ON MORPHOGENESIS OF LETTUCE LEAVES IN RELATION TO LIGHT AND TEMPERATURE

J. BENSINK

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Dit proefschrift met stellingen van

JAN BENSINK,

Landbouwkundig ingenieur, geboren te Rotterdam op 11 mei 1923, is goedgekeurd door de promotor, Dr. E. C. WASSINK, hoogleraar in het Plantenphysiologisch Onderzoek en de Physiologie der Planten.

De Rector Magnificus van de Landbouwhogeschool
J. M. POLAK

Wageningen, 29 april 1971

ON MORPHOGENESIS OF LETTUCE LEAVES IN RELATION TO LIGHT AND TEMPERATURE

(with a summary in Dutch)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, MR. J. M. POLAK,
HOOGLERAAR IN DE RECHTS- EN STAATSWETENSCHAPPEN
VAN DE WESTERSE GEBIEDEN,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP WOENSDAG 30 JUNI 1970 TE 16.00 UUR

DOOR

J. BENSINK

STELLINGEN

I

De breedte van een slablad is een indicatie voor de photosynthesecapaciteit van de plant ten tijde dat het blad werd aangelegd.

Dit proefschrift.

II

De eindige groei van bladen is niet genetisch maar phaenotypisch bepaald.

III

Tijdens de phase van meristematische groei wordt de toename in volumen door factoren die de celstrekking beïnvloeden bepaald.

A. H. HABER and D. E. FOARD, Amer. J. Bot. 50, 937–944 (1963).
Dit proefschrift.

IV

Morphogenetisch onderzoek dient vergelijkenderwijs bij verschillende uitwendige omstandigheden te geschieden.

V

Meer aandacht dient geschonken te worden aan de physiologische motivering van de verlangde nauwkeurigheid bij de conditionering van de klimaatruimten voor planten.

P. GAASTRA, Proc. IBP/PP Technical Meeting, Trebon, 14–21 september 1969, pp 387–398.

VI

De ontdekking van de betekenis van absciscinezuurachtige stoffen voor de sluiting van huidmondjes biedt nieuwe perspectieven voor de plantenteelt in aride gebieden.

WRIGHT, S. T. C. Planta 86, 10–20 (1969).
JONES, R. J. and T. A. MANSFIELD, J. Exp. Bot. 21, 714–719 (1970).

VII

Het is niet waarschijnlijk dat de primaire effecten van IAA op de celstrekking verlopen via het RNA systeem.

BURSTRÖM, H. G., I. UHRSTRÖM and B. OLAUSSON, Phys. Plant. 23, 1223–1223 (1970).

VIII

De landbouw zal voor een evenwichtige bestemming van de open ruimte zelf
een fundamentelere inbreng moeten leveren.

SLUIS, P. A. VAN DER, Omvang van een landbouwgebied. Voor-
dracht voor de Ver. Hoger Landbouw-Onderwijs 26-1-1971 te
Groningen.
BEEREN, J.Th. J. and J. W. VAN DEN BERG, Stedebouw en volks-
huisvesting 7, 251–258 (1970).

IX

De door Posthumus voorgestelde veranderingen in het Hoger onderwijs kunnen
het behoud van de niet doelgerichte wetenschapsbeoefening in Nederland zijn.

X

Men zal de Waddenzee droogleggen, niet omdat het moet, maar omdat het kan.

VOORWOORD

Bij het schrijven van dit laatste, maar als eerste bedoelde woord, besef ik hoe velen, in welke vorm dan ook, hebben bijgedragen aan het tot stand komen van dit proefschrift. Ik hoop dat zij allen overtuigd zijn van mijn zeer grote dank.

Het onderwerp van mijn onderzoek verraadt mijn tuinbouwkundige vóóropleiding. Daarvan ondervind ik nog dagelijks de grote waarde, vooral vanwege het aandeel dat U, hooggeleerde WELLENSIEK, daarin hebt gehad. Door U werd voor het eerst mijn belangstelling voor de plantenphysiologie gewekt, met voor mij verstrekkende gevolgen. Daar kan ik U niet anders dan dankbaar voor zijn.

Aan U, hooggeleerde WASSINK, hooggeachte promotor, dank ik grotendeels mijn verdere 'specialisatie' in de plantenphysiologie. De veelzijdige kennis die U op dit gebied hebt en het zeer gevarieerde onderzoekprogramma dat door Uw toedoen en onder Uw leiding op ons laboratorium aanwezig is, hebben daar in belangrijke mate toe bijgedragen. Zeer erkentelijk ben ik U voor Uw nooit verflauwde belangstelling in de resultaten van mijn werk. De kritische aandacht die U aan mijn manuscript hebt besteed werkte op mij zeer inspirerend.

De geografische spreiding tussen het 'oude' en 'nieuwe' gebouw en de graad van gemeenschappelijke interesses brachten met zich mee dat de contacten met U, collega's en oud-collega's van het laboratorium, verschillend van aard en intensiteit waren, maar ik dank U allen voor de grote mate van collegialiteit, de adviezen en steun die ik steeds van U ondervonden heb. Deze dank geldt ook zeker alle technische medewerkers op wie ik nooit tevergeefs een beroep deed.

Hoewel al geruime tijd geleden, Uw aandeel, mevrouw E. VOS-TIMMER, in de beteugeling van 'epidermitis' ben ik allerminst vergeten.

De daadwerkelijke en daarmee morele steun tijdens de laatste fase van voorbereiding van het manuscript ondervonden van DR. IR. J. P. VAN DEN BERGH en IR. F. KUIPER, is voor mij van groot belang geweest. Paul, Fred, mijn hartelijke dank. De heer D. STEDELAAR dank ik in het bijzonder voor de wijze waarop hij in korte tijd de tekeningen heeft verzorgd.

CONTENTS

INTRODUCTORY

1.1. INTRODUCTION

Lettuce is a common garden vegetable, grown in particular for its leaves. Although different types of lettuce are known, classified partly on base of the organisation of the leafy shoot, those in which relatively large leaves are arranged in dense rosettes, eventually developing into more or less compact 'heads', are economically the most important ones.

Head formation or hearting, evidently, is a process closely related to leaf morphogenesis. Since leaf growth is strongly influenced by light, hearting is likely to be light dependent. This appears when lettuce is grown during winter in Holland, when light conditions are poor, as well as head formation. On the other hand, since lettuce is consumed in a fresh state only and, therefore, in demand the whole year round, a winter crop production is attempted at. Hence, efforts have been and still are made to improve the growth of lettuce under glass during the winter months. New varieties have been bred which, genetically, seem to be more adapted to poor light conditions (HUYSKES, 1968, RODENBURG, 1960, 1965, SMITH, 1964). Culture methods have also been improved, notably by means of extra CO_2 supply (VAN BERKEL, 1964, HARTMANN, 1966). As a result, winter crop production of lettuce in The Netherlands has very much increased during the last ten years (VAN SOEST and KEMMERS, 1959, VAN DER HOEVEN and GROENEWEGEN, 1970). Yet, if one would ask by the improvement of which qualities of the plant these higher yields are being obtained, and whether still further improvements might be achieved, an answer will be difficult to give, because of lack of factual knowledge in this respect. BROUWER and HUYSKES (1968) found the more rapid growth of a promising hybrid to be due to a better exposition to light of its leaves rather than to enhanced photosynthetic capacity. As to this, it should be remarked that hearting as such can be considered incompatible with the requirements of unhampered photosynthesis of the leaves, as this supposes easy access for light and CO_2 to the photosynthetically active sites within the leaves. Hence, under restricted light conditions, it seems difficult to combine satisfactory plant growth as measured by weight increase with adequate head formation. One of the aims of the present study is to obtain a better insight into the external and internal conditions of the plant, controlling head formation. This has been attempted in the first place by studying leaf morphogenesis under different conditions of light and temperature.

Previous to experimental research it seems useful to consider the construction of a lettuce head more closely, in particular to obtain more information about the relationship between leaf shape and head formation.

On plate IA, a plant of the variety 'Meikoningin' (syn. May Queen) is shown which, for six weeks after planting has grown in a cold frame in the laboratory garden at Wageningen in April and May. Although the plant is not yet full-grown, it clearly has started to form a head which only has to be 'stuffed' with more leaves in order to reach a marketable weight.

In a 5/13 spiral phyllotaxy, 37 leaves longer than 1 cm have been formed on a stem of only 2.5 cm length. This means an average internode length of 0.7 mm. Except for the first three leaves which were lost at the time of harvest, the leaves are shown separately on plate IB. In the apical bud, a further 27 leaf primordia are present, making a total of 65 leaves formed. Heading appeared to have started with the outgrowth of the 12th leaf. A close-up of leaf 17 is shown on plate IIA. One of the most striking features of this leaf is a conspicuous surplus of meso-phyll development relative to midrib elongation. This causes folding and crink-ling of the lamina, in particular along the basic part of the midrib, a type of development described by HELM (1954, p. 93), as: 'Die Spreite der meisten (Buttersalat) Sorten ist infolge Stärkeren Flächenwachstums auffallend blasig-runzelig. Die daran nicht beteiligten, begrenzend wirkenden, grösseren Leit-bundelstränge bewirken eine Aufteilung in eine Anzahl Auftreibungen und führen zu einer Reihung der Spreite entlang der Mittelrippen'.

When the leaf blade is cut along the midrib and flattened thereafter, as has been done for one half of the leaf on plate IIB, the deficit of midrib length, necessary for a flat leaf surface is evident. There is no doubt, that this relative surplus of mesophyll development promotes head formation and gives a sub-stantial contribution to the solidity of the formed head. Differential growth of midrib and leaf blade, therefore, can be regarded as an essential element of hearting.

In fig. 1.1, A the course of the average length and width is shown for the first 37 leaves taken from ten plants identical to the one shown on plate IA. Both length and width strongly increase in the first 12 to 15 leaves, remain con-stant for a number of leaves thereafter, and eventually decrease again. The latter phenomenon, evidently, represents the successive growth stages the younger leaves had reached at the time of harvest. Periodic measurements revealed that the first 20 leaves had stopped growth, and hence could be considered as having reached their final dimensions, although the leaves 16 to 20 had not reached the size of the preceding ones, 12 to 16. It seems that for some reason they have stopped growing before they have realized their potential growth capacity. With respect to this, it may be noted that it is a common feature of rosette plants to show a continuous decrease in leaf size over a large number of leaves.

Increase in leaf size of the first leaves of the plant, evidently, displays the in-
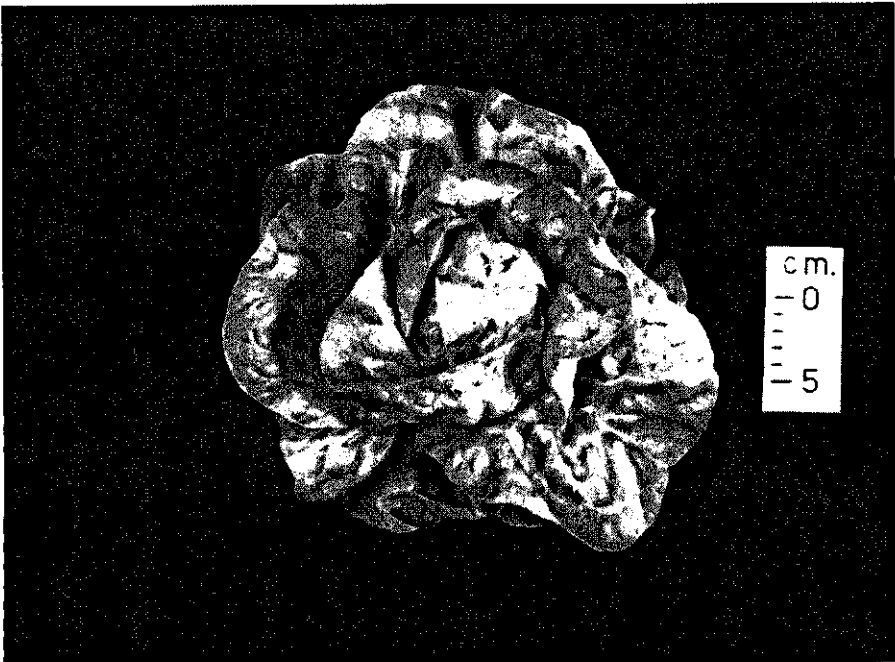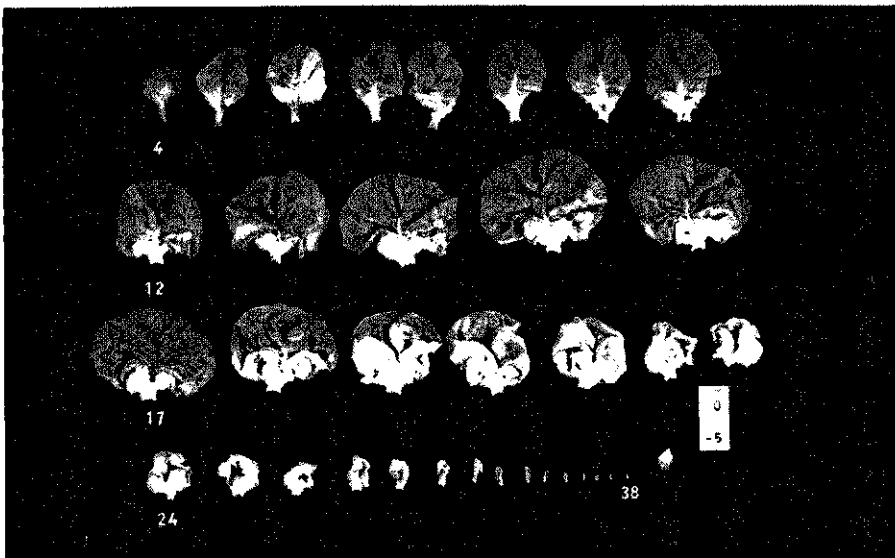
PLATE IA

PLATE IB

PLATE I. A plant of the variety 'Meikoningin' grown under natural conditions during April and May (IA). Separate leaves arranged according to leaf number from old to young (IB).
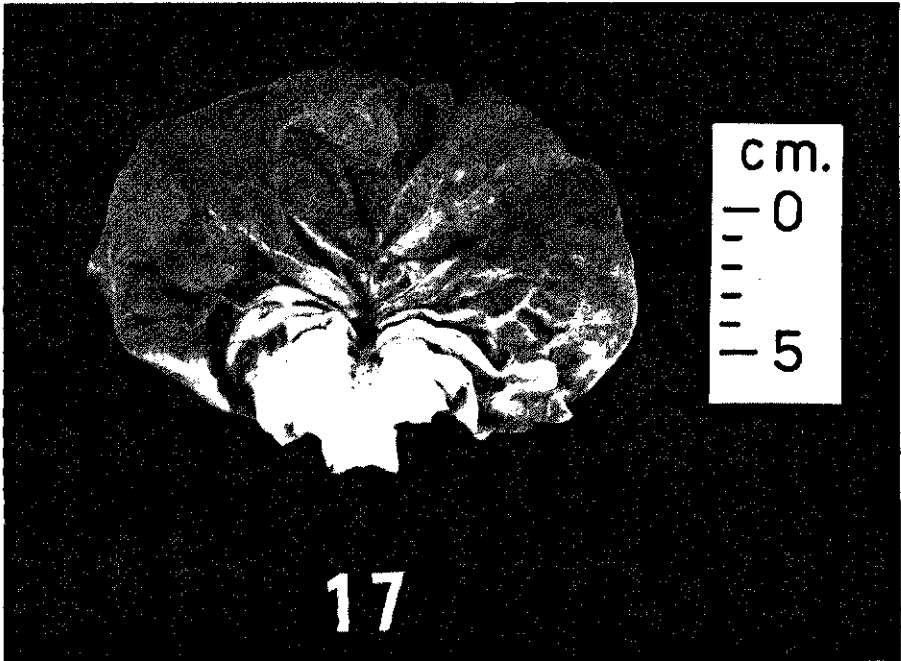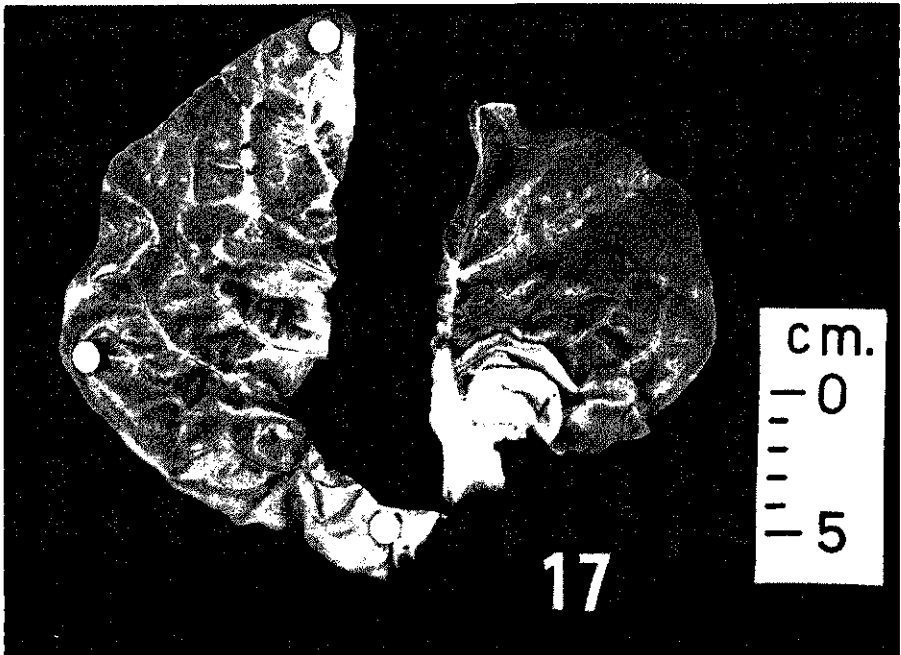
PLATE IIA

PLATE IIB

PLATE II. Close-up of leaf 17, intact and left half cut from midrib and flattened.

Fig. 1.1. A: Length (●) and width (×) of the successive leaves of a plant grown under natural conditions during April and May. Leaves numbered stem upwards, from old to young.
B: Length-width ratio of the same leaves.
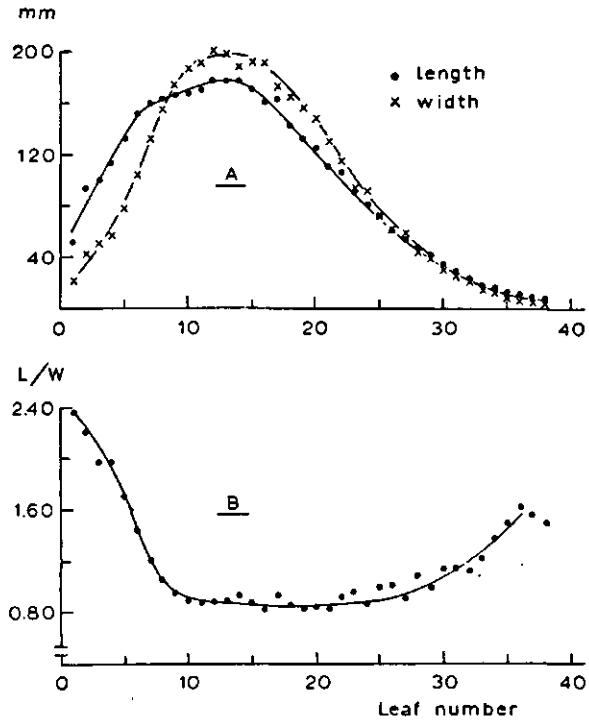
creasing growth capacity which characterizes early plant development. Apart from an increase in leaf size, a change of leaf shape is found, yielding a gradual decrease of the length to width ratio (L/W). This is shown in fig. 1.1,B. Starting at a value of about 2.40 for the first leaf, L/W decreases to a value below unity in the next 12 leaves. For the then following leaves it remains constant at this low value, but increases again for the very young, still expanding ones. Since hearting became manifest from leaf 12 onwards, development of a low L/W ratio may be taken as favourable for the onset of head formation.

Some points of interest appear when for all leaves, used for fig. 1.1, length is plotted against width, as has been done in fig. 1.2. The result is a continuous loop-shaped curve, of which the upper, curvi-linear half is made up by the leaves 1 to 10, and the straight lower half by the leaves 18 to 38. The leaves 11 to 18 are located at the meeting point of both parts of the curve, indicating the maximum leaf size present.

Linearity of the lower leg suggests that during leaf expansion a constant pro-portionality between increase in length and in width is maintained. The line does not pass the origin but cuts the vertical (length) axis somewhat higher, since very young leaves show appreciably increasing L/W ratios, as demonstrated in fig. 1.1,B. The intersection of the length axis may be interpreted as representing the length of the leaf primordium at the moment leaf blade extension properly starts.

In fig. 1.2, the straight part of the length to width relationship runs at an angle
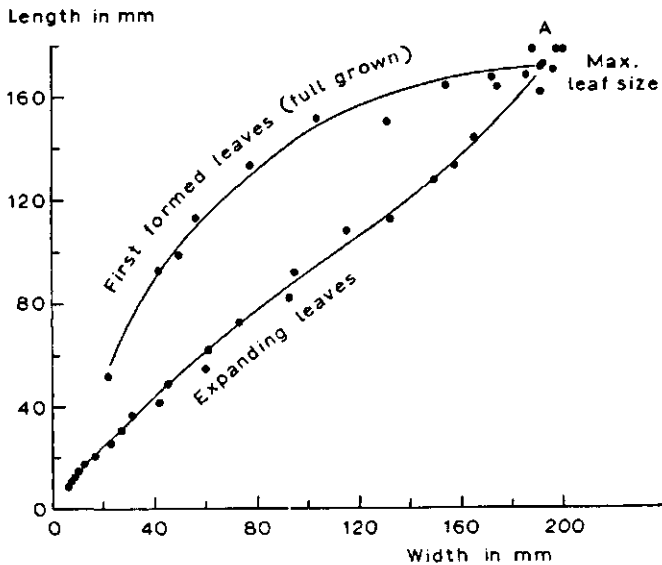
FIG. 1.2. Length-width diagram for the leaves presented in fig. 1.1. A represents the maximum leaf size reached under the given conditions.

of 40°, indicating that leaves grow faster in width than in length. If this line may be considered as indicating the course of growth of all leaves above leaf 12, *it is quite clear that the leaves 1 to 10 cannot have developed along the same line but are likely to have grown along possibly similar straight lines for the L/W relationship, only with steeper slopes.* Actually, the slope should have been steepest for leaf 1, decreasing gradually for each following leaf untill, with leaf 10, an L/W relationship is reached which, apparently, holds for the growth of all further leaves. In order to investigate this, we have periodically harvested plants from the moment of planting onwards, and L/W relationships were determined for the growth of the leaves 4, 6, 8, 10 and 12. As shown in fig. 1.3, the leaves in this sequence indeed display an increasing leaf blade development, resulting in a decreasing slope of the L/W lines as expected. A fact of great importance, evidently, is formed by the number of leaves, making up a head, determined by the rate at which leaves are initiated and the rate at which they grow out and substantially contribute to head formation. For the plant shown on plate I, in total 65 leaf initiations have been counted. Considered over a growth period of six weeks, this implies an average production rate of 1.5 leaves per day. It should be noted, however, that from these 65 leaves, 27 are still less than 1 cm long and do not, as yet, substantially contribute to the formation of the head. Further outgrowth of leaves apparently proceeds at a much lower rate than that at which they are initiated. The more the rate of outgrowth of leaves matches a high rate of initiation, the better the conditions are for head formattion.

At the same time, elongation of the stem on which these leaves are produced,
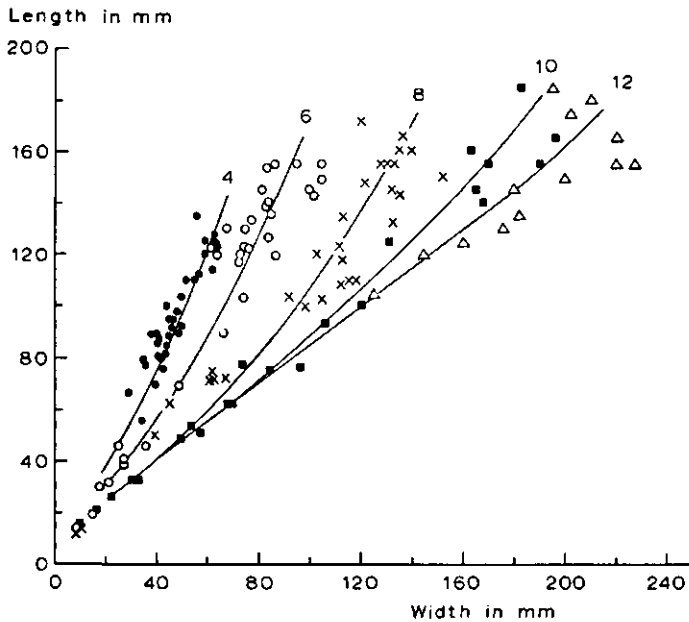
FIG. 1.3. Length-width relationships for leaves 4, 6, 8, 10, and 12 as measured at subsequent harvests. Conditions as in fig. 1.1.

appears very much restricted. A length of 2.5 cm, developed during a six weeks growth period, implies an average daily extension of only 0.6 mm. It needs no further comment that, together with the high rate of leaf production observed, the low rate of stem elongation favours the density of the head.

Normally, for most rosette plants, a strong elongation of the stem introduces the formation of a flower stalk, and marks the transition from vegetative to reproductive growth. This, necessarily, inhibits further head development, so that premature 'bolting' is one of the reasons of insufficient heading. Since, for several lettuce varieties, including 'Meikoningin', flower initiation is promoted by long photoperiods (ERNST-SCHWARZENBACH, 1936), this easily happens during growth in the long days of early summer.

Finally, it should be noted that leaves developing inside the head often show a hyponastic (adaxial) curvature, in particular of the basal part of the leaf axis or midrib. As these curvatures are becoming fixed in the course of growth, they may greatly contribute to the formation of a solid head. It cannot be decided beforehand whether these leaf curvatures should be considered as a consequence or as a cause of head formation.

For the plants, analized above, they are less prominent, although they may arise at a later stage of growth. It is observed that the degree of hyponasty shows a great varietal diversity. An example of this is given on plate III, showing partly defoliated heads and single leaves of two varieties. Left, heads are shown after removal of the outer 24 leaves. In the middle, the leaves 25 to 30 are shown on
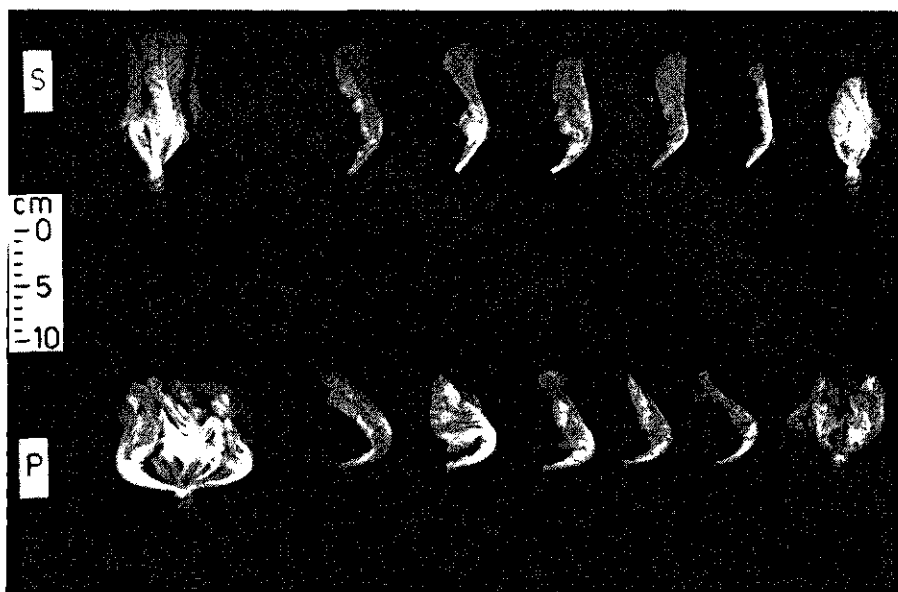
PLATE III. Varietal difference in hyponastic curvature of the midrib. Upper row: partly defoliated heads and single leaves of 'Secura'. Lower row: same for 'Profos'.

their side (one half of the leaf having been cut off), while the remainder of the head is shown on the right The bottom row represents the variety 'Profos', characterized by a 'square'-head (sic), originating from strongly curved leaves, whereas the top row represents a 'slender'-head variety, 'Secura', the leaves of which are only slightly curved

For the butterhead varieties these leaf curvatures seem to be restricted to the leaf base only, and develop gradually in the course of growth.

In contrast to this, leaves of the so called crisp-head varieties, a type of lettuce grown especially in the U.S.A., show hyponastic curvatures along the entire leaf axis. Since these curvatures are noticeable already at the primordial stage, the successive leaves overlap each other rather closely from the beginning. Consequently, rather compact heads are formed, resembling those of cabbage. When grown for seed production, it often is necessary to quarter the tight heads to allow normal development of the flower stalk (JONES, 1927), a method which can be replaced by spraying the plants with gibberellin at an early stage (HARRINGTON, 1960). The nature of these leaf curvatures has not been examined further in this study; they, possibly, are related to auxin metabolism in the midrib.

Coming to a preliminary conclusion of this introductory survey, it may be stated that hearting is not a monofactorial effect, but should be considered as the ultimate result of different processes operating simultaneously. As has been indicated by DULLFORCE (1962), hearting seems to depend upon a relatively high rate of leaf production, a relatively slow rate of stem elongation, a relatively large size of individual leaves, and a relatively short length of petioles.

8   *Meded. Landbouwhogeschool Wageningen 71-15 (1971)*

The last two indications directly refer to matters of leaf morphogenesis and seem to be ultimately due to a control of the ratio of mesophyll to midrib development in favour of the first. The way this is affected by both internal and external growth conditions is the main basis of the experiments to be dealt with in the following chapters.

1.3. DISCUSSION OF SOME RELATED LITERATURE

Under the heading 'Lettuce and other salad crops', Horticultural Abstracts annually refers to more than 100 articles which, although all bearing on lettuce as a research object, are of widely different nature, from simple field experiments to elaborate studies on the mode of action of phytochrome in lettuce seed germination or hypocotyl lengthening. Those dealing with leaf morphogenesis, however, are scarce.

A well documented survey from the morphological and taxonomical viewpoint of the different types of lettuce is given by HELM (1954, 1955); special attention being paid to probable ontogenetic relationships between the different forms of lettuce leaves, varying from long and narrow to round and wide, either with smooth or lobed margins.

The presence of discrete marginal meristems ('Fiederprimordiën') should be basic to the development of more or less divided or lobed leaves. Leaves with entire or slightly serrate margins may be understood to be formed as a result of a reduction of these meristems. At the same time a primarily longitudinal growth tendency may change into one resulting in a more prominent surface extension. Long and narrow leaves yield the loaf-shaped heads of different Cos or Romaine varieties (*Lactuca* provar. *longifolia* Lam.). Large, round to kidney-shaped leaves are formed in the true heading types of lettuce (*Lactuca* provar. *capitata* L.).

The true origin of *Lactuca sativa* as plant species is unknown, although it is generally accepted to have originated through domestication of a wild form, *Lactuca scariola* L, possibly out of two already different forms, one with divided lobed leaves (f. *serriola*) the other with entire leaf margins (f. *integrifolia*). For full, historical details see HELM (l.c.).

DULLFORCE (1962) has summarized the requirements for heading as mentioned before. Hearting of the variety Cheshunt 5[B] further appeared associated with a critical value of the leaf area ratio of the plant (dm^2/g), being satisfactory at 8 dm^2/g, marginal at 9 to 11 dm^2/g, and absent at still higher values. As is found also for other plant species (BLACKMAN, 1958), temperature and light intensity had an opposite effect on leaf area ratio, *viz*, showing a positive correlation with light intensity and a negative one with temperature. Hence, light and temperature may exert a compensatory influence on hearting as was found for the length to width ratio developed by the leaves (BENSINK, 1958). Both high light intensity and low temperature tend to decrease the L/W ratio. Hearting may occur at high temperature (21 °C.) provided light intensity is sufficiently high,

(DULLFORCE, 1969). Similar to the effects of light intensity are those of light duration, as observed by OLSON (1968). Growth at a 16 hours photoperiod results in wider leaves that are only slightly longer than those developing under an 8 hours light period, the greater leaf lamina under the long days being mainly the result of a larger number of cells.

For the morphogenetic effects of light, the nitrate concentration at which the plants are grown appears important (BENSINK, 1960). The effects of a low light intensity, both on leaf shape and on stem elongation (etiolation) could be strongly suppressed by growing the plants at a very low nitrate concentration. Flower initiation and subsequent shooting was speeded up by low nitrate supply which seems in accordance with old views on the importance of the C/N ratio of the plants for the vegetative and reproductive growth (KRAUS and KRAYBILL, 1918). JAGER, VAN DER BOON and PAUW (1968 a,b; 1970) found that soil steaming led to accumulation of ammonium and manganese which, together with poor light conditions, stimulated poor heading, while the length-width ratio of the leaves was increased.

Several physiological and ecological investigations on lettuce growth have been carried out in Japan. Since most of the results are published in Japanese language, though with English summaries, they are less accessible for many readers. ITO, already in 1936, made a study of changes in nitrogen and carbohydrate contents of the leaves during growth. Both were found to be higher for leaves forming the head. Heading did not occur at too high and too low a carbohydrate nitrogen ratio of the plants. According to MIYAZAKI (1960), moisture content of the leaves increases at the time the plants start to heart which seems to agree with findings of KATO et al. (1963) who stated that transpiration of the plants decreases after they have started to form heads. KATO (1964a,b) studied also the auxin and gibberellin metabolism in relationship to stem elongation and flowering of three varieties including May King (syn. Meikoningin, ROODENBURG 1960). Of interest is that under natural conditions gibberellin-like substances were hardly detectable up to the time of flower bud differentiation, but increased rapidly thereafter. High temperatures induced the appearance of gibberellin-like substances, and also enhanced flowering. Whereas application of gibberellin induced stem elongation both at high and at low temperature, it induced flowering only at high temperature. The auxin (IAA) content of apical buds showed a tendency similar to that of gibberellin, while IAA-oxidase activity was reduced by all combinations of gibberellin application and high temperatures. At low light intensities, stem elongation occurred, while the gibberellin level in the apical bud increased, and IAA oxidase decreased.

SHIBUTANI and KINOSHITA (1966 and 1968), in a study on the ecological adaptation of lettuce, concluded that the optimum temperature for the crisphead varieties Great Lakes and Imperial was 17° to 18 °C. Above 18 °C, leaf weight declined and above 20 °C the plants rapidly flowered. Also HIRAOKA (1967a,b, 1969), studying the effects of temperature, photoperiod and gibberellin sprays on bolting, budding and flowering of the cultivar Wayahead (a butterhead type), found that flowering was accelerated by high temperatures and long days,

whereas short days and temperatures between 15° and 20°C were favourable for heading. Gibberellin application promoted stem elongation but did not accellerate flowering.

Valuable information on the growth of lettude can be found also in publications of a more practical conception, such as given by BANGA (1940), VAN KOOT and GROENEWEGEN (1955), WHITAKER et al. (1962), and others, while a full account on the anatomical structure of different parts of the lettuce plant is given by HAYWARD (1938).

Clearly, a vast literature on leaf morphogenesis exists when not restricted to the growth of lettuce leaves. Thus, similar problems on the influence of light and temperature on the growth of leaves of *Fragaria* are dealt with by ARNEY (1954–1956), of cucumber by MILTHORPE, NEWTON, and WILSON (1959–1966), of *Phaseolus vulgaris* by DALE (1964–1970), a list which may be extended at will; however a full account of this seems to be outside the scope of this Chapter. To some of them we will refer at more appropriate places in the course of this paper.

In 'The Growth of Leaves', MILTHORPE (1956) has edited a series of papers which are still of great value, while HUMPHRIES and WHEELER (1963) have reviewed literature on that subject up to that time. Much work of the type dealt with in the present paper is going on in the U.K. which may be related to the circumstance that expansion of the leaf surface is an essential detail of the method of growth analysis introduced and advocated in particular there (GREGORY, 1921, 1952).

To conclude this brief introduction into the literature, it might be stated that *Lactuca sativa* is a plant species which displays a great sensitivity to light in its growth and even in the early developmental processes going on during seed germination. For that reason it is generally used as a research object in the study of photomorphogenesis. Thus, lettuce has also been used as a test plant in studies of plant growth under light of different spectral composition, carried out in this laboratory since 1948 (WASSINK, STOLWIJK and BEEMSTER, 1951). The present study may be considered as a logical continuation of this type of research, in a wider context also of studies on the relationship between production and morphogenesis of plants as carried out mainly by WASSINK and others in this laboratory, on different types of plants (KAMEL, 1959, BUTT, 1968, SANCHEZ, 1967, WASSINK, 1960, 1963, 1969).

MATERIAL AND METHODS

2.1. PLANT MATERIAL

Only the growth of so called 'butterhead' lettuces has been investigated. Although, in the course of this study different varieties have been used, most experiments were carried out with 'Meikoningin' and 'Rapide'. For a long time, Meikoningin, a relatively old variety, well known all over the world (RODENBURG, 1960), has been the only variety suitable for growth under glass during winter and early spring in The Netherlands. During the last 15 years, however, it has been replaced almost completely by an ever increasing number of new varieties yielding better results in this type of growth (ANON., 1970); Rapide is one of these new varieties. Occasionaly, also other varieties have been used, mainly to investigate possible differences in growth type.

Originally, seeds of all varieties were obtained from Dr. J. A. HUYSKES of the Institute of Horticultural Plant Breeding (IVT) at Wageningen. For further use, each year a few plants of each variety have been raised for seed production, the production of one plant, generally, being sufficient to supply all experimental plants needed in one yaer.

Seeds were sown in seed boxes, at 20°C. As soon as both cotyledons had fully expanded and the first leaf started to elongate, seedlings were pricked out at 2 cm distance in fertile soil. Further growth occurred under fluorescent light at about 30,000 ergs/cm^2 sec, given during 12 hours per day, at a temperature of 20°C throughout. At the time the sixth leaf had reached a length of about 1 cm, plants were selected for uniformity to enter the experiments.

2.2. EXPERIMENTAL CONDITIONS

2.2.1. *Culture methods*

Plants are grown either in pots or in PVC-plastic containers of $80 \times 60 \times 20$ cm. In the latter case, a number of plants were grown together. Pots or container are placed on wheeled trucks of 80×60 cm surface area, adjustable in height. When necessary, trucks with plants were moved daily to different rooms.

Fertile soil, a mixture normally prepared and used in this laboratory, or fine gravel (2–4 mm) together with a nutrient solution, was used.

A pot size of 13 cm diameter (0,7 L content) proved to be the minimum size to avoid influences on growth to be ascribed to pot limitation of the root system. Recently, square pots of 17×17 cm and 17 cm height of black PVC have been successfully used.

The gravel used had a free air space of 36%. Through a hole in the bottom of

the container nutrient solution could be pumped up from a reservoir placed on the lower floor of the truck. By means of an overflow, 2 cm below the gravel surface, the solution was circulated as long as the pump was in operation and drained back into the reservoir as soon as the pump was switched off. Using a time switch, the solution was circulated for five minutes every hour.

A nutrient solution of half the strength of the four-salt mixture given by HOAGLAND and ARNON (1938) was used. Iron was given as a mono-sodium ferri salt of E.D.T.A.

2.2.2. *Environmental control*

Most of the experiments to be described were made in growth rooms of the climatized department of this laboratory. In these rooms (300 × 400 cm and 190 cm high) temperature can be controlled within 1 °C. between 10° and 30 °C. Relative humidity can be varied, a facility, however, not used so far, as it was kept constant at about 75 % in all experiments. Light is provided by fluorescent tubes (Philips 120 Watt, TL 55), mounted parallelly as individual units on top of a double layer of perspex panes which form the ceiling of the growth rooms. Every month, a quarter of the lamps is renewed in order to make light intensities less dependent on lamp age. Light intensity can be varied either by changing the number of lamps used or by adjusting the distance between plants and ceiling. Intensities are measured in ergs/cm²sec (1 erg/cm²sec = 0.24. 10^{-7} gcal/cm²sec = 0.1 mW/cm²) with a cosine corrected photocell, calibrated for the spectral energy distribution of the lamps used, with the aid of a standarized thermopile. See for details STOLWIJK 1954, and GAASTRA 1959. Thirty cm underneath the perspex ceiling an intensity of 100,000 ergs/cm²sec can be obtained. When necessary, the plants are moved to dark rooms, identical to the light rooms apart from the illumination.

2.2.3. *Application of higher CO_2 concentrations*

Some experiments were carried out in which plants were grown in an enriched CO_2 atmosphere. Two leak-proof cabinets of 100 × 100 × 70 cm were used for these experiments. Plants were grown in 1 liter glass jars, subirrigated with nutrient solution. The jars were covered with plastic foil. In both cabinets, air was pumped through at a rate of 1000 l per hour and in one of them CO_2 gas was added at a rate of 15 l per day, calculated to maintain a CO_2 concentration of about 1000 ppm. Small fans were used for proper mixing of air and CO_2*

2.3. MEASUREMENTS

2.3.1. *Macroscopical measurements*

Since, for obvious reasons, leaf areas (see plate II) are difficult to be measured ac-

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* Technical advice was kindly supplied by DR. P. GAASTRA, at that time a collaborator of this laboratory.

curately, growth effects will be related to differences in total leaf length and greatest measurable leaf width. The first represents midrib elongation, the latter is a measure for leaf blade extension.

Growth effects have been measured in the first place in leaves which represent the maximum attainable leaf dimensions under the given conditions, i.e., those indicated by A in figure 1.2. They are produced only after a different number of leaves of gradually increasing size, demonstrating the increasing growth capacity of the plant.

Besides the dimensions of mature, full grown leaves, also time courses of growth in length and in width have been measured under different experimental conditions. For this, leaf number 12 or 15 was chosen counted from below. It will be clear that, when growth is to be measured on the plant, measurements can start only after the leaf has reached a certain seize. Hence, early growth will not be covered by this kind of measurements. To meet this difficulty, the course of growth has also been derived from measurements made on leaves of one particular serial number taken from different plants which were periodically harvested. Plants were selected carefully on uniformity beforehand. In this way, information about primordial leaf development could be obtained which, otherwise, was imposible to collect.

Leaves are numbered in the order of appearance, and accordingly plotted in the graphs. When counting leaf numbers, a difference has been made between total number, including all the primordial leaves, and the number of leaves having reached a length of more than one cm. Actually, in the latter case the plastochron-index of the plants has been determined, as defined by ERICKSON and MICHELINI (1957), see page 20.

2.3.2. *Microscopical observations*

In order to compare differences in leaf size on a cellular level, the number and the size of epidermal cells covering midrib and leaf blade have been determined. The whole leaf or samples of it (leaf blade discs of 10 mm diameter cut half way from both leaf halves) were preserved in 70% alcohol. The epidermis of the midrib could easily be stripped off and examined under the microscope; samples of the leaf blade were left intact, epidermal cells and the underlying palisade cells could easily be seen.

On the midribs, epidermal cells extend primarily in longitudinal direction, so that they appear as long, elongated cells, usually with tapering ends. The lengths of ten cells or more in one row have been measured; per sample hundred cells were measured. Average cell lengths were determined for subsequent sections of the midrib from base to tip. Cell number per single row was estimated by dividing midrib length by average cell length.

On the leaf lamina, mature epidermal cells show wavy anticlinal walls, well known for the leaf epidermis. *Camera-lucida* drawings of a group of cells were made, and their surface areas measured with a planimeter. After calibration with an object micrometer, planimeter readings were converted to μ^2 cell surface. Dependent on cell size and magnification used, each drawing comprised ten to

fifty cells. Two or more drawings were made per leaf blade sample, four or more over the entire leaf width.

Always, cells of the adaxial (upper) leaf side were measured, which cells were clearly distinguishable from those on the abaxial (lower) side, as they show more strongly undulated anticlinal walls. Stomata which occur on both leaf sides, together with adjacent subsidiary cells, were excluded from measurement.

Since cells of the mesophyll parenchyma were clearly distinguishable as round cells when leaf tissue was cleared in alcohol, growth of these cells could be measured as well.

For the estimation of cell number over the leaf width, leaf width was divided by the square root of the average cell surface. As will be discussed in Chapter 4, cell surface is rather constant over the leaf blade, so that differences in leaf width are fairly well correlated with differences in cell number.

ASPECTS OF LEAF DEVELOPMENT AS AFFECTED BY LIGHT AND TEMPERATURE

In this chapter, the influence of light and temperature conditions will be examined with respect to leaf production (3.1), maximum leaf size (3.2), growth-time relationships (3.3.), differential growth (3.4), and leaf shape development (3.5).

3.1. LEAF PRODUCTION

A feature of rosette plants is that they possess only one, terminal, vegetation point at which all leaves are produced. Their number, therefore, is easily determined by peeling off all the leaves from the rosette, beginning with the oldest, outermost leaf formed immediately after the two cotelydons. In order to arrive at the total number of leaves produced, leaf primordia are to be counted also, up to the most recently formed one which is noticeable only microscopically as a small swelling, the leaf buttress, at the apical dome. Since the plant has to be sacrificed, rates of leaf production can be determined only indirectly from the
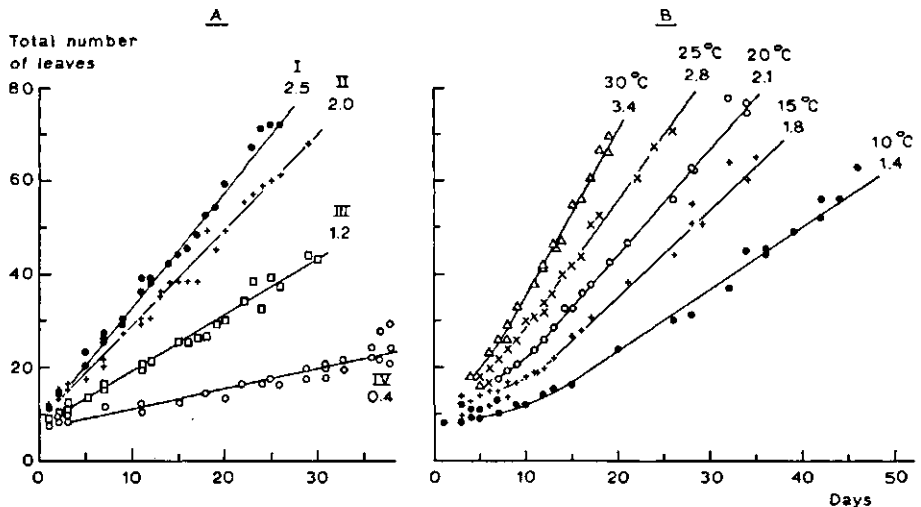
FIG. 3.1. A: Increase of total number of leaves with time, for plants grown at different light intensities: I : 100,000 (●), II: 40,000 (+), III: 18,000 (□), and IV: 7,500 ergs/cm² sec. (○). Leaf production expressed in leaves per day is indicated at the top of each curve. Temperature for all light intensities 20 °C.
B: The same as A, for plants grown at different temperatures and at a constant light intensity of ca. 40,000 ergs/cm² sec.

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total number of leaves of periodically harvested plants. Thus, in fig. 3.1,A, increase in total number of leaves in the course of time is shown for plants growing at four different light intensities between 7,500 and 100,000 ergs/cm²sec. From the linear increase in leaf number it may be concluded that leaves are produced at constant rates which differ for the different light intensities. Production rates are represented by the slope of the straight lines, and are indicated as 'leaves per day' at the top of each line.

At a light intensity of about 100,000 ergs/cm²sec, an average of 2,5 leaves is initiated per day, which means an average plastochron (i.e. time interval between the initiation of two successive leaves) of 0,4 days. At 7,000 ergs/cm²sec only 0,4 leaves per day are formed, corresponding with a plastochron of 2,5 days.

The first leaf countings were made when all plants possessed equal numbers of leaves, viz., about 8, which had been initiated during the three weeks period in which the plants were raised from seeds, all under the same conditions, before they entered into the experiment. The question arises whether constant leaf production rates exist from the very beginning onwards. In fig. 3.2, therefore, the average increase in leaf number of three to four plants is shown during early growth. Leaf countings started one day after seed emergence, i.e. four days after the seeds were sown in soil at a temperature of 20 °C. Both cotyledons then have been just released from the seed envelope and start to expand to full size in the subsequent two days.

All apices of seedlings investigated at that time possess already the first two leaves. During the first 8 days the leaf number increases to five, and then remains constant for a couple of days before leaf production increases again. At the time the plants are 19 days old, at least 8 leaves are present, a number which agrees very well with the initial number of leaves represented in fig. 3.1. To explain the
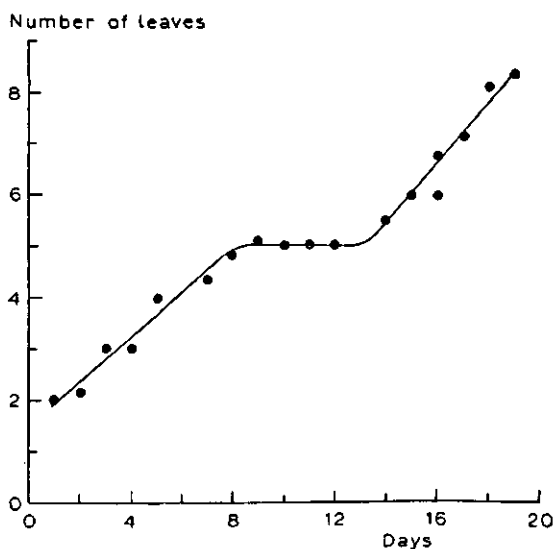

Number of leaves

Days

FIG. 3.2. Average number of leaves of three to four plants plotted against time. Observations started at seed emergence. Cotyledons not included.

curve of fig. 3.2, we may assume that the first 5 leaves are produced at the expense of seed reserves and the first two leaves, possibly, were present already in the plumule of the embryo in the seed. The apparent intermission in leaf production between leaves 8 and 13 can be understood as a lag-phase in which the supply of energy to the growing point shifts from seed reserves to photosynthates produced by the young seedling. The reason why leaf production eventually should lead to constant rates as shown in fig. 3.1, can only be guessed. Fig. 3.1,A (and especially fig. 3.3,A, discussed later) suggests that the slope which indicates the rate of leaf formation, depends on the rate of photosynthesis, and, hence, on light intensity. The rather smooth curves of fig. 3.3,A may be due to the fact that lettuce plants are light absorbers of a rather complex structure.

The second feature to be explained is the remarkable fact that in fig. 3.1, the slopes of the lines remain the same during the development of the plant, notwithstanding the fact that the plant continually produces increasing amounts of photosynthates. It must, therefore, be assumed that the amount of photosynthates delivered to the growing point which is pace setting for the early leaf increment and which reflects the effect of light intensity, is independent of the size of the plant. Two mechanisms might be visualized. First, it might be assumed that a certain, small part of the photosynthetic capacity of the plant, remaining constant in size during development, is responsible for providing the vegetation point with the required assimilates. Another possible mechanism is that the independence of the actual size of the plant is mediated by way of some, morphogenetic, trigger mechanism. Since total plant growth, in each individual case, probably is near exponental for some time, the effect of the trigger mechanism with regard to the supplies to the vegetation point may be denoted as 'logarithmation', an effect which is found in many stimulus effects, as is shown by many reports indicating the validity of the 'WEBER-FECHNER law.'

Regarding the high production rates observed, such as 2,5 leaves per day at 100,000 ergs/cm^{2}sec, one may ask to what extent leaf number will continue to increase linearly with time. In the case of fig. 3.1,A, about 70 leaves are present after 26 days at the highest light intensity. At that time no plants were left to continue the experiment, but in other experiments numbers of 150 and more have been counted which still fitted a linear relationship.

Since the outgrowth of the leaf initials, however, does not keep pace with the rate of initiation of new leaves, the number of primordia in the apical bud increases continuously (see later). This may yield a problem of space and it is indeed observed that, at that time the vegetation point often changes from circular into oval or oblong, in extreme cases leading to the formation of fasciated stem growth, thus increasing the available sites for new leaf primordia. Eventually, the apex shifts to reproductive development, becomes more convex and soon is covered by numerous protuberances forming the floral organs, (JONES, 1927).

Apart from light intensity, leaf production also depends on temperature. In fig. 3.1,B, increase in total leaf number is shown for plants grown at five different temperatures, at a light intensity of about 80,000 ergs/cm^{2}sec. On the whole,

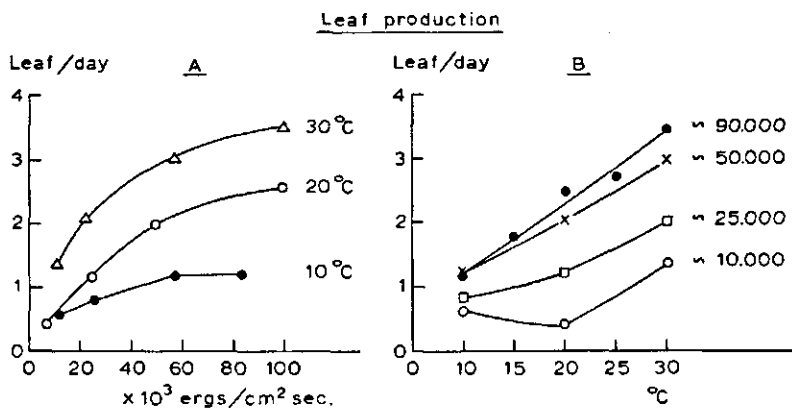18

FIG. 3.3. A: Leaf production in leaves per day plotted against light intensity at 10 °C, 20 °C, and 30 °C.
B: Leaf production plotted against temperature at four different light intensities, in ergs/cm² sec.

leaf number increases again linearly with time, but, as may be expected, at a lower rate when temperature is lower. At 10 °C and 15 °C, leaf production rates initially increase. Since these plants, before they entered the experiment, had grown at 20 °C, this may be due to adaptation to the new, lower temperature.

Together with results of other experiments, the interaction of light and temperature was examined. Fig. 3.3,A shows light dependence curves for leaf production at three different temperatures, viz., 10 °C, 20 °C and 30 °C. Leaf production reaches light saturation, however, over the whole range of intensities, it is clearly temperature dependent. The latter also follows from the more or less linear temperature gradients for leaf production at different light intensity levels, as shown in fig. 3.3,B. The dip at 20 °C at the lowest intensity is to be ascribed to the fact that this point was obtained at a light intensity of 7,500 ergs/cm²sec instead of 11,000 ergs/cm²sec.

Linear temperature curves are characterized by a gradual decrease in Q_{10}, indicating that in the various parts of the plant material processes with low Q_{10} values (e.g. diffusion processes) gradually preponderate over those with higher Q_{10} values (e.g. enzymatic reactions) as rate limiting; a phenomenon that rather commonly occurs in plant material of more or less complicated structure, WASSINK (1934), BOTTELIER (1935).

So far, leaf number refers to the total of leaves initiated. It has already been remarked that, in general, further leaf development does not proceed at the same high rate as new leaves are initiated at the apical dome. In order to study this more quantitatively, not only the total number of leaves initiated, but also the number having reached a length of at least 1 cm has been determined. As a matter of fact, for each plant the Plastochron-Index (PI) has been determined as defined by ERICKSON and MICHELINI (1957). Based on the number of leaves which have reached an arbitrary chosen reference length (e.g. 10 mm), this PI

is a numerical index to specify the developmental stage of the vegetative plant. It has been used in studies on the growth of *Xanthium* leaves (MAKSYMOWYCH, 1959; MAKSYMOWYCH and ERICKSON, 1960). If the reference length is 10 mm, a plant is considered to be *n* plastochrons old when leaf *n* has just reached a length of 10 mm, and (*n* + 1) plastochrons old when leaf (*n* + 1) has reached this length. In case leaf *n* is longer than 10 mm and leaf (*n* + 1) shorter than 10 mm, PI is found by interpolation between *n* and (*n* + 1). For *Xanthium* and for lettuce this appeared to be rather easy, since it was found, as will be demonstrated and discussed in chapter 4, that there is a constant ratio between the lengths of two successive young leaves, independent of the node number. Thus, plotted logarithmically against node number, the successive leaf lengths fall along a straight line, the slope of which represents the value of $\log L_n - \log L_{n+1}$. PI then can be
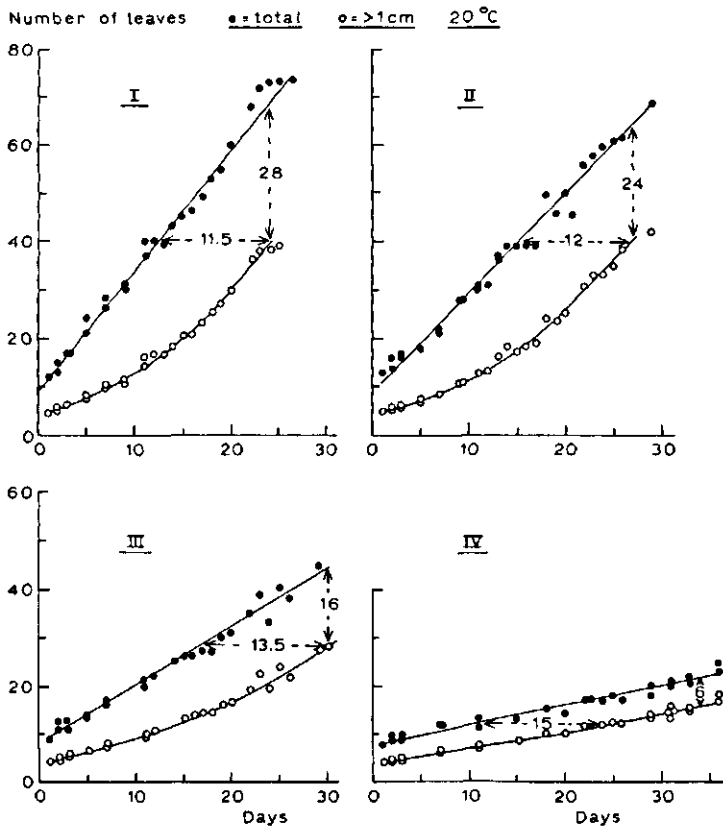
FIG. 3.4. Total number of leaves (●), and number of leaves longer than 1 cm (○), plotted against time, for plants grown at different light intensities, I: 100,000, II: 40,000, III: 18,000, and IV: 7,500 ergs/cm²sec, at 20°C. Horizontal distance between the two curves indicates, for each leaf, the time interval between initiation and the moment of reaching a length of 1 cm. Vertical distance between the two curves indicates the number of leaves smaller than 1 cm. Numbers indicated in the graphs represent finally reached values.

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read as the intersection point between n and $n + 1$ of this straight line and a horizontal line indicating the reference length of 10 mm.

PI can also be calculated by the following formula derived by ERICKSON c.s.:

$$PI = n + (\log L_n - \log 10) / (\log L_n - \log L_{n+1})$$

in which n is the serial number of the leaf which just exceeds 10 mm length, L_n the length of that leaf, and L_{n+1} the length of the next leaf which is just shorter than 10 mm.

Thus, under different conditions of light and temperature, in the nine graphs presented in figures 3.4 and 3.5, increase in PI with time has been compared with increase in total leaf number, as already presented in fig. 3.1. In contrast to the linear increase of total leaf number, PI of the plants, at first, increases at a much lower rate, which, however, gradually increases with time until, finally, PI increases almost at the same rate as does total leaf number. Consequently, the
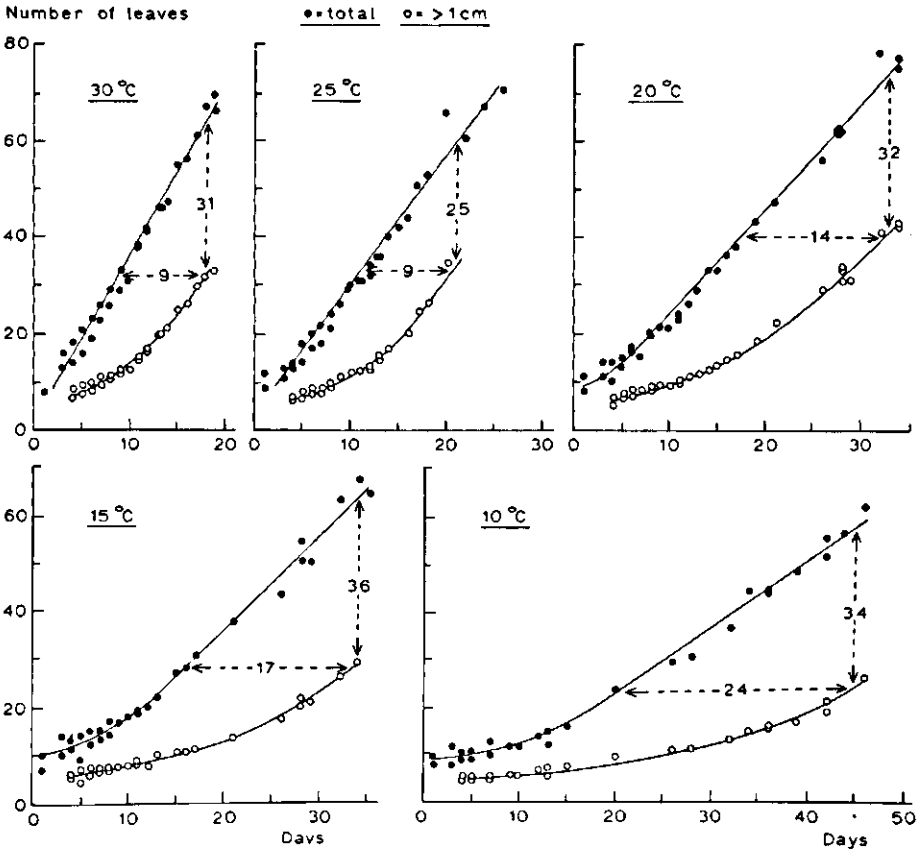

FIG. 3.5. The same as fig. 3.4., for different temperatures as indicated. Light intensity ca. 40.000 ergs/cm²sec.

number of leaf primordia, shorter than 1 cm in the apical bud, increases with the age of the plant. Graphically, this can be easily seen from the increasing distance between the two curves in the vertical direction. Thus, an older plant possesses a larger number of small leaves than a young plant. In direct relation to this, it may be concluded that primordia on a young plant elongate more rapidly than those on an old plant. This is demonstrated by the distance between the two curves on a horizontal line which, for each serial leaf number, represents the duration of growth from initiation to a length of 1 cm. It should be noted, however, that the lower growth rate of young leaves on an old plant goes to-gether with a simultaneous increase of their number. Thus, the slower growth of the individual leaf may be a consequence of increased competition for available 'growth-capacity' at the apical dome.

Based on the graphs of the figures 3.4 and 3.5, and some other experiments, both the number of primordia shorter than 1 cm, and the duration of growth from leaf initiation to a length of 1 cm, has been estimated for different light and temperature conditions. The results are summarized in fig. 3.6. The number of leaf primordia (A), has a distinctly positive correlation with light intensity; with more light an increasing 'stock' of primordia is thus formed. At all light inten-sities, the accumulation of leaf primordia is greatest at 10 °C, which indicates that leaf initiation is less affected by low temperature than further growth of the leaves. In this connection, results of MILTHORPE (1956) are of interest. Studying the growth of cucumber plants, transferred from low to high temperature, he found an increased rate of leaf appearance, as compared with plants grown
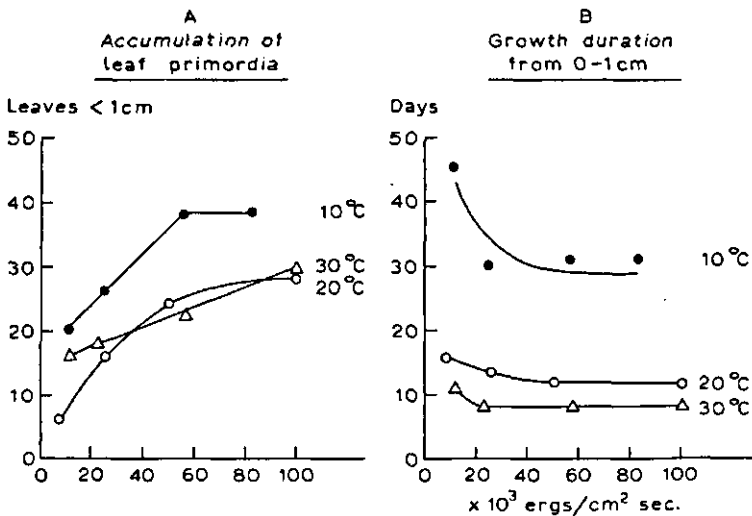

FIG. 3.6. A: Final number of leaves smaller than 1 cm plotted against light intensity at 10 °C (●), 20 °C (○), and 30 °C (△).
B: Time interval between leaf initiation and moment of reaching a length of 1 cm, plotted against light intensity at 10 °C (●), 20 °C (○), and 30 °C (△).
Values in A and B determined as in fig. 3.4.

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throughout at the high temperature. This may well have been due to an accumulation of leaf primordia during the period of low temperature.

Temperature and light intensity effects on the duration of growth from 0 to 1 cm (indicated by the horizontal lines in figures 3.4 and 3.5) are summarized in fig. 3.6,B. From the linear course of the curves, it follows that the effect of light intensity over a large range is much the same. Temperature, on the other hand, has a large effect, since growth duration at all light intensities is greatly increased at lower temperatures. Since the reciprocal of the duration of growth, evidently, is a measure for the rate of growth from 0 to 1 cm, it may be concluded also that leaf elongation in early stages is much more determined by temperature than by light intensity. Only at very low light intensities a large decrease in growth rate is observed.

3.2. MAXIMUM LEAF SIZE

Maximum leaf size refers to length and greatest width of a series of leaves formed on the plant after the early ones which follow the two cotelydons and demonstrate the increasing growth capacity of the plant. In a length-width plot, as presented in fig. 1.2, they are found in the region indicated by A and are to be considered as the greatest leaf size which can be reached under the given experimental conditions.

3.2.1. *Effects of light intensity* .

In figure 3.7, graphs are presented indicating the dependence of leaf length (A) and leaf width (B) on light intensity. Results of several experiments are presented
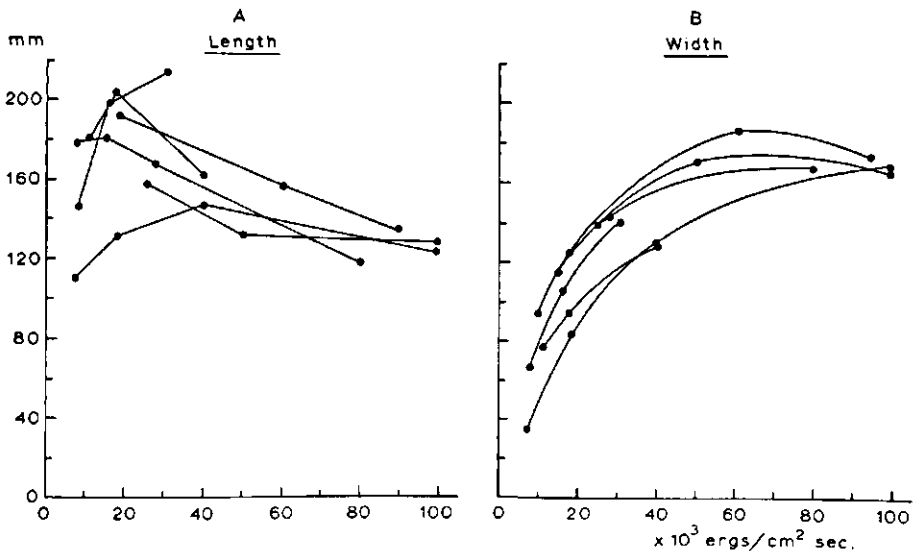

FIG. 3.7. A: Maximum leaf length. and B: Maximum leaf width as related to light intensity. Data from various experiments.

together. In spite of quantitative differences which may be ascribed to different experimental conditions, the general picture is one of a decreasing leaf length and increasing leaf width when the plants receive more light.

Leaf width relationships clearly are of the type of light saturation curves which even seem to pass through the origin, indicating that leaf blade expansion will only occur when the plant receives some light. The effect of darkness is well-known as etiolation, particularly in dicot leaves, MacDougal (1906). The curves thus suggest a positive relation between leaf width expansion and light energy. However, the higher the light intensity, the more conditions other than light seem to limit further growth, e.g., water, minerals, hormones. That this is so, is even more clear from the light intensity dependence of leaf length (A). At low light intensities a similar positive relation with light intensity is found as observed for leaf width. The curves for leaf length, however, do not pass through the origin but cut the vertical, length axis at some height, in accordance with the general finding that leaves also in darkness grow out to some length. Further, leaves reach their maximum length at light intensities much lower than those at which maximum leaf width occurs; at high intensities leaf length clearly decreases again. Apparently, at high light intensities, midrib elongation is limited by a factor not operative at low intensites. In this respect, elongation of lettuce leaves seems to be another example of photo-inhibition, as also found for elongation in other parts of the plant, viz., hypocotyls, epicotyls, stem internodes, petioles. It has been demonstrated, v. d. Meer (1968) that a large part of this growth reduction at high light intensities can be removed by gibberellin, suggesting photo-inhibition to include a factor of hormonal nature.

3.2.2. *Effects of daylength*

Plants can be given more light, either by increasing light intensity or by extending the duration of illumination (the daylength). Daylength as such is well-known for controlling developmental processes in the plant, e.g., the onset of flowering. In these types of light effects, low energy supply usually suffices for full response. In this section we are interested mainly in the interaction of daylength and light intensity from the viewpoint of total energy supply to the plant.

In the first experiment to be discussed, plants have been grown at a series of daylengths between 6 and 24 hours per day at a high light intensity (80,000 ergs/cm^2sec) and at a low one (30,000 ergs/cm^2sec). Results are shown in fig. 3.8. Leaves at all daylengths applied are shorter but wider at the higher light intensity which is in agreement with what one would expect from the light dependence of leaf length and leaf width as shown in fig. 3.7. With increasing daylengths leaf width approaches saturation in a way similar to that demonstrated for increasing light intensities. This indicates that leaf blade development is affected primarily by the amount of light energy received by the plant.

In general, leaf length is not much affected by daylength, so that light intensity seems more important in controlling leaf elongation than daylength. Applied to natural conditions, it may perhaps be concluded that during the winter season, when days are short and light intensities are low, leaf width expansion is
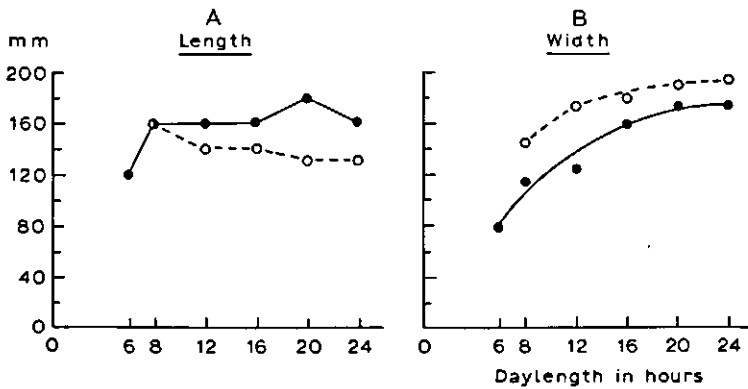
FIG. 3.8. A: Maximum leaf length, and B: Maximum leaf width, for plants grown at different daylengths at light intensities of 30,000 (●), and 80,000 ergs/cm² sec. (○).

reduced both by the shorter days and the lower intensities, whereas leaf elongation is stimulated mainly as a result of the low intensity. Obviously, both tendencies will result in an increase of the length to width ratio of the leaves, earlier characterized as an unfavourable condition for heading.

In another type of experiment, plants have been grown at a series of three different light intensities (25,000, 50,000 and 100,000 ergs/cm²sec) and three different daylengths (6, 12 and 24 hours). Of the nine possible combinations, there are three in which plants receive the same amount of light, though differing in intensity and duration. In fig. 3.9, the maximum lengths and widths have been plotted against total daily energy (time × intensity). The same results are presented in two different ways, *viz.*, by focussing attention to light intensity (A and B) or to daylength (C and D). Once more, the differences in response of leaf length and leaf width are quite obvious. At the lower light intensity (25,000 ergs/cm²sec) only, leaf length and leaf width respond similarly to increasing daylength, *viz.*, by a strong increase. It lays at hand to assume that growth in length and in width at this low intensity both are limited by lack of energy, i.e. supply of photosynthates.

At the higher intensities (50,000 and 100,000 ergs/cm²sec) however, the situation becomes quite different. Leaf width still responds positively to increased light duration up to 12 hours (B). Daylengths above 12 hours do not give much further increase. The three curves in B together, suggest that leaf width reaches saturation at about 200 × 10⁷ ergs/cm². Some of the points are not in accordance with this view, e.g., that observed in a 6 hour day at 100,000 ergs/cm²sec. Evidently, a limiting factor other than the amount of light energy supplied becomes of importance, causing light saturation at a much lower level. This is still more clear, when considered in relation to light intensity, as shown in D.

With respect to leaf length, it is quite clear from A and C that leaf elongation is inhibited by high light intensities. Consequently, a positive effect of light energy, noticeable at 25,000 ergs/cm²sec does not occur at 50,000 and 100,000 ergs/-
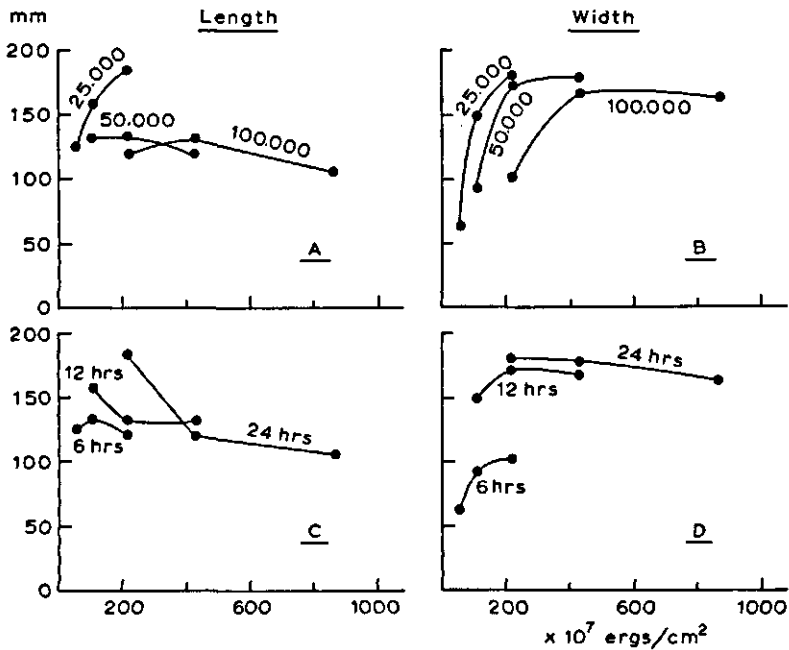
Fig. 3.9. A and C: Maximum leaf length, and B and D: Maximum leaf width for plants grown at light intensities of 25,000, 50,000 and 100,000 ergs/cm² sec., and daylengths of 6, 12, and 24 hours, in relation to daily energy dose in ergs/cm². Same data presented in A and C and in B and D respectively. In A and B points are connected according to equal light intensities, in C and D according to equal daylengths.

cm²sec. Evidently, also here, a factor other than light energy limits leaf elongation.

It is tempting to suggest, also in view of results to be presented in Chapter 5, that the other limiting factor under discussion both in relation to leaf length and leaf width, is of a hormonal nature and reaches optimal concentrations at much lower light intensities than photosynthesis does.

3.2.3. *Light-temperature relationships*

The preceding discussion of effects of light refers only to experiments at 20 °C. In fig. 3.10 graphs, similar to those shown in fig. 3.7 are given for three different temperatures, *viz.*, 10 °C, 20 °C and 30 °C. Qualitatively, the curves are very similar. In general, greater lengths and widths are reached when temperatures are higher. The shape of the leaves does not change, since the L/W ratio shows the same course against light intensity, at least at 20°C and 30°C. At 10°C, some difference is found, mainly because the reduction in length and width, usually found at low light intensities, is suppressed. Consequently, at low light intensities, larger leaves are produced at low temperature than at high temperature.
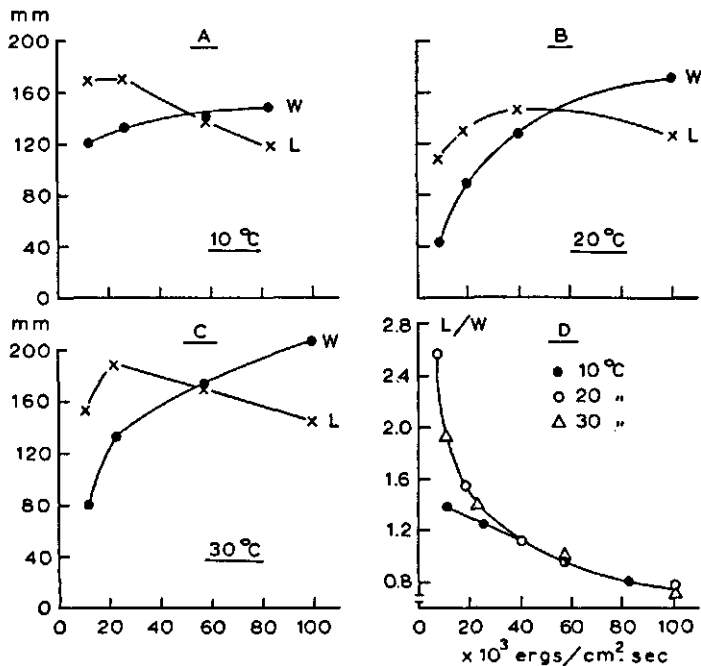
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FIG. 3.10. Maximum leaf length (×) and maximum leaf width (•) as related to light intensity, at 10°C. (A), 20°C. (B) and 30°C. (C). Length width ratio's given in D.

In A and B of fig. 3.11, the average lengths and widths are shown at different temperatures between 10°C and 30°C for a high light intensity and a low one. In general, linear temperature gradients are found which, in particular for leaf width, have a negative slope at low light intensity, and a positive one at high light intensity. In other words, leaves decrease in width with increasing temperature at low light intensities, but increase in width at high light intensities. Although more or less the same trend is observed for leaf length, a negative temperature response at low light intensity is much less evident.

A striking difference between length and width appears, in as much as temperature gradients at high and low light intensity converge with decreasing temperatures in the case of leaf width, whereas for leaf length they diverge. Evidently, the greatest effects of light on leaf width are found at high temperature, whereas for leaf length, light intensity effects are greatest at low temperature.

From this negative temperature response to low, and the positive response to high light intensity, it may be predicted that at some intermediate light intensity the effect of temperature on leaf development will be small. Evidence for this has been collected in another experiment, in which plants were grown at four different light intensity levels at 10°C and 30°C. Based on the average length and width developed at these temperatures (fig. 3.11,C,D) straight temperature gradients are drawn which have different slopes for each light intensity. Particularly
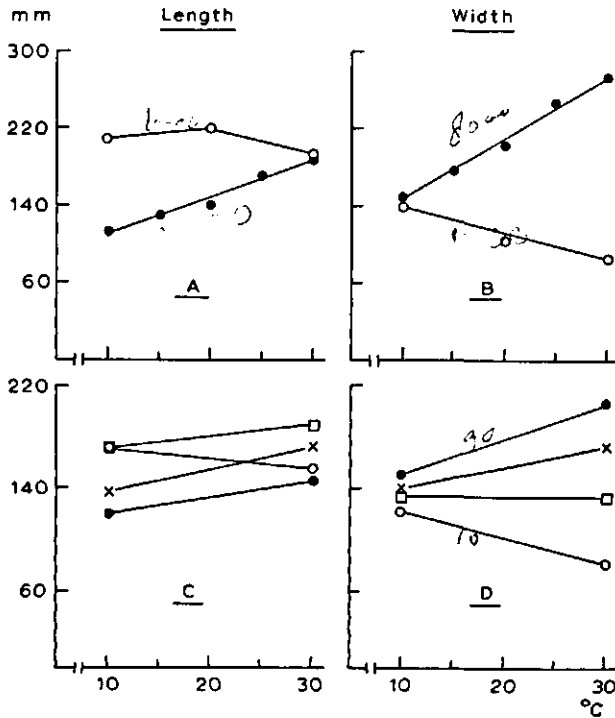
FIG. 3.11. Maximum leaf length (A and C), and maximum leaf width (B and D) plotted against temperature for different light intensities. Light intensities in A and B: ca. 10,000 (○), and ca. 80,000 ergs/ cm² sec. (●), in C and D: ca. 10,000 (○), ca. 25,000 (□), ca. 55,000 (×), and ca. 90,000 ergs/cm² sec. (●).

for leaf width it is quite clear that the slopes gradually change from negative to positive when light intensity increases from low to high. For leaf length, the situation is different in so far as a clear-cut change in slope occurs only at the lowest light intensity used.

In fig. 3.12, the different slopes, expressed as change of leaf length or width per unit temperature difference (mm/°C), have been plotted against light intensity. Thus, tentatively, two curves are drawn, indicating for each light intensity the effect of a change in temperature of 1°C. Although too much weight should not be attached to the actual values, there is no doubt that a critical light intensity exists above which leaf growth will be enhanced by raising the temperature, and below which it is decreased. The largest response is found for leaf width.

With regard to the negative temperature response, observed at a low light intensity, it may be noted that it has been demonstrated before (see fig. 3.3), that leaf production increases with temperature at all light intensities. Consequently, it may be understood that, at a low energy level of the plant, i.e., at a low light intensity, an increase in leaf number will reduce the growth of the individual leaves, owing to increased competition for the available growth substrates. At high light intensities, on the other hand, it seems that an excess of energy permits growth of each leaf to be enhanced by increasing the temperature, notwithstanding leaf number will increase as well.

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FIG. 3.12. Increase or decrease in leaf length (×), and leaf width (○) in mm per °C., as a function of light intensity. Points represent the gradients of the curves in C and D of fig. 3.11.
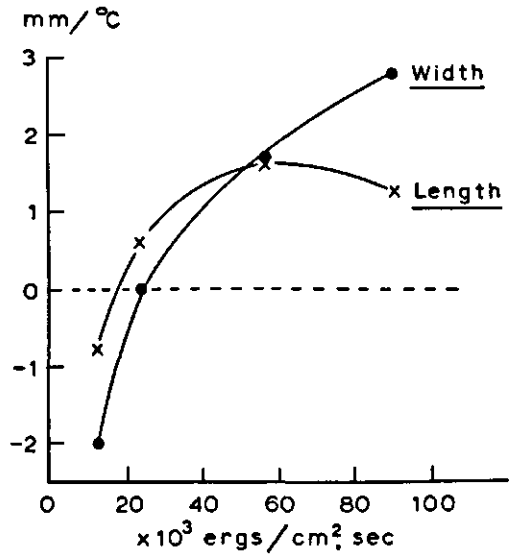
3.3. GROWTH-TIME RELATIONSHIPS

Differences in the growth-time relationships under the various experimental conditions are at the base of differences in leaf dimensions as discussed in the preceding sections. Therefore, length and width expansion of a given leaf has been followed in the course of time. For obvious reasons, however, accurate measurements cannot be started before the leaf has reached a certain size. This implies that measurements do not include early phases of growth, necessary to obtain an idea of its complete course. Moreover, it should be taken into account that measurements cannot be made without some handling of the leaves, which, however careful, may well interfere with the natural course of growth. For these reasons, and in particular to collect information about primordial leaf growth, direct measurements have been supplemented by indirect ones, by means of periodically harvested plants, in which development of a leaf of a definite serial number (usually no. 12, counted from below) has been followed in the course of time. In this way, records could be collected from a leaf length of about 0.2 mm onwards. It is required that plants are carefully selected for uniform development at the start of the experiment, so that growth of a particular leaf proceeds equally in all plants, and measurements of length and width made at subsequent harvests reveal the same course of growth as when measured on one and the same leaf.

In figure 3.13, an example is given of measurements on the plant, of the increase in length and width of the 12th leaf at a high light intensity and at a low one. At the time the measurements started growth was found to proceed at a constant rate, yielding a linear growth-time relationship. Conform to what generally was found in all our experiments, leaf length reached a higher growth rate at the
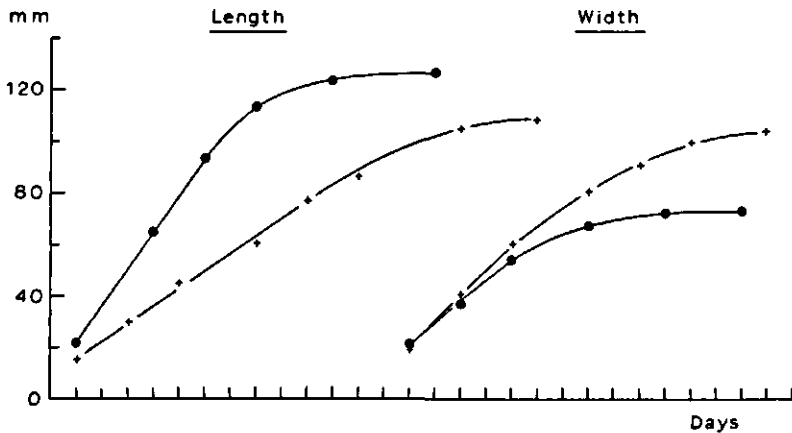
FIG. 3.13 Growth in length (A) and in width (B) of a leaf (leaf number 12) measured on one plant grown at a light intensity of 20,000 (●) and 40,000 ergs/cm² sec. (+) respectively.

low light intensity. Leaf width, on the other hand, usually, increases more slowly at low light intensity. Both growth in length and in width are maintained for a longer period at high light intensity. Thus, the effect of low light intensity on the final length of the leaf is positive through enhancement of the rate of growth, but negative by reducing the duration of growth. For a high light intensity the reverse is true: decreased rate of growth is counterbalanced by increased growth duration. Consequently, differences in leaf length at different light intensities will be small or moderate. For leaf width growth rate and growth duration are influenced in the same direction by light intensity giving rise to much greater differences in the ultimately reached widths (cf. also fig. 3.10).

In figure 3.14, the logarithms of leaf length and width of the 12th leaf are shown against time, as measured in subsequent harvests which started at an early stage, including the phase of primordial growth. In this experiment, plants have been grown at four different light intensities, viz., of 7,500, 18,000, 40,000 and 100,000 ergs/cm²sec respectively from left to right. From the linear course of the curves of the first stages, it can be concluded that the early increase of both length and width proceeds exponentially. Mathematically, this can be expressed by the well known formula for 'compound-interest' growth:

$$L = L_0 e^{kt} \tag{1}$$

or written in its linear form

$$\ln L = \ln L_0 + kt \tag{2}$$

in which L is leaf length (or width) at time t, L_0 the same at t_0, e the base of the natural logarithms, and k the relative rate of growth. According to 2, the slopes of the straight lines in fig. 3.14 are proportional to k.

FIG. 3.14. Growth in length (A) and in width (B) of a leaf (leaf number 12) as measured on different plants in subsequent harvests. I, II, III, and IV are light intensities of 100,000, 40,000, 18,000, and 7,500 ergs/cm²sec. respectively. Numbers at top of the straight lines represent relative growth rates (i.e. the slopes of the lines), during the phase of exponential growth, expressed on the base of log *e*. Arrows indicate estimated end of exponential growth.
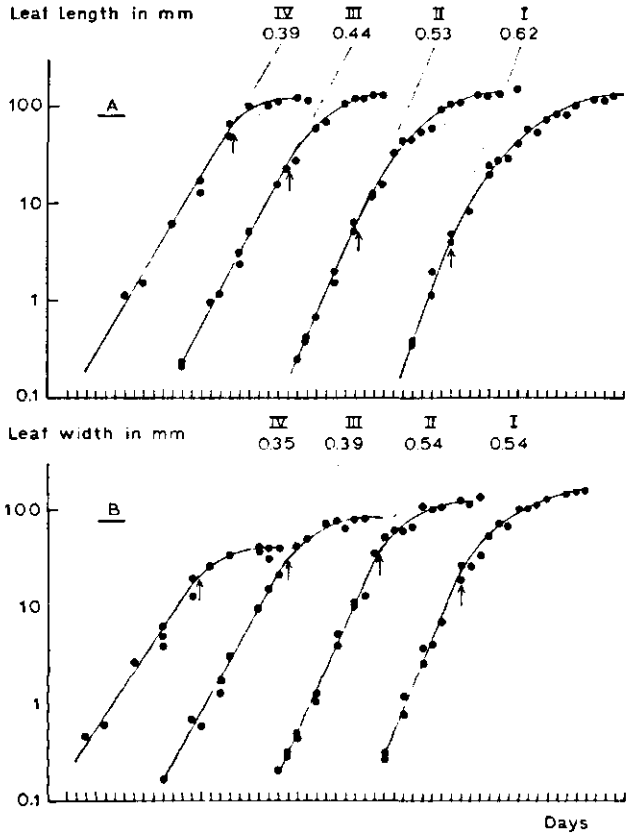
The steeper slopes of the lines at higher light intensities indicate that relative growth rate increases with light intensity, both for length and width. However, length and width differ with respect to the duration of exponential growth. For leaf length, the phase of exponential growth, clearly becomes shorter when light intensity increases, whereas for leaf width this phase is much less affected by light intensity. Fig. 3.15 shows values for k(a), and the duration of exponential growth (b) in relation to light intensity, both determined by graphical estimation. We may conclude that there are mainly quantitative differences between growth in length and width. Relative growth rate (a) for both length and width shows a positive relation to light, whereas duration of exponential growth (b) shows a negative relationship. However, the effects of light are much more pronounced for growth in length than for growth in width. These quantitative differences appear to give rise to quite different growth-time curves for length and width as shown in fig. 3.16. Curves have been adjusted so that for each light intensity they start at the same length or width of 1 cm. In accordance with the higher values for k, leaves at the primordial stage will grow faster at a high light intensity, both in length and in width. However, since for leaf length the period of ex-
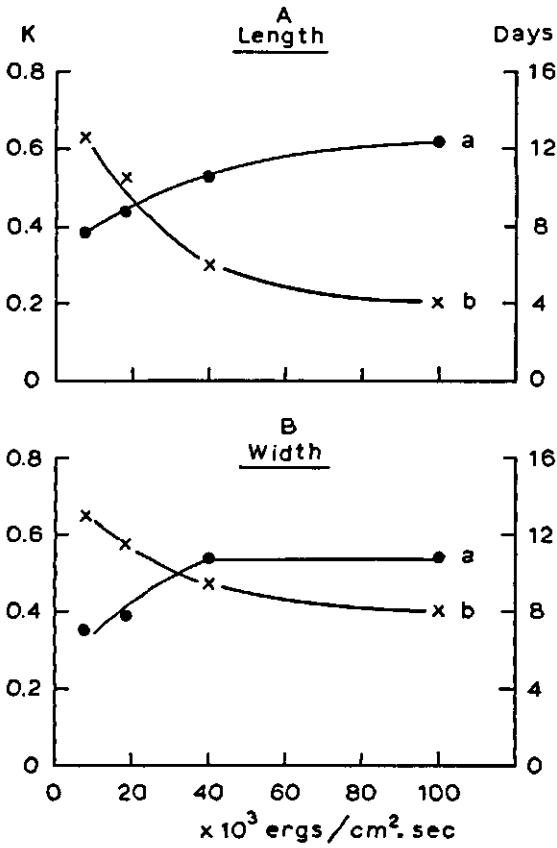
FIG. 3.15. Relative growth rate k (curves a: ●) and duration of exponential growth (curves b: ×), as related to light intensity. A: Leaf length, B: Leaf width. Values graphically derived from the curves of fig. 3.14. Growth is considered to start at a length or width of 0.3 mm.
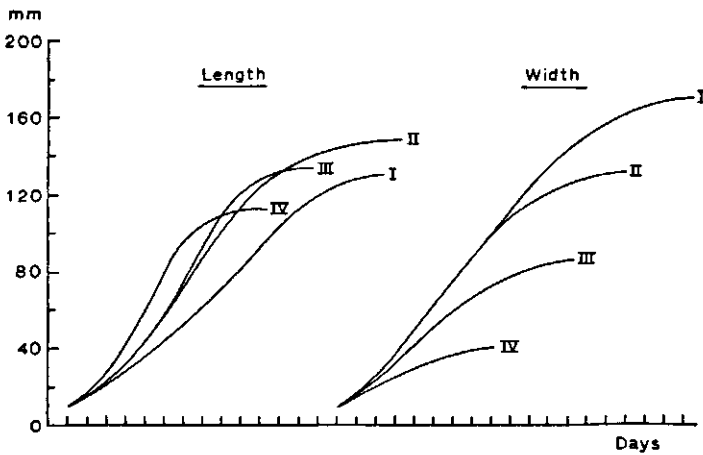
FIG. 3.16. Sigmoid growth curves for leaf length and leaf width, as derived from the logarithmic curves of fig. 3.14.

ponential growth is maintained longer at low light intensity, leaves eventually elongate at a higher rate at a low light intensity which is in agreement with results shown before in fig. 3.13. On the other hand, the curves indicating growth in width are reduced over the whole range at lower light intensities, leading to a greater ultimate effect of light intensity on leaf width.

In fig. 3.17, growth curves are presented for different light intensities at 10 °C and 30 °C, for leaves measured on the plant. As to the effect of light intensity, the curves are in accordance with what has been demonstrated above: growth in width decreases when light intensity decreases, whereas for leaf length higher growth rates are measured at lower light intensities.
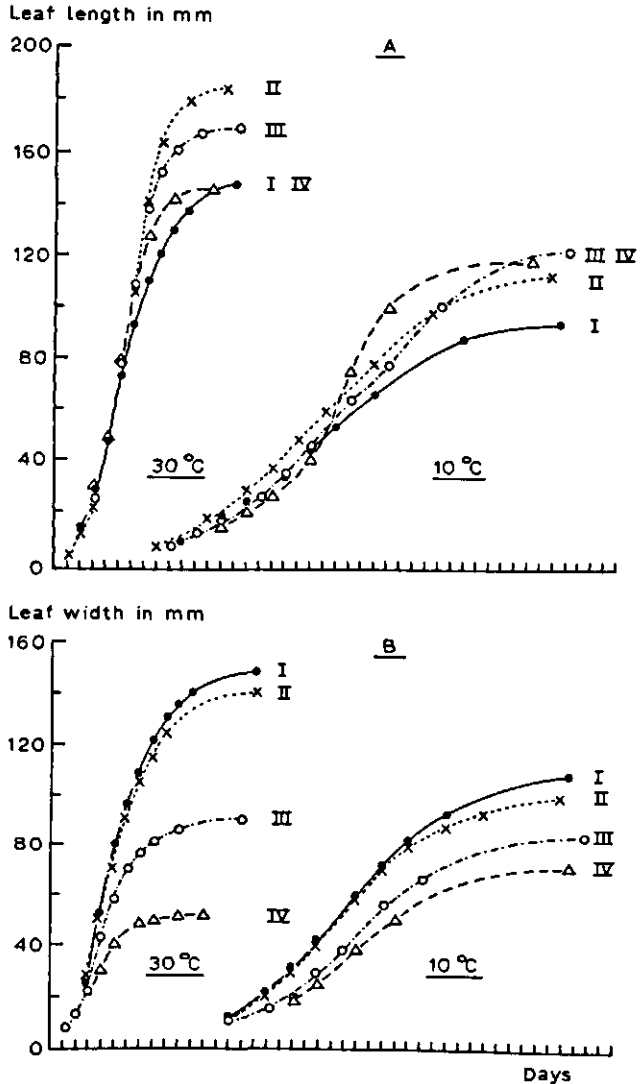
Fig. 3.17 Growth in length and in width of a leaf (leaf 12) of plants grown at different light intensities at 10°C and at 30°C, I, II, III, and IV represent light intensities of 100,000, 40,000, 25,000, and 10,000 ergs/cm²sec. respectively.

At all light intensities, at 30 °C, the leaves elongate faster than at 10 °C, though for a shorter period. For leaf width at 30 °C, effects of light intensity are more pronounced than at 10 °C (cf. also fig. 3.11). The influence of temperature also in this case is affected by light intensity, and changes from a negative effect (decreasing leaf width with increasing temperature) at low light intensity, into a positive effect (increasing leaf width with increasing temperature) at high light intensity (cf. fig. 3.12).

Although we have suggested a more or less similar light-temperature relationship for leaf length, a negative effect of temperature probably occurs only when light intensity is very low. In the present case (fig. 3.17,A) at 10,000 ergs/cm²sec leaves are still somewhat longer at 30 °C than at 10 °C, although the difference is smaller than at the higher light intensities.

Variation in temperature can be applied during the day or during the night. In early experiments (BENSINK, 1958), it has been demonstrated that a low temperature given during the night can partly compensate for the effects of a low light intensity during the day which is in accordance with the evidence discussed above. In general, in effects of temperature during light and dark periods the energy balance of the plant, and especially the light intensity, is important.

The effect of daylength on the rate of leaf growth has not been examined in particular, but in all cases where plants have been grown at different photoperiods, it was evident that growth was speeded up by increasing the daylength, a trend which went on up to continuous light. A question closely connected herewith is whether growth rates are different during day and night. For daylengths of 12 and 16 hours, reliable differences could never be established between growth rates during the light and the dark period of the 24-hours cycle. However, since dark growth, eventually, occurs at the expense of energy accumulated during the light period, previously presented data (fig. 3.9) render it conceivable that with light periods shorter than 12 hours, a difference will appear between the growth rates during day and night.

3.4. DISTRIBUTION OF GROWTH ALONG THE MIDRIB AND OVER THE LEAF BLADE

In this paper, so far, leaf growth has been considered merely in terms of total length or greatest width, but there is no doubt that a leaf, and in particular a lettuce leaf, will grow at different rates in different places. A simple method to investigate this is to measure the differential displacement of originally equally spaced marks of Indian ink. Introduced by SACHS (1874) in his classical study on the growth of the primary root, this technique has been widely used since then, also for leaves, AVERY (1933), MAKSYMOWYCH (1962), SAURER and POSSINGHAM (1970).

Thus, midribs of young lettuce leaves were divided by three Indian-ink marks into four equal parts, indicated as A, B, C, and D from base to tip. After growth had stopped, the length of each part was measured again and its increment expressed as percentage of total growth. In fig. 3.18, the relative elonga-

FIG. 3.18. Increase in length of four originally equal parts of the midrib as indicated. Increases in percentages of total leaf elongation at different light intensities and at 10°C., 20°C., and 30°C.
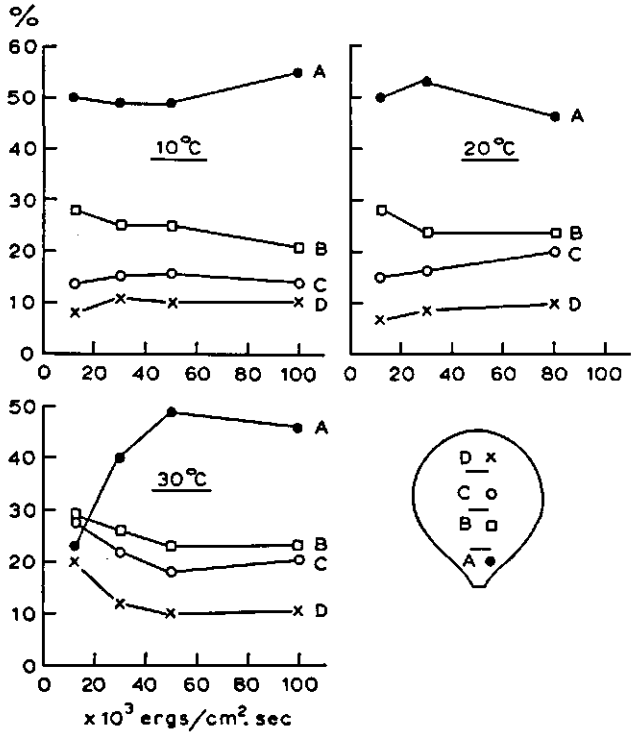
tion of the four parts is presented for four different light intensities, and for plants, grown at 10°C, 20°C or 30°C. Each point of these graphs represents an average of 12 leaves. They show that at 10°C and 20°C, 50% of all growth in length is made up by the lowest quarter of the midrib, whereas only 10% is due to increase of the upper quarter; the two middle parts showing values in between. Most strikingly, this distribution of elongation along the midrib is not, or very little affected by light intensity at least at 10° and 20°C. Since it was found previously that light intensity has an effect on midrib elongation as a whole (fig. 3.7), it seems that this is not accompanied by a change in the pattern of growth. At 30°C, however, a clear influence of light intensity also on the distribution of elongation is observed. At high light intensities, the situation is the same as found at 10° and 20°C., but at low intensities elongation is more equally distributed over the whole length of the midrib, each of the four parts, A, B, C and D, contributing about 25% of the total growth.

For other leaves, extension of different parts of the midrib has been followed during growth. Figure 3.19, A presents growth-time curves of three subsequent parts of the midrib. The leaf was marked at a length of 21 mm so that each section was about 7 mm long. Adding up the three curves should yield the course of the increase of total leaf length. It appears that the basic part of the midrib grows faster and continues growth longer than the more distal parts do. Both
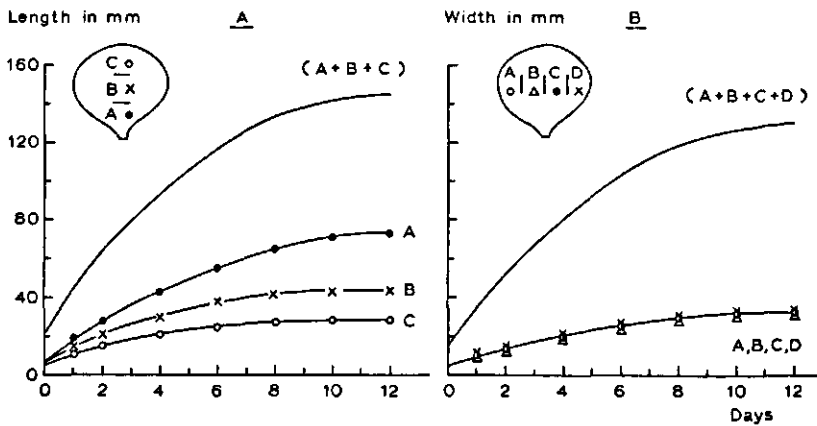
FIG. 3.19. Growth in length (A) and width (B) of various parts of the midrib and leaf blade as indicated. Curves on top represent increase of total length (A + B + C) or width (A + B + C + D).

effects together bring about the relatively greater extension of the proximal end of the midrib as presented in fig. 3.18. The measurements of fig. 3.19 were only made at a light intensity of about 80,000 ergs/cm^{2}sec. It may be of interest to carry out similar measurements at low light intensity and high temperature, as in that case one would expect a more equal distribution of growth along the midrib (cf. also fig. 3.18).

When midribs of young leaves are divided into more and smaller parts, it appears that it is the very base which elongates most (fig. 3.20). The lower 5 mm of the originally 30 mm long leaf yields 30 to 40% of the total ultimate elongation.

The leaf blade has also been marked; both halves of young leaves were marked with Indian-ink in the middle of the greatest width. Increase in width of each
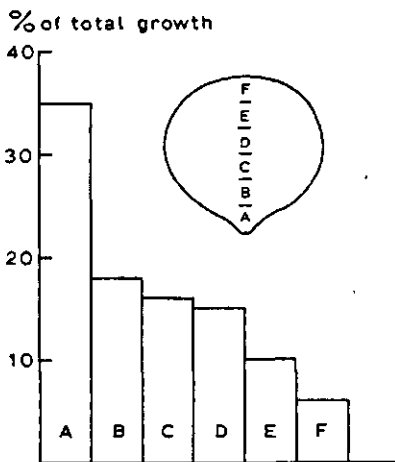
FIG. 3.20. Increase in length of 6 subsequent parts of the midrib as indicated. Increases in percentages of total leaf elongation.

part was followed in the course of time. Figure 3.19,B shows that identical
growth-time curves are obtained for each of the four parts, which, added up,
represent increase of total leaf width. This indicates that leaf blade expansion
in the middle of the greatest width, is equally distributed over the leaf. In longi-
tudinal direction, however, differences in growth are likely to occur, considering
the relative surplus of leaf blade extension, demonstrated, e.g., on Plate II. To
investigate this further, young leaves have been marked by some parallel, 5-mm
spaced horizontal and vertical lines, the course of which was examined again
after the leaf had reached full size. Since leaves grow in a rosette, and often have
a very irregular surface it appeared difficult to derive quantitative expressions of
differential growth for the different segments of the leaf, such as given, e.g., by
AVERY (1933) and RICHARDS and KAVANAGH (1943) for the tobacco leaf. How-
ever, the obtained results are quite conclusive and illustrated on Plate IV which
presents two such leaves grown at low and at high light intensity. Both leaves were
marked by four horizontal and two vertical lines when about 25 mm long and
20 mm wide. For the leaf grown at the low light intensity, the lines remained
parallel until the end, indicating that the pattern of differential growth has re-
mained unchanged from the moment of marking. A gradient of decreasing
growth, both in the midrib and the leaf blade is evident from the base to the tip

PLATE IV. Example of different outgrowth of a leaf grown at low light intensity (left) and at
high light intensity (right). Leaf blades marked with 5-mm squares of Indian ink at a young
stage.

of the leaf. Lamina extension in the longitudinal direction, evidently, kept pace with the elongation of the midrib, yielding a flat and smooth leaf surface.

At the high light intensity, the original rectangular network becomes deformed: the horizontal lines show an upward bending near the leaf margins, whereas the vertical ones diverge the more they approach the leaf base. A longitudinal gr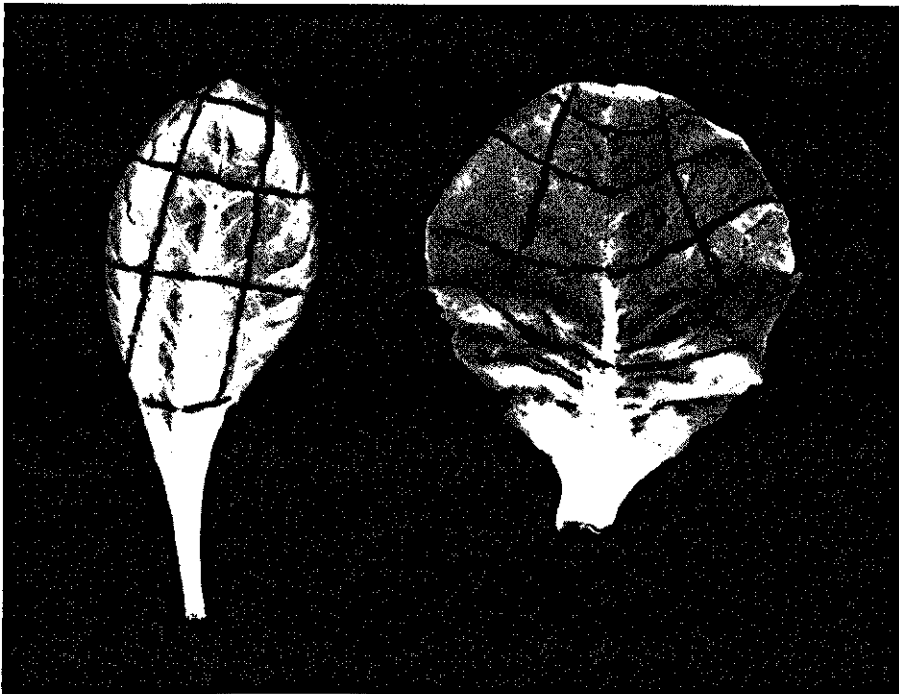adient in growth intensity is observed, similar to that found for the low-intensity leaf. Clearly, leaf blade expansion has increased towards the leaf base, both in longitudinal and transversal directions. Similar results have recently been published for spinach leaves by SAURER and POSSINGHAM (1970). The evident surplus of lamina development yields the well known folds already demonstrated on plate II. It is quite clear that the ratio between midrib and leaf blade development greatly depends on light intensity. The more light the plants receive, the more this ratio shifts in favour of the leaf blade.

3.5. LEAF SHAPE DEVELOPMENT

As an indication for differences in leaf shape, the length to width ratio may be used, although data on L/W ratio's do not give full justification to the differential development of midrib and leaf lamina (c.f, e.g., Plate II and the preceding section).

It has been demonstrated earlier in this paper, that L/W gradually decreases in the first 12 to 15 leaves and a more or less constant level is reached in later ones (see fig. 1.1). Development of a low L/W ratio has been considered as a necessary condition for the onset of heading. In fact, the L/W ratio ultimately reached, greatly depends on the intensity of light at which the plants are grown. Temperature appears to be a factor of minor importance as is demonstrated by the almost similar light intensity dependence of L/W at 20 °C and 30 °C shown in figure 3.10. Only at 10 °C, leaves will develop a lower L/W, in particular at low light intensity, owing to a decreased reduction in leaf width as compared with the situation at higher temperatures.

Since differences in leaf size are not expressed in the L/W ratio, preference has been given to construct plots of length against width as presented in fig. 3.21. In A the length-width relationships are shown for the 12th leaf during its growth at four different light intensities. The increase in slope with decreasing light intensity represents the higher L/W ratio's developed at low light intensity. At each light intensity, increase in length and in width proceeds in a more or less proportional way, yielding linear relationships. Towards the end of the growth period, however, growth in length, evidently, prevails over growth in width, as demonstrated by the upward bend of the curves. Earlier cessation of increase in leaf width seems the most probable explanation herefor. In B it is demonstrated that exactly the same L/W relationships apply also for the length and widths of leaves 13 and higher, when measured simultaneously on the plant. Therefore, it may be concluded that from leaf 13 onwards leaves display the same pattern of growth, as indicated for leaf 12 in A.

FIG. 3.21. A: Relation between length and width of a leaf (leaf 12), as measured during its growth at different light intensities. I: 100,000 (●), II: 40,000 (+), III: 18,000 (□), and IV: 7,500 ergs/cm² sec (○).
B: Length-width relationships of leaves 13 and higher as measured simultaneously on the plant. Curves similar to those in A, representing the same light intensities.

Leaf length in mm   A   II

Width in mm
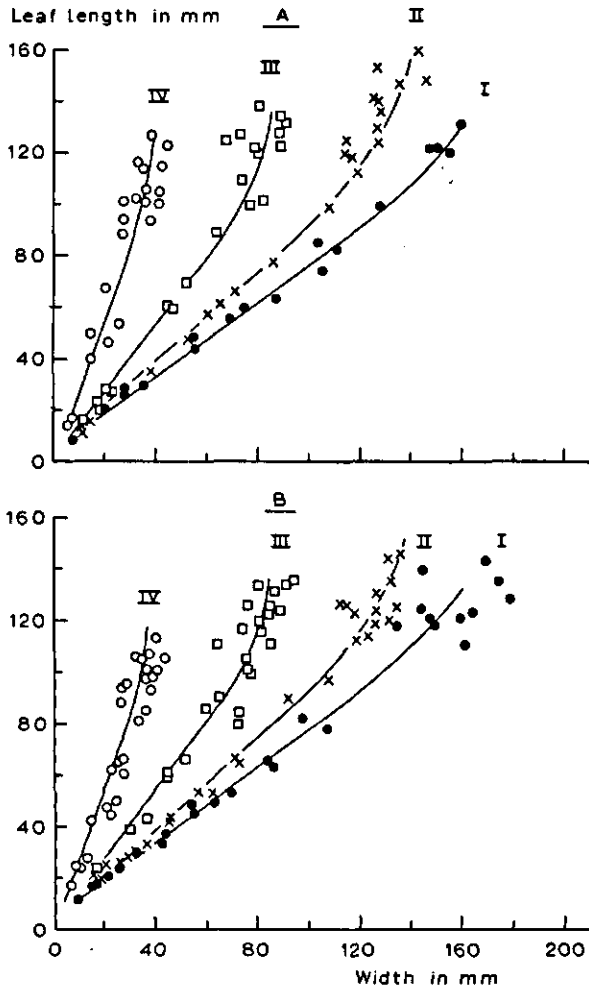
When the presented graphs are supplemented with the corresponding data for the first 11 leaves as has been done in fig. 3.22, loop-shaped curves originate of the type presented before in fig. 1.2 on page 6. It follows from fig. 3.22 that the lower the light intensity, the more the ultimate L/W ratio reached by subsequent leaves resembles that of the first leaves. In other words, the first leaves apparently show the characteristic development of a low-light-intensity leaf. This conclusion is in accordance with a concept of GOEBEL (1908), viz., that early, juvenile leaf development is related to the nutritional status, i.e., the photosynthetic capacity of the plant. In the present case it mainly reflects to what extent leaf width development is allowed to occur.

In many cases in which growth in two different directions is compared, the proportion between the relative growth in both directions remains constant.

Length in mm
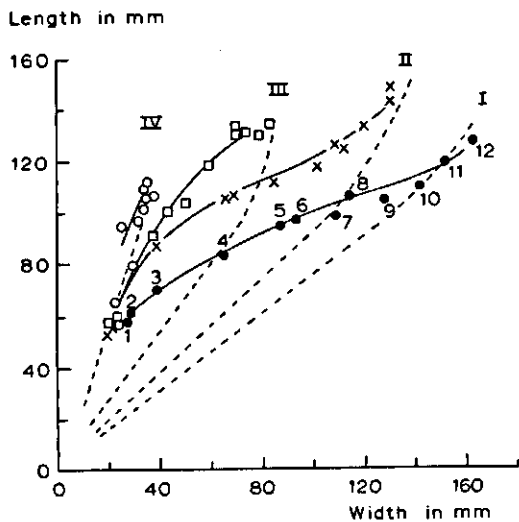
FIG. 3.22. Length-width relationships of the first 12 leaves of the same plants as in fig. 3.21. Curves of fig. 3.21 reproduced as dotted lines.

This phenomenon, known as *allometry* can be expressed by a formula given by HUXLEY (1932). When Y and X are two directions considered, e.g., length and width of a leaf, showing allometric growth, then

$$Y = b \; X^k \tag{1}$$

or

$$\ln Y = \ln b + k \ln X \tag{2}$$

in which b is a constant (representing the value of Y when $X = 1$), and k the ratio between the relative growth of X and Y. This formula has often been used to determine differences or changes in leaf shape, HAMMOND (1941), WHALEY and WHALEY (1942), JONES (1956), HABER and FOARD (1964). When plotted logarithmically, the values for X and Y fall along a straight line, the slope of which represents k. From the value of k, one may obtain an idea about changes in leaf shape that occur during leaf expansion. For $k > 1$, leaves become more elongated during growth for; $k < 1$ they become rounder in shape, whereas for $k = 1$, leaf shape remains unchanged. In the last case a graph of Y against X yields a straight line also in a linear plot, the slope of which is determined by the value of b in the above formula. In cases when k deviates from unity, a linear plot of Y against X will produce curved lines, running upwards for $k > 1$ and downwards for $k < 1$.

We have seen that in lettuce leaves, increase in length and width, at all light intensities, over a long period of growth, shows a linear relationship, the slope of which differs, however, according to light intensity. From the foregoing discussion we may conclude, that differences in growth at different light intensities are only due to differences in b, k being unity at all intensities. Usually, no special biological significance is attributed to the value of b, however, since it indicates

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the relation between the initial values of X and Y, it represents the length-width ratio of the leaf primordium at the moment at which marginal meristems, initiating the leaf lamina, start differentiation.

Hence, it may be concluded from fig. 3.21 that leaf blade formation starts relatively earlier, i.e., at a shorter length of the primordium, at high light intensity.

A similar reasoning appears to hold for the first leaves of the plant, for which the successively lower L/W ratio's may be basically explained by an earlier start of leaf blade development in subsequent leaves. This does not contradict the above suggestion that differences in the growth pattern of these leaves are controlled mainly by the energy supply, but fits quite well into this view.

From the fact that the curves in figure 3.22 eventually bend upwards, it can be concluded that k increases above unity towards the end of leaf expansion. By means of linear regression calculations of the logarithms of length and width, values for k have been determined which tend to increase with decreasing light intensity indicating that leaf elongation should increase relatively more than leaf width expansion at low light intensity. However, since calculations for k show a great adaptability to rather large variations in length and width, the significance of small differences in k may be doubted. For this same reason use of the allometric formula in demonstrating small differences in leaf expansion seems of limited analytical value.

For lettuce, length-width diagrams, as presented in fig. 3.22 may demonstrate the influence of variations in light conditions, as may be illustrated by an example from plants grown under natural conditions. In A of fig. 3.23, loop-shaped curves as discussed before, are presented for plants grown either in the open during spring or in an unheated glasshouse during winter; measurements were made May 11 and December 3, respectively. As is shown, in both cases the same course of development is followed by the first leaves. However, in May increase in leaf width continues up to much higher values than in December. Consequently,
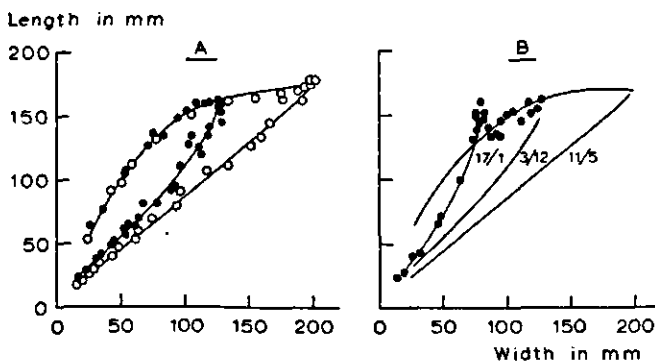

FIG. 3.23. A: Length-width diagrams for leaves of plants grown under natural conditions during spring, measured May 11 (O), or grown in an unheated glasshouse during winter, measured December 3 (●).
B: The same for a plant, measured January 17.

the lower leg also has a much steeper slope in December because of the higher L/W ratio of the leaves developing in winter. In B, a plant of the same set has been measured again on January 17. Owing to still poorer light conditions in the period prior to this measurement, in particular leaf width development has been further reduced, yielding a slope still steeper than that of the curve measured in early December.

3.6. CONCLUSIONS

Leaf production
Leaves are produced at a constant, relatively high rate; 1 to 2 leaves per day may be considered as normal, rates increase both with light intensity and temperature. Leaves are produced at a higher rate than that at which they expand; consequently, leaf primordia and young leaves in the centre of the plant accumulate in the course of time. This accumulation is greatest at high light intensities and low temperatures, indicating a difference in the effects of light intensity and temperature on leaf initiation and subsequent growth: leaf production increases relatively more than primordial expansion at high light intensity, whereas the latter appears to be more affected by temperature.

From the linear increase in total leaf number and the non-linear increase in the number of leaves of a certain length, e.g., 1 cm, it has further been derived that leaves on a young plant grow faster than those developing on an old plant. Interfoliar competition seems the most probable explanation for this phenomenon.

Leaf size
Light intensity has different effects on midrib elongation and leaf blade expansion, measured as leaf length and leaf width respectively.

Leaf width is positively affected by light energy, either in terms of higher light intensities or longer daylengths. In both cases the relation is represented by saturation curves which show the strongest light dependence at intensities below 20,000 ergs/cm²sec and at daylengths shorter than 12 hours.

Leaf length shows a positive relation with light energy at low light intensity only, whereas at a high intensity, midrib elongation is clearly suppressed. A hormonal factor may be thought of as a limiting factor, operating particularly at high light intensities. Thus, it can also be understood that effects of daylength on leaf lengths are noticeable only at relatively low light intensites; at high intensities they appear rather ineffective.

Temperature effects are evident, but greatly dependent on the prevailing light intensity. Thus, a negative response to temperature may gradually change into a positive one at light intensities increasing from low to high. Relative to this, light intensities may occur at which leaf growth appears only little affected by temperature, which was especially clear for leaf width. Since leaf production at all light intensities is higher at high temperatures, it seems likely that at a low energy level of the plant, i.e., at low light intensity, reduction in width may result

from increased competition for growth substrates. At high light intensities, on the other hand, the energy situation allows increased growth of the leaves at higher temperatures, notwithstanding leaf number has increased as well.

Growth rates

The different behaviour of leaf length and leaf width towards light intensity is reflected also in growth-time relationships. Leaves elongate faster at low light intensities, but leaf blade expansion then is slower. This difference, at least partly, can be understood from differences in growth already present in the primordial stage, when length and width both increase exponentially. It is observed that exponential growth increases with increasing light intensity but that at the same time its duration is shortened. This effect is much more pronounced for the growth in length than for that in width. Consequenty, during the primordial leaf stage, leaves elongate faster at high light intensity, whereas at a later stage elongation is faster at low intensity. Increase in width, on the other hand, occurs more slowly over the whole period of growth, when light intensity is low. It may be understood herefrom that light intensity effects are much more pronounced as differences in width than in length of the leaves.

The leaf base is the part showing the strongest elongation. This is clearly so for the midrib, for which it is true at all light intensities. For the leaf blade, expansion in the lower part is excessive only at high light intensities and then causes the leaf lamina to produce the well known horizontal folds and crinkles along the midrib.

Leaf shape

Differences in leaf shape are best presented in length to width diagrams. When the dimensions of subsequent leaves of a plant are plotted that way, curves are produced which indicate the developmental sequence of leaf shape and differences induced, e.g., by differences in light intensity. The different length-width relationships in leaves growing at different light intensities or between successive early leaves of the plant, can be understood on the basis of the relative early formation of the lamina in the primordial stage.

ON CELL MEASUREMENTS

4.1. INTRODUCTION

Since the whole plant is made up of cells, increase in total cell mass is basic to all growth. Therefore, differences in number and size of the constituent cells account for all differences in leaf growth, discussed in the previous chapters. Leaves, in general, have a relatively simple anatomy, nevertheless they still contain a great number of different cell types.

In many studies on leaf growth, cell measurements have been restricted to determination of the surface area of epidermal cells (ASHBY and WANGERMANN, 1950, ARNEY, 1954, MILTHORPE, 1956. Besides the circumstance that epidermal cells can be measured by relatively simple techniques, this procedure seems more or less justified because surface extension is a characteristic feature of leaf growth. On the other hand, estimates of the total number of cells of which a leaf consists have been aimed at by macerating samples of the entire leaf, followed by cell counting with the aid of a haemocytometer (SUNDERLAND, 1960, MILTHORPE and NEWTON, 1963, DALE 1964a,b). Some discrepancy appeared as to the conclusions derived from both methods. Studies on epidermal cells seem to suggest cell division to be restricted to the primordial stage of leaf development, leaf unfolding being due exclusively to cell extension. Results obtained with maceration, however, seem to indicate that cell division continues during a much longer period of growth, notably during leaf unfolding. For tobacco leaves AVERY (1933) found that epidermal cells are the first to stop division which may explain the observed differences.

In the present study, measurements are made on epidermal cells only, *viz.*, of the adaxial leaf surface, although clarification of leaf tissue in 70% alcohol enabled exact measurement of the cross sections of cells of the palisade parenchyma as well, so that correlation of these data with those of the surface area of adjacent epidermal cells was possible (fig. 4.12).

Epidermal cells covering the veins, in particular the midrib, are very different from those in the interveinal areas. On the midrib, they appear in longitudinal rows of more or less elongated cells, usually with tapering end walls, whereas on the lamina of the mature leaf they appear as irregularly shaped cells with strongly undulated anticlinal walls (fig. 4.11), which is characteristic for many dicotelydonous leaves (ESAU, 1962).

When leaves are not too young, the epidermis on the midrib can be easily stripped off and mounted on slides. Under a microscope, cell length then can be measured with an ocular micrometer. Usually, the length of 5 to 10 cells in one row is measured. Thus, at different positions along the midrib, the average length of 100 cells has been determined. Younger leaves and leaf primordia are

mounted intact, usually after having been 'flattened' by cutting off parts of the abaxial side of the midrib.

From the epidermal cells, covering the leaf blade, *'camera-lucida'* drawings were made, and their surfaces determined with a planimeter. Stomata and their small neighbour cells were excluded from the measurements.

4.2. CELL MEASUREMENTS ON MATURE LEAVES GROWN AT DIFFERENT LIGHT AND TEMPERATURE CONDITIONS

4.2.1. *Cells of the midrib*

In fig. 4.1, the outlines of three leaves are shown from plants raised at three different light intensities, *viz.*, from left to right at about 10,000, 20,000 and 40,000 ergs/cm^{2}sec. Along the axis of each leaf, the average cell length in μ has been indicated as measured at that particular section of the midrib. Full data are given in Table I. There appears to be a certain sequence of cell length along the midrib: cells are short at the very base, increase to a maximum length higher up the leaf axis and decrease again towards the leaf tip, as shown in figure 4.2, in which for the three leaves of fig. 4.1, positions on the midrib are indicated in percents of total leaf length. Cell length increases when light intensity decreases, particularly in the lower part of the midrib. Likewise, maximum cell length increases at lower intensities and, moreover, occurs at a relatively lower position on the leaf axis.
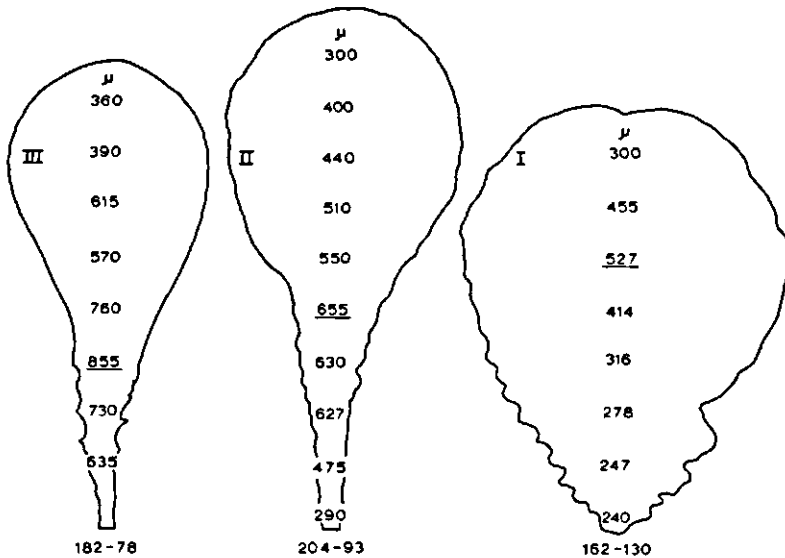

FIG. 4.1. Three leaves grown at different light intensities. I: 40,000, II: 20,000, and III: 10,000 ergs/cm^2 sec. Average length of epidermal cells along the midrib in μ. Bottom: length and width of each leaf in mm.

TABLE 1. Epidermal cell length along the midrib of three leaves of plants grown at different light intensities. Outlines of the leaves shown in fig. 4.1. Graphical presentation in fig. 4.2.

Light intensity I 40,000 ergs/cm²sec.				Light intensity II 20,000 ergs/cm²sec.				Light intensity III 10.000 ergs/cm²sec.			
A mm	B %	C μ	D n	A mm	B %	C μ	D n	A mm	B %	C μ	D n
0–15	3	240	63	0–15	3	290	52	0–15	3	415	75
15–35	16	247	81	15–35	12	475	42	15–35	14	635	32
35–55	28	278	72	35–55	22	627	32	35–55	25	730	27
55–75	40	316	63	55–75	32	630	32	55–75	36	855	23
75–95	53	414	48	75–95	42	655	30	75–95	47	760	26
95–115	65	527	38	95–115	51	550	36	95–115	58	570	35
115–135	77	455	44	115–135	61	510	39	115–135	69	615	33
135–162	90	300	90	135–155	71	440	45	135–155	80	390	51
				155–175	81	400	50	155–182	90	360	75
				175–204	91	300	97				

maximum cell length 527 μ	maximum cell length 655 μ	maximum cell length 855 μ
average cell length 320 μ	average cell length 450 μ	average cell length 540 μ
total number of cells 499	total number of cells 455	total number of cells 388

Column A: Section along the midrib expressed in mm distance from base.
Column B: The same, but expressed as percentage of total leaf length.
Column C: Average cell length in μ per midrib section, data used for the graphs of fig. 4.2.
Column D: Number of cells (n) per longitudinal row determined by dividing average cell length of midrib section (A/C).
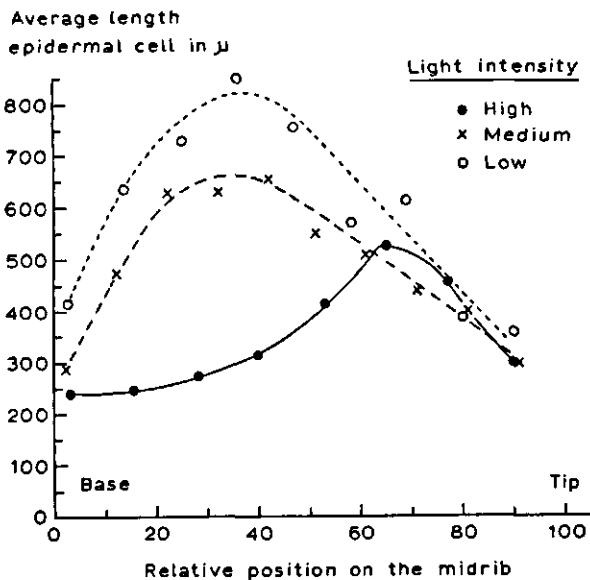

FIG. 4.2. Distribution of average length of epidermal cells along the midrib for the leaves of fig. 4.1.

Towards the tips of the leaves there are no differences in length; cells equally decrease in length at the three light intensities applied.

On the whole, this picture of increased cell length in the lower half of the leaf is consistent with the general finding of an increased extension of the leaf base at reduced light intensities. Conversely, the short cells found at higher intensities agree with an apparent inhibition of midrib extension, which we considered as a primary cause of the crinkling of the leaf lamina along the basic part of the midrib (Plate II).

The number of cells per single longitudinal row may be estimated by dividing the length of the corresponding section of the midrib by the average cell length. Clearly, distribution of cell number along the midrib can be represented by graphs which are the reverse of those in fig. 4.2.

In fig. 4.3, we have summarized the influence of light intensity on leaf length (*a*), number of cells per single row over the whole length of the leaf (*c*), and average cell length (*b*) as found by dividing total leaf length (*a*) by cell number (*c*). Regarding leaf length, the general picture of previous graphs (fig. 3.7) is obtained showing a positive effect at low light intensities changing into a negative one at high intensities, so that at a certain intermediate light intensity leaves reach their maximum length. From curve *c* it appears that cell number increases over the whole range of light intensities, although strongest at low intensity. Average cell length (*b*), on the other hand, shows a continuous decrease with increasing light intensity. Since the length of a leaf, at any time, is determined by the product of cell number and cell length, the course of curve *a* results from those of curves *b* and *c*. It may be concluded, therefore, that at low intensities leaf length is restricted mainly because of a limitation of cell division, whereas the reduction at high intensities should be ascribed to a limitation of cell extension. In this respect, however, it should be noted that HABER and FOARD (1964) have pointed out that differences in cell size may only be used as an indication
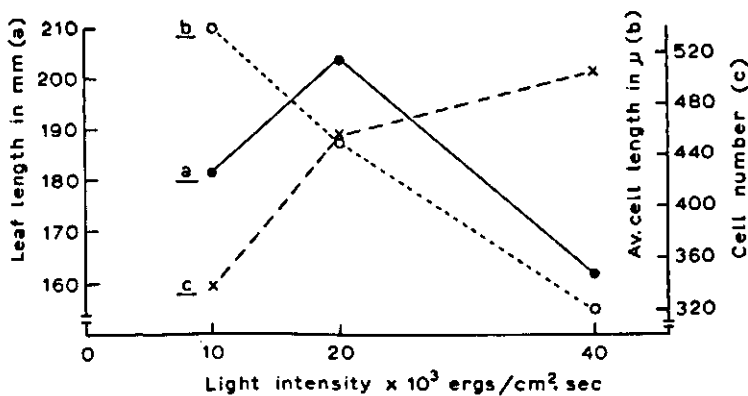

FIG. 4.3. Leaf length in mm (*a*: ●——●), average cell length in μ (*b*: ○——○), and number of cells per longitudinal row (*c*: ×———×), in relation to light intensity. Data of Table 1.

TABLE II. Epidermal cell length along the midrib of leaves of plants grown at a light intensity of 40,000 ergs/cm²sec, at 10° and 30°C. Same data as used for fig. 4.4.

Temp. °C	Midrib section in mm	Midrib section expressed as percentage of total length	Average cell length in μ	Number of cells per longitudinal row
30	0– 20	9	374	54
	20– 40	25	466	43
	40– 60	43	490	41
	60– 80	60	514	39
	80–100	77	380	53
	100–116	90	206	78

Total number of cells : 308
Average cell length : 377 μ

10	0–10	7	169	59
	10–20	21	228	44
	20–30	36	296	34
	30–40	50	280	36
	40–50	64	298	33
	50–60	79	250	40
	60–73	93	164	79

Total number of cells : 325
Average cell length : 225 μ

for enhanced or limited cell extension when the cells do not divide at the same time. As will be demonstrated below, increase in cell number, at least as far as the epidermis is concerned, remains restricted to very early stages of leaf development. After that, leaf expansion proceeds without further increase in cell number, which means that growth increments are entirely due to a proportional increase in cell size. Therefore, short cells at the base of the midrib, evidently, reflect limited extension growth in that part of the midrib, in comparison to parts in which the cells are longer.

In Table II, similar cell measurements are given for two leaves from plants grown at 10°C and 30°C, at the same light intensity of about 40,000 ergs/cm²sec. According to what could be expected, the leaf at 30°C reached a greater length than the one at 10°C. At a cellular level, this can be explained for the greater part from differences in cell length, as cell number of both leaves is almost the same. In fig. 4.4, the distribution of cell length along the midrib of the same two leaves is shown. The general trend is the same as in fig. 4.2, but at 30°C the cells in all parts are longer. In fig. 4.3 it was shown that light had an effect on both cell length and cell number. It may be asked, whether the fact that the cell number is the same in the leaves of Table II is due to the circumstance that these leaves were grown at the same light intensity. It is to be mentioned that they both have produced fewer cells than those at the lowest intensity shown in

FIG. 4.4. Distribution of average cell length along the midrib for leaves of plants grown at 10°C, and at 30°C Light intensity 40,000 ergs/cm²sec.

Average length epidermal cell in μ

- 30 °C
- × 10 °C

Base

Tip

Relative position on the midrib
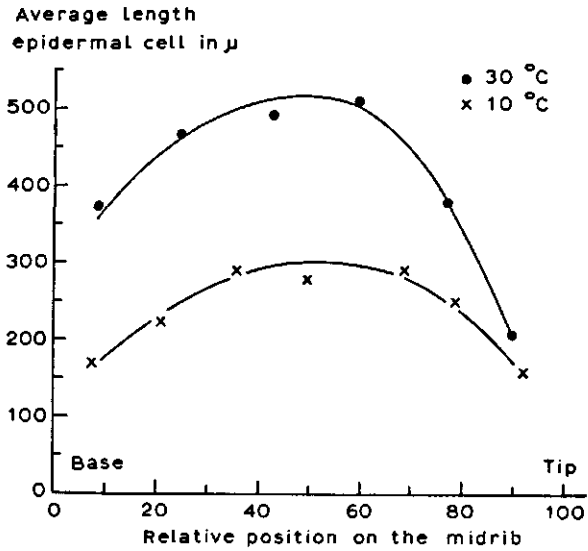
TABLE III Leaf length and total number of epidermal cells in the midrib per single longitudinal row. Leaves of plants grown at different light intensities at 10°C. and 30°C.

Light intensity ergs/cm²sec	Temperature °C	Leaf length mm	Total number of cell per single longitudinal row
40,000	30	139	303
		165	323
		116	373
		47	431
25,000	30	90	221
		94	298
		95	237
		63	260
		41	229
40,000	10	70	307
		70	325
		50	331
		36	345
		33	327

Table I, and also leaf lengths reached are smaller. However, in general, we found that leaves of different experiments are difficult to compare in absolute values. Therefore, in Table III, cell numbers are given for leaves taken from plants grown in a single experiment, at high and low light intensity, both at 10° and 30°C. These results also suggest that cell number depends more on the light

intensity than on the temperature at which the leaves were grown. Not all leaves had reached their final length at the time of cell measurement. That they, nevertheless, did not greatly differ in cell number, suggests that all had passed the stage of cell division.

It seems, therefore, that cell number, i.e. the activity of cell division, in the first place depends on the intensity of the light the plant receives, and is not much affected by temperature, whereas cell length is influenced by both temperature and light. Hence, the effect of temperature on final growth cannot be considered apart from the prevailing light intensity, as has already been demonstrated previously in Chapter 3.

4.2.2. *Cells of the leaf blade*

To investigate the distribution of cell size over the leaf surface, cells were measured at 47 places along two strips, one parallel to the leaf axis, the other at right angles to that, in the area of greatest leaf width. Results shown in fig. 4.5 suggest that cell size varies only little over the entire leaf. A clear difference appears only between the average cell size in the left and right halves of the leaf. This difference, when found, is usually associated with unequal dimensions of the two leaf halves. Yet, a general conclusion may be that differences in width in different parts of a leaf are due to differences in cell number rather than in cell size. In order to estimate the number of cells present in distinct parts of a leaf, leaf width was divided by the square root of the average cell area, as determined in that part of the leaf. The square root of cell area has been taken as the average linear cell dimension, since the cells were either isodiametric, or, when oblong in shape, their longitudinal axes were in all possible directions. Only in the vicinity of large veins they were elongated in the direction of the vein. In fig. 4.6, the outline of a leaf is shown, the left half drawn according to a mm scale, the right
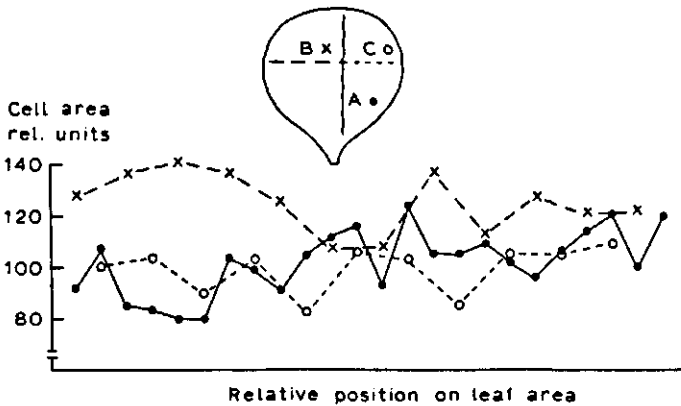
FIG. 4.5. Distribution of average surface area of epidermal cells over the leaf blade. Measurements at 24 places along A (●———●), at 12 places along B (×——×), and at 11 places along C (○———○), as indicated.

FIG. 4.6. Outline of a leaf. Left half drawn according to a scale in mm, right half according to one indicating number of cells.
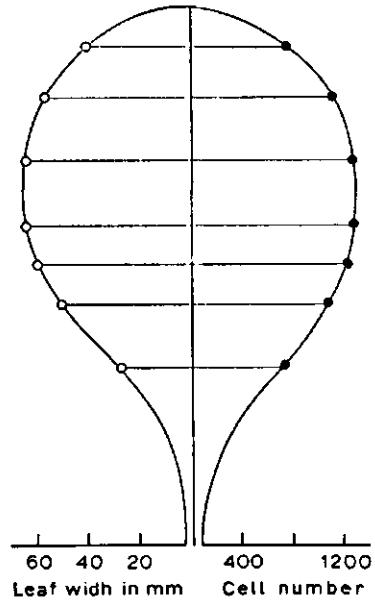
half according to cell number at the different positions in the leaf. The two leaf halves thus drawn match nicely, indicating that leaf width varies mainly by difference in cell number. Below, data on cell size and cell number always refer to the area of greatest leaf width. The average cell area was derived from four to six drawings of cell groups in either leaf half.

In fig. 4.7, leaves of plants grown at three different light intensities are compared for leaf width (a), cell size (b), and cell number (c), representing averages of three leaves.

FIG. 4.7. Leaf width in mm (a: ●———●), average surface area of epidermal cells in μ² (b: ○———○), and number of cells (c: ×——— ×) in relation to light intensity.

Leaf width increases with increasing light intensity (fig. 3.10), this increase is almost directly proportional to increase in cell number, while cell size differs only little for the three light intensities. In this respect the midrib is different (fig. 4.3), for which there 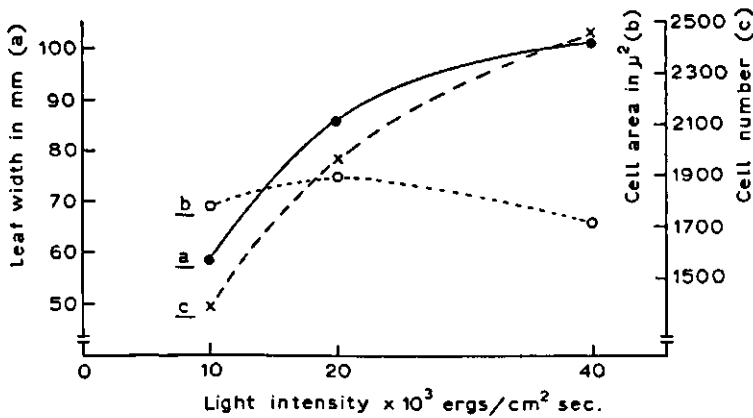is a positive effect of light intensity also on cell number, with a simultaneous decrease in cell length, resulting in a decrease in leaf length with increasing light intensity.

In Tables IV and V, cell measurements are given for the area of maximum width in leaves of plants grown at 10° and 30°C and at three different light intensities. Table V contains average values of Table IV expressed in percents either of those reached at the highest intensity, or of those reached at 10°C. As already demonstrated in chapter 3, leaves become wider at 30°C than at 10°C, except for very low light intensities when the reverse is true. On the whole, differences due to differences in light intensity are more pronounced at 30° than at 10°C. Thus, the average decrease in width between the highest and the lowest intensity, amounts to 63% (from 210 to 78 mm) at 30°C, but only to 34% (from 155 to 102 mm) at 10°C. The average cell surfaces at all light intensities are greater at 30° than at 10°C. Cell number increases with increasing light intensity at both temperatures. There is hardly any difference between the two tempe-

TABLE IV. Greatest leaf width, average surface area of the epidermal cells, and number of cells. The latter calculated by dividing leaf width by the square root of the average cell area. Data for leaves of plants grown at different light intensities at 10° and 30°C.

Light intensity ergs/cm²sec	Temperature °C	Leaf width mm	Cell area μ^2	Number of cells
100,000	30	210	3427	3680
		206	3358	3550
22,000	30	127	2300	2650
		138	2310	2870
		156	3360	2690
11,000	30	84	2630	1640
		68	2270	1430
		82	3660	1350
85,000	10	150	1760	3570
		159	2300	3320
		156	2170	3350
25,000	10	113	1690	2750
		110	1640	2720
		109	1590	2740
11,000	10	85	1990	1900
		77	2345	1590
		86	1610	2130
		129	2335	2680
		132	3025	2400

TABLE V. Average values taken from Table IV, expressed as percentage either of the values at the highest light intensity, or of those at the lowest temperature.

Light intensity ergs/cm²sec	Leaf width		Cell area		Number of cells	
	10°C	30°C	10°C	30°C	10°C	30°C
85–100,000	100	100	100	100	100	100
22– 25,000	72	67	79	78	81	76
11,000	66	37	99	83	62	41
85–100,000	100	135	100	164	100	106
22– 25,000	100	155	100	162	100	100
11,000	100	78	100	137	100	69

ratures at the two higher light intensites, but at the lowest intensity cell number is greater at 10°C than at 30°C which, in particular, accounts for the greater leaf width reached at this light intensity and 10°C, the more so since cell size at 10°C is smaller.

Table VI shows data of leaves of plants grown at a larger series of temperatures and one light intensity only, in the range of the highest one of Table V. There is again a clear-cut positive effect of temperature on cell size, with a corresponding increase in leaf width. Cell number varies to a much smaller extent, although, at 30°C, it seems clearly increased. Yet, on the whole, it may be concluded that cell size is more affected by temperature than cell number, the latter depending more on light intensity.

TABLE VI. Greatest leaf width, average surface area of the epidermal cells, and number of cells. The latter, calculated by dividing leaf width by the square root of the average cell area. Data for leaves of plants grown at a light intensity of about 80,000 ergs/cm²sec, and at different temperatures.

Temperature °C	Leaf width mm	Average cell area μ²	Numbers of cells
10	120	1780	2850
10	100	1630	2480
10	130	1860	3000
15	155	2800	2950
15	147	2400	3000
20	173	2870	3240
25	185	3890	2970
30	240	3800	3900
30	230	3600	3830

4.3. Cell measurements in growing leaves at different light and temperature conditions

Cell measurements, reported so far, have been made on full grown leaves. Before discussing measurements made during leaf expansion, and starting at an early stage of development, in fig. 4.8 a few successive stages of primordial leaf growth are shown in diagram. In early developmental morphology, lettuce leaves agree in broad lines with the description given by AVERY (1933) for tobacco leaves, an example which has also been used by ESAU (1953) to illustrate primordial development of most single dicotelydonous leaves: 'After the initiation of a 'leaf buttres' near the top of the apical dome, this grows out to an erect like protuberance' (a in fig. 4.8) 'often somewhat flattened on the adaxial side. This protuberance is the axis of the young leaf. It may be regarded as consisting of the midrib-petiole part of the primordium, bearing the meristematic initials of the future lamina. This lamina is initiated in the early stages of elongation of the leaf axis from two strips of meristematic cells, located along two margins of the leaf axis' (b in fig. 4.8) 'and called the marginal meristems' (ESAU, lc., pp. 444–445).
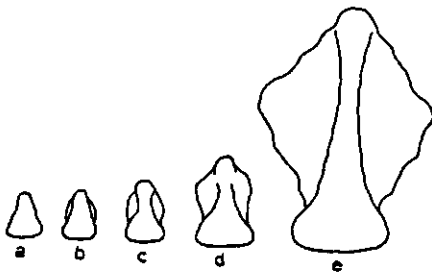

FIG. 4.8. Diagrams of successive stages of primordial leaf development.
a: initial outgrowth of leaf axis and future midrib;
b: development of two marginal meristems initiating the lamina;
c—e: further outgrowth of midrib and leaf blade.
(Different stages on different scales, a is about 0.5 mm, e about 5 mm long).

Since the activity of these leaf blade meristems starts at about 1/3 from the tip of the leaf and gradually extends along the whole axis, the primordium obtains the well known arrow-like shape (fig. 4.8,c,d).

During the course of further expansion ample opportunity exists to modify the ultimate leaf shape. In the case of lettuce the separate study of the elongation of the leaf axis, i.e., the midrib, and of the expansion of the leaf blade, is of particular interest.

4.3.1. Midrib elongation

For plants, grown at a high and a low light intensity respectively, increase in leaf length has been correlated with the average length of the epidermal cells on the midrib (fig. 4.9). Each point represents a single primordium or young leaf, taken from four plants, two at either light intensity.

The slope of the line which connects each point with the origin represents the number of cells in a single row along the midrib. At first, growth of the leaf axis is accompanied by a strong increase of cell number; however, already at a relati-
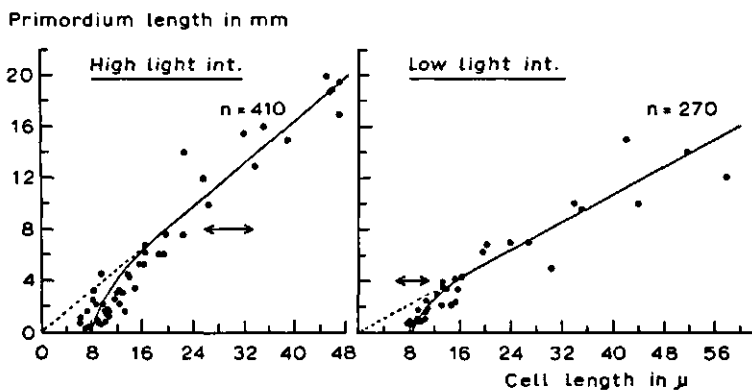
Primordium length in mm

FIG. 4.9. Relation between increasing length of subsequent leaf primordia on the plant and average length of the epidermal cells in the midrib, for plants grown at a high light intensity (40,000 ergs/cm² sec) and at a low one (10,000 ergs/cm² sec). Slopes of the linear relationships represent number of cells (n) per longitudinal row. Arrows indicate presumed end of cell division.

vely early stage, a constant linear relationship is found between increase in leaf length and increase in cell length, indicating that growth occurs without further increase in cell number. The steeper slope at the higher light intensity appears consistent with previous findings of greater cell numbers at higher intensities. In this case, the slope results from the measurement of 270 cells at the low light intensity and 410 cells at the higher one, because at the high intensity cell division, evidently, continues during growth of the first 8 mm, whereas, at the lower intensity, cell multiplication already stops at a length of 4 mm (see arrows in fig. 4.9). Thus, the effects of light intensity on cell number are already determined at an early stage of leaf development. Therefore, a change in light intensity will have no effect on cell number in leaves larger than around 1 cm. However, light intensity remains important for the degree of cell extension, as shown by its effect on final cell length (fig. 4.2).

When subsequent leaf lengths of a plant are measured, including as many primordia as possible, it appears that they show a series of exponentially increasing values yielding a straight line on a logarithmic scale. An example is given in fig. 4.10. Primordia usually were not measured when smaller than 7 mm, although they were counted, including the most recently initiated leaf buttres at the apical dome. Assuming the same exponential relationship to hold for the lengths of these very small primordia, a linear extrapolation of the curve of fig. 4.10 leads to an estimate of $30-50 \mu$ for the length of the youngest primordium which seems a reasonable approximation of the actual length, so that the exponential relationship between the length of successive leaves is likely to hold from the very first leaf onwards.

It should be noted that the slope of the straight line in fig. 4.10, depends on the number of leaves present in a certain size interval, i.e., on the rate at which leaves
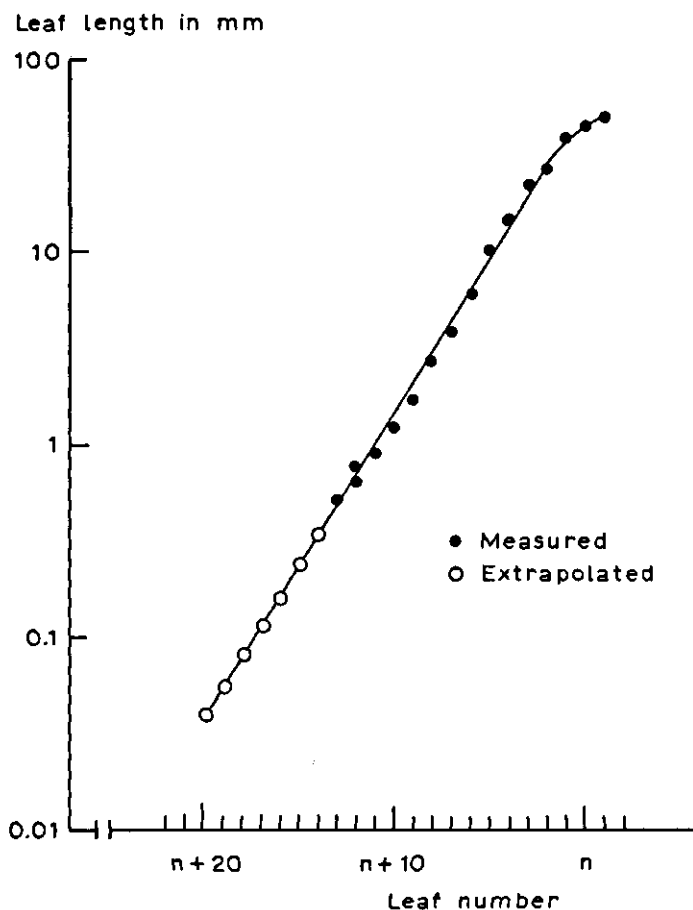
FIG. 4.10. Log length of subsequent leaves and leaf primordia up to the last visible leaf buttres in relation to leaf number. Leaves numbered stem upwards from right to left. Closed circles (●) actual measurements, open ones (○) values estimated by extrapolation.

are produced. Therefore, for plants grown at high light intensity or at high temperature, the slope of these lines will be flatter than for plants grown at low intensity or low temperature, owing to higher or lower leaf production rates under the various conditions.

When, besides leaf length, also average cell length is plotted logarithmically against leaf succession, graphs like that in fig. 4.11 are obtained. Now, the period of apparent meristematic growth (cell multiplication) appears as the more or less horizontal initial part of the line, whereas the straight increase, proportional to that of increase in leaf length, indicates the phase of predominant cell extension. The special interest of this presentation is in the fact that the conspicuous nick in the course of the curves of cell length, which marks the transition between the

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Fig. 4.11. Similar data as presented in fig. 4.10 for two plants (●) and corresponding average length of epidermal cells (○) in the midrib.
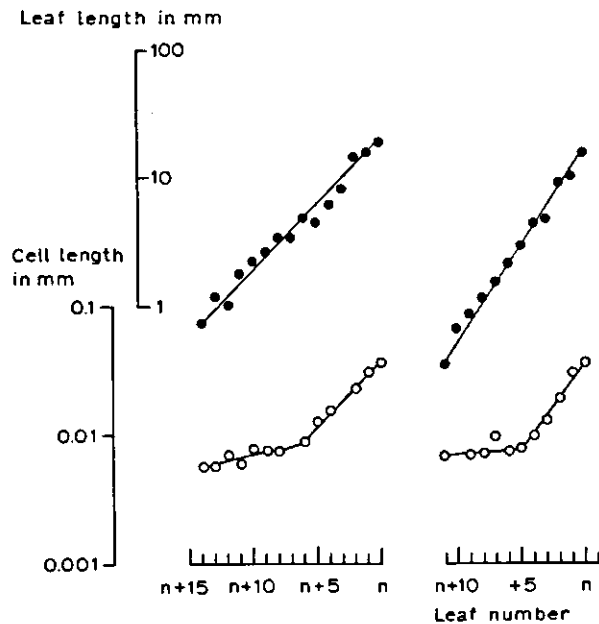
presence and the absence of cell division, is not reflected in the course of leaf length. It may be concluded that growth of the midrib as such is not affected by the occurrence of cell division. Or, extension of meristematic cells including mitoses, occurs at the same rate as extension of cells which do not divide further. SINNOTT (1960, p. 32, fig. 3.7) gives an example of the relation of cell division and cell enlargement in the growth of ovaries of *Cucurbita* fruits which is essentially the same as fig. 4.11.

In literature and textbooks, growth *by* cell division and *by* cell enlargement are usually referred to as two separate phases of growth. HABER and FOARD (1964), however, made it clear that such a distinction is incorrect, since in both cases cell extension is the primary cause of measurable growth. They state that, instead, it should be preferred to distinguish between growth *with* and *without* concurrent cell division. From results obtained with gamma-irradiated wheat seedlings, they concluded that concurrent cell division was not essential in determining the rate of leaf elongation. The same conclusion can be drawn also from SINNOTT's fig. 3.7 and from our fig. 4.11. A further demonstration of the apparent lack of relation between growth and cell division can be found in the way gibberellin affects the curves of fig. 4.11, as will be shown in chapter 5.

4.3.2. *Leaf blade expansion*

In fig. 4.12, drawings of epidermal cells of the leaf blade are presented, made at different moments during the growth of the 12th leaf, the corresponding leaf width being indicated by bars. Initially, the cells are small, rectangular and clearly meristematic. When the cells extend, their anticlinal walls become undu-
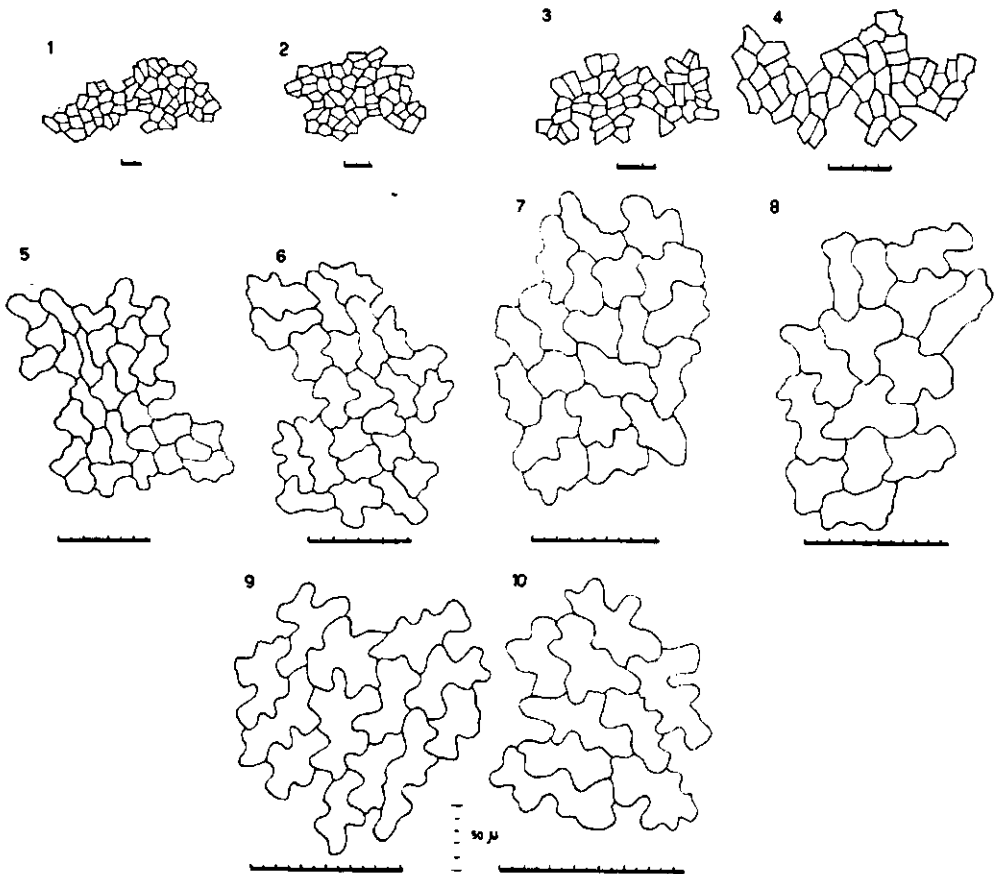
FIG. 4.12. *Camera-lucida* drawings of epidermal cells of the leaf blade, for leaves of different age. Corresponding leaf widths indicated by bars under each drawing; scale unit of bars 0.5 cm. Cell sizes, cf. 50 μ scale.

lated. In all leaf samples, the underlying palisade cells were visible and round in cross section. By measuring their diameter, the growth of these cells could be followed as well, and compared with the surface areas of the epidermal cells. In all cases a linear relation between the size of both cell types was found (fig. 4.13). It seems likely, therefore, that the sequence measured in cells of the epidermis to some extent also holds for other cells of the leaf.

Fig. 4.14 shows the relationship between leaf width and the square root of the average surface area of epidermal cells, as measured in the midst of the lamina and at the greatest leaf width. This relation is essentially the same as the one shown earlier for the midrib in fig. 4.10. The vertically ascending line in the beginning marks the period of meristematic growth, in which the average cell size does not increase and increase in leaf width, consequently, goes along with an increase in cell number. Although cell multiplication continues during a

FIG. 4.13. Increase of epidermal cell area related to increase in cross section of underlying cells of the palisade parenchyma.

Cross section palisade cells in μ^2

Epidermal cell area in μ^2
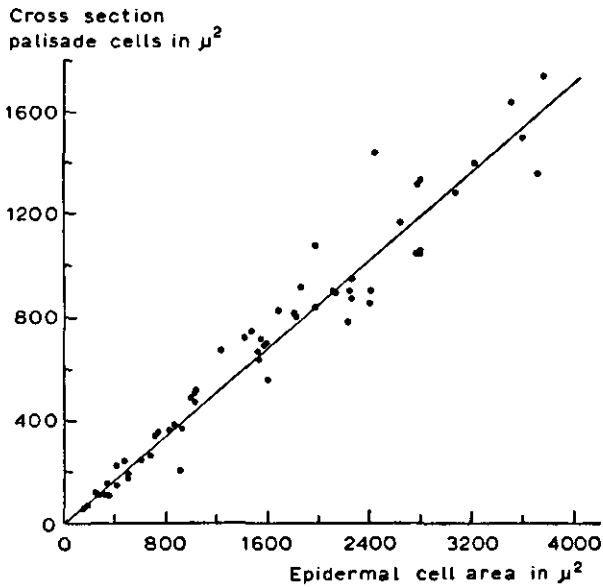
longer period than in the case of the midrib, evidently also in the leaf blade cell division remains restricted to a relatively early stage.

It is clear that in fig. 4.14, the final width reached is represented by the slope of the line and its length, thus, by the number of cells produced at the primordial stage, and the ultimate size the cells reach. As it was found earlier (fig. 4.6) that cell number and leaf width are greatly influenced by light intensity, it is to

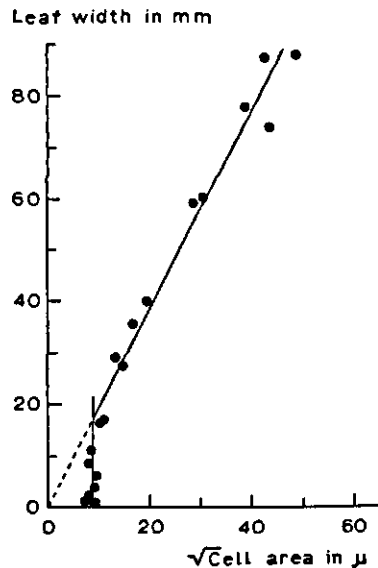Leaf width in mm

$\sqrt{C}$ell area in μ

FIG. 4.14. Relation between leaf width and epidermal cell size as determined during growth of a leaf (leaf 12). Cell size expressed as the square root of the average surface area of the cells. Slope of the linear relationship represents number of cells per single row.
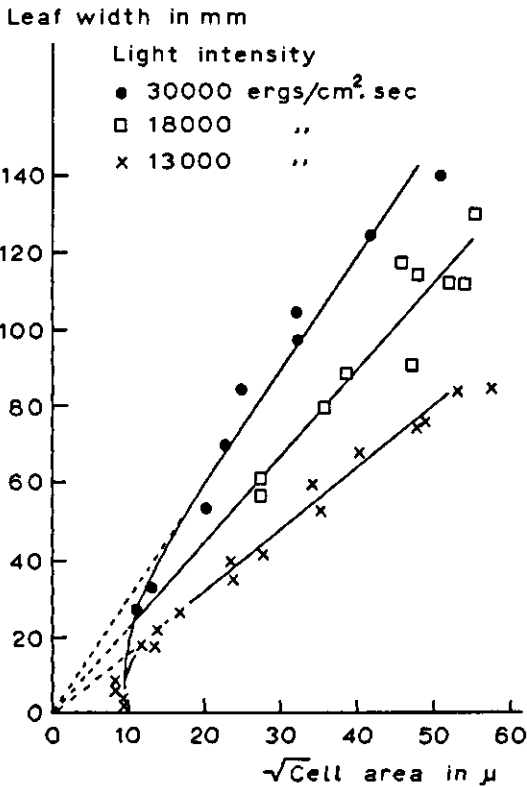
Leaf width in mm

Light intensity

- ● 30000 ergs/cm². sec
- □ 18000 ,,
- × 13000 ,,

√Cell area in μ

be expected that, in fig. 4.14, the slope will depend on light intensity. Fig. 4.15 indeed shows that steeper slopes are obtained with increase in light intensity which means a greater number of cells because cell division continues for a longer period. It is also clear that the increased cell number accounts for the greater leaf width at higher light intensities, since final cell size usually does not show large differences between different light intensities. Where there is a difference, cell number seems to be negatively correlated with cell size.

Fig. 4.16 shows an example in which of three light intensities applied, no difference in final leaf width was found between the two higher ones, notwithstanding the fact that, at the higher one, a greater number of cells had been produced than at the lower one. However, at the highest intensity the cells remained smaller. By extrapolation, it can easilly be seen that about 25 % greater leaf width would have resulted if cell extension at the highest intensity had continued up to the size reached at the lower intensities. It is of interest that this apparent restriction of cell extension could be annihilated when, instead of 12 hours continuous light at a high intensity, the daily light period was given as 2 hours low, 8 hours high, and 2 hours low intensity. In fig. 4.17 it is shown that in this way the extrapolated cell extension envisaged in fig. 4.16 indeed could be reached
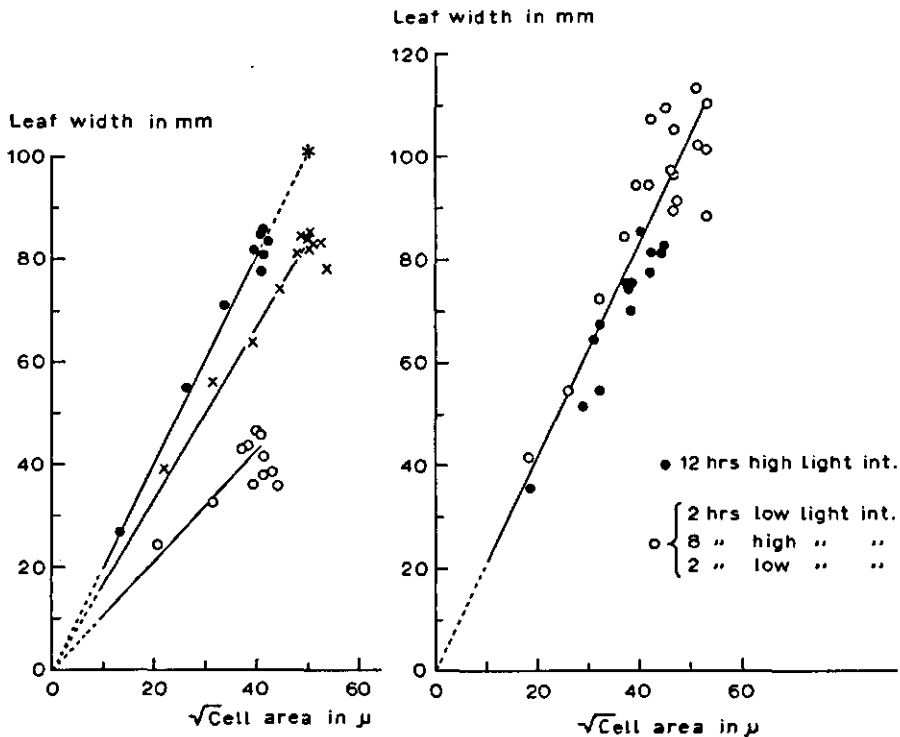
Leaf width in mm

FIG. 4.16. (left). Same as in fig. 4.14, for light intensities of 50,000 (●), 30,000 (×), and 10,000 ergs/cm²sec (○). Curve of the highest light intensity extrapolated to same cell size (*) as reached at the medium light intensity.

FIG. 4.17. (right). Relation between leaf width and epidermal cell size for leaves growing at two different light regimes as indicated.

experimentally. These effects of light intensity distribution are not necessarily direct light effects on growth processes; it is quite conceivable that secondary effects, e.g. the induction of too great a water-stress in expanding leaf tissue during an uninterrupted period of high light intensity interferes with the requirements for optimal cell extension (SCHWABE, 1956). Another possibility is that at high light intensity a hormonal factor, important for cell expansion, acts as a limiting factor.

The foregoing has made it sufficiently clear that cell number is the main determinant of differences in leaf width induced by differences in light intensity. The first 12 to 15 leaves of a plant also show, as we saw earlier (fig. 1.1) increasing leaf widths, and we may ask whether also here increase in cell number is the decisive factor. In fig. 4.18, the relationship between leaf width and epidermal cell size is shown for 27 successive leaves of the same plant. The leaves are numbered stem upwards, so that leaf 27 represents the youngest leaf measured. In fig. 4.18, a straight line fits the points representing the leaves 27 to 12 like the
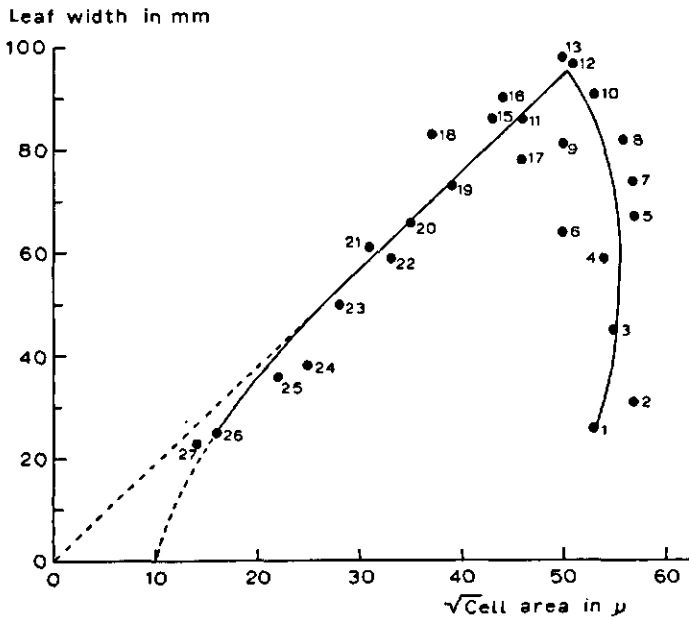
Leaf width in mm

FIG. 4.18. Relation between leaf width and epidermal cell size for 27 successive leaves of a plant, numbered from old to young.
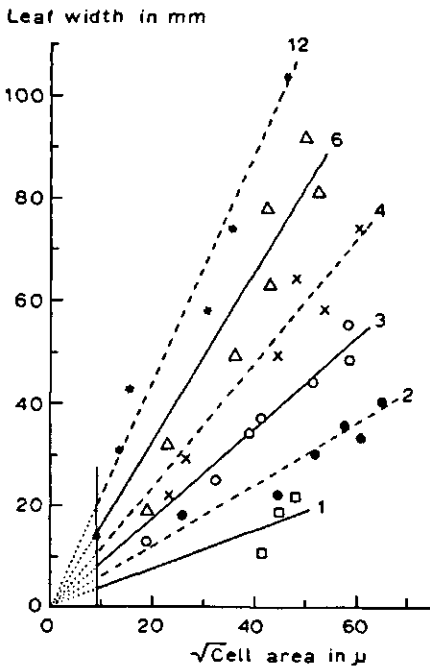
Leaf width in mm

FIG. 4.19. Relation between leaf width and epidermal cell size as measured during growth of leaf number 1 (□), 2 (●), 3 (○), 4 (×), 6 (△), and 12 (*).

linear leaf width-cell size relation found for the growth of a single leaf (fig. 4.14). The slope of the line, again, represents the number of cells and, according to the position of the different leaves, we may conclude that cell number remains constant in leaves wider than ca. 2 cm, whereas, leaves narrower than 2 cm still comprise dividing cells. It seems most likely, therefore, that from leaf 12 onwards all leaves follow the same growth characteristic. The first 12 leaves, show successively increased leaf width in their full-grown stage, however, having all cells of the same size. Thus, it is clear that here increase in leaf width is due to increase in cell number. Likewise, we can expect that these first leaves during their expansion will also follow straight lines, only with a flatter slope than that for the leaves 12 to 27, and the flatter, the lower the serial number of the leaf is.

This has been checked by measuring cell size during growth of the first 13 leaves of a plant. The result (fig. 4.19), shows a series of straight lines, with increasing slopes representing subsequent leaves, the same as occurs for leaves of a specific number at a series of light intensities (cf. fig. 4.15). Evidently, cell numbers at the primordial stage increase for subsequent leaves in the young plant. This continues for a number of leaves until at a certain stage of development of the plant, a constant cell number is reached for all further leaves. In fig. 4.18 this clearly is so from leaf 12 onwards. The final number of cells depends on light intensity.

Fig. 4.20 presents the final cell number as reached by the first 13 leaves of plants grown at three different light intensities.

In these leaves which never reach the maximum cell number, cell size increases with increasing light intensity, as well as the final number of cells. It should be noticed that this experiment started with young plants which already possessed 3 or 4 leaves; experiments should rather have started at the stage of seed emergence.
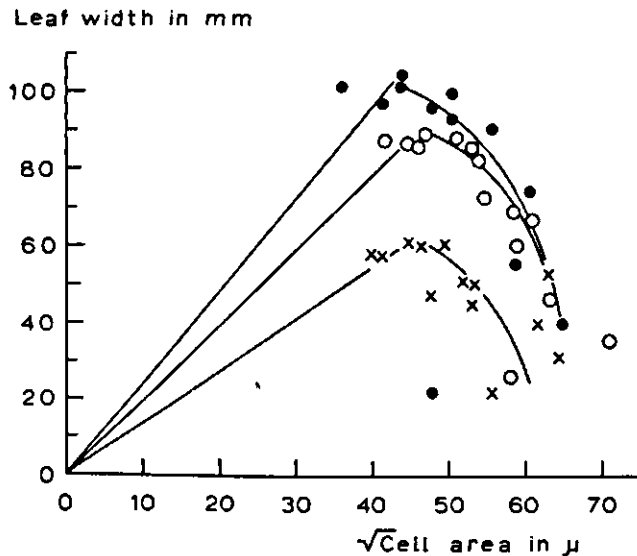

Leaf width in mm

FIG. 4.20. Relation between leaf width and epidermal cell size as measured for the first 13 leaves of plants grown at light intensities of 45,000 (●), 22,000 (○), and 11,000 ergs/cm² sec (×) respectively.

√Cell area in µ

4.4. CONCLUSIONS

1. Measured on epidermal cells, a distinctly positive relation exists between light intensity and cell number, both for midrib and leaf blade.
2. Cell multiplication remains restricted to an early, primordial stage of leaf development. Although the collected data refer exclusively to epidermal cells, a close correlation was found between extension of epidermal cells, and that of the underlying palisade cells.
3. From a size of ca. 2 cm onwards, leaf expansion is the result of cell enlargement, whereas, in connection with the previous conclusions, final leaf size also depends on the number of cells.
4. Leaf length mainly depends on cell extension in the midrib. Cell extension in the midrib is different at different positions along the leaf axis. Maximum cell length occurs somewhere half-way along the leaf axis, decreasing both basi- and acropetally. The pattern of cell length distribution, however, depends strongly on the prevailing light intensity. In particular, at high intensities, cell extension at the base of the midrib appears restricted. The average cell length shows a continuous decrease with increasing light intensity. The relation between leaf length and light intensity, as demonstrated in Chapter 3, can be understood from the positive influence of light intensity on cell number and its negative effect on cell length.
5. The successive leaves of one plant, as long as they still grow, show exponentially increasing lengths, including the very young initials at the apex. Cell length, however, shows a clear-cut distinction between a period of none or little average increase, covering the stage of cell multiplication, and a subsequent period in which average cell length and total leaf length increase almost proportionally, indicating that cell division has stopped. Together, both curves confirm earlier findings of SINNOTT and conclusions of HABER and FOARD, who state that cell division is not necessarily reflected in the rate of growth. It follows that, at least in this material, dividing and non-dividing cells extend at the same rate.
6. In contrast to the midrib, epidermal cell size on the leaf blade, in general, is not much affected by light intensity. Therefore, observed differences in leaf width owing to different light conditions result from differences in cell number.
7. Increasing widths in the first leaves of a plant also are clearly correlated with an increase in cell number. The obvious analogy with the effects of light intensity on leaf width, respectively cell number, may be ascribed to the circumstance that in both cases the photosynthetic capacity of the plant is increased, either by more light or by the gradually increasing total leaf area of the young plant. This implies a relation between the supply of photosynthates and the degree of cell division at the primordial stage of the leaf.
8. There are indications that continuous exposure to a high light intensity during the entire photoperiod hampers optimal cell extension of the leaf blade. As has been shown, this could be overcome by giving two hours of low intensity at the beginning and at the end of the daily light period (artificial dawn and twilight). These effects should be further examined since one may well suppose

that they express a shift of balance between factors of energetic and hormonal control of leaf growth. On the other hand, the possibility that the observed effects might be artefacts, due to experimental conditions which result in sub-optimal growth responses, cannot be excluded. In this respect the water balance in the leaves should be seriously taken into consideration.

ADDITIONAL EXPERIMENTS

5.1. INTRODUCTION

In this Chapter, effects on leaf growth are discussed, produced by means other, than variation in light and temperature conditions. These experiments still are on a rather small scale, and partly of a provisional character. They were designed mainly to interfere with the energy and hormonal balance of the plant. Thus, effects were investigated of partial defoliation (5.2), increase in CO_2 concentration (5.3), external supply of sugar (5.4) and application of gibberellin (5.5). The same sort of data were collected as in the previous chapters.

5.2. EFFECTS OF DEFOLIATION

In literature, defoliation has been used as a method to investigate possible influences which mature and not quite mature leaves may have on the growth of younger ones. These influences are supposed to operate through supply or withdrawal of substances controlling cell division and/or cell extension. Defoliation, therefore, may cause a temporary enhancement or reduction of the growth of young leaves. Defoliation experiments are difficult to interpret since little is known about the mode of action and interaction of the substances concerned (photosynthates, auxins, gibberellins, kinetins, etc.), the sites at which they are produced, how they are transported, and where they are used (ASHBY, 1948b, MORTON and WATSON, 1948, ARNEY, 1955).

In our experiments, old and young leaves were removed in variable numbers from either young or old plants. Growth in length and width of the remaining leaves were measured thereafter. On the whole, clear-cut effects are obtained only when defoliation causes a drastic reduction of the photosynthetic capacity of the plant, i.e., when more or less full grown leaves are removed. Removal of a number of not yet unfolded leaves, on the other hand, had no significant inhibitory or promotive effect on the growth of subsequent leaves. In some cases, when very young leaves were cut away, growth disturbances occurred which could be identified as secondary effects caused by latex, released from the wounds made. Latex is known to induce this kind of effects (TIBBITS et al., 1965).

In case relatively young plants are partly defoliated, the pattern of leaf growth clearly is set back temporarily to that of an earlier stage. This, e.g., is demonstrated by the three graphs of fig. 5.1. Removal of leaves 6 to 12 at the time leaf 15 has reached a length of 1 cm, causes the normal trend of decreasing L/W for the first formed leaves (cf. fig. 1.1), to be interrupted by a temporary increase. After a few leaves L/W starts to decrease again to the same level of that of untreated

FIG. 5.1. Effect of defoliation. Leaves 6 to 12 removed at the moment leaf 15 had a length of 1 cm, as indicated by hatched areas. Effects measured two weeks after defoliation.

A: Length width ratio (L/W) in relation to leaf succession for a defoliated plant (○) and a non-defoliated plant (×).

B: Length of successive leaves of the same plants.

C: Width of successive leaves.

Arrows in B and C indicate outgrowth of leaves 13, 14 and 15 measured also at the moment when leaves 6 to 12 were removed (lower curve).
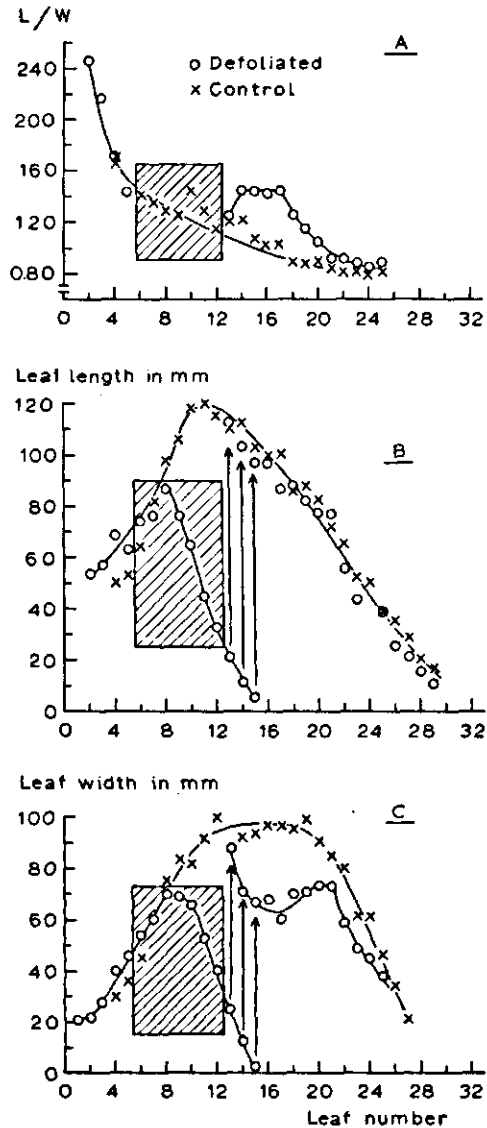
plants. Thus, the effect of defoliation may be characterized as causing a temporary regression of leaf development.

According to the graphs presented in B and C, leaf width development is especially reduced while leaf length is rather unaffected. Decrease in leaf width is associated with decrease in cell number, as determinted in the way discussed in Chapter 4. An example of this is given in fig 5.2. In A it is shown again how removal of the first 12 leaves causes a temporary decrease in width of subsequent ones, in B the course of cell number for the same sequence of leaves is presented.
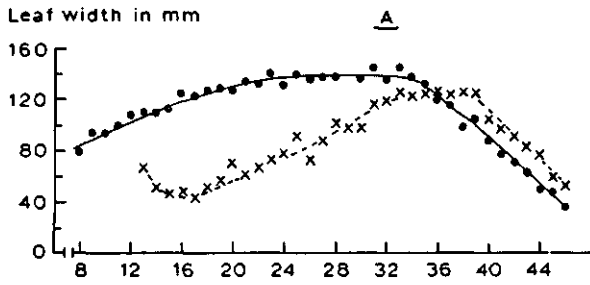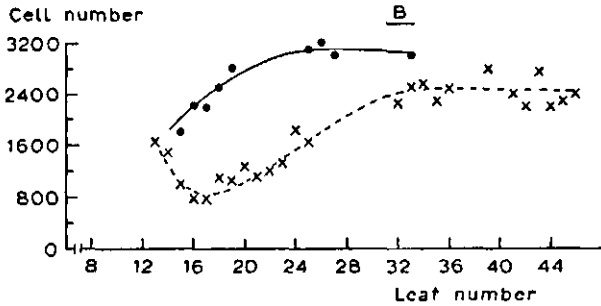
FIG. 5.2. A: Effect of defoliation on the width of subsequent leaves (×), as compared to leaf width of a non-defoliated plant (●).
B: Effect on cell number of corresponding leaves.

The close resemblance of the graphs A and B is striking. Since it has been shown previously (fig. 4.14) that cell number is determined at an early meristematic stage, it seems clear that the effect of defoliation upon the leaves left on the plant will depend on the extent to which these leaves have completed meristematic growth at the moment of defoliation. This may explain the gradual decrease in width, and in cell number, of the leaves 13 to 17, in fig. 5.1 and 5.2. As soon as the plant has again developed a sufficiently large total leaf area, cell division activity in the primordial leaves, apparently, is restored, causing an increase in leaf width again.

Fig. 5.3 shows that completely similar results are obtained, when the prevailing light intensity suddenly drops, followed by gradual restoration to the original level in the course of, e.g., two weeks.

Thus, the results of defoliation are quite consistent with ideas advanced earlier, *viz.*, that the close relationship between leaf width development and light intensity, basically represents a close relationship between energy supply to the plant and cell division activity.

5.3. EFFECTS OF EXTRA CO_2 SUPPLY

A method to increase photosynthesis, growth and production of plants without changing the light regime, under certain conditions is to grow them at CO_2 concentrations higher than that of normal air. In particular for glasshouse crops,

FIG. 5.3. Effects of a sudden decrease in light intensity followed by gradual increase to the original level (×), as compared with constant light intensity (●).
A: Effect on subsequent leaf width development.
B: Effect on cell number.
C: Diagram of light intensity-time relation applied.
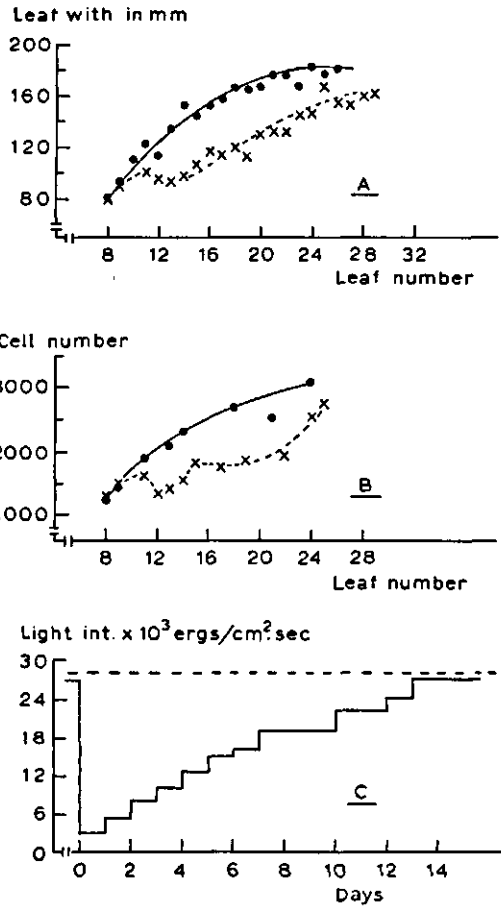
like lettuce, much research has already been done on the practical and economical implications of extra CO_2 supply (VAN BERKEL, 1964, DULLFORCE, 1965, HARTMAN, 1966, HEATH et al., 1967).

In most cases, faster growth and greater yields are reported with extra CO_2. In accordance with what has been mentioned before, we were particularly interested in effects CO_2 might have on leaf development, as compared to the effects induced by light.

So far, two experiments on a limited scale have been made. In one of these, extra CO_2 (1000 ppm) was given continuously, during day and night. In the other one, CO_2 was applied either during the dark or the light period only. During the dark, CO_2 appeared to have no measurable effect on leaf and plant growth, whereas, when given during the light period, the effects were similar to those obtained when CO_2 was supplied continuously. Consequently, it seems quite clear that the extra CO_2 is mediated through a light effect, and most probably through enhancement of photosynthesis. A CO_2-effect is noticed even under
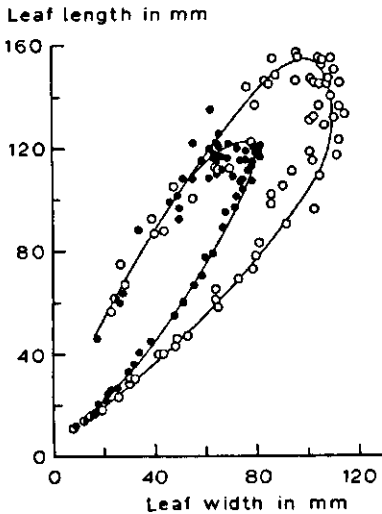
Leaf length in mm

FIG. 5.4. Length-width diagrams for leaves of plants grown in normal air (●) and in air with 0.1 % CO_2 (○).

Leaf width in mm

conditions of apparent light limitation. We will not enter in any detail into this matter here, but only remark that the explanation for this phenomenon no doubt is related to the relatively complicated structure of the lettuce plant as used in horticultural practice, and the smooth segregation between limitation and light saturation of photosynthesis which is the consequence of this complex structure.

Plants, grown at 20,000 ergs/cm²sec and 1000 ppm CO_2 developed as if they grew in normal air but at a higher light intensity. Leaf production was higher, and in particular, larger leaves developed. This is demonstrated in fig. 5.4, showing a length-width diagram for the leaves of two plants grown either in normal air or with extra CO_2. The difference between the two curves is quite similar to that found between a low and a high light intensity.

The greater leaves obtained with extra CO_2 are due to a greater number of cells both for midrib and leaf blade. In fig. 5.5, the increase in width of leaves grown in normal air or at 1000 ppm CO_2 has been correlated with cell size. Since the slopes of the straight lines represent the number of cells formed at the primordial stage, it follows that the extra CO_2, apparently, has increased cell division. The difference is the same as that found between a high and a low light intensity (fig. 4.14), so that it may be concluded that the extra CO_2 has had the same effect on cell number as an increase in light intensity. Obviously, the increased leaf area as such will have a favourable effect on the growth of subsequent leaves, so that the ultimate effect of extra CO_2–supply has the character of a self–amplifying process.

FIG. 5.5. Relation between leaf width and epidermal cell size for leaves grown in air (●) and in air with 0.1 % CO_2 (○).
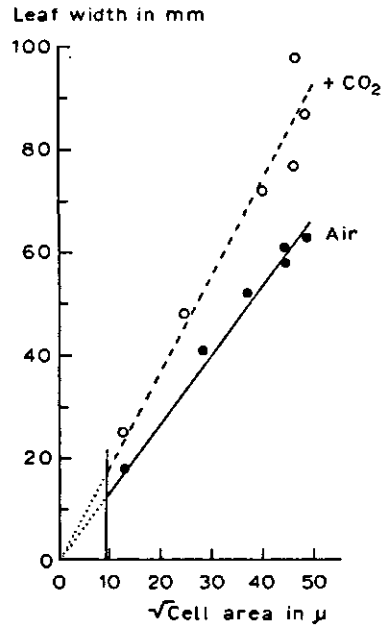
5.4. EFFECTS OF EXTERNAL SUGAR SUPPLY

Thirty years ago, SPOEHR (1942) reported the successful culture of albino maize by immersing the tip of leaves in a sucrose solution. FREDERICQ (1958) used this technique to keep *Hyoscyamus* plants alive which were grown in a CO_2 free atmosphere.

Similarly, we have tried to feed lettuce with externally supplied sugar. Plants were grown at low light intensity, two or three detipped leaves were immersed in a 10% sucrose solution containing 0.025% sulfanilamide. The intention was to investigate a possible favourable effect on leaf blade expansion which, owing to the low light intensity, showed the typical features of a low intensity leaf. In none of the cases, however, any effect of sugar on leaf growth could be detected Some plants also have been sprayed with the same solution, also without response. As it appears doubtful whether appreciable amounts of sucrose have been really taken up by the leaves, the results of these experiments remain uncertain.

5.5. EFFECTS OF GIBBERELLIN

A characteristic feature of gibberellin is to stimulate elongation of intact plants. Well-known are the pictures in textbooks of rosette plants like lettuce, cabbage or *Hyoscyamus* which have formed excessively long flower stalks after application of gibberellin.

Notwithstanding the fact that one should make abstraction here from possible

flower promoting effects, an influence on elongation per sé is evident in most of these cases. Dwarf mutants of several plants as corn, peas, cucumber and beans will grow to the normal size when treated with gibberellin (PHINNEY and WEST, 1960).

Lettuce is very sensitive to most of the known gibberellins (RAI and LALORAYA, 1967), possibly indicating a relatively low level of effective endogeneous gibberellin (KATO, 1966b). Some Russian papers, available only in abstract (KENTZER, 1960, ZUKOVA, 1962), report increased leaf yields of lettuce after application of gibberellin, though leaves and stems appeared elongated. Elongation of lettuce hypocotyls is often used as a bio-assay for gibberellin or gibberellin-like substances.

Effects of gibberellin on internode elongation have been ascribed to a stimulation of cell division (CLELAND, 1969). SACHS, BRETZ and LANG (1959), indeed found for the rosette plants *Samolus* and *Hyoscyamus* that gibberellin induced and increased mitotic activity in the stem apex and sub-apical meristem in the same way as occurs when the plants are placed at the right photoperiod for flowering. Final cell length was unaffected by gibberellin. Evidence exists that gibberellins act through an effect on IAA metabolism (BRIAN and HEMMING, 1958, OCKERSE, 1970). In some cases, gibberellin was found to stimulate leaf expansion, however, according to most authors, gibberellin is not primarily active in leaf growth (CLELAND, 1969).

In the course of the present study, a few experiments have been made with gibberellin to investigate its effect on leaf development.

A sodium salt, containing 10% pure gibberellin was dissolved in water. During one week, 5 drops of this solution, together 10 μg gibberellin, were administered daily to the centre of young plants, the 9th leaf of which had an average length of 1.5 cm. Control plants were supplied with the same amount of distilled water. The plants were grown at an intensity of about 25,000 ergs/cm²sec. It appeared later, that clear effects of gibberellin are obtained already at doses of 0.1 μg every two days (VAN DER MEER, 1968).

Effects became visible within two or three days after the first dose. In particular, young leaves start to elongate and acquire a more yellowish-green colour. In fig. 5.6, the course of length and width in successive leaves is indicated for a treated and a non-treated plant, measured two weeks after the first and one week after the last daily dose of 10 μg gibberellin. In particular, leaf elongation appears enhanced, whereas leaf width presents almost the same course as that of the control. However, it would be erroneous to conclude that gibberellin has no effect on leaf width. For, it should be noted that the effect of gibberellin observed greatly depends on the stage of development of the leaf at the moment of gibberellin application. As shown, gibberellin has slightly increased the width of the leaves 6, 7 and 8, whereas it seems to have had no effect on that of the following ones. However, eventually, leaves will decrease in width with gibberellin. An effect which, although not yet apparent in the leaves shown in fig. 5.6, is demonstrated on Plate V and in Table VII, presenting later leaves of the same serial order both for a plant given gibberellin and for a control.

FIG. 5.6. Length and width of successive leaves of a plant treated with gibberellin (GA, ○) as compared to those of a non-treated one (●).
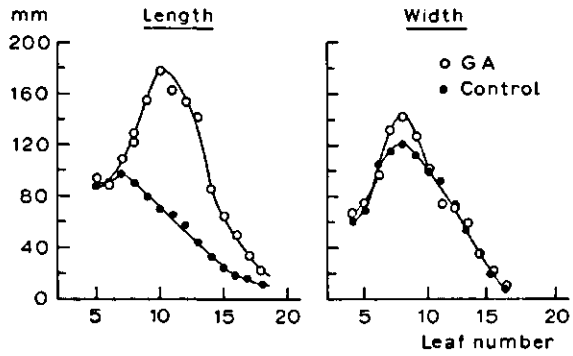
This apparent shift from a positive response to a zero and even negative one becomes intelligible when the effects of gibberellin are considered at a cellular level. It then appears that gibberellin in all cases has increased cell size. On the midrib they are much longer, on the leaf blade their areas are larger (Table VII). At the same time, cell number, as derived from the quotient of leaf size and cell size, is decreased. It seems reasonable to assume that leaves which have passed the stage of cell multiplication (chapter 4), at the moment gibberellin is applied, will increase in size, owing to increased cell extension only. Evidently, this goes at the expense of energy otherwise available for cell division in the younger leaves which will lead to a corresponding decrease in cell number. Initially, the

PLATE V. Leaves of corresponding serial number taken from a plant treated with gibberellin (GA) and from a non-treated one. Leaf discs were taken for cell measurements (see Table VII).

TABLE VII Effect of gibberellin on length and width, size and number of epidermal cells in midrib and leaf blade of the three leaves shown on plate VI.

	Gibberellin		Control	
Leaf	length mm	width mm	length mm	width mm
1	241	78	143	102
2	220	53	107	90
3	180	45	78	74

Average cell length in μ and calculated number of cells (n), per single longitudinal row in the midrib

	μ	n	μ	n
1	560	430	420	340
2	590	375	238	450
3	585	310	161	485

Average surface area in μ^2 and calculated number of cells per single transversal row in the leaf blade

	μ^2	n	μ^2	n
1	3030	1420	2380	2100
2	2040	1170	1820	2110
3	1600	1125	920	2440

effect of a lower cell number on final growth will be compensated by increased cell extension, so that no difference in width may be observed as compared with the control. However, in subsequent leaves, the probably more severe reduction in cell number may not be fully compensated by increased cell size and thus will lead to a decrease in width. Although the same reasoning should hold also for the midrib – subsequent leaves indeed become shorter – leaf shape more and more presents the character of etiolated growth, due to the strongly increased length to width ratio.

In Chapter 4, it was demonstrated (fig. 4.19) that successive, growing leaves of a plant show an exponential increase in length. This remains true also for plants treated with gibberellin; successive leaves, however, then increase in length at a higher rate. For the corresponding cell length it has been demonstrated (fig. 4.11) that a clear-cut distinction can be made between an initial phase of meristematic development, characterized by only a small increase in average cell size, followed by one in which cell length increases almost at the same rate as the overall leaf length. As a point of special interest it was remarked that the evident change from meristematic to non-meristematic cell development did not affect the overall rate of leaf elongation, indicating that cell extension is independent of the occurrence of cell division.

It seemed of special interest to investigate whether and how this picture is

affected by the application of gibberellin. With respect to the phase of meristematic growth, when average cell size remains more or less constant, two possibilities exist: average cell size may either be unaffected or increased by gibberellin. The first case would imply that gibberellin has increased the rate of cell division, and the period between two subsequent cell divisions or the generation time is decreased. In the second case, the generation time may have been unchanged, but cells with gibberellin reach greater size before mitosis.

In fig. 5.7, an example is given of the logarithmically linear increase of successive leaf lengths and corresponding cell lengths, for a plant treated with gibberellin and a control. Both plants at the time of harvest had initiated 40 leaves, and for both 0,05 mm is found as the length of the youngest primordium, notwithstanding the different rate at which leaf length appears to increase. For all leaves investigated, longer cells were found in the gibberellin treated plant, also at the phase of meristematic growth, which indicates that the second possibility above applies in this case.

Internode elongation and, consequently, stem formation is another evident response of lettuce plants to gibberellin which leads to advanced flower initiation and bolting. If gibberellin application is stopped before the plants become reproductive, stem elongation also stops. Rosettes of much shorter leaves than those produced during the gibberellin treatment, are produced on top of earlier formed stems. Flower initiation and subsequent bolting for these plants occurs at the same moment as in untreated plants. This is demonstrated in fig. 5.8, showing average internode length with leaf succession. After seven daily doses of gibberellin, internode length (leaf 25) has increased to 8.7 mm as compared with
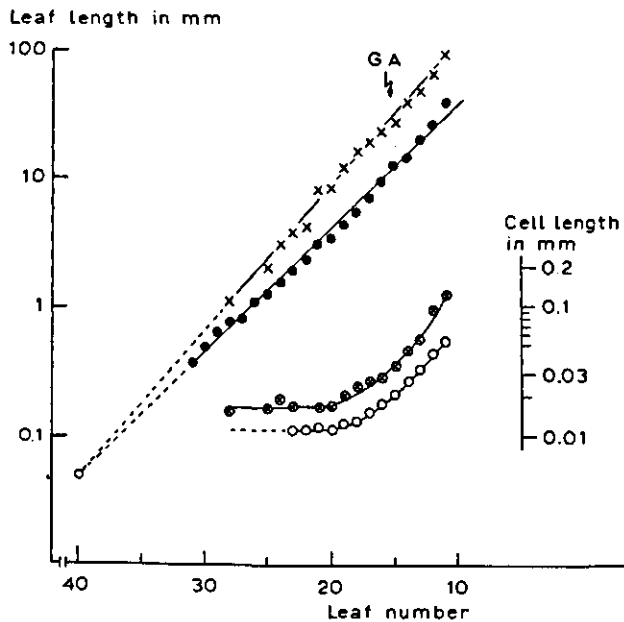
FIG. 5.7. Log leaf length and primordium length in relation to leaf number, for a plant treated with gibberellin ($\times$) and for a non-treated one ($\bullet$). Leaves numbered stem upwards from right to left. Both plants had initiated 40 leaves at the moment of measurement For some leaves the average lengths of the epidermal cells in the midrib are given, with ($\otimes$) and without gibberellin ($\bigcirc$), (see fig. 4.11).

Internode length in mm
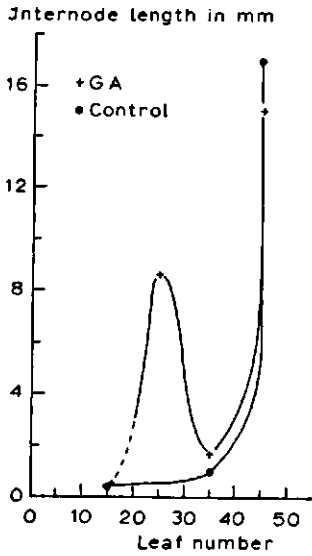
FIG. 5.8. Effect of a short period of gibberellin application on internode elongation. Flower initiation and subsequent shooting occurs at the same internode for the plant treated with gibberellin (+) and for the non-treated one (●).

0.5 mm in the control. After gibberellin application is stopped, internode elongation at leaf 35 is reduced to the same level of that in the control. After both plants have become reproductive, internode length rapidly increases in a similar way in both.

PLATE VI. Four different stages of leaf development after gibberellin application to the plant is stopped.

It was already remarked that concurrent with a stop of stem elongation, leaf elongation also decreases again. Four subsequent stages are shown on plate VI. It is of interest to note that the laminas of these leaves exhibit the same kind of crinkling along the midrib as shown on plate I which represents the 'normal' development. Also in this case it is to be interpreted as indicating unequal growth of midrib and adjacent leaf blade. Apparently, enough leaf blade is still available to from a long leaf, while midrib elongation is checked earlier. A complete restoration of the original pattern of leaf growth has not been observed experimentally, the plants became too old and started to initiate flowers.

The results obtained justify a more extensive research on the action of gibberellin upon the ontogenesis of midrib and leaf blade, in particular in relationship to light. Results of some preliminary experiments on the effects of different doses of gibberellin at different light intensities (VAN DER MEER, 1968), are in a line with LOCKHART's (1961) views on a light intensity-gibberellin interaction of stem growth of Pinto beans. According to this view, slow growth at low light intensities is primarily due to reduced photosynthesis, while growth inhibition at high intensities is supposed to be due to decrease in the effective levels of endogenous gibberellin. Consequently, the strongest growth promotion with gibberellin should be obtained at high light intensity.

GENERAL DISCUSSION

It has been one of the aims of this study to acquire a better understanding of the process of head formation in lettuce under various environmental conditions.

Heading mainly seems a matter of the arrangement of the leaves around the shoot of the plant, in which growth of the individual leaf plays a major rôle. As to this, many of the experimental results can be understood on the basis of the three diagrammatic leaf pictures, presented in fig. 6.1. Although representing quite different types of leaves, they nevertheless can be understood as to have originated from the same kind of primordium (cf. fig. 4.8) by differences in the relative outgrowth of the midrib and the leaf blade.

Leaf A represents a leaf in which midrib elongation has strongly exceeded lamina expansion, (cf. also the leaves presented on Plate V, taken from a plant treated with gibberellin). 'Spoonshaped' leaves are produced, which can be spread out flatly only by making one or more incisions. It represents the typical features of etiolated growth. On the contrary, leaf B represents a leaf in which development of the midrib or in general of the vein skeleton, and that of the leaf blade (mesophyll tissue) have just balanced each other, yielding a flat leaf surface which may be considered normal for most leaves in other plants. Eventually, continuing along the same trend, in leaf C the situation occurs where leaf blade development is relatively greater than that of the midrib; it turns out that in this case the leaf can be flattened by merely cutting the lamina from the midrib and by stretching its folds (cf. Plate II). Leaf C represents the typical features of a mature lettuce leaf.

Experimentally, the leaves A, B, and C can be obtained by growing plants at increasing light intensities. However, the same sequence, though less extreme as to type A, can be observed in the first 12 to 15 leaves of the plant. Together, this strongly suggests that photosynthesis to a large extent controls this development of the leaves; increased photosynthesis, either achieved by higher light inten-
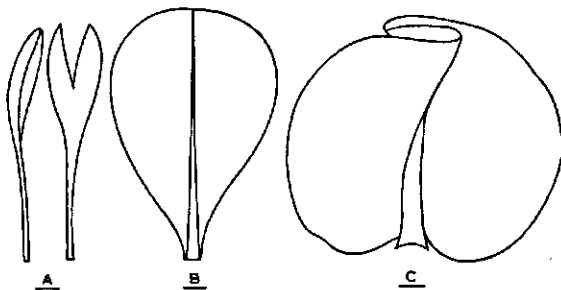

FIG. 6.1. Diagrammatic pictures of three types of leaves. Further explanation, see text.

sities or being the result of a gradually increasing leaf area of the young plant, may be considered to govern the change in leaf shape development from A to C. Experiments with increased CO_2 concentration and defoliation treatments have given further support to this view.

The effects of the production of leaves of the type C on head formation seems quite obvious. The more so, if they are produced in a great number, and at the same time internode elongation is greatly suppressed. Although no further details have been given concerning this last aspect, it can be stated that in all cases where elongation of the midrib was found to be either stimulated or suppressed, the same was found for elongation of the internodes. In an extreme case, e.g., at a very low light intensity, this could give rise to the formation of stems of appreciable lengths. Elongation immediately stops when light intensity is increased, and a new rosette is formed again on top of the preformed stem. Fig. 5.8 illustrates a similar situation for plants which have been treated with gibberellin for a short period only.

By a more close examination of heading in young plants, it is observed that leaves initially form an open rosette. However, when the leaves successively become larger and leaf blade extension starts to exceed midrib development, the lamina begins to show folds. There is a strong impression that especially the upper part of the leaf is being 'pushed-up' by the extension of the lower part. Therefore, folds usually originate at the upper part of the leaf. In this respect it is of interest that SAURER and POSSINGHAM (1970) in spinach leaves, observed a continued mitotic activity at the base of the leaf, providing new portions of leaf blade during leaf expansion. The situation was reported to remind of that in leaves of monocotyls, where the leaf blade elongates through the activity of intercalary meristems.

The first leaves, folding together in this way, will cover the centre of the still open rosette, including the young leaves. Since subsequent leaves do the same, the vegetation point will be gradually covered by an increasing number of leaves. This, in essence, typifies the mode in which heads of butterhead varieties of lettuce are built up. This clearly differs from the heading of cabbages as described by NORTH (1957), where hyponastic curvatures of the leaf primordia seem to play an important rôle. In cabbage, newly formed leaves grow more and more arched over the growing point producing an embryonic head which may continue to grow in size and amount of leaves, without changing essentially in form until maturity. In contrast to this, the heads of lettuce develop from the outside to the inside, although in some varieties, in particular the crisphead lettuces, head formation shows resemblance with that of cabbage. On Plate III, an example is given how hyponastic curvatures, in particular of the lower part of the midribs greatly determine the shape of the heads formed. Since varietal differences in this respect are evident, the nature of these curvatures are worth to be further examined.

A point of special interest is that the younger leaves developing inside the head will be increasingly cut off from light. Nevertheless, they display the morphological features of leaves growing at a high light intensity. It seems, therefore,

that the older leaves which are exposed to light, can supply the inner ones with all the essentials for their growth. As to their chlorophyll synthesis, however, they respond to their actual situation of darkness. On the other hand, stem and midribs of the inner leaves respond with an immediate elongation when the light intensity, received by the outer leaves, is strongly decerased. This seems to indicate that also the site of perception of the light stimulus, inducing elongation in the younger leaves, is in the older ones.

Heading, evidently, will increase the amount of the non photosynthetically active parts of the plants. Moreover, it is clear that a close relationship exists between photosynthetic activity of the plant and the onset of heading. Therefore, under conditions of limited energy supply, e.g., during winter time, head formation will be more difficult to achieve. But it would seem advantageous if it should not start at a too early stage since the effect of the lower light intensity might be compensated by a greater area of leaves exposed to light. In this respect, the results of BROUWER and HUYSKES (1968) are illustrative, who found that the faster growth of one of two varieties of lettuce tested, could be ascribed solely to a better exposition of its leaves to light.

Besides the pattern of growth of the individual leaves, also the number of leaves which take part in head formation is of great importance. It has been demonstrated in the previous chapters that leaves are initiated at a constant rate, different according to conditions of light and temperature. Subsequent growth of the leaves, however, occurs at a lower rate, leading to an accumulation of young leaves. Differences in the rates of leaf production and leaf unfolding are rather common in plants (MILTHORPE, 1959, CLOWES, 1961) and in the case of cabbage it has been denoted as a condition leading to the onset of heading (NORTH, 1957). Also for lettuce it seems important that, as soon as head formation has started, the head is filled up by leaves which should be produced at a high rate, but which should not grow so rapidly that they will break through the enclosing sheats of older leaves.

In this respect, varietal differences occur. 'Meikoningin', e.g., is a lettuce variety of which the leaves show the right morphological features for proper head formation. However, owing to a low leaf production rate at low light intensities, growth in particular during the winter will give less favourable results. On the other hand, the variety 'Proeftuin's Blackpool' seems to give an example in which a less favourable leaf morphology is partly compensated by increased leaf production. The most ideal situation arises when both qualities are present at the same time.

On several occasions in the course of this paper, it has been stated that differences in leaf growth may be successfully understood on the basis of a balance of energetic and non-energetic processes. Both may operate as a limitation for further growth at a given time.

Energetic processes are likely to be closely related to the process of photosynthesis. Non-energetic processes may be of different nature, and concerned with, e.g., the supply of water, minerals, and hormones. The importance of this

balance can be further illustrated by examining the differences in leaf growth observed on a cellular level. With the restriction that only the size and number of epidermal cells have been measured, it has been found that in all cases cell number is positively related to light intensity; this holds for both midrib and leaf blade. But, while cells in the leaf blade grow out to almost the same size under all conditions, cells in the midrib show a large variation in length. This variation was found to occur at various positions along the midrib, but also between the midribs of leaves grown under different environmental conditions, e.g., different light intensities. Consequently, differences in cell number account almost completely for the differences in leaf width observed, whereas the length of the midrib appears more dependent on the length of the cells, the average cell length decreasing, e.g., with increasing light intensity. Evidently, conditions for cell extension become less optimal at high light intensity. For an appropriate cell extension, water and minerals should be supplied at optimal concentrations. In particular at high light intensities it may easily occur that either one, or both limit growth. However, for the experiments presented here, special care has been taken to grow plants at ample supply of water and minerals. Therefore, a negative relation between cell length and light intensity, as found for cells in the midrib, seems to indicate a light induced limitation in the process of cell extension itself.

Cell extension, evidently, requires metabolic energy for the synthesis of protoplasm, cell wall formation, etc. On the other hand, increase in cell size is also accompanied by uptake of water by the cell. This requires that the water potential inside the cell be lower than that outside and at the same time a pressure potential that exceeds the resistance of the cell wall to plastic deformation (for a recent, detailed discussion of this matter the reader may be referred to, e.g., SALISBURY and ROSS, 1969).

Hence, plastic properties of the cell wall will play a major rôle in the process of cell extension; they may be denoted as a 'resistance'-factor in the growth process. Hormones are known to act as chemical regulators in this, in the sense that they may increase the plastic (HEYN, 1931) or elastic (BURSTRÖM et al., 1970) properties of the cell wall. On the other hand, increased metabolic activity in the cells, e.g., induced by high light intensity, may have a decreasing effect on cell wall plasticity (THOMSON, 1950, LOCKHART, 1960, 1961). This could be a helpfull explanation for the negative relation between cell extension and light intensity, mentioned above. In this respect it might also be mentioned that we found a prolonged exponential growth of the midrib at low light intensity as compared with high light intensity. At high light intensity a relatively higher hormonal activity may be required to keep the cell in optimal conditions for elongation than at low light intensity. Results of preliminary experiments indeed, suggest that at a high light intensity, higher concentrations of gibberellin are required than at a low light intensity to obtain the same relative increase in length of the leaves (VAN DER MEER, 1968). In this respect also the effects of gibberellin on photo-inhibited stem elongation of beans obtained by LOCKHART (1961) should be mentioned.

The effects of gibberellin as demonstrated in this paper, show that the pattern

of growth of the leaf is fundamentally changed. It strongly suggests that the excessive elongation of the midrib observed occurs at the expense of the leaf blade, however, gibberellin has increased the size of leaf blade cells as well. (see Table VII on page 74) This seems to indicate that midrib and leaf blade, though structurally integrated, may be regarded as two distinct parts, competetive in growth. At a low light intensity, i.e., at a low energy supply, the midrib, apparently, is in the most favourable situation. This is changed in favour of the leaf blade when light intensity increases, i.e., energy supply is high, and the hormonal balance for the midrib, presumably, becomes less favourable. This will again be completely reversed, if the plant is treated with gibberellin.

Competetive growth also seems to give the most acceptable description for the differences in temperature response of the leaves at different light intensities. In this case, competition for available growth substrates seems to occur mainly between the leaves, in which the leaf blades show the greatest response.

In general, it can be concluded that leaf blade expansion is mainly controlled by energy supply (light intensity), and midrib extension by energy and hormone supply. However, the ultimate shape of the leaves will be determined by the influence of light intensity on the balance between energy, providing metabolites, and hormonal effects.

SUMMARY

The growth of leaves of some butterhead type varieties of lettuce has been investigated under different light intensities and temperatures, with special reference to the process of head formation. Most experiments were carried out with the varieties 'Meikoningin' and 'Rapide' in climatized growth rooms.

In Chapter 1, a typical feature of lettuce leaves is demonstrated, *viz.*, that lamina extension may largely exceed that of the corresponding midrib, yielding the caracteristic folds and crinkles of the leaf blade (Plate II). Therefore, length and greatest width of the leaves have mainly been chosen as criteria for differences in leaf growth.

In Chapter 3, the effects of different light intensities, light duration (daylength) and their interaction with temperature are presented. Leaf production increases both with light intensity and with temperature (fig. 3.1), but appears to remain fairly constant with plant age. Since subsequent leaf development occurs at a lower rate, a number of leaf primordia and young leaves of the plant accumulate with time (figs. 3.4 and 3.5). Results suggest that primordial growth is more affected by temperature than by light intensity (fig. 3.6).

In many respects clear-cut differences are found between the response of leaf length and leaf width under various experimental conditions. Based on the maximum leaf dimensions reached, leaf width generally responds positively to increasing light energy, either given as a higher light intensity or a greater daylength (figs. 3.7 and 3.9.) In both cases relationships are represented by saturation curves which, for light intensity, tend to go through the origin. Effects of daylength become particularly evident for periods shorter than 12 hours.

For leaf length, a positive relation to light energy is only found at a low intensity level, since at high light intensity midrib elongation appears clearly suppressed. Effects of different daylengths, also are only evident at a low light intensity (fig. 3.9).

Temperature effects greatly depend on the prevailing light intensity: a negative response observed at low light intensity changes into a positive one at high light intensity, in particular for leaf width (fig. 3.11). This implies that the effect of temperature on leaf width is small at intermediate light intensities (fig. 3.12). It further appeared that light intensity effects on leaf width are especially manifest at high temperature, whereas for leaf length they are more pronounced at low temperature (fig. 3.11).

Growth-time relationships appear to be quite different for leaf length and leaf width. Leaves elongate fast at low light intensity, but growth is maintained for a longer period at high light intensity. As a consequence hereof light intensity effects on the final length of the leaves remain restricted. In contrast to this, the effects on leaf width are much more pronounced, since both growth rate and growth duration are greatly reduced at low light intensity (fig. 3.16).

From the linear, but greatly different length-width relationships, measured

during leaf expansion at different light intensities (fig. 3.21), it may be concluded that an important factor in determining the ultimate shape of the leaves is the moment at which leaf blade expansion is initiated during primordial leaf development.

In Chapter 4, the foregoing results are examined on the base of differences in number and size of epidermal cells in the midrib and the leaf blade. Both for the midrib and the leaf blade there is a positive relation between light intensity and cell number. Differences in cell number largely determine differences in leaf width, whereas in the midrib differences in cell length are much more important (figs. 4.3 and 4.7). In general, average cell length in the midrib decreases with increasing light intensity. This may explain the reduction of the midrib observed at high light intensities. Cell division appears restricted mainly to the early stage of growth.

In chapter 5, results of some additional experiments are presented, concerning defoliation, extra CO_2, and gibberellin application. Defoliation causes a temporary reduction of leaf width of subsequent leaves (fig. 5.1) which can also be brought about by a temporary reduction in light intensity (fig. 5.3). At a higher CO_2 concentration larger leaves are produced than in normal air. In all these cases, differences in leaf width are closely related to changes in cell number. Gibberellin, in particular, induces a strong elongation of the midrib which eventually may go at the expense of leaf blade development.

It has been suggested that differences in leaf growth may be understood on the base of a balance between energetic and non-energetic processes. Both may operate as a limitation for further growth. Energetic processes (i.e. photosynthesis) seem to control to a large extent cell division, consequently expansion of the leaf blade, whereas non-energetic processes (e.g. hormon activity) seem important in particular for cell extension, and therefore play a major rôle in midrib elongation. In this respect it is tempting to assume that at high intensity a relatively higher hormonal activity is required to keep the cells in optimal condition for extension.

ACKNOWLEDGEMENTS

SAMENVATTING

Een onderzoek werd verricht naar de invloed van lichtintensiteit, lichtduur (daglengte) en temperatuur op de groei van bladen van kropsla, in het bijzonder met betrekking tot het tot stand komen van de kropvorming. De meeste experimenten werden uitgevoerd met de varieteiten Meikoningin en Rapide in geklimatiseerde groeikamers.

Hoofdstuk 1 bevat enkele inleidende beschouwingen over bladvorm en kropvorming. Kenmerkend voor het volwassen slablad is de relatief sterke ontwikkeling van de bladschijf t.o.v. de middennerf (plaat II.) Samen met een hoge bladproductie, en een sterke remming van de stengelgroei, ontstaat kropvorming als vanzelf uit een ruimteprobleem bij de ontplooiing van de opeenvolgende bladen. Afhankelijk van de varieteit kunnen hyponastische krommingen van de middennerf de stevigheid van de krop sterk bevorderen (plaat III). De aard van deze krommingen werd niet nader onderzocht. Als belangrijke criteria voor de bladgroei onder de verschillende experimentele condities zijn de bladlengte (strekking van de middennerf) en de grootste bladbreedte (ontwikkeling van de bladschijf) gebruikt.

Hoofdstuk 3 bevat de belangrijkste gegevens over de invloed van lichtintensiteit, daglengte en temperatuur op verschillende aspecten van de bladgroei. De bladproductie neemt zowel met de lichtintensiteit als met de temperatuur toe, doch wijzigt zich niet met de leeftijd van de plant (fig. 3.1). Aangezien de groei van de bladen geen gelijke tred houdt met de bladaanleg, heeft er een geleidelijke ophoping van bladprimordia en jonge bladen plaats (fign. 3.4 en 3.5). Uit de resultaten verkregen met verschillende lichtintensiteiten en temperaturen kan worden afgeleid dat de primordiale groei meer door de temperatuur dan door de lichtintensiteit wordt beïnvloed (fig. 3.6).

In menig opzicht blijken middennerf en bladschijf verschillend te reageren op de uitwendige omstandigheden. Gemeten aan de grootste bladen (fig. 1.2), vertoont de bladbreedte een positieve relatie met de lichtenergie, hetzij gegeven in de vorm van een hogere lichtintensiteit of een grotere daglengte (fign. 3.7 en 3.9). In beide gevallen wordt het verband door verzadigingskrommen voorgesteld, die voor het geval van de lichtintensiteit duidelijk door de oorsprong gaan, m.a.w. bij lichtintensiteit 0, dit is donker, ontwikkelt zich geen bladschijf. In dit verband dient opgemerkt te worden dat bladen die zich ontwikkelen binnen een gesloten krop in hun groei reageren op de lichtintensiteit die door de buitenste bladen wordt ontvangen. Voor de daglengte geldt, dat pas bij belichtingstijden korter dan 12 uur de bladbreedte duidelijk afneemt.

De bladlengte vertoont alleen bij lage lichtintensiteit een positieve relatie met de lichtenergie. Bij hoge lichtintensiteit wordt de groei van de middennerf duidelijk geremd. In overeenstemming hiermee is dat alleen bij lage lichtintensiteit de daglengte een duidelijke, bevorderende invloed heeft op de groei.

Temperatuureffecten hangen grotendeels van het lichtniveau af. Vooral voor

de bladbreedte geldt, dat een verhoging van de temperatuur afname veroorzaakt bij lage lichtintensiteit, maar toename bij hoge lichtintensiteit. De hieruit voortvloeiende verwachting, dat bij een bepaalde lichtintensiteit de invloed van de temperatuur op de bladbreedte gering zal zijn werd experimenteel bevestigd (fign. 3.10 en 3.11). Bij de meeste lichtintensiteiten neemt de bladlengte met de temperatuur toe, alleen bij zeer lage intensiteit wordt een duidelijke afname gevonden.

Voor het groeiverloop in de tijd blijken er ook duidelijke verschillen tussen bladlengte en bladbreedte te bestaan. De grootste groeisnelheden worden voor de bladlengte gemeten bij lage lichtintensiteit, doch aangezien de groei bij hoge lichtintensiteit langer voortduurt, blijven de verschillen in de uiteindelijke lengten bij de verschillende lichtintensiteiten beperkt. In tegenstelling hiermee neemt voor de bladbreedte zowel de groeisnelheid als de groeiduur sterk met de lichtintensiteit af, met als gevolg veel grotere verschillen in uiteindelijke bladbreedte tussen de verschillende lichtintensiteiten.

Uit de vrijwel lineaire, maar wel duidelijk verschillende relaties tussen lengte en breedte tijdens de groei bij verschillende lichtintensiteiten (fig. 3.21), kan worden afgeleid dat een belangrijke factor in de bepaling van de uiteindelijke bladvorm (lengte/breedte verhouding) gelegen is in het moment waarop de ontwikkeling van de bladschijf in het primordiale groeistadium begint (fig. 4.8). Ontwikkeling van de bladvorm bij opeenvolgende bladen en de invloed daarop van de uitwendige omstandigheden blijken duidelijk uit lengte-breedte grafieken (fign. 1.2 en 3.23).

In Hoofdstuk 4 zijn de hiervóór besproken resultaten verder geanalyseerd naar aantal en grootte van de epidermiscellen in middennerf en bladschijf. Zowel voor de middennerf als voor de bladschijf geldt, dat het aantal cellen toeneemt met de lichtintensiteit. Verschillen in bladbreedte blijken vooral te berusten op verschillen in aantallen cellen (fign. 4.6 en 4.7). Voor de middennerf spelen verschillen in cellengte een veel grotere rol (fign. 4.2 en 4.4). De gemiddelde cellengte neemt af met toenemende lichtintensiteit (fig. 4.3), wat de afname in bladlengte bij hoge lichtintensiteit kan verklaren. Celvermeerdering (celdeling) blijkt beperkt tot een vroeg stadium van de groei, waarin de potentiele bladgrootte grotendeels wordt bepaald.

In Hoofdstuk 5 worden de resultaten vermeld van enkele aanvullende proeven, die betrekking hebben op ontbladering, extra CO_2 en gibberellinebehandeling. Vermindering van het photosynthetisch werkzame bladoppervlak van de plant door ontbladering veroorzaakt in de daarna gevormde bladen een tijdelijke teruggang van de bladbreedte, geheel analoog aan die, welke verkregen kan worden door een tijdelijke verlaging van de lichtintensiteit. In beide gevallen is de afname in bladbreedte duidelijk gecorreleerd met een afname in het aantal cellen. (fign. 5.2 en 5.3). Bij een hogere CO_2 concentratie (0.1 %), neemt de bladgrootte toe door toename in celaantal (fign. 5.4 en 5.5). Toediening van gibberelline doet vooral de middennerf sterk uitgroeien als gevolg van een vergrote celstrekking; ook in de bladschijf neemt de celgrootte toe (Tabel VII), maar als gevolg van een sterke reductie in het aantal cellen neemt de bladbreedte in de later

gevormde bladen sterk af. Aangenomen wordt dat de verhoogde strekkingsgroei ten koste gaat van energie die anders beschikbaar zou zijn voor de celdeling.

In een slotbeschouwing wordt gesteld dat bij de groei van het blad zowel energetische (photosynthese) als niet energetische processen (opname van water, mineralen, werking van groeistoffen) betrokken zijn. Voor het uiteindelijke resultaat zal het heersende evenwicht tussen beide processen van groot belang zijn. Hierbij lijken duidelijke verschillen te bestaan tussen bladschijf en middennerf. Energetische processen lijken vooral voor de bladschijfontwikkeling van belang, mogelijk vanwege een grotere relatie met de celdelingsactiviteit. De niet energetische processen lijken vooral van belang bij de beheersing van de celstrekking, en spelen daardoor mogelijk een grotere rol bij de groei van de middennerf. Op grond van de betekenis die groeistoffen in het algemeen bij celstrekkingsprocessen hebben, wordt verondersteld dat bij hogere lichtintensiteit een relatief hogere groeistofconcentratie vereist wordt voor een optimale strekkingsgroei van de middennerf. De verschuiving in de verhouding middennerf/bladschijf ten gunste van de bladschijf, wanneer de lichtintensiteit toeneemt, wordt mogelijk hiermee verklaard.

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