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G. A. PIETERS

THE GROWTH OF SUN AND SHADE LEAVES OF *POPULUS EURAMERICANA* 'ROBUSTA' IN RELATION TO AGE, LIGHT INTENSITY AND TEMPERATURE

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF. DR. H. A. LENIGER,
HOOGLERAAR IN DE TECHNOLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP DINSDAG 11 JUNI 1974
DES NAMIDDAGS TE VIER UUR IN DE AULA VAN DE
LANDBOUWHOGESCHOOL TE WAGENINGEN.

H. VEENMAN & ZONEN B.V. - WAGENINGEN - 1974

THE GROWTH OF SUN AND SHADE LEAVES OF *POPULUS EURAMERICANA* 'ROBUSTA' IN RELATION TO AGE, LIGHT INTENSITY AND TEMPERATURE

Dit proefschrift met stellingen van

GEORGE ALBERT PIETERS

landbouwkundig *ingenieur, geboren te 's-Gravenhage op* 23 october 1928, is goedgekeurd door de promotoren Dr. E. C. WASSINK, hoogleraar in het Plantenfysiologisch Onderzoek en de Fysiologie der Planten en Dr. Ir. C. T. DE WIT, buitengewoon hoogleraar in de Theoretische Teeltkunde.

De Rector Magnificus van de Landbouwhogeschool,
H. A. LENIGER

Wageningen, 26 April 1974

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VOORWOORD

Gaarne begin ik dit proefschrift met een woord van dank aan allen die hebben bijgedragen aan mijn wetenschappelijke vorming. Daartoe reken ik ook mijn richtingshoogleraren in de bosbouw, die mij tijdens de vele excursies in zo'n nauw contact brachten met de ongerepte en de georganiseerde natuur.

Tijdens mijn eerste schreden op het wetenschappelijke pad ben ik begeleid door U, hooggeleerde PRAKKEN en hooggeleerde WASSINK. Reeds toen leerde ik hoe moeilijk het is en hoeveel arbeid het kost om een klein steentje bij te dragen aan de kennis en het inzicht.

Het bij U, hooggeleerde WASSINK, hooggeachte promotor, door mij als student aangevangen werk mondde uit in een langjarig onderzoek, met vele ups en downs, dat tenslotte toch enigszins kon worden afgerond in de zin, die daaraan ten grondslag lag. Ik ben mij diep bewust van het voorrecht dit in zo'n grote vrijheid en zo langdurig te hebben mogen doen onder Uw voortdurende kritische begeleiding. Ook tijdens de voorbereiding van het manuscript, hooggeachte promotor, heb ik zeer veel geleerd van Uw kritische opmerkingen en stilistische gaven.

Hooggeleerde DE WIT, hooggeachte 2e promotor, het contact met U in het laatste stadium van de voorbereiding van het manuscript, kan het best worden gekarakteriseerd als kort en intensief. Ik ben blij dat dit leerzame contact met U zo duidelijk in de vormgeving van het proefschrift tot uitdrukking komt.

Gelukkig staat men bij de bewerking van een wetenschappelijk probleem niet alleen en kan men terugvallen op de kennis en het inzicht van vele collega's. Dr. J. BENSINK, met dankbaarheid zal ik de vele discussies met U memoreren. Ook U, Dr. J. C. WESSELIUS, Dr. Ir. P. J. C. KUIPER, Dr. W. LINDEMAN en Ir. F. KUIPER ben ik zeer veel dank verschuldigd voor hulp en steun in zeer vele vormen. Dr. R. E. KENDRICK ben ik zeer erkentelijk voor taalkundige verbeteringen in het manuscript.

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Tenslotte, Ans en kinderen, jullie hebben door je toewijding en geduld veel bijgedragen aan dit proefschrift.

Aan Ans

CONTENTS

1. INTRODUCTION

1.1. STATEMENT OF THE PROBLEM

This study was initiated as an attempt to elucidate the differences in the photosynthetic rates of sun and shade leaves in relation to their environment. The incentive to this research was an experiment, published in 1956 (WASSINK, RICHARDSON and PIETERS), which demonstrated that sun and shade leaves of sycamore could be produced by cultivation of young plants at different light intensities. This was confirmed by data of BÖHNING and BURNSIDE (1956) and BURNSIDE and BÖHNING (1957) on the photosynthetic characteristics of leaves of a number of ombrophilic and heliophilic plants, grown at high and low light intensities.

The problem of sun and shade leaves has been treated phenomenologically by a large number of investigators, and a huge amount of literature on the subject exists. Direct experimental approaches to find a basis to explain physiologically the development of these differences between sun and shade leaves are few (cf. BJÖRKMAN and HOLMGREN, 1963). It therefore appeared justified to make a new attempt and to continue our earlier work.

The main problems, encountered during this research, were in the measurement of photosynthesis in CO_2-enriched air, and in the cultivation of the plants under conditions, controlled as fully as possible. Initially, apparatus to measure CO_2 concentrations continuously with appropriate accuracy was not available. The katharometer was, therefore, developed into a very accurate piece of laboratory apparatus for this purpose (PIETERS, 1971). The large variability found in the maximum rates of photosynthesis of the leaves compelled us to spend a considerable time in finding its causes. It soon became clear that there was a parallelism between this variability and that of other leaf characteristics (PIETERS, 1962). The mentioned variability may be ascribed partly to the cultural conditions of the plants and partly to difficulties encountered with the assimilation chamber climate during the measurement of photosynthesis. When improvement of the cultural conditions was achieved, larger plants with larger leaves and higher photosynthetic capacities and a higher chlorophyll content were produced. The irradiation intensities, needed to saturate the photosynthetic apparatus, increased steadily and enlarged the problems of cooling the leaves in the assimilation chamber. This led to a study of the errors involved in the measurement of leaf temperature with thermocouples in the assimilation chambers (PIETERS, 1972a; PIETERS and SCHURER, 1973; SCHURER and PIETERS, in preparation).

The main problem, however, has been that the cultivation of a plant is an art, which is basic for the progress of developmental physiology (cf. HEATH, 1969; WAREING and PHILLIPS, 1970). One of the main elements is the adequate control of the root environment. Stresses in the root environment cause variability in

the characteristics of the leaves. It is not improbable that this variability can be used as an indication of the degree of deficiency of the root environment and thus also as a guide to the improvement of this environment, necessary for the cultivation of what has been called in this paper 'optimal plants', viz. plants, the growth of which is not limited by supplies from the roots. Only when the growth of a plant is restricted by its own genetically determined physiological limitations, it reveals the characteristics of its own growth pattern. Any limitation imposed by root environment, induces deviations of this primary developmental pattern so that the mature plant characteristics are greatly deteriorated.

In this paper, mainly the data on growth of leaves, petioles and internodes of 'optimal' plants grown at different light intensities and temperatures are presented. Results of a study of the development of the photosynthetic apparatus of the leaves will be presented in Ch. 7. The data obtained suggest that leaf thickness and photosynthetic capacity are linearly related, as if the cells making up the thickness of the leaf, possess similar photosynthetic properties.

1.2. Discussion of some related literature

This thesis deals mainly with the growth and photosynthesis of leaves, but also includes data on the growth of petioles and internodes. To produce here a review on the growth of leaves would not be very useful, because this has been done several times (Milthorpe, 1956; Humphries and Wheeler, 1963; Cutter, 1965 and Allsopp, 1965). The review of heteroblastic development in Cormophytes by Allsopp is of special interest, because his reasoning is similar to that developed here. This discussion may, therefore, be limited to some main properties of sun and shade leaves and the controversies in the literature about them.

Differences between sun and shade leaves are found in their morphology, anatomy and physiology, and are due to the interaction between the plant and its environment. The way in which a plant interacts with its environment is an outcome of its genetic make-up (Nelson and Postlethwait, 1954; Cutter, 1965; Wareing and Phillips, 1970). According to Björkman and Holmgren (1963), the possibility of genetic adaptation to the habitat also exists.

Sun leaves are commonly thicker than shade leaves. This is due to greater elongation of the palisade parenchyma or by the formation of more layers of cells in the mesophyll of the leaf (Dufour, 1887; Pieters, 1962; Björkman and Holmgren, 1963; Zimmermann, 1971). Therefore, sun leaves possess higher specific leaf weights (Milthorpe, 1956; Wassink, 1969; Bensink, 1971; Callagher and Lof, pers. comm.) or lower leaf area / leaf weight ratios. It is generally accepted that light is one of the main factors determining the relative development of the mesophyll (Esau, 1962), although competition may also be involved (Milthorpe, 1956). Xeromorphic leaves also have a more strongly developed palisade tissue than mesomorphic leaves (Watson, 1942; Shields, 1950; Esau, 1962). The way in which water shortage is connected with these

effects is not at all clear (WANGERMAN, 1961). BJÖRKMAN and HOLMGREN (1963) report that the rate of light saturated photosynthesis increases with increasing xeromorphy.

Leaf area is also influenced by light intensity, but the reports in literature are not unequivocal. HUMPHRIES and WHEELER (1963) mention that leaf area may increase with decreasing illumination and may not decrease until a level of 12% of daylight has been reached (BLACKMAN and WILSON, 1951), but MILTHORPE (1943) found that the leaf area of flax consistently declined with decrease of light intensity. The size of the leaf is often found to show an optimum with light intensity (WASSINK, 1969; BENSINK, 1971). BJÖRKMAN and HOLMGREN (1963) investigated the growth and development of several leaf characteristics in plants adapted to sun-exposed or shaded habitats. They found that the leaf size of strains of plants, adapted to shaded habitats, was negatively correlated with irradiation during cultivation, and that in plants, adapted to sun-exposed habitats, leaf size was positively correlated with irradiation level during cultivation. At the same time xeromorphy is related to smaller leaf sizes by smaller mature cell sizes and smaller number of cells. A similar effect is seen in leaves of plants having nutrient deficiencies (FERNANDO, 1958; PEARSE, 1960; NEWTON, 1963).

Chlorophyll content per unit area also depends on light intensity and a great many other conditions such as temperature, age, nutrition and water availability. The reaction to irradiation is ambiguous; it is generally accepted that the chlorophyll content of the leaves decreases with increasing irradiation levels, at least on a leaf weight basis, although the opposite has also been reported. These opposing reactions compelled MONTFORT and KRESS-RICHTER (1950) to distinguish between photolabile and photostable plants. Also BJÖRKMAN and HOLMGREN (1963) established these differences in type of reaction in their plants from different habitats: plants adapted to exposed habitats (photostable type) increased their chlorophyll content (on a leaf area basis) with increasing light intensities, while plants from shaded habitats (photolabile type) showed the opposite reaction.

Size of the leaf also shows an optimum in relation to position on the stem, usually the largest leaves being in the middle region of the stem. However it is not known whether this is because of leaf position or plant age (HUMPHRIES and WHEELER, 1963; GROEN, 1973). It may be of interest to investigate the development of leaf characteristics under such conditions that mutual shading of the leaves is largely excluded.

In our work, variable reactions of leaf characteristics have also been found in one and the same species or clone: e.g., in seedlings of sycamore (which are held to be ombrophilic) and in clones of poplar (which are held to be heliophilic), and in many other species. This variability was one of the greatest difficulties encountered in this investigation, but gradually over the years, variability diminished as a consequence of improvements in the root environment. Now, our thickest, greenest and largest poplar leaves (length 24 cm) with the highest photosynthetic capacity can be cultivated only at our highest light

intensity, and it may be questioned whether the different reactions on exposure to different light intensities of ecotypes, as described by BJÖRKMAN and HOLMGREN (1963), are really a direct effect of light on the growth of plants or a secondary effect on growth via the water and ion balance of the plants, resulting in xeromorphy (see their fig. 10 with the deteriorated chloroplasts).

DAUBENMIRE (1947) produced a list of properties of sun and shade leaves (the light factor) and of mesomorphic and xeromorphic leaves (the water factor). The similarity between the properties of sun leaves and xeromorphic leaves is striking, and stresses, once more, the conclusion that it is not easy to distinguish correctly between the direct growth stimulating effect of light and the secondary growth retarding effect via the induction of xeromorphism. The interaction of light and water stress are thought to explain a number of the discrepancies in the literature about the growth of leaves.

2. METHODS

2.1. THE CULTIVATION OF THE PLANTS

2.1.1. *Plant materials*

During the investigations the following plants were used: birch, limetree, lettuce, bean, tomato, duckweed, sycamore and poplar. The results, presented in this study are mainly based on *Populus euramericana* (DODE) GUINIER 'Robusta' and partly on *Populus raverdeau*, *Acer pseudoplatanus* L., and *Acer platanoides* L.

2.1.2. *Cultivation*

Initially, the plants were cultivated on subirrigated gravel culture in glass tubes (50 cm long and 5 cm diameter), as described by RICHARDSON (1953). This system only functioned well at low levels of irradiation. High levels of irradiation led to a gradual reduction in area of successive leaves to such an extent that ultimately only bractae remained at the stem, leading to death of the plant (sycamore). Cultivation in soil improved the quality of the plants at high light intensities, although a large variability in the plant characteristics remained. A further improvement could be obtained by changing over to a subirrigated gravel culture in 12 liter black buckets. Later on buckets were replaced by square containers (25 × 25 × 33 cm), made of thermosealed polyethylene plate (Hostalit Z, HOECHST, Plates I and II). A side tube was molded into the buckets or containers near the base through which nutrient solution was pumped into the containers and could flow out afterwards. Four containers with one plant in each were placed in a row on a trolley, together with a large polyethylene container from which nutrient solution could be pumped (water pump: CEM parvex, France) via a manifold of sufficient diameter (3,5 cm) and narrow plastic tubing (1.6 cm bore) into the plant containers. The amounts of solution pumped into each plant container were equal because of the symmetry of the system. The large container was filled with nutrient solution to a grade mark and contained just enough solution to fill the plant containers to a predetermined height. Once or twice a day, the large container was replenished to the grade mark with tap water. This method functioned satisfactorily and its simplicity was an advantage. Still, there were also some disadvantages: part of the root system could be damaged, when the nutrient solution did not reach the predetermined height, because the large container was not adequately replenished with water, owing to technical disturbances, or root growth blocked the tubing; all this causing variability in the growth of stem and leaves. For these reasons, an overflow was added to the plant container (2.1 cm bore), and the amount of nutrient solution doubled, which added security to the system.

To prevent gravel entering the pump and tubing system, the outlets were

provided with oblong filters, which added little resistance to flow of the nutrient solution. Initially, these filters were made of fine bronze gauze, which sometimes corroded severely. Recently, they have been replaced by plastic filters (+GF+, type 21.305 N), in order to prevent corrosion products to harm the plant growth and thus add to variability.

Initially, subirrigation was applied 3 times a day; later on the plants were irrigated automatically two times per hour for 5 minutes. The solution flowing back via the overflow returns into the large container by a free fall of 30 cm. When the pump stops, the nutrient solution flows back via the pump-system, sucking air into the gravel.

The plant shoots used for the experiments were grown from adventitious buds on stumps in the gravel culture. One bud per stump was allowed to develop into a shoot. When a shoot reached a length of 80 cm, it had to be cut back to the stump, because of the limited height of the growth chamber. A new bud was then allowed to grow. Therefore, the successive shoots were supported by a stump and root system of increasing size. The first shoot on a stump always grew at a lower rate than the successive ones. Shoots on older stumps had ample supply of water and ions and are, also physiologically, well characterized by the old expression: 'watershoots'.

The importance of the root environment for the stability of the growth pattern of the poplar was only gradually recognized during this investigation (PIETERS, 1962), although attention was directed constantly to the improvement of the conditions in the root system. On the basis of experience with optimally growing plants, variability in plant growth and leaf characteristics could be attributed to variations in the root environment. A quantitative study of the effects of root environment on the characteristics of growth remains to be done. First the root environment will have to be adequately defined in chemical and physical terms. The use of a phytotron may become senseless, unless adequate attention is also paid to the root environment, and, in this respect, cultivation of plants in gravel culture is not a guarantee for good growth. The plants need continuous intensive care to produce at best. Still it seems inevitable to encounter small differences in growth of the plants, which sometimes can be traced back to their individual life history. It may be stated here that temporary deficiencies in the cultural system can limit the growth of the plants during a rather long time, especially at low light intensities. The faster growing plants from higher light intensities recover more rapidly from damage caused by deficiencies.

In many cases, the low growth rate owing to deficiencies in the root environment results in an increase of carbohydrate reserves in such plants, e.g. starch, causing those plants to be more liable to attack by insects and fungi. At very low light intensities, the growth of poplar may become discontinuous and proceed in flushes. This phenomenon is accompanied by appearance and disappearance of starch reserves.

Finally, we may observe that large trees often exhibit a growth pattern which is similar to the growth pattern of optimally growing plants in the phytotron.

They produce branches with leaves of steadily increasing size, and chlorophyll content increases until the end of the growing season.

2.1.3. *Nutrient solution*

Initially, the Hoagland A–Z solution was used. The composition is given by HOAGLAND and SNIJDER (1933). Iron was added in the form of ethylenediamine tetra-acetic acid ferri sodium salt (JACOBSON, 1951). The stock solution contains 131.36 g FeNa-EDTA per 20 l, of which 5 ml is added to 1 l nutrient solution. For convenience, the solution of micro elements has been changed some years ago according to a recipe given by STEINER* (pers. comm.):

$Mn\ SO_4 \cdot 1\ H_2O$	–	20 g
$H_2\ BO_3$	–	27 g
$Zn\ SO_4 \cdot 7\ H_2O$	–	5 g
$Cu\ SO_4 \cdot 5\ H_2O$	–	0.8 g
$Na_2MoO_4 \cdot 2\ H_2O$	–	1.2 g
Distilled water to	–	1.000 ml

This change was based on the mean ion content of the local tap water. Of this solution 0.1 ml is added to 1 l of nutrient solution. The composition of the macro elements remained unchanged.

The nutrient solution was renewed once a week.

2.1.4. *Irradiation*

The plants were irradiated from the sides as well as from above to prevent intermixing of effects of age and mutual shading of the leaves. To accomodate this type of irradiation, four containers with one plant each were placed in a row on a trolley, placed between three banks of fluorescent tubes (with reflectors), two on the sides and one on top, forming together a growth cabinet (150 × 75 × 90 cm). Five of these cabinets (Plate I and II) were used in most experiments. Initially, Philips TL 55, 40 Watt fluorescent tubes were used and later, in the phytotron, Philips TLMF 33, 120 Watt.

Light intensities were measured with a calibrated, cosinus corrected, flat barrier layer photocell (GAASTRA, 1959; KUIPER, 1961) in the middle of the growth cabinet after removal of the plants. The light distribution in the growth cabinets measured at different heights horizontally as well as vertically was reasonably even. The irradiation levels measured, decreased with the age of the lamps, but the ratios of the light intensities in the three light cabinets remained reasonable constant if a scheme of exchanging lamps was maintained. The measurement of the prevailing light intensity was complicated by the fact that the plants were irradiated from three sides. The irradiation levels given in Table I are obtained by putting the cosinus corrected flat photocell in the middle of the growth cabinet and aiming it at one of the vertical banks with fluorescent tubes. No incandescent light was added; the daylength was 16 hours and the night length 8 hours.

*A. A. STEINER, C.P.O., Bornsesteeg 47, Wageningen.

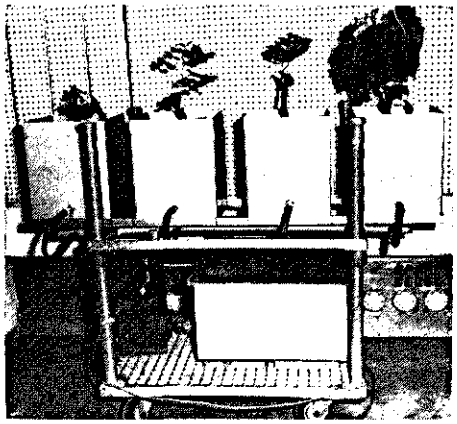


PLATE I. The trolley and the subirrigation system.

PLATE II. The growth cabinet, the trolley and the subirrigation system.

TABLE I. Irradiation levels in the growth cabinets, specified according to the type of fluorescent lamps used.

Irradiation level	Number, type and lengths of tubes	Intensity W/m²	Number, type and length of tubes	Intensity W/m²
I	25 TLM 120W/33RS, 150 cm	40	30 TL 40W/55, 120 cm	40
II	12 TLM 120W/33RS, 150 cm	20	16 TL 40W/55, 120 cm	20
III	6 TL 65W/33 , 150 cm	10*	4 TL 40W/55, 120 cm	10*

* Use was made of straylight entering this cabinet from lamps of irradiation levels I and II.

2.1.5. *Air conditioning*

Initially, the plants were grown in a darkened greenhouse with artificial illumination (Plate III). These plants were ventilated in such a way, that the leaves were moving gently, and the temperature was controlled as accurately as possible. In autumn or winter, the temperature sometimes fell below the threshold value that induced sycamore to shed its leaves. In summer, the temperature could rise above that desired, because no cooling equipment was available. Normally, the system functioned reasonably well. As soon as the phytotron became at our disposal (1963), the temperature could be held within 1–2 °C of the desired value. Air temperature at high levels of irradiation normally was 1–2 °C higher than that at low levels of irradiation. Air humidity could also be regulated, but has little influence if the root system is held in an 'optimal' condition.

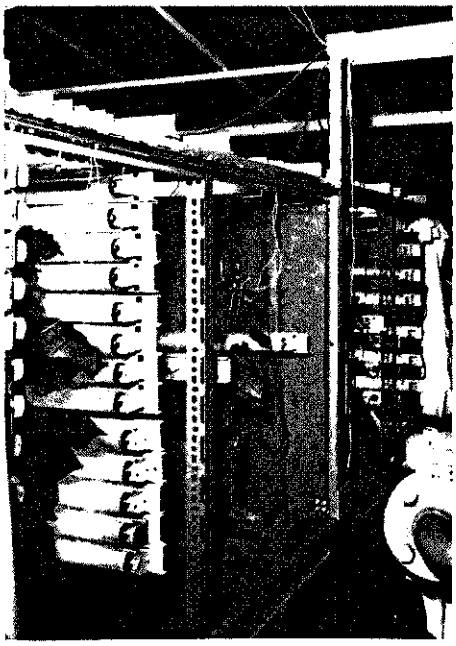


PLATE III. View of the growth cabinets in the darkened greenhouse.

2.2. MEASUREMENTS

2.2.1. *Measurement of plant dimensions*

Leaf length: measured with a ruler from the junction of the midrib and the petiole to the leaf tip.

Leaf width: measured with a ruler as the largest width at right angles to the midrib.

Leaf thickness: measured with an ocular micrometer under the microscope on transversal sections of the leaf or measured mechanically with a micrometer gauge.

Petiole length: measured with a ruler from the base of a stipulum to the junction of petiole and midrib.

Primordium length: the successive detached primordia were measured with a ruler under the binocular loupe, and if very small, with a micrometer under the microscope.

Leaf or primordium number: the number was counted from the base of the plant upwards, unless otherwise stated. Sometimes, the first 5 to 10 leaves were already shed, before counting began; then the first remaining leaf was counted as number 1. Each fifth leaf was colour coded with a strip of tape or plastic coated wire.

Internode diameter: measured with a micro-calliper. The accuracy is diminished by the irregular form and the softness of the tissue of young internodes.

2.2.2. *Silicone rubber technique*

Silicone rubber is very suitable for making replicas of fine structures like the epidermis of a leaf (SAMPSON, 1961; ZELITCH, 1961). The type of silicone rubber used was 'Silicoset 105', together with the polymerizing agents 'A' (slow) and 'D' (quick) obtained from 'ICI'. These agents are poisonous, especially to very young plant tissue. To minimize damage, the concentration of the agent must be as low as possible, without making the polymerization time excessively long (not longer than 10–15 minutes). The rubber and the polymerizing agent have to be mixed thoroughly. The polymerization time begins during the mixing and the mixture can be spread in a thin layer onto the leaf surface with a spatula, as soon as its consistency is such that it will not drip from the leaf and is still sufficiently fluid to replicate the epidermis. The replica can easily be removed from the leaf, as soon as the mixture has polymerized. The leaf then is immediately rinsed with water. The replica is covered with a thin layer of nail varnish and, after this has been allowed to dry for about 30 minutes, a second layer is applied. Then, the varnish is removed from the rubber replica when it is practically dry and laid upside down on a glass slide and pressed gently with the thumb to stick it flat onto the glass. The preparation is now ready to be analysed under the light microscope. The nail varnish preparations can be projected onto drawing paper at a fixed magnification. A square portion of this projection with sides of 14 cm was analyzed; this corresponds to an actual field with sides of 250 μ. The analysis consisted of drawing cell groups, consisting of the stomata with surrounding cells and determine these areas with a planimeter. Subtraction of the planimetered areas from the total area of the analysed square (256 cm^2), yielded the area of the other epidermal cells. The other cells were counted, and their mean diameter calculated.

It is possible to repeatedly make replicas of the epidermis of a leaf during its expansion. This can even be accomplished at one and the same position on the leaf, if a reference point, such as a recognisable arrangement of veins or a small hole punched in the leaf is used. This method is used with sycamore and poplar leaves, and the results are satisfactory. It is not too difficult to find back the position under analysis at the cellular level. A problem is that the curing agent used in the silicone rubber procedure is poisonous. The primordial cells of sycamore, especially, are very sensitive to the heavy metal compound it contains, while poplar is more resistant. The silicone rubber itself is harmless. This method still is not fully reproducible because damage cannot always be avoided. There is a check on the degree of damage by following the growth of the leaf concerned in relation to its neighbours. An example of this type of investigation is given in section 4.6. Other types of silicone rubber with non-poisonous harders are being manufactured by WACKER-Chemie G.M.B.H. München. Until now we have no experience with them. A new method is described very recently by WILLIAMS (1973).

2.2.3. *Photosynthesis*

Photosynthesis of leaf parts, attached to or detached from the plant, was

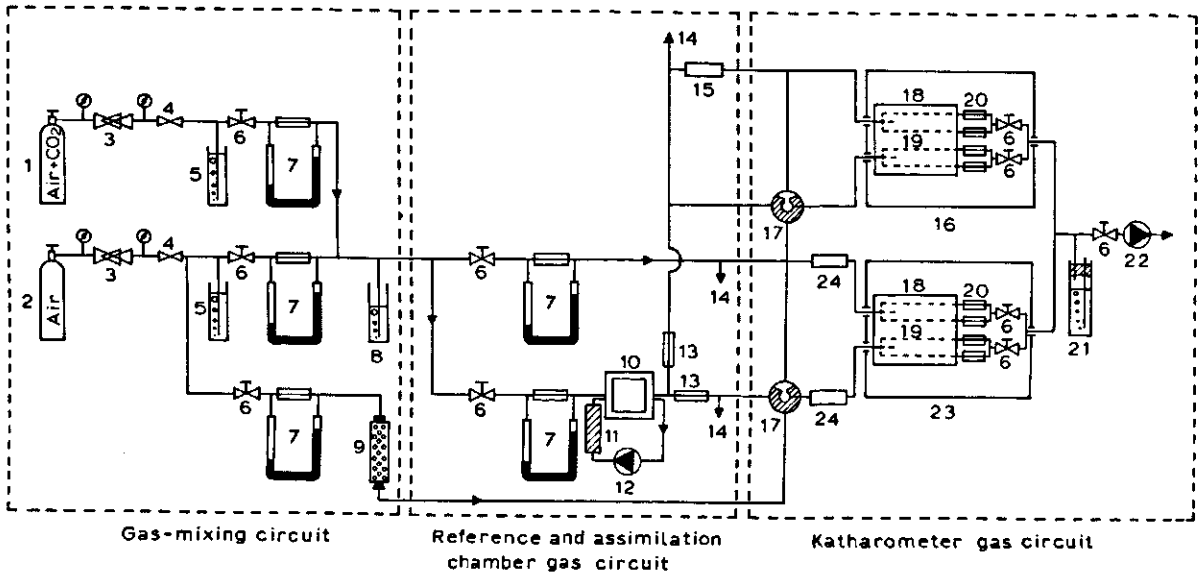


FIG. 2.1. *Gas mixing circuit.* 1 Cylinder with air and CO_2; 2 cylinder with air; 3 pressure reducing valve; 4 valve; 5 overflow pressure regulator; 6 needle valve; 7 resistance flowmeter; 8 overflow pressure regulator; 9 CO_2-absorber.

Reference and assimilation chamber gas circuit. 6 Needle valve; 7 resistance flowmeter; 10 assimilation chamber; 11 heat exchanger in recirculation circuit; 12 recirculation pump; 13 capillaries to distribute gas and to damp vibration; 14 outflow; 15 large drying tube (P_2O_5).

Katharometer gas circuit.
Transpiration meter (16).
17 three way valve; 18 katharometer block; 19 2 reference channels, 2 measuring channels; 20 flow regulating capillaries; 21 suction pressure regulator; 22 suction pump;
Carbon dioxide analyser (23).
17–22 as above; 24 drying tubes (P_2O_5).

measured in assimilation chambers of different constructions at 20°C in air with 5% CO_2 on a volume basis. With detached leaves, their petiole was cut under water with a razor blade and placed in water for the measurement. The gas analysis was performed with a katharometer. Fig. 2.1. shows a scheme of the equipment used for the measurement of photosynthesis, in its present form (PIETERS, 1971), but the earlier measurements were made with a simpler system (PIETERS, 1960). Temperature was measured with thermocouples; gradually, it became evident that the use of thermocouples for the measurement of leaf temperature may introduce large systematic errors (PIETERS, 1972a; PIETERS and SCHURER, 1973). Leaf temperature was controlled manually by changing the temperature of the double walls of the assimilation chamber, or automatically with the infrared compensation system (PIETERS, 1972b). The maximum irradiation level was 700 W/m².

Initially high pressure mercury lamps (PHILIPS, HPLR 700), mounted in a waterbath for cooling, irradiated via three glass walls the leaf in the assimilation chamber. The UV-content of this light appeared in some cases to be harmful for the physiological functions of the leaf. The sensitivity of a leaf seems to depend on its condition. For this reason the HPLR-lamps were replaced by 500 W Prado projectors (LEITZ).

2.2.4. *Chlorophyll content*

The chlorophyll content was determined on a leaf area basis. Areas of leaf surface (3 to 6 cm²) were punched from fresh leaves, killed in boiling water, immediately transferred to hot ethanol 70% (70°C), and extracted 3 to 6 times. The resulting chlorophyll solutions were decanted in 50 ml volumetric flasks, chilled in ice or in a refrigerator and covered with black cloth. The solutions were made up to volume, and the relative chlorophyll content characterized by the extinction of the extract in the colorimeter at 665 nm. Quantitative estimation of chlorophylls was made in the way described by WINTERMANS (1969).

3. GENERAL DESCRIPTION OF THE SHOOTS

3.1. Introduction

In this chapter a general description is given of the shoot of *Populus euramericana* 'Robusta', under different conditions of light and temperature. Measurement of leaf lengths alone produces a rough picture of the characteristics of this plant, because it is correlated with the dimensions of other plant parts, e.g. leaf width, petiole length, internode length, and internode diameter.

Without the application of destructive methods, measurements of leaf length can only be made in the period of growth after emergence of the leaves from the apex, i.e. mainly in the linear phase of growth.

3.2. Leaf width and leaf length

Leaving the apex intact, the length of the growing leaves can be measured from 1 cm onward. Their width then cannot yet be measured accurately, because the lamina is rolled. The unrolling of the lamina is completed when the leaf is between 2 and 5 cm long, dependent on the ultimate size of the leaf. Data on leaf width between 0.2 and 3 cm, may not be correct in case unrolling was incomplete. Fig. 3.1a shows the sigmoidal increase of length and width of the individual leaf with time and in fig. 3.1b the relation between the developing

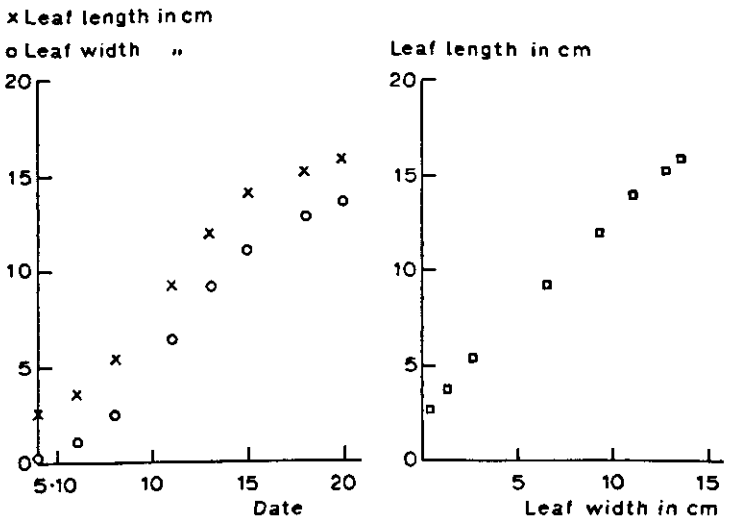


FIG. 3.1.a The increase of length (x) and width (o) of an individual leaf versus time.
FIG. 3.1.b Relation between length and width of the same leaf during growth (□).

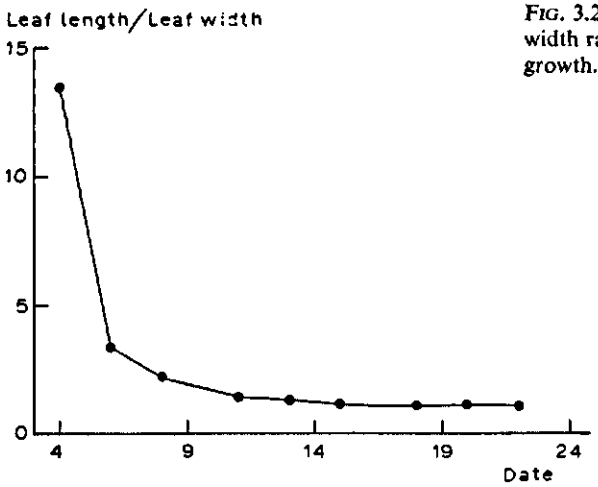


FIG. 3.2. The change of the length/width ratio of an individual leaf during growth.

length and width of the same leaf. The rate of increase of both is similar. The development of width lags behind that of length, and stops increasing approximately at the same moment. This growth habit of the poplar leaf is also reflected in its length-width ratio (L/W) in relation to time (fig. 3.2). Initially, the L/W ratio is high, but this value rapidly diminishes and approximates 1 when the leaf matures. Plotting leaf length versus width may be the most useful representation of the development of leaves, because it shows that growth of length and width is similar, and that the width is 2 cm smaller than the length, thus from a length of 2 cm onwards,

$$W = L - 2 \qquad (1)$$

This relationship proves to be the same for all the conditions under which the plants were cultivated. Fig. 3.3 illustrates this point clearly for plants cultivated at light intensities of 40 (I), 20 (II) and 10 (III) W/m^2 at 20°C and in 40 W/m^2 at 16°C and 25°C; consequently variation of the L/W ratio during growth of the individual leaves follows the same course for all; small leaves, however, stop at an earlier stage. The rolling of the lamina clearly influences the picture; it is present from early stages of development. Unrolling gradually starts, and lamina development becomes apparent above a length of ca. 2 cm; it should be noted, however, that if the lamina is unrolled per force (e.g. by killing the young leaf in hot water) the shape of the leaf is virtually the same as in the later more expanded states, although with a much higher L/W-ratio.

3.3. PETIOLE LENGTH AND LEAF LENGTH

Petiole length has been compared with the leaf length during growth under different conditions, as is shown in fig. 3.4. As in the case of leaf length and leaf

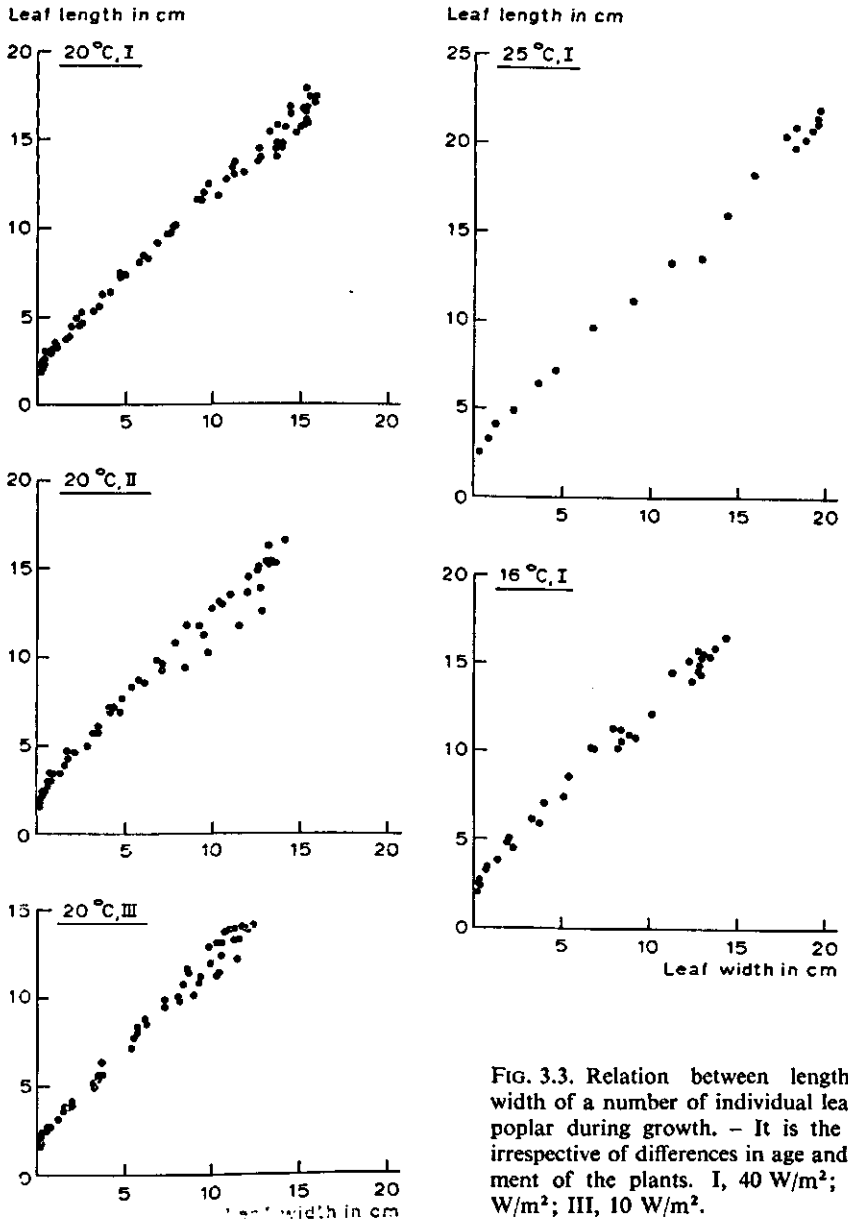


FIG. 3.3. Relation between length and width of a number of individual leaves of poplar during growth. – It is the same, irrespective of differences in age and treatment of the plants. I, 40 W/m²; II, 20 W/m²; III, 10 W/m².

width, a reasonably linear relationship is found. Light seems to have a weak influence on the relative development of petiole and leaf lamina. When grown at higher temperatures and low light intensities, the petioles tend to be somewhat longer.

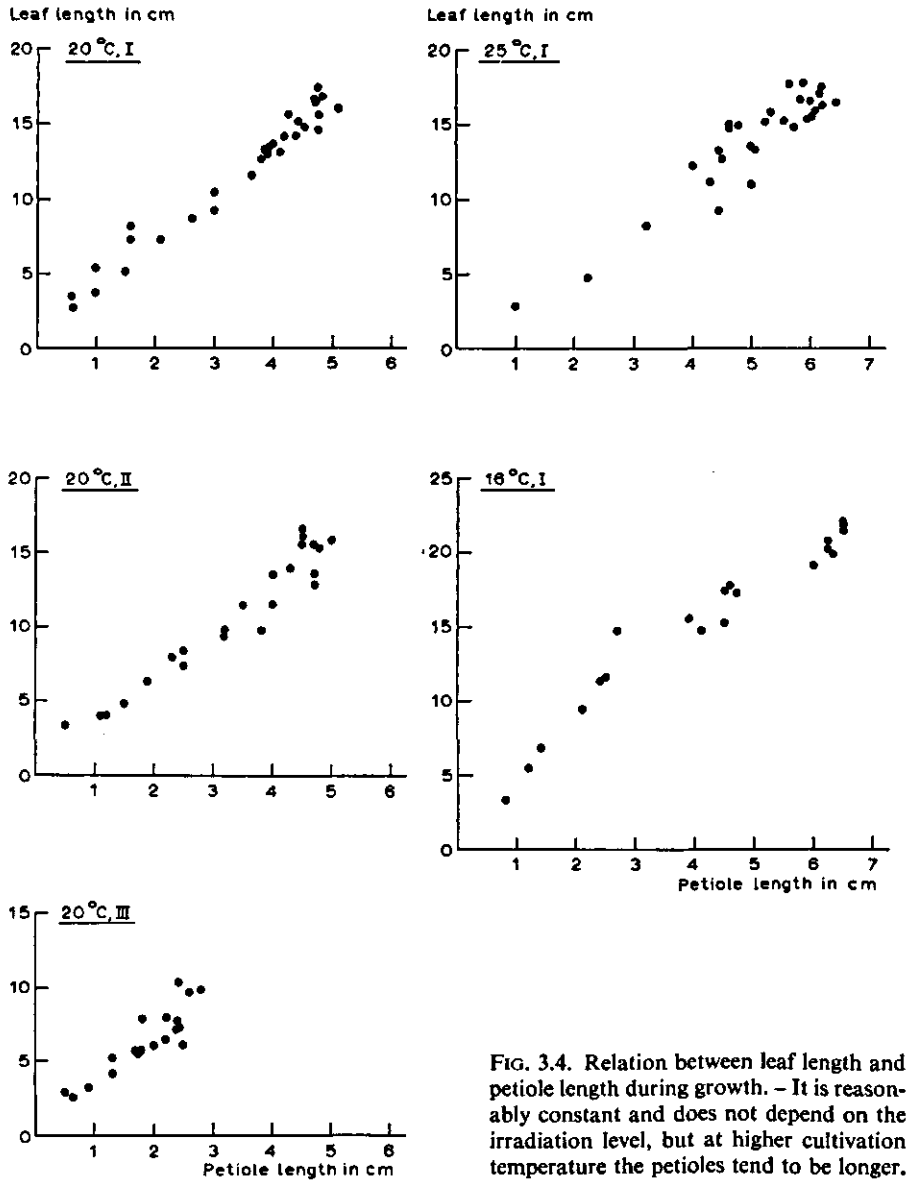


FIG. 3.4. Relation between leaf length and petiole length during growth. – It is reasonably constant and does not depend on the irradiation level, but at higher cultivation temperature the petioles tend to be longer.

3.4. INTERNODE LENGTH AND LEAF LENGTH

The relation between internode length and leaf length appears to be more complicated than the previous ones. Fig. 3.5 shows the relationship between the lengths of growing internodes and leaves. Clearly, the relationship depends on the light conditions of the plant: shade leaves grow relatively more slowly and

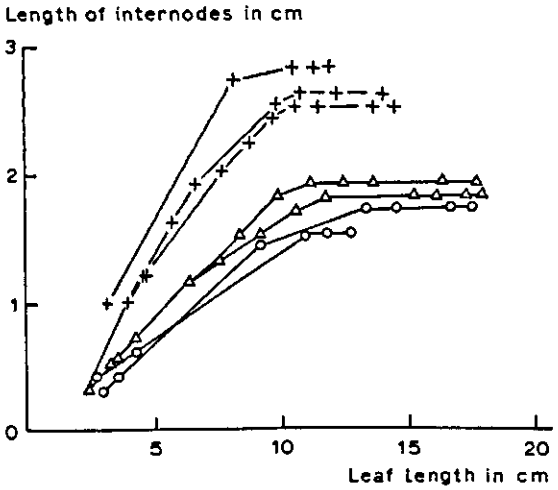


FIG. 3.5. The growth correlation between length of the subtending internode and leaf length shows that internode growth stops earlier than leaf growth. Leaves growing in higher light intensities tend to grow relatively faster and those growing in lower intensities slower than the internodes. Growth conditions: temperature 20°C, light intensities 40 W/m² (○), 20 W/m² (△) and 10 W/m² (+).

sun leaves faster than the subtending internode. The increase of leaf length continues some time after the internode has attained its ultimate size. This is shown more clearly by the length versus time relationship of the growing internodes, leaves and petioles (fig. 3.6). The ultimate (average) lengths of the internodes are more or less constant as is shown in Table II, and independent of treatment. Mature internode length seems to be much less affected by light intensity and temperature than leaf length (and leaf size in general). The reduction of the length of the internodes with higher light intensities, as indicated in fig. 3.5 and 3.6 may perhaps be ascribed to adverse conditions in the root environment.

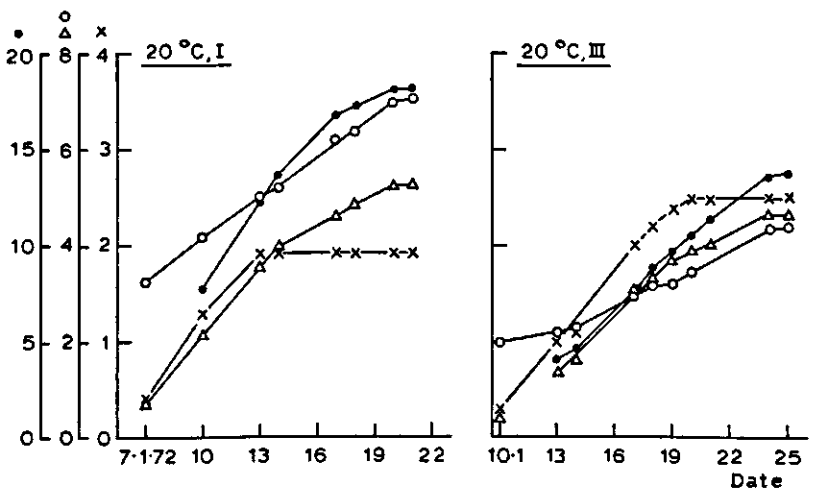


FIG. 3.6. The growth against time relationship of leaf length (•) in cm, petiole length (△) in cm, internode length (x) in cm and internode diameter (o) in mm of a leaf growing on a plant in high (I) or low irradiation level (III) at 20 °C.

TABLE II. The lengths (in cm) of mature internodes of poplars grown in different light intensities (I, II and III) and at different temperatures (16, 20 and 25°C), measured at different dates.

Date	2/11/'71	10/12/'71	24/12/'71	3/2/'72	Mean
Plant*					
25-I-1	1.5		1.7	2.3	1.87
25-I-2	2.0				
16-I-4	1.8			1.4	1.62
20-I-1	1.9	2.1	2.1	2.3	2.08
20-I-2	2.0				
20-I-3	1.9				
20-I-4	2.1	2.3			
20-I-5	1.6	2.3			
20-I-6		2.3	2.3		
20-I-7	1.8				
20-I-8			2.1		
20-II-1	2.2			2.1	2.13
20-II-2	1.9	2.2	2.2		
20-II-4			2.2		
20-III-1	1.9	2.3		2.6	2.16
20-III-2			2.5		
20-III-3	1.5				
20-III-4			2.2		

* Code indicates temperature – light intensity – plant number.

3.5. INTERNODE LENGTH AND INTERNODE DIAMETER

It is concluded from fig. 3.7 that light can exert a powerful influence on the relation between internode length and internode diameter: internodes of old sunplants being thicker than those of shade plants. This phenomenon can be observed easily on plants, growing in the different environments (see Plate IV–VI, p. 20 and 21). It appears that the mode of growth of young sunplants resembles that of shade plants. However, when sun plants grow older, there is a gradual increase in diameter of the successive internodes, in area of the successive leaves and in length of their petioles. The lengths of the internodes, however, remain approximately the same.

3.6. INTERNODE DIAMETER MEASURED AT THE EARLIEST POSSIBLE STAGE AND MATURE LEAF LENGTH

Internode diameter can be measured with a microcalliper with some accuracy. Very young internodes cannot be measured in this way, because the young growing leaves and the stipulae totally cover the internode. When the internode elongates, there is a moment at which measurement becomes possible. The diameter of that youngest measurable internode can be correlated with the

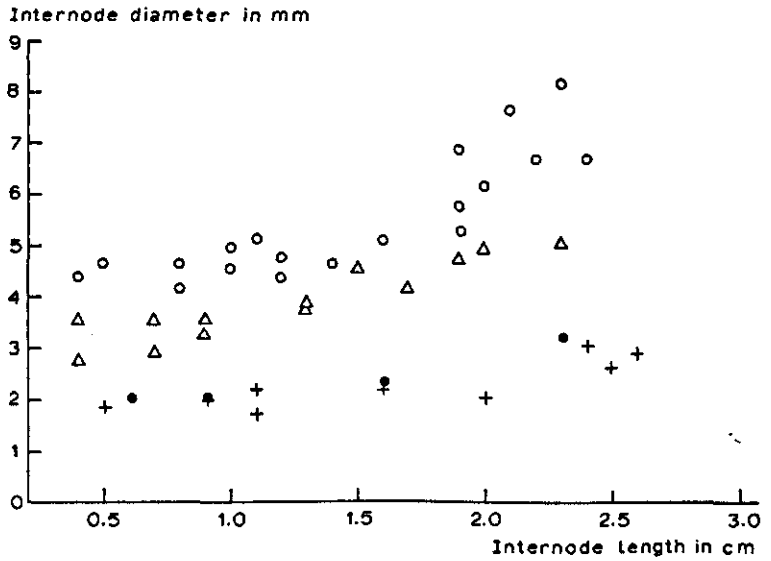


FIG. 3.7. Correlation between diameter and length of internodes during growth. – It depends on the irradiation level and on the age of the plants. Growth conditions: as in fig. 3.8, except (•), which denotes the relation in a very young sun plant.

mature length of the attached leaf. Fig. 3.8 shows that this correlation is linear and independent of the irradiation levels to which the plants are exposed. However, the diameter of the youngest measurable internode and mature leaf length both are similarly influenced by irradiation level and age.

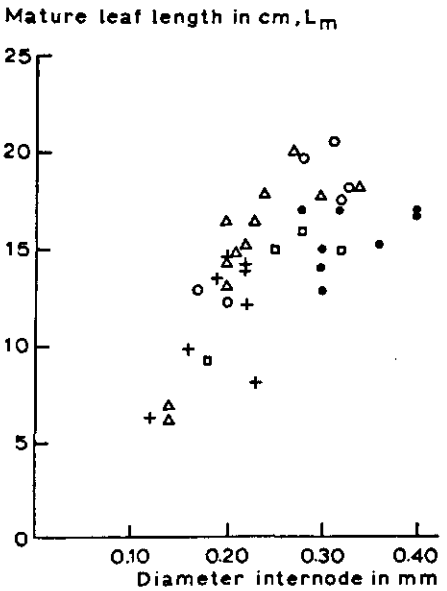
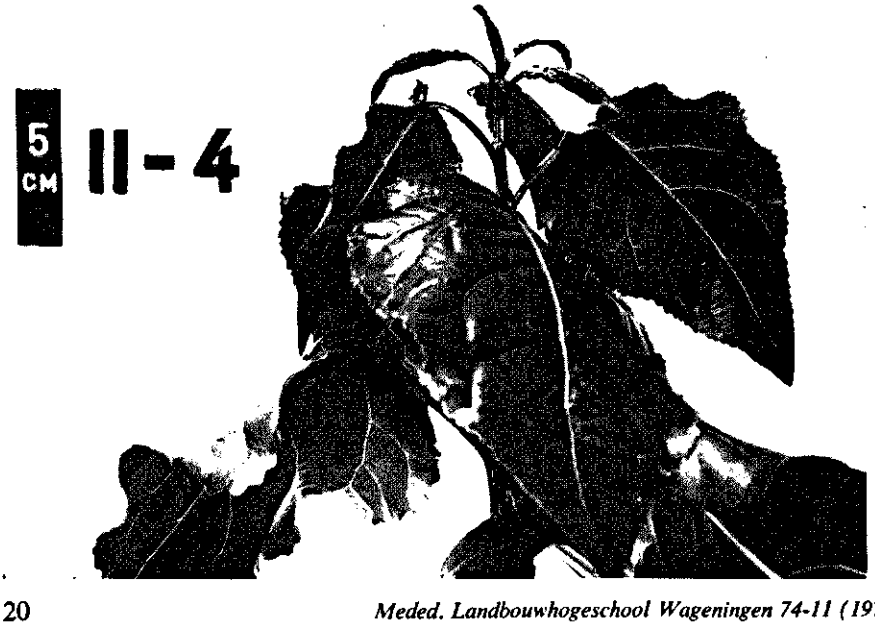
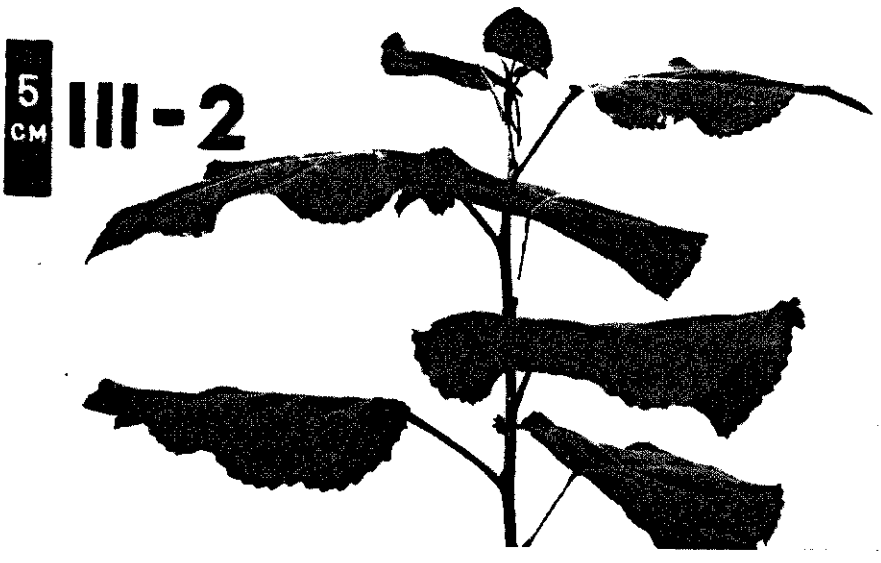


FIG. 3.8. Relation between internode diameter, measured at the earliest possible stage, and the mature length of the attached leaf. – It is linear and independent of irradiation level. Growth conditions: temperature 20°C at light intensities of 40 W/m² (○), 20 W/m² (△) and 10 W/m² (+), temperature 25°C (□) and 16°C (•) at 40 W/m².


5
CM
I-6


5
CM
II-4



PLATES IV-VI. Representative pictures of the sizes of the growing tips of large poplar plants, grown in the three light intensities I, II and III. I, 40 W/m², II, 20 W/m² and III, 10 W/m² at 20°C.

3.7. DISCUSSION

It is observed that in *Populus euramericana* 'Robusta' several other characteristics are clearly correlated with leaf lengths. The development of leaf length and width of large and small leaves proceeds according to a similar pattern, although growth rates may be very different. The growth rates of the internodes appear to be far less dependent on environmental conditions. These conditions, however, affect the diameters of the internodes which in the most vigorously growing plants become much thicker than those under conditions of low energy supply.

In the subsequent chapters an attempt is made to study some of these correlations separately, supplying elements for a model for growth to be eventually made. The relative rigidity of such growth patterns, evidenced by growth correlations, is a common occurrence in plant physiology. Well known examples are: leaf length – leaf number relation (ERICKSON and MICHELINI, 1957), top-root ratio, in which the nutritive balance is involved (BROUWER, 1963) and apical dominance (PHILLIPS, 1969), in which the auxin-balance may be involved. In short, the whole habit of the plant is the result of growth correlations (WARDLAW, 1967). DORMER (1964), STEWART (1968), and many others have given a critical consideration on growth correlations. Dormer warns against two obvious fallacies often occurring in literature viz., that a correlation proves

a direct physiological connection between processes, and that a simple form of a correlation indicates the physiological connection to be simple also. Growth correlations only require some form of communication between the correlated phenomena via internal or external factors and some comparable way of reacting upon these. AVERY (1933) used the concept of growth correlations by drawing a map of the distribution of growth in the developing tobacco leaf. MAKSYMOWICH (1963, 1973) presented a beautiful example of correlative growth on a cellular level in his study of the growth of leaves of *Xanthium pensylvanicum*, showing that epidermis and mesophyll cells, each can be characterized by a specific mode of growth, differing in duration, rate and direction. Many of these studies necessarily are of a descriptive nature, because in fact it is not known which regulative principles underly the correlation between these physiological phenomena. It is generally accepted that genetic control of developmental processes has to be assumed to interpret the correlation phenomena. POSTLETHWAIT and NELSON (1964) concluded that there are numerous 'switch points' in the development of plants, where a group of genes evoke particular types of development (CUTTER, 1965). In the case of poplar, the constant relation between the growth of leaf length and width suggests that the start of developing of the leaf lamina is such a 'switch point', followed by a closely correlated growth of width and of length.

Sometimes, the length-width relationship of the poplar leaves shows changes and the leaves become oblong. The causes of this abnormal growth must be looked for in the root environment. Further study of such 'abnormal' growth may lead to a better understanding of the normal growth pattern of the leaf.

The relation of the length of the petiole and the leaf can be influenced by total darkening of the apex: the petioles become longer than normal (fig. 3.9). This type of experiment may be useful in further elucidating processes involved in the morphogenesis of the plant.

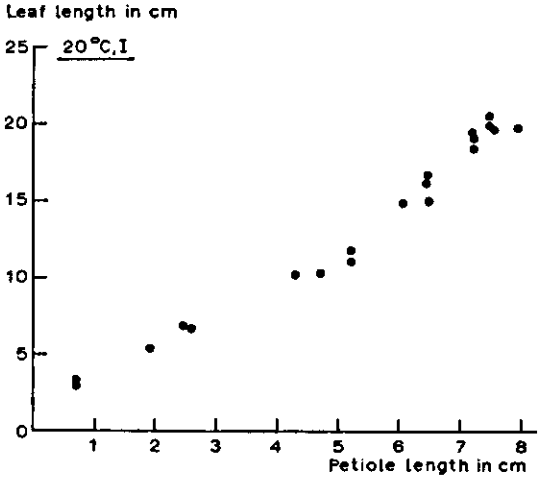


FIG. 3.9. The relation between leaf length and petiole length during growth of a darkened sun plant.

4. THE SIZE OF THE EPIDERMAL CELLS OF GROWING AND MATURE LEAVES

4.1. INTRODUCTION

The area of the leaf is determined by number and size of the cells in its epidermis. The ultimate size of a cell and the number of cells in the leaf depend on its genetics and its environment.

In some plants it appears necessary to distinguish between the developmental pattern of epidermal cells of midrib and lamina. In lettuce the elongation of midrib cells tends to be inhibited by higher light intensities (BENSINK, 1971) or in *Phaseolus* with increasing daylength (DALE, 1968). The effect of light intensity on cell elongation in the midrib of lettuce can be correlated with the position on the midrib (BENSINK, 1971). Normally, the size of the mature cells of the lamina is hardly influenced by light intensity, unless this intensity is very low. Indirectly, light intensity may exert an influence via the water balance of the plant, since water strain (SCHWABE, 1956) often causes small epidermal cells. Also shortage of mineral nutrients generally depresses the ultimate size of the epidermal cells (MILTHORPE and NEWTON, 1963). Cell size is sometimes largest in mature lower leaves on a plant (HUMPHRIES and WHEELER, 1963; BENSINK, 1971).

A basipetal trend in the maturation of leaf cells is very common (AVERY, 1933; MAKSYMOWICH, 1963); this is due to differences in growth rate or duration of growth in the leaf, both factors being responsible for leaf shape. Often the size of the epidermal cells of the lamina tends to become constant over the entire leaf surface as the leaf matures (HUMPHRIES and WHEELER, 1963; MAKSYMOWICH, 1963; BENSINK, 1971). Mature cell size is sometimes positively correlated with temperature (DALE, 1967; BENSINK, 1971). It is generally accepted that cell number is the main determinant of leaf size (HUMPHRIES and WHEELER, 1963).

4.2. DISTRIBUTION OF THE CELL SIZES OVER THE EPIDERMIS OF MATURE LEAVES

An analysis of cell sizes in the mature epidermis was made with the aid of the silicone-rubber technique. Replicas were taken from seven different positions, regularly distributed over the leaf surface. Cell measurements were made in each replica at two restricted areas. The results are shown in fig. 4.1. Mathematical analysis of the data of Table III shows that the duplicates are correlated and that the mature cell size is determined by factors which differ locally, giving a mosaiclike 'mini' pattern. Therefore, a reliable measure of mean cell area of the epidermal cells of the leaf blade can only be obtained by measuring of mean cell area at different positions distributed over the epidermis of the

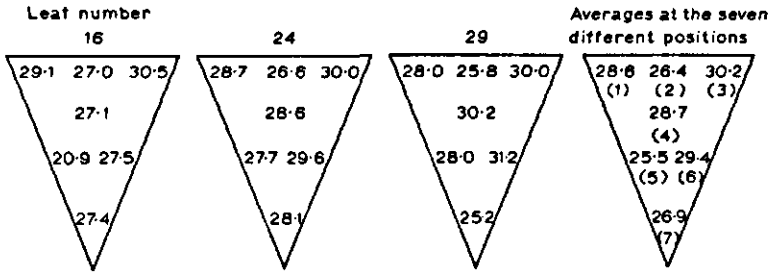


FIG. 4.1. Mean mature size, in μ, of the epidermal cells at seven different positions on the surface of each of three leaves of the same poplar plant, and their averages. Between the parentheses the numbers of the positions (see Table III).

TABLE III. Mean mature cell diameter in μ in the epidermis, measured by the silicone rubber technique and analyzed in duplicate (a and b) at seven positions, evenly distributed over the leaf surface, and their averages (AV).

Leaf number	16			24			29		
Position	a	b	AV	a	b	AV	a	b	AV
1	29.2	29.0	29.1	29.2	28.2	28.7	26.9	29.0	27.9
2	27.3	26.7	27.0	26.3	26.8	26.5	27.2	24.3	25.7
3	28.1	32.8	30.4	30.1	29.9	30.0	29.2	30.8	30.0
4	26.2	27.9	27.1	28.7	28.4	28.5	30.5	29.8	30.1
5	20.0	21.7	20.8	27.9	27.5	27.7	27.5	28.4	27.9
6	26.9	28.0	27.4	28.6	30.5	29.5	30.6	31.8	31.2
7	27.0	27.8	27.4	28.3	27.0	27.6	24.5	25.8	25.1

leaf. A decision about the question, whether a distinct pattern in the distribution of cell sizes over the leaf surface is present, cannot be made on the basis of the presented data; for such a purpose the number of measurements was too small. It could be of interest to investigate the distribution of cell size in connection to asymmetry in the shape of the poplar leaf. The few figures available suggest that in general, the values in the midrib, especially at the base, and the data obtained for the left sides of the leaves as represented in fig. 4.1 are somewhat lower than those recorded at other spots on the leaf.

4.3. MEAN MATURE EPIDERMAL CELL SIZE AS INFLUENCED BY THE LEVEL OF LEAF INSERTION

Measurements of the mean diameter of epidermal cells were taken from a sequence of leaves along the axis of plants which had been submitted to different treatments. Results are shown in Table IV. There is no distinct relationship between the position of the leaves and the mean epidermal cell diameter in *Populus euramericana* 'Robusta'.

TABLE IV. Mean mature epidermal cell diameter (in μ) of leaves of *Populus euramericana* 'Robusta' from different treatments as influenced by the position of the leaves, subirrigated gravel culture, 1970–1971. Plant code see Table II.

Plant: 20-I-4		20-I-1		20-II-1		20-II-3		20-III-3		20-III-2		20-III-1	
Leaf no.	Size	Leaf no.	Size	Leaf no.	Size	Leaf no.	Size	Leaf no.	Size	Leaf no.	Size	Leaf no.	Size
37	27.2	38	24.6	29	27.1	20	30.1	50	21.4	32	22.3	25	28.0
36	29.2	37	25.2	20	29.7	18	32.4	47	24.7	27	25.8	21	26.1
21	22.8	29	24.6	17	31.3	17	30.5	44	27.7	22	28.3	19	24.1
10	26.5	25	29.8	12	32.1	15	30.5	41	24.5	19	27.2	18	21.1
		24	24.5	8	21.4	14	28.5	38	26.3	17	25.9	16	26.3
		20	27.7			13	28.9	35	25.4	14	27.7	15	26.2
32	25.9	15	29.3			12	31.6	32	27.3	12	28.4	13	25.0
24	25.2	10	25.7			10	24.7	29	23.8	7	25.3	10	32.0
16	26.6	9	26.5			9	29.2	26	25.6				
11	22.4	5	25.8					23	25.7				
10	24.4	4	29.1					20	26.2				
								17	27.6				
								15	29.9				
								13	26.0				

Plant: 25-I-4		25-I-2		15-I-2	
Leaf no.	Size	Leaf no.	Size	Leaf no.	Size
20	26.3	14	29.0	37	32.8
19	24.6	6	26.6	32	27.1
9	22.2			13	30.4
4	26.2				

Comparable measurements of mean epidermal cell diameter on a sequence of leaves, taken some years earlier from *Populus raverdeau* plants, showed that the epidermis of older leaves is composed of larger cells, cf. Table V.

What has been found for *Populus euramericana* 'Robusta' thus has no general validity. It may be noted, however, that the conditions under which the first mentioned plants were grown, were considerably more favourable than those for *Populus raverdeau*. Therefore, it can not be excluded that in the earlier experiments the steadily increasing leaf area of the growing plants inhibited leaf cell growth by water or ionic desequilibration especially at high light intensities. That the demands on the root system for the uptake of water and ions increase with the size of the plants is evident and illustrated by the observation that especially at high light intensity death resulted when failure of the irrigation system, longer than 6 hours, occurred, and that the younger and smaller plants readily survived such an interruption. From the figures of Table V it is obvious that the largest cell sizes are found in the best growing plants, which were obtained at light intensity II. Obviously, at light intensity I an elongation suppressing tendency was manifest, the cause of which we provisionally are inclined to locate in deficiencies in the root environment.

TABLE V. Leaf width (cm), mean mature cell diameter (in μ) and cell number in the width of leaves of *Populus raverdeau* from different light intensities at 20 °C as influenced by level of leaf insertion, large pots with soil, 1962.

Plant:	20-I-5			20-II-4			20-III-4		
Leaf number	Width	Cell size	Cell number	Width	Cell size	Cell number	Width	Cell size	Cell number
27	3.7	growing		12.5	23.9	5200			
25	7.0	growing	4630	12.9	25.1	4640			
23	8.8	growing	4520	13.4	27.4	4880	7.5	25.3	2960
21	9.1	growing	4940	14.4	28.5	5040	9.2	31.0	2950
19	11.6	25.30	4580	13.6	28.8	4710	8.4	26.2	3210
17	12.4	27.0	4570	13.9	32.6	4260	7.8	–	–
15	12.7	28.3	4360	13.3	31.3	4250	8.4	24.5	3430
13	11.4	27.3	4180	12.5	32.1	3920	8.6	26.6	3230
11	10.2	26.0	3930	12.5	32.0	3900	9.2	26.7	3450
9	10.1	27.7	3640	11.8	36.1	3280	9.2	26.9	3430
7	9.3	31.3	2920	10.7	32.6	3290	8.7	–	–
5	9.1	21.2	2830	9.7	38.5	2490	9.8	31.2	3160
3	8.0	33.6	2350	7.9	34.8	2270	11.0	–	–
1	6.3	–	–	5.8	38.5	1500	8.2	33.6	2450

4.4. MEAN MATURE CELL SIZE OF LEAVES FROM DIFFERENT CULTURAL TREATMENTS

The conclusion from the preceding section justifies direct comparison of the mean epidermal cell diameters of leaves of plants from different cultural treat-

ments. As demonstrated, mean cell diameter may differ somewhat from one position to another over the leaf surface. However, since these differences are fairly small, it was considered sufficient to analyse one position on a large number of leaves which then could be taken as representing the mean cell diameter per plant.

Some results are summarized in Tables VI and VII. Only small differences in mean epidermal cell diameter were found between various treatments. Some deviations appeared, e.g. the 15 °C plants had larger average cell diameters while plants from light intensity II also had slightly larger cell diameters. Since cell size thus appears fairly constant irrespective of treatment, it may be concluded that leaf size is determined by cell number. This conclusion is in agreement with that of previous workers. The outline of a mature poplar leaf is a graphical representation of the distribution of cell number over the leaf.

TABLE VI. Mean mature epidermal cell diameter (in μ) of leaves of *Populus raverdeau* from different treatments. Large pots with soil, 1962.

Light treatment	I	II	III
Cell size	31.8	32.8	29.3

TABLE VII. Mean mature epidermal cell diameter (in μ) of leaves of *Populus euramericana* 'Robusta', from different treatments, gravel culture, 1970–1971.

Temperature Light intensity	16	20	25	°C
I	30.77	25.28	26.30	
II		28.29		
III		27.39		

4.5. THE BASIPETAL TREND IN THE EXPANSION OF THE LEAF

In order to investigate the expansion of a poplar leaf, a square pattern of holes was made in the leaf lamina at an early stage of development (leaf size at that time about 6×4 cm^2, and 4×2 cm^2 respectively). After maturation, a linear distortion from the tip downward has developed in the square pattern (Plates VII and VIII). This demonstrates a basipetal trend in the development of leaf area, a common feature of leaf growth. Probably, this is connected with leaf shape: where the leaf is broadest cell expansion persists longest. The same is expressed by the observation that the leaf tip is the first region to mature.

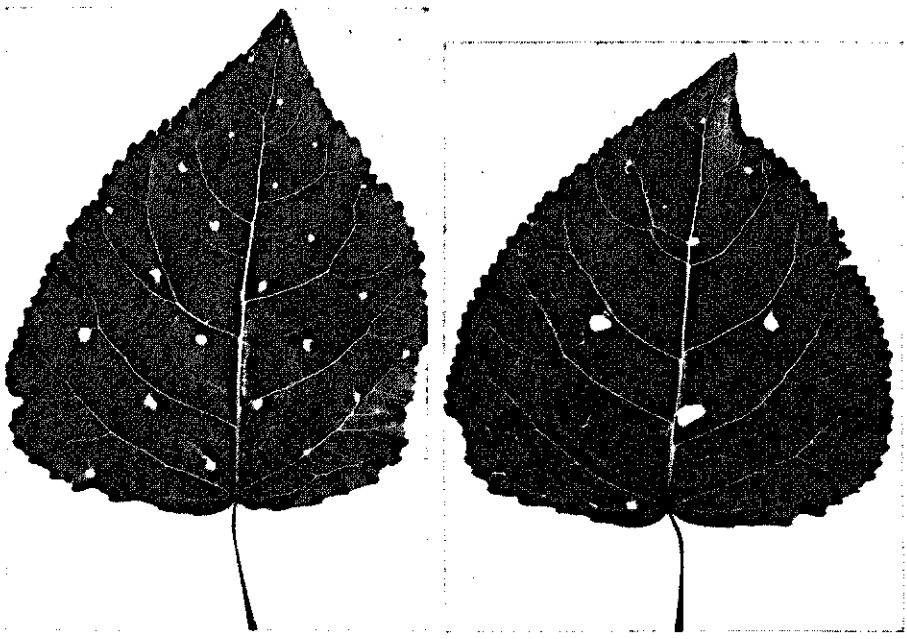


PLATE VII. The basipetal trend in the development of poplar leaves demonstrated as a distortion of a square pattern of holes during the growth of the leaf. – The square pattern was punched in the leaf, when its dimensions were 4 × 6 cm². The development of the leaf has been disturbed at its right side, possibly because a vein has been destroyed.
PLATE VIII. As plate VII, but the square pattern of holes is punched when the leaf was 2 × 4 cm².

4.6. THE EXPANSION OF INDIVIDUAL CELLS OF THE EPIDERMIS IN THE LINEAR PHASE OF GROWTH

At 3 stages of development (every three days) of the same leaf, replicas of one and the same region of the epidermis were made. The replicas were photographed and are reproduced on Plate IX. The magnification is the same in the three stages. A number of questions are of interest:

1. What is the distribution of cell sizes in the tissue?
2. What cell types divide and to what extent?
3. How much linear expansion of the tissue occurs, and is there a preference for expansion with regard to cell type or direction?

The amount of linear expansion of the whole tissue and the stomata is summarized in Table VIII. The stomata grow more slowly than the other epidermal cells, the more so, the older they are. The mean cell size of laminal epidermal cells, measured at right angles along the lines in Plate IX indicates that the cells grow at equal rates in the measured directions (Table IX). The distribution of relative cell sizes is shown in fig. 4.2: cell sizes are distributed mainly in a range between the smallest and 2 × the smallest values of each stage.

TABLE VIII. Linear expansion of the whole tissue and of the stomata in the epidermis in plate IX at stages II and III as percentages of the value at stage I.

	Stage II	Stage III
Tissue	172	229
Stoma A	168	220
Stoma B	136	161

TABLE IX. Mean cell diameter measured in two directions at right angles (rel. units).

Stage	Direction	↔	↕	Mean
I		62.13	63.13	62.63
II		108.41	106.71	107.56
III		141.95	144.51	143.23

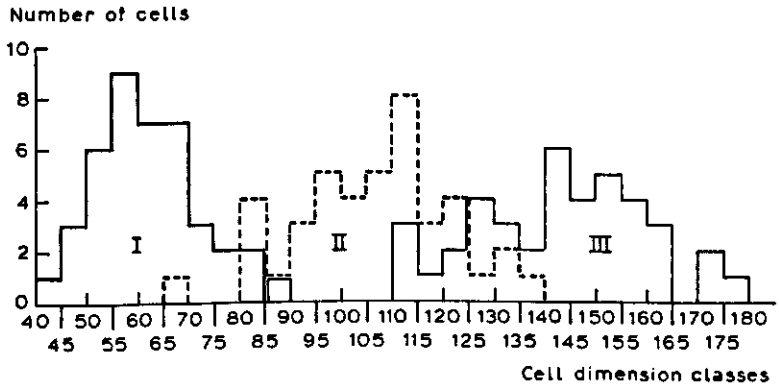
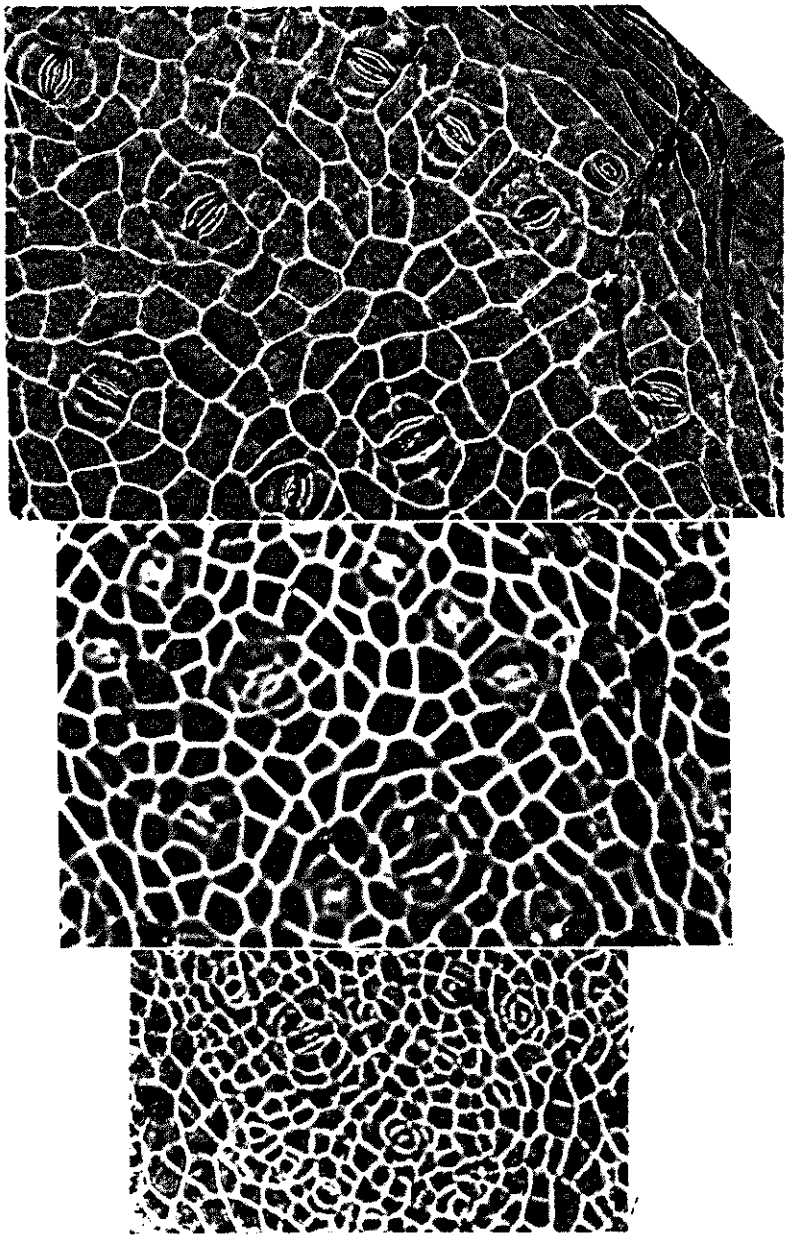


FIG. 4.2. The distribution of cell diameters (as cell dimension classes in rel. units) at three stages of development of the same region of the epidermis of a poplar leaf. Data taken from Plate IX.

A careful inspection of Plate IX shows that during this stage of development no cell division takes place in the epidermis of the lamina, except for regions around the stomata. In the epidermal cells above the veins numerous divisions are seen. In agreement with data of the literature (BÜNNING, 1957), most of the stomata are formed during the earlier phases of linear expansion. Even in stage II, a square cell bordering the vein is seen in a stage preceding the formation of a stoma. In stage I, a row of cells on the right hand side of the plate, is also in a stage preceding the formation of stomata, although situated only two cells apart.

To obtain some rough idea how the growth rate is divided over the cells, the individual cells have been sorted out in growth classes, designating the mean diameter of the individual cells in relative units in stage I as percentage of their final mean diameter in stage III. The smaller this percentage, the stronger the


Stage
III
II
I

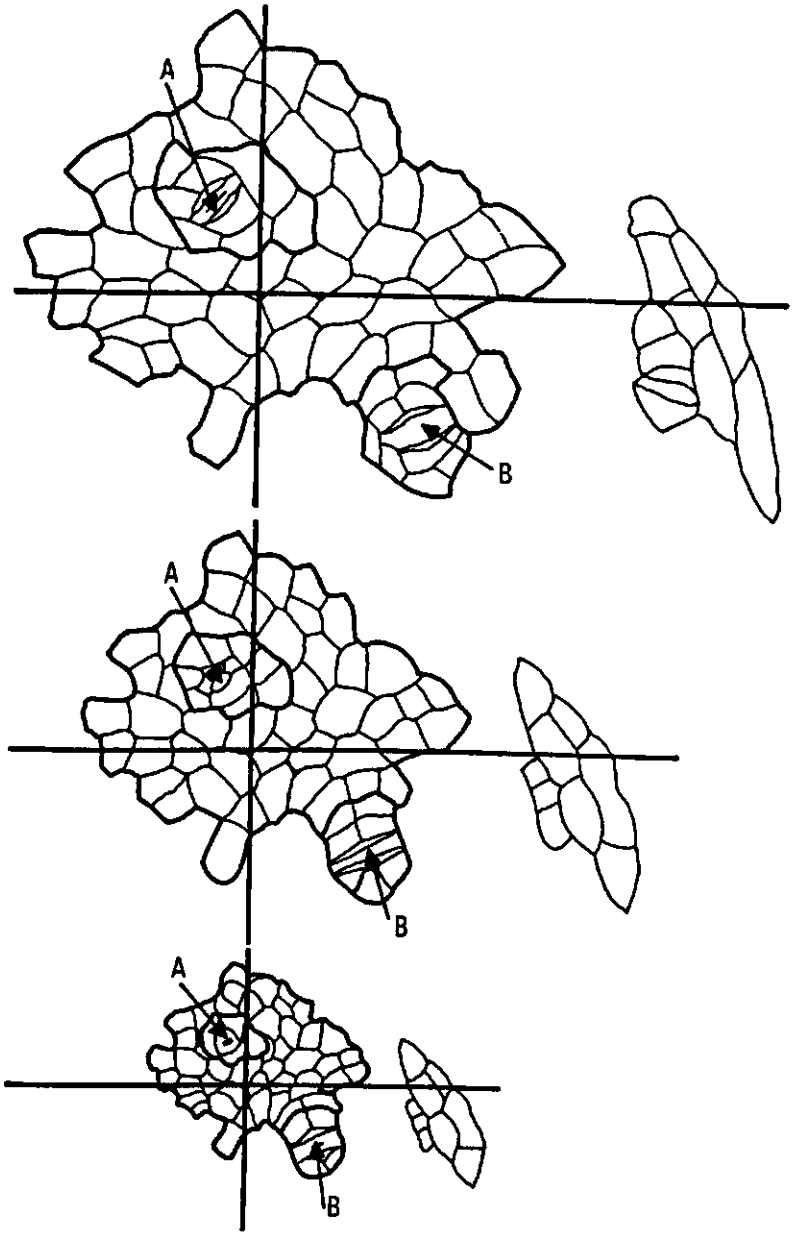


PLATE IX. The expansion of the epidermis followed at cellular level with the aid of the silicone rubber technique. The magnification of the preparations is constant and the three pictures show the same region of the epidermis. Stage I is young, stage II intermediate, and stage III old.

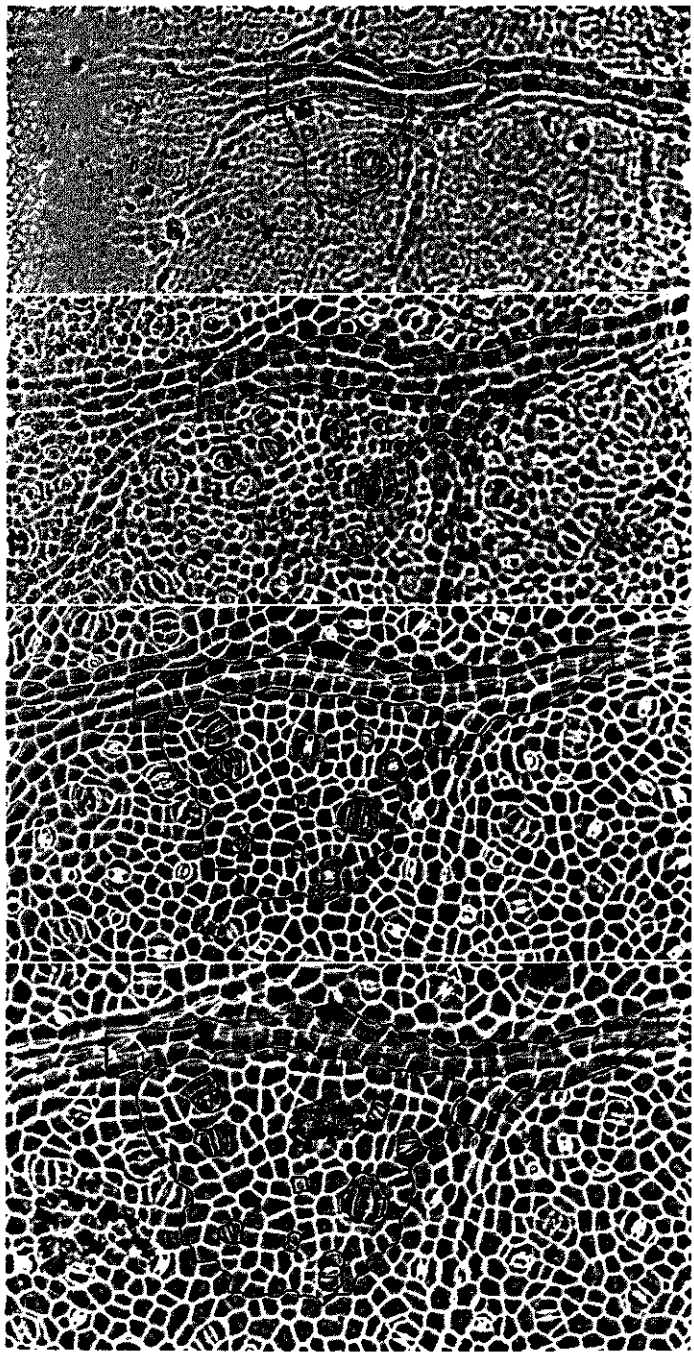
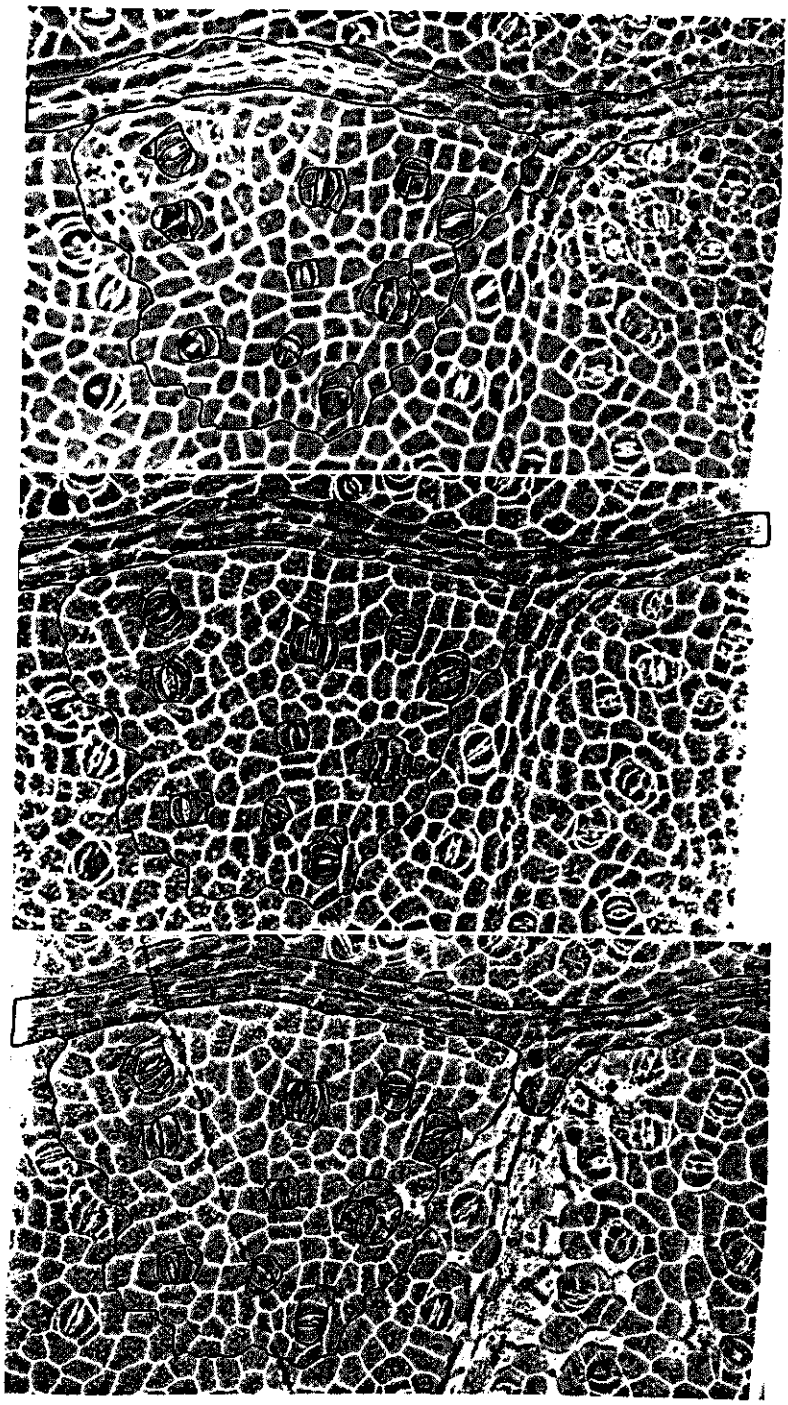


PLATE X. The expansion growth of the epidermis followed at cellular level with the aid of the silicone rubber technique. The magnification of the preparations is constant. The seven pictures show the same region of the epidermis.



V

VI

VII

TABLE X. The relation between the diameter of the cell and its growth rate expressed as the difference in size at stage III and stage I.

Growth rate	Growth class	Number of cells per class	Rel. mean diameter			
D_3-D_1	$D_1/D_3 \times 100$		Stage I	Stage II	Stage III	Remarks
1.35	25–30	1 (2×)	0.55	1.20	1.90	2 cells
1.06	30–34.9	2	0.54	1.09	1.60	(divided)
0.91	35–39.9	7	0.54	1.06	1.45	
0.82	40–44.9	16	0.62	1.08	1.44	
0.76	45–49.9	11	0.68	1.08	1.44	
0.70	50–54.9	1	0.72	1.15	1.42	
0.56	55–59.9	2	0.75	1.08	1.31	

growth afterwards has been (see Table X). The table clearly shows that some order can be detected in the growth rate of the individual cells. The causes of the fact that the smaller cells grow faster is not known and may just be an expression of the tendency to reach a constant mature size.

Another set of replicas of 7 successive stages in the expansion of the epidermis of a poplar leaf (Plate X) may be used to illustrate the important differences in growth pattern between the veins and the leaf blade. Table XI shows that

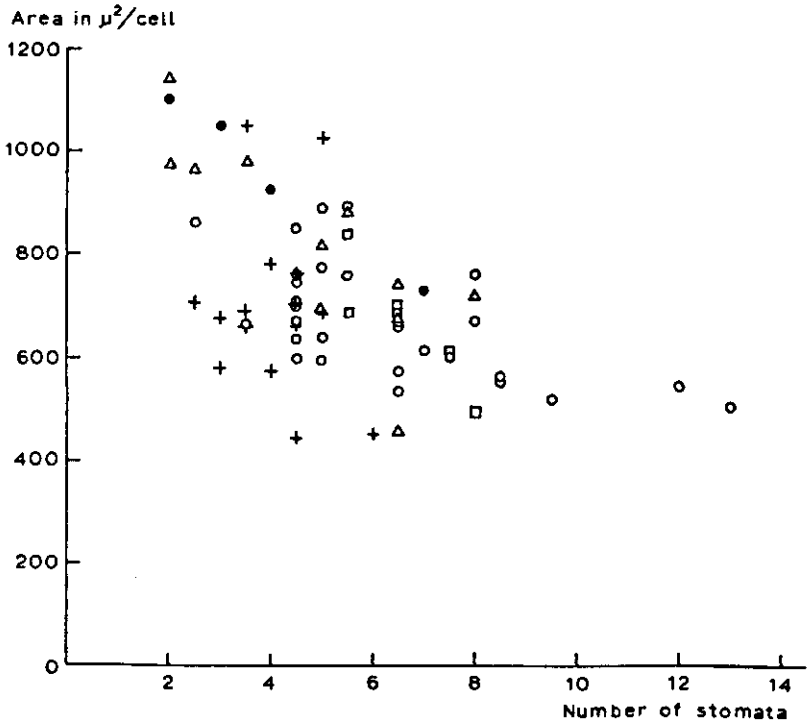


FIG. 4.3. The relation between the area per cell (μ^2) and the number of stomata per unit area. For symbols: see fig. 3.8.

TABLE XI. Increase of area and linear dimensions in relative units of epidermal tissues of leaves of *Populus euramericana* 'Robusta'.

Stage	Number		Area			Linear dimensions							
						Vein				Lamina		Stomata	
	of cells	of stomata	Vein	Blade minus stomata	Stomata	length		width (= 0/L)		blade minus stomata			
						L	%	W	%	A‡	%	A‡	%
I		3	6.10	5.65	0.4	5.1	28.3	1.19	87.0	2.38	29.4	0.63	18.3
II		10	12.07	15.09	2.1	9.2	51.1	1.31	95.3	3.88	48.0	1.46	42.2
III	143		14.32	23.96	3.8	11.8	65.5	1.21	88.0	4.89	60.4	1.94	45.2
IV			16.05	33.03	5.0	13.8	72.2	1.22	89.0	5.75	71.0	2.25	65.0
V			24.45	62.89	9.4	17.6	97.7	1.38	101.0	7.93	98.1	3.06	88.7
VI			24.45	65.21	10.8	18.0	100.0	1.36	99.0	8.08	99.0	3.29	95.4
VII	151		24.80	65.50	11.9	18.0	100.0	1.37	100.0	8.09	100.0	3.45	100.0

the area of the vein increases about 4.1 fold and that of the lamina 11.6 fold during the development of the leaf from stage I to stage VII. This difference is due to the fact that the growth of the vein proceeds largely in one direction, while that of the lamina proceeds in all directions. The expansion of these tissues, however, is adjusted to each other in such a way that the leaf remains nearly flat during expansion, contrary to what occurs e.g. in lettuce (BENSINK, 1971). The area occupied by the stomata increases 29.8 fold. This large increase is due to the fact that 7 new stomata arise. Older stomata contribute less to the formation of leaf area, indicating that a newly initiated stoma develops directly and independent of the other epidermal tissue to maturation. The contribution of the stomatal tissue (however, including possibly here also some epidermal cells) to leaf area is not unimportant. Factors stimulating the initiation of new stomata, thus may also influence leaf area formation to some extent. It may be added here that there are extraordinarily large differences in the number of stomata per unit leaf area (fig. 4.3). It may be of interest to investigate how these large differences arise.

4.7. CONCLUSIONS

The conclusions of chapter 4 on epidermal cell measurements of leaves of *Populus euramericana* 'Robusta' may be summarized as follows:

1. There is no clear systematic pattern in the distribution of mean mature epidermal cell sizes over the leaf blade, but it cannot be excluded that there is a mosaic-like mini-pattern causing differences from spot to spot.
2. In poplar plants cultivated under the conditions described, mean mature size is not correlated with leaf position. In many other plants such a correlation is found and it is suggested that this is partly due to a water and ion deficiency when the plants have grown large.
3. Cell division in the epidermis of the lamina stops at an early phase of leaf development.
4. Mean mature epidermal cell size of the leaf blade is weakly influenced by light or temperature, although in plants from low temperature (16 °C) mean mature cell size tends to be somewhat larger; the mature leaf size is a measure of the number of cells, irrespective of treatment.
5. In expanding leaves, there is a definite pattern in the maturation of the cells. The leaf tip matures first and the region, where the leaf develops its largest width, matures last. Cell division must continue longer in that region, since the leaf blade is broader.
6. In contrast with the epidermal cells above the parenchymatous parts of the leaf, the epidermal cells above the midrib and the veins do not expand isodiametrically, but become elongate.
7. It has been found that the development of the stomata, by the induction of meristemoids, contribute not unimportantly to the area formation of the leaf. There are extraordinarily large differences in the number of stomata per unit leaf area.

5. THE GROWTH OF SUCCESSIVE LEAVES IN RELATION TO AGE, LIGHT INTENSITY AND TEMPERATURE

5.1. INTRODUCTION

In chapter 3 it was concluded, that by measuring lengths of leaves at regular intervals, a good picture of the growth of *Populus euramericana* 'Robusta' could be produced. Therefore, in this and subsequent chapters, it appeared unnecessary to pay attention to the width of the leaves.

5.1.1. *The relation of increase in leaf length to light intensity at 20 °C*

Fig. 5.1 summarizes the increase in length of successive leaves of healthy plants, cultivated at three light intensities (I, II and III). The figure shows that large differences in growth rate exist between the plants at these light intensities. Under the heading 'mean linear growth rates' in Table XII this is illustrated by the mean linear growth rate values (m, in cm/day) of a number of successive leaves at the point, where they have reached half their final lengths. The magnitude of the differences is even somewhat underestimated because at high light intensity, growth rate increases over a larger number of leaves. From the point of view of leaf area formation, these differences are large and important.

The relative rate of growth in length, S (cm/cm. day), when the leaf has attained half its length is defined as the ratio of the linear growth rate and half the mature length of the individual leaf,

$$S = \frac{2m}{L_m} \qquad (2)$$

L_m is also a measure of the number of cells in the leaf as has been shown in chapter 4 and irrespective of treatment, the number of cells per cm mature

TABLE XII. Mean linear growth rates m, in cm/day, and mean relative growth rates, $S = 2m/L_m$, in cm/cm. day of leaves at half their final length of plants from various treatments of light and temperature.

Light intensity	40 W/m² (I)		20 W/m² (II)		10 W/m² (III)	
Temperature	m	S	m	S	m	S
16	0.567	0.1160				
20	1.479	0.1598	1.328	0.1580	0.877	0.1404
25	1.670	0.1872				

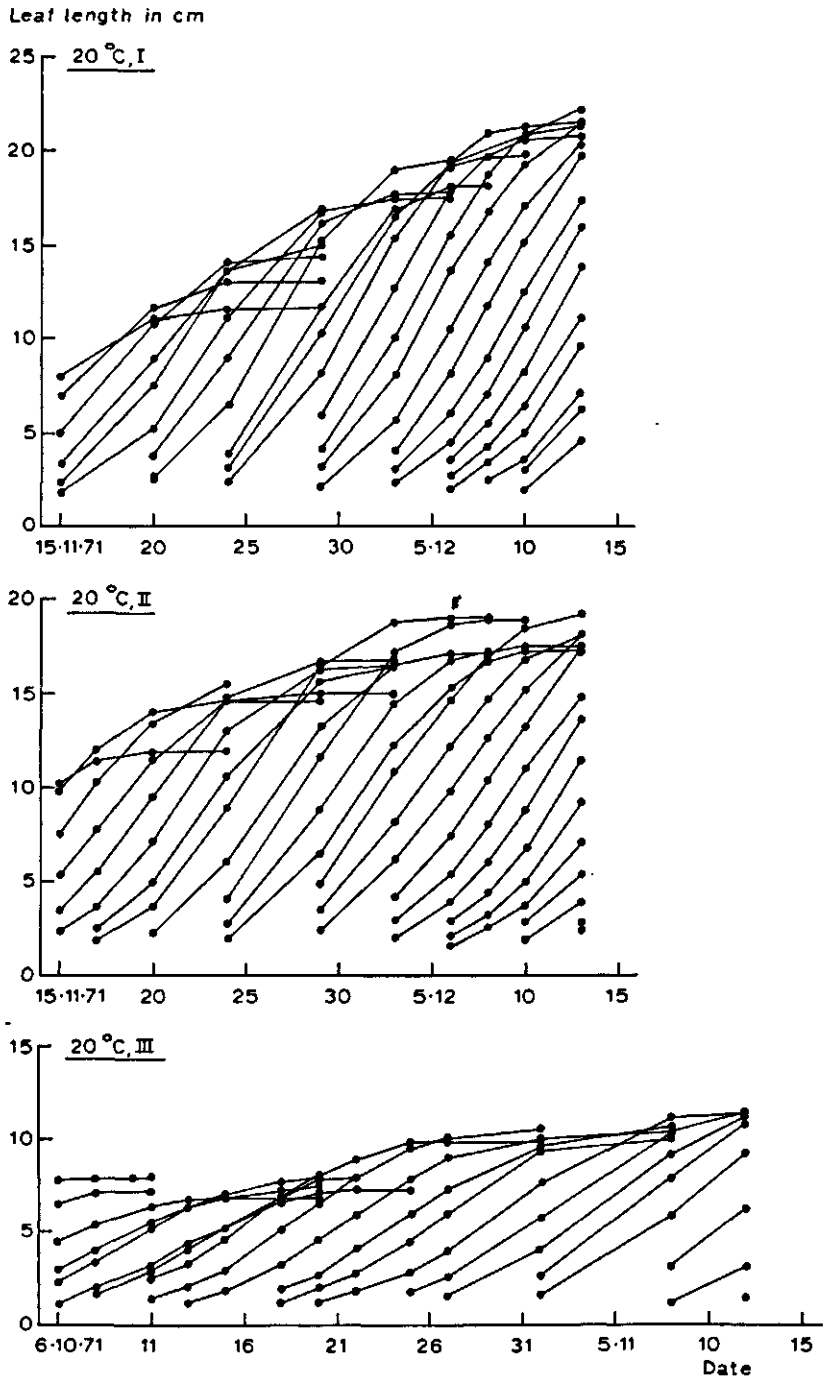


FIG. 5.1. Leaf length against time of poplar leaves on plants cultivated at different light intensities at 20 °C. Light intensities: I 40 W/m², II 20 W/m², III 10 W/m².

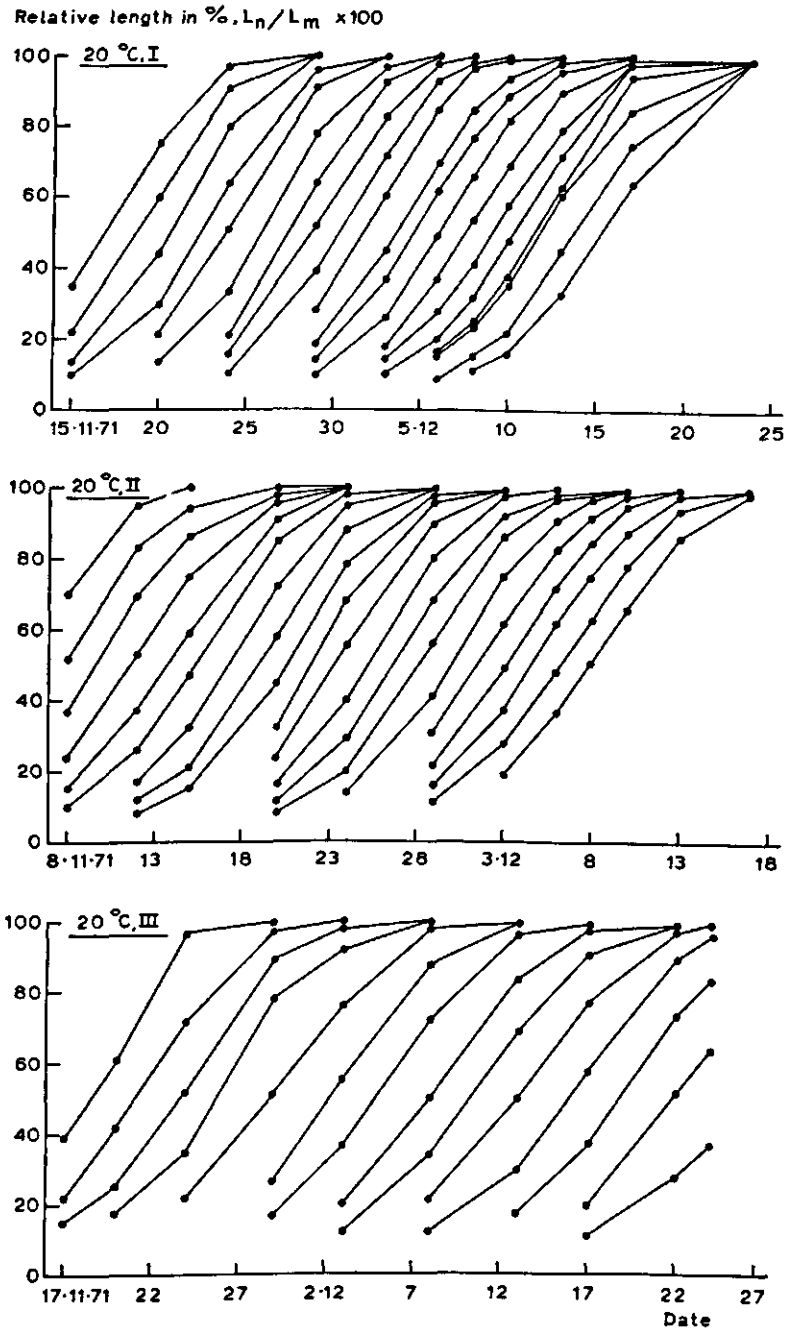


FIG. 5.2. Leaf length (L_n), expressed as a percentage of the individual mature leaf length (L_m) against time of poplar leaves of plants cultivated at the indicated light levels. – The inclination of the lines is constant and the number of leaves, produced per unit time is higher at higher irradiation levels.

leaf is constant (± 350 cells/cm). Division of the relative growth rate S by the number of cells/cm mature leaf length, yields a new constant, viz.: the absolute growth rate per cell.

The small differences, found in the value of S (see Table XII) between the three light intensities, at 20 °C, may be interpreted as an indication that in the linear phase of growth, light intensity has little influence on the growth rate of the cells. This is illustrated once more by fig. 5.2 in which the length against time relationships of the different leaves of fig. 5.1 are transformed into percentages of their individual final sizes against time. It is clear that there are no large differences in the growth rates per cell between sun and shade leaves or between large and small leaves. The differences in linear growth rates of leaves, grown at constant temperature, have to be accounted for by the differences in the number of cells. The wider spacing of the curves of fig. 5.2 at low light intensity, against data, as compared with high light intensity reflects the effect of light intensity on the rate of leaf formation in the apex.

The conclusion that the expansion of the cells is only slightly influenced by light intensity, can be confirmed by experiments in which individual leaves were shaded. This resulted in only a slight decrease of growth rate and final size. In other experiments whole plants were transferred from high to low light intensity which resulted in an immediate, but slight decrease of growth rate (fig. 5.3).

Assuming that the small differences in relative growth rate are really due to light intensity, it may be concluded that the rate of cell expansion at 20 and 40 W/m² is light saturated as is illustrated in fig. 5.4.

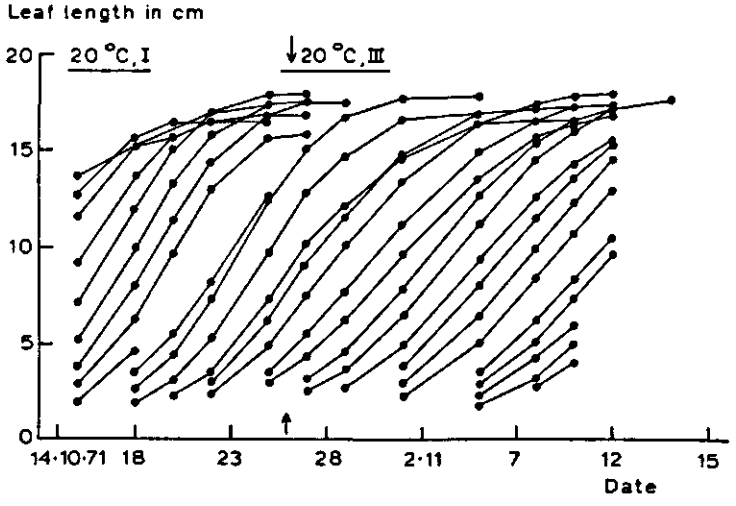


FIG. 5.3. Leaf length against time of poplar leaves of a plant grown at high irradiation level and then transferred to low irradiation level at the moment indicated by the arrows. – An immediate slight decrease in the rate of expansion of the leaves occurs, but an immediate reaction of mature leaf length is absent.

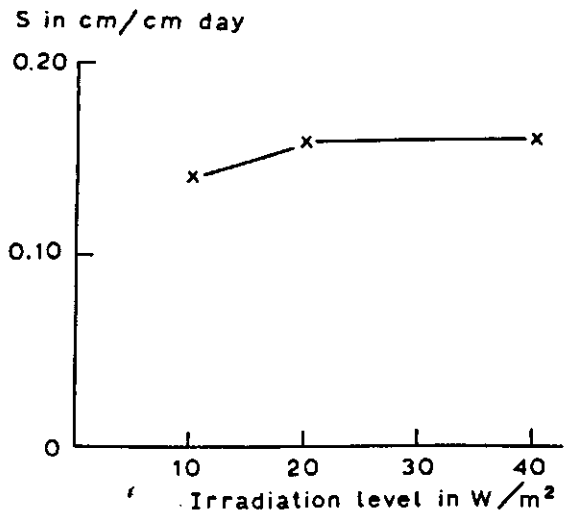


FIG. 5.4. The relation between relative growth rate ($S=2\ m/L_m$) of leaf length and the irradiation level at which the poplar plants were cultivated.

5.1.2. *The relation of increase in leaf length to temperature*

In the linear phase of growth the relative growth rate at half the final length of the leaf, S, of the individual leaves depends only weakly on irradiation and this dependency on light shows light saturation above 20 W/m². This means that it is advantageous, when investigating the influence of temperature on S, to use only data from plants exposed to high light intensity to prevent interference of light.

Fig. 5.5 shows the length against time relationships of leaves of three plants grown at more or less constant light intensity (above 20 W/m²) at temperatures of 16, 20 and 25 °C respectively. Also in this case large differences in linear growth rate (m) are seen, as is summarized in Table XII. At the same time we see that the relative growth rates show appreciable differences.

The important influence of temperature on cell expansion is clearly demonstrated (fig. 5.6), if, also here, the length against time relationships of fig. 5.5 are plotted as percentage of their individual final leaf lengths against time.

Fig. 5.7 shows the relation between relative growth rate and temperature. Like in many other physiological processes, 'Q_{10}' decreases over an extended temperature range (cf. WASSINK, 1972); in the present case Q_{10} between 20° and 16 °C was 2.24, Q_{10} between 25° and 20 °C was 1.38, the average Q_{10} was 1.70.

5.1.3. *Discussion*

In the preceding sections it has been established that in the linear phase of growth, cell expansion in poplar depends only slightly on light intensity (cf. WASSINK, 1969, with gladiolus and tulip), and strongly on ambient temperature. This conclusion may have some general validity, because the growth of poplar leaves possesses characteristics which are commonly found in leaf growth (cf.

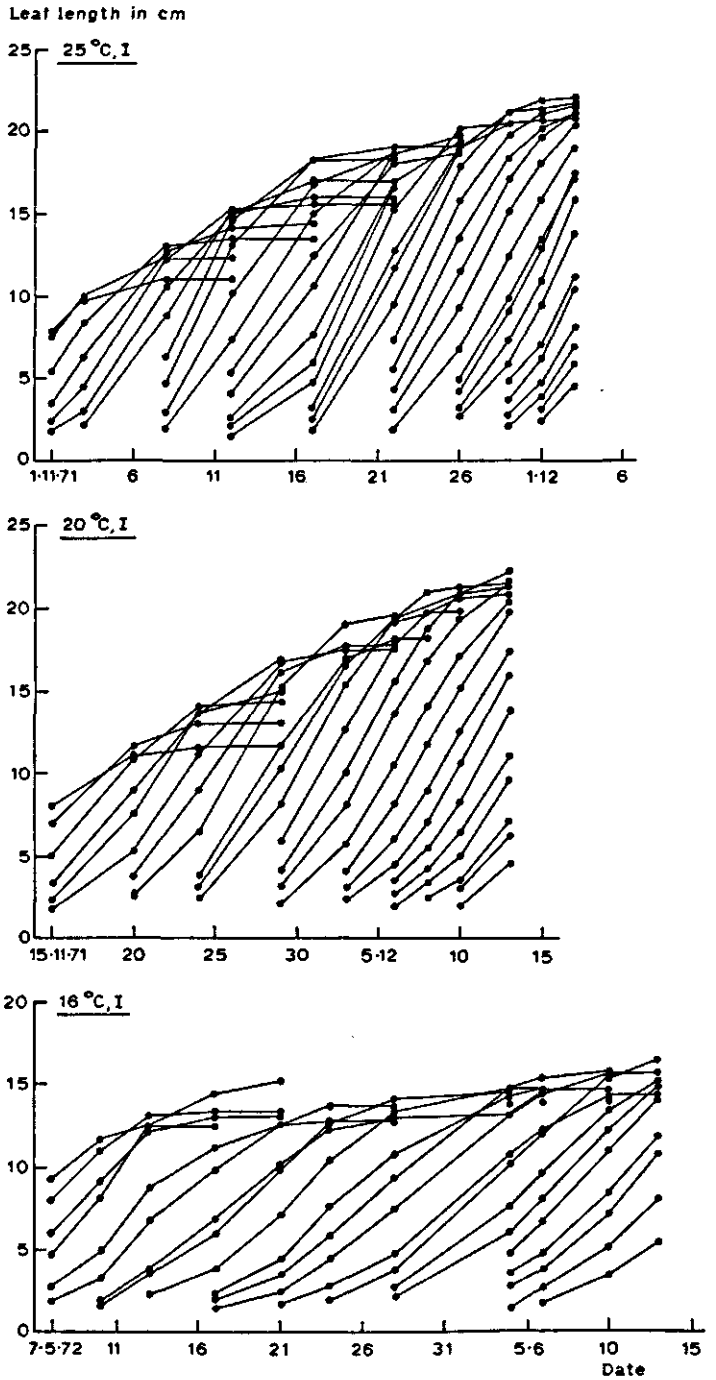


FIG. 5.5. Length against time of poplar leaves of plants cultivated at a light intensity of 40 W/m², and 16°, 20°, and 25°C

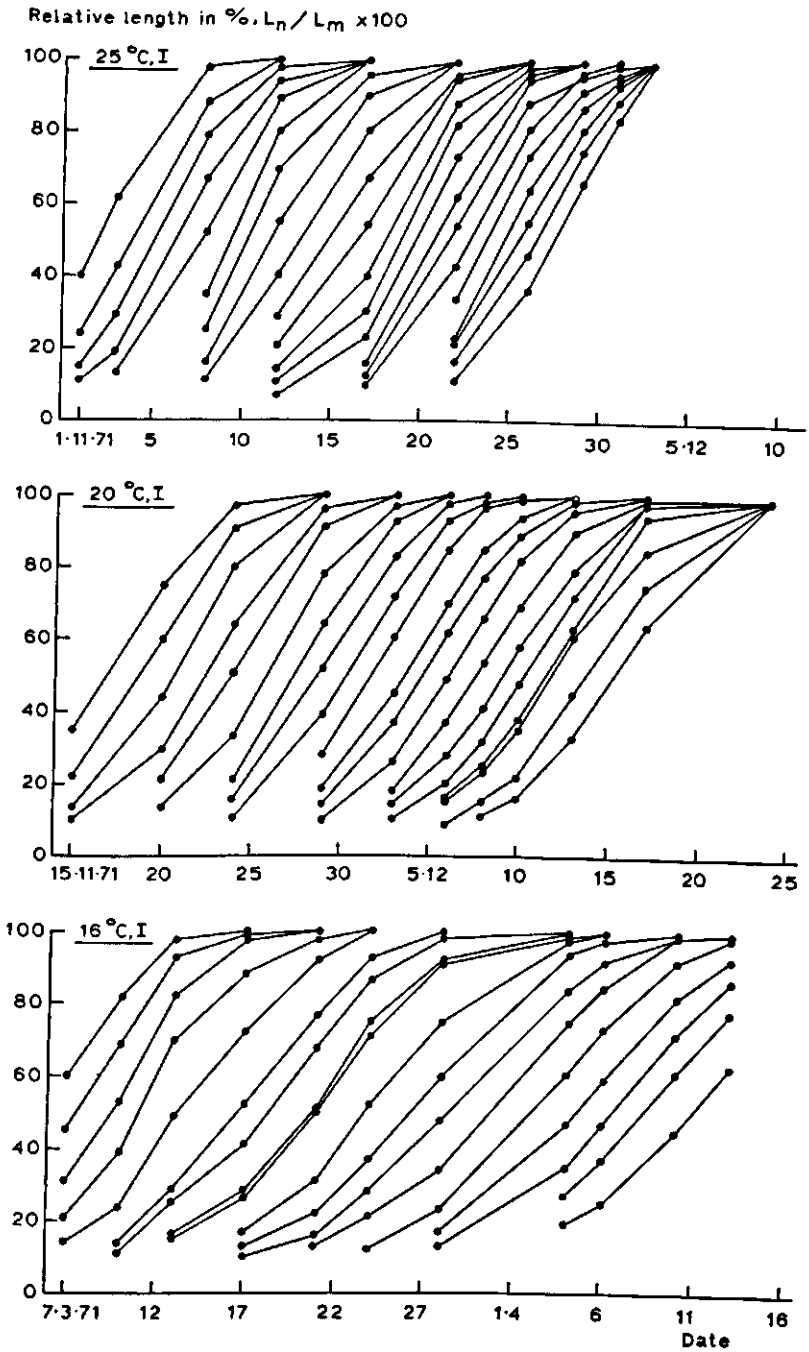


FIG. 5.6. Leaf length (L_n) expressed as a percentage of the individual mature leaf length (L_m) against time of poplar leaves of plants cultivated at a light intensity of 40 W/m² and at the temperatures of 16°, 20°, and 25°C. – The inclination of the lines and the number of leaves, produced per unit time, both depend on temperature.

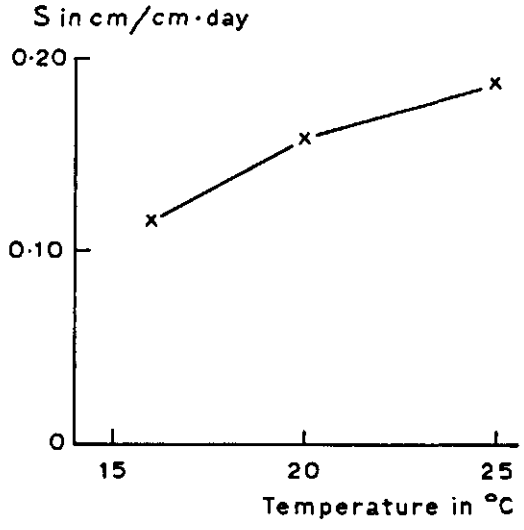


FIG. 5.7. The relation between relative growth rate (S) of leaf length and temperature at which the poplar plants were grown. Irradiation level 40 W/m² and temperatures 16°, 20° and 25°C.

DALE, 1965; MILTHORPE, 1956; MILTHORPE and NEWTON, 1963; NEWTON, 1963). The results also indicate that the time needed for the growth of a leaf in the linear phase depends mainly on temperature. WOLF (1947) reached similar conclusions on the growth of tobacco leaves.

In chapter 4, it was established that cell division stops early in the development of the leaf area and of the epidermis (cf. MAKSYMOWICH, 1963). This does not imply that in this phase of growth no cell division in the leaf occurs. The development of leaf thickness, for instance, goes on until and even after the end of leaf expansion, and is accompanied by cell division in the mesophyll. The divergence of opinion in literature over the moment at which cell division stops, may be due to the fact that cell division in different tissues have not always been properly distinguished (MILTHORPE and NEWTON, 1963). Some cell division in the epidermis still takes place during the linear phase of growth, but this is confined to the meristemoids of the stomata and to the parts covering the midrib and the veins. The contribution of the stomata to total leaf area is fairly important and varies considerably from leaf to leaf, even on the same plant. The initiation of stomata seems therefore to be under the control of factors which show considerable variation, cf. fig. 4.3. This may in part explain the variability found in the rates of leaf expansion.

At constant temperature, differences in the growth rates of leaves have to be ascribed mainly to differences in cell number. Hence, the differences in the growth rates of the area of sun and shade leaves, or of large and small leaves can be traced back to differences in the rates of cell division in the growing tip. After cell division has ceased in an early stage of leaf development, the rate of leaf growth equals the rate of cell expansion times the number of cells and, vice versa, the rate of leaf growth divided by the number of cells ($\sim$ mature leaf length) represents the rate of expansion per cell. This is shown to be fairly

independent of irradiation in fig. 5.4 and dependent on temperature in fig. 5.7. It is clear that the later parts of the sigmoid growth curve of the leaf when division has ceased are based on the average characteristics of cell expansion, so that leaf growth appears essentially a problem of cell physiology.

Leaf growth (increase in length) and thus cell expansion was found temperature dependent to an extent, suggesting the partial limitation by enzymatic processes especially at the lower temperatures [Q_{10} (16–20 °C) = 2.24, Q_{10} (20–25 °C) = 1.38]. This is in agreement with the idea that cell expansion involves more than absorption of water. It has been demonstrated that the synthesis of proteins continues until the end of cell expansion. HABER and FOARD (1962) studied the growth of gamma irradiated seeds of wheat. The irradiation of the material destroyed a system controlling cell division and the seeds germinated and grew without cell division taking place. In this way investigation of expansion growth per se was possible. It was concluded that many functions in these plants were performing reasonably normal: there was a net increase of dry matter, protein and RNA. The photosynthetic apparatus and the pigment system developed and photosynthesis occurred, yielding sugar phosphates, sucrose, amino acids and organic acids. Cell size was somewhat larger than normal. The protein content of normal plantlets of comparable size was similar to that of the irradiated ones.

DALE (1968) measured the synthesis of proteins and cell wall material during leaf expansion. Total nucleic acid content, protein content and cell wall components per cell increase parallel with expansion. The rate of increase of these products was the same whether cell division occurred or not. The level of nucleic acids and proteins reached a maximum with the attainment of full size of the lamina (cf. SMILLIE and KROTKOV, 1961; NIEMANN, 1965). This substantiates earlier evidence that cell expansion is not merely water uptake but increase in protoplasmatic material as well.

Finally, it must be emphasized that large deviations of the proposed scheme of leaf growth results, as soon as the mean diameter of the mature epidermal cells of the lamina changes. This may be a consequence of the position of the leaf on the plant (cf. ZALENSKI, 1929; ASHBY and WANGERMANN, 1950; MARENKOV *et al*, 1971), of water strain or xerophytism (cf. SCHWABE, 1956; WHALEY, 1965; WANGERMANN, 1961; NIEMANN, 1965; MARENKOV *et al*, 1971; WOOLLEY, 1973), of shortage of mineral nutrients (cf. FERNANDO, 1958; MILTHORPE and NEWTON, 1963), of a relative change in shape of the epidermal cells (cf. DALE, 1968) or of reduced length growth by light inhibition (cf. BENSINK, 1971).

5.2. THE LENGTHS OF SUCCESSIVE LEAVES ALONG THE MAIN AXIS IN THE LINEAR PHASE OF GROWTH

5.2.1. *The succession of leaf lengths in the growing region*

Fig. 5.8 shows the lengths of successive leaves, numbered from the base of the plant, along the main axis of plants under different treatments of light and

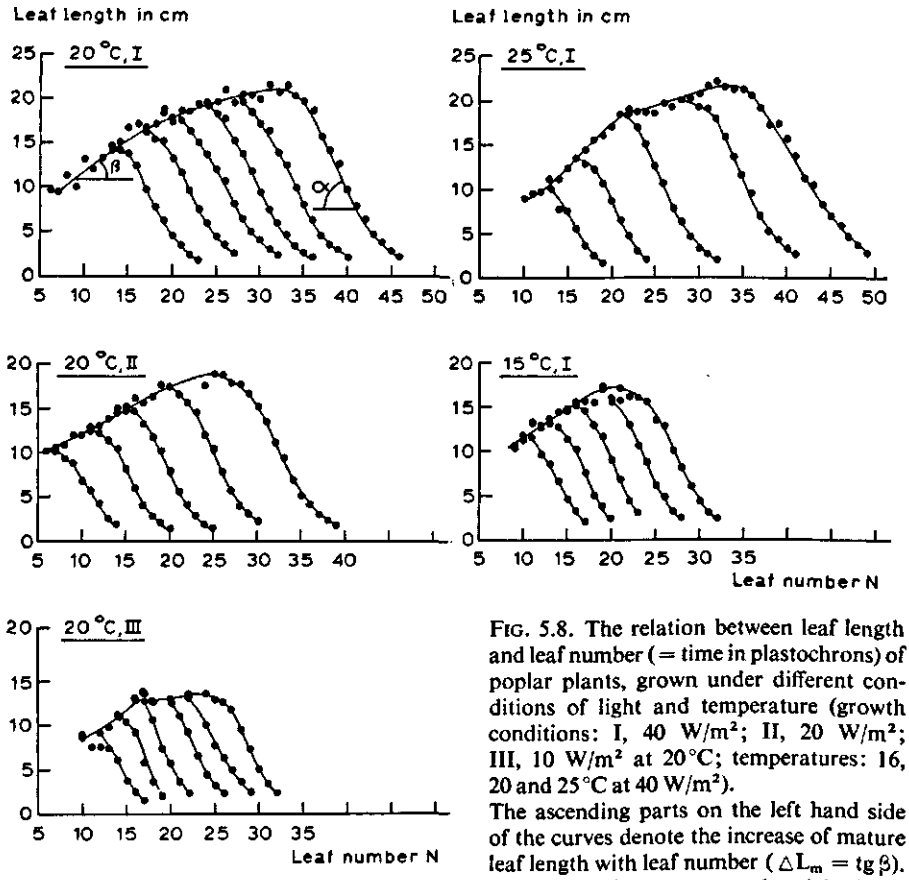


FIG. 5.8. The relation between leaf length and leaf number (= time in plastochrons) of poplar plants, grown under different conditions of light and temperature (growth conditions: I, 40 W/m²; II, 20 W/m²; III, 10 W/m² at 20 °C; temperatures: 16, 20 and 25 °C at 40 W/m²).
The ascending parts on the left hand side of the curves denote the increase of mature leaf length with leaf number ($\triangle L_m = tg\,\beta$). The descending parts on the right hand side denote the decreasing lengths of the younger leaves ($\triangle L = tg\,\alpha$). – The values of tg α and tg β are not noticeably influenced by the treatments.

temperature, at various stages of development. The ascending part on the left hand side of the curves describes the increase of mature leaf length with leaf number. This increase, characterized by tg β, is approximately constant and independent of treatment in the early stages of development, but later the mature leaves are of the same size. The descending parts on the right hand side each indicate the lengths of the growing leaves in their successive stages of development, at different moments of measurement. The constancy of the slopes of successive lines indicates that the difference in length of successive leaves is more or less constant notwithstanding differences in the developmental stage of the entire plant, and in environmental conditions. (It should be observed here, that the presentation of the data against leaf number entails that effects of rates of development in principle are absent in graphs of this type.)

Obviously, the above relationship is largely linear, representing a descending arithmetical progression. Its common difference (ΔL) represents the difference in length of two successive leaves at any moment of measurement, and determines the slope of the relation (denoted by tg α in fig. 5.8).

The length of the n^{th} leaf behind the youngest mature leaf with length L_m thus can be expressed by the formula:

$$L_n = L_m - n \cdot \Delta L \qquad (3)$$

TABLE XIII. Mean values of ΔL (in cm) in plants from various treatments of light and temperature.

Temperature (°C)	Light intensity		
	40 W/m²	20 W/m²	10 W/m²
16	2.069		
20	1.823	2.044	1.672
25	1.940		

Some mean values of ΔL for plants of different treatments are given in Table XIII. It may be concluded, that there are no important differences in ΔL between the various treatments. It should be observed that there is still a variation of ΔL within each treatment, which cannot be connected to the special treatment given to a plant and must be of a more general nature. From experience it is concluded that the conditions in the root environment have a great influence on this variation. An example of such a variation of ΔL is shown e.g. by plant III-3 (fig. 5.9a), which was growing poorly on a deficient nutrient solution. This variability of ΔL is often found in poorly growing plants from all treatments. The effect of transfer of a plant from light intensity III to I is shown in fig. 5.9b.

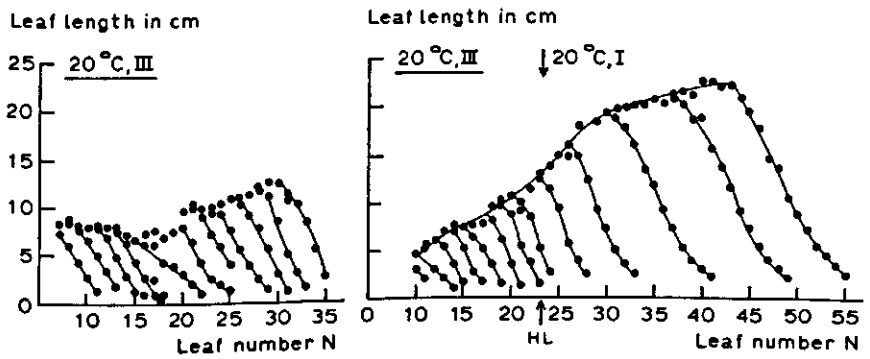


FIG. 5.9a. The relation between leaf length and leaf number of a plant from irradiation level III, growing on a deficient nutrient solution. Between the 15th and 20th leaf the deficient nutrient solution has been changed. – Note the variability in ΔL and leaf length.
FIG. 5.9b. The relation between leaf length and leaf number of a plant from irradiation level III and then transferred to irradiation level I.

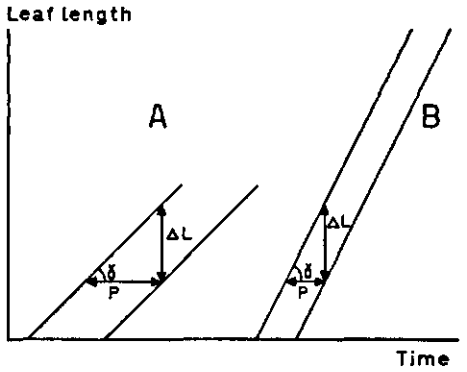


FIG. 5.10. Schematic representation of the increase in leaf length against time of two successive leaves on a plant, growing either slowly (A), or vigorously (B), to clarify the relation between linear growth rate m, the time elapsing between the formation of the leaves (plastochron duration, = P), and the difference in length of successive leaves, ($\Delta L = m.P$).

In general it may be concluded that the lengths of successive growing leaves represent a descending arithmetical progression, in which the common difference = ΔL is independent of light and temperature treatments. This is a simplification which is justified because especially the vigourously growing larger leaves contribute strongly to the area formation.

5.2.2. *The significance of the constancy of* ΔL

It is instructive to analyse in some detail the relation of leaf length against time, which is represented schematically in fig. 5.10, to understand the significance of the constancy of ΔL. The first two parallel lines represent leaf length against time of two successive leaves on a slowly growing plant; the second pair of lines the same for a vigorously growing plant. It is easily seen that the vertical distance between the parallel lines of each single pair equals ΔL, since this distance represents the difference in length between two successive, growing leaves at one moment of measurement. It follows that

$$\Delta L = m \cdot P = \frac{S \cdot L_m \cdot P}{2} \qquad (4)$$

in which m ($\text{tg}\,\gamma$ in fig. 5.10; see also section 5.1, Table XII) is the linear growth rate (cm/day) and P is the plastochron duration, which is the number of days between the appearance of two successive leaves of a particular length (see also section 5.7 on leaf appearance). The shorter the duration of P, the faster the age of the plant increases in plastochrons and thus the higher the rate of leaf production.

It is concluded, that, because of the constancy of ΔL, there exists a linear relation between the rate of leaf production (1/P) and the growth rate (m) in the linear period of growth of the leaves. There exists also a linear relation between the rate of leaf production and the cell number of the leaves, because the linear growth rate of the leaves is determined by the number of cells in the leaf and thus by cell division in the growing tip.

ONDOK (1968) attempted an analysis of leaf growth of *Typha* spp. and *Scirpus*

spp. on the basis of the autocatalytic growth formula and also concluded to a parallelism between growth rate and leaf production.

5.3. Results of mathematical analysis of growth in the linear phase

A mathematical analysis of the data on growth of poplar has been presented in the appendix. In this section the results of this analysis will be discussed. The initial assumption of the mathematical treatment was that the leaves are growing linearly from initiation till final size within a constant growth period for all leaves and that each plant is forming leaves of constant size. Shade plants growing in low light intensity produce small leaves and sun plants growing in high light intensity large leaves. The pathway along which these differences in size are reached are not yet considered here.

5.3.1. *Relative growth rates*

Length. The relative growth rate S with respect to the length of a leaf, that has reached half its final length, has been shown to depend on temperature and to be fairly independent of light intensity (see section 5.1). In the appendix it has been shown that the average relative growth rate of total leaf length of a plant is also S and thus equals that of the individual leaf at half its final size. The conclusion is that differences in mature leaf length and also in absolute growth rate of leaves in the linear phase of growth in sun and shade plants are due to differences in number of cells which originated in earlier phases of growth.

Width. The appendix shows that the average relative growth rate of total leaf width of a plant is equal to:

$$RGR_w = S \cdot \frac{L_m}{L_m - 2}$$

The equation shows, that relative growth rate of width is highest for small leaves and approximates S for large leaves. In the early linear phase of growth cell division is confined to the epidermis above the veins and to the stomatal regions, so that the number of cells in the leaf blade at half its final width is equal to that in the mature leaf. The relative growth rate divided by the number of cells in the width of the leaf equals the absolute rate of growth per cell. The dependence of relative growth rate on the width of the leaf suggests that cells in leaves with a small mature width are growing faster than in leaves with a large mature width. This may be explained by assuming that the initiation of the growth in width starts later in small leaves than in large leaves, so that at the moment considered the mean age of cells in small leaves is lower than in large leaves.

The average relative growth rate in the larger leaves cannot explain the higher absolute rates of growth in these leaves. Again the causes of these differences in absolute growth rate are due to differences in the number of cells present.

Area: Calculation of the mean relative growth rate of total area (see appendix) gives:

$$RGR_s = \frac{3}{2} \cdot S \cdot \frac{L_m}{L_m - 1}$$

The mean relative growth rate of total area thus is not equal to the average relative growth rate of total leaf length, because the leaf that has reached half its final area in the succession of growing leaves does not coincide with the area of the leaf that has attained half its final length. When the mature length of the leaves becomes large the mean relative growth rate of total area of growing leaves approximates $^3/_2$ S. Again the higher absolute growth rates of the area in large sun plants cannot be explained by differences in their relative growth rates. The causes of differences in absolute growth rates have to be found in earlier stages of growth, i.e. when the number of cells is determined.

5.3.2. *Absolute growth rates per plant and the area of growing leaves*

The absolute growth rates in the various dimensions of a plant growing at a constant rate have been calculated in the appendix. The average increase of total leaf length per day equals $L_m^2 \cdot S/(2 \cdot \Delta L)$, that of total width $L_m \cdot (L_m - 2) \cdot S/(2 \cdot \Delta L)$ and that of total area $K \cdot L_m^2 \cdot (L_m - 2) \cdot S/(2 \cdot \Delta L)$. The average total length of growing leaves amounts to $L_m^2/(2 \cdot \Delta L)$, that of total width $(L_m^2 - 4 \cdot L_m + 4)/(2 \cdot \Delta L)$ and that of total area $2 \cdot K \cdot L_m \cdot (L_m^2 - 3 \cdot L_m + 2)/(6 \cdot \Delta L)$. All these values are averages, because in the model of growth adopted here, the absolute growth rates per plant and the total growing length, width and area are discontinuous. The total growing length, width and area and their rates of growth are highest just before maturation of a leaf and lowest just after its maturation. The period of this discontinuity is one plastochron duration. It thus appears possible to express total dimensions and growth rates as cubes and squares of L_m, the length of the youngest mature leaf. The total growing area, A_g, just before maturation of the largest leaf, e.g., is:

$$A_g = K \cdot (1/6 \cdot L_m^3 - 2/3 \cdot L_m) \approx 1/6 \cdot K \cdot L_m^3 \qquad (5)$$

and the rate of increase of total leaf area is:

$$R_s = K \cdot S/(2 \cdot \Delta L) \cdot L_m^2 \cdot (L_m - 2) \approx K \cdot C \cdot L_m^3 \qquad (6)$$

in which $C = S/(2 \cdot \Delta L) = 0.035$.

These approximations are only valid, when leaf length is not too small, e.g. 10 cm, which is usual for poplar leaves. It may be emphasized that the above formulas were derived for plants growing at a constant rate and thus forming leaves of constant size. Such cubic relations are also found in some anatomical characteristics of *Gladiolus* (WASSINK, 1963). To establish, whether the relations found, have a wider significance, we applied them to data, collected from plants, which were growing in a non-stationary state.

Fig. 5.11a demonstrates the relation between L_m and the total area of growing

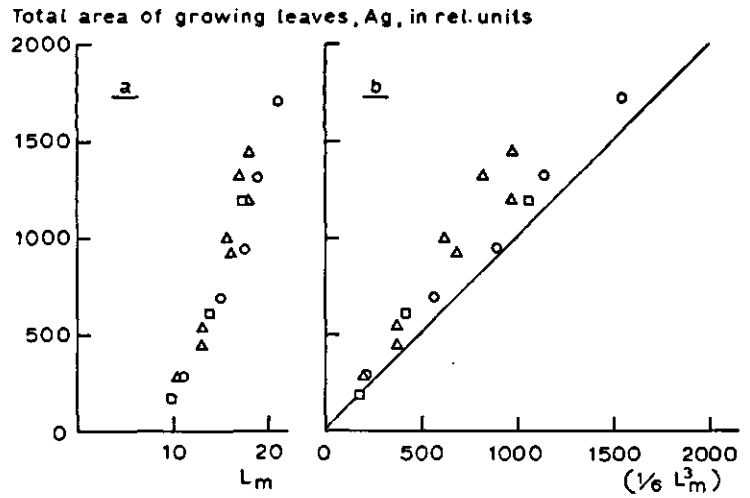


FIG. 5.11a. Relation between total area of growing leaves (A_g, in relative units) and length of the youngest mature leaf L_m. L_m is defined as the length of the first leaf, in which linear growth rate clearly declines. – This relation is independent of light and temperature. Growth conditions: 25 °C, I (□), 20 °C, I (o) and II (△).
FIG. 5.11b. Same data, as in fig. 5.11a on total area of growing leaves (A_g, in relative units) plotted against 1/6 L_m^3 (see formula 5).

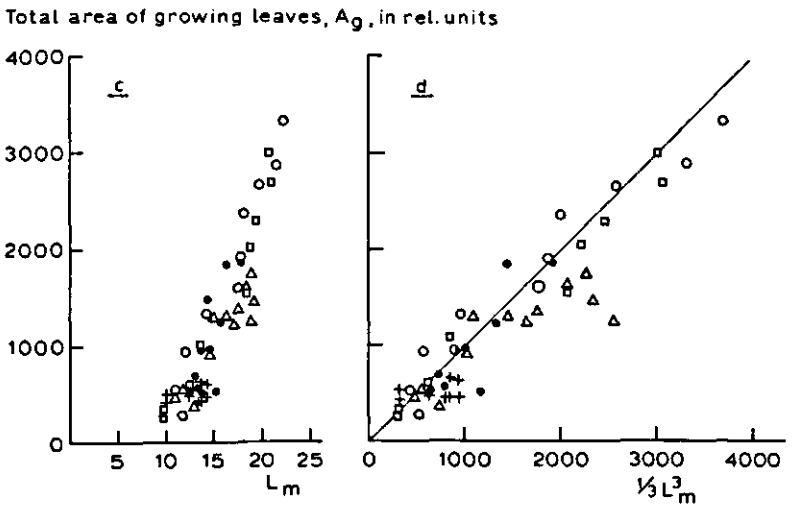


FIG. 5.11c. Relation between total area of growing leaves (A_g, in relative units) and length of the youngest mature leaf L_m. L_m is defined as the length of the first leaf, in which linear growth rate has declined to zero. – This relation is independent of light and temperature. Growth conditions: 25 °C, I (□); 20 °C, I (o); 20 °C, II (△); 20 °C, III (+); 16 °C, I (●).
FIG. 5.11d. Same data, as in fig. 5.11c on total area of growing leaves (A_g, in relative units) plotted against 2 × 1/6 L_m^3 (cf. formula 5).

leaves. As expected, the relation between L_m and the total area of the growing leaves is similar for all treatments, since the treatments applied do not or hardly affect the shape of the leaves. The areas of the individual leaves have been approximated by calculation using the formula:

$$A_n = K \cdot L_n \cdot (L_n - 2), \text{ in which K is put to 1} \quad (7)$$

The length of the youngest mature leaf is defined more arbitrarily as the length of the first leaf in which the linear growth rate declines and the summation of areas includes the area of that leaf and all the younger ones. Fig. 5.11b shows, that the measured area approximately equals that expected on the basis of formula (5). The deviation may be due to the fact that leaves have been included in the summation that no more belonged to the theoretical arithmetical progression. However, when the length of the youngest mature leaf is defined as the length of the leaf, in which growth was totally finished, a larger number of (large) leaves is necessarily included in the summation, that is assumed in formula (5), and the area of growing leaves thus becomes larger than in the first case. In this case also, the relation between A_g and L_m (Fig. 5.11c) is similar for all treatments, while plotting A_g against $1/3\ L_m^3$ reveals, that on the basis of the latter assumption the accumulated areas of growing leaves, is about twice as large as in the first case (Fig. 5.11d) and still remains linear to L_m^3.

The increase of total leaf area against time can be determined by the measurement of the total leaf area at intervals. This might be accomplished by measuring the actual area of each successive leaf, although this was not easily possible in a non-destructive way. Therefore, the relative area of each growing leaf has again been calculated, with formula (7), in which K is put to 1, on the basis of the measured leaf lengths L_m. Fig. 5.12 shows the accumulated relative leaf area versus time of plants from different treatments. As expected, the rate of increase of area increases with light and temperature. Initially, the growth of the accumulated area progresses exponentially, but ultimately changes into linear growth.

When plotting the actual increase in area of the succession of leaves (in rel. units) against the third power of the length of the youngest mature leaf (fig. 5.13), the relation found agrees with the expectation: despite the large variability the tendency of the relation is a straight line and the slope of the line is about 0.035 or similar to the constant C of formula (6). A large part of the variability may be caused by the difficulty in the determination of L_m from the protocols containing the measurements of leaf length.

5.4. Discussion

In the preceding sections it has been shown that in the linear phase of growth the relative growth rate S with respect to length, which is proportional to the absolute growth rate per cell, is independent of the irradiation level and of the

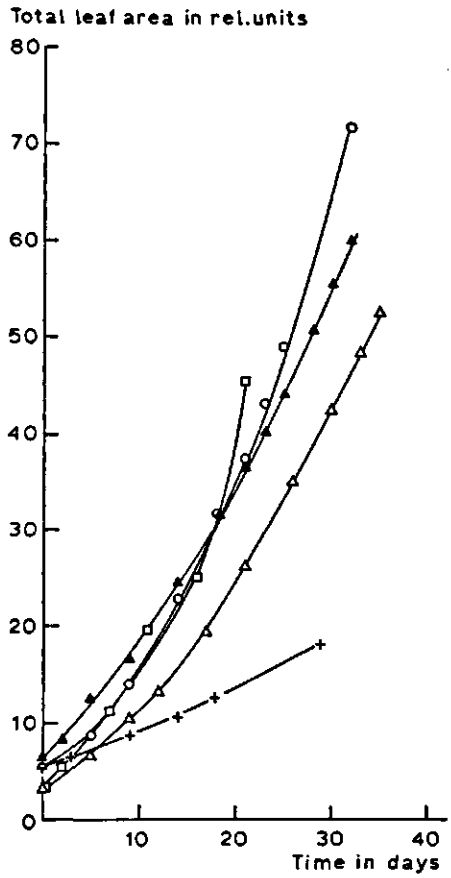


FIG. 5.12. Relation between total leaf area (A_g, in relative units) and time (days). Initially the increase of total leaf area proceeds exponentially and sooner or later becomes linear. The rate of increase of total leaf area increases with temperature and light intensity. Growth conditions: 25°C, I (□); 20°C, I (o); 20°C, II (△); 20°C, III (+); 16°C, I (▲).

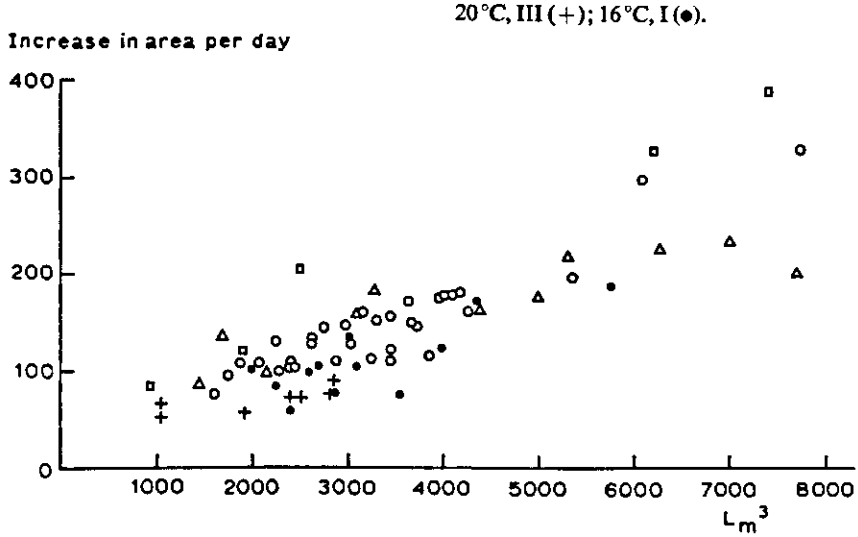


FIG. 5.13. Relation between increase in leaf area per plant per day in relative units and the third power of the length of the youngest mature leaf, L_m^3. L_m is defined as the length of the first leaf, in which linear growth rate has declined to zero. Growth conditions: 25°C, I (□); 20°C, I (o); 20°C, II (△); 20°C, III (+); 16°C, I (●).

length of the mature leaf. The length of the mature leaf is a measure of the absolute growth rate of the plant, because the increase of total leaf area per day is proportional to the cube of L_m. High and low rates of growth of plants, grown at different light intensities, cannot be based on differences in relative growth rates or absolute growth rates of cells. The cause for increasing growth rates is that a larger number of cells is involved in growth and the number of cells involved is determined in a phase preceding the linear phase of growth.

The mathematical relations for growth are derived under the assumption of a stationary state of growth. Nevertheless, application of these formulas to the data obtained from plants in a non-stationary state of growth and growing under various conditions of irradiation and temperature, resulted in a reasonable agreement between the calculated and the measured characteristics of the plants.

5.5. The relation between cell division and cell expansion

5.5.1. *The lengths of the successive leaf primordia along the main axis of the apex*

Lengths of the primordia in the apex can only be measured by destructive methods. Therefore, it is not possible to determine the growth against time relationships of individual primordial leaves, and it seems necessary to be satisfied with the measurement of the relation between primordium length and primordium number. Fig. 5.14 summarizes what has been found in the apex of the experimental plants. The following conclusions can be drawn:

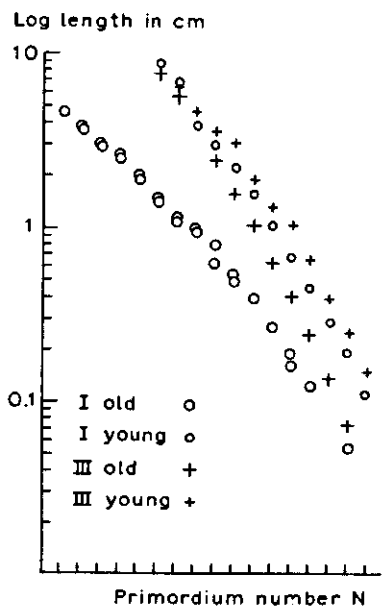


FIG. 5.14. The relation between log length of the primordia and their serial number. – This relation is approximately linear for primordia smaller than 1 cm. The slope of the line is characterized by the relative growth rate on plastochron basis, k_p, which is higher in shade plants and young sun plants than in old sun plants, owing to differences in the plastochron duration.

1. Young plants from low and high illumination levels possess the same sequence of primordia lengths in the apex.
2. In shade plants this sequence (slope) does not change, when they grow older and larger.
3. In plants exposed to high light intensities the slope becomes less steep when the plants grow older; they take longer to reach a stationary state of growth than shade plants do.

These phenomena can be linked qualitatively with the growth rate of the plants: the faster a plant grows, the larger the number of leaves in the apex (cf. also HUMPHRIES and WHEELER, 1963). A visual comparison of the size of the growing tips of large sun and shade plants gives the same impression (see Plate IV–VI), and also shows that the apices of vigorously growing plants have a greater diameter. The apex of a young sun plant, however, equals that of a shade plant. Initially, there is no quantitative difference between a sun plant and a shade plant; the sun plant distinguishes itself from the shade plant by the fact that its growth rate gradually increases during a longer period.

Assuming that the logarithms of the lengths of all the primordia below 10 mm (fig. 5.14) against primordium number form a straight line and that growth is exponential (see appendix), the following relation holds for the relative growth rate per plastochron, k_P:

$$k_P = \frac{\log L_n - \log L_{n+N}}{N} \tag{8}$$

in which N represents the number of leaf primordia produced or the number of plastochrons passed. Conversion to clock time, yielding the relative growth rate of the primordial leaves, results when k_P is multiplied with the reversal of the plastochron duration 1/P. The k_P's are 0.339 and 0.479 and the P's are 1.041 and 1.695 days for sun and shade leaves respectively, so that the relative growth rates are 0.325 day^{-1} and 0.282 day^{-1}. These figures are practically equal (Table XIV).

The relative growth rate per plastochron k_P has been estimated by measuring $(\log L_n - \log L_{n+N})/N$ of the approximately straight lines in fig. 5.14 and multiplying this figure with $\ln 10 = 2.303$. The choice of the values of L_m is somewhat arbitrary and needs further confirmation.

Their ratio is 0.325/0.282 = 1.15 and for the rates of leaf initiation 22/14 = 1.57. This illustrates well that there is a divergence between the ratios of relative growth rates and of primordium initiation rates in sun and shade plants, which fact results in a relatively large accumulation of leaves in the apex of the sun plants. It may be useful to add that the ratio of the relative growth rates of the leaves in the linear phase of growth, is about 1.14. This indicates that the ratios between relative growth rates of the primordia in sun and shade plants in the primordial phase of growth and that between the relative growth rates in the linear phase of growth are practically the same in spite of the fact that cell division is more intensive in the sun plants.

TABLE XIV. Approximation of the relative growth rates per unit time of the primordial leaves of plants from light intensity I and III at 20 °C and the ratio's of the relative growth rates of sun and shade plants in the linear and primordial phase of growth.

Light intensity	k_p	S	ΔL	L_m	$\frac{1}{P} = \frac{SL_m}{2\Delta L}$	k_t
	p^{-1}	$days^{-1}$	cm	cm	$days^{-1}$	$days^{-1}$
I (large sun plants)	0.339	0.1598	1.823	22.0	0.96	0.325
III (shade plants)	0.479	0.1404	1.672	14.0	0.59	0.282
	$\frac{k_t\ (sun)}{k_t\ (shade)} = 1.15$		$\frac{S_{sun}}{S_{shade}} = 1.14$			

5.5.2. *Discussion and conclusions*

The results, presented in the preceding section, have been profoundly discussed from the mathematical point of view in the appendix. It has been shown that in poplar, cultivated in the way described at different light intensities and growing in a stationary state, the relative growth rate in the primordial phase of growth remains constant and growth is exponential. This relative growth rate is independent of light intensity and the differences in absolute growth rate of sun and shade plants cannot be explained on that basis. The causes of these differences have to be traced back to the phase of initiation, in which the cell number of the primordia is determined.

A cell grows exponentially if its growth at any moment is proportional to its size. The same is true for a tissue, when it is composed of cells which grow exponentially; then its growth is independent of cell division. In vigorously growing sun plants and in shade plants of poplar, the relative growth rate in the initial phase is similar, and cell growth proceeds exponentially, notwithstanding the fact that the amount of cell division in sun plants is clearly higher (Table XIV, see also section 5.5.1).

The relative growth rate k_t thus can be considered as a characteristic of the growth potential of the individual cells and is only slightly influenced by the irradiation of the plants. It is of interest that the ratio of relative growth rates in sun and shade leaves in the exponential phase of growth is practically equal to the ratio of the relative growth rates during the linear phase of growth, because it shows that environment influences the relative growth rate equally in both phases.

Higher rates of cell division by shorter generation times in tissues growing exponentially at a constant relative growth rate might imply smaller cells. Sun leaves may contain 2 times as many cells as shade leaves and the mean cell volume in the apex of the sun plant could be half that in the shade plant. Such a difference in cell volume is not easily observed as a smaller diameter, because the difference in diameter is only $2^{-1/3} = 0.8$. Literature reports that apices of vigorously growing plants do not contain smaller cells. This is substantiated by

the well-known fact that the apex of a vigorously growing plant is larger in size than that of a more slowly growing plant.

Exponential growth of a tissue, therefore, can be due to the exponential growth of the cells themselves, independent of the occurrence of cell division, or by a constant rate of cell division in tissues, in which the cells each are in the linear phase of growth. The last part of the 'exponential' growth of a leaf, just before its linear phase begins, may therefore be due to cell growth or to cell division. Only a careful analysis of the rate of cell division in this stage of growth may clarify this point and at the same time produce an exact picture of the growth against time relationship of the individual cell which is basic for a better understanding of growth.

The rate of cell division is correlated with the rate of leaf initiation. Increased leaf initiation, together with the constant cell expansion causes the accumulation of leaf primordia in the apex of sun plants. Exponential relationships between the dimensions of successive primordia lengths, frusta radii and frusta heights are very common (RICHARDS, 1948, 1951; DALE, 1965; BERG and CUTTER, 1969; BENSINK, 1971). The succession of frustum heights is not always exponential (BERG and CUTTER, 1969); the exponential nature of growth can be hidden by a changing relative growth rate. BENSINK (in preparation) has established that in lettuce the relative growth rate declines steadily with age and explains this on increasing competition for food. This situation does not appear to occur in *Populus euramericana* 'Robusta' in our experiments.

The exponential nature of the early growth of cells is established in many plant cells (BRUMSFIELD, 1942; BURSTRÖM, 1942, 1957; GOODWIN and STEPKA, 1945; GREEN, 1954; ERICKSON and SAX, 1956). It is likely that in that case synthesis of the apparatus necessary for protein formation is proceeding at a constant rate per unit of mass with time. The cell may remain in this phase of development, when cell division is going on. When cell division stops, cell growth will become more or less linear, sooner or later, until final cell size, which is commonly reasonably constant, is reached more or less abruptly (LOCKHART, 1971). The rate of expansion of dividing cells is not always exponential. COOPER (1971) describes that in *Schizosaccharomyces pombe* and *S. cereviseae*, the increase in dry weight of a single cell with time is linear and he explains this by assuming that 'the synthesis is controlled by cytoplasmic particles which remain constant in number and activity throughout the life of the cell and which double at cell division, being shared equally between the daughter cells.' Certainly, it should be remembered that these yeasts are cells with structures and properties, strongly differing from those in higher plants.

The amount of information about the influence of environmental conditions such as light, temperature and supply of nutrients on the rate of cell division in the apex is relatively scarce. GIFFORD and CORSON (1971) state: 'It would seem esssential to the present authors that, when mitotic activity is to be compared between certain regions of the shoot apex, the effects of factors such as light, temperature, time of day and apical stage must be carefully controlled, so that the results can be compared with others.' To the present author it seems neces-

sary to evaluate carefully the influence of these factors themselves on the mitotic activity and the expansion of the cells of the apical dome, frusta and leaf primordia, and to analyse the relationship between cell expansion and division.

5.6. Maximum leaf length

5.6.1. *The steady increase of mature leaf length onto a stationary state*

As was shown in fig. 5.8 mature leaf size increases steadily with leaf number until a stationary state of growth is reached. The increase in mature leaf length per leaf number (tg β_1) is given in Table XV. It is concluded that these differences show no consistent relation with light intensity nor with temperature. Both for the growing leaves and for the mature leaves the lengths of suc-

Table XV. The increase in length of the mature leaves with leaf number, tg β_1 and tg β_2, (see fig. 5.8), in plants from various treatments of light and temperature.

Plant	tg β_1	tg β_2
20-I-1	0.649	–
I-2	–	0.414
I-3	1.018	0.268
I-4	0.713	0.185
I-5	0.885	0.167
I-6	0.854	–
I-7	–	0.176
I-8	0.869	–
mean	0.831	0.242
20-II-1	0.713	–
II-2	0.566	0.158
II-3	0.839	–
II-4	0.700	–
mean	0.702	0.158
20-III-1a	1.000	0.259
III-1b	0.767	–
III-3	0.455	–
III-4	0.687	
mean	0.727	0.259
25-I-1	0.869	–
I-2	0.900	–
I-3	0.754	–
mean	0.841	–
16-I-1	0.649	–
I-2	0.700	–
I-4	0.740	–
mean	0.696	–

cessive leaves form an arithmetical progression, and the areas approach an arithmetical progression of the second order with a constant common difference, irrespective of treatment. Consequently, the total area of the *mature* leaves is a (somewhat complicated) function of L_m, containing terms in L_m^3, L_m^2 and L_m, while the area of the growing leaves approaches a relation in L_m^3. Therefore the photosynthesizing leaf area in the initial phase increases equally with time in all treatments.

The rate of photosynthesis per plant, however, is proportional to light intensity, because the efficiency of photosynthesis in sun and shade leaves is practically equal over extended periods of time (unpublished results) and photosynthesis is light limited at the irradiation levels used in our experiments. Hence, it may be concluded that during this period the increase in total leaf area is not limited by carbohydrate production.

At a certain stage in the development of the plant, the increase of mature leaf length stops. In shade plants (light intensity III) this happens when the leaves reach a length of 14 to 15 cm, for the plants from light intensity II, when they reach a length of 18 to 19 cm and for the plants of the highest light intensity, when they reach a length of 23 to 24 cm. Thus the maximum mature leaf length is related to light intensity. Moreover, the rate of increase in total leaf area is related to the third power of mature leaf length, and is found to be proportional to light intensity (Table XVI).

TABLE XVI. Maximum rate of leaf area growth (rel. units), calculated as the third power of maximum leaf length in relation to irradiation level.

(I) Irradiation level W/m²	(II) Maximum leaf length in cm	(III) Rel. rate of leaf area growth	(IV) Col. (III)/col. (I)
40	23–24	12200–13800	300–345
20	18–19	5850–6800	249–344
10	14–15	2750–3380	275–338

5.6.2. *The increase of total leaf area in the stationary state*

Despite the fact that total leaf area continually increases further by the formation of new leaves of maximum mature size, and thus, total photosynthesis of the plant continually increases further, it appears remarkable that the area of single mature leaves does not appreciably increase beyond the maximum size indicated above (Table XV, under tg β_2). It looks as if only a restricted leaf area provides the energy for the growth processes going on in the apex (cf. also BENSINK, 1971), the size of which according to the presentation given in these pages determines the ultimate mature size of the successive leaves. Competition with other growing règions of the plant may be involved. Experiments, in which several branches were allowed to grow on one stump, showed that under these conditions, the distribution of assimilates was changed, and the production of

TABLE XVII. Comparison of total growing leaf area (A_g) and total stem area (A_s) in plants consisting of one stem or five stems. Illumination: 40 W/m², temperature 20 °C.

Plant	A_g cm²	A_s cm²	A_g/A_s
with 5 stems	4022	3.613	1114.1
with 1 stem	1656	2.600	636.9

leaf area per stem was reduced, but far less than the secondary growth of the stem (Table XVII). The reduction of stem diameter resulted in some stems falling over (Plate XI). Also in this case it is possible that maximum mature leaf size is related to the irradiation level which is lowered by mutual shading, and that stem thickness is reduced by the lower availability of carbohydrates.

5.6.3. *Tentative further evaluation of data*

The maximum rate of increase of total leaf area is about proportional with the irradiation level and thus, at first sight, may appear proportional with carbohydrate production. The explanation of this simple correlation, however, is rather difficult. Three factors at least are involved: the irradiation level (differing a factor 4 in our sun and shade plants), the photosynthesizing area, and carbohydrate distribution over leaves and other parts.

The leaf area increase, being proportional to L_m^3, proceeds 4 times faster in the sunplants since L_m^3 is proportional to light intensity. The internodes also grow 4 times faster, because their cross section is related to L_m^2 and the number

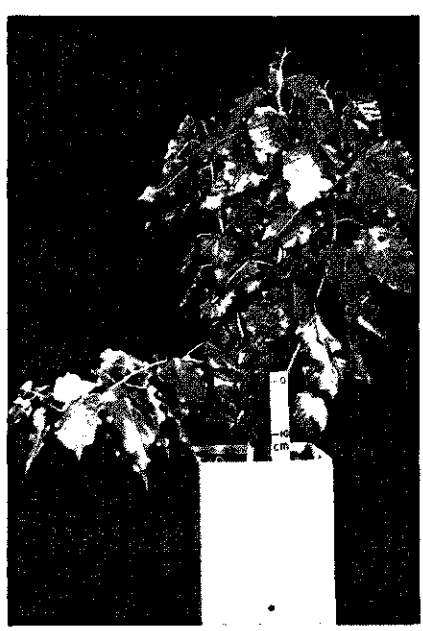


PLATE XI. The growth of several shoots on one stump reduces the secondary stem growth so that some stems fell over.

produced per unit time to L_m so their total growth is also proportional to L_m^3. At the same time, however, the leaves become thicker, from 136 μ in shade plants to 223 μ in sun plants, secondary stem growth is much more intensive and probably also the top-root ratio decreases, so that the total mass produced in the sun plants is at least $223/136 \times 4 = 6.5$ times higher. At present there are not enough data on carbohydrate distribution of poplar to make up a complete energy balance of growth. Furthermore, it is unknown in these poplars, how many mature leaves contribute to the nutrition of the growing apex and what is the efficiency of energy conversion in growing leaves (presumably on the average much lower than in the mature leaves). Nevertheless, some more detailed estimation of characteristics of mutual shading and photosynthesis of both groups of leaves may be attempted.

With regard to the rate of photosynthesis per plant, this rate can be calculated as the product of total leaf area, the mean efficiency of energy conversion and the prevailing light intensity. The total leaf area of plants in the stationary state of growth per 10 cm stem length, putting the mean length of the internodes to 2 cm and the shape factor K to 0.73 in all treatments, is 2016 cm^2 in sun plants and 767 cm^2 in shade plants. To calculate the leaf area index (LAI) we may compare this area with the area of a cylinder around the stem with, rather arbitrarily, a radius equal to the sum of the length of the petiole and half the length of the leaf. These areas are 1196 cm^2 and 744 cm^2 and the LAI's 1.69 and 1.03 in sun and shade plants, so that after this rough estimation, the effect of mutual shading is nearly absent in shade plants and of importance in sun plants.

According to our above estimations the ratio between total mature leaf areas in sun and shade plants is 2016:767 and the ratio between 'effective' total mature leaf areas is 1196:744. Considering that the ratio between the incident light intensities in both cases is 4:1, the ratio between the average rates of photosynthesis over 10 cm stem length may be estimated to be $4 \times 1196:744$, or 6.4. This can be assumed as a lower limit, the upper limit being $4 \times 2016:767 = 10.5$. On the basis of this rough estimate, the rate of biosynthesis in the poplar plants in our experiments is about 8.5 times higher at light intensity I (40 W/m^2) than at light intensity III (10 W/m^2).

Attention may be drawn to the observation that the lower limit of the ratio of total mass produced per unit time by photosynthesis in the stationary state of leaf growth in high and low light intensity (viz., 6.4) emerged from the estimate based on leaf thickness as well as from the estimate based on effective total mature leaf area. Notwithstanding the fact that not too much value should be attached to this coincidence, it might be evaluated as an indication that effective leaf area and leaf thickness react in a similar manner to the light intensity.

Corresponding values can be calculated for the growing leaves. According to the formula $A_g = {}^1/_3 \cdot K \cdot L_m^3$, their areas in sun and shade plants are 3158 cm^2 and 742 cm^2. The areas of the right circumscribing cones are 1315 cm^2 and 486 cm^2, taking as the height of the cone 5.5 cm in shade plants and 11 cm in sun plants. The LAI's in sun and shade plants are then 2.40 and 1.53. The

ratio between total growing area in sun and shade plants then is 3158/742 = 4.26 and between effective total growing leaf area 1315/486 = 2.71. Hence, the ratio between the average rates of photosynthesis in the growing leaves of sun and shade plants is 4 × 2.71 = 11.84 as a lower limit and 4 × 4.26:1 = 17.04:1 as an upper limit. The actual ratio of total photosynthesis of growing leaves in sun and shade plants might approximate more the lower limit than the upper limit, because of the strong mutual shading.

5.6.4. *Conclusion*

The preceding considerations may show that it is for the present impossible to calculate the mean contribution of mature and growing leaves to the nutrition of the growing point. Clearly a comparative study of the rates of photosynthesis and respiration along the stem in situ and of transport of carbohydrates are required to improve our understanding of the determination of maximum leaf size.

The picture presented here, deviates from what has often been reported in literature, because no optimum in growth has been observed in relation to irradiation level. However, also in the plants used, such optima can be found when limitations in the root environment occur. This would explain why in many cases a correlation is found in several characteristics, such as maximum final leaf area, chlorophyll content per unit area, maximum photosynthetic rate per unit area and time, epidermal cell size etc. In Table XVIII some data are given illustrating this. In these experiments, root environment was found to limit growth as soon as light intensity rises above a certain level. In this case, the intermediate light intensities were most favourable for growth, because all the leaf properties shown, except leaf thickness, were highest. This type of growth reaction to irradiation has been found generally (DAUBENMIRE, 1947; NEWTON, 1963; BJÖRKMAN and HOLMGREN, 1963; BENSINK, 1971).

As shown in the preceding sections however, the rate of growth can be linearly related to the irradiation level, in the same range of light intensities in which earlier not only light saturation was reached but even an optimum showed up.

5.7. INITIATION AND APPEARANCE OF LEAVES

The appearance with time of successive leaves of plants from different treatments is given in fig. 5.15. Leaf appearance for the period of observation is linear with time. This is in agreement with the results of several other investigations about the rate of leaf initiation and of appearance. (SUNDERLAND and BROWN, 1956; MILTHORPE, 1959; SCHWABE, 1959; POPHAM, 1965; BERG, 1966, 1970; BERG and CUTTER, 1969; BENSINK, 1971). The mean rates of leaf appearance (N/d) and the mean linear growth rates (m) of the leaves of plants from different treatments are given in Table XIX. Both characteristics show the same type of dependency on light intensity and temperature. They increase with light

TABLE XVIII. Some characteristics of the successive leaves of three plants of *Populus raverdeau*, grown in three light intensities under constant conditions. I highest light intensity, III lowest light intensity. Leaves are numbered from top to base. Plant I is limited in its growth by the volume of the pot. Plant III is in rest because of the low light intensity.

Light intensity	Leaf number	Leaf width cm	Leaf thickness μ	Chlor. content	Mean cell width μ	Mean cell number 10^3
I	1	3.7	149.7	0.087		
	3	7.0	159.0	0.139	15.36	4.56
	5	8.8	180.0	0.162	19.53	4.50
	7	9.1	210.3	0.174	18.44	4.94
	9	11.6	221.8	0.178	25.30	4.58
	11	12.4	236.0	0.198	26.97	4.60
	13	12.7	258.1	0.213	28.32	4.48
	15	11.4	251.3	0.186	27.25	4.18
	17	10.2	260.7	0.168	26.00	3.93
	19	10.1	247.6	0.217	27.67	3.64
	21	9.3	252.5	0.221	31.30	2.97
	23	9.1	251.5	0.180	32.19	2.83
	25	8.0	234.5	0.167	33.56	2.38
	27	6.3	238.8	0.162	–	–
II	1	12.5	173.0	0.205	23.95	5.20
	3	12.9	171.8	0.203	25.08	5.14
decapitated	5	13.4	177.0	0.199	27.39	4.88
	7	14.4	185.8	0.202	28.51	5.04
	9	13.6	195.0	0.210	28.83	4.71
	11	13.9	187.5	0.185	32.57	4.26
	13	13.3	187.1	0.208	31.31	4.25
	15	12.5	188.3	0.209	32.05	3.90
	17	12.5	210.2	0.225	31.99	3.90
	19	11.8	219.5	0.217	36.06	3.28
	21	10.7	204.0	0.200	32.57	3.29
	23	9.7	203.7	0.199	38.47	2.52
	25	7.9	207.7	0.194	34.82	2.27
	27	5.8	222.5		38.49	1.50
III	1	7.5	138.0	0.173	25.28	2.96
	3	9.2	132.7	0.198	31.01	2.97
	5	8.4	133.3	0.203	26.20	3.21
	7	7.8	132.0	0.179	–	–
	9	8.4	134.3	0.172	24.50	3.43
	11	8.6	145.3	0.166	26.57	3.23
	13	9.2	147.0	0.180	26.66	3.45
	15	9.2	148.2	–	26.86	3.43
	17	8.7	150.7	–	–	–
	19	9.8	175.3	–	31.23	3.14
	21	11.0	193.2	–	–	–
	23	8.2	199.5	–	33.59	2.45

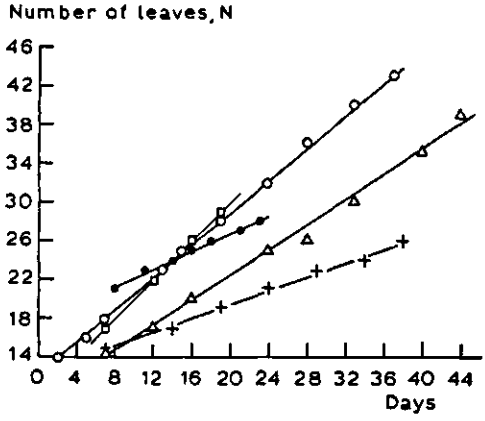


FIG. 5.15. Leaf appearance against date in sun and shade plants.
Growth conditions: 20 °C, I (○); 20 °C, II (△); 20 °C, III (+); 16 °C, I (●). 25 °C, I (□).

TABLE XIX. Mean rate of leaf appearance (number/day, N/d) and mean growth rate (cm/day, m) of plants from various treatments of light and temperature. Between parentheses the various values as percentages of the treatment at 25 °C and 40 W/m².

Temp. °C	Light intensity in W/m²					
	40		20		10	
	N/d	m	N/d	m	N/d	m
16	0.353 (38.8)	0.567 (34.0)				
20	0.830 (92.1)	1.497 (89.6)	0.695 (77.1)	1.328 (79.5)	0.370 (41.1)	0.877 (52.6)
25	0.901 (100.)	1.670 (100.)				

intensity, but this effect saturates above 20 W/m². The influence of temperature is most marked between 16 and 20 °C. MILTHORPE and NEWTON (1963) using cucumber and BENSINK (1971) using lettuce found a similar dependence of the rate of leaf initiation on light intensity. Moreover, BENSINK (1971) with lettuce showed a similar dependence on temperature.

In the stationary state of growth of poplar such a linearity is expected. Shade plants are soon in the stationary state; sun plants, however, reach this state not before an extended period of time. During the preceding non-stationary period of growth it is expected that along with the increasing linear growth rate, the rate of leaf initiation increases, because

$$m \cdot P = \Delta L = \text{constant} \qquad (4)$$

This equation was derived for plants in the stationary state of growth, but can also be applied to plants in the non-stationary state of growth, as is shown in section 5.2.2. The disagreement between the observed constancy of the rate of leaf appearance and the expected increase of the rate of leaf initiation, raises the question, which relation between both rates exists. The solution of this problem is rather simple, when it is considered that the period of growth of a small and a large leaf from initiation onto maturation is the same at constant

temperature. The arbitrary length of e.g. 2 cm, which is the criterion of appearance of a leaf, represents a later stage of development in a small than in a large leaf. The rate of appearance should be measured at equal stages of development of leaves of different size.

The easiest way to do this is to plot leaf length as a percentage of its final value against time and count leaf number along a horizontal line, e.g. the 50% line. The slope of the line representing N against date represents the leaf appearance rate. Fig. 5.16 (see pg. 66) shows an example of this procedure and, clearly, the rate of appearance increases steadily with time. In fig. 5.17 the relation between m and the rate of leaf appearance is seen to be approximately constant, as is expected on the base of formula 4, denoting the relation between the linear growth rate and leaf initiation rate.

ONDOK (1968), ASHBY and WANGERMANN (1950) proposed a definition of the plastochron as the time difference between the inflexion points of the growth curves of successive leaves. SAEKI (1960) defines the plastochron as the time interval between the moments of maturation of successive leaves. These suggestions are in full agreement with our proposal to measure rates of leaf appearance at equal developmental stages.

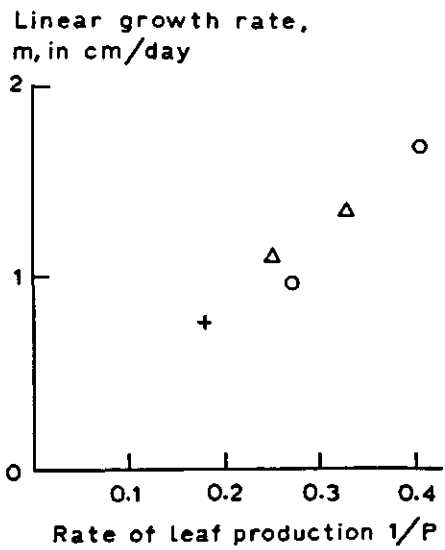
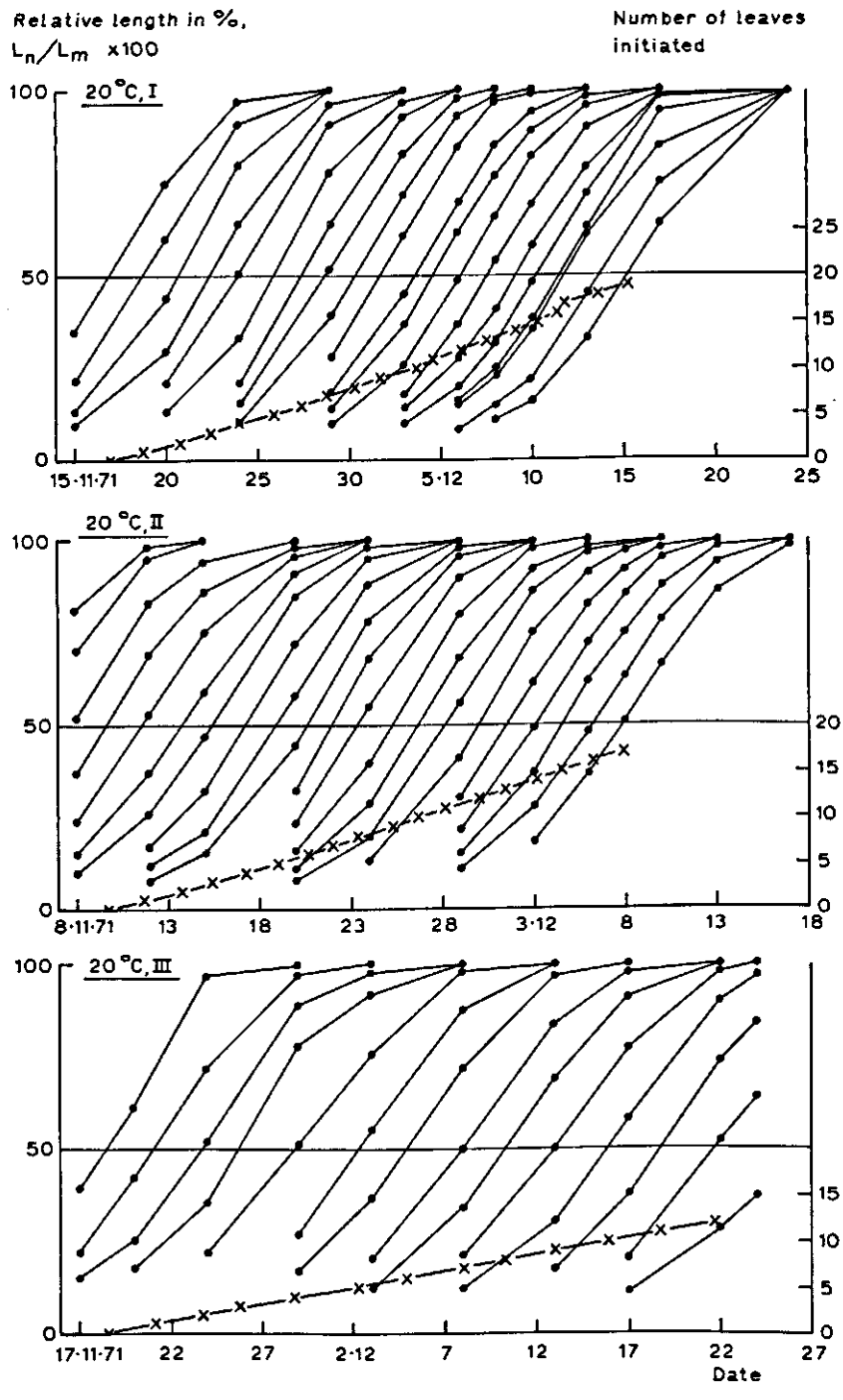


FIG. 5.17. The relation between linear growth rate, m, according to data of fig. 5.1, and the rate of leaf initiation 1/P, according to data of fig. 5.16. Growth conditions: 20°C, I (o); 20°C, II (Δ); 20°C, III (+).


Relative length in %,
L_n/L_m x100
Number of leaves initiated
20°C, I
100
50
0
25
20
15
10
5
0
15·11·71
20
25
30
5·12
10
15
20
25
20 °C, II
100
50
0
20
15
10
5
0
8·11·71
13
18
23
28
3·12
8
13
18
20 °C, III
100
50
0
15
10
5
0
17·11·71
22
27
2·12
7
12
17
22
27
Date

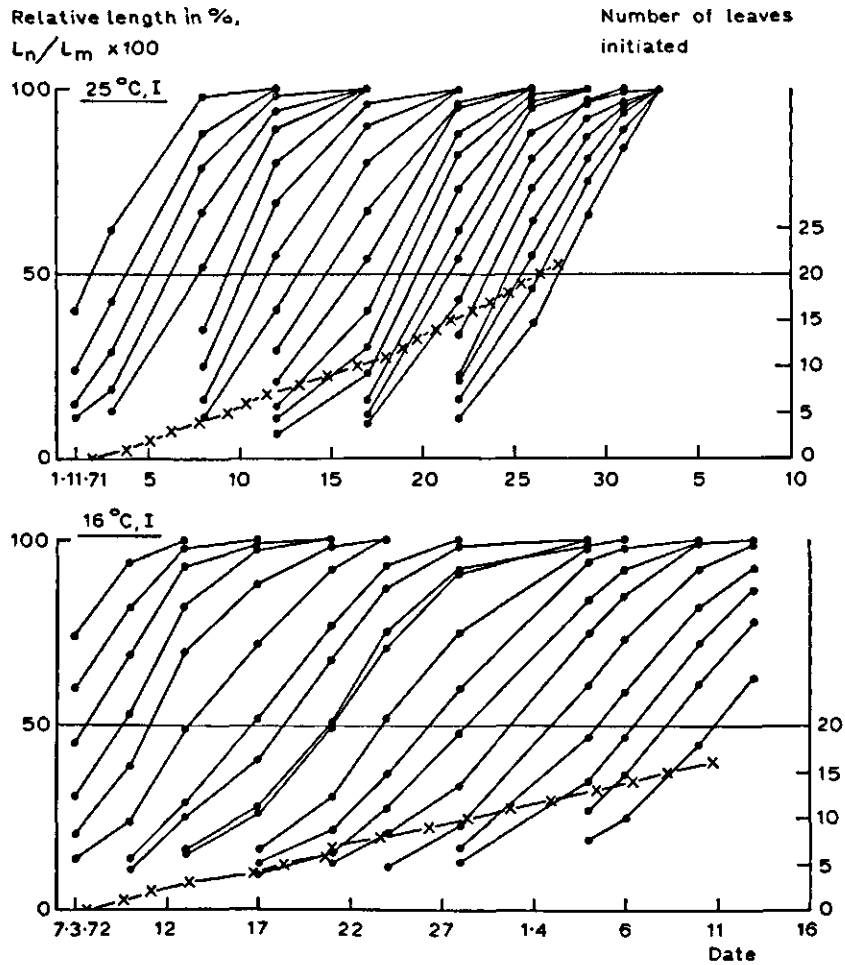


FIG. 5.16. Initiation of successive leaves, as determined by the time at which they reach 50% of their individual mature length. Left hand scale: relative length of each leaf in % of its mature length (•—•). Right hand scale: number of leaves initiated. – Rate of leaf initiation = slope of the line, representing number of leaves against date (= 1/P) (x—x).

6. THE GROWTH OF THE INTERNODES

6.1. Several characteristics of the growth of internodes

The growth rates of internodes of plants from different light intensities and their final lengths are similar (Table II), as shown already in chapter 3. However, variability of internode growth is large, in agreement with previous reports in literature (cf. Greathouse, Laetsch and Phinney, 1971).

The growth rates of the internodes of plants from different treatments are summarized in Table XX. As was the case in leaves, in internodes growth rate is correlated with final size. It may be speculated on the basis of the theory on growth developed in chapter 5, that the growth rate in the length of the internode is based on the number of cells elongating times a constant mean growth rate per cell.

On the basis of the observation that the ultimate length of the internode is more or less constant and independent of treatment, it may be suggested that

Table XX. Mature internode length, number of days between maturation of leaf and subtending internode, growth rate and relative growth rate of internodes of plants of various treatments.

Treatment	Mature length	Mean	Earliness of internodes	Mean	Growth rate	Rel. growth rate	Mean
	cm		days		cm/day	cm/cm. day	
25-I-1	1.30		6–7		0.162	0.248	
25-I-1	1.50		7		0.204	0.272	
		1.40		6.7			0.260
16-I-4	0.90		± 6		0.055	0.123	
16-I-4	0.80		± 6		0.041	0.102	
		0.85		6.0			0.112
20-I-3	1.70		7		0.222	0.262	
20-I-3	1.90		7		0.247	0.260	
20-I-5	1.80		6		0.153	0.168	
20-I-5	1.70		6		0.173	0.204	
20-I-1	2.00		?		0.314	0.314	
		1.82		6.5			0.223
20-II-2	1.90		8		0.342	0.247	
20-II-2	1.80		?		0.214	0.238	
		1.85		8.0			0.242
20-III-2	2.75		6–7		0.361	0.263	
20-III-2	2.30		6		0.211	0.184	
20-III-4	2.60		6		0.238	0.184	
20-III-4	2.50		5		0.238	0.191	
		2.54		5.7			0.205

the number and the dimension of the mature cells in vertical direction is more or less constant and, therefore, also its rate of elongation is constant. It has to be observed that internodes of plants cultivated at 40 W/m² in 20 °C often are longer than at 15 and 25 °C. There is also a tendency for internodes being longer when cultivated at low light intensity. For the present it may be permitted to neglect these differences, because it is not al all sure that limitations in the root environment are not involved, and it makes no large difference in the theoretical treatment of the growth of internodes presented here.

Further experimentation will be required to confirm this view. For example it could be useful to measure mature cell length with the silicone rubber technique. In this respect it is of interest to mention, that ALLSOPP (1965) states: 'Since the early work of MOLL (1876), it has been recognized that the differences in internode length are due almost entirely to differences in cell number rather than in cellular extension.' The situation in mature internodes seems therefore comparable to that found in mature leaves.

It is observed that the linear portion of internode growth continues over a more extended period than that of the leaf (see fig. 3.6). The cause for this difference in growth has not been investigated, but it is plausible to suggest that the growth of the internode proceeds in a similar manner to roots. To quote BUCHANAN (LOOMIS 1953): 'the rate of increase in length of the root or stem is determined by the rate of formation of apical cells and the rate at which the cells enlarge and elongate. The growth of the individual differentiating cells is undoubtedly sigmoid. The overall effect is that of regular or constant increase in length of the root.' It may also be of interest to use the silicone rubber technique to see, if an acropetal gradient in epidermal cell lengths exists, and to study the growth of the individual cell length with time in an attempt to explain the extended linear growth.

Another remarkable property of the internode growth is that growth stops suddenly, whereas in the case of leaves growth stops gradually. The same growth pattern as in the internodes is seen in the roots (LOCKHART, 1971). It is possible that the individual cells of root or internode stop growth suddenly when reaching their ultimate size. If this is the case, why is this type of cell growth not seen in the leaf? It has been shown in chapter 4 that in the leaf lamina all developmental stages of the cells occur simultaneously. The characteristics of growth of the individual cell, therefore, may be hidden in the characteristics of growth of the population of cells of the leaf, whereas in the internode the presence of an acropetal gradient may permit to some extent to recognize still the characteristic pattern of the growth of the individual cell.

ESAU (1962) remarks about the growth of internodes that 'it is not known whether the elongation is terminated first and, consequently, the formation of rigid types of wall (in the xylem elements) becomes possible, or whether the elongation ceases because inextensible elements differentiate'.

The relationship, mentioned above between elongation rate and final length of internodes is less easy to understand if the growth of the internodes stops suddenly by the mechanical resistance of differentiating elements of the vascular

tissue. This phenomenon may be an expression of the rigid growth pattern found in poplar, as is also the observation that the leaf terminates its growth 6 to 7 days later than the internode (see Table XX). This time relationship between the growth of leaves and subtending internodes is independent of treatment or age.

Finally, in chapter 3, the relation between internode length and diameter and between the diameter of the youngest measurable internode and the length of the attached leaf, after it has matured, was given in fig. 3.7 and fig. 3.8. It was concluded that the growth of the young sun plant resembles that of a shade plant and that a gradual increase of the mature area of successive leaves, mature petiole length and diameter of the youngest measurable internodes occurs. The lengths of the mature internodes are, however, the same. This leads to the apex crowded with leaf primordia characteristic of an old sun plant. It has been suggested several times in literature that the girth of the youngest measurable internode reflects the increasing diameter of the apical dome of the shoot apex (SINNOT, 1921; WHALEY, 1939; ABBE, RANDOLPH and EINSET, 1941).

6.2. DISCUSSION AND CONCLUSION

The growth of the internodes follows a rigid growth pattern as do other plant parts, although this can be hidden by variability. As in the leaves, this variability is probably due to deficiencies in the root environment. Variability is largest in the internodes, less in the petioles and least in the leaves.

Since leaf production is correlated with the growth rate of the leaves m, with the ultimate leaf size L_m, thus with the number of cells in the leaves, and with the diameter of the apical dome, it may be concluded, that ultimately these correlations are determined by the amount of cell division in, or the volume of the apical dome.

It may be assumed that the amount of cell division in the apical dome determines the growth in height of the stem. Because the amount of cell division is also correlated with the rate of leaf initiation, this would lead to a constant number of cells in each mature internode. The variability in the length of internodes may be due to effects on the growth rates of the cells themselves or on the initiation rate of the leaves.

The constant time lapse between the maturing of leaf and internode suggests that the time needed for the development of an internode, as well as of a small and a large leaf is constant, a conclusion also reached in section 5.1.3. In agreement with this suggestion is the observation that a small leaf grows relatively more slowly and a large leaf faster than the subtending internode.

7. LEAF THICKNESS

7.1. LEAF THICKNESS, ITS DEVELOPMENT AND ITS RELATION TO THE MAXIMUM RATE OF PHOTOSYNTHESIS

Important factors, determining the thickness of leaves are light (ANDERSON, 1955; MILTHORPE, 1956), soil moisture conditions (CLEMENTS and LONG, 1935; DAUBENMIRE, 1947; BJÖRKMAN and HOLMGREN, 1963) and temperature (ANDERSON, 1955). BLACKMANN (in MILTHORPE, 1956) concluded that besides light, competition for substrates between leaf and other plant parts determines leaf thickness. NORDHAUSEN (1903) suggested that sun and shade leaf characteristics are already predetermined in the bud.

Fig. 7.1 shows the development of leaf thickness in oak and sycamore in the open. Directly after emergence from the bud, leaf thickness is between 80 and 100 μ. After an initial small decrease, leaf thickness develops to an extent which is related to the light conditions of the environment. The leaves in the shade are 80 to 100 μ thick, whereas in bright light they may be double this value or even more.

The increase of leaf thickness and leaf area proceed at different periods in the development of the leaf (cf. MAKSYMOWICH, 1963). In fig. 7.2 width and thickness of leaves of *Populus raverdeau* are compared. The increase of leaf area stops earlier than that of leaf thickness. In the shade leaf the dip in the growth of leaf

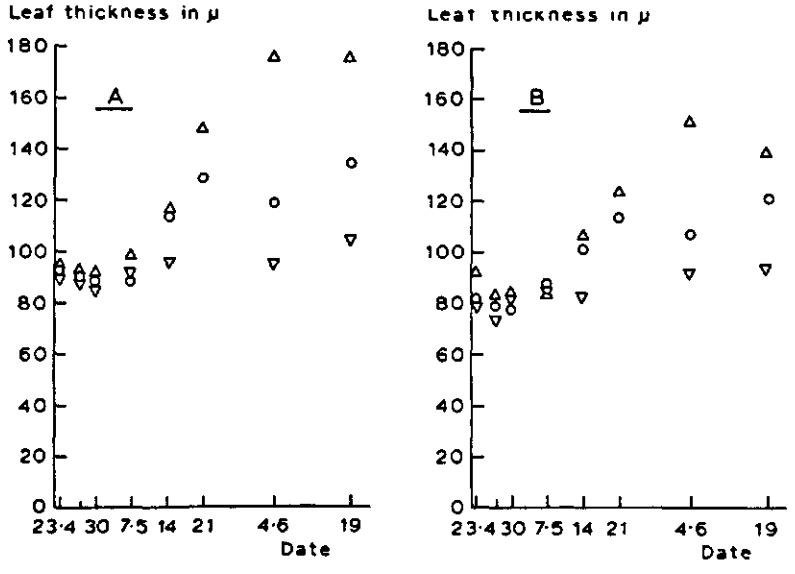


FIG. 7.1. Increase of leaf thickness of *Quercus borealis* L. (A) and *Acer pseudoplatanus* L. (B), measured at different depth in the crown. △ sun leaf; ▽ shade leaf; ○ halfshade leaf.

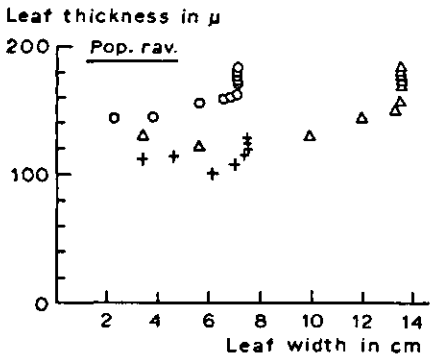


FIG. 7.2. Differences in increase in leaf width and in leaf thickness (*Populus raverdeau*). – Increase in leaf width has finished, while increase of leaf thickness still continues. Light intensity: high (○); medium (△); low (+).

thickness is seen again, notwithstanding the fact that the experimental conditions are constant. Sun leaves are found to be thicker than shade leaves, but the difference between the two highest intensities (○ and Δ) is small. In fig. 7.3 the increase of length and thickness of leaves of sycamore is plotted against time. The length growth proceeds in a smooth manner to the final size, but leaf thickness increases irregularly. The method used for the measurement of leaf thickness introduces some variability of mechanical origin, but in fig. 7.4 it is seen that the variation in leaf thickness is parallel in the successive leaves. The apparent decrease in leaf thickness observed on June 27th was the result of a failure of the watering of these potplants (soil culture). The irregularity is

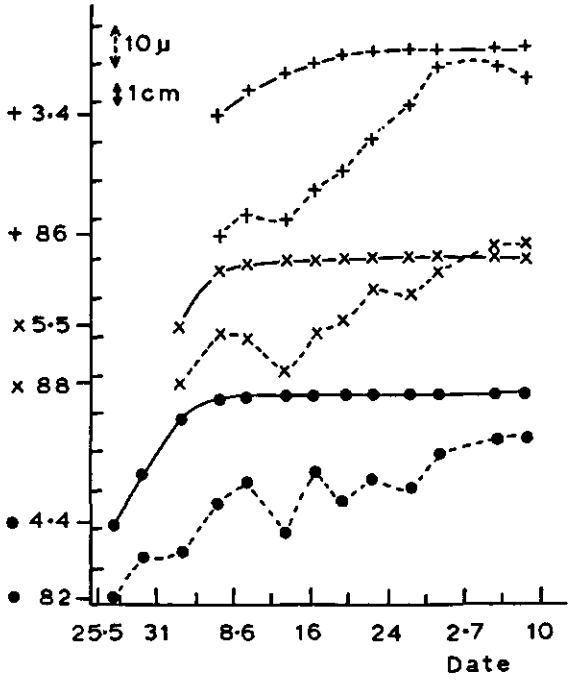


FIG. 7.3. The growth of leaf length (—) in cm and leaf thickness (---) in μ against date in *Acer* spec. Graphs shifted vertically for the sake of clearness.

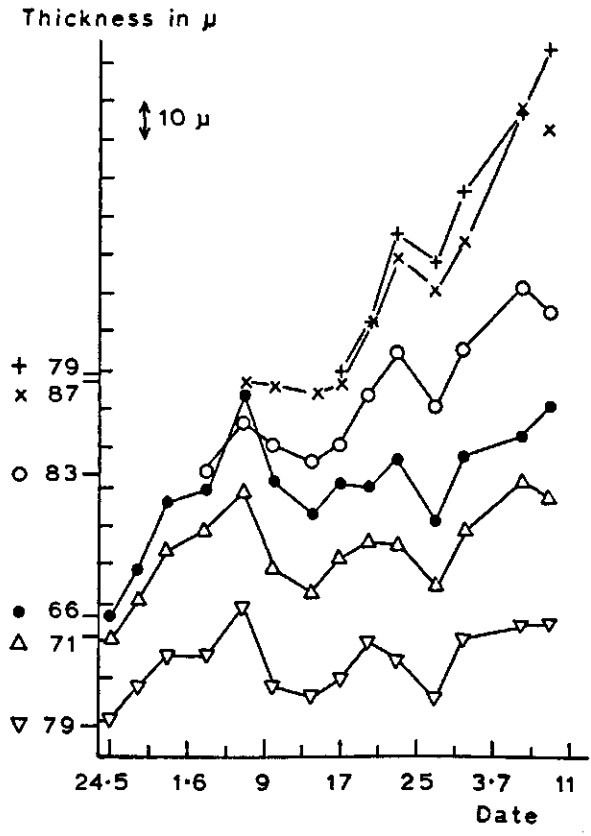


FIG. 7.4. Development of thickness of successive leaves in *Acer* spec. and variation of thickness with date also in mature ones. The dip at 27 June coincides with water deficiency. Increasing leaf age from top to bottom. Note initial values for the different leaves. Graphs shifted vertically for the sake of clearness.

therefore at least partly ascribed to differences in the water content of the leaves (cf. ANDERSON, 1955). Conversely, poplar leaves do not decrease in leaf thickness under conditions of (experimental) water stress.

In poplar, the growth of leaf thickness may continue for a rather long time as is shown in fig. 7.5 for plants cultivated at different light intensities, and, thus, leaf thickness increases with leaf age.

Leaf thickness and the maximum rate of photosynthesis (at light and carbon dioxide saturation) have been shown to be correlated in some cases (PIETERS, 1962). Fig. 7.6 shows the maximum photosynthetic rate and leaf thickness for three large sycamore trees. The leaves were collected from different parts of the crown. Initially, this relationship between leaf thickness and photosynthesis could not be reproduced by cultivation of sycamore, birch, poplar etc. under controlled environments. A large variability in the photosynthetic rates was observed. This is shown in fig. 7.7, where maximum photosynthetic rate and leaf thickness of birch are given. The variation in photosynthetic rates is much larger than that in leaf thickness, and this variation increases with increasing illumination, as do the highest possible rates of photosynthesis. In Table XXI maximum rates of photosynthesis, leaf thickness and leaf number of *Populus*

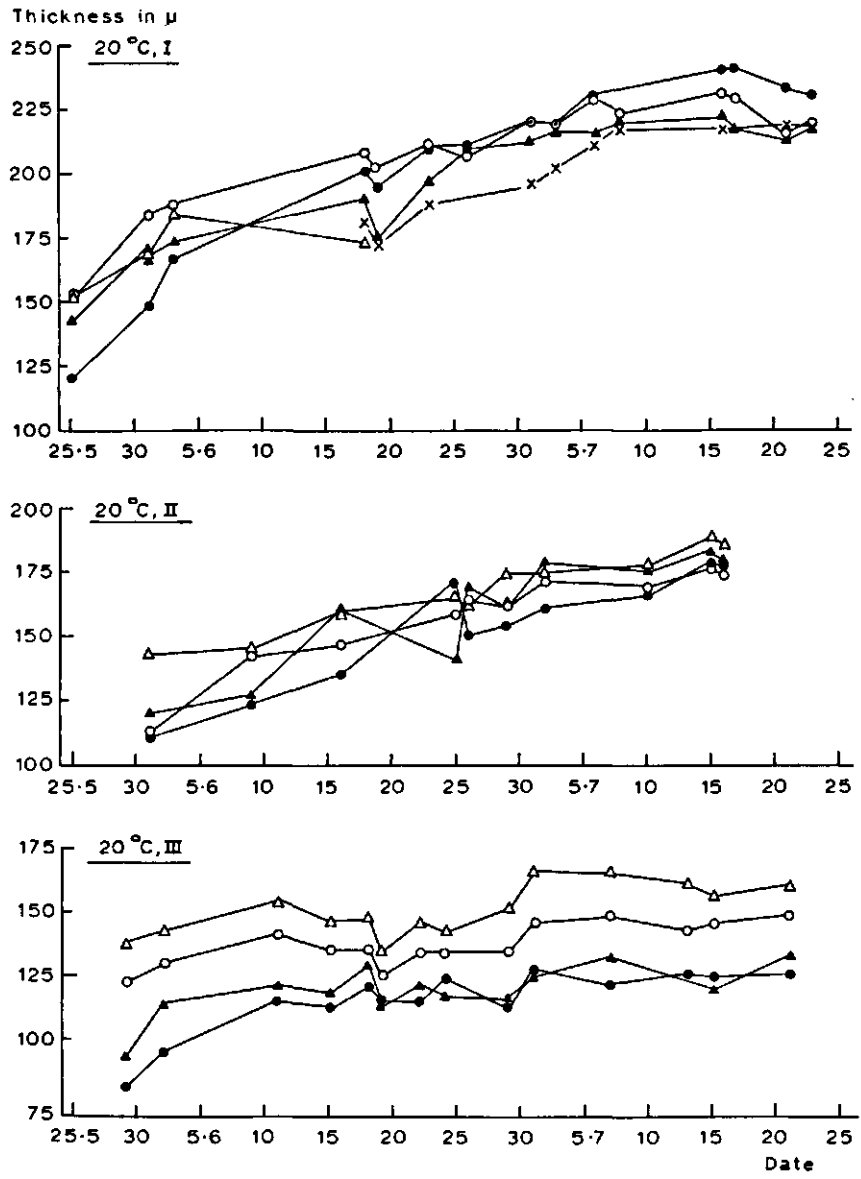


FIG. 7.5. The development of leaf thickness in poplar (*Populus eur.* 'Robusta') at different light intensities against date (from 25 May to 25 July). – These leaves were full-grown in area, cf. also fig. 7.2.

raverdeau cultivated in controlled environment in large pots, in soil, are given. Large and irregular variations in the values of the maximum photosynthetic rates exist. One conclusion is possible, viz., that the largest leaves found on a plant at a certain moment have the highest rates of photosynthesis. The varia-

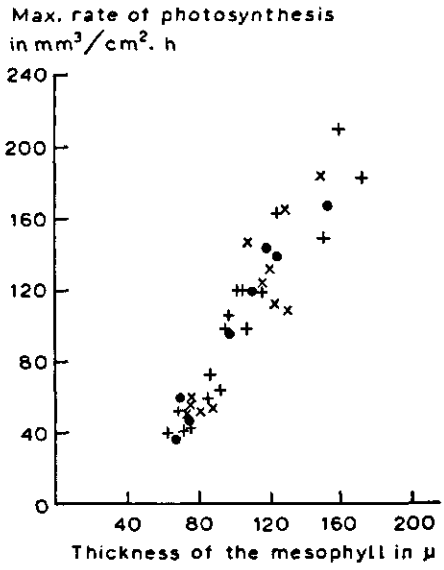
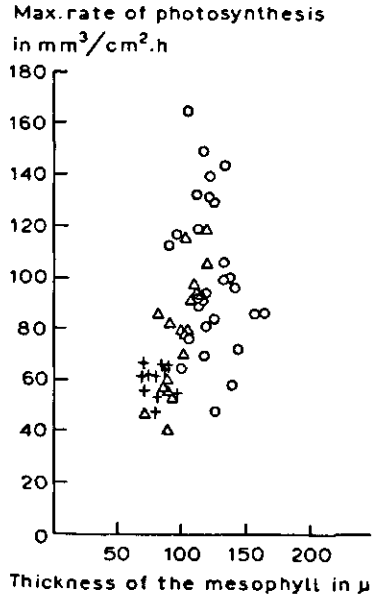


FIG. 7.7. The relation between maximum rate of photosynthesis (mm³ CO_2. $cm^{-2} \cdot hr^{-1}$) at 20°C and thickness of the mesophyll (leaf thickness minus epidermis) in leaves of birch, cultivated in controlled conditions at three light intensities (I,o; II, △; III, +). Note the large variability in the photosynthetic rates especially of plants cultivated at the highest light intensity. – Later experience has shown that this probably is due to deficiencies in the root environment. ▶

FIG. 7.6. The relation between maximum rate of photosynthesis (mm³ CO_2.cm^{-2}. hr^{-1}), measured at 20°C, and thickness of the mesophyll (leaf thickness minus epidermis) in leaves of three large *Acer* trees. Mature leaves from various parts of the crown, from inside to outside. ◀



tions in size, and photosynthetic capacity appear to be related. This was the result of an experiment in which it was hoped to depress variability by changing from seedplants to a clone of poplar. An explanation might be that at high light intensities the root environment limits the growth and development of several leaf characteristics in a very irregular way. This is supported by the fact, that variability is greatly diminished when the plants are cultivated in irrigated gravel culture. Even with gravel culture, however, the plants are not automatically under optimum conditions. In Table XXII, the photosynthetic capacity of sun and shade leaves of an experiment of 1964 in gravel culture are compared with similar data from an experiment of 1971 in gravel culture. Clearly, variability has been suppressed in the latter experiment and this appears partly due to the improvement of the root environment. This suggests that the photosynthetic capacities of birch leaves, shown in fig. 7.7, are to various extents underestimates of those under optimal conditions.

A parallel between chlorophyll content and maximum photosynthetic rate

TABLE XXI. Maximum photosynthetic rate at 20°C and leaf thickness of *Populus raverdeau*, cultivated under various light intensities (I and II). Each set of figures is measured at the same date, the different sets of figures at different dates. The marked leaves (*) were the largest ones on the plant at the date of measurement.

Nr. plant	Nr. leaf from top	Photos. mm^3/CO_2 / $cm^2 \cdot hour$	Thickness in μ	Nr. plant	Nr. leaf from top	Photos. mm^3/CO_2 / $cm^2 \cdot hour$	Thickness in μ
I-1	3	127	239	I-3	3	110	244
	4	137	265		6	194	276
	6	178	287		9	245	269
	7	200	275				
					*8	346	274
	4	258	254		*10	386	305
	9	270	241				
	13	256	289		4	309	261
					8	327	285
	*13	388	299		13	326	281
					17	164	264
I-2	3	140	235				
	6	216	283	I-4	4	238	289
	9	236	302		8	284	300
	11	245	286		12	308	272
	11	307	276		10	417	311
	14	366	268		14	319	283
	14	218	273				
	*12	326	261	II-3	6	254	247
					9	127	184
II-1	3	166	169				
	5	256	182		2	76	159
	8	246	184		4	178	192
	18	265	201		7	213	204
	22	267	214		11	314	269
					16	192	241
	*10	261	192		20	155	189
	*12	258	195				
					*18	301	272
II-2	6	140	250				
	7	144	230				
	10	251	236	II-4	4	226	252
	13	258	243		8	250	265
					10	230	210
	4	189	219		13	198	218
	8	260	241				
	14	316	268		3	124	181
	19	238	265		7	188	231
	24	334	235		11	274	271
					15	354	277
	*17	310	244		21	290	257
					26	262	248
II-3	11	222	200				
	13	187	219		*8	252	220

TABLE XXII. Comparison of mean maximum photosynthetic rates Ph (mm^3 $CO_2.cm^{-2}.h^{-1}$) of leaves of poplar plants from different cultivation conditions (25,20 and 16 °C and 40 (I), 20 (II) and 10 (III) W/m^2), their standard deviations, the number of measurements n, variance s^2, variance ratio F, and the relevant 1 % level of variance ratios from the Snedecor Table for the variance ratio F_s.

Treatment	Experiment 1964				Experiment 1971				$F = \frac{s_1^2}{s_2^2}$	F_s
	Ph	s_1	n_1	s_1^2	Ph	s_2	n_2	s_2^2		
25-I					235.75	7.71	2			
20-I	309.17	82.17	137	6720	275.30	49.98	52	2500	2.69	1.60
20-II	275.32	79.13	184	6280	182.04	44.11	18	1920	3.28	2.67
20-III	181.96	65.59	201	4225	121.91	35.31	30	1240	3.38	2.01
16-I					406,10	81.66	6			

is shown in fig. 7.8 within the light treatments, whereas between the light treatments a negative correlation between both is found. This negative correlation is a result of the increasing xeromorphism of the plant under the influence of increasing irradiation and pot limitation. In 'optimally' growing plants of *Populus euramericana* 'Robusta' the chlorophyll content increases steadily, also at high light intensity (fig. 7.9). Such an increase in chlorophyll content can be observed also under natural conditions during the growing season.

Whereas the increase in area of the leaves was shown to be a long term reaction (the size of the apex has to adapt itself), the increase in leaf thickness is a short term reaction. This can be shown easily by transferring the plants from high to low light intensity or vice versa. In Table XXIII examples are given of the two cases, showing that light intensity exerts a rapid effect on leaf thickness. This reaction seems to be of an energetic, rather than of a stimulatory nature. When a single leaf on a plant in high light intensity is shaded, its leaf thickness will become somewhat lower, but even darkness does not create a shade leaf. This, moreover, is in accordance with the idea of BLACKMAN, already mentioned, that

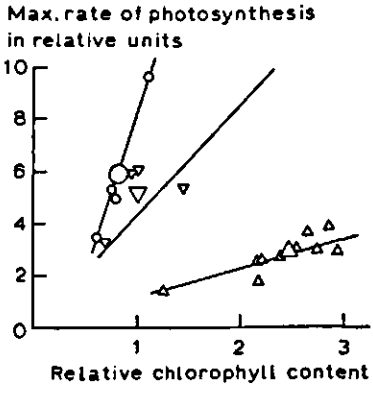


FIG. 7.8. The relation between maximum rate of photosynthesis and relative chlorophyll content in *Acer* leaves, grown under controlled conditions at different light intensities. Light intensities: high (○), medium (▽) and low (△).
Large symbols are the mean values of photosynthetic rate and chlorophyll content per treatment.

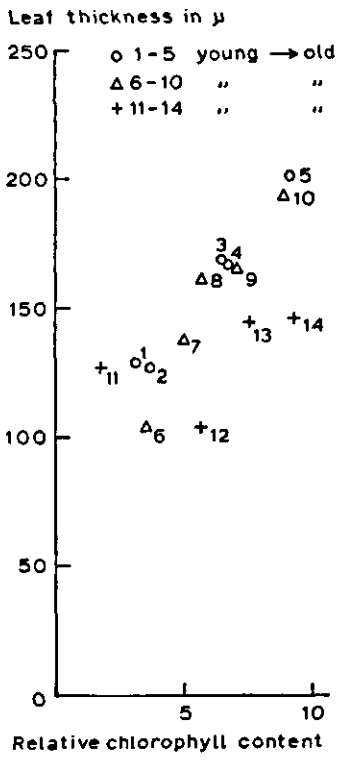


FIG. 7.9. The increase of the chlorophyll content per unit area in leaves of increasing age. Irradiation levels: I, (○); II, (△); III, (+). – Ordinate: leaf thickness, gradually increasing with leaf age, the least at the lowest light intensity (III).

competition is involved, maybe for carbohydrates. A similar type of reaction is found in growing roots. If no storage material is present, as e.g. in *Acer* seedlings or seedlings of oak without acorns, illumination of the above grounds parts induces the growth of the roots in a short term reaction. The relation between the rate of root growth and illumination intensity resembles a photosynthesis – light intensity curve (RICHARDSON, 1953).

In fig. 7.10 the average maximum photosynthetic rate measured at light

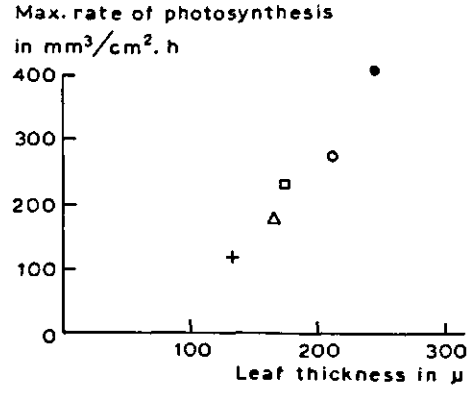


FIG. 7.10. The relation at between the average maximum photosynthesis per unit area in $mm^3\ CO^2 \cdot cm^{-2} \cdot hr^{-1}$ measured at 20 °C and at light- and CO_2-saturation and leaf thickness in 'optimally' growing leaves of *Populus eur.* 'Robusta'. Growth conditions: 40 W/m² (o), 20 W/m² (△) and 10 W/m² (+) at 20°C, and 25°C (□) and 16°C (●) at 40 W/m².

TABLE XXIII. Leaf thickness in the succession of leaves of plants at a constant irradiation level (a) and of plants transferred from high to low irradiation level and vice versa (b), (*denotes youngest measurable leaf, † single leaf shaded). Light intensity I = 40 W/m², II = 20 W/m² and III = 10 W/m².

a

Plant:	I-1		I-2		I-3		I-4				I-5		I-6		
Date:	15/11		28/9		29/9		12/11		10/12	16/12	10/11		10/12		16/12
Leaf number:	Nr		Nr		Nr		Nr				Nr		Nr		
Leaf thickness:		μ		μ		μ		μ	μ	μ		μ		μ	μ
	13	190	42	192	13	193	9	225	229		18	204	10	233	235
	16	213	43	179	14	190	11	†193	210	221	20	226	15	233	244
	18	194	44	181	15	176	13	223	248		22	219	20	248	256
	20	197	45	167	16	182	15	205		249	24	200	25	210	258
	21	178	46	160	17	163	16	198			26	183	28	180	221
	22	175	47	162	18	163	17	191			28	168	30	187	193
	23	164	48	144	19	149	18	184			30	162	31	179	195
	24	161	49	161	20	143	19	174			31	153	32	*193	183
	25	163	50	*151	21	*150	20	173			32	160			
	26	161					21	171			33	163			
	27	*173					22	175			34	*155			
							23	173							
							24	*168							
							30		231	248					
							35		237	243					
							40		*199	245					

→ continued on p. 80

TABLE XXIII (continued).

Plant	II-3			III-2				III-4					
Date	12/11		17/12	10/12		16/12	17/12	17/8		20/8	24/8	5/10	
Leaf number	Nr			Nr				Nr				Nr	
Leaf thickness		μ	μ		μ	μ	μ		μ	μ	μ		μ
	20	148	157	10	150	160	158	2	130	136	136	54	139
	23	143		15	133	134	142	3	130	137	140	55	138
	25	133	157				133	4	130	139	139	56	136
	26	134		20	119	124		5	126	134	137	57	134
	27	133		21	128	122		6	130	136	140	58	128
	28	105		22	*130	119		7	*132	134	137	59	126
	29	122		23			118	8		*136	132	60	128
	30	129	158	24			*128	9			131	61	131
	31	*148										62	*138
	35		159										
	37		143										
	40		144										
	41		150										
	42		*140										

→ continued on p. 81

TABLE XXIII (continued).

b

Plant	I-1				I-2			♀				
Date		15/11	10/12	17/12		28/9	7/10		26/10	10/11	12/11	25/11
Transfer		I→III							I→III			
Leaf number	Nr				Nr			Nr				
Leaf thickness		μ	μ	μ		μ	μ		μ	μ	μ	μ
	8		224	217	42	192		7	203			
	10		218	222	43	179		11	215			
	12		208	208	44	181	242	14	220		223	
	13	190			45	167	199	19	205	218	212	
	14		196	199	46	160	209	23		197		
	16	213	188	195	47	162		24			198	228
	18	194	178	176	48	144		26		184	183	204
	20	197	171	169	49	161		28	*		166	196
	21	178			50	*151		29		164		
	22	175	158	160				30			158	183
	23	164						32		148	146	175
	24	161						34		143	135	165
	25	163	148	155				35			134	
	26	161						36			133	173
	27	*173						37			138	
	30		135	143				38			140	168
	35			132				39			145	
	37			138				40			148	166
	38			141				41			149	
	39			148				42			*153	

→ continued and concluded on p. 82

TABLE XXIII.

Plant	II-3				III-1				
Date		6/10	4/11	15/11		26/10	12/11	10/12	16/12
Transfer		II→III	III→II			III→I			
Leaf number	Nr				Nr				
Leaf thickness		μ	μ	μ		μ	μ	μ	μ
	27	178			10		181		
	28	175			14				198
	29	165			15	123			
	30	168	187	203	16			200	198
	31	156			18	122	177	189	191
	32	157			20		193	237	199
	33	160	181	199	21		208		
	34	153			22		224	244	
	35	*161			23		241		262
	37		163	182	24		243	274	
	40		155	169	25	*	219		266
	42		148	167	26		199	292	
	44		138	165	27		182		
	46		138		28		174	268	275
					29		171		
					30		*168	261	266
					33			256	
					35				264
					36			247	252
					39			239	252
					40				263

and CO_2 saturation at 20°C against leaf thick ness, is given for plants growing optimally, under different conditions. A very good correlation is found. The plants that have been growing at 16, 20 and 25°C and at low, medium and high irradiation level show fairly indentical relationships between maximum rate of photosynthesis and leaf thickness. This supports the notion, that the growth reactions resulting in increased leaf thickness at high light intensity and low temperature are responsible for the differences in the photosynthetic rates of the various leaves.

7.2. Conclusion

Leaf thickness is controlled by light and temperature. High irradiation levels and low temperature create thicker leaves. High light intensity and low temperature often have similar effects on plant growth. This supports the idea that competition for carbohydrates is involved: high light intensity renders competition less important, high temperatures accelerate leaf area growth, which may act as a sink and compete with the growth in thickness.

Increase in leaf thickness, by division and growth of the mesophyll cells, continues for extended periods of time, although eventually at a low rate. After transfer of shade plants to a higher light intensity the older leaves increase in thickness to some extent, suggesting that the capacity for growth in thickness is preserved long after the leaf area has been completed.

The correlation between leaf thickness and maximum photosynthetic rate suggests that the higher photosynthetic capacity of sun leaves and leaves that have developed at low temperature is due to the increased thickness of the leaves. This growth reaction is part of the growth pattern, which is only expressed when sufficient energy is available. Deviations of this primary pattern are due to deviations in the normal development of leaf thickness and photosynthetic apparatus by deficiencies in the root supply. Deficiencies lead to smaller epidermal cells, increased leaf thickness and lower photosynthetic rates.

Finally, it may be stressed that during our investigation it has become clear that the correlation between maximum rate of photosynthesis and leaf thickness, in plants grown under controlled conditions of light and temperature, nevertheless may be masked by limitations, e.g. imposed by inadequate root environments. In an early stage of our work, using leaves of large trees outside, such deviations were less obvious, and it may well be so, that provision with water and minerals in a large tree, are more adequate than can be easily produced under laboratory conditions.

GENERAL DISCUSSION

The problem of sun and shade leaves or the growth and development of leaf characteristics at different light intensities and temperatures was investigated in *Populus euramericana* 'Robusta' growing under optimal conditions. Optimal conditions have been defined as those conditions in which the root environment does not impose limitations on the growth of the plants.

Strong correlations exist between length and width of a growing leaf and between leaf length and petiole length. A rather rigid time relation exists between the moments of maturation of the leaf and the subtending internode: the internode reaches maturation 6-7 days earlier than the leaf. Mature leaf length shows a correlation with the diameter of the youngest measurable internode. This diameter is supposed to be an estimate of the diameter of the apical dome (cf. WHALEY, 1939; ABBE, RANDOLPH and EINSET, 1941; ABBE and PHINNEY, 1951). The ultimate size of the apical dome is determined by light intensity. As such, the above mentioned growth correlations are independent of the light intensity or the temperature under which the plants are cultivated. Clearly, a rigid growth pattern underlies the growth of these plants. The whole seems quite according to a conclusion of STEEVES and SUSSEX (1957) that 'in both ferns and Angiosperms the complete pattern of leaf development is selfcontrolled, once it has been determined at the shoot apex.'

Mean mature cell size in the epidermis of the leaf lamina is constant and nearly independent of light intensity, temperature and level of leaf insertion. Mature cell size in the leaf lamina is generally found to be characteristic for a species (HAMMOND, 1941). The size of the mature leaves is, therefore, a function of cell number.

The relative growth rate of leaf length, S, of an individual leaf at half its final length and thus in the linear phase of growth, can be calculated as the ratio of the linear growth rate of length, m, and half the final length, $\frac{1}{2}L_m$, thus: $S = (2 \cdot m)/L_m$. The results show that the relative growth rate, S, depends only weakly on light intensity, but more strongly on temperature, Q_{10} being 1.70. In the linear phase of growth no cell division takes place in the leaf blade, except around the stomata. Therefore, the absolute growth rate per cell can be calculated as the ratio of the relative growth rate, S, and the number of cells per cm leaf length at half its final length. The upper part of the sigmoid growth curve of a leaf (when divided by the number of cells) may be considered to be an average characteristic of the growth of the individual cells. The study of leaf growth is, therefore, ultimately a study of cell growth.

The mean relative growth rate of total leaf length per plant is also equal to S, that of total width is equal to $S.L_m/(L_m-2)$ and that of total area to $3/2\ S.L_m/(L_m-1)$. With larger mature leaf lengths the relative growth rates of total leaf width and area become practically independent of leaf length. Consequently, differences in absolute growth rate in the linear phase of growth cannot be ex-

plained by differences in average relative growth rate, but only by the larger number of cells which is determined in earlier phases of growth.

There is a constant difference in length ΔL of the successive leaves along the stem during the linear phase of growth, irrespective of treatment; the leaves thus form a descending arithmetical progression, with a common difference ΔL. The constancy of ΔL (about 2 cm) means that the linear growth rate of the leaves (m) and the initiation rate (1/P) are related as: $\Delta L = m \cdot P \approx 2$ cm (see form. 4).

Making use of the relations found, it is possible to relate total leaf area growth, R_s, to the length of the youngest mature leaf, viz.: $R_s \approx KCL_m^3$ (form.6), in which K is a shape factor and C is a coefficient which is slightly dependent on light intensity and highly dependent on temperature, because $C = S/(2.\Delta L)$ and S has a Q_{10} of 1.70. The relation of R_s with L_m^3 is understandable, since it is correlated with length, width and the number of growing leaves which are also mutually correlated.

The primordia of poplar exhibit exponential growth. The logarithms of the lengths of the primordia smaller than 1 cm in the apex approximate a linear relationship with their serial number. Logarithmic relations are rather common in primordial growth (ERICKSON and MICHELINI, 1957; BERG and CUTTER, 1969), but do not directly imply exponential growth. The slope of this relation is related to the ratio of relative growth rate and leaf initiation rate and is connected with accumulation of primordia in the apex. The accumulation of primordia in the apex increases with increasing growth rate of the plant. This is due to an increase of the leaf initiation rate owing to the increasing size of the apex combined with a nearly constant relative growth rate in the phase of primordial growth.

In young plants the mature length of successive leaves increases steadily with serial number. This means that the rate of increase of leaf area increases ($\sim L_m^3$). This constant increase of mature length stops first in low light intensity, then in intermediate light intensities and last in high light intensity. Because the length of the mature leaf is also correlated with the diameter of the youngest measurable internode, it can be inferred, that the diameter of the apical dome has increased proportionally. This is in agreement with the observation that, at constant temperature, differences in growth rate cannot be ascribed to differences in the average relative growth rate in the primordial phase, which is found to be independent of the irradiation level. The increase of leaf length and thus also of the rate of leaf area formation, is ascribed to the increasing size of the apex. In this picture the apex decides over the amount of cells to be incorporated into each initiated primordium. The size of the apex adapts stepwise to the prevailing light intensity. The leaf primordia grow according to a self determined pattern, and reflect the properties of the apex in their growth rate, in the mature leaf size ultimately reached, and in the rate of initiation.

Maximum leaf length and maximum increase in total leaf area per unit time is proportional to the irradiation level. Maximum leaf length then remains the same, the plant has attained a stationary state of growth, and leaf area further increases linearly. This indicates that only a restricted number of leaves provide

the products necessary for growth in the apex of the plant.

It has been observed that the time interval between the initiation of a leaf and its maturation is nearly constant and independent of the level of irradiation or of the leaf size. The time needed for the development of a small leaf to maturation is the same as the time needed for a large leaf. This affects the relation between the rate of leaf appearance and the rate of leaf initiation. It is usual to measure the rate of leaf appearance as the number of leaves per unit time passing an arbitrary length, e.g. 1 cm. If indeed mature leaf size increases with leaf number, this means that the leaf appearance is counted at an ever earlier stage in the development of the successive leaves. Therefore, it is suggested that a better measure of the initiation rate is gained by measuring their appearance at a constant stage of their development, e.g. when they have reached half their ultimate length (cf. ASHBY and WANGERMAN, 1950; SAEKI, 1960; ONDOK, 1968). This entails that the rate of initiation can be estimated only when ultimate mature leaf size is known. However, this implies that no other restrictions are imposed on the development of the leaves and mature cell sizes are similar.

The mature length of internodes is rather variable, but seems to be only weakly affected by irradiation and temperature. The reasons for this variability have not been investigated, but may be related to the conditions in the root environment. There is a difference in the mode of growth of leaves and internodes: leaf growth is described by the well-known sigmoid curve, but internodes show an extended period of linear growth, which stops suddenly (cf. LOCKHART, 1971). It may be of interest to investigate the causes of this different behaviour in growth, e.g. on the basis of a study of gradients in developmental stages of cells along the growing axis. If indeed the length of the internodes is constant and independent of irradiation level and temperature, the growth in height of the poplar plants is proportional to the initiation rate of the leaves and therefore to the rate of cell division in or the size of the apex.

Leaf thickness reacts rapidly on a change in light intensity. This reaction to light is possibly a reaction to carbohydrate level. This agrees with results reported by BLACKMANN, G. E. (in MILTHORPE, 1956) who stated with respect to leaf thickness that 'for leaves, in which the lamina is still expanding, the response to light is centred in the individual leaves, but that the magnitude of the changes is determined by competition for substrates between the different parts of the plants'.

The different reaction of the expansion of the leaf lamina and of the increase in thickness of the mesophyll may be due to differences in the relationship between carbohydrate level and growth rate of the tissues involved. Differences in the relation between substrate level and growth rate of different tissues may occur quite generally: 'source-sink relationships and substrate levels and gradients may form an integral part of the growth pattern' (CUTTER, 1965). The observed differences in reaction to irradiation level may be exemplified by the epidermal cells of the leaf lamina at one side and the mesophyll cells at the other side.

Also the influence of temperature on the development of leaf thickness may

be seen as a growth response to carbohydrate level. High irradiation level and low temperature produce the thickest leaves. The high maximum rates of photosynthesis in sun leaves and leaves from low temperature may be seen as a result of leaf growth: thicker leaves contain more photosynthetically active cells. The correlation between leaf thickness and maximum photosynthesis supports this, but was only found in optimally growing plants. The deviations of this primary pattern of growth are due to deficiencies in the root environment, resulting in a limited nutrient supply to the growing tissues. These deficiencies prevent the normal development of the chloroplasts and lead to various degrees of chlorosis, accompanied by lower photosynthetic capacities and to deterioration of chloroplasts (DUFOUR, 1887; BJÖRKMAN and HOLMGREN, 1963).

Homoblastic leaf growth is based on the regular increase of the size of the apical dome (until this size is adapted to the prevailing light intensity) and on the self-determined growth of the primordia after their inception. If the apical dome does not change its shape, its volume is related to the cube of its diameter. Hence, it may be assumed that at constant temperature there is a constant ratio between the increase of total leaf area per plant per unit time and the apical volume. To put it in another way: the return of the cell capital, deposited in the apical dome, only depends on temperature. This indicates once more that the growth of the leaves of a plant follows a rigid growth pattern from initiation to maturation and that its rate depends on the number of cells in each primordium times a constant average relative growth rate per cell. In the linear phase of growth the same is expressed by the constant absolute growth rate per cell.

It has been shown that in the primordial phase, the growth of a poplar leaf proceeds exponentially and can be characterized by the relative growth rate k_t, which represents the slope of the exponential part, when plotted logarithmically. The relative growth rate is shown to remain constant irrespective of the age of the plant and of the irradiation level to which it is exposed. Only at the lowest light level used, the growth rate per cell is 14% lower in the exponential as well as in the linear phase of growth. This indicates that at this irradiation level, shortage of energy flow expresses itself to a certain extent also in the growth rate of the leaves, which means for this energy level a certain amendment of the above conclusion.

Exponential growth of an individual leaf can be formulated as (BUCHANAN, 1953):

$$L_t = L_o.e^{k_t.t} \qquad (9)$$

in which:
L_t is the length of the primordium at time t
L_o is the length of the same primordium at time 0
k_t is the average relative growth rate and
t is the time interval considered.

It has been shown in the preceding sections, that the growth of either a small or a large leaf proceeds in a constant interval of time, in the exponential as well

as in the linear phase of growth. Taking t to be the constant interval of time between initiation and the end of exponential growth, this, together with a constant relative growth rate results in constancy of the term $k_r.t$ in formula (9). In that case differences in absolute growth rate have to be due to differences in L_o, a measure of the volume of cells given over by the apex to the newly initiated primordium.

Small differences in the relative growth rate, k_r, or in the duration of growth, t, both may have a large effect on the ultimate size of a leaf. The 13% lower growth rate observed in leaves growing at the lowest light intensity, in principle might be sufficient to explain the smaller size of the shade leaves, if t and L_o were assumed to be equal in sun and shade leaves. It is, however, improbable that L_o in the small shade leaves and the large sun leaves is equal, because of the relation existing between the diameter of the youngest measurable internode (i.e. the size of the apical dome) and the length of the mature leaf. Another possibility is that in shade leaves the duration of growth increases as much as the relative growth rate declines.

In connection with these questions direct observations of the behaviour of the apical dome under various culture conditions will be of great importance.

SUMMARY

The growth and development of sun and shade leaves was investigated in *Populus euramericana* 'Robusta', growing under various light intensities and temperatures and on a substrate optimal for the roots.

Strong correlations exist between the dimensions of the various plant parts. The width of an individual growing leaf is always 2 cm smaller than its length. Leaf length and petiole length are strongly correlated. Mature leaf length is correlated with the diameter of the subtending internode, as measured at the earliest possible stage. The mature length of the internodes is similar in all treatments (Chapter 3).

The mean mature size of the epidermal cells in the leaf lamina is practically constant, irrespective of treatment or of level of leaf insertion. The individual cells too tend to grow to a constant final size. The basipetal trend in the expansion of the leaf is the consequence of the shape of the leaf: where the leaf is broadest, the expansion lasts longest and the cells mature last (Chapter 4).

In the linear phase of growth, the growth rate of the length of the individual leaf is correlated with its final size. The relative growth rate of leaf length depends only weakly on irradiation level but rather strongly on temperature. The average relative growth rate of total leaf length, of total leaf width and of total leaf area per plant is fairly independent of irradiation level and leaf length. The differences in growth rate have to be ascribed to differences in the number of cells involved in leaf growth. This number of cells is determined in phases preceding the linear phase of growth (Chapter 5.1.1).

The difference in length of successive, growing leaves is more or less constant, irrespective of treatment or of the age of the plant and these lengths form a descending arithmetical progression with a common difference of about 2 cm. This is due to the fact that the linear growth rate of the leaves is proportional to the initation rate of the leaves (Chapter 5.2).

The rather rigid relation between leaf length, leaf width and initiation rate allows to relate total growing leaf area and the growth rate of total leaf area per plant to the cube of the length of the youngest mature leaf (Chapter 5.3).

The logarithms of the lengths of the primordia along the growing axis are linearly related to their serial number. The slope of this relation is steeper in shade plants than in sun plants, when they are both in their stationary state of growth. This indicates that the primordia grow exponentially and that a high growth rate of the plant is accompanied by accumulation of primordia in the apex. It is shown that this is due to the leaf initiation rate, being higher in sun plants than in shade plants, together with a constant relative growth rate of the primordia. The higher growth rate is due to the higher number of cells in the primordia and this higher number of cells is determined during the phase of initiation: the larger the apical dome, the larger the number of cells initiating a primordium (Chapter 5.5 and appendix).

In young plants mature leaf length increases steadily with leaf number until a stationary state is reached. Shade plants reach this state soon, plants from intermediate light intensities somewhat later and sun plants last. It appears that the rate of increase of total leaf area in the stationary state of growth is linearly related to the irradiation level. The causes of this fact are discussed (Chapter 5.6).

The time interval between initiation and maturation of a leaf is constant at constant temperature, irrespective of leaf size. This has consequences for the relation between leaf initiation rate and leaf appearance rate. It is suggested to define the plastochron as the time interval between the appearance of successive leaves at equal developmental stages (Chapter 5.7).

Homoblastic leaf growth is defined as growth, based on a steadily increasing size of the apical dome, combined with a selfdetermined growth of the leaf primordia, once initiated at the apex. In the internode this growth pattern is also recognized: the growth in height of the stem is proportional to the diameter of the apical dome. Because also leaf initiation is proportional to the size of the apical dome, the mature length of the internodes is more or less constant (Chapter 6).

Leaf thickness may be the result of competition for substrates of the mesophyll and other tissues. The higher the irradiation level or the lower the temperature, the thicker the leaves are and the higher also the rate of light- and CO_2-saturated photosynthesis per unit leaf area. The photosynthetic capacity of the mesophyll cells, composing the thickness of the leaf, is mutually equal (Chapter 7).

The various observations are discussed and compared with the literature. The mathematical aspects of the observations are discussed in the Appendix.

SAMENVATTING

De groei en ontwikkeling van zon- en schaduwbladen werd onderzocht aan planten van *Populus euramericana* 'Robusta', opgekweekt in verschillende licht intensiteiten en temperaturen en op een substraat, dat als optimaal voor de wortels wordt beschouwd.

De afmetingen van verschillende plantendelen vertonen nauwe correlaties. De breedte van een groeiend blad is 2 cm korter dan de lengte. Bladlengte en de lengte van de bladsteel zijn nauw gecorreleerd, evenals de volwassen bladlengte en de diameter van het bijbehorend internodium, gemeten in het vroegst mogelijke stadium. De volwassen lengte van het internodium is ongeveer gelijk in alle behandelingen (Chapter 3).

De gemiddelde diameter van de volwassen epidermis cellen van de bladschijf is practisch constant en onafhankelijk van de behandeling en van het bladnummer. In de individuele cellen bestaat de tendens tot een zelfde volwassen diameter uit te groeien. De bladstrekking verloopt basipetaal als gevolg van de bladvorm: daar waar het blad het breedste is, gaat de strekking het langst door en bereiken de cellen het laatst hun volwassen afmeting (Chapter 4).

De snelheid van de lengte groei van het individuele blad vertoont een lineaire relatie met de volwassen grootte van dat blad. De relatieve groeisnelheid van de bladlengte wordt in geringe mate door de lichtintensiteit beïnvloed en tamelijk sterk door de temperatuur. De gemiddelde relatieve groeisnelheid van de totale bladlengte, van de totale bladbreedte en van het totale bladoppervlak per plant is eveneens nagenoeg onafhankelijk van de lichtintensiteit en de bladlengte. De verschillen in groeisnelheid moeten daarom worden toegeschreven aan het aantal cellen in het blad. Dit celaantal komt tot stand in groeifasen die aan de lineaire groei vooraf gaan (Chapter 5.1).

Het verschil in lengte van opeenvolgende bladen is tamelijk constant en onafhankelijk van de behandeling of de leeftijd van de plant. Deze lengten vormen samen een rekenkundige reeks met als verschil het verschil in bladlengte ($\approx$ 2 cm). Dit is het gevolg van het feit, dat de lineaire groeisnelheid van de bladlengte evenredig is met de snelheid van bladafsplitsing (Chapter 5.2).

De vrij nauwe relatie tussen bladlengte, bladbreedte en de snelheid van bladafsplitsing heeft tot gevolg dat er een relatie bestaat tussen het totale groeiende bladoppervlak en de groeisnelheid van het totale bladoppervlak per plant met de derde macht van de lengte van het jongste volwassen blad (Chapter 5.3).

De logarithme van de lengte van het primordium staat in een lineair verband tot het primordium nummer. De helling van deze relatie is steiler in schaduwplanten dan in zonneplanten, wanneer beide in hun stationaire groeifase zijn. Hieruit volgt dat de primordia exponentieel groeien en dat een hoge groeisnelheid samen gaat met ophoping van primordia in de apex. Dit wordt veroorzaakt doordat de snelheid van afsplitsing van primordia in zonneplanten hoger is dan in schaduwplanten en de relatieve groeisnelheid van de primordia gelijk. De

hogere groeisnelheid is toe te schrijven aan het grotere aantal cellen in de primordia en dit grotere aantal cellen ontstaat in de fase van de aanleg van de primordia: des te groter het groeipunt is, des te groter het aantal cellen in het geïnitieerde primordium (Chapter 5.5 en de appendix).

In jonge planten neemt de volwassen bladlengte regelmatig met het bladnummer toe, totdat de fase van stationaire groei wordt bereikt. Schaduwplanten bereiken dit stadium het eerst, planten uit de middelste lichtintensiteit wat later en planten uit de hoogste lichtintensiteit het laatst. Het blijkt dat de groeisnelheid van het totale bladoppervlak per plant in de stationaire fase evenredig is met de lichtintensiteit. De oorzaken hiervan worden aan een beschouwing onderworpen (Chapter 5.6).

Bij constante temperatuur is de tijd, die verloopt tussen de initiëring en de afrijping van het blad constant en onafhankelijk van de bladafmeting. Om deze reden wordt voorgesteld de plastochron te definiëren als het tijdsinterval tussen het bereiken van een gelijk ontwikkelings-stadium door opeenvolgende bladen (Chapter 5.7).

Homoblastische bladgroei kan worden gedefinieerd als groei op basis van een toenemende afmeting van het groeipunt, gevolgd door een autonome uitgroei van de primordia na hun afsplitsing. Hetzelfde groeipatroon is aanwezig in de internodiën: de hoogtegroei van de plant is evenredig met de diameter van het groeipunt. Omdat ook de bladafsplitsing evenredig is met de diameter van het groeipunt, is de volwassen lengte van de internodiën min of meer constant (Chapter 6).

De bladdikte lijkt het gevolg van concurrentie om substraat van het mesophyl en andere weefsels. Hoe hoger de lichtintensiteit en hoe lager de temperatuur, des te dikker zijn de bladen en des te hoger ook de licht- en koolzuurverzadigde photosynthese per eenheid van bladoppervlak. De photosynthesecapaciteit van de mesophylcellen, die tezamen de bladdikte opbouwen, is onderling gelijk (Chapter 7).

De waarnemingen worden besproken en met de literatuur vergeleken. De wiskundige aspecten ervan worden in een appendix behandeld.

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APPENDIX

MATHEMATICAL ASPECTS OF LEAF GROWTH

1. Introduction

When a poplar plant is grown for some time at constant environmental conditions, a stationary state of growth develops which is characterized by a constant rate of growth of the length of the shoot and of the leaf area, a constant initiation rate of new leaves, a constant total area of all the leaves that are growing and a constant size of the mature leaf.

For each leaf, three distinct periods of growth can be distinguished. Most growth takes place in the last phase, which is characterized by a linear increase in leaf length. This phase is preceded by a phase of primordial growth in which the leaf size increases much more than proportional with time. The first phase is the initiation at the growing point.

Each phase can be characterized by a number of growth parameters that concern the growth of the leaves in length, width and surface, but these parameters are very much interrelated, so that the entire growth process in the stationary state may be characterized by only a few parameters.

The purpose of this appendix is to develop the expressions that characterize the growth and the interrelations of growth parameters within each phase of growth and between them. For this purpose, the main phases are treated at first independently of each other and then in combination.

2. The linear phase of growth

It was observed that starting from a size of roughly 5 cm the length and the width of the leaves increase linearly at the same rate, but that the width is, irrespective of the growth conditions, always about 2 cm smaller than the length. Moreover, it appeared that within this linear phase the length of the successive growing leaves differ also about 2 cm in size, irrespective of the growth conditions. Such a constant difference in size means that the time interval between the initiation of two successive leaves, the plastochron duration P, is closely related with the linear growth rate m:

$$\Delta L = m \cdot P \approx 2 \text{ cm} \tag{1}$$

Another main characteristic is the relative growth rate of a leaf at the moment it reaches half its mature length. This parameter is defined by

$$S = 2 \cdot m/L_m \tag{2}$$

in which L_m is the mature leaf length. This relative growth rate appeared to be independent of the light intensity. Since the size of the mature epidermal cells is independent of the growth conditions, this means that the average linear growth rate of the cells is constant.

The absolute growth rates of the length, width and area of all growing leaves per plant and the total length, width and area of the growing leaves are now considered. Their quotients are the relative growth rate of these characteristics for all leaves together.

2.1. *The growth in length*

In the stationary state of growth one mature leaf is formed during the time interval between the initiation of two successive leaves, the plastochron duration, so that the average rate of growth of the total leaf length is

$$R_L = L_m/P = S \cdot L_m^2/(2 \cdot \Delta L) \tag{3a}$$

The latter expression is obtained by substituting equation (1) and (2).

The total length of all growing leaves is obtained by summation of an arithmetical progression with a common difference of ΔL.
This sum equals just before a leaf matures

$$L_m^2/(2 \cdot \Delta L) + 0.5\, L_m$$

and just after a leaf matures

$$L_m^2/(2 \cdot \Delta L) - 0.5\, L_m$$

so that the length of all growing leaves on the average equals:

$$T_L = L_m^2/(2 \cdot \Delta L) \tag{4a}$$

Hence, the relative growth rate of the total length equals:

$$RGR_L = R_L/T_L = S \tag{5a}$$

This is an approximate formula, because it is assumed that from initiation onwards the leaves grow at a constant linear rate and this is only true for a size of about 5 cm or more.

2.2. *The growth in width*

The final width of the leaves is $L_m - 2$ and the average growth rate of the width of all leaves together is given by

$$R_W = S \cdot L_m(L_m - 2)/(2 \cdot \Delta L) \tag{3b}$$

This equation is otherwise derived in the same way as equation 3a.

The average total width of all growing leaves is again obtained by summation of an arithmetical progression with common difference ΔL resulting in

$$T_W = (L_m^2 - 4 \cdot L_m + 4)/(2 \cdot \Delta L) \tag{4b}$$

Hence, the relative growth rate of the total width equals

$$RGR_w = \frac{L_m(L_m-2)}{L_m^2-4\cdot L_m+4} \times S = \frac{L_m}{L_m-2} \times S \qquad (5b)$$

This expression for the relative growth rate is somewhat more complicated than the similar expression for the length, because the growth in width of each leaf starts only when a length of 2 cm is reached. With increasing final length the relative growth rate of the width approaches the relative growth rate of the length.

2.3. *The growth in leaf area*

Since

$$\frac{d(W\cdot L)}{W\cdot L}/dt = \frac{dL}{L}/dt + \frac{dW}{W}/dt$$

it could be concluded that the average relative growth rate of the area is the sum of the relative growth rates of length and width and therefore approximates $2\cdot S$. However, the above differential equation is only valid for one leaf. If the total growth of the successive leaves is considered, the relative growth rate is defined by

$$\frac{d\,\Sigma(W\cdot L)}{\Sigma W\cdot L}/dt$$

and the value of this expression can only be found by going through the proper arithmetics.

The average growth rate of the area is again found by considering the rate of formation of mature leaves and is

$$R_S = K\cdot L_m(L_m-2)/P = K\cdot S\cdot L_m^2(L_m-2)/(2\cdot\Delta L) \qquad (3c)$$

in which K is a coefficient of shape.

If the poplar leaf would be of triangular shape, then this coefficient would be 0.5. Its value is about 0.73, which means that the sides of the triangle are curved outwards. It is experimentally established that the coefficient of shape changes little with leaf size.

The area of the n^{th} growing leaf is

$$A_n = K\cdot L_n\cdot W_n = K\cdot L_n(L_n-2) = K(L_m-n\cdot\Delta L)(L_m-n\cdot\Delta L-2)$$

The sum of the areas of all p growing leaves is

$$T_s = K\{p\cdot L_m^2 - q\cdot L_m\cdot\Delta L + r(\Delta L)^2 - 2\cdot p\cdot L_m + q\cdot\Delta L\} \qquad (6)$$

in which:

$q = p(p+1)$

is the sum of an arithmetical progression of the first order with common difference 2 and

$$r = {}^1/_3 \cdot p^3 + {}^1/_2 \cdot p^2 + {}^1/_6 \cdot p$$

is the sum of an arithmetical progression of the second order with common difference 1: $1^2 + 2^2 + \ldots + p^2$. This summation looks cumbersome, but a lucid geometric method of summing arithmetical progressions of higher order is given by GARDNER (1973).

The area of the growing leaves just after maturation of a leaf, expressed in L_m and ΔL only, is found by substituting for p the value $L_m/\Delta L$. The area just before maturation of a leaf is found by substituting for p the value $(L_m/\Delta L) + 1$. In this way it is found that the mean area that grows equals:

$$T_s = \frac{K \cdot L_m \{2 \cdot L_m^2 - 6 \cdot L_m + (\Delta L)^2\}}{6 \cdot \Delta L} \tag{4c}$$

and the relative growth rate equals:

$$RGR_s = \frac{L_m (L_m - 2)}{L_m^2 - 3 \cdot L_m + 0.5 (\Delta L)^2} \times \frac{3}{2} S$$

This seems a cumbersome expression, but by substituting for ΔL the value of 2 cm, which value holds for all plants cultivated at any temperature or irradiation level, it is found that the total area of the growing leaves depends mainly on L_m^3 and the relative growth rate equals

$$RGR_s = \frac{L_m}{L_m - 1} \cdot \frac{3}{2} \cdot S \tag{5c}$$

Hence, the relative growth rate of the total area approaches with increasing L_m very soon the value $(3/2) \cdot S$ which is smaller than $2 \cdot S$ as would be found by using the attractive, but incorrect approach in the beginning of this section. This difference and the more complicated form of the expression for the total area are of course due to the fact that the average area of all growing leaves is not equal to the area of a growing leaf at half of its final length.

2.4. *Discussion*

The importance of the above formulas is that they illustrate the intrinsic simplicity of the growth pattern of poplar in the linear phase. All relative growth rates are directly dependent on S, the relative growth rate of length of the leaves at half their final length. Since the number of cells per centimeter in the mature leaf is always equal, this means that all relative growth rates are only dependent on the absolute linear growth rate of each individual cell, which is always the same in all growing leaves of the plant. Moreover, it has been found experimentally that this linear growth rate of the cells is only dependent on temperature and within a wide range independent of light intensity. In its most simple way, the

phenomena are summarized by stating that a leaf starts to grow in width when its length is 2 cm and that a new leaf is initiated at the same moment. If then a leaf grows two times faster it reaches this critical length in half the time, so that the initiation rate is doubled. The only reason that poplar under high light intensity grows faster than under low light intensity is a larger amount of growing tissue. Why this is the case has to be found by an analysis of the growth of the leaves in the earlier phases of growth.

3. The primordial phase of growth

Leaf growth in width becomes apparent only when they are longer than 2 cm. Hence, in the earlier phases of growth, only length can be considered. The analysis of growth in length is often done by considering the relation between the lengths of successive leaves in the primordial stage at a certain moment.

It appears that irrespective of growth conditions and plant species (Erickson and Michelini, 1957; Berg and Cutter, 1969; Bensink, 1971), often a linear relationship is found between the logarithms of length of the successive leaves of a plant, as illustrated in figure 5.14 for sun and shade plants of poplar at two light intensities. It is often concluded from this linearity on a logarithmic scale that the growth of the leaves is exponential, with a relative rate proportional to the slope of the line. Bensink (pers. comm., cf. also Erickson and Michelini, 1957) has evidence that this is a simplification of the existing relations and that this conclusion is only true under very special circumstances. In the following this type of inference is discussed in some detail.

The linearity in figure 5.14 means only that at time t

$$\ln L_n^{(t)} - \ln L_{n-1}^{(t)} = C^{(t)}$$

and that at time $t + \Delta t$

$$\ln L_n^{(t+\Delta t)} - \ln L_{n-1}^{(t+\Delta t)} = C^{(t+\Delta t)}$$

These relations hold for any value of n, but since the slope of the line in the semilogarithmic graph generally changes with time, the following unequality exists: $C^{(t)} \neq C^{(t+\Delta t)}$

Irrespective of whether the growth of a leaf is exponential or not, the length of leaf n at time $t+\Delta t$ equals

$$L_n^{(t+\Delta t)} = L_n^{(t)} \cdot e^{R_n \cdot \Delta t}$$

in which R_n is the mean relative growth rate over the period Δt.

Hence,

$$\ln L_n^{(t+\Delta t)} - \ln L_{n-1}^{(t+\Delta t)} = \ln L_n^{(t)} - \ln L_{n-1}^{(t)} + (R_n - R_{n-1})\,\Delta t$$

or

$$(R_n - R_{n-1}) = \frac{C^{(t+\Delta t)} - C^{(t)}}{\Delta t} = \frac{\Delta C}{\Delta t} = K \qquad (6)$$

which is independent of the value of n.

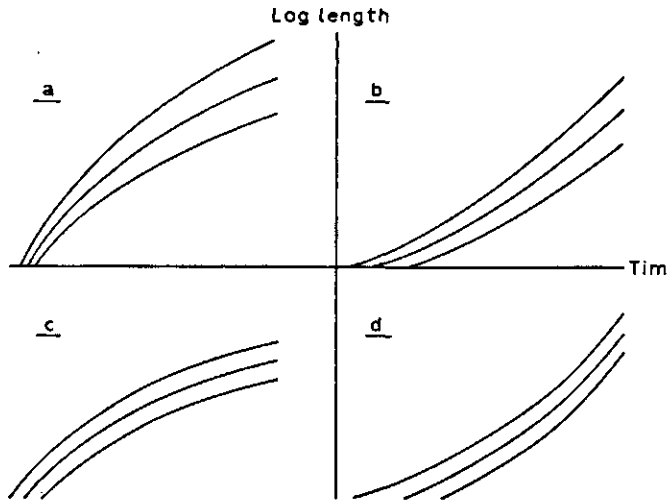

FIG. 1. Schematic representation of primordial growth. Explanation in text.

Therefore, if the relative growth rate of the n^{th} leaf equals R_n, then the relative growth rate of the $(n-1)^{th}$ leaf equals $R_n - K$ and of the $(n-2)^{th}$ leaf $R_n - 2 \cdot K$ and so on, all at the same moment. The relative growth rates of successive leaves decrease or increase therefore in arithmetical fashion. However, the relative growth rate of each leaf may change with time. This seems difficult to visualize and therefore two situations are illustrated in fig. 1 (a and b). The logarithm of the length of successive leaves is given as a function of time. In graph a, the relative growth rate decreases with time and in graph b the relative growth rate increases with time. In both cases the difference of the logarithms of the lengths of any pair of successive leaves at an arbitrary moment is constant, so that linear relations result when the logarithm of the length of successive leaves is plotted against serial number like in figure 5.14. In both cases $\Delta C/\Delta t$ is unequal to zero, but with opposite sign. If, however, $\Delta C/\Delta t$ is zero, then the relative growth rate of the successive leaves is the same at any moment. Two examples of this situation are given in the graphs c and d in fig. 1. In graph c the relative growth rate of each leaf decreases with time and in graph d it increases with time. Hence, even in this situation the growth of individual leaves is not necessarily exponential. In case of graph c the relative growth rate of the successive leaves decreases continuously with time, so that at last a plant remains which does not grow at all. In graph d a plant develops which grows in due course in an explosive fashion. In our experiments it has been observed with poplars at high and low light intensity that their growth reaches in due course a stationary state. Movereover, it has been shown that then $\Delta C/\Delta t$ does not change with time, i.e. that the slopes of the lines in figure 5.14 do not change. It must be concluded that in a poplar plant in its stationary state all leaves in their primordial phase grow exponentially and at the same relative growth rate. In

this and only in this case, the relative growth rate can directly be read from the slope of the line in figure 5.14, taking into account the plastochron duration. It is seen that the slopes of the lines differ a factor 1.6 while the plastochron duration differs also a factor 1.6 for high and low light intensity. Hence, the relative growth rates of the leaves of sun and shade plants in their primordial phase is practically the same, irrespective of the light intensity.

Exponential growth of the leaves in the primordial phase of growth is not self-evident and it can only be demonstrated when a stationary state of growth develops as in the case of poplar. In this case the relative growth rate of all leaves is necessarily the same in all stages of primordial growth. However, this does not mean that this relative growth rate is within wide ranges independent of light intensity. This is again characteristic for poplar, at least when grown as in the present experimental series.

Each poplar leaf proceeds from a phase of exponential growth into a phase of linear growth. The length at the transition point is obtained by division of the growth rate in the linear phase by the relative growth rate in the primordial phase. For shade plants, this length is $0.98/0.28 = 3.5$ cm and for sun plants $1.76/0.325 = 5.4$ cm. Both lengths are $\pm$ 25 per cent of their final length, so that the transition occurs at the same cell size. This is not necessarily at the time that the cells stop with division.

4. The initiation phase of growth

In the stationary state of growth the relative growth rate of the leaves does not change with time in the primordial phase. To obtain a start of the growth, the leaf has to be initiated. It is an arbitrary decision at which moment or what size the leaf is recognized as a part of the apex or as a primordium. Hence, there is no reason at all to assume that the relative growth rate of the leaf cells is different during the period that they are still classified as part of the apex. Or more in general there is no reason to suppose that the relative growth rate of the apex itself is different from the relative growth rate of the primordial leaves.

Now it has been observed that the relative growth rate is the same for sun and shade plants. Therefore the sun plant can grow only faster than the shade plant because its apex is larger.

Considering the length of leaves, it has been shown that the total length of the growing leaves is proportional to the square of the length of the mature leaf (eq. 4a). Hence, the initiation rate of new tissue resulting in length of leaf primordia is for the sun plants $23.5^2/14.5^2 = 1.62^2 = 2.62$ times larger than for shade plants, 23.5 and 14.5 being the lengths of mature sun and shade leaves respectively. Obviously this is achieved by an initiation rate for both the number and the length of the leaf primordia, which is 1.62 times larger in sun plants than in shade plants. Thus the apex is 2.62 times larger with regard to the formation of total length of leaves. Considering the area of the leaves, it has been shown that the total growing area of the leaves is practically proportional to the cube

of the length of the mature leaf. Hence, the initiation rate of new tissue resulting in area is $1.63^3 = 4.24$ times larger in sun plants than in shade plants and the apex has to be also 4.24 times larger.

Since it has been observed that the diameter of the internode in its youngest measurable stage is proportional to final leaf length, it must be concluded that the diameter of the apex is 1.63 times larger which makes the surface $1.63^2 = 2.65$ times larger and the volume 4.32 times larger in sun plants than in shade plants, assuming that the apical dome can be represented by half a sphere. In this way it is shown, that there are many geometric relations between the size of the apex and the characteristics of leaf growth. The length of the youngest mature leaf is linearly correlated with the diameter of the apex. The growth rate in leaf length per plant and the total growing length per plant is correlated with the surface of the apex and the growth rate in leaf area per plant and the total growing area per plant correlated with the volume of the apex.

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De auteur is geboren op 23 oktober 1928 te 's-Gravenhage. In 1947 behaalde hij aldaar het einddiploma β van het Gymnasium Haganum. Daarna ving hij zijn studie in Wageningen aan en koos als hoofdvak Tropische Bosbouw met als keuzevakken Erfelijkheidsleer en Plantenfysiologie.

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