highest rates were shown by the youngest leaves and the lowest rates by the oldest leaves. This confirms the general information that in young leaves the rate of respiration is normally higher than in old leaves, due to the greater proportion of protoplasm in meristematic tissues.

It is of interest to compare the absolute levels of CO_2 assimilation, with those found by other workers. Few measurements of photo-

Species	Maximum rate of photosynthesis ($\text{mm}^3\text{CO}_2/\text{cm}^2$ leaf surface/hour) ¹⁾
<i>Aesculus hippocastanum</i>	178.8
<i>Acer Negundo</i>	216.8
<i>Sambucus nigra</i>	184.2
<i>Acer pseudoplatanus</i> ("young leaves")	86.7
<i>Acer pseudoplatanus</i> ("old leaves")	140.9
<i>Tilia cordata</i> ("young leaves")	97.5
<i>Tilia cordata</i> ("old leaves")	151.7
<i>Quercus robur</i>	222.2

¹⁾ WILLSTÄTTER and STOLL actually present their results as mg CO_2 absorbed /100 cm^2 leaf surface/hr. Their results have been recalculated here as $\text{mm}^3\text{CO}_2/\text{cm}^2/\text{hr}$, so as to make them directly comparable with our data.

synthesis in woody species have been made, and even these lack standardisation in methods of measurement or expression of results. The most extensive data for trees measured under laboratory conditions are those given by WILLSTÄTTER and STOLL (1918). In detached leaves in air enriched with 5 % CO_2 at 20° C, they obtained the following maximum rates of photosynthesis (light and CO_2 saturated.¹⁾

In an atmosphere containing 6.46 % CO_2 and at a temperature of 21° C, the present author (1952) found maxima in the region of 120.0 for both *Acer pseudoplatanus* and *Quercus robur*.

The data given in Table I show that the maximum assimilation values measured for detached leaves of *Acer saccharinum* (ca. 138.0 for fully-expanded leaves) and *Acer pseudoplatanus* (150.6) are of the same order as those found by Willstätter and Stoll for old leaves of *Acer pseudoplatanus*, but are somewhat higher than those recorded earlier (RICHARDSON, 1952) for the latter species. The *Quercus robur* and *Quercus borealis* figures (mean maximum = 116.2 and 73.3 for fully-expanded leaves) are markedly lower than those found previously for *Quercus borealis* (RICHARDSON, 1952) and very much lower than those recorded by Willstätter and Stoll for *Quercus robur*. The maxima recorded here for leaves of *Quercus borealis* are some 40 % lower than those found in 1951 (RICHARDSON, 1951). However, the seedlings used in 1951 had been grown under field conditions and were, presumably, adapted to the much higher light intensities than those in the present experiments. Photosynthetic adaptation of this kind has been found by previous workers (BOYSEN-JENSEN and MUTTER (1929), GESSNER (1937), BECK (1938), BOHNING (1949) and also in our laboratory WASSINK *et al.* (1956).

On present evidence the conclusion is probably justified that variations in the maximum assimilation level are at the specific rather than the generic level. Cultural conditions (especially light intensity) have a marked influence on the photosynthetic capacity of leaves.

SUMMARY

Determinations of CO_2 assimilation per unit leaf area in relation to light intensity were made with detached leaves of various physiological ages of the following species: *Acer pseudoplatanus* L., *A. platanoides* L., *A. saccharinum* L., *Quercus robur* L., and *Q. borealis maxima* (MARSH) ASHE. Measurements were made using the diaferometer technique, at a temperature of 25° C. and on first year material only.

In general, maximum assimilation level and saturation light intensity increased with physiological age of plant material up to full leaf expansion and then fell off during senescence. In *Quercus borealis*, however, the maximum assimilation level was higher in young than in half-expanded leaves; it was concluded that, in this species, leaf expansion outstrips the rate of formation of an internal factor limiting photosynthesis, thus giving rise to an initial decrease in photosynthesis when expressed per unit leaf area.

An experiment using seedlings of *Acer saccharinum* grown under different daylength conditions and, consequently, in different stages of development, demonstrated

¹⁾ See table bottom p. 455.

that physiological age is more important than actual age in determining the rate of photosynthesis.

In general, the maximum recorded rates of assimilation were markedly higher in species of *Acer* than in the *Quercus* species. From a comparison of these results with those of previous workers it was concluded that variation in photosynthesis potential are at the specific rather than the generic level and are determined to a great extent by the cultural conditions, especially light intensity.

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