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PHOTOSYNTHESIS OF CALENDULA OFFICINALIS L. AND IMPATIENS PARVIFLORA DC., AS INFLUENCED BY LIGHT INTENSITY DURING GROWTH AND AGE OF LEAVES AND PLANTS

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1. INTRODUCTION

It is a well known fact that the photosynthetic capacity of leaves of higher plants depends on the light intensity to which the plants are exposed during growth. Also is it known that the photosynthetic capacity varies with the age of the leaf. In the present investigation an attempt was made to find out how these two factors interfere and to determine the contribution of each specific leaf to the production process.

1.1. HISTORICAL REVIEW

1.1.1. Influence of light on the rate of photosynthesis

The influence of differences in light intensity on growth and photosynthesis has been the subject of numerous studies for many years. The first studies were mainly concerned with the influence of light on leaf anatomy. As early as 1754, BONNET compared the growth type of pea seedlings grown in the open air, under a glass cover, and under a wooden shed, and with that he probably was the first to carry out definite experiments regarding this subject. STAHL (1883) described how the level of irradiance causes differences in leaf structure: larger cells and more layers of palisade cells occurring under high light intensities. DUFOUR (1887) confirmed these observations. He, too, attributed the greater thickness of a sun leaf to elongation of the palisade cells and the development of a second layer of palisade cells and, to a smaller degree, to additional cell layers in the spongy parenchyma. Before, BATALIN (1871) had already shown that very thin, nearly chlorophyll-free leaves developed on plants exposed to very low amounts of light, suggesting that leaf thickness as well as chlorophyll content were related to the level of irradiance. Later on, it became evident that the chlorophyll content may also drop when light intensity becomes too high. For instance, RANDALL (1953) found a gradual drop in the chlorophyll content in leaves of different herbs in a Southern Wisconsin forest border from high forest to savannah, i.e. from low to high light intensity conditions.

WASSINK, RICHARDSON and PIETERS (1956) also found higher chlorophyll contents in *Acer pseudoplatanus* leaves, outdoors when, by reducing the light with the aid of gauze screens, they were grown under low light intensities, as well as indoors by varying the distance of the plants to the light source. BJÖRKMAN and HOLMGREN (1963) grew ecotypes of *Solidago virgaurea* from sunny and shaded habitats under different light regimes. In all cases they found a higher chlorophyll content per unit fresh weight under low light intensities. Per unit leaf area, chlorophyll contents were highest in leaves from sunny habitats grown under high light intensities and in leaves from shaded habitats grown under low light intensities. *Acer pseudoplatanus* as well as *Solidago virgaurea* showed higher maximum photosynthetic rates, when grown under high light intensities, except some *Solidago* plants from shaded habitats, which suffered from destruction of

the chloroplasts, a phenomenon already described by DUFOUR (1887). That the rate of photosynthesis at light saturation is not related to the chlorophyll content is easily understood from the fact that any fully green leaf contains an excess of chlorophyll, so that at high light intensity, the chlorophyll content will not easily act as a limiting factor for light saturation. Only at low light intensities, the extra light absorbed by a high concentration of chlorophyll may become important and ecologically adventageous for the shade leaves (GABRIELSEN, 1948).

Studies on the influence of light intensity during growth on the rate of photosynthesis started much later. Most studies were carried out with trees, since here sun and shade leaves may be taken from the same plant, and, moreover, tree seedlings generally grow in the deep shade of a forest floor. Rather than with an influence of light intensity on the photosynthetic capacity, the first studies were concerned with the inhibition of photosynthesis upon exposure to high light intensities. This effect was found to occur at lower light intensities in shade plants than in typical sun plants, the maximum photosynthetic rate being reached, e.g., at 10,000 lux or less in shade plants and at 50,000 lux in sun plants (WEIS, 1903; HARDER, 1930, 1933). At light intensities higher than the saturation level, photosynthesis is easily inhibited in shade plants. This might, however, partly be due to overheating. The mentioned authors were also among the first to observe differences between the shapes of the light curves of sun and shade plants. WEIS (1903) compared the light curves of Polypodium and Oenothera (a shade and a sun plant), and observed a higher saturation rate and a higher saturating light intensity for the latter.

BOYSEN-JENSEN and MÜLLER (1929) were the first to compare, in this respect, sun and shade leaves from one plant, *Fagus silvatica*. As in earlier comparisons between sun and shade plants of different species, they found a higher maximum rate of photosynthesis, a higher saturation light intensity and a higher compensation point for the sun leaves. Experiments where the plants were artificially exposed to different light intensities during growth, keeping the other external factors constant as much as possible, were carried out by BLACKMAN et al. (1946, 1951) and by WASSINK, RICHARDSON and PIETERS (1956).

Since then, a great number of studies has been performed, mainly with plants known as species or ecotypes from sunny and shaded habitats (e.g. BJÖRKMAN and HOLMGREN, 1963, 1966) and with sun and shade leaves of trees (e.g. PIETERS, 1960, 1962).

It is now well established that most plants are more or less able to develop a higher photosynthetic capacity when grown under conditions of high light intensity, except for possible damage done to the chloroplasts. Recent studies are mainly dealing with the problem what the reasons are for the differences in the light curves of plants grown under different light regimes. Although the most simple reason seems to be the presence of more assimilatory tissue in the sun leaves, because they are thicker, this explanation does not always hold. Other reasons might be a reduced mesophyll resistance owing to smaller and more densely packed mesophyll cells in sun leaves (HOLMGREN, 1968) or a reduction in stomatal resistance which may be due either to an increase in the number of stomata or to a larger stomatal pore width or to both (HOLMGREN, 1968; WILSON and COOPER, 1969d). BJÖRKMAN (1968) found higher activities of the enzyme carboxydismutase (ribulose-1,5-diphosphate carboxylase), wich is active in photosynthetic carboxylation, in plants with a higher photosynthetic capacity; this could be due to genetic factors or to exposition to high light intensities. All these possibilities may be active to some degree. Little attention has been paid to the amount of assimilating tissue, although it is known that, e.g. in rice, a linear correlation exists between the maximum rate of photosynthesis, the protein, phosphate and potassium content, and respiration (MURATA, 1961). Certainly, the latter properties may, in their turn, be proportional to leaf mass.

1.1.2. Influence of age on the rate of photosynthesis

It is a general assumption that the maximum rate of photosynthesis varies with the age of the plant or the leaf, reaches its maximum at full leaf expansion and, after a longer or shorter period, gradually declines. WILLSTÄTTER and STOLL (1918) found a maximum assimilation rate which, expressed per unit leaf area, was 60% higher in old leaves than in young, not fully expanded ones, of Acer pseudoplatanus and Tilia cordata. However, the rate of assimilation was over 100% higher in the young leaves, when expressed per unit chlorophyll. They concluded, as did also BRIGGS (1920), that some protoplasmic factor limits photosynthesis in older leaves. DASTUR (1924, 1925) found the same effects in herbaceous plants. Since the differences in photosynthesis were correlated with the differences in the water content in the leaves, he concluded that the limiting factor was water. Even in cultures of unicellular algae a reduction in maximum rate of photosynthesis with age was found by WASSINK and KATZ (1939). Here it is difficult to be certain that the reduction is not caused by exhaustion of the growth medium. However, PRATT (1940, 1942, 1943) succeeded in isolating an inhibitor of photosynthesis from aged Chlorella cells, 'chlorellin', which was proven to occur in amounts that ran parallel with the decline of photosynthetic activity.

RICHARDSON (1957) concluded from his experiments on seedlings grown under different daylengths that physiological age is more important than actual age in determining the rate of photosynthesis. He also found that an initial decrease in photosynthesis of young leaves may occur if leaf expansion exceeds the rate of formation of an internal factor limiting photosynthesis. On the other hand, it has been found that photosynthesis may show a small initial rise after complete leaf expansion and before the decline starts (JEWISS and WOLEDGE, 1967). Here, probably, the formation of the same internal factor goes on after the leaf has reached its final size. The low rate of apparent photosynthesis in young leaves is partly due to very high respiration rates (RICHARDSON, 1957; HOFFMANN, 1962).

The decline of the photosynthetic capacity connected with senescence coincides with some other properties: decline of respiration, loss of protein, reduc-

tion of the RNA, DNA, and chlorophyll content, export of Ca and K, already found by SINGH and LAL (1935), P, and N, and finally yellowing of the leaf. Furthermore, there is a loss of semipermeability of membranes (LEOPOLD, 1961, 1964).

Lack of light, water and minerals accelerates the process of senescence. Also the development of flowers and fruits, including bulbs and tubers, causes increased ageing, probably because nutrients from the leaves are mobilized in favour of the developing organs (MOLISCH, 1938).

Senescence of leaves can be retarded by a number of auxins and kinins (MOTHES, 1960; OSBORNE, 1962). Kinetin, e.g., temporarily arrests the senescent changes of protein, RNA, DNA, and chlorophyll levels in attached leaves as well as in leaf discs. Since kinetin is produced in the root system and distributed by the transpiration stream, it may have a regulating function in senescence. The production of kinetin is only performed by an actively functioning root system (KULAEVA, 1962). The effect of kinetin may maintain the synthesis of nucleic acids and protein.

1.2. SCOPE OF THE INVESTIGATION

Few studies seem to have been made on the decline of the photosynthetic rate with increasing age. The present study may be considered as a mutual supplement of investigations made since 1966 at the Laboratory for Plant Physiological Research of the Agricultural University at Wageningen on the influence of light intensity on photosynthesis and on dry matter production of various plant organs, morphogenesis and anatomical features of a shade plant (*Impatiens parviflora*) and a sun plant (*Calendula officinalis*), (WASSINK et al., in preparation). The development of higher plants is characterized by a rather complicated interaction of photosynthesis and morphogenetic effects. For a better understanding of the data on production and morphogenetic features, the present investigations on the rates of photosynthesis under various conditions of light intensity during growth were made.

The numerous experiments have led to the presentation of a great number of figures. As far as possible, comparable data were averaged in summarizing figures. These figures are outlined and provided with a heavy-typed letterpress.

These studies were carried out in the frame of the Production Process Subsection Photosynthesis (P.P.photos.) of the International Biological Programme.

2. MATERIAL AND METHODS

2.1. MATERIAL

The experiments were carried out with two species: Impatiens parviflora (Balsaminaceae) and Calendula officinalis (Compositae). The first species is mainly found under trees and can thus be considered as a shade tolerant species. The plants used in our experiments originated from seeds collected in the neighbourhood of the Laboratory for Plant Physiological Research, where it grows abundantly.

The second species is a common garden plant, which prefers a sunny habitat and at our latitude is regarded as a sun loving species. In southern regions however, e.g. in Israel, the plant seems to flower during the winter (YAHEL, personal comment).

During the summer season, the plants were grown in plots of 2×2 meter in the experimental field of the laboratory, at four different light intensities: 100%, 75%, 37% and 12% daylight (KAMEL, 1959; WASSINK, 1960 and 1968). Light was reduced with the aid of metal gauze screens, iron gauze with a mess width of 6 mm reducing the light intensity to 75%, and one or two layers of phosphorbronze gauze with a mesh width of 0.9 mm, reducing the light intensity to 37% and 12% respectively. One upper screen of 2×2 meter and four side screens, 50 cm high, were used. The whole construction hang between four wooden posts and could be moved upwards as the plants elongated. In order to avoid as much as possible additional differences in temperature and humidity, after the first season a space of about 5 cm was left between the upper screen and the side screens, to stimulate air circulation.

The seedlings were invariably planted at 20 cm distance. Around the plots an additional row was planted in order to avoid borderline effects.

The plants were grown in single and mixed plots.

During winter, the plants were grown on trolleys in the phytotron, again at different light intensities. Light is provided by a set of 20 Philips TLMF No W/33 RS fluorescent tubes, which in the way the trolleys were used, yields a light intensity of ca. 11.4×10^4 ergs \cdot cm⁻² · sec⁻¹ at a height of 25 cm above pot level, which is about the maximum height of *Calendula* under these conditions, and of ca. 6.5×10^4 ergs \cdot cm⁻² · sec⁻¹ at pot level. Lower light intensities were obtained either by using metal gauze screens or by reducing the number of lamps, which facilitated temperature control. Temperature was kept constant at 20° \pm 1°C, air humidity was 65%. Plants were grown in soil and provided weekly with ca. 50 ml nutrient solution, apart from daily watering.

Unfortunately, it proved to be very difficult to break the seed dormancy of *Impatiens parviflora* within four weeks after collecting the seeds. The seeds were soaked in water during one week, and stored in moist sand at a temperature of 5° C. Germination occurred after a storage of at least seven weeks. Once the

seeds had germinated, the plants sometimes grew rather poorly; therefore, compared with *Calendula officinalis*, only few data from indoor experiments are available. To compensate this, in outdoor experiments more data were collected from *Impatiens* than from *Calendula*.

2.2. Methods

2.2.1. Measurement of photosynthesis

Photosynthesis was measured with the aid of the WARBURG technique. This method, applied to measurements on higher plants, has been described by WASSINK (1946) and VERHOEKS (1965).

The apparatus used in our experiments is the same as that used by VERHOEKS. A shaking frame, supporting ten single manometers, provided with vessels, is mounted in front of a water thermostate. The water is circulated by a small pump, ensuring an even temperature. Four lamps are mounted under the bath with two fans for heat removal.

Light is provided by high pressure mercury lamps (Philips HO, 450 W, type 57103 G). For obtaining lower light intensities, the light was reduced by means of various small phosphorbronze screens of definite transmission values, attached underneath the vessels. An envelope of black cloth excluding all light was used in determinations of respiration. The light intensity (ergs \cdot cm⁻²· sec⁻¹) was determined with a calibrated MOLL's microsurface thermopile, adapted for measurements under water. Correction was made for the infrared radiation, which was taken apart by application of a SCHOTT RG 8 filter in a separate measurement.

The vessels (with a volume of about 40 ml) consist of a bottom part and a lid so that the leaf material can easily be introduced. The two parts are held together with small metal spirals attached to small protuberances. Vaseline is used for air tight closure of the vessels. Small protuberances at the inner side of the bottom part support a perforated plexiglass plate, on which leaf discs are arranged. The leaf material is thus prevented from contact with the buffer solution at the bottom of the vessel. To avoid CO₂-limitation of photosynthesis, the CO₂-concentration in the vessels was maintained at 1.26% during the measurement. This was achieved by the application of 0.5 M WARBURG buffer No. 9 (cf. WARBURG, 1928; UMBREIT et al., 1964), a mixture of carbonate and bicarbonate in the ratio 15:85 (v/v).

Although an experimental measurement confirmed this theoretically calculated value, yet it is possible that, in spite of shaking, there is a decline in the CO_2 -concentration from the surface of the buffer solution towards the top of the vessel. It may be observed, however, that, even with the stomata closed, the concentration of 1.26% should ensure a non-rate limiting CO_2 -supply to the leaf.

Yet, two series of comparative gas-exchange measurements with different CO_2 -concentrations were made on leaves of *Calendula officinalis*, one in 1967, and one in 1969. See Tables 1 and 2.

TABLE]	I. Maximum	rates of pho	tosynthesis	of C	'alendula o	officinal	is usin	g differe	nt WARB	URG
buffers,	expressed as	percentages	of the rate	in V	VARBURG	buffer	No. 9,	0.5 M.	Experim	ents
without	agar slices (1	967).								

Buffer No.	3	5	6	7	8	9	10	11
Phot. rate	14	30	37	70	85	100	97	87

TABLE 2. Maximum rates of photosynthesis of *Calendula officinalis* using different WARBURG buffers, expressed as percentages of the rate in WARBURG buffer No. 9, 0.5 M. Experiments with agar slices (1969).

Buffer No.	7	8	9	10	11
Phot. rate	94	99	100	90	81

The first experiments were carried out with leaf discs lying free on the perforated plexiglass, the others with the leaf discs embedded in agar (see p. 10). The results justify the use of the 0,5 M WARBURG buffer No. 9. The rather low photosynthetic rates found at lower CO₂-concentrations in the 1967 experiments may partly be attributed to stomatal closure owing to water deficit or even wilting, since the photosynthetic rate at CO₂-saturation should be about twice as high as that in normal air (GAASTRA, 1959) whereas the difference between lower and higher CO,-concentrations in the 1967 experiments was much larger. In the 1969 experiments, where wilting was prevented by means of agar discs (see p. 10), photosynthetic rates at lower CO₂-concentrations differed less from the rates obtained at 1.26% CO₂. In these experiments, probably, stomatal closure occurred to a smaller degree. In both cases, gas exchange dropped at CO₂-concentrations higher than 1.26%. This may be due to damage caused by an excess of carbon dioxide. However, MÜLLER (1958), with Asparagus, required 10% CO₂ to obtain saturation. Another reason might be a still further stomatal closure at high CO2-concentrations, although this should not be of influence at concentrations of this magnitude.

Although the carbon dioxide concentration used is sufficiently high for non-CO₂-limited photosynthesis, yet CO₂ could be rate limiting, because the buffer solution at higher photosynthetic rates cannot replace sufficiently fast the CO₂ taken up by the leaves. This may be the case if too much leaf material is used, and total photosynthesis is too high. To examine this, photosynthetic rates were measured at different temperatures, using 6, 4 or 2 leaf discs of *Calendula officinalis* (area resp. 4.71, 3.14 and 1.57 cm²).

In the experiments with 6 leaf discs little or no differences occurred between maximum photosynthetic rates per unit leaf area at different temperatures (Table 3A, B, fig. 1). The apparent Q_{10} here is around 1.2, which indicates that the CO₂-concentration is rate limiting. If not, a temperature-sensitive biochemical process would be limiting, causing marked differences between the

Temp.	Α	В	С	D	Е	F
		Plants ra	ised at 6 \times	10 ⁴ erg · cm [−]	2. sec - 1	
25°	442	104.5	411	131	_	-
20°	466	99	314	100		-
15°	-	-	251	80	-	-
		Plants rai	sed at 2,9 $ imes$	10 ⁴ erg · cm ⁻	- 2. sec - 1	
25°	476	101	385	122,5	182	116
20°	424	90	283	90	140	89
15°	_	-	204	65	-	-

TABLE 3. Maximum rates of photosynthesis of *Calendula officinalis* at different temperatures and with different amounts of leaf material.

A. Gas exchange per hour of six leaf discs.

B. Gas exchange of A per cm^2/h .

C. Gas exchange per hour of four leaf discs.

D. Gas exchange of C per cm²/h.

E. Gas exchange per hour of two leaf discs.

F. Gas exchange of E per cm^2/h .



FIG. 1. Calendula officinalis. Rates of photosynthesis per cm² leaf area at 2 temperatures with 6 leaf discs (together 4.71 cm²). — plants, grown at 60,000 ergs · cm⁻²· sec⁻¹: ● photosynthesis measured at 25 °C, ■ at 20 °C; - - - plants, grown at 29,000 ergs · cm⁻²·sec⁻¹: ○ photosynthesis measured at 25 °C, □ at 20 °C.



FIG. 2. Calendula officinalis. Rates of photosynthesis per cm² leaf area at 3 temperatures with 4 leaf discs (together 3.14 cm²). — plants, grown at 60,000 ergs \cdot cm⁻²·sec⁻¹: • photosynthesis measured at 25 °C, • at 20 °C, • at 15 °C; – – – plants, grown at 29,000 ergs \cdot cm⁻²·sec⁻¹: • photosynthesis measured at 25 °C, □ at 20 °C, △ at 15 °C.

maximum photosynthetic rates at different temperatures. This is the case when four or two leaf discs are used (Table 3 C-F, fig. 2). The results of these two experiments are identical. In this case Q_{10} is calculated to be around 1.8, which is well in accordance with the findings of other authors, e.g. GAASTRA (1959). If a weak buffer solution (0.025% CO₂) is used, however, CO₂-supply is ratelimiting, causing a higher photosynthetic rate per cm² leaf area when two leaf discs are used instead of four.

Although not rate limiting, the CO_2 -concentration might gradually decrease, because of insufficient replacement by the buffer solution of the CO_2 assimilated. In that case the rise of O_2 -pressure on the manometer is partly compensated by the drop in CO_2 -pressure.

From the former experiments it was concluded that four leaf discs (with an area of 0.79 cm^2 , each) represent a suitable amount of material per vessel. The discs were arranged on the plexiglass plates in 8 of the vessels, using two manometers for thermo-barometers. The upper surface of the discs was turned towards the light sources. Care was taken that the composition of the material was approximately the same in each vessel, by dividing the leaf discs according to the scheme, presented in fig. 3, (the discs were taken from four equivalent leaves). The measured rate of photosynthesis thus may be considered to represent that of an average leaf. While punching out the leaf discs, the mid rib and the larger side ribs were avoided as much as possible.

In a single measurement, as mentioned before, all the material was of the same origin. Four manometers were used for measuring photosynthesis at reduced levels of light intensity. Respiration was occasionally measured in one of the manometers. Usually, five readings were made with intervals of 15 minutes. At the beginning of an experiment, the manometers were shaken during one hour to equilibrate CO_2 -concentrations. After 40 minutes of shaking, the lamps were lighted, and the readings started 20 minutes thereafter. This was done to avoid the effect upon the readings of the initial low rate of CO_2 -absorption,



FIG. 3. Scheme of the division of leaf discs from 4 leaves over 8 vessels (for experiments with 4 leaf discs per vessel).

reported in literature. This low rate probably is due to stomatal opening still being incomplete (NATR, 1969). At the applied high CO_2 -concentration, this effect, however, should not be of great importance.

As by VERHOEKS (1965), it was noticed in most measurements that at higher light intensities, the leaf discs tended to wilt. This was especially the case with the relatively thin leaves of *Impatiens parviflora*, which per unit area have about half the water content of the leaves of *Calendula officinalis*.

Wilting apparently results from evaporation upon absorption of radiant energy. This means also that leaves under high light intensities assume a higher temperature than those under low light intensities. Since the measurement of the absolute temperature of leaves by means of thermocouples attached to their surface is open to criticism (IDLE, 1968; PIETERS and SCHURER, 1973), no attempts were made to do so. Moreover, since the measurements were mainly comparative, it was not considered a great disadvantage not to know the exact leaf temperature upon a certain irradiation. The air temperature in the vessels when exposed to the light, however, was found to rise 1 °C per 8 × 10⁴ ergs · cm⁻². sec⁻¹. Since the maximum light energy was 20×10^4 ergs · cm⁻². sec⁻¹, the difference in temperature between a fully illuminated vessel and one kept in dark, was $2\frac{1}{2}$ °C. This means that the data had to be recalculated for one temperature (20° or 25°C), assuming Q₅ to be 1.30.

Since wilting resulted in a drop of the rate of photosynthesis in the course of the measurements, a way was sought to prevent wilting. In accordance with a method described by BÁRTOŠ, KUBÍN and ŠETLÍK (1960) and ŠETLÍK, BARTOŠ and KUBÍN (1960) halfway our experimental period a slice of 2% agar (circa 3.2 cm diameter, 0.4–0.45 cm high) was placed on the plexiglass plate. In the slice four holes (1 cm diameter) were punched, in each of which a leaf disc was adjusted. Now, water content was maintained for several hours and so was the maximum photosynthetic rate, except for a very gradual decline after two hours in the high light intensities. This may be due to an accumulation of photosynthates in the isolated tissue (NÁTR, 1969). However, NÁTR and LUDLOW (1970) found no influence from photosynthate accumulation on photosynthesis and transpiration in barley leaf segments.

The application of agar slices resulted in a rise of up to 12-15% of the maximum rate of photosynthesis in mature leaves; in very young, thin leaves of *Impatiens* a rather dramatic rise of over 100% sometimes was found. At lower light intensities, up to 4×10^4 ergs \cdot cm⁻² · sec⁻¹, however, there was no difference in photosynthesis whether agar was used or not.

2.2.2. Estimation of chlorophyll

In some cases, chlorophyll contents of the leaves were estimated. This was done comparatively in leaves grown under different light conditions and in leaves of different ages. Equal surface areas of leaves were extracted with a fixed volume of ethanol. Then the extinction values of the extracts were determined in the colorimeter at 665 nm.

2.2.3. Morphological differences

2.2.3.1. Numbers of stomata. Stomata per unit leaf area were counted under the microscope after stripping off the upper and lower epidermis of different leaves or after making prints with the aid of the silicone rubber technique.

2.2.3.2. Leaf anatomy. Differences in leaf thickness, assimilating tissue, etc. were estimated microscopically after making cross sections with the aid of a microtome.

2.2.4. Estimation of leaf age

As will be shown in chapter 4, the photosynthetic capacity of a leaf strongly depends on its age. The leaves along the main stem were numbered upwards, starting with the first leaf to develop above the cotyledons. Since our experiments are concerned with actually photosynthesizing leaves, leaf age was considered to be 1 day, when the leaves were just visible, which means that they were approximately 1.5 cm long for *Impatiens parviflora* and 2.5 cm for *Calendula officinalis*. No attempts were made to examine the exact leaf age by investigating the primordia.

The 'first' day of leaf age was noted for the first leaf and for about three other, not directly sequential ones along the stem. For the other leaves, the leaf age was calculated by interpolation. Since not all plants generally develop at the same speed, and since from only a few plants the leaf age was noted, especially the age indicated for the upper leaves often is the average of four slightly different values. For the same reason, leaves from lateral branches generally are only characterized by their position on the branch and not by their age.

2.3. EXPRESSION OF THE DATA

In literature, the rate of photosynthesis mostly is expressed as volume or weight of carbon dioxide absorbed per unit leaf area and per unit time. This is the most plausible way, since, when e.g. studying the photosynthesis/light curves of two leaves of different ages, it is essential that equal leaf areas receive equal amounts of light energy. In this way, the physiological characters are best comparable. However, differences in photosynthetic rates between two leaves, e.g. a sun leaf and a shade leaf, are often connected with differences in leaf thickness and, therefore, in fresh or dry weight. To examine possible differences between photosynthetic capacities, rates of photosynthesis can also be expressed per unit fresh or dry weight. One should remember, however, that in that case equal amounts of leaf mass represent different leaf areas. So, although photosynthetic rates at light saturation may be very well comparable in this way, the parts of the curves, where light is limiting, show misleading differences in the slopes, owing to unequal amounts of light energy received.

From an ecological point of view, a unit leaf area from leaves of different species, ecotypes of individuals, grown under different light regimes, may not

mean the same. DECKER (1955) suggested for studies of this kind to put the rate of photosynthesis under light saturation at 100% in each case and to express photosynthesis in limiting light intensities as a percentage of the maximum value. The same ecological value is thus given to the maximum rate of photosynthesis per unit leaf area. It is easy to see that in this way of expression e.g. a shade leaf which reaches light saturation at a relatively low light intensity is relatively more effective in light intensities, where light is limiting.

An objection against this way of expression might be that nothing is known of the absolute values of the photosynthetic rates. Therefore, for an ecological comparison of photosynthetic rates it may be more reliable to take the whole leaf as an ecological unit. Shade leaves often appear to have a larger surface than sun leaves, which might be considered as an adaptation to low light intensities.

In the present study photosynthesis/light curves are generally related to unit leaf area. The maximum rates of photosynthesis are also given per unit fresh or dry weight. For comparative ecological purposes, the photosynthetic rate, in a number of cases, is expressed per entire leaf.

3. PHOTOSYNTHESIS AS INFLUENCED BY LIGHT INTENSITY DURING GROWTH

3.1. INTRODUCTION

The experiments were carried out from 1967 till 1970. This chapter deals with the influence of light intensity during growth on the photosynthetic capacity of the leaves without regard to the age of leaf and plant. For this purpose, data are taken either from measurements of photosynthesis of leaves of a definite position on the stem (the '12th' leaf) or from measurements on leaves of different ages along a stem. Since shading slows down the development of a plant, a leaf at a definite position on the stem grown in 100% light, in general, will not correspond in actual age and even less in physiological age, with a leaf in the same position, grown in e.g. 12% light. In this chapter, leaves grown at different light intensities are compared by taking the leaves with the highest maximum rates of photosynthesis per unit leaf area from each of the above mentioned series of measurements.

As far as *Calendula officinalis* is concerned, the data are mainly taken from young plants, which were still in the vegetative phase or had started flowering very recently, because light intensity strongly influences flowering while, vice versa, flowering influences photosynthesis. In *Impatiens parviflora*, flowering starts at a very early stage and is much less influenced by light intensity. For the purpose of the present chapter, therefore, data from older plants are better comparable than in *Calendula officinalis*.

3.2. CALENDULA OFFICINALIS

In the figures 4-14, photosynthesis/light curves are given for comparable leaves grown at different light intensities according to the procedure described above (Section 3.1).

As can be seen and as can be expected from a plant originating from a sunny habitat (cf. BJÖRKMAN and HOLMGREN, 1963; BOURDEAU and LAVERICK, 1958) there is a general trend that the maximum rate of photosynthesis rises with the light intensity at which the plants are grown. In some cases the differences are small and sometimes the maximum rate of photosynthesis is highest in leaves grown at 75% or 37% light. This may be explained either by the fact that the measurements were made shortly after the plants were brought into different light intensities (figs. 5, 7, 9) or because the measurements started at a relatively old age of the leaves, so that the values found are less than the highest rates of photosynthesis at an earlier stage (fig. 13).

The average values for the indoor experiments (fig. 14) clearly show an increase of the maximum rate of photosynthesis with increasing light intensities during growth.



FIG. 4. Calendula officinalis, 1967, outdoors Maximum rate of photosynthesis per cm^2 leaf area at 25°C of the 10th leaf from the main stem of plants grown at different light intensities.



FIG. 6. Calendula officinalis, 1969, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 25 °C of the 12th leaf on the main stem of plants grown at 100% (\bullet) and 12% (\triangle) daylight. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 5. Calendula officinalis, 1968, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 25 °C of the leaf with the highest photosynthetic capacity from the main stem, as determined in separate measurements, of plants grown at different light intensities. • plants, grown at 100% day-light, \bigtriangledown at 75% daylight, \square at 37% day-light.



FIG. 6a. Calendula officinalis, 1972, outdoors, single plots. Rates of photosynthesis per cm² leaf area at 25 °C of the 11th and 12th leaf on the main stem of plants grown at light intensities of 100% (\bullet), 75% (\bigtriangledown), 37% (\square), and 12% (\blacktriangle) daylight. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 7. Calendula officinalis, 1967–68, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the leaf with the highest photosynthetic capacity from the main stem, as determined in separate measurements, of plants placed under different light intensities 18 days before the measurements started. \bullet plants, grown at 60,000 ergs \cdot cm⁻². sec⁻¹, \bigtriangledown at 35,000 ergs \cdot cm⁻². sec⁻¹, \square at 20,000 ergs \cdot cm⁻². sec⁻¹, \blacktriangle at 10,000 ergs \cdot cm⁻². sec⁻¹.



FIG. 10. Calendula officinalis, 1968–69, phytotron. As fig. 9, however, plants placed under different light intensities 26 days before the measurements started.

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FIG. 8. Calendula officinalis, 1967–68, phytotron. As fig. 7, however, plants placed under different light intensities 40 days before.



FIG. 9. Calendula officinalis, 1968–69, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the leaf with the highest photosynthetic capacity from the main stem, as determined in separate measurements, of plants placed under different light intensities 12 days before the measurements started. • plants grown at 81,000 ergs \cdot cm⁻². sec⁻¹, \bigtriangledown at 57,000 regs \cdot cm⁻². sec⁻¹, \square at 41,000 ergs \cdot cm⁻². sec⁻¹, \blacktriangle at 20,000 ergs \cdot cm⁻². sec⁻¹.

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FIG. 11. Calendula officinalis, 1968-69, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 9th and 10th leaf on the main stem of plants grown at different light intensities. • plants grown at 67,000 ergs \cdot cm⁻²· sec⁻¹, \bigtriangledown at 48,000 ergs \cdot cm⁻²· sec⁻¹, \square at 27,000 ergs \cdot cm⁻²· sec⁻¹, \blacktriangle at 16,000 ergs \cdot cm⁻²· sec⁻¹. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 12. Calendula officinalis, 1968–69, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 15th and 16th leaf on the main stem of plants grown at different light intensities. • plants grown at $67,000 \text{ ergs} \cdot \text{cm}^{-2} \cdot \sec^{-1}$, \bigtriangledown at 48,000 ergs $\cdot \text{cm}^{-2} \cdot \sec^{-1}$, \square at 27,000 ergs $\cdot \text{cm}^{-2} \cdot \sec^{-1}$. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 13. Calendula officinalis, 1969–70, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 15th leaf on the main stem of plants grown at different light intensities (leaf age 50–60 days). • plants grown at 72,000 ergs \cdot cm⁻² sec⁻¹, \bigtriangledown at 48,000 ergs \cdot cm⁻² sec⁻¹, \square at 28,000 ergs \cdot cm⁻² sec⁻¹, \blacktriangle at 14,000 ergs \cdot cm⁻². sec⁻¹. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 14. Calendula officinalis, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of leaves of plants grown at different light intensities. Average values of figs. 7–12.

Furthermore, in most cases, photosynthesis at limiting light intensities, i.e. in the straight part of the slope, is higher in plants grown at low light intensities. This may be due only to the fact that respiration per unit leaf area is stronger in plants grown at high light intensities, probably because these plants have thicker leaves and therefore more respiring tissue (cf. e.g. figs. 6 and 6a). Another difference sometimes found between sun and shade leaves is the steeper slope in the light limited part of the photosynthesis/light curve of shade leaves (e.g. OSIPOVA, KHEIN and NICHIPOROVICH, 1971). The latter difference does not seem to occur in *Calendula* leaves. Steeper slopes mostly are due to considerably higher chlorophyll contents (GABRIELSEN, 1948). Since the chlorophyll contents found in *Calendula* differed only slightly (see chapter 5) a large variation in the slope could not be expected.

As mentioned above, the leaves grown at higher light intensities are generally thicker, which results e.g. in a higher respiration rate. The greater thickness may also be a reason for the higher saturation level, but there may also be other reasons for difference between leaves from different light intensities. To examine this, a great number of the maximum photosynthetic rates given in the figures 4–14 is given in block diagrams as maximum photosynthesis per unit fresh weight and per unit dry weight (figs. 15, 16). Since the water content of the leaf varies with the light intensity during growth, photosynthesis per unit dry weight seems to be a better measure than photosynthesis per unit fresh weight. Differences appear to be small and it is difficult to draw a definite conclusion.

Considering the averages, (figs. 17, 18) one might conclude that maximum photosynthesis per unit leaf mass is hardly influenced by the light intensity at which the plants grow.



FIG. 15. Calendula officinalis. Maximum rates of photosynthesis per unit fresh weight for leaves of plants grown at different light intensities. Figs. 4-6A; outdoor experiments; figs. 7-13; indoor experiments.



FIG. 16. Calendula officinalis. Maximum rates of photosynthesis per unit dry weight for leaves of plants grown at different light intensities. Fig 6A: outdoor experiments; figs. 9-13: indoor experiments.



FIG. 17 (left). Calendula officinalis. Mean values of fig. 15, with indication of the highest and the lowest values.

FIG. 18 (right). Calendula officinalis. Mean values of fig. 16, with indication of the highest and the lowest values.

3.3. IMPATIENS PARVIFLORA

In this section a number of photosynthesis/light curves (rates per unit leaf area) from comparable *Impatiens* leaves is given in the figures 19–27, in the same way as for *Calendula* in the figures 4–14. It might be possible that the shade plant *Impatiens* reacts in a different way, because the high light intensities could destroy the chloroplasts and thus cause an even lower maximum rate of photosynthesis than that of the plants grown at the lowest light intensities, as it is the case with ecotypes of *Solidago virgaurea* (BJÖRKMAN and HOLMGREN, 1963). However, from the figures it can be seen that this is not so. Without exception, the maximum rate of photosynthesis rises when the light intensity during growth is higher. In all cases there is a clear difference between the photosynthetic levels,



FIG. 19. Impatiens parviflora, 1967, outdoors. Maximum rate of photosynthesis per cm^2 leaf area at 25°C of the 9th leaf from the main stem of plants, grown at different light intensities.





FIG. 20. Impatiens parviflora, 1968, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 25°C of the leaf with the highest photosynthetic capacity from the main stem, as determined in separate measurements, of plants grown at different light intensities. • plants grown at 100% daylight, \bigtriangledown at 75% daylight, \square at 37% daylight, \blacktriangle at 12% daylight.

FIG.21. Impatiens parviflora, 1969, outdoors, single plot. Rates of photosynthesis per cm^2 leaf area at 25 °C of the 12th leaf on the main stem of plants grown at different light intensities. • plants grown at 100 % daylight, \bigtriangledown at 75 % daylight, \square at 37 % daylight, \blacktriangle at 12% daylight. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 22. Impatiens parviflora, 1969, outdoors, mixed plot. Rates of photosynthesis per cm² leaf area at 25 °C of the 12th leaf on the main stem of plants grown at different light intensities. • plants grown at 100% daylight, ⊽ at 75%
⊥ daylight, □ at 37% daylight, ▲ at 12% daylight.
²⁰ (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG.23. Impatiens parviflora, 1970, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 20 °C of the 14th leaf on the main stem of plants grown at different light intensities. \bullet plants grown at 100% daylight, \triangledown at 75% daylight, \square at 37% daylight, \blacktriangle at 12% daylight. (Maximum values out of a number of measurements at different leaf ages; see text.)

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FIG. 24. Impatiens parviflora, 1970, outdoors, mixed plot. Rates of photosynthesis per cm² leaf area at 20°C of the 14th leaf on the main stem of plants grown at different light intensites. \bullet plants grown at 100% daylight, \bigtriangledown at 75% daylight, \square at 37% daylight, \blacktriangle at 12% daylight. (Maximum values out of a number of measurements at different leaf ages; see text.)



FIG. 25. Impatiens parviflora, 1967-68, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the leaf with the highest photosynthetic capacity from the main stem, as determined in separate measurements, of plants, placed under different light intensities 19 days before the measurements started. • plants grown at 60,000 ergs \cdot cm⁻². sec⁻¹, \bigtriangledown at 20,000 ergs \cdot cm⁻². sec⁻¹, \square at 20,000 ergs \cdot cm⁻². sec⁻¹, \square at 10,000 ergs \cdot cm⁻². sec⁻¹.



FIG. 26. Impatiens parviflora, 1967-68, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the leaf with the highest photosynthetic capacity from the largest lateral branch, as determined in separate measurements, of plants, grown at different light intensities • plants grown at 60,000 ergs · cm⁻² · sec⁻¹, \bigtriangledown at 35,000 ergs · cm⁻² · sec⁻¹, \square at 24,000 ergs · cm⁻² · sec⁻¹.



FIG. 27A. Impatiens parviflora, 1969-70, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 11th till 14th leaf on the main stem of plants, 19 days after their being placed at light intensities of 72,000 ergs \cdot cm⁻² · sec⁻¹ (\bullet), 48,000 ergs \cdot cm⁻² · sec⁻¹ (∇), 28,000 ergs \cdot cm⁻² · sec⁻¹ (\square), and 14,000 ergs \cdot cm⁻² · sec⁻¹ (\blacktriangle).



FIG. 27B. Impatiens parviflora, 1969–70, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 10th till 13th leaf on the main stem of plants, 11 days after their being placed at light intensities of 72,000 ergs \cdot cm⁻²· sec⁻¹ (\bullet), 48,000 ergs \cdot cm⁻²· sec⁻¹ (\bigtriangledown), 28,000 ergs \cdot cm⁻²· sec⁻¹ (\square), and 14,000 ergs \cdot cm⁻²· sec⁻¹ (\blacktriangle)

even though, as in the previous section, in a few cases the measurements were made rather shortly after the plants were brought into different light intensities.

In the light limited part of the curve, *Impatiens* reacts in a way similar to *Calendula*: A higher respiration rate in leaves which were exposed to high light intensities, owing to more respiring tissue, depresses the apparent photosynthesis. Also in this case, no clear differences between the slopes of the curves are found, which indicates that no severe destruction of the chloroplasts in high light intensities occurs. Also the chlorophyll contents of the leaves do not differ much.

The average values for the outdoor experiments (fig. 28A) and the indoor experiments (fig. 28B) confirm the observations made above: maximum rate of photosynthesis as well as respiration rate are clearly influenced by light intensity during growth.





FIG. 28A. Impatiens parviflora, outdoors. Rates of photosynthesis per cm² leaf area of leaves of plants grown at different light intensities. Averages values of figs. 20-24.

FIG. 28B. Impatiens parviflora, phytotron. Rates of phytosynthesis per cm^2 leaf area at 20 °C of leaves of plants grown at different light intensities. Average values of figs. 25-27.

In fig. 29 some of the maximum photosynthetic rates per unit leaf area are given as a function of the light intensity during growth. As could be concluded from the foregoing figures, the maximum photosynthetic rates of *Impatiens* are more influenced by the light intensity during growth than those of *Calendula*, at least in the outdoor experiments (fig. 29C and D). This is especially clear in fig. 29E and F, where the maximum rates of photosynthesis are expressed as a percentage of those from plants grown in 100% daylight. In the indoor experiments these differences between the two species are less significant (fig. 29A and B). Furthermore, it may seem surprising that the leaves from plants grown at the highest light intensity in the phytotron, though exposed to much lower maximum light intensities and receiving less total irradiation, show photosynthetic capacities that are only slightly lower (*Impatiens*) or even exceed (*Calendula*) those from leaves grown in the experimental field in full daylight. A possible explanation for this might be the fact that the plants in the phytotron.



FIG. 29A. Maximum rates of photosynthesis per cm² leaf area against light intensity during growth. Indoor experiments. • *Calendula officinalis*; \Box --- *Impatiens parviflora*. Data obtained from figs. 8, 10, 11, 12, 25, 27.



FIG. 29B. Averages of fig. 29A.

have a more constant supply of water and nutrients and therefore are in a better condition than the plants in the outdoor experiments, which are, moreover, submitted to various, often unfavourable, climatical conditions. Apart from that, in the outdoor experiments, unlike in the indoor experiments, it is difficult to determine to what absolute light intensity the photosynthetic apparatus is adapted.

In the figures 30 and 31, the maximum rates of photosynthesis per unit fresh weight and per unit dry weight, calculated from data of the figures 19-27, are given. Averages from the data of the figures 30 and 31 are given in figs. 32 and 33. From these averages some conclusions may be drawn. In the outdoor experiments as well as in the indoor experiments, the maximum rate of photosynthesis per unit dry weight seems to rise slightly as the light intensity during growth decreases, with an exception however for the lowest light intensity. One might assume that this light intensity is too low for good adaptation of the photosynthetic apparatus. However, the intensity of 12% of daylight equals, even on a

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FIG. 29C. As fig. 29A. Outdoor experiments. Data obtained from figs. 4, 6, 6a, 20-24.





FIG. 29E. Outdoor experiments. Maximum rates of photosynthesis per cm^2 leaf area expressed as percentages of the rates of leaves of plants grown at 100% daylight.



FIG. 29F. Averages of fig. 29E.

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FIG. 30. *Impatiens parviflora*. Maximum rates of photosynthesis per unit fresh weight for leaves of plants grown at different light intensities. Figs. 19-24: outdoor experiments; figs. 25-27: indoor experiments.



FIG. 31. Impatiens parviflora. Maximum rates of photosynthesis per unit dry weight for leaves of plants grown at different light intensities. Figs. 20-24: outdoor experiments; figs. 26-27: indoor experiments.



FIG. 32 (left). *Impatiens parviflora*. Mean values of fig. 30 with indication of the highest and the lowest values.

FIG. 33 (right). *Impatiens parviflora*. Mean values of fig. 31 with indication of the highest and the lowest values.

cloudy, day, 40% rather than 20% of the artificial light, as applied in the indoor experiments. Looking at the highest values for the maximum rate of photosynthesis, there seems to be no influence of the light intensity during growth on the maximum rate of photosynthesis per unit dry weight.

For the outdoor experiments, the maximum rate of photosynthesis on a fresh weight base, yields a picture very much like that of photosynthesis per unit dry weight. On the other hand, in the indoor experiments, photosynthesis per unit fresh weight resembles very much photosynthesis on leaf area basis.

This is because in the phytotron the fresh weights remained very much the same throughout the light intensity series, whereas the dry weights and therefore the percentage of dry matter decreased with decrease in light intensity.

In the outdoor experiments, however, both fresh and dry weight decrease about equally as the light intensity decreases, thus yielding a fairly constant percentage of dry matter.

In later indoor experiments (1970–1971), however, the plants developed much better than in the preceding year and fresh and dry weights resemble more the values found in the outdoor experiments (cf. chapter 5, fig. 96).

3.4. DISCUSSION

From the data of this chapter we may first conclude that both species react towards higher light intensities during growth with higher maximum rates of photosynthesis per unit leaf area (fig. 29), although they normally occur in different habitats with regard to the irradiance. The same holds for the growth rates (WASSINK et al., in prep.).

In the indoor experiments, the curves of the maximum rates of photosynthesis per unit leaf area against light intensity during growth show fairly equal slopes for both species.

Remarkably, in the outdoor experiments the slope is much flatter for Calendula than it is for Impatiens.

There neither is a significant difference between the slopes of the photosynthesis/light curve in both species. The advantage a shade grown *Impatiens* has over a *Calendula* grown under the same conditions, might result from a lower compensation value, owing to a lower rate of respiration. In fact, during summers with subnormal solar irradiation, *Calendula* in the 12% daylight experiments tended to die, which was never the case with *Impatiens*.

It must be pointed out, however, that in *Impatiens* the leaves are wider spaced (*Impatiens*: 15 leaves on 50 cm stem length, *Calendula*: 15 leaves on 12 cm stem length) and have petioles, and thus the lower leaves receive more of the incident light than those of *Calendula* which, with its densely arranged sessile leaves is more troubled with self shading. In fact, the light intensity under a *Calendula* plant can be less than 5% of that above the plant, whereas at the bottom of the *Impatiens* crop light intensity is rather homogeneous and about 15% of that on top of the plants. A further disadvantage caused by the dense arrangement of the leaves makes both plants adapted to their habitat: *Impatiens* has flat thin leaves with stomata almost exclusively at the abaxial side; *Calendula* possesses rather thick leaves with about equal numbers of stomata on both sides. Therefore, *Calendula* is better suited to endure the heat irradiance of high light intensities: firstly because of the more favourable surface/volume ratio, and secondly because of the better possibilities for transpiration.

It will be discussed in chapter 5 how the anatomical features change when the plants are exposed to different light intensities. The present findings do not contradict other investigations. BLACKMAN and WILSON (1951a and b) found that shading to 50% of full daylight did not reduce the relative growth rate of *Geum urbanum*, and concluded that this species can thus justly be called a shade plant. Twenty-one other species, however, all showed reduction of the growth rate, although many of them are also known as shade plants. BJÖRKMAN and HOLMGREN (1966), investigating ecotypes of *Geum* and *Rumex* from sunny and shaded habitats, found higher maximum photosynthetic rates inasmuch as light intensity during growth was higher. The maximum photosynthetic rate of the shadow-type of *Rumex* was even higher than that of the sun-type, when both were grown at high light intensities. Only ecotypes of *Solidago virgaurea* from

shaded habitats (BJÖRKMAN and HOLMGREN, 1963) showed a decline of their maximum photosynthetic rates, when grown at high light intensities. As mentioned earlier, this was due to their low tolerance to high light intensities, which caused destruction of the chloroplasts. Likewise, two populations of *Dactylis glomerata*, originating from Portugal (sun-type) and from Norway (shade-type) responded in different ways to various light treatments (EAGLES and TREHARNE, 1969). In the Portugese population, maximum rates of photosynthesis increased with increase in irradiance during growth; in the Norwegian type there was hardly any difference, and even a small decline at the highest level of irradiance if photosynthesis was expressed per mg chlorophyll.

In tree seedlings there often is an increase in photosynthetic activity, when the light intensity during growth is higher, e.g. in *Pinus strobus*, *P. resinosa*, *Tsuga canadensis* (BOURDEAU and LAVERICK, 1958), *Acer pseudoplatanus* (PIETERS, 1960), *Alnus rubra*, *Pseudotsuga menziesii*, *Picea sitchensis*, *Tsuga heterophylla* (KRUEGER and RUTH, 1969), *Betula alleghaniensis* (LOGAN, 1970), *Eucalyptus fastigata* (CAMERON, 1970).

It may, therefore, be concluded that both *Impatiens parviflora* and *Calendula* officinalis in their photosynthetic capacity react much the same as most other species when grown under different light intensities. However, they show some morphological and anatomical features, which make them especially suited for shaded and sunny habitats respectively.

4. PHOTOSYNTHESIS AT DIFFERENT AGES OF LEAF AND PLANT

4.1. INTRODUCTION

With a view to production processes, it is of interest to know what the contribution of any leaf at any moment to the total photosynthesis of a plant can be. This was explored by determining the photosynthetic rates of as many leaves as possible in a few days, or by repeatedly measuring the photosynthetic rate of a single leaf during its existence.

Since flowering seems to influence senescence of the leaves, a brief description of the anatomy of both species, especially with regard to the position of the flowers seems indicated.

In Impatiens parviflora flowers originate from the axils of almost each leaf. Flowering starts already at a very early stage, about five weeks after germination when only a few leaves are formed, which are, at that moment, too small for photosynthetic measurements with the methods used in the present experiments. Since flowering occurs so soon after leaf formation, its influence on the photosynthetic capacity is hard to separate from that of ageing as such. Lateral branches arise all from the axils of the cotyledons and the lower leaves. Yellowing starts in the lowest leaves.

In Calendula officinalis flowering starts at the top of the stem. First, all the leaves on the main stem are formed, the lowest with very short internodes. When flowering starts at the apical end of the main stem, where one head is formed, most leaves have attained their final sizes. There is only a further elongation of the upper internodes. Flower formation then descends to the upper lateral branches, which bear only a few small leaves, and downward to the lower lateral branches, which bear more and larger leaves. Yellowing starts in the lowest leaves and often is enhanced because these leaves are lying on the moist soil. Yellowing occurs also from the top leaves downward when the flowers have died. This process proceeds rapidly downward so that, in Calendula, ageing as a whole seems to be somewhat more complicated than it is in Impatiens: two 'waves' of senescence move in opposite directions along the stem. The downward wave concerns only a number of about ten top leaves, which are very small and give only a minor contribution to total photosynthesis. The last leaf to die among the main stem will generally be the one in the axil of which the uppermost lateral branch originates. Dying of the leaves along the lateral branches occurs in the same order as along the main stem. Because flowering occurs last in the lowest branches, the leaves of these branches generally have the longest life.
4.2. PHOTOSYNTHESIS AND AGE IN Calendula officinalis

4.2.1. Outdoor experiments

4.2.1.1. Photosynthesis of simultaneously for med leaves at different ages. The experiments started in the summer of 1967 with an introductory investigation of the 8th leaf along the main stem. Photosynthesis was measured on separate leaf halves of different plants; a second measurement using the 2nd half leaf was made after about twelve days. Formation of these leaves started around June 7th, that is, at that date the leaves were just visible (cf. 2.2.4.), five days before the metal screens were placed. So, all the leaves passed the first days of their development in full daylight. Photosynthetic rates were measured at a light intensity of 15×10^4 ergs \cdot cm⁻². sec⁻¹. As can be seen from figure 34, these rates show more difference in the second measurement (at the age of 34 days) than in the first one (at the age of 22 days). This seems to be due to a slow adaptation to conditions of shade rather than to an ageing effect. There is no significant difference between photosynthetic rates of leaves from the single crop and the mixed crop. In the mixed crop the first measurements show somewhat more differentiation in photosynthetic rate between leaves from different light



FIG. 34. Calendula officinalis, 1967, outdoors. Photosynthetic rate per cm² leaf area at 150,000 ergs \cdot cm⁻² \cdot sec⁻¹ at 25 °C of the 8th leaf on the main stem of plants grown at 4 different light intensities, at two different leaf ages. Screens placed at a leaf age of 6 days.

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levels than in the single crop. In fact, the measurements were carried out four days after the measurements of the single crop, so differentiation could have proceeded somewhat further. One may ask, however, why there is at the first measurements, which were 17 days after shading started, still no differentiation in the single crop.

A comparable measurement was performed, three months after planting, on leaves of equal positions on a primary lateral branch, which were, however, of different age, due to the retardation in development caused by shading. No strictly comparable leaves were available on the plants grown in 100% daylight, owing to advanced senescence. Here leaves were taken from a secondary lateral branch. From figure 35 it can be seen that differences in leaf age affect the picture of possible differences in photosynthetic rate of these leaves, grown under different light conditions from the beginning. Furthermore it appears that leaves grown under high light intensities show a steeper decline in their maximum photosynthetic rate between the two measurements at one week interval. These first experiments gave only a rough idea of how photosynthetic rates change with time.

In a more detailed study of the change of the photosynthetic rate with increasing leaf age, performed in 1969, the photosynthetic rate of the 12th leaf of



FIG. 35. Calendula officinalis, 1967, outdoors, single plot. Photosynthetic rates per cm² leaf area at 25 °C on two different days (I and II), 7 days apart, for leaves of plants grown at 4 different light intensities $(12\% (\triangle), 37\% (\Box), 75\%(\bigtriangledown)$, and $100\% (\bullet)$ natural daylight). Data at 12%, 37%, and 75% are for leaves on a primary lateral branch, those at 100% for leaves on a secondary lateral branch. For the sake of clearness only the first series of measurements (I) is indicated by symbols.

the main stem was measured at weekly intervals, starting at a leaf age of 25 days. The leaf age was estimated as indicated in subsection 2.2.4. At the time of emergence of the 12th leaf, the screens had already been placed, so the leaves developed at different light intensities.

For reasons of time shortage, only leaves from plants grown at 100% and 12% daylight were measured. In fig. 36A, presenting measurements with plants grown at 12% daylight, it can be seen from the first four measurements that the maximum rate of photosynthesis of the 12th leaf gradually decreases, and that the slope of the light limited part of the photosynthesis/light curve also becomes less steep. The same holds for leaves of plants grown at 100% daylight (fig. 36B), although here the decrease of the maximum rate of photosynthesis is stronger. Leaves of equal age but with different maximum photosynthetic rates owing to growth in different light intensities, however, show no differences in the slope of their curves (cf. Chapter 3).

As can be seen from figure 37A, the maximum photosynthetic rate of the 12th leaf of plants grown at 100 % daylight shows a faster decrease than that of plants grown at 12 % daylight, so that leaves of 6-7 weeks old have almost the same rates of photosynthesis. The leaves grown at 100 % daylight died soon after their



FIG. 36A. Calendula officinalis, 1969, outdoors, single plot, plants grown at 12% daylight. Rates of photosynthesis per cm² leaf area at 25 °C of the 12th leaf on the main stem at four different times. (Leaf age 26 (\bullet), 33 (\bigcirc), 40 (\bigcirc) 47 (\triangle) days).

FIG. 36B. Calendula officinalis, 1969, outdoors, single plot, plants grown at 100% daylight. Rates of photosynthesis per cm² leaf area at 25 °C of the 12th leaf on the main stem at four different times. (Leaf age 26 (\bullet), 33 (\Box), 40 (\bigcirc), 47 (\triangle) days).



FIG. 37A. Calendula officinalis, 1969, outdoors. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different leaf ages for plants, grown at different light intensities. Data for 25-70 days: 12th leaf on main stem; 65-95 days: 15th leaf on main stem. Single lines: single plots; double lines: mixed plots.

photosynthetic rates had decreased to the same values as the leaves from the 12% light experiment, in which the leaves lived a few weeks longer. Measurements of photosynthesis of the 15th leaf from the same plants, carried out a few weeks later, however, revealed that these leaves, generally, had a longer life, although it must be pointed out that, especially on the 100% plants, only few leaves were available. Since these measurements began after the decrease in maximum photosynthesis had started, no difference between the different light intensities could be demonstrated.

No significant differences were found between the maximum photosynthetic rates of leaves from plants grown in single plots and that of leaves from plants grown in the competition experiments together with *Impatiens parviflora*. In fig. 37B we have attempted some streamlining and averaging of certain groups of data from fig. 37A, to bring out the features discussed as clearly as possible. It is clear that at high light intensities the decrease of the photosynthetic rate proceeds faster than at low light intensities, so that old leaves have about the same photosynthetic capacity, irrespective of the light intensity, in which they had grown. It is not quite clear why the 12th leaf of the plants grown at 12% light has, at an old age, a lower photosynthetic rate than the 15th leaf, although it is remarkable that the leaf dry weights in this case also show a clear difference in favour of the 15th leaf.



FIG. 37B. Calendula officinalis, 1969, outdoors. Average values of fig. 37A.



FIG. 38. Calendula officinalis, 1972, outdoors, single plot. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different leaf ages, of the 11th and 12th leaf on the main stem of plants grown at different light intensities.

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A comparable series of measurements, now made on leaves grown at four different light intensities, was performed in 1972. The photosynthetic rate of the 11th and 12th leaves on the main stem of plants grown in the single plot was measured, again at weekly intervals, starting at an average leaf age of 17 days. The screens had been placed at the time that these leaves just became visible, i.e. when they were one to three days old according to the estimation indicated in subsection 2.2.4. Fig. 38 shows the decrease of the maximum photosynthetic rate with increasing age, which agrees well with the data obtained in 1969. Once again, the decrease is strongest in the 100 % light series, so that, at a leaf age of 6 to 7 weeks, no clear differences exist between the maximum rates of photosynthesis of plants grown at the various light intensities. The rather low values of the maximum rates for all light intensities at young stages might be ascribed to rather low daylight intensities during the development of the leaves. As in the former experiment, these low photosynthetic rates coincide with low leaf dry weights. The differences between the maximum rates of photosynthesis at these young stages owing to differences in light intensity during growth are well in accordance with the data presented in Chapter 3.



FIG. 39A. Calendula officinalis, 1969, outdoors, single plot. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different times, of leaves on a secondary lateral branch of plants grown at different light intensities.



FIG. 39B. Same as fig. 39A, mixed plot.

A late series of measurements was carried out in 1969 on leaves from young lateral branches from September 29 until October 24. During this period, flowering had largely stopped, probably owing to lower temperatures and/or lower irradiation, or to internal factors.

Reduction of flowering was accompanied by a kind of 'vegetative revival', which was manifest by the formation of larger leaves at the higher light intensities. The increased differences in differentiation of plants grown under various light intensities made comparison difficult. Leaves formed under equal light intensities, in single and mixed plots were best comparable. From figs. 39A and B it can be seen that mean photosynthetic rates show a small but consistent difference in favour of leaves grown in mixed plots. Here, the competing plants of *Impatiens parviflora* had already died off, so there was a greater stand width, causing higher light intensities in the crop.

4.2.1.2. Photosynthesis of successively formed leaves. In order to investigate the photosynthesis of a plant at a certain point of time, the photosynthetic rates of a number of leaves along the main stem or along a lateral branch were successively determined in as short as possible a period. Supposing that leaves on one stem should not differ much in their ultimate maximum photosynthetic capacity, these measurements also give an idea how the photosynthetic rate of a leaf behaves as time proceeds. However, since removing a leaf may influence the photosynthetic rate of the remaining ones, the picture thus obtained may be somewhat misleading. MOKRONOSOV and IVANOVA (1971) e.g. found that removal of 30-50% of the leaf area in potato plants was associated with intensification of the photosynthetic rate in the remaining leaves, whereas the loss of 80% of leaves led to considerable depression of photosynthesis.

Experiments of the above mentioned type were mainly performed in 1968. The first one was concerned with the leaves along the main stem and was carried out between July 2 and July 19. The results are presented in fig. 40, where the maximum rate of photosynthesis is plotted as a function of leaf age (cf. sub-



FIG. 40. Calendula officinalis, 1968, outdoors, single plot. Maximum rate of photosynthesis per cm^2 leaf area at 25 °C of a number of leaves along the main stem of plants grown at different light intensities.

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section 2.2.4.). As can be seen, the maximum rate of photosynthesis is fairly constant up to a leaf age of over 50 days in plants grown at 100% or 75% daylight. No data are available from plants grown at 37% light. At 12% daylight, the reduction of the photosynthetic rate seems to occur earlier. This might be due to shading conditions, although generally, as was e.g. found in fig. 37, at low light intensities physiological processes proceed at a slower rate, thus retarding the decrease of the photosynthetic capacity. It should be mentioned, however, that the metal screens were placed only 18 days before measuring of the 12% light plants started. Immediately after the placing of the screens a period with low light intensities occurred, so that at 12% light especially the lowest leaves may have passed long parts of the day in light conditions below their compensation points. In this way, the plants may have degraded to the poor conditions in which they were found later in the season (see below). Since the lowest (= oldest) leaves receive the smallest amounts of light and also tend to senesce sooner (DICKMANN, 1971), these leaves show the lowest photosynthetic capacities.

Another series of measurements of this kind was made from August 12 to 30, on leaves of a lateral branch. These leaves had grown up entirely under the different light conditions. Unfortunately, the plants in the mixed plots grown at 12% daylight were in such a poor condition, that no data could be obtained. Figures 41A and B clearly show that photosynthetic rates decrease inasmuch as the leaves are older. The shift to the right of the curves for plants grown in 37% and 75% daylight respectively, corresponds with the data of the measurements.



FIG. 41A. Calendula officinalis, 1968, outdoors, single plot. Maximum rate of photosynthesis per cm^2 leaf area at 25 °C of a number of leaves along a primary lateral branch of plants grown at different light intensities. Numbers indicate position of leaf on the stem.



FIG. 41B. Same as fig. 41A, mixed plot.

It is surprising that senescence proceeds more slowly at 75% light intensity than at 37% light intensity. Looking at leaves of corresponding numbers (positions along the stem) at different light intensities, one might conclude that senescence proceeds at a slower rate than is suggested by the measurements of leaves of different physiological ages at each light intensity.

Influence of competition does not seem to occur in the 37% light experiment, where the competition of the shade plant *Impatiens parviflora* might be expected to be of most importance. In 75% daylight, the maximum photosynthetic rate of the leaves grown in the competition experiment is considerably lower than in the single plot. In fact, the plants were smaller in the competition experiment, with fewer and smaller leaves on the lateral branches. Since this was not the case in other years, another factor than competition probably has played a rôle.

4.2.2. Indoor experiments

4.2.2.1. Photosynthesis of simultaneously formed leaves at different ages. The first measurements of this kind were carried out in 1967 on two sets of plants, originally grown both at a light intensity of 66,000 ergs \cdot cm⁻² ·sec⁻¹. After 16 days, one of these sets was brought at a light intensity of 20,000 ergs \cdot cm⁻² ·sec⁻¹. Measurements were carried out on the 10th and 11th leaf of 8 plants in each set. From each leaf two discs were punched out at a time for determining the photosynthetic rate. The measurements started one week after the above mentioned second set of plants was transferred to low light intensity. The results, presented in fig. 42, suggest a differentiation in photosynthetic rate, i.e., over a period of about ten days, a decrease of the maximum rate of photo-



FIG. 42. Calendula officinalis, 1967–68, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C of the 10th/11th (\bullet , \bigcirc) and the 12th/13th leaf (\bullet , \square) on the main stem at different times. Plants brought into two different light intensities one week before the first measurements. Closed symbols: plants grown at 66,000 ergs \cdot cm⁻². sec⁻¹; open symbols: at 20,000 ergs \cdot cm⁻². sec⁻¹.

synthesis of the plants moved into the low light intensity. The 10th and the 11th leaf of these plants were already formed, but not yet full-grown at the time of the move. A decrease of the photosynthetic rate owing to senescence seems to occur earlier at the highest light intensity. The drop of photosynthesis in the high light intensity series corresponds with the onset of flowering as well as with a 'Karathan' treatment against mildew. Data from the 12th and 13th leaf, however, suggest a more synchronous senescence of corresponding leaves in both light intensities.

As time proceeds, in the high light intensity series, the slope of the curve of O_2 -evolution against light intensity decreases, as is shown in figs. 43A and 43B. (These figures represent only the first and the last measurement.) This was not found for the low light intensity series which may be due to the fact that senescence had proceeded less far (cf. fig. 42).

Notwithstanding the fact that the values are small, and, therefore, cannot claim a high degree of accuracy, respiration does not seem to be much influenced by senescence in the 10th and 11th leaf at high light intensity, as can be seen in fig. 43B, representing the lower part of fig. 43A on a larger scale. The plants which were transported to a lower light intensity, however, show a larger decrease in respiration. It may seem surprising that O_2 -uptake in one case had a negative value. In fact, this was found more often, when WARBURG buffer solution was used. In case a sodium hydroxide solution was used to keep the CO₂-concentration at zero, higher respiration values were found. However, when the measurements with the buffer solution lasted some hours, O_2 -uptake as measured increased and reached the same value as with the NaOH-solution.



FIG. 43A. Calendula officinalis, 1967-68, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of the 10th/11th leaf on the main stem of plants, after their being placed at light intensities of 66,000 ergs \cdot cm⁻² · sec⁻¹ (closed symbols) and of 20,000 ergs \cdot cm⁻² · sec⁻¹ (open symbols). •, \bigcirc 1 week after transportation; \blacksquare , \Box 5 weeks after transportation. Entire range of light intensities.



FIG. 43B. Data from fig. 43A in the light limited range (enlarged).

In all other experiments of this kind, photosynthesis was measured on equivalent leaves of different plants at about weekly intervals. Figs. 44A, B and C show the maximum photosynthetic rates over a period of about two months (March and April 1969), of three pairs of leaves along the main stem. There is a general trend that photosynthesis reaches a higher maximum rate in plants grown at higher light intensities. As was mentioned in the foregoing chapter, the differences are rather small. Furthermore it appears that photosynthesis in the lowest leaves (nrs. 3 and 4) decreases earlier than in the younger ones (leaf nrs. 9 and 10, 15 and 16). This is in agreement with the general assumption that senescence is increased by extreme shortage of light. Moreover, as stated in the introduction of this chapter, the lower leaves are often in touch with the moist soil, which accelerates dying.

Evidence that senescence is increased by flowering is rather weak. The only indications are a somewhat faster decrease of the maximum photosynthetic rate of the plants grown at 100 % light, and a somewhat faster decrease in the leaves nrs. 15 and 16, as compared with the leaves nrs. 9 and 10. Here, a downward wave of senescence after flowering might be manifest. However, this cannot affect the results for the lowest light intensity where no flowering occurred.

Finally, it attracts attention that the highest photosynthetic rate reached by the leaves nrs. 15 and 16 surpasses that of the leaves nrs. 9 and 10. It should be mentioned, however, that this can partly be explained by the fact that after



FIG. 44A. Calendula officinalis, 1968–69, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 $^{\circ}$ C, at different leaf ages, of the 3rd and 4th leaf on the main stem of plants grown at different light intensities. Arrows indicate date after which agar slices were used during the measurement of photosynthesis.



FIG. 44B. As fig. 44A. 9th and 10th leaf.



FIG. 44C. As fig. 44A. 15th and 16th leaf.

March 20th, agar slices were used to prevent wilting during the measurements of photosynthesis (cf. p. 10). Since no obvious irregularities occur in the photosynthesis-time curves after this date, the influence of application of agar should not be overestimated, because it did not change the general shape of the curves. Moreover, the highest photosynthetic rates reached by the 9th and 10th leaves might have been missed, because during two weeks no measurements were made.

The average values of figs. 44B and 44C and some more data are presented in fig. 45. To avoid possible irregularities, only measurements where agar slices were used are included. Here, the general trend, already mentioned earlier, becomes evident: The highest photosynthetic rates are reached by leaves grown at the highest light intensities. These leaves also show the fastest decrease of their maximum photosynthetic rates owing to senescence.

Similar measurements, made with leaves of lateral branches, are presented in the figures 46–51. As in the outdoor experiments, leaves of equal position on the plant are not quite comparable in age. Therefore, no leaf age is given in the



FIG. 45. Calendula officinalis, phytotron. Maximum rate of photosynthesis per cm^2 leaf area at 20°C, at different leaf ages, of leaves on the main stem of plants grown at different light intensities. Average values of figs. 44B, 44C, and some other data, not presented in separate figures. (Measurements with the use of agar slices).

figures. Since comparable leaves are probably younger in the lower light intensities, one might expect that these leaves have a relatively high photosynthetic rate at a certain time, because the photosynthetic rate in the higher light intensities may already decrease. This seems to be the case in the figures 46 and 47A, but is not apparent in the other ones.

In figs. 47A and 49 the photosynthetic rate of the leaves grown in 100% light again seems to show the greatest decrease. In the other figures, however, only small differences occur between leaves from various light intensities, which, moreover, can hardly be attributed to effects of these light intensities.



FIG. 46. Calendula officinalis, 1968-69, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different times, of the 1st and 2nd leaf (double lines) and of the 9th and 10th leaf (single lines) on a primary lateral branch of plants grown at different light intensities.



FIG. 47A. Calendula officinalis, 1968-69, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different times, of the 7th and 8th leaf on a primary lateral branch of plants grown at different light intensities.



FIG. 48. Calendula officinalis, 1969-70, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different times, of young leaves on a primary lateral branch of plants grown at different light intensities.



Fig. 49. Calendula officinalis, 1969–70, phytotron. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C, at different times, of the 7th and 8th leaf on a primary lateral branch of plants grown at different light intensities.

From fig. 50, representing the average values of the data from figs. 46–49, it can be seen, as was already concluded from the separate figures, that the decrease of the maximum photosynthetic rate of leaves from primary lateral branches owing to senescence proceeds at a slower rate, as compared with leaves from the main stem (fig. 45). The lower physiological age of plants grown at the lowest light intensity is manifest by their leaves reaching later the highest photosynthetic rates.

Leaves from secondary lateral branches (fig. 51) show a still slower decrease in the maximum photosynthetic rate as compared with leaves from primary lateral branches and leaves from the main stem (summarized in the figures 50 and 45 respectively).

Figs. 52 and 53, representing two different experiments, show the photosynthesis/light curves of simultaneously formed leaves measured at different ages; the decrease of the maximum rates of photosynthesis with increasing age is clear. The photosynthetic rates at lower light intensities decrease also, causing decline of the slope of the curves with age. In this case, there is no significant change of the respiration values.



FIG. 50. Calendula officinalis, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different leaf ages, of leaves on primary lateral branches of plants grown at different light intensities. Average values of figs. 46, 47A, 47B, 48, and 49.



Fig. 51. Calendula officinalis, 1969–70, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different times, of young leaves on a secondary lateral branch of plants grown at different light intensities.



FIG. 52. Calendula officinalis, 1968–69, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of leaves of plants grown at 41,000 ergs \cdot cm⁻² · sec⁻¹, from fig. 47B. Numbers indicate order of measurements. • 1st, \bigcirc 3rd, \bigtriangledown 5th measurement, \times 2nd and 4th measurement.



FIG. 53. Calendula officinalis, 1969–70, phytotron. Rates of photosynthesis per cm² leaf area at 20 °C of leaves of plants grown at 72,000 ergs \cdot cm⁻² · sec⁻¹, from fig. 48. Numbers indicate order of measurements: • 1st, × 2nd, \bigcirc 3rd, \triangle 4th \checkmark 5th measurement.

4.2.2.2. Photosynthesis of successively formed leaves. The first experiments of this kind are concerned with photosynthesis of leaves along the main stem. The measurements were carried out in early and late 1968 and are quite comparable with the outdoor experiments of this kind. In the figures 54A and 54B the maximum photosynthetic rates are presented of a number of leaves along the main stem. The data are plotted as maximum rate of photosynthesis against leaf age which was again estimated as indicated in subsection 2.2.4. The two series of measurements were carried out with 3-4 weeks interval on comparable plants, grown under four different light intensities.

Like in the outdoor experiments, one might conclude that the maximum photosynthetic rate shows a gradual decrease, starting at a leaf age of about seven weeks. Earlier, in most cases, there is a very slow increase of the maximum photosynthetic rate. The differences between the photosynthetic capacities of plants grown under various light intensities appear to be small and rather inconsistent. It should be mentioned, however, that all plants grew under the same light conditions of about 4×10^4 ergs \cdot cm⁻² · sec⁻¹, until 18 days before the measurements started. This may also be the reason why in the second series of measurements (fig. 54B), the leaves of plants grown at the highest two light intensities, maintain their maximum rate of photosynthesis for more than ten weeks, and the leaves of the plants grown in 100% light even show an increase up to the age of 9-10 weeks. The differences between the four light intensities are clearer in the second series of measurements, probably owing to the longer period of exposure to various light conditions.



FIG. 54A. Calendula officinalis, 1967–68, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different leaf ages, of a number of leaves along the main stem of plants grown at different light intensities. Numbers indicate position of leaves on the stem.



Fig. 54B. Calendula officinalis, 1967-68, phytotron, As fig. 54A, 3-4 weeks later. However, whilst in fig. 54A measurements of leaves of the same age, grown at different light intensities were taken shortly after each other, in fig. 54B the various ages were first measured at the same light intensity, and subsequently at the other intensities, explaining their different positions with regard to leaf age.

Senescence appeared enhanced by extremely low light: ntensities, and a condition of the plants which permitted no further photosynthetic measurements was reached at a younger stage in plants grown at low light intensities, whereas this more often occurs in plants grown at high light intensities.

An identical series of measurements, performed late in 1968, is presented in the figures 55A, B and C. Since the measurements started only 12 days after transport of the plants to different light intensities, again there are small and inconsistent differences in fig. 55A. Decrease of photosynthesis seems to start already after 30 days. However, as in the previous series, this may rather be explained as a lower maximum rate of photosynthesis of the oldest leaves. The data presented in fig. 55B, obtained two weeks later, suggest a further decrease of photosynthesis, owing to senescence in leaves grown at high light intensities. At this stage, flowering which might be of influence, does not vet occur. Therefore, it seems more likely to assume that the low values obtained at this stage are due to a lower photosynthetic rate in the oldest leaves. Leaves of equal positions, grown at different light intensities, were measured at different times. This causes an apparent divergence of the photosynthesis - leaf age curves for these different light intensity series. When the maximum rate of photosynthesis is plotted against the position number instead of the age of the leaf, the curves for different light intensities coincide better, because old leaves grown in equal



FIG. 55A. Calendula officinalis, 1968–69, phytotron. Maximum rate of photosynthesis per cm^2 leaf area at 20°C, at different leaf ages, of a number of leaves along the main stem of plants grown at different light intensities. Measured as indicated with fig. 54b, however, on pairs of leaves.



FIG. 55B. Calendula officinalis, 1968-69, phytotron. As fig. 55A, 14-20 days later.



FIG. 55C. Calendula officinalis, 1968-69, phytotron. As fig. 55A, 60-70 days later.



FIG. 55D. Calendula officinalis, 1968-69, phytotron. Data from fig. 55B plotted against leaf number instead of against leaf age.

positions at different light intensities don't differ much in their photosynthetic rates (fig. 55D).

A reason why the values of the photosynthetic rates in these series are so much higher than those measured in the previous season must be in the fact that during the second season higher light intensities were used, so that the highest light intensity series in the first season corresponds with the second highest of the second season. The youngest leaves in the second series in part show rather low photosynthetic rates, probably because in a young, thin stage, they tend to wilt sooner during the measurements.

The data presented in fig. 55C were obtained about 10 weeks after those presented in fig. 55A. In contradiction with the experiment shown in fig. 54B, here leaves grown under high light intensities died sooner than those grown under low light intensities. However, the plants were five weeks older in this case, and senescence of the leaves was influenced by flowering. Apart herefrom there were too few leaves left to give a clear picture.

Although the data from figs. 54A and B and 55A, B, C are rather inconsistent, the average values, presented in fig. 56, give a clear picture. The maximum rates of photosynthesis decrease with decreasing light intensities at which the plants have grown, and, moreover, a faster decrease of the maximum photosynthetic rate of leaves grown at the highest light intensity is apparent.



FIG. 56. Calendula officinalis, phytotron. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C, at different leaf ages, of leaves on the main stem of plants grown at different light intensities. Average values of figs. 54A, 54B, 55A, 55B, and 55C. (Measurements without the use of agar slices.

In order to investigate photosynthesis of leaves which developed entirely under different light intensities, photosynthetic rates were measured of a great number of leaves from primary and secondary lateral branches. The maximum rates of photosynthesis are given in schematic drawings of the plants (figs. 57-59). There are a few general, although not always occurring trends:

1. On young branches, the lowest leaves have the highest photosynthetic rates, the young leaves having not yet reached their maximum. On older branches



FIG. 57A. Calendula officinalis, 1968–69, phytotron. Maximum rate of photosynthesis in mm³ O_2 per cm² leaf area per hour at 20 °C of a number of leaves on primary lateral branches of a plant grown at 81,000 ergs · cm⁻². sec⁻¹. ?: no measurements; †: leaf died.



FIG. 57B. As fig. 57A. Plant grown at 57,000 ergs \cdot cm⁻². sec⁻¹.



FIG. 57C. As fig. 57A. Plant grown at 41,000 ergs \cdot cm⁻². sec⁻¹.



FIG. 57D. As fig. 57A. Plant grown at 20,000 ergs \cdot cm⁻² · sec⁻¹.



FIG. 58A. Calendula officinalis, 1968-69, phytotron. Maximum rate of photosynthesis in mm³ O_2 per cm² leaf area per hour at 20 °C of a number of leaves on primary and secondary lateral branches of a plant grown at 81,000 ergs \cdot cm⁻² · sec⁻¹. Measurements performed 6 weeks after fig. 57. ?: no measurements; †: leaf died or branch died.



FIG. 58B. As fig. 58A. Plant grown at 57,000 ergs \cdot cm⁻². sec⁻¹.



FIG. 58C. As fig. 58A. Plant grown at 41,000 ergs \cdot cm⁻² sec⁻¹.



FIG, 58D. As fig. 58A. Plant grown at 20,000 ergs \cdot cm⁻² · sec⁻¹.



FIG. 59A. Calendula officinalis, 1968–69, phytotron. Maximum rate of photosynthesis in mm³ O_2 per cm² leaf area per hour at 20°C of a number of leaves on secondary lateral branches, growing on one primary lateral branch of a plant grown at 81,000 ergs \cdot cm⁻². sec⁻¹. Measurements performed 9–11 weeks after fig. 57. ?: no measurements; †: leaf died.



FIG. 59B. As fig. 59A. Plant grown at 57,000 ergs \cdot cm⁻², sec⁻¹.



FIG. 59C. As fig. 59A. Plant grown at 41,000 ergs \cdot cm⁻². sec⁻¹.

the youngest leaves have the highest photosynthetic rates, the older ones already ageing. This is perfectly in accordance with our observations on the leaves along the main stem.

2. The oldest leaves of the lowest branches tend to die first, although flowering occurs first in the upper branches. This is probably due to the deep shade to which these leaves are submitted. Although these leaves in majority have grown for their whole life under the different light intensities, there is hardly any relation between maximum rate of photosynthesis and light intensity during growth. The average maximum photosynthetic rates per plant, presented in table 4, illustrate this. Here, probably, the faster senescence of plants at high light intensities counteracts the originally higher photosynthetic capacities.

Unfortunately, no sufficient data are available to discuss these features for plants, grown in the field.

Light intensity during growth	Primary leaves	Secondary leaves	Primary leaves	Secondary leaves	Primary leaves	Secondary leaves
100 %	118	_	96	_		120
70%	132	-	71	140	_	138
40%	107	_	40	113	-	135
20%	114	-	58	-	-	-

TABLE 4. Average maximum rates of photosynthesis in mm³ $O_2 \cdot cm^{-2} \cdot h^{-1}$ of a number of leaves of *Calendula officinalis* along primary and secondary lateral branches, measured three times, with about 6 and 4 weeks intervals respectively.

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4.3. PHOTOSYNTHESIS AND AGE IN Impatiens parviflora

4.3.1. Outdoor experiments

4.3.1.1. Photosynthesis of simultaneously formed leaves at different ages. The preliminary observations were made in 1967 on leaves from which the photosynthetic rate of the two halves was measured with an interval of about two weeks.

The results are presented in figures 60-62. The 9th leaves of the main stem (fig. 60) show a decrease of their maximum photosynthetic rates already after 39 days, except for the 100% grown in the single plot. It is improbable that the adaptation to low light intensities after placing the screens is not yet complete, since this happened well over three weeks before, and there is no greater difference in the photosynthetic levels in the 2nd measurements. There are no great differences between the leaves from plants grown in single plots and those mixed with *Calendula officinalis*.

The 8th leaves of the older lateral branches which were formed later, show lower maximum rates of photosynthesis than the leaves from the main stem (fig. 61). The plants growing in full daylight show a decrease of the photosynthetic rate. In the other light intensities there is no clear relation between leaf age and photosynthetic rate. Taking into account the different times of measure-



FIG. 60. Impatients parviflora, 1967, outdoors. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at two different leaf ages, of the 9th leaf on the main stem of plants grown at different light intensities. Screens placed at a leaf age of 6 days.



Fig. 61. *Impatiens parviflora*, 1967, outdoors. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at two different leaf ages, of the 8th leaf on the oldest lateral branch of plants grown at different light intensities.



FIG. 62. *Impatiens parviflora*, 1967, outdoors. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different leaf ages, of leaves from secondary lateral branches of plants grown at different light intensities.

ment, there again is no clear difference between plants from single and mixed plots.

Measurements with leaves from secondary lateral branches are presented in fig. 62.

The data, obtained with the 5th leaves of these branches, are rather irregular. Probably, the leaf material was too heterogeneous in quality, due to the age of the plants, to give clear results. A certain retardation of the development at lower light intensities may have also played a rôle, although this is less pronounced than with *Calendula officinalis*. The general increase in photosynthetic rates from leaves in the single plots might be ascribed to weather conditions, viz. a decrease in temperature and sunlight after the first measurements; however, this does not generally hold for the findings in the mixed plots.

More detailed studies were performed in 1969 and 1970, when photosynthesis of a distinct leaf was determined with weekly intervals, both in the single and in the mixed plots. In 1969, the 12th leaf from the main stem was measured, in 1970, when the experiments started two weeks later, the 14th leaf was taken. At the beginning of the measurements, both leaves were just large enough for the purpose. The results are presented in the figures 63-66.

As can be seen, there is a clear effect of light intensity during growth on the maximum photosynthetic rate, as was already discussed in Chapter 3. This effect generally persists when the photosynthetic rates decrease owing to age. Yet there are some marked differences between the two successive years. The measurements in 1969 were carried out at 25°C, those in 1970 at 20°C, which explains the differences in the maximum levels of the rates of photosynthesis. In 1969, there is a striking decrease in the photosynthetic rate after three weeks of measurements. Soon afterward the leaves vellowed and died rapidly. Leaf age was then about 50 days. This rapid decrease can be ascribed to external factors. The maximum day temperatures after the first three weeks rose to a mean value of about 30 °C during the remaining period of measurement. The maximum day temperatures at 10 cm above the soil are presented in fig. 63. This must have caused the drop in photosynthesis, probably by indirect factors, e.g. water deficit. Though based on fewer observations, this same phenomenon can be found at the same time in Calendula officinalis (fig. 37A). In 1970, there is a far more gradual decrease of the photosynthetic rate, so that the leaves stay alive for about 80 days. Yet there is again a decrease in the photosynthetic rate of a number of leaves, again coinciding with a period of high temperatures (viz. fig. 65). Provided such a period does not last too long, the plants are evidently able to recover. The leaves were collected in the morning and conserved in water until they were used for the measurement of photosynthesis. Although the leaves thus well had the opportunity to take up water, this did not lead to a complete recovery of the photosynthetic capacity. So, water deficit should not be the only reason for the temporary drop in photosynthesis. In general, the decrease in photosynthetic rate with increasing age is stronger in the plants grown at high light intensities. In percents, however, the decreases seem to be very much alike for all light intensities.



FIG. 63. Impatiens parviflora, 1969, outdoors, single plot. Maximum rate of photosynthesis per cm^2 leaf area at 25 °C, at different leaf ages, of the 12th leaf on the main stem of plants grown at different light intensities, with indication of the maximum day temperatures at 10 cm above the ground during the period of measurements.

Neither in 1969, nor in 1970 there seem to be important differences between the data obtained from single and mixed cultures. This goes especially for 1969. In 1970, the data from the mixed plots are somewhat irregular. It is remarkable, e.g., that the leaves from the plants grown at 12% light intensity show a slight increase of the maximum rate of photosynthesis up to a rather old stage. The reason for this is not clear. The last observations made on the 100% plants should not be taken into account, since here the leaf material was obtained from a plant with a different habit, having thick, bumpy leaves with a high chlorophyll content, the only plant still available at that stage. These leaves also yellowed much later. Attempts to germinate seeds from this plant have failed so far.



FIG. 64. Impatiens parviflora, 1969, outdoors, mixed plot. Maximum rate of photosynthesis per cm² at 25 °C, at different leaf ages, of the 12th leaf on the main stem of plants grown at different light intensities.

In fig. 67, the data from the figures 63–66 are averaged. The general trend clearly shows a faster decrease of the maximum rate of photosynthesis when the plants are grown at higher light intensities, so that ultimately the maximum rates of photosynthesis of leaves grown at different light intensities do not show the same proportion as in a young stage. In addition it may be remarked that, especially owing to the data of fig. 66, the plants grown at 12 % light show hardly any decrease of the photosynthetic capacities of their leaves. Moreover, fig. 67 clearly shows, apart from the effect of age, also the effect of light intensity during growth. The latter effect has been already discussed in Chapter 3.

From fig. 68, presenting the successive photosynthesis/light curves numbered 1-10 from the plants grown at 100% light in 1970, in single plots, it can be seen that the curve representing the photosynthesis measurements against light intensity also decreases in the lower light intensity range when the maximum rate of photosynthesis drops. In fig. 69, the comparable data from plants grown at
FIG. 65. Impatiens parviflora, O_2 -1970, outdoors, single plot. Maximum rate of photosynthesis per cm² leaf area at 250 20°C, at different leaf ages, of the 14th leaf on the main stem of plants grown at different light intensities, with indication of the maximum 200 day temperatures at 10 cm above the ground during the period of measurements.





FIG. 66. Impatiens parviflora, 1970, outdoors, mixed plot. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C, at different leaf ages, of the 14th leaf on the main stem of plants grown at different light intensities.

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FIG. 67. Impatients parviflora, outdoors. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different leaf ages, of leaves on the main stem of plants grown at different light intensities. Average values of figs. 63–66, with exclusion of the obviously by external factors caused low values in the right part of figs. 63 and 64.

12% daylight are given on a larger scale, showing identical results. It is also obvious that a reversible drop of photosynthesis at light satuaration owing to external factors (4th measurement), goes together with an equally reversible decrease of photosynthesis in limiting light intensities. Again, the small dark respiration rates, owing to the methods used (cf. page 42) are striking. In order to eliminate certain unavoidable temporary deviations, figs. 70 and 71 present averages from series of 3-4 successive measurements each. They strongly endorse the conclusions already drawn from figures 68 and 69.



FIG. 68. Impatiens parviflora, 1970, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 20 °C, at a selection of different leaf ages, of the 14th leaf on the main stem of plants grown at 100% daylight (cf. fig. 65). (Numbers indicate order of measurements; leaf ages: (1) 23, (2) 29, (3) 37, (5) 52, (6) 58, (8) 72 and (10) 85 days.)



FIG. 69. Impatiens parviflora, 1970, outdoors, single plot. Rates of photosynthesis per cm² leaf area at 20°C, at different leaf ages, of the 14th leaf on the main stem of plants grown at 12% daylight (cf. fig. 65).



FIG. 70. Impatiens parviflora. Averaged values of fig. 68.



FIG. 71. Impatiens parviflora. Averaged values of fig. 69.

We still attempted to measure maximum rates of photosynthesis of leaves of increasing age from a lateral branch. These measurements were performed in 1969 and started at the end of the warm period, from which the temperatures were given in fig. 63. These leaves were then about 40 days old. The data of the single culture showed that with decreasing day temperatures there was a slight recovery of the maximum photosynthetic rates. Yet, many leaves started yellowing at the beginning of the period of measurements, especially at the higher light intensities and in the mixed plots, and leaf age rarely exceeded 60 days. In the mixed plots too little material was available to obtain enough results for comparison with the single plot. For these various reasons, the results obtained with these measurements were so inconsistent, mutually as well as with results obtained under more favourable conditions, that it seems hardly worthwhile to deal with them further.

4.3.1.2. Photosynthesis of successively formed leaves. This type of investigation was only performed in 1968 in two series, related to photosynthesis of leaves along the main stem and along a lateral branch respectively. The measurements of the leaves along the main stem started at the 5th leaf, since the older leaves had already yellowed or even fallen off (fig. 72). Leaves



FIG. 72. *Impatiens parviflora*, 1968, outdoors, single plot. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different leaf ages, of a number of leaves along the main stem of plants grown at different light intensities, measured as indicated with fig. 54B. Vertical lines indicate the first leaves that developed entirely after the screens were placed.

of plants grown at a given light intensity were measured in series, subsequently those grown at another light intensity, and so on. The vertical lines indicate the 10th leaf, which was the first to develop entirely after the screens were placed: from their position along the time scale the sequence of the measurements of the various series may be read. In general, there is little or no decrease with age in the older leaves. The excessively low values of the youngest leaves of plants grown at 100% light are probably due to the fact that these thin leaves have suffered more from wilting during the measurements of the photosynthetic rates. The data from the plants brought into 75% and 12% daylight lead to the conclusion that the photosynthetic capacity of leaves, which have partly developed in full daylight, still may be reduced when the plants are moved into lower light intensities. The leaves which have developed fully after the transportation to low light intensities have still lower photosynthetic capacities. (The data from the plants grown in 37% light do not fit into this conclusion; there are, however, very few data in this case.) The finding that the maximum photosynthetic rate remains fairly constant up to a leaf age of about 70 days is rather remarkable, also as compared with the repeated measurements on a specific leaf in 1970 (fig. 65) which showed a gradual decrease with increasing age, this being the more 'normal' behaviour (fig. 67).

The photosynthetic rate of leaves from lateral branches was measured when the leaves were already over 70 days old, so that a decrease in the maximum rate of photosynthesis was found. As can be seen in fig. 73A, which represents results obtained with the leaves from the single plot, grown at different light intensities, these leaves show no significant differences in photosynthetic rate. There seems to be, however, a difference in the decrease of photosynthesis, which corresponds with the order of measurement and not with the light intensities during growth. The leaves from a set of plants were measured one after another. It is known that removing a leaf may increase the photosynthetic capacity of the neighbouring leaves. Since the oldest leaves were measured first, the younger ones should therefore show an increase in photosynthetic rate, the younger the leaf, the higher the increase, which causes an apparent steeper decrease in photosynthetic rate with increasing age. However, there is not such an indication with the leaves along the main stem (fig. 72). This may be explained by the fact that the influence of leaf removal is less when the leaves are still at their maximum photosynthetic capacity.

The comparable data of the leaves from the mixed plots (fig. 73B), in general show a slighter apparent decrease with age in the maximum rates of photosynthesis, somewhat more difference between the plants grown at various light intensities and, except for the 12% light intensity, a significantly higher photosynthetic rate than the leaves from the single plots. The explanation of these differences is difficult. There is no reason to believe that monocultures and mixed cultures cause such big differences in persistance of maximum photosynthetic rates. It should be noted that the differences in photosynthesis between the leaves from single and mixed plots go together with differences in fresh and dry weight (cf. fig. 84).



FIG. 73A. Impatients parviflora, 1968, outdoors, single plot. Maximum rate of photosynthesis per cm² leaf area at 25 °C of five leaves along a primary lateral branch of plants grown at different light intensities, measured as indicated with fig. 54B.



FIG. 73B. Impatients parviflora, 1968, outdoors, mixed plot. Maximum rate of photosynthesis per cm² leaf area at 25 °C of five leaves along a primary lateral branch of plants grown at different light intensities, measured as indicated with fig. 54B.

4.3.2. Indoor experiments

4.3.2.1. Photosynthesis of simultaneously formed leaves at different ages. It proved to be difficult to break the dormancy of *Impatiens* seeds (cf. p. 5) and, though less, to raise plants under artificial conditions. Therefore, only a few series of measurements were performed, all in 1970. The first series, presented in fig. 74, is concerned with photosynthesis of the four sufficiently large top leaves along the main stem, i.e. the leaves nrs. 11–14, from which the photosynthetic rate was determined at weekly intervals. Twelve days before the measurements started, the plants were brought into the different light intensities. At that moment, the mentioned leaves were about fully expanded. As can be seen, there is a differentiation in the maximum photosynthetic rates as time proceeds, which probably should be ascribed to an influence of the differences in light intensity during growth rather than to the increasing leaf age, which would tend to the opposite. Up to the last measurement there was no clear decrease in the photosynthetic rate.

Especially at the lowest light intensities, however, in several leaves suddenly necrotic spots appeared which enlarged rapidly, so that in a number of cases whole plants died within a few days. This might be due to too low amounts of light, although the compensation point of *Impatiens* should be very low and probably does not exceed 2% of June solar radiation (EVANS and HUGHES, 1961). Therefore, the daylength was prolonged from 9 to 12 hours, which did not prove to be an amelioration. No explanation can be given for the rather inconsistent data from the plants grown in 100% light, except for the last high value, which has been obtained from a plant with similar thick, bumpy, dark green leaves as described in subsection 4.3.1.1. (fig. 66). The photosynthetic rates of the leaves just beneath the ones mentioned above are also presented in fig. 74. Owing to bad condition of several plants, only few data could be obtained, which, however, are rather well in accordance with the data of the other leaves. If the maximum photosynthetic rates of the second series were shifted ten days to the left), the data of the two series would still better harmonize. This suggests that conditions or age of the whole plant are as important as leaf age.

Identical measurements were performed two months later on the 10th-13th leaves of a new set of plants. Eleven days before the measurements started, the plants had been moved to different light intensities; the leaves then were already full-grown. As can be seen from fig. 75, differentiation of the photosynthetic rates had already occurred within eleven days from the move to the required light intensities. From the first measurements onwards, there is a gradual decrease of the maximum photosynthetic rate at all four light intensities. The condition of the plants again was rather unfavourable, so that only four measurements were possible before the plants had declined so much that no further experiments could be performed.







FIG. 75. Impatients parviflora, 1969-70, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different leaf ages, of the 10th till 13th leaf (averaged at each point) on the main stem of plants grown at different light intensities.



FIG. 76. Impatiens parviflora, phytotron. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C, at different leaf ages, of leaves on the main stem of plants grown at different light intensities. Average values of figs. 74 and 75.

The results presented in figs. 74 and 75 are averaged in fig. 76. As mentioned, the most striking difference with the outdoor experiments, presented in fig. 67, viz., the sharper decrease of the maximum rate of photosynthesis of leaves of plants grown at the lowest light intensity, should be ascribed to transport of the plants from high to low light intensities.



FIG. 77. Impatiens parviflora, 1967-68, phytotron. Maximum rate of photosynthesis per cm² leaf area at 20 °C, at different leaf ages, of a number of leaves along the main stem of plants grown at different light intensities, measured as indicated with fig. 54A. Right side: the first formed leaves; left side: the last formed leaves.

4.3.2.2. Photosynthesis of successively formed leaves. These experiments were carried out in 1968. The first were concerned with all leaves along the main stem. Measurements started with the top leaf. The results are presented in fig. 77 (right part). When the lowest leaf had been measured, a number of younger leaves had developed in the meantime, photosynthesis of which was also measured. The results obtained with these youngest leaves are given in the left part of fig. 77.

Fifteen days before the first determination of CO_2 -exchange, the plants had been moved to the different light intensities, so that photosynthetic adaptation might be expected to have already taken place. In general, the results confirm these expectations.

The leaves show a rather constant rate of photosynthesis until the age of about six weeks, after which a gradual decrease starts in plants grown at the higher light intensities. With a view to other experience this decrease in plants grown at the highest light intensity may in part be connected with accelerated senescence.

The apparent gradual increase with age of the photosynthetic rate of leaves grown at the lowest light intensity may lead to the conclusion that a change in the photosynthetic capacity as a result of a change in light intensity during growth is smaller when the leaf is older, i.e. the less a leaf is expanded when it is transported to another light intensity, the more its photosynthetic capacity is



FIG. 78. Impatients parviflora, 1967–68, phytotron. Maximum rate of photosynthesis per cm² leaf area at 25 °C, at different leaf ages, of a number of leaves along a primary lateral branch of plants grown at different light intensities, measured as indictated with fig. 54A.

influenced by the new light intensity. The youngest leaves also show a somewhat lower photosynthetic rate, which may partly be ascribed to the thin constitution which enhances wilting, and partly to the fact that leaves which have not yet fully expanded, have not yet reached their maximum photosynthetic capacity, For the rest, the data from the older and the younger five leaves correspond well.

The leaves along a primary lateral branch (fig. 78) show a more consistent photosynthetic capacity over a longer time than is shown in fig. 77 (right part). However, the differences between the four plant series, grown under various light conditions, though consistent, are smaller. The maximum rates of photosynthesis are lower, which is especially evident since these measurements were carried out at 25° C. Since, however, at this time agar slices (see section 2.2.1.) were not yet used, the increase in photosynthetic rate to be expected at the higher temperatures during these measurements may have been counteracted by more severe wilting.

4.4. DISCUSSION

From the results presented in this chapter, a general trend can clearly be distilled. Like the leaves of other plants (RICHARDSON, 1957; HOFFMANN, 1962; JEWISS and WOLEDGE, 1967) the leaves from *Calendula* and *Impatiens* reach the highest value of their maximum rate of photosynthesis at the time they are fully expanded, i.e. after about 20 days in the outdoor experiments, and after about 30 days in the phytotron, where the plants develop somewhat more slowly, mainly owing to lower light intensities.

Calendula leaves, thereafter, show a rather rapid decrease of their maximum rates of photosynthesis. This decrease is most obvious in the plants grown at

high light intensities, which results in about the same maximum photosynthetic rate of circa $100 \text{ mm}^3 \text{ O}_2 \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ for all light intensities at a leaf age of 50–60 days. Here, one may suspect an influence of differences in the degree of flowering: flowering enhances the decrease of the photosynthetic rate (MOLISCH, 1938) and is most abundant at the highest light intensities for growth. A further, more gradual decrease of the maximum photosynthetic rate then occurs and is roughly the same for all light intensities. In this connection it should be observed that the leaves of plants grown at low light intensities, generally, have a longer life with an almost constant, low final rate of photosynthesis.

As can be seen from the figures 57-59, the rôle of the leaves on the main stem with respect to photosynthesis is successively taken over by those on the primary lateral branches, the secondary lateral branches, etc. It is striking that the leaves on lateral branches show little difference in their maximum photosynthetic rates, when grown at different light intensities. Probably, the effect of flowering and seed formation counteracts the effect of light intensity, since, in young plants, photosynthetic rates actually vary with the light intensities during growth (which was the special subject of Chapter 3).

Impatiens leaves show a somewhat more gradual decrease with age after the maximum photosynthetic capacity has been reached. This decrease is more proportional to the highest value reached, so that also in old leaves, grown at different light intensities, a difference remains between the maximum photosynthetic rates which are, at a leaf age of about 10 weeks, circa 50% of the maxima observed in the beginning. Although only few data from leaves on lateral branches are available, it is obvious that the leaves of the main stem are most important; the number of primary lateral branches is limited, their leaves are smaller, and the leaves on the secondary lateral branches are still smaller, with lower photosynthetic rates. Moreover, *Impatiens* plants have a rather short life, and the leaves on the main stem are photosynthetically active during the greater part of the plant's life. Since flowering occurs throughout the whole range of light intensities, there is no special influence on the photosynthetic capacity. Consequently, the later leaves show the same differences in maximum photosynthetic rate as the oldest ones.

5. MORPHOGENETIC EFFECTS IN RELATION TO LIGHT INTENSITY

5.1. LEAF THICKNESS

5.1.1. Morphological differences in relation to light intensity

5.1.1.1. Introduction. Together with most measurements of photosynthesis, fresh and dry weights were determined of the leaf samples used. Leaf thickness, the measurement of which takes more effort and is less accurate, was determined in only few cases. From these few determinations, however, a rather good correlation between leaf thickness and dry weight was found and an even better correlation between leaf thickness and fresh weight. During photosynthesis, an increase in dry matter takes place in the leaf discs. In most cases, this increase is too small to be detected. Only after several hours of exposure to the light, differences in leaf weight are found between the various samples, which are proportional to the photosynthetic rate, as measured by gas exchange (cf. fig. 79). The fresh weight determinations of leaf samples, in experiments in which no agar slices were used, yielded values which were too low, owing to wilting. The decrease in fresh weight was about proportional to the light intensity to which the leaf discs were exposed during the measurements. The mean decrease of eight leaf samples of 3.14 cm², exposed to different light intensities, was about 35 mg for *Calendula*, and 15 mg for *Impatiens*.

5.1.1.2. Effects of light intensity. In the next figures (80-85), the average fresh and dry weights and the percentages of dry matter are presented for most of the leaves, the photosynthesis data of which were discussed in the foregoing chapter.

In general, there is a clear difference between the weights per unit area of leaves grown at different light intensities. Fresh weights of leaves of both species grown at the highest and at the lowest light intensities mostly show differences of the same order of magnitude. This implies that for leaves of *Impatiens*, at least when grown outdoors, the percentual differences are larger than for *Calendula* leaves, because the absolute values for *Impatiens* are lower.

Dry weights per unit area of leaves increase with light intensity, both in indoor and outdoor experiments for *Calendula* and *Impatiens*. For both species they are of the same order of magnitude. The largest differences between the highest and the lowest light intensities are shown by *Impatiens*, grown outdoors. This may be seen from fig. 86, where average fresh and dry weights are plotted against light intensity during growth. The average values were calculated from weights per unit area of leaves of different ages which seems allowed, since fresh and dry weights proved to change hardly with increasing leaf age, as can also be seen from the figures 80–85. The percentages dry matter in *Impatiens* are about twice as high as in *Calen*dula. In either species they show small differences in the various light intensities, with the highest values generally for the plants grown at the highest light intensities.



FIG. 79. Impatiens parviflora, 1969, outdoors. Rates of photosynthesis per cm² leaf area at 20 °C during six hours of measurement, and leaf fresh weight after measurement of photosynthesis of a leaf from the main stem of a plant grown at 100% daylight. • Rates of photosynthesis during the first three hours; \bigcirc rates of photosynthesis during the second three hours; \triangle leaf fresh weight after photosynthesis.

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FIG. 80. Calendula officinalis, outdoors. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on the main stem of plants grown at different light intensities. Data obtained from leaves as measured in fig. 37A. Plants grown at 100% (\odot), 37% (\Box), and 12% (\blacktriangle) daylight.



FIG. 81. Calendula officinalis, phytotron. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on the main stem of plants grown at different light intensities. Data obtained from leaves as measured in figs. 44, 55A, 55B, 55C, and some other data. Plants grown at 100% (\bullet), \pm 70% (\heartsuit), \pm 40% (\square), and \pm 20% (\blacktriangle) light.



FIG. 82. Calendula officinalis, phytotron. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on primary lateral branches of plants grown at different light intensities. Data obtained from leaves as measured in figs. 46, 47A, 47B, 48, 49. Plants grown at 100% (\bullet), \pm 70% (\bigtriangledown), \pm 40% (\square), and \pm 20% (\blacktriangle) light.



FIG. 83. Impatiens parviflora, outdoors. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on the main stem of plants grown at different light intensities. Data obtained from leaves as measured in figs. 63, 64, 65, 66, 70. Plants grown at 100% (\odot), 75% (\bigtriangledown), 37% (\Box), and 12% (\blacktriangle) daylight.



FIG. 84. Impatiens parviflora, outdoors. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on primary lateral branches of plants grown at different light intensities. Data obtained from leaves as measured in figs. 71, 73 and some other data. Plants grown at 100% (\bullet), 75% (\bigtriangledown), 37% (\Box), and 12% (\blacktriangle) daylight.



FIG. 85. Impatiens parviflora, phytotron. Average fresh weights (A), dry weights (B), and percentages dry matter (C) of leaves of different ages on the main stem of plants grown at different light intensities. Data obtained from leaves as measured in figs. 74 and 75. Plants grown at 70,000 (\bullet), 48,000 (\bigtriangledown), 27,000 (\Box), and 15,000 (\bigstar) ergs \cdot cm⁻² · sec⁻¹.



FIG. 86. Average fresh weight (A), dry weight (B), and percentage dry matter (C) of leaves plotted against light intensity during growth. \bullet —outdoor experiments; \bigcirc --- indoor experiments. Averages calculated from several values, also presented in the figs. 80–85.

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FIG. 87. Calendula officinalis, 1969, outdoors, single plot. Leaf samples from fig. 37A (left side). A: fresh weight, B: dry weight. C: percentage dry matter.

Some exceptions from the general trends may be mentioned. In the dry season 1969, the dry weights per unit area from both *Calendula* leaves (fig. 87), and *Impatiens* leaves (figs. 88, 89), grown at high light intensities show a tendency to decrease with increasing age. In *Calendula*, this decrease is less in the fresh weight while in *Impatiens*, there is even a small increase in fresh weight of the leaves. At some points these exceptions are still to be seen in the averages which cover various years. A similar decrease in dry weight with age together with a small increase in fresh weight was found in the leaves along the main stem of *Impatiens* grown in full daylight in the field in the 1968 season (fig. 90). In this case, the data were collected successively in a period of moderate temperatures and some rainfall.

As already mentioned in Chapter 3, both *Calendula* (fig. 91) and *Impatiens* (figs. 92, 93), grown in the phytotron in early 1970, show only very small differences in fresh weight, and the normal differences in dry weight, so that the dry matter percentages differ in the same way as the dry weights. These small differences were not reproduced to that extent in later measurements (e.g. fig. 96).

Some additional data from fresh and dry weight determinations are given in the figures 94-96. This is concerned with all the leaves along the main stem of *Calendula* and *Impatiens* plants, grown in the phytotron, in 1970/71.

Calendula already had four full-grown leaves when the plants were moved into different light intensities. Yet, a fortnight later there is some difference in fresh and dry weight in these leaves, between those from the highest and the lowest light intensities, though smaller than in the younger leaves (fig. 94). Most of these old leaves had already died at the time of the second measurement, 3 weeks later, so that the behaviour of these leaves could not be followed further. Yet, it is clear that at the lowest light intensity the few remaining old leaves still had higher fresh and dry weights than the other leaves of the same plant, in which fresh and dry weights mutually differ hardly, except for a slight decrease in fresh weight with decreasing age. The same trend is observed in the plants at the highest light intensity (figs. 94, 95).



FIG. 88. Impatiens parviflora, 1969, outdoors, single plot. Leaf samples from fig. 63. A: fresh weight. B: dry weight. C: percentage dry matter.



FIG. 89. Impatiens parviflora, 1969, outdoors, mixed plot. Leaf samples from fig. 64. A: fresh weight. B: dry weight. C: percentage dry matter,



FIG. 90. Impatiens parviflora, 1968, outdoors, single plot. Leaf samples from fig. 70. A: 'fresh weights' of wilted leaves. B: dry weights.



FIG. 91. Calendula officinalis, 1969-70, phytotron. Leaf samples from fig. 48 (primary lateral branches). A: fresh weight. B: dry weight. C: percentage dry matter.



FIG. 92. Impatiens parviflora, 1969-70, phytotron. Leaf samples from fig. 74 (11th till 14th leaf). A: fresh weight. B: dry weight. C: percentage dry matter.



FIG. 93. Impatiens parviflora, 1969–70, phytotron. Leaf samples from fig. 75 (10th till 13th leaf). A: fresh weight. B: dry weight. C: percentage dry matter.





FIG. 94. Calendula officinalis, 1970-71, phytotron. Fresh weight (A), dry weight (B), and percentage dry matter (C) of all the leaves along the main stem of plants grown at 2 different light intensities. (Average of three plants).

FIG. 95. Calendula officinalis, 1970-71, phytotron. Fresh weight (A), dry weight (B), and percentage dry matter (C) of all the leaves along the main stem of plants grown at 2 different light intensities. Data collected 3 weeks after those from fig. 94. (Average of three plants).

Impatiens leaves, which were measured 19 days after transfer to different light intensities, show a differentiation in both fresh and dry weight, which is greater inasmuch as the leaves have passed a larger part of their life at different light intensities (fig. 96). This means a fairly constant level for the plants, which have grown permanently at the highest light intensity, except for a slightly lower value for the oldest and the youngest – not yet fullgrown – leaves. Plants which have been moved into lower light intensities show a more or less sharp decrease, especially in fresh weight, of the leaves along the stem upwards.

5.1.1.3. Structural differences related with leaf thickness. It has been known for a long time that the differences in leaf thickness for different



FIG. 96. Impatiens parviflora, 1970-71, phytotron. Fresh weight (A), dry weight (B), and percentage dry matter (C) of all the leaves along the main stem of plants grown at 4 different light intensities. (Average of two plants).

light intensities are mainly due to elongation of the palisade cells or to the formation of an extra layer of palisade parenchyma (DUFOUR, 1887). We have investigated this for the two species used in this paper (Plate I). *Impatiens* forms thicker leaves at the high light intensities by elongation of the palisade cells, without, however, forming an extra layer of cells. Some indication of an extra layer can sometimes be observed near the veins. In *Calendula* leaves the situation is less clear. There seem to be more layers of cells in the palisade parenchyma. The differentiation in palisade and spongy parenchyma is not very sharp; likewise, even from outside the adaxial and abaxial sides of the leaves are hard to distinguish.

5.1.2. Maximum rate of photosynthesis in relation to fresh and dry weight per unit leaf area

In general, there is a remarkable correlation between fresh weight per unit leaf area and maximum photosynthetic rate. In leaves of *Calendula* plants, grown at the lowest light intensity, the fresh weight is about 60 to 70% from that of leaves grown in 100% light; the same relation holds for the maximum photosynthetic rates. The highest values for fresh weight are found for leaves of

PLATE I. Cross-sections of leaves. \times 110.



I A. Calendula officinalis, grown at 100% daylight.

I B. Calendula officinalis, grown at 12% daylight.





I C. Impatiens parviflora, grown at 100% daylight.



I D. Impatiens parviflora, grown at 12% daylight.



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FIG. 97. Impatiens parviflora, outdoors. Maximum rate of photosynthesis per cm² leaf area at 20 °C (in part recalculated from 25 °C) of several leaves of plants grown at different light intensities, plotted against leaf fresh weight.

plants grown in the phytotron, again in accordance with the values found for the photosynthetic rates. For the leaves of lateral branches, the differences between high and low light intensity are much smaller. The highest values of dry weight are found in leaves grown in 100% daylight; there are only small differences between leaves grown in the field and indoors. The differences in fresh weight per unit area mentioned above thus are due to differences in the dry matter percentages.

Impatients leaves show greater differences than Calendula leaves in their fresh weights, those of leaves grown at the lowest light intensity being about 45% of those of leaves grown at the highest light intensity, corresponding with similar differences in maximum photosynthetic rates. As in Calendula, the leaves from lateral branches have lower fresh and dry weights per unit area than those of the main stem. The highest values for both fresh and dry weights are found in the outdoor experiments, whereas the dry weight values for leaves grown at the lowest light intensities are about equal for outdoor and indoor experiments.

The correlation between maximum rate of photosynthesis and fresh weight as a function of leaf thickness is presented in figures 97–99. The photosynthetic rates have, if necessary, been recalculated for 20 °C, using a Q₅-value of 1.30 which was determined experimentally. Fig. 97 shows a number of data from



FIG. 98. Impatiens parviflora. Maximum rate of photosynthesis per cm² leaf area at 20°C of several leaves of plants grown at different light intensities, plotted against leaf fresh weight. • leaves of plants grown outdoors (cf. fig. 97); $\bigcirc --$ leaves of plants grown in the phytotron in 1969-70; $\times \cdots$ leaves of plants grown in the phytotron in 1967-68.

outdoor experiments with Impatiens parviflora. There is a highly significant correlation (correlation coefficient = 0.95, p<0.001) between leaf thickness and maximum photosynthetic rate, with, as is already known from the previous chapters, only a small overlap between the data from different light intensities. In the indoor experiments, this correlation is less obvious, though again highly significant (corr. coeff. = 0.95, p<0.001, fig. 98). The relation between leaf thickness and maximum rate of photosynthesis was quantitatively variable in the seasons 1967/68 and 1969/70. In 1969/70, the differences in the maximum rate of photosynthesis were accompanied by only very small differences in leaf thickness. The situation is reflected by a very steep line in fig. 98 (corr. coeff. = 0.65, p<0.01). This indicates that photosynthesis is not related in a simple way to leaf thickness or rather to mesophyll thickness, i.e. to the amount of assimilating tissue.

In *Calendula* leaves (fig. 99), the correlation between fresh weight per unit area and maximum rate of photosynthesis again differs according to the growth conditions. Plants grown in the field seem to reach their maximum rates of photosynthesis with a thinner leaf than those grown in the phytotron.



FIG. 99. Calendula officinalis. Maximum rate of photosynthesis per cm² leaf area at 20 °C of several leaves of plants grown at different light intensities, plotted against leaf fresh weight. • leaves of plants grown outdoors (recalculated from 25 °C); $\times --$ leaves of plants grown in the phytotron (leaves on the main stems); $\bigcirc \cdots$ leaves of plants grown in the phytotron (leaves on lateral branches).

Both in the field and in the phytotron, the correlation between leaf thickness and maximum rate of photosynthesis is slightly less significant than with *Impatiens*; apart from that, the same trend is found. In relation to light intensity, plants grown in the phytotron show smaller differences in fresh weight. This results in a steeper slope for the correlation between fresh weight and maximum rate of photosynthesis. This is especially so with leaves from lateral branches. The leaves, photosynthesis of which has been measured without the use of agar slices (see p. 14) have not been taken into account, although here again the same trend is visible.

The maximum rates of photosynthesis for Impatiens and Calendula as plotted



FIG. 100. Impatients parviflora. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C of several leaves of plants grown at different light intensities, plotted against leaf dry weight (cf. fig. 98).

against fresh weight per unit leaf area in the figs. 98 and 99, have also been plotted against dry weight per unit leaf area in figs. 100 and 101, although dry weight was somewhat less strictly correlated than fresh weight with leaf thickness. Comparing figure 100 with figure 98, it can be seen that the relation between dry weight and maximum rate of photosynthesis in *Impatiens* is much more the same for indoor and outdoor experiments than in the case of fresh weight. This indicates that the dry weights of leaves of plants from the field and from the phytotron change in the same way in relation to light intensity. The water content, however, appeared much more dependent on light intensity in the field experiments.



FIG. 101. Calendula officinalis. Maximum rate of photosynthesis per cm^2 leaf area at 20 °C of several leaves of plants grown at different light intensities, plotted against leaf dry weight (cf. fig. 99).

5.2. LEAF SIZE

As already mentioned in Chapter 3, different light intensities during growth do not only lead to different photosynthetic capacities and leaf weight, but also influence the size of the leaf.* To investigate this, the size of most of the leaves which were used for measurements of photosynthesis was determined and averaged. As far as successive measurements on different leaves in the same position are concerned, the mean values after the respective leaves had reached their final size, are taken. Each value that contributes to an average in itself, generally is a mean of four leaves. In case a number of leaves in different positions along a stem were measured, all values are used in calculating the average.

* Total leaf area is also influenced by light intensity; data on this are available (WASSINK et al. in preparation) but are not included in this paper.



FIG. 102. Calendula officinalis. Average area of several leaves plotted against light intensity during growth. —— outdoor experiments: • leaves on the main stem, \blacksquare leaves on lateral branches; - - - indoor experiments: \bigcirc leaves on the main stem, \Box leaves on lateral branches.

In Calendula officinalis, in field experiments as well as in the phytotron, leaves grown in 75% light tend to have the largest leaf area (fig. 102). This might be explained by assuming that at lower light intensities too little energy is received to form larger leaves, whereas in the highest light intensity the general rule is followed that sun leaves are smaller in area, but thicker. It is also possible that the excessive flowering at the highest light intensity goes at the expense of leaf expansion. Since leaf expansion precedes flowering, this explanation does not hold for the leaves along the main stem. Fig. 103 shows the mean of all the leaves present along the main stem of plants grown at four different light intensities in the phytotron. Again, the differences are small, with the lowest light intensity staying behind in leaf number as well as in area per leaf.



FIG. 103. Calendula officinalis, 1970–71, phytotron. Leaf area of successive leaves along the main stem of plants grown at different light intensities (\bullet 67,000 ergs · cm⁻²· sec⁻¹; \bigtriangledown 47,000 ergs · cm⁻²· sec⁻¹; \Box 28,000 ergs · cm⁻²· sec⁻¹; \blacktriangle 16,000 ergs · cm⁻²· sec⁻¹).

Impatients parviflora shows a clear reaction to light intensity in the field experiments, as far as the area of single leaves is concerned. The area of a leaf decreases with increasing light intensity in such a way that a leaf in 12% light may be three times as large as a leaf in corresponding position on a plant grown in full daylight (fig. 104). Evidently, Impatiens has a greater capacity to react to shade conditions by forming larger leaves. These observations support those made by HUGHES (1959). In the phytotron, however, the reaction is even weaker than in Calendula. Lower light intensities lead to the formation of smaller leaves (figs. 104 and 105). Here, obviously, the plants receive too small amounts of light to react in the same way as outdoors (compare HUGHES and EVANS, 1962).

It is clear that the large areas of the shade leaves compensate for a great deal their lower photosynthetic capacities. This is demonstrated in figures 106 and 107, in which the data from figs. 63 and 65 are presented as maximum photosynthesis per whole leaf.


FIG. 104. Impatients parviflora. Average area of several leaves plotted against light intensity during growth. —— outdoor experiments: \bullet leaves on the main stem, \blacksquare leaves on lateral branches; ---- indoor experiments: \bigcirc leaves on the main stem, \Box leaves on lateral branches.

Their larger area implies that shade leaves are more efficient in rate limiting light intensities. This can be seen in the figures 108A and 108B, where the photosynthesis/light curves are shown from leaves grown in 100% and 12% daylight, presented as photosynthesis per unit leaf area and per leaf respectively. These leaves were measured third in the series, given in fig. 65. Light saturation is reached at much lower light intensities by the shade leaf. Considering the entire leaf as a physiological unit, this means about the same effectivity for both types of leaves under light saturation, but an advantage for the shade leaf in light intensities below saturation.



FIG. 105. Impatiens parviflora, 1970-71, phytotron. Leaf area of successive leaves along the main stem of plants grown at different light intensities (\bullet 67,000 ergs · cm⁻² · sec⁻¹; \bigtriangledown 47,000 ergs · cm⁻² · sec⁻¹; \square 28,000 ergs · cm⁻² · sec⁻¹; \blacktriangle 16,000 ergs · cm⁻² · sec⁻¹).



FIG. 106. *Impatiens parviflora*, 1969, outdoors, single plot. Data from fig. 63, recalculated as maximum rate of photosynthesis per leaf.



FIG. 107. Impatiens parviflora, 1970, outdoors, single plot. Data from fig. 65, recalculated as maximum rate of photosynthesis per leaf.



FIG. 108A. Impatients parviflora, 1970, outdoors, single plot. Photosynthetic rate per cm² leaf area of the 14th leaf on the main stem of plants grown at 100% (--) and 12% (--) daylight. Same data as presented in figs. 68 and 69 (3rd measurement).



FIG. 108B. As fig. 108A. Data presented as rate of photosynthesis per leaf.

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FIG. 109A. Calendula officinalis, 1969, outdoors, single plot. Photosynthetic rate per cm² leaf area of the 12th leaf on the main stem of plants grown at 100% (---) and 12% ($\bigcirc ---$) daylight. Data from leaves as measured second in fig. 37A, left part.



FIG. 109B. As fig. 109A. Data presented as rate of photosynthesis per leaf.

For comparison, similar data are given for a sun and a shade leaf of *Calendula* officinalis (figs. 109A and 109B). (Data from fig. 37A, second measurement.) Since *Calendula* is less capable of reacting to low light intensities by the formation of larger leaves, the photosynthetic capacities per leaf show roughly the same difference as those per unit leaf area. Also there is little difference in photosynthesis in the rate limiting light intensities.

5.3. NUMBERS OF STOMATA

Some investigations have shown that light intensity during growth influences stomatal resistance. BJÖRKMAN and HOLMGREN (1963) found an increase of number of stomata in *Solidago virgaurea*, with increasing light intensities during growth. WILSON and COOPER (1969d) found a small decrease in the number of stomata with increasing light intensities during growth of *Lolium perenne*, but the stomata were much larger. Although, owing to the high carbon dioxide concentrations used in the measurements, the stomatal resistance will not be of much influence on the photosynthetic rates, yet in a few cases a count was made of the number of stomata from both species, from the series grown at the highest and at the lowest light intensities. The results are presented in Tables 5 and 6.

Although all the counts were made on full-grown leaves, there often is a great variation in the number of stomata and epidermal cells per unit leaf area. Yet, it is clear that at high light intensities the number of stomata is higher, in *Calen*-

	adaxial side		abaxial side		
S	E	S/E	S	E	S/E
		High li	ght intensity		
82	504	0.16	91	452	0.20
71	364	0.20	110	560	0.20
103	528	0.20	73	368	0.20
85	464	0.18	105	560	0.19
ıv. 85	465	0.18	av. 95	485	0,20
		Low li	ght intensity		
75	624	0.12	79	568	0.14
52	528	0.10	105	588	0.18
51	448	0.11	117	732	0.16
50	356	0.14			
.v. 57	489	0,12	av. 100	629	0.16

TABLE 5. Calendula officinalis. Stomatal frequency of leaves of plants, grown in the phytotron at light intensities of 67,000 and 16,000 ergs \cdot cm⁻²· sec⁻¹, respectively. S = number of stomata per mm², E = number of epidermal cells per mm².

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TABLE 6. Impatients parviflora. Stomatal frequency of leaves of plants, grown in the phytotron at light intensities of 67,000 and 16,000 ergs \cdot cm⁻² · sec⁻¹ respectively, and in the field in 100 and 12% daylight.

·	muoou	n experiments	,	o outdoor experiments.			
adaxial side			1		abaxial side		
	S	Е	S/E	S	E	\$/E	
				High light intensity			
I :	4	288	0,01	I : 128	352	0.36	
I:	19	365	0.05	I : 140	358	0,39	
av.	11	326	0.03	av. 135	355	0.38	
0:	4	384	0.01	O: 170	448	0.38	
O :	15	388	0.04	O: 175	416	0.42	
0:	15	408	0.04				
0:	80	744	0.11				
av.	28	481	0.05	av. 172	432	0.40	
				Low light intensity			
I :	0	285	0.00	I: 60	272	0.22	
I :	4	315	0.01	I: 99	324	0.30	
av.	2	300	0.01	av. 80	298	0.26	
0:	25	356	0.07	O: 180	648	0.28	
0:	15	408	0.04	O: 145	664	0.22	
0:	0	296	0.00	O: 44	152	0.29	
0:	8	168	0.05	O: 52	248	0.21	
0:	12	184	0.06				

av. 105

428

0.25

S = number of stomata per mm², E = number of epidermal cells per mm², I = indoor experiments.

dula owing to a reduction of the number on the adaxial side of the leaf at low light intensities, in *Impatiens* owing to a reduction on both sides. As was already mentioned in Chapter 3, *Impatiens* has the bulk of its stomata on the abaxial side, whereas, at high light intensities, *Calendula* shows a more or less equal distribution over both sides; at low light intensities stomata are found in greater number on the abaxial side. The mentioned difference is in harmony with the more distinct differentiation in palisade and spongy parenchyma of *Impatiens* leaves. The ratio stomata/epidermal cells, which shows less variation than the numbers of stomata and epidermal cells themselves, is influenced in the same way by light intensity both in *Calendula* and *Impatiens*. Their larger numbers of stomata enable the sun leaves to profit even more from their higher capacities. It also ensures a better possibility for transpiration in situations of higher irradi-

av. 12

282

0.04

ation. The stomatal size is not significantly influenced by light intensity during growth: for both species, and under all light conditions, the pore length varies between 30 and 40 μ , with an average of about 35 μ .

5.4. CHLOROPHYLL CONTENTS

The chlorophyll content of leaves was determined in relation to light intensity during growth, and to leaf age. In general, a fully green leaf contains enough chlorophyll to absorb a sufficient amount of light to saturate photosynthesis at high light intensities and to secure an efficient use of incident quanta at low light intensities. In the latter case, however, a high chlorophyll content which is often found in shade leaves might be important to lower situated leaves for absorbing green and far-red light remaining after passage through other leaves (HEATH, 1969).

TABLE 7. Calendula officinalis. Chlorophyll contents of leaves of different ages of plants, grown in the phytotron at light intensities of 67,000 ergs $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ (100%), 47,000 ergs $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ (10%), 28,000 ergs $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ (40%) and 16,000 ergs $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ (20%).

A. Values per unit l	eaf area (extincti	on coefficients, ave	rage of several val	ues).
Light intensity	100%	ca. 70%	ca. 40%	ca. 20%
Young leaves	0.382	0.440	0.470	0.342
Adult leaves	0.510	0.538	0.544	0.377
Old leaves	0.529	0.542	0.478	0.387
B. Relative values p	er unit fresh weig	ght (average of seve	eral values).	
Light intensity	100%	70 %	40 %	20 %
Young leaves	26	36	40	29
Adult leaves	39	43	36	20
Old leaves	39	32	31	27

Table 7 shows that in *Calendula* leaves there is an increase in chlorophyll content after the young stage. Afterwards, it shows little change when the leaves grow older, until yellowing occurs. As mentioned above, shade leaves often have higher chlorophyll contents. This is most obvious in young leaves per unit leaf area as well as per unit fresh weight, except for the lowest light intensity. Here, probably, too little energy is received to ensure the normal level of chlorophyll development.

In *Impatiens* leaves (Table 8), there is a slight indication of chlorophyll content per unit leaf area to decrease with decreasing light intensity, which appears somewhat surprising for a shade plant. Chlorophyll content on a fresh weight basis, however, shows, in most cases, an increase with decreasing light intensity. Moreover, the chlorophyll content decreases with age more conspicuously than in *Calendula* leaves. The decrease with age is more pronounced when the light intensity is higher. Here, obviously, the same damaging effect of light is

TABLE 8. Impatients parviflora. Chlorophyll contents of leaves of different ages of plants, grown in the phytotron at light intensities of 67,000 ergs \cdot cm⁻²· sec⁻¹ (100%), 47,000 ergs \cdot cm⁻²· sec⁻¹ (70%), 28,000 ergs \cdot cm⁻²· sec⁻¹ (40%) and 16,000 ergs \cdot cm⁻²· sec⁻¹ (20%).

A. Values per unit l	eaf area (extincti	on coefficients, aver	rage of several value	ues).
Light intensity	100 %	ca. 70%	ca. 40%	ca. 20%
Young leaves	0,372	0,373	0,336	0,331
Adult leaves	0,565	0,504	0,521	0,463
Old leaves	0,300	0,319	0,390	0,354
B. Relative values p	er unit fresh wei	ght (average of seve	eral values).	
Light intensity	100 %	70%	40%	20 %
Young leaves	56	67	71	82
Adult leaves	73	81	103	102
Old leaves	48	52	65	61

found, though less strong, as described by BJÖRKMAN and HOLMGREN (1963) for ecotypes from a shade habitat of *Solidago virgaurea*. The maximum rate of photosynthesis of *Impatiens* as compared with *Calendula*, generally shows a less sharp decrease as the leaves grow older (figs. 38 and 67, 45 and 76). The discrepancy between the decrease in chlorophyll content and the decrease in maximum rate of photosynthesis with age is another indication that part of the chlorophyll is sufficient for photosynthesis at light saturation.

6. GENERAL DISCUSSION

6.1. BRIEF SURVEY OF RESULTS

In this paper it was investigated whether the maximum rate of photosynthesis (the rate at light saturation with sufficient supply of CO_2) is affected by growth of plants at different light intensities. The following results were described in the Chapters 3, 4 and 5, and may be briefly surveyed first.

The maximum rate of photosynthesis is reduced in both species investigated, viz. *Calendula officinalis* and *Impatiens parviflora*, and most clearly in *Impatiens*, with reduction of the light intensity at which the plants are grown (Chapter 3).

Moreover, there is a decrease of the maximum photosynthetic rate with increasing age. In *Impatiens parviflora*, this decrease is rather gradual and proportional to the maximum rate. In this plant species, flowering occurs at all light intensities and does not visibly influence the plant's appearance, nor the maximum photosynthetic rate.

In Calendula officinalis, flowering is highly dependent on light intensity and influences leaf size. In connection herewith it appears that the decrease of the maximum photosynthetic rate is sharper inasmuch as this rate was higher, so that old leaves of plants grown at different light intensities show very little difference in their maximum photosynthetic rates (Chapter 4).

In *Impatiens parviflora*, the reduced maximum photosynthesis by growth under low light intensities is compensated to a large extent by the formation of larger leaves. Furthermore, the maximum rate of photosynthesis in both species is related to anatomical properties, as leaf thickness and stomatal frequency (Chapter 5).

Subsequently, we will discuss these results in relation to those obtained by other investigators.

6.2. INFLUENCE OF LIGHT INTENSITY ON THE DEVELOPMENT OF THE PHOTOSYNTHETIC CAPACITY

Plants grown at different light intensities differ in various properties. For the distinction of sun and shade plants, various criteria can be used. Most obvious is the criterion of their natural habitats. This, however, does not give any physiological explanation. A physiological characterization of both types might be by way of a higher or lower compensation point. Since this may change rapidly upon transfer to another light intensity, this criterion is also doubtful (BURNSIDE and BÖHNING, 1957; McCREE and TROUGHTON, 1966). The latter authors suggest the adaptability of the light intensity at which the compensation point occurs as a criterion, sun plants being slower in adapting their respiration to low light intensities. This is based on the finding of a rapid change in respiration

rate in the shade plant *Trifolium repens* upon transfer to another light intensity. Gross photosynthesis, however, did not change in this case.

In the present experiments, most measurements were made over much longer periods. However, one week after transportation into a lower light intensity, *Calendula* shows only a small reduction of its respiration rate (fig. 43), whereas *Impatiens* has its respiration reduced to half its value after 12 days. In the following weeks, the reduction of respiration in the *Calendula* leaves goes on, so that in the long run also here a good adaptation is found.

It is questionable whether a classification in sun and shade plants can be based on one criterion. Probably, a number of factors, such as the plant's habitus, the distribution of stomata, mentioned in Chapter 3, the formation of larger leaves in the shade, mentioned in chapter 5, together with physiological properties, such as the lack of adaptability to high light intensities in shade plants (BJÖRKMAN and HOLMGREN, 1963) or the quick adaptability of the respiration rate, mentioned above, together determine whether a plant can be considered as a sun plant or a shade plant. On the other hand, one might ask whether it is not rather the absence of such specific adaptabilities, which decides whether a plant is a sun or a shade plant. One might make two distinctions, viz. between sun and shade plants on a genetic base and between sun and shade leaves, on physiological and anatomical bases.

Our experiments have led to the conclusion that, in general, there is a correlation – although quantitatively somewhat variable – between the maximum rate of photosynthesis and leaf thickness (see pp. 96 to 101 and figs. 97 to 101). Since maximum rate of photosynthesis and dark respiration rate of leaves of plants grown at different light intensities roughly differ in the same proportion (see e.g. figs. 36a and b), the same correlation seems to hold for the respiration rate and leaf thickness, respiration as well as the maximum rate of photosynthesis being determined by the amount of tissue. MEIDNER (1970) found a correlation between leaf thickness and compensation point at various temperatures in sun and shade leaves of Impatiens parviflora. This can also be explained by a correlation between leaf thickness and respiration rate, since in our experiments the slopes of the photosynthesis/light curves of sun and shade leaves run parallel. The very rapid change of the respiration rate in Trifolium repens (MCCREE and TROUGHTON, 1966) upon change of irradiance makes the assumption of a correlation between respiration rate and leaf thickness very doubtful. Also a certain variability in the correlation between leaf thickness and photosynthesis, as discussed in Chapter 5, does not suggest a simple way of dependence of photosynthesis and respiration on the amount of tissue.

PIETERS (1960) suggests that the lack of correlation between leaf thickness and photosynthesis in pot grown plants is due to the poor root environment. Another suggestion is that the differences in leaf thickness do not exclusively reflect direct effects of different light intensities, but may in part be due to other effects, linked with the irradiance. In fact, leaf shape can be considered as a compromise. With a fixed volume a leaf as a photosynthesizing unit can best be as large and consequently as thin as possible in order to intercept the maximum amount of

light. In order to maintain its water content, however, it can limit its transpiration losses to a minimum by taking a spherical form, thus having the most favourable surface/volume ratio and the largest heat capacity (WHITEHEAD, 1970). Shade leaves of *Impatiens parviflora* e.g., are found at one end of this range of compromises, and desert plants, such as succulents, at the other.

Impatiens parviflora, as a shade plant, is able to adapt itself to a range of light intensities from very low to at least the average summer intensities in our climate, resulting in either large, thin leaves or leaves two to three times as small and thick. Calendula officinalis is more rigid in its adaptability; it does not form larger leaves under shade conditions which, morover, have a less reduced thickness, and it can hardly survive an intensity of 12% daylight. However, as a sun plant, probably originating from the eastern mediterranean area, it might well be capable of adapting itself to much stronger light conditions by forming still thicker and possibly smaller leaves.

The supposed dependence of leaf thickness on conditions concurring with the intensity of radiation might also explain the differences in correlation of photosynthesis and leaf thickness between plants grown outdoors and those grown in the phytotron. Outdoors, the plants are exposed to sunlight with e.g. a large heat load, over a wide range of light intensities. Consequently, the amounts of heat irradiation received are very different which, by indirect effects, may well lead to a great variation in leaf thickness. This goes together with differences in dry weight and to a large extent with differences in water content. In contrast with this are the results of SHARMA and SEN (1971), who found a higher percentage of moisture and *thicker* leaves in *Solanum surattense* BURM. when grown in the shade.

In the phytotron, the plants receive artifical light with less heat radiation and over a smaller range of light intensities. Moreover, the plants are permanently well ventilated, so that indirect environmental effects will occur to a minor degree. (The air temperature at the highest and the lowest light intensity differed $0.8 \,^{\circ}$ C only). So, as was indeed found in our experiments, one would expect only small differences in leaf thickness. It is interesting to note that the differences in leaf thickness go along with differences in dry weight, whereas fresh weight in some cases is virtually the same.

WILSON and COOPER (1969c) found that in *Lolium perenne* plants, grown at different temperatures, mesophyll thickness generally increased with decreasing temperature. Leaf surface, however, often varied in an opposite way, so that leaf volume showed only small variation, not related with temperature. We might suppose that temperature affects growth of a leaf in the various directions in different ways, so that, at low temperatures, leaf expansion may proceed at a slower rate than the increase of leaf thickness. It is striking that, together with leaf thickness, the maximum rate of photosynthesis, according to WILSON and COOPER, l.c., increases as the plants are grown at lower temperatures. Both the increase of leaf thickness and the increase of the maximum rate of photosynthesis may be explained by an increase in leaf dry weight. It has also been observed elsewhere that reactions of size and shape of leaves to decrease in tempera-

ture are analogous to those to increase in light intensity (see e.g. BENSINK, 1971).

It must be concluded that the amount of assimilating tissue is better represented by leaf dry weight than by its fresh weight, this amount is, at least in part, responsible for the differences in the maximum photosynthetic rate. The fact that increased leaf thickness under influence of high light intensities is mainly due to the elongation of the photosynthetically important cells of the palisade parenchyma, seems to support this idea.

HOLMGREN (1968), and WILSON and COOPER (1969d) tried to explain the differences in photosynthetic capacity from differences in diffusion resistance with respect to carbon dioxide. This may be due to different mesophyll resistances, linked with mesophyll cell size, or to increase of stomatal conductivity, linked with stomatal pore width and/or increase of stomatal frequency upon stronger illumination. This explanation might also be valuable for Calendula and Impatiens, where stomatal frequency at the highest light intensity is about one and a half times higher than at the lowest light intensity. Indeed, this may enhance photosynthesis as well as transpiration in cases of strong irradiation, and thus be considered as an adaptation to high light intensities. However, this applies only in natural, rate limiting CO₂-concentrations. Since the differences in photosynthetic capacity were found under conditions of saturation with light as well as with carbon dioxide, their cause can neither be sought in CO_2 -concentration dependent diffusion processes nor in light dependent photochemical processes, but must be due to adaptation of biochemical (dark) processes. BJÖRKMAN (1968) demonstrated a higher activity of the enzyme catalyzing the fixation of CO₂, viz. ribulose-1,5-diphosphate carboxylase (carboxydismutase), in sungrown plants, compared with shade-grown plants. This might as well be so with the plants of the present experiments; the amount of carboxydismutase may well be proportional to leaf dry weight. Also the activities of other photosynthetic enzymes may show similar differences in sun and shade leaves as does carboxydismutase. The higher content of soluble protein in sun plants than in shade plants is an indication in this direction (BJÖRKMAN, 1969). There is a general trend that, on the basis of leaf weight, shade plants have higher chlorophyll contents than sun plants, (e.g. EVANS and HUGHES, 1961). Together with the lower enzyme activites of shade plants, this supports the idea that the fraction of available chemical energy used for the synthesis of compounds determining the efficiency of light absorption (i.e. chlorophyll) is larger among shade plants as compared with the fraction used for the synthesis of compounds determining the capacity of the dark-enzymatic steps (BJÖRKMAN, 1969). That this higher chlorophyll content per unit fresh weight was not found in the shade leaves of *Calendula officinalis* may be one more reason for the lack of capability to compensate for low light intensities.

Resuming it can be stated that the adaptability in several features determines whether a plant will be more or less able to adapt itself to the light intensity of an environment. Which forces determine the direction in which these physiological and morphological properties develop, still remains uncertain.

6.3. INFLUENCE OF AGE

For calculating photosynthesis of an entire plant, which is of considerable ecophysiological interest, it is necessary to know the contribution of the individual leaves. From the present investigation, the photosynthetic capacity of any leaf at any moment can be predicted and so the photosynthetic capacity of a whole plant can be calculated. This does not yet mean that the actual rate of photosynthesis of a whole plant can simply be predicted. Apart from carbon dioxide limitation under the culture conditions, many older leaves do not receive a saturating amount of light. This will even never be the case in the low light intensity experiments. Since, in ageing leaves, the photosynthetic rate in limiting light intensities also decreases, the contribution to total photosynthesis of the plant by the old leaves, will generally be very small. Moreover, in field experiments light intensities change, with daytime and cloudiness; the position of the sun, moreover, causes variations in shading of the lower leaves. An examination of these factors will be a subsequent step in this investigation.

Most of our results in relation to leaf age in the plants used in our experiments are well in agreement with previous observations. With the methods used in the present investigation, it is difficult to say whether the moment of complete leaf expansion coincides exactly with reaching the highest photosynthetic capacity. Completion of leaf expansion may come slightly before (JEWISS and WOLEDGE, 1967) or after (TICHÁ, 1968) the moment the highest photosynthetic capacity is reached. The gradual decrease of the maximum rate of photosynthesis with increasing age, as observed in *Calendula* and *Impatiens*, has as well been observed elsewhere. The effect of flowering on the senescence of leaves may explain the differences found between leaves from different light intensities, especially in *Calendula officinalis*. As already mentioned in the introductory chapter, flowering may influence the senescence of the leaves through withdrawal of nutrients from the leaves in favour of the flowers (MOLISCH, 1938).

Compared with the changes in photosynthetic rate with increasing age, the changes in respiration rate are small, just as the respiration rate itself is small as compared with the rate of photosynthesis. Therefore, in general, the changes of the net assimilation rate with age are only to a very small extent influenced by the respiration rate. This is also in agreement with earlier observations in other plants, (see e.g. WATSON et al., 1966).

No observations have been made on the change of the maximum rate of photosynthesis of full-grown leaves upon transfer to another light intensity. It is generally stated that this causes no change of the maximum photosynthetic rate, whereas the respiration rate may change very rapidly (MCCREE and TROUGHTON, 1966). Some of our measurements do not seem to support this idea. In fig. 70, e.g., there is a great difference in the photosynthetic rates of leaves moved into different light intensities when they were already developing. These leaves were, however, not yet completely full-grown. Since the photosynthetic apparatus obviously develops alongside with leaf expansion, it is very likely that its definite capacity, which is determined by the light intensity, can be

changed more easily by changing the light intensity at a younger stage, i.e. when the apparatus is in an earlier phase of its development.

In itself it is not an advantage for a shade grown plant, to have a lower photosynthetic capacity, though a high capacity should be useless. The energy available for building up the photosynthetic apparatus, at high light intensities is obviously used in the first place for developing a strong 'dark system', whereas, at low light intensities it is used for the formation of larger leaves, There may, therefore, perhaps be a relation between the rather high photosynthetic capacity of 'shade' leaves of *Calendula officinalis* and its lack of capability to form large leaves at low light intensities.

7. SUMMARY

In the present investigation, the rate of photosynthesis and some morphological aspects have been studied on leaves of *Calendula officinalis* and *Impatiens parviflora*, from plants grown at four different light intensities both in the field and in the phytotron.

The rate of photosynthesis was measured on leaf discs with the aid of the WARBURG technique, while relatively high CO_2 -concentrations were applied (Chapter 2.2.1).

Different light intensities during growth lead to different maximum rates of photosynthesis per unit leaf area at light saturation so that in leaves of plants grown at 12% daylight it is 40% of that in leaves of plants grown in full daylight for *Impatiens parviflora* and 60% for *Calendula officinalis*. In both species the respiration rates are also higher when the plants are grown at higher light intensities. Since no or only small differences could be observed in the slopes of the light limited parts of the photosynthesis-light curves, the plants grown at low light intensities are somewhat more effective at rate limiting light intensities, with their compensation points at lower light intensities. These differences are clearest in leaves from the main stems; leaves from lateral branches generally have lower maximum rates of photosynthesis and show smaller differences when grown at different light intensities (Chapter 3).

The highest maximum rates of photosynthesis are found at about the time the leaves are full-grown. Soon afterwards, a decrease is observed. This decrease occurs at all light intensities at which photosynthesis was measured, so that the slope of the light limited part of the photosynthesis-light curve becomes less steep. Since the respiration rate decreases also, the light-saturation point shifts slightly to the left. The decrease of the photosynthetic rate with age is most severe in leaves growing at high light intensities, so that old leaves grown at different light intensities show no or only small differences in their maximum photosynthetic rates. This goes especially for *Calendula officinalis*, where flowering, which does not or hardly occur at the lowest light intensity, probably speeds up senescence of the leaves at high light intensities. In Impatiens parviflora, where flowering is less influenced by light intensity, the decrease of the maximum rate of photosynthesis of plants grown at high light intensity, proceeds more slowly as compared with *Calendula officinalis*. Consequently, the differences in photosynthetic rate between leaves grown at different light intensities remain over a longer period. Leaves from lateral branches, which generally have lower maximum rates of photosynthesis, show a slower decrease of their photosynthetic capacity as well (Chapter 4).

Different light intensities during growth lead also to morphological differences in the leaves; as with other plants, the leaves grow thicker at higher light intensities. In *Impatiens parviflora* this is mainly due to elongation of the palisade cells, which, moreover, are more densely packed, whereas in *Calendula officinalis*

which has a less differentiated internal structure of the leaf, no clear qualitative differences could be detected.

There is a good correlation between dry weight and the maximum rate of photosynthesis when the leaves are at the optimum of their capacity. A similar correlation exists between fresh weight and maximum rate of photosynthesis of leaves from plants grown in the field. Differences in fresh weight of leaves of plants grown in the phytotron at different light intensities, are small and correspond to the differences in dry weight. These observations lead to the idea that both in the field and in the phytotron the maximum rate of photosynthesis is related with leaf dry weight and depends on the light intensity during growth. In the field, the water content of the leaf, as an adaptation to wilting, increases with increased heat absorption linked with increased sunlight intensities (Chapter 5.1).

The low maximum photosynthetic rates of *Impatiens parviflora* leaves at light saturation for plants grown at low light intensities in the field is compensated by an increase in leaf surface, so that the photosynthetic capacity of a leaf as a whole at light saturation is rather independent of the light intensity during growth.* Under conditions of light limitation, however, a shade leaf is capable of much more photosynthesis, both by its higher efficiency at those light intensities and by its larger area. *Calendula* leaves do not show significant differences in surface, when grown at different light intensities (Chapter 5.2).

Both species show an increase in numbers of stomata per cell of about 50% in leaves of plants grown at the highest light intensity as compared with leaves of plants grown at the lowest light intensity (Chapter 5.3).

Chlorophyll contents of leaves in *Calendula* per unit area as well as per unit fresh weight showed small differences when grown at different light intensities; at the lowest light intensity, the chlorophyll content was much lower, reflecting the general poor condition of the plants. In *Impatiens*, a decrease in chlorophyll content per unit fresh weight was found if the plants were grown at light intensities above the lowest one (which was 1.6×10^4 ergs \cdot cm⁻² · sec⁻¹) (Chapter 5.4).

From the present investigation it was concluded that the shade plant *Impa*tiens parviflora has a rather good adaptability for high light intensities, whereas the sun plant *Calendula officinalis* is much less able to adapt itself to conditions of low light intensity. The adaptability is based on morphological rather than on physiological characteristics.

* This does not go for the experiments in the phytotron, where lower light intensities were used.

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De fotosynthesesnelheid werd gemeten aan bladschijfjes met behulp van de WARBURG-methode, waarbij betrekkelijk hoge koolzuurconcentraties (1,26%) werden gebruikt (Hoofdstuk 2.2.1).

Verschillende lichtintensiteiten tijdens de groei hebben verschillende maximum fotosynthesesnelheden bij lichtverzadiging ten gevolge en wel zodanig dat bladen van *Impatiens* planten, die bij 12% licht gegroeid zijn, een fotosynthesesnelheid per eenheid van bladoppervlak hebben, die ca. 40% bedraagt van die van bladen, die bij 100% licht zijn opgekweekt, terwijl de fotosynthesesnelheid van *Calendula* slechts tot 60% gereduceerd wordt, wanneer deze in 12% licht wordt gekweekt. Bij beide soorten wordt ook de ademhaling sterker naarmate de planten in een hogere lichtintensiteit groeien. Er werden geen of slechts zeer geringe verschillen geconstateerd in de hellingen van het lichtgelimiteerde deel van de fotosynthese-lichtkrommes, zodat de planten, die bij lage lichtintensiteiten gegroeid zijn, iets effectiever zijn bij limiterende lichtsterktes, waarbij hun compensatiepunten bij een lagere intensiteit liggen. Deze verschillen zijn het duidelijkst bij bladen van de hoofdstengels; bladen van zijstengels hebben in 't algemeen lagere fotosynthesemaxima en laten hierin ook kleinere verschillen zien, wanneer ze onder verschillende lichtintensiteiten gegroeid zijn (Hoofdstuk 3).

De bladen bereiken hun hoogste fotosynthesesnelheden omstreeks het tijdstip, waarop ze volgroeid zijn. Spoedig daarna treedt er een daling op van de maximum fotosynthese. Deze daling vindt plaats bij alle lichtintensiteiten en geldt ook voor de ademhaling, zodat de helling van het lichtgelimiteerde gedeelte van de fotosynthese-lichtkromme allengs minder steil wordt, waarbij het compensatiepunt en de verzadigingsintensiteit iets lager komen te liggen. De afname van de fotosynthesesnelheid is het sterkst bij bladen, die bij de hoogste lichtintensiteiten groeien, zodat er uiteindelijk slechts weinig of in 't geheel geen verschillen meer bestaan tussen de fotosynthesesnelheden van oude bladen, die bij verschillende lichtintensiteiten zijn gegroeid. Dit geldt vooral voor Calendula officinalis, waar het bloeiproces, dat in de laagste lichtintensiteit niet of nauwelijks optreedt, waarschijnlijk de veroudering van bladen in de hoogste lichtintensiteit bespoedigt. Bij Impatiens parviflora, waar de bloei veel minder door de lichtintensiteit wordt beïnvloed, daalt de maximum fotosynthesesnelheid in de hoogste lichtintensiteit jets langzamer. Daardoor blijven de verschillen in fotosynthesesnelheid tussen bladen, die bij verschillende lichtintensiteiten zijn gegroeid, ook langer bestaan. Bladen van zijstengels, die in 't algemeen een lagere maximum fotosynthesesnelheid hebben, vertonen ook een langzamere afname van die maximum snelheid (Hoofdstuk 4).

Verschillende lichtintensiteiten tijdens de groei leiden ook tot morphologische verschillen in de bladen: Zoals ook bij andere planten is gevonden, worden de bladen dikker bij hogere lichtintensiteiten. Bij Impatiens parviflora vloeit dit voornamelijk voort uit een verlenging van de palissadecellen, die bovendien dichter opeengepakt zitten, terwijl bij Calendula officinalis, waar de inwendige structuur veel minder duidelijk is, geen kwalitatieve verschillen konden worden waargenomen. Er bestaat een duidelijke correlatie tussen drooggewicht en maximum fotosynthesesnelheid, wanneer de bladen hun maximale vermogen bezitten. Een dergelijke correlatie werd ook gevonden tussen versgewicht en maximum fotosynthesesnelheid bij bladen van planten, die buiten waren gekweekt. Wanneer planten in het phytotron worden opgekweekt bij verschillende lichtintensiteiten, ontstaan er slechts zeer geringe verschillen in het versgewicht per eenheid bladoppervlakte, die voornamelijk veroorzaakt worden door de verschillen in drooggewicht. Dit heeft tot de veronderstelling geleid dat de maximum fotosynthesesnelheid is gecorreleerd met het drooggewicht van het blad, die beide afhankelijk zijn van de lichtintensiteit tijdens de groei, terwijl het watergehalte van het blad, als een adaptatie tegen te sterke uitdroging door verdamping, toeneemt met toeneming van de warmtestraling, die mede door de zon wordt uitgezonden (Hoofdstuk 5.1),

De lage fotosynthesesnelheid per eenheid van oppervlak bij lichtverzadiging van *Impatiens* bladen, die bij lage lichtintensiteit zijn gegroeid, wordt gecompenseerd door een toename van het bladoppervlak, zodat de fotosynthesecapaciteit van een blad als geheel bij lichtverzadiging betrekkelijk onafhankelijk is van de lichtintensiteit tijdens de groei. (Dit geldt niet voor de experimenten in het phytotron, waar met lagere lichtintensiteiten werd gewerkt.) Bij lage, beperkende lichtintensiteiten is een schaduwblad als geheel echter tot een veel sterkere assimilatie in staat dan een zonneblad, enerzijds door zijn grotere effectiviteit per eenheid van oppervlakte, anderzijds door zijn grotere oppervlakte. Bladen van *Calendula* vertonen geen duidelijke verschillen in bladoppervlak, wanneer ze bij verschillende lichtintensiteiten worden gekweekt (Hoofdstuk 5.2).

Bij beide soorten is het aantal huidmondjes t.o.v. het aantal bladcellen ongeveer 50% hoger in bladen van planten gegroeid bij de hoogste lichtintensiteit in vergelijking met bladen van planten gegroeid bij de laagste lichtintensiteit (Hoofdstuk 5.3).

Verschillende lichtintensiteiten leiden bij bladen van *Calendula* slechts tot geringe verschillen in chlorophylgehalte, behalve in de laagste lichtintensiteit, waar de vaak slechte algehele conditie van de plant zich ook weerspiegelt in een lager chlorophylgehalte. Bij *Impatiens* werd een daling van het chlorophylgehalte per eenheid van versgewicht gevonden, indien de planten werden gekweekt in lichtintensiteiten, hoger dan de laagst gekozene (d.i. $1,6 \times 10^4$ ergs \cdot cm⁻²· sec⁻¹) (Hoofdstuk 5.4).

Dit onderzoek heeft tot de conclusie geleid dat de schaduwplant Impatiens parviflora zich redelijk goed kan aanpassen aan hoge lichtintensiteiten, terwijl de lichtminnende plant Calendula officinalis een veel minder sterk aanpassings-

vermogen heeft aan lage intensiteiten. Het aanpassingsvermogen vloeit eerder voort uit een verandering van morfologische eigenschappen dan van fysiologische.

- BARTOŠ, J., S. KUBÍN and I. ŠETLIK (1960). Dry weight increase of leaf disks as a measure of photosynthesis. Biol. Plant. 2, 201–215.
- BATALIN, A. (1871). Ueber die Wirkung des Lichtes auf die Entwicklung der Blätter. Botan. Ztg. 29, (40), 669–686.
- BENSINK, J. (1971). On morphogenesis of lettuce leaves in relation to light and temperature. Meded. Landbouwhogeschool Wageningen 71-15, 1-93.
- BJÖRKMAN, O. (1968). Carboxydismutase activity in shade-adapted and sun-adapted species of higher plants. Physiol. Plant. 21, 1-10.
- BJÖRKMAN, O. (1969). Characteristics of the photosynthetic apparatus as revealed by laboratory measurements. Proc. IBP/PP Technical Meeting, Třeboň, 14–21 september 1969, 267–281.
- BJÖRKMAN, O., and P. HOLMGREN (1963). Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. Physiol. Plant. 16, 889-914.
- BJÖRKMAN, O., and P. HOLMGREN (1966). Photosynthetic adaptation to light intensity in plants native to shaded and exposed habitats. Physiol. Plant. 19, 854–859.
- BLACKMAN, G. E., and A. J. RUTTER (1946). Physiological and ecological studies in the analysis of plant environment I. Ann. Bot. N.S. 10, 361–390.
- BLACKMAN, G. E., and G. L. WILSON (1951a). Physiological and ecological studies in the analysis of plant environment VI. Ann. Bot. N.S. 15, 63-94.
- BLACKMAN, G. E., and G. L. WILSON (1951b). Physiological and ecological studies in the analysis of plant environment VII. Ann. Bot. N.S. 15, 373-408.
- BONNET, C. I. (1754). Recherches sur l'usage des feuilles dans les plantes et sur quelques autres sujets relatifs à l'histoire de la végétation. Gottingue et Leide.
- BOSIAN, G. (1968). Die Bedeutung der Stomata, der Luftfeuchte und der Temperatur für den CO₂- und Wasserdampfgaswechsel der Pflanzen. Photosynthetica, 2, 105–125.
- BOURDEAU, P. F., and M. L. LAVERICK (1958). Tolerance and photosynthetic adaptability to light intensity in white pine, red pine, hemlock and *Ailanthus* seedlings. Forest Sc. 4, 196-207.
- BOYSEN-JENSEN, P., and D. MÜLLER (1929). Die maximale Ausbeute und der tägliche Verlauf der Kohlensäureassimilation. Jahrb. wiss. Botan. 70, 493-502.
- BRIGGS, G. E. (1920). Experimental researches on vegetable assimilation and respiration. XIII. Proc. Roy. Soc. B. 91, 249–268.
- BURNSIDE, C. A., and R. H. BÖHNING (1957). The effect of prolonged shading in the light saturation curves of apparent photosynthesis in sun plants. Plant Physiol. 32, 61–63.
- CAMERON, R. J. (1970). Light intensity and the growth of *Eucalyptus* seedlings I. Ontogenic variation in *E. fastigata*. Australian J. Bot. 18, 29–43.
- DASTUR, R. H. (1924). Water content, a factor in photosynthesis. Ann. Bot. 38, 779-788.
- DASTUR, R. H. (1925). The relation between water content and photosynthesis. Ann. Bot. 39, 769-786.
- DECKER, J. P. (1955). The uncommon denominator in photosynthesis as related to tolerance. Forest Sc. 1, 88-89.
- DICKMANN, D. I. (1971). Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* BARTR.) Bot. Gaz. 132, 253–259.
- DUFOUR, L. (1887). Influence de la lumière sur la forme et la structure des feuilles. Paris.
- EAGLES, C. F., and K. J. TREHARNE (1969). Photosynthetic activity of *Dactylis glomerata* L. in different light regimes. Photosynthetica 3, 29–38.
- EVANS, G. C., and A. P. HUGHES. (1961). Plant growth and the aerial environment I. Effect of artificial shading on *Impatiens parviflora*. The New Phytol. 60, 150–180.
- GAASTRA, P. (1959). Photosynthesis as influenced by light, CO₂, temperature and stomatal diffusion resistance. Meded. Landbouwhogeschool, Wageningen 59 (13), 1-68.

- GABRIELSEN, E. K. (1948). Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. Physiol. Plant. 1, 5–37.
- HARDER, R. (1930). Über die Assimilation der Kohlensäure bei konstanten Aussenbedingungen. Planta 11, 263-293.
- HARDER, R. (1933). Ueber die Assimilation der Kohlensäure bei konstanten Aussenbedingungen II. Das Verhalten von Sonnen- und Schattenpflanzen. Planta 20, 699-733.
- HEATH, O. V. S. (1969). The physiological aspects of photosynthesis. Heinemann Educational Books Ltd., London.
- HOFFMANN, P. (1962). Untersuchungen über Photosynthese und Atmung von Laubblättern verschiedenen Alters. Flora 152, 622-654.
- HOLMGREN, P. (1968). Leaf factors affecting light-saturated photosynthesis in ecotypes of Solidago virgaurea from exposed and shaded habitats. Physiol. Plant. 21, 676-698.
- HUGHES, A. P. (1959). Effects of the environment on leaf development in *Impatiens parviflora* DC, J. Linn. Soc. Bot. 564, 161-165.
- HUGHES, A. P., and G. C. EVANS (1962). Plant growth and the aerial environment II. Effects of light intensity on *Impatiens parviflora*. The New Phytol. 61, 154-174.
- IDLE, D. B. (1968). The measurement of apparent surface temperature. In: The measurement of environmental factors in terrestrial ecology, R. M. WADSWORTH ed., Blackwell Sc. Publ., Oxford and Edinburgh, pp. 47–57.
- JEWISS, O. R., and J. WOLEDGE (1967). The effect of age on the rate of apparent photosynthesis in leaves of tall fescue (*Festuca arundinacea* SCHREB.) Ann. Bot. N.S. 31, 661–671.
- KAMEL, M. S. (1959). A physiological study of shading and density effects on the growth and the efficiency of solar energy conversion in some field crops. Meded. Landbouwhogeschool Wageningen 59 (5), 1-101.
- KRUEGER, K. W., and R. H. RUTH (1969). Comparative photosynthesis of red alder, Douglasfir, Sitka spruce, and western hemlock seedlings. Can. J. Bot. 47, 519–527.
- KULAEVA, O. N. (1962). The effects of roots on leaf metabolism in relation to the action of kinetin on leaves. Sov. Plant Physiol. 9, 182–189.
- LEOPOLD, A. C. (1961). Senescence in plant development. Science 134, 1727-1732.
- LEOPOLD, A. C. (1964). Plant growth and development. Mc Graw Hill, New York.
- LOGAN, K. T. (1970). Adaptations of the photosynthetic apparatus of sun- and shade-grown yellow birch (*Betula alleghaniensis* BRITT). Can. J. Bot. 48, 1681–1688.
- MCCREE, K. J., and J. H. TROUGHTON (1966). Prediction of growth rate at different light levels from measured photosynthesis and respiration rates. Plant Physiol. 41, 559–566.
- MEIDNER, H. (1970). Light compensation points and photorespiration. Nature 228, 1349.
- MOKRONOSOV, A. T., and N. A. IVANOVA (1971). Characteristic features of the photosynthetic process following partial defoliation of plants. Sov. Plant Physiol. 18, 563-570.
- MOLISCH, H. (1938). The longevity of plants. Science Press, Lancaster.
- MOTHES, K. (1960). Ueber das Altern der Blätter und die Möglichkeit ihrer Wiederverjüngung. Naturwiss. 47, 337–351.
- Müller, J. (1958). Ueber die Verwendung von Magnos- Sauerstoffschreibern für Gaswechselregistrierungen in der Biologie. Ber. deutsch. bot. Ges. 71, 205–223.
- MURATA, Y. (1961). Studies on the photosynthesis of the rice plant and its culture significance. Bull. Nat. Inst. Agr. Sci. Series D, 9, 1-169, Nishigahara, Tokyo (English summary).
- NÁTR, L. (1969). The use of leaf tissue samples for studying the characteristics of the photosynthetic apparatus. Proc. IBP/PP Techn. Meet. Třeboň, 331-337.
- NATR, L., and M. M. LUDLOW (1970). Influence of glucose absorption and photosynthate accumulation on gas exchange of barley leaf segments. Photosynthetica 4, 288–294.
- OSBORNE, D. J. (1962). Effect of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. Plant Physiol. 37, 595-602.
- OSIPOVA, O. P., K. H. KHEIN and A. A. NICHIPOROVICH (1971). Activity of the photosynthetic apparatus of plants grown under different light intensities. Sov. Plant Physiol. 18, 257–263.
- PIETERS, G. A. (1960). On the relation between the maximum rate of photosynthesis and the thickness of the mesopyhll in sun- and shade leaves of *Acer pseudoplatanus* L. Meded. Landbouwhogeschool Wageningen 60 (17), 1-6.

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- PIETERS, G. A. (1962). Some aspects of the problem of sun and shade leaves. Proc. XVIth International Horticultural Congress, Brussels 393-399.
- PIETERS, G. A., and K. SCHURER (1973). Leaf temperature measurement. I. Thermocouples. Acta Bot. Neerl., in prep.
- PRATT, R. (1940). Influence of the size of the inoculum in the growth of *Chlorella vulgaris* in freshly prepared culture medium. Am. J. Bot. 27, 52-56.
- PRATT, R. (1942). Studies on Chlorella vulgaris V Am. J. Bot. 29, 142-148.
- PRATT, R. (1943). Studies on Chlorella vulgaris VI Am. J. Bot. 30, 32-33.
- PRATT, R. (1943). Studies on Chlorella vulgaris VII Am. J. Bot. 30, 404-408.
- RANDALL, W. E. (1953). Water relations and chlorophyll content of forest herbs in southern Wisconsin. Ecology 34, 544-553.
- RICHARDSON, S. D. (1956). Photosynthesis and root growth in tree seedlings. Landbouwk. Tijdschr. 68 (9), 775-782.
- RICHARDSON, S. D. (1957). The effect of leaf age on the rate of photosynthesis in detached leaves of tree seedlings. Acta Bot. Neerl. 6, 445-457.
- ŠETLÍK, I., J. BARTOŠ and Š. KUBÍN (1960). Photosynthesis in leaf disks as a measure of photosynthetic capacity in crop plants. Biol. Plant. 2, 292–307.
- SHARMA, K. D., and D. N. SEN (1971). Sun and shade tolerance in the ecophysiology of Solanum surratense BURM. Zeitschr. Pflanzenphys. 64, 263-266.
- SINGH, B. N., and K. N. LAL (1935). Investigation of the effect of age on assimilation of leaves. Ann. Bot. 44, 291-307.
- STAHL, E. (1883). Ueber den Einfluss des sonnigen und schattigen Standortes auf die Ausbildung der Laubblätter. Jen. Zeitschr. f. Naturwiss. 16, 162-200.
- TICHÁ, I. (1968). Ontogenetische Veränderungen der Photosyntheseintensität bei Blättern verschiedener Insertionshöhe von *Plectranthus fructicosus* L'HERIT. – Pflanzen aus zwei künstlichen Klimas. Photosynthetica 2, 167–171.
- UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER (1964). Manometric Techniques (4th edition) Burgers Publishing Company, Minneapolis.
- VERHOEKS, J. L. (1965). Photosynthesis and carbohydrate metabolism of leafroll diseased potato plants. Thesis Agricultural University, Wageningen. LEB-Fonds publicatie. H. Veenman en Zn., Wageningen.
- WARBURG, O. (1928). Ueber die katalytischen Wirkungen der lebendigen Substanz. Julius Springer, Berlin.
- WASSINK, E. C. (1946). Experiments on photosynthesis of horticultural plants with the aid of the WARBURG method. Enzymologia 12, 33-35.
- WASSINK, E. C. (1960). The effect of light intensity on growth and development of *Gladiolus*. In: Progress in Photobiology, B. CHR. CHRISTENSEN and B. BUCHMANN (Eds.). Proc. 3rd Int. Congr. Photobiology, Copenhagen, pp. 371–378.
- WASSINK, E. C. (1968). Effects of light intensity and plant density on dry matter production and morphogenesis of higher plants. 5th Intern. Congr. on Photobiology, Hanover NH, USA, August 26–31, p. 12.
- WASSINK, E. C. (1969). Effects of light intensity on dry matter production and morphogenesis of *Iris* 'Wedgwood', as compared with *Gladiolus* and Tulip. Meded. Landbouwhogeschool Wageningen 69-20, 1-17.
- WASSINK, E. C., and E. KATZ (1939). The initial changes of chlorophyll fluorescence in Chlorella. Enzymologia 6, 145–172.
- WASSINK, E. C., S. D. RICHARDSON and G. A. PIETERS (1956). Photosynthetic adaptation to light intensity in leaves of *Acer pseudoplatanus*. Acta Bot. Neerl. 5, 247–256.
- WATSON, D. J., J. H. WILSON, M. A. FORD and S. A. W. FRENCH (1966). Changes with age in the photosynthetic and respiratory components of the net assimilation rates of sugar beet and wheat. New Phytol. 65, 500–508.
- WEIS, FR. (1903). Sur le rapport entre l'intensité lumineuse et l'énergie assimilatrice chez des plantes appartenant à des types biologiques différents. Compt. rend. hebd. des séances acad. sc. 137, 801–804.

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- WHITEHEAD, F. H. (1970). The effect of adaptation to low light intensity on reproductive capacity. Unesco Symposium on plant response to climatic factors. Uppsala. SC/Conf. 44/51.
- WILLSTÄTTER, R., and A. STOLL (1918). Untersuchungen über die Assimilation der Kohlensäure. Springer, Berlin.
- WILSON D., and J. P. COOPER (1969a). Effect of light intensity and CO_2 on apparent photosynthesis and its relationship with leaf anatomy in genotypes of *Lolium perenne L*. New Phytol. 68, 627-644.
- WILSON, D., and J. P. COOPER (1969b). Apparent photosynthesis and leaf characters in relation to leaf position and age, among contrasting *Lolium* genotypes. New Phytol. 68, 645-655.
- WILSON, D., and J. P. COOPER (1969c). Effect of temperature during growth on leaf anatomy and subsequent light-saturated photosynthesis among contrasting *Lolium* genotypes. New Phytol. 68, 1115-1123.
- WILSON, D., and J. P. COOPER (1969d). Effect of light intensity during growth on leaf anatomy and subsequent light-saturated photosynthesis among contrasting *Lolium* genotypes. New Phytol. 68, 1125-1135.

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