A formal template for the development of cucumber in its vegetative stage. I

by T. Horie[1,2], C. T. de Wit[1,3], J. Goudriaan[1] and J. Bensink[4]

1. Department of Theoretical Production Ecology, Agricultural University, Wageningen.
2. National Institute of Agricultural Sciences, Nishigahara, Tokyo, Japan.
3. Centre for Agrobiological Research (CABO), Wageningen.
4. Department of Plant Physiological Research, Agricultural University, Wageningen.

SUMMARY

Inspired by the common observation that the form of plants is very stable in spite of large changes in size and chemical composition, an attempt has been made to describe the development of cucumber by means of a limited number of inter-relations that are largely invariant to environmental changes.

In this study, developmental stage is fully characterized by the plastochron index of Erickson and Michelini, the development rate itself being a function of radiation and temperature.

It is shown that the relative length growth rates on plastochron basis of the leaves in the primordial and expanding phase are linearly related with leaf number, according to age functions which are independent of radiation and temperature, provided that the plastochron age of the plant is used as the independent variable. Also, the transition between the primordial and expanding phase of a leaf or its unfolding is governed only by the plastochron age.

The final leaf length with optimal supply of water and nutrients is fully determined by the number of epidermal cells along the midrib. The growth of the cell number is governed by the phenomenon that the fraction of dividing cells in the leaves decreases about linearly from hundred to zero percent from the moment of unfolding onwards. The number of unfolded leaves with dividing cells is almost independent of the plastochron age of the plant. It increases however with radiation, which is the only reason why plants with the same plastochron age have larger leaves when grown under more light.

1. INTRODUCTION

In a recent monograph (De Wit et al., 1978), the work of the Department of Theoretical Production Ecology of the Agricultural University in

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Wageningen on simulation of assimilation, respiration and transpiration of crop canopies was presented. Apart from these main aspects of crop production, the simulation program hand'es also some important aspects of the root environment and the functional balance for the distribution of growth between shoot and root. The program, however, does not treat morphogenesis, the development of form and function. The important relation between dry weight growth of the shoot and the development of the leaf area is not considered, and the leaf area growth in course of time is introduced as a forcing function. Likewise the functional relationship between net assimilation of CO_2 and light absorption is introduced as a forcing function and not related to simulated properties of the leaf, as there are leaf thickness, content of carboxylating enzymes and so on.

An earlier attempt to relate leaf area growth to the growth in weight of shoot (De Wit, Brouwer and Penning de Vries, 1970) was based on the introduction of a specific leaf weight and a leaf weight-shoot weight ratio as a function of time or of development stage and of the conditions during growth. This approach failed because neither the distribution factors of dry weight, nor specific leaf weights are variables that are controlled as such by physiological processes. They are rather the final result of different growth and development processes that proceed to some extent independent of each other. Dry weight growth of leaves is governed by photosynthesis, the costs of synthesizing chemical plant constituents and transport of these constituents over the organs. Leaf area growth on the other hand is governed by rate of leaf initiation, cell division in the more primordial stage and the subsequent possibilities of cell expansion.

The working hypothesis that growth of weight and area have to be considered in first instance independently of each other is based on the common observation that the form of plants at the same development stage is very much the same in spite of large differences in size and chemical composition. This constancy of form is often characterized by so called allometric relationships which reflect that the relative growth rate of the main dimensions of the plant are often linearly related. Classical allometry does not consider, however, interrelations between dynamic parameters such as rate of leaf production, weight growth rates and cell division and enlargement rates, although it is clear from the extensive literature on leaf development (see for instance Maksymowych, 1973) that these have to be also closely interlinked in situations where form is maintained in spite of variations in size.

The present work combines information from various sources with the explicite purpose to extend allometric analyses to more growth attributes and to obtain interrelations that are largely invariant to environmental changes. Otherwise formulated, the purpose is to characterize the formal template of development, a template being, according to Webster's dictionary, a pattern for testing the accuracy of form.

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Interest in the problem was stimulated by a novel interpretation of a well known allometric observation: a linear relation between the logarithm of the length of younger growing leaves and their sequential number at any moment. This linear relation appears to hold even when plants are grown under conditions that vary with time. Since new leaves are often formed at constants intervals, this logarithmic relation has often been assumed to signify that all leaves grow exponentially with the same relative growth rate. Bensink, as first published by Pieters (1974) in an appendix to his paper on leaf growth of poplars, showed that a necessary and sufficient condition for maintaining this linearity is that the relative growth rates of the leaves at any moment are linearly related with their sequential number. Hence, growth is not necessarily exponential, nor is the relative growth rate constant over time.

Many experiments on leaf growth are reported in literature, but some more information was necessary to relate the results of leaf area analyses with classical growth analyses, to study time-lags in response to changes in growing conditions and to obtain a consistent data set. Cucumber was chosen as an experimental plant for various reasons. The crop is of large economic importance in Japanese and Dutch horticulture and its diurnal pattern of growth has already been analysed in great detail (Challa, 1976). Moreover, Milthorpe and his co-workers (1963) analysed in detail the interrelations between leaf production rate, cell division rate and growth of the leaves of this species. Since his and our data sets appeared to be consistent, it was not necessary to repeat this excellent and cumbersome work.

2. AN EXPERIMENT WITH CUCUMBER

Cucumber seedlings (cultivar Sporu) were germinated on moist perlite in the dark at an air temperature of 25 °C and a relative humidity of 60%. The time of harvests and subsequent treatments is reported in days from the moment of sowing. After germination, the seedlings were grown further at a visible radiation intensity (400–700 nm) of 65 J m^{-2} and a daylength of 8 hours. The first growth measurements were made on the 7th day. On the same day, the seedlings were transplanted to one liter containers with a half strength Hoagland solution with extra iron and a pH of 6. Around this time, the initiation of the fourth leaf was observed microscopically and the size of the cotyledons were about a quarter of their final area.

Growth conditions at high (H: 65 J m^{-2} s^{-1}), intermediate (I: 33 J m^{-2} s^{-1}) and low (L: 18 J m^{-2} s^{-1}) light levels were obtained with cheese cloth and maintained for 8 hours per day, so that the total light flux was 1.87, 0.95 and 0.52 MJ m^{-2} day^{-1}. These fluxes refer to the top of the canopy which was kept in the same position with respect to light by adjusting the level of the pots.

The experimental area of 2.4 m^2 at each light condition contained

75 plants initially, but this number gradually decreased due to periodic harvests. Twice a week, four randomly chosen plants were harvested from each treatment. At the same time the remaining plants were randomized again to promote a more even light distribution and the nutrient solution was changed. After the 26th day, the solution was changed three times a week with a full-strength Hoagland solution. Axillary buds and flower buds were removed upon appearance as done in practice in The Netherlands during this early stage of growth. On the 34th day, immediately after the 8th harvest half of the plants of each treatment were transferred to another light intensity, resulting in three other treatments: high to intermediate (HI), intermediate to high (IH) and low to high (LH). The experiment was terminated at the 55th day. All treatments were fully analysed, but in the subsequent chapters only relevant data will be reported.

Length, width, area and dry weight of the individual leaves as well as the weight of other organs were measured for each treatment by destructive methods and when possible also by nondestructive methods. The non-destructive measurements of length and width of the leaves were made at 2–3 days intervals on ten plants at each treatment, which were specially reserved for this purpose. After the 34th day, the number of treatments were doubled, so that the measurements had to be continued on 5 plants for each treatment. Apart from length and width of the leaves, their area and weight were determined as well as the dry weight of roots and stems. The length of the young leaf primordia and their number was determined by means of a stereoscopic microscope with a magnification of 50. All results are reported as simple arithmetic averages over 4 plants in case of the destructive harvests and first over 10, but later on over 5 plants in case of the nondestructive measurements.

3. GROWTH AND ALLOMETRIC ANALYSES

3.1. *Growth analyses*

Total dry weight, leaf dry weight and leaf area per plant in course of time are given in Figs. 1 and 2. Weight and area increased with increasing light intensity, but leaf weight was much more affected than leaf area. The response to the sudden change in light regime was much more rapid in leaf weight than in leaf area. After transfer from high to intermediate light, the leaf weight was almost completely adapted to the intermediate light situation after 20 days, whereas the leaf area showed no appreciable adaptation. After transfer from low to high light, the adaptation in leaf area did not manifest itself within 10 days. This difference in velocity of adaptation of leaf weight and area shows that these two growth attributes respond differently towards environmental conditions.

The results of a growth analysis for about 10-day intervals are given in Table 1. Apart from the net assimilation rate (NAR), the leaf area
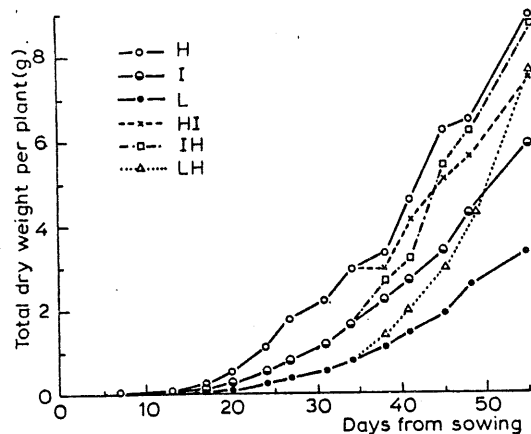
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Fig. 1. Increase with time of total dry weight per plant of cucumber grown under different irradiance conditions for 8 hours per day. H: high irradiance, $65 \ J \ m^{-2} \ s^{-1}$. I: Intermediate irradiance $33 \ J \ m^{-2} \ s^{-1}$. L: Low irradiance, $18 \ J \ m^{-2} \ s^{-1}$. HI: Plants transferred from high to intermediate at day 34 after sowing. IH: Plants transferred from intermediate to high. LH: Plants transferred from low to high.
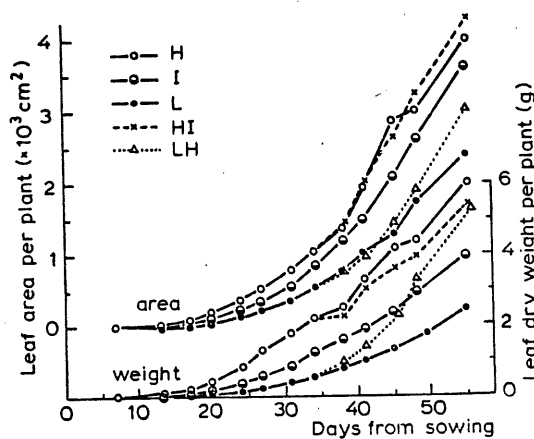

Fig. 2. Increase with time of Leaf Area and Leaf Dry Weight per plant of cucumber grown under different light conditions. Symbols used as in Fig. 1.

ratio (LAR, that is leaf area over total plant weight) and the mean relative growth rate of the total dry weight RGR_{td}), the relative growth rates of the leaf area (RGR_{la}) and of the leaf weight (RGR_{ld}) are also reported. Initially, the NAR at the highest light level was 2.5 times larger than that at the lowest level which is in fair agreement with the 3.5 times difference in light intensity. This difference, gradually decreased and at the end no difference in NAR between the high (H) and low (L) irradiance plants was observed. However, the transferred plants did not have the same

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Table 1. Net assimilation rate (NAR), leaf area ratio (LAR), and relative growth rate of total dry weight (RGR_{td}), of leaf dry weight (RGR_{ld}) and of leaf area (RGR_{ta}) at different light treatments and at different times.

	treatments	time period				
		7–15th day	15–25th day	25–35th day	35–45th day	45–55th day
NAR 10^{-4} g cm^{-2} day^{-1}	H	6.88	7.83	2.51	1.57	0.73
	I	4.06	5.12	2.01	1.25	0.55
	L	2.70	3.03	1.63	1.22	0.77
	HI	–	–	–	1.22	0.50
	IH	–	–	–	2.19	1.16
	LH	–	–	–	2.18	2.21
LAR cm^2 g^{-1}	H	298	290	390	402	589
	I	394	359	527	568	1018
	L	411	475	705	737	921
	HI	–	–	–	402	920
	IH	–	–	–	507	422
	LH	–	–	–	569	425
RGR_{td} g g^{-1} day^{-1}	H	0.205	0.227	0.098	0.063	0.043
	I	0.160	0.184	0.106	0.071	0.056
	L	0.111	0.144	0.115	0.090	0.071
	HI	–	–	–	0.049	0.046
	IH	–	–	–	0.111	0.049
	LH	–	–	–	0.124	0.094
RGR_{ta} cm^2 cm^{-2} day^{-1}	H	0.273	0.235	0.103	0.086	0.032
	I	0.227	0.227	0.115	0.090	0.032
	L	0.192	0.184	0.124	0.086	0.057
	HI	–	–	–	0.082	0.026
	IH	–	–	–	0.096	0.039
	LH	–	–	–	0.086	0.074
RGR_{ld} g g^{-1} day^{-1}	H	0.212	0.273	0.115	0.056	0.036
	I	0.166	0.205	0.103	0.063	0.053
	L	0.120	0.160	0.111	0.078	0.060
	HI	–	–	–	0.039	0.026
	IH	–	–	–	0.103	0.053
	LH	–	–	–	0.120	0.094

NAR at the end of the experiment. Transfer from high to intermediate light resulted in a distinct decrease of NAR, whereas the transfer from intermediate or low to high light resulted in a considerable increase of the NAR. These changes are probably related to changes in light distribution with depth.

The leaf area ratio (LAR) was highest at low light intensity and this difference became more and more pronounced in course of time, although it has to be remarked that the LAR of the intermediate light intensity between the 45th and 55th day deviated towards the high value. The LAR's of the treatments with a sudden light change are, however,
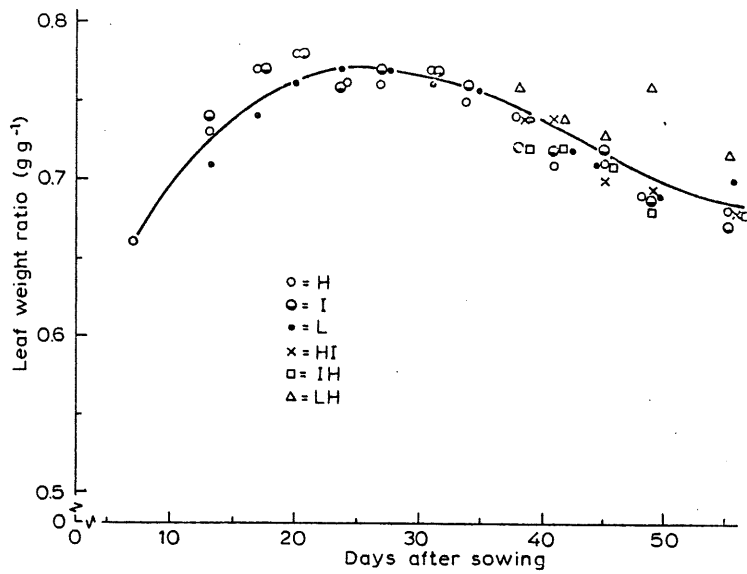
Fig. 3. Leaf Weight Ratio in the course of time of cucumber plants grown under different light conditions. Symbols used as in Fig. 1.
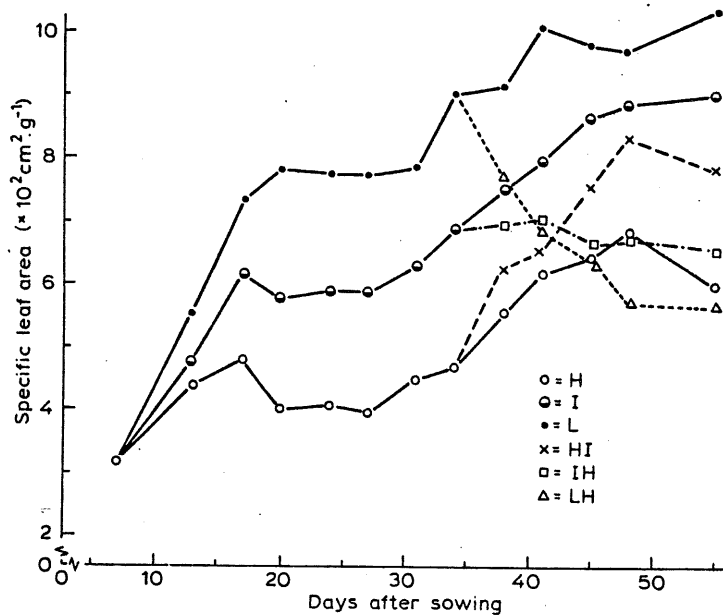

Fig. 4. Specific Leaf Area (SLA) in the course of time of cucumber plants grown under different light conditions. Symbols used as in Fig. 1.

practically fully adjusted to the new situation. The mean relative growth rate of the total dry weight (RGR_{td}) is the product of LAR and NAR in the same period. At the beginning, RGR decreased with decreasing light, but later on roles were reversed as a consequence of the adjustment

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in LAR and NAR. The leaf area ratio may be considered the product of the leaf weight ratio (LWR, leaf weight over total weight) and the specific leaf area (SLA, leaf area per unit leaf weight). Both are given in course of time in Fig. 3 and Fig. 4. LWR shows a small ontogenetic drift, but is practically independent of the light intensity. SLA on the other hand is highly sensitive to the light level and showed at the end, like the LAR, a difference of almost a factor of two between the low (L) and high (H) light treatment. It is also seen in Fig. 4 that upon the change in light conditions at the 34th day, the adjustment is, except perhaps for the HI treatment, almost complete within ten days.

The relative growth rate of the leaf weight (RGR_{ld}) is practically equal to the relative growth rate of the whole plant weight for all treatments, which illustrates the conservative character of the dry weight distribution pattern as shown in Fig. 3 in another way. On the other hand, the differences between the treatments are fully reflected in the difference in the relative growth rate for the leaf area (RGR_{la}).

3.2. Allometric relations between leaf dimensions

The relation between the length and the width of leaves expanding on different nodes and grown under different light treatments is given in Fig. 5. Independently of position, age, treatment and stage of growth,
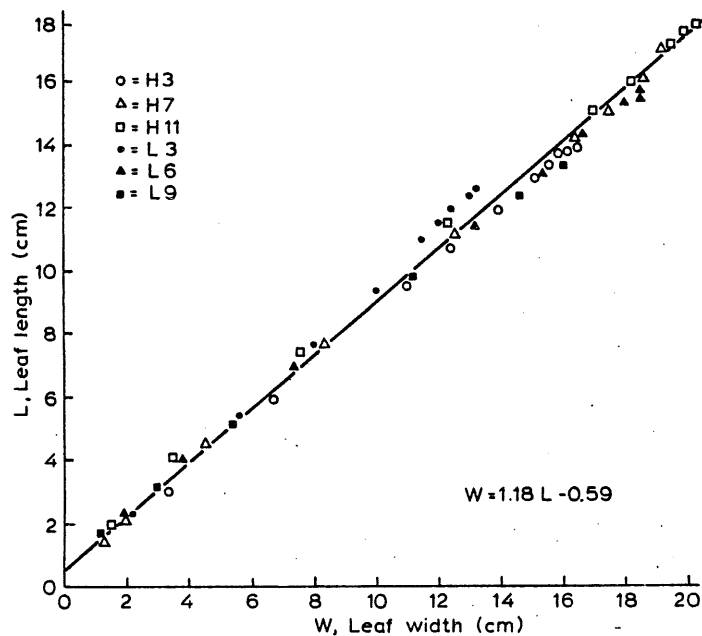

Fig. 5. The relation between the length and the width of leaves expanding on different nodes of cucumber plants grown under different light conditions. H is high irradiance, L is low irradiance and the numbers are leaf numbers.

the result may be presented by the linear relation:

(3.1) $W = 1.18(L - 0.5)$ cm

in which the length L is measured over the midrib and W is the largest width of the leaf. Since rapid width growth starts later than rapid length growth, the line does not pass through the (0,0) point and the relation is only valid for leaves that are larger than 1 centimeter. Similar relations between length and width of leaves are also found for other plant species, for instance poplar (Pieters, 1974). The relation between the area and the product WxL for these leaves is given in Fig. 6. Although the deviations are larger than in Fig. 5, it may still be assumed that a straight line relation:

(3.2) $A = 0.75LW = 0.89 (L - 0.5)L$ cm^2

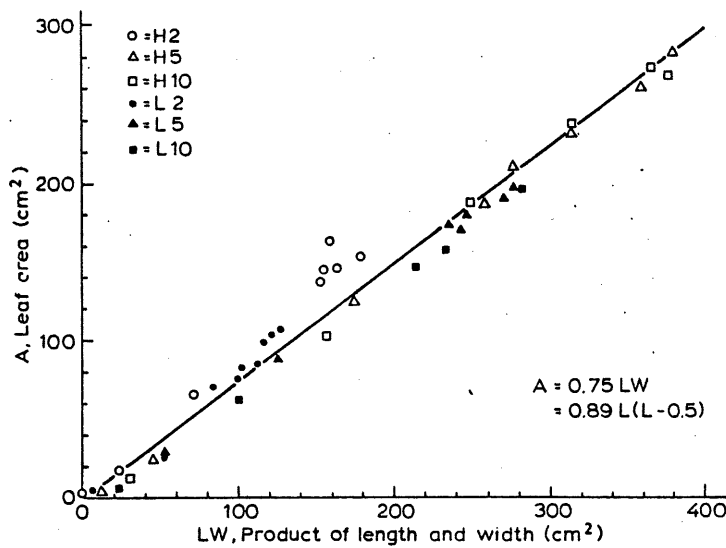holds for all the leaves with a length of more than 1 cm.

Fig. 6. The relation between Leaf Area (A) and the product of leaf length and leaf width (LW) for some expanding leaves. For symbols see Fig. 5.

Because of these stable allometric relations, it suffices to measure only leaf length of unfolded leaves. The relative growth rate of the width of the leaf is:

(3.3) $RGR_w = \dfrac{1}{W}\dfrac{dW}{dt} = \dfrac{1.18}{1.18(L-0.5)}\dfrac{dL}{dt} = \dfrac{L}{L-0.5}RGR_l$

which means that with increasing leaf length the relative growth rate of the width approaches the same rate as for the length (RGR_l). Similarly it is found by means of eq. (3.1) and (3.2) that the relative growth rate

of the area equals

$$
(3.4) \qquad RGR_a = \frac{1}{A} \frac{dA}{dt} = \frac{0.75}{0.75WL} \frac{d(WL)}{dt} = \frac{1}{WL} \frac{(LdW + WdL)}{dt} =
$$

$$
= \frac{2(L - 0.25)}{L - 0.5} RGR_l
$$

and thus approaches 2 times the relative length growth rate with increasing L. These relations hold for any leaf in any position, grown under any light treatment.

4. GROWTH PATTERNS OF THE LEAVES

4.1. *Leaf production rate*

Leaf initiation from the apex is a continuous process and its rate should be calculated, based on a clear, operational definition of the moment of appearance of a new leaf. This could be done by using a distinct morphological stage, as appearance of the leaf buttress, or the procambium. Whether meaningful or not, this method needs the analysis of sectioned samples of the apex and is often too laborious. The rate of leaf production may also be obtained by periodic counting of the number of leaves up to the smallest visible primordium under a stereoscopic microscope (Milthorpe, 1959; Newton, 1963; Bensink, 1971). However, considerable ambiguity arises from differences in quality and magnification of the microscope and personal judgement.

Ambiguity is excluded without introducing laborious methods by defining the moment of appearance of a leaf as the moment when its length passes a reference length LR. Hence the total number of leaves increases by 1 at the moment that the next leaf attains the length LR. However, the development stage of plants, is a continuous variable and if this stage is characterized by leaf number, its value must be found by a suitable interpolation between the last appeared leaf and the one that is next to appear. On basis of the observation that there is a linear relation between leaf number and the logarithm of the length of the leaves when they are in the primordial phase, Erickson and Michelini's (1957) definition of the plastochron index P is accepted

$$
(4.1) \qquad P = n + \frac{\ln L_n - \ln LR}{\ln L_n - \ln L_{n+1}}
$$

where n is the leaf number (counting from the base onwards) of the leaf which is just longer than the reference length LR. L_n and L_{n+1} stand for the lengths of n-th and (n + 1)-th leaf. The defining equation of the age in plastochrons implies an intimate relation between leaf production rate

and relative length growth rate of the primordial leaves around reference length. This is shown by differentiating eqn. 4.1 with respect to time

$$\frac{dP}{dt} = k = \frac{d\ln L_n/dt}{\ln(L_n/L_{n+1})} - \frac{d\ln(L_n/L_{n+1})/dt\ \ln(L_n/LR)}{\ln^2(L_n/L_{n+1})}$$

The last term of this expression is zero at the moment that the length of the n–th leaf passes the reference length. Because $d\ln L_n/dt$ represents the relative length growth rate RT of the leaf on basis of time, the leaf production rate k can also be written as

(4.2) $k = RT/\ln(L_n/L_{n+1})$

The relative length growth rate on basis of plastochron, RP, is defined by

$$RP = d\ln L_n/dP$$
or
$$RP = d\ln L_n/dt \times dt/dP$$
or

(4.3) $RP = RT/k$

Combining 4.2 and 4.3 shows that

(4.4) $RP = \ln(L_n/L_{n+1})$

Thus, upon passing the reference length, the relative length growth rate on basis of plastochrons of any leaf at reference length is equal to the logarithm of the ratio between its own length and the length of the next older leaf. This identity is of fundamental importance because it relates a time-variable (RP) with a position variable $(\ln(L_n/L_{n+1}))$.

The plastochron ages of the cucumber plants in the present experiment, are given in Fig. 7 as a function of the time since sowing for a reference length of 1 mm and 10 mm. The plastochron age for the smallest reference length is of course the highest. It increases linearly with time for each light treatment except for a short time after germination. This initial lapse phase is longer for the reference length of 10 mm, but later the leaf production rate is the same as for the 10 times smaller reference length. In this paper, the reference length of 1 mm is further used as a standard. The constant leaf production rate under constant environmental conditions has often been observed (Sunderland and Brown, 1956; Milthorpe, 1959; Newton, 1963; Bensink, 1971) for lupine, cucumber and lettuce. However, there are exceptions. For instance, the leaf production rate of fasciating varieties of lettuce increases exponentially with time (Spitters, pers. comm.).

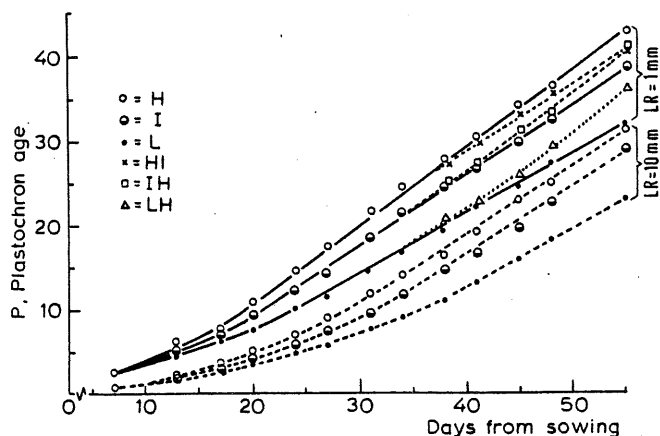The rate of leaf production at the stationary phase, k_s, was 0.88, 0.83

Fig. 7. Increase of Plastochron age (P) of cucumber plants grown under different light conditions. A Leaf Reference length (LR) of either 0.1 or 1 cm is used. For further explanation see text. Symbols used as in Fig. 1.

and 0.69 day^{-1} for the H, I and L light treatments, respectively, and after adjustment to the change, these rates were 0.84, 0.89 and 0.87 day^{-1} for the HI, IH and LH treatment. Hence, the adjustment is complete. For older plants, the lines for the two reference lengths are parallel, so that these stationary rates do not depend on the reference length. This means that young leaves accumulate at first in the size class 1 to 10 mm, but not so later on.

The duration of the initial lag phase in leaf production is longer under lower radiation when expressed in real time, but appears to be the same when expressed in plastochrons. Its duration is about 7 plastochrons for the reference length of 1 mm and about 10 for the reference length of 10 mm.

It may be seen in Fig. 7, that the value of k($=$dP/dt) is about 0.2 at plastochron age 1; the adjustment may now be described by:

(4.5) $k = (1 - \exp(-P/\lambda_p))k_s$ for $P > 1$

in which λ_p is the plastochron constant of adaptation and k_s is the stationary value of k. The value of λ_p is about 3 plastochrons for a reference length of 1 mm and is independent of the radiation intensity. After the initial lag phase, the adaptation to k to changing conditions appears to be instantaneous. This is more or less confirmed by measurements of Milthorpe (1959) with cucumber and measurements of Bensink (unpublished) with lettuce.

Newton (1963) found the following relation for cucumber under constant conditions between the rate of leaf production, k, and the visible daily total radiation ID in cal (4.18 Joule) cm^{-2} day^{-1}:

(4.6) $k_s = 0.584 + 1.02 \times 10^{-2} \text{ID} - 5.45 \times 10^{-5} \text{ID}^2$

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The leaf production rates calculated with this equation without adaptations of constants equal within 5% the observed production rates in the present experiment.

The leaf production rate is constant over time in spite of the large changes in net assimilation (Table 1), so that the leaf production rate is not affected by the light level through the assimilation rate of the plant as a whole. Perhaps it depends on the assimilation rate of the just unfolded leaves and the leaves that enclose the terminal bud (Milthorpe, 1959; Bensink, 1971).

Milthorpe (1959) determined the influence of temperature on the leaf production rate at two light intensities. His data are presented in Fig. 8, the data being normalized by setting the value at 24 °C at 1. The curves are extrapolated to 12 °C because Milthorpe found that below this temperature the rate of unfolding of the leaves was zero. The curves for the two light intensities are the same so that any interaction between the influence of light and temperature on leaf production rate appears to be negligible.
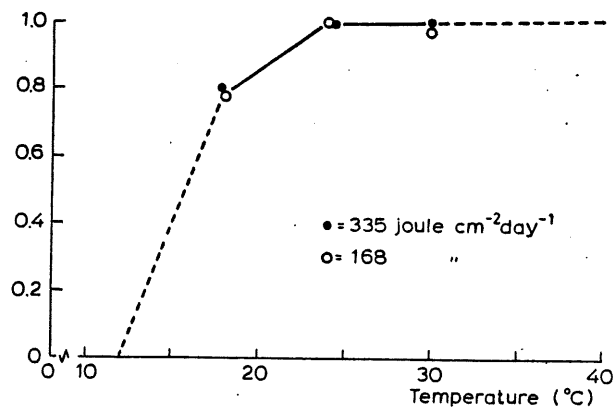

Fig. 8. The relative leaf production rate of cucumber plants at two light intensities as a function of temperature. Data of Milthorpe (1959) are used. Leaf production at 25° is given the value 1.

4.2. GROWTH IN LENGTH OF SUCCESSIVE LEAVES

The growth in length and the growth in weight of successive leaves along the main axis as a function of time are given in Figures 9 and 10. The weights are given on a logarithmic and the length on a linear scale for the various treatments of radiation.

The length growth at each treatment is initially exponential, subsequently linear and then levels off. The length growth curves for the successive leaves at all treatments are initially widely spaced, then their distances narrow until a stationary phase is attained where the curves are spaced at even distances in time. The leaf production rate exceeds the unfolding
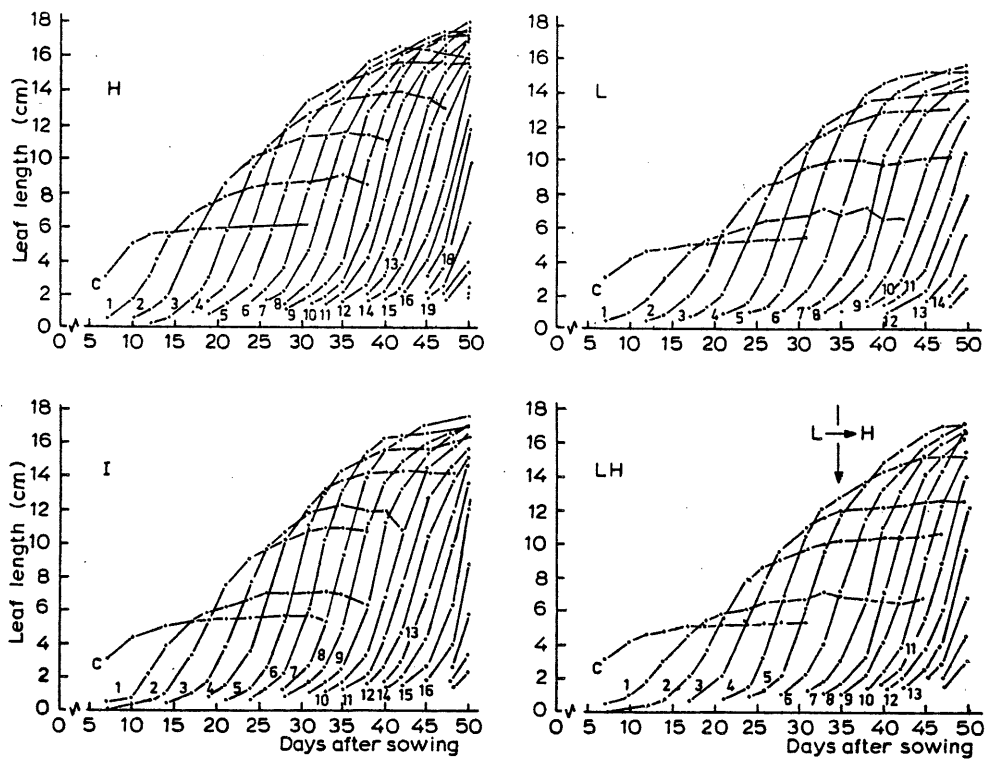
Fig. 9. The growth in length of successive leaves along the main axis of cucumber plants grown under a high (H), an intermediate (I), and a low (L) irradiance, and after a change from a high to a low irradiance at the 34th day after sowing (LH).
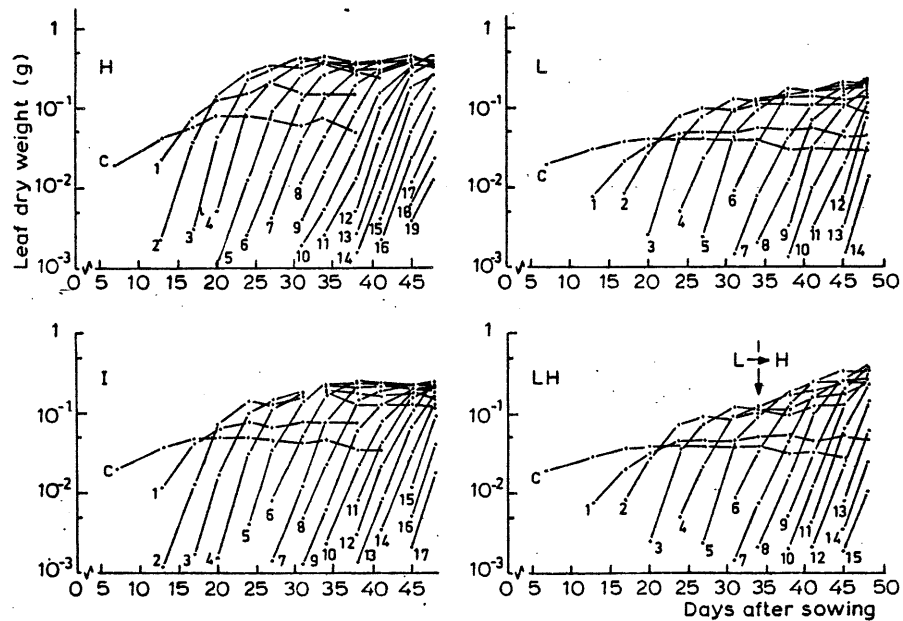

Fig. 10. The growth in dry weight of the same leaves of Fig. 9. Weights presented on a logarithmic scale.

in the earlier phase of growth which leads to an accumulation of primordial leaves on the apex. In the later stationary stage the production and the unfolding rates are the same so that no further accumulation occurs. It should also be noted that the length of the period of exponential growth and the final leaf size increase with increasing leaf number, but these growth aspects also reach a stationary stage.

The influence of the light treatment on the final length is rather small but much larger for the weight growth (Fig. 11). The sudden change of the radiation intensity had only a small effect on the length growth of actively growing leaves, but no effect on that of the maturing and matured leaves. Its influence on the weight of the actively growing leaves was, however, large. Moreover, it affected appreciably the weight growth of maturing and seemingly matured leaves.
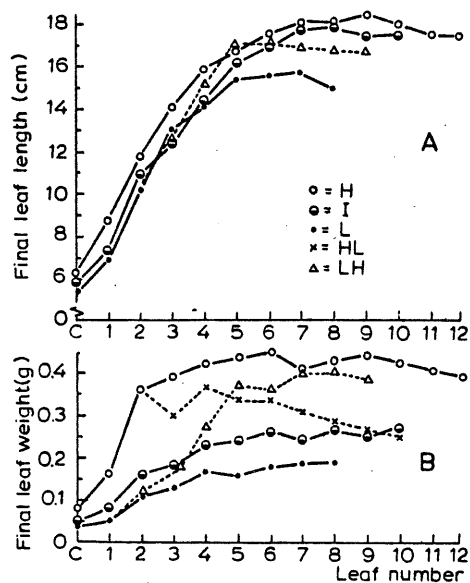

Fig. 11. The final length (A) and final dry weight (B) of successive leaves along the main axis of cucumber plants grown under different light conditions. Symbols used as in Fig. 1.

At first, the final dimensions increase with time but later on, that is the right hand side of the figure, a constant level is reached. This reflects again the stationary character of growth at this stage. The large effect of the light level on the growth of weight and its small effect on the growth in length is again apparent in the end results.

After the change in light level on the 34th day, the younger leaves

(higher leaf number) were exposed for a longer time of their growth period to the new light regimes than the older leaves. That was reflected in the more fully completed adjustment of their final weight. The weights of the 7th, 8th and 9th leaf of LH treatment adapted almost completely to the new light level, but their adaptation in length is still far from complete.

(To be continued)

A formal template for the development of cucumber in its vegetative stage.   II

by T. Horie[1,2], C. T. de Wit[1,3], J. Goudriaan[1] and J. Bensink[4]

1. *Department of Theoretical Production Ecology, Agricultural University, Wageningen.*
2. *National Institute of Agricultural Sciences, Nishigahara. Tokyo, Japan.*
3. *Centre for Agrobiological Research (CABO), Wageningen.*
4. *Department of Plant Physiological Research, Agricultural University, Wageningen.*

4.3. *Interdependences of leaf growth rates*

Linear relations result for many species when the logarithm of the length of successive leaves is given as a function of leaf number, as has been shown by Sunderland and Brown (1956) for *Lupinus*, Erickson and Michelini (1957) for *Xanthium*, Bensink (1971) for *Lactuca*, Pieters (1974) for *Populus* and at the Department of Theoretical Production Ecology by Lof (not published) for *Zea maize*.

This phenomenon is illustrated in **Fig. 12** for the H and LH treatments of cucumber for the various harvest dates. Leaves are numbered stem upwards, and presented along the horizontal axis from left to right. It should be noted that the leaf pattern curves in the left hand side of the figure hold for young plants and in the right hand side of the figure for old plants, but that along each curve, the points at the left and in the top of the figure concern old leaves and at the right and at the bottom concern young leaves.

Two linear parts and one curvilinear part may be distinguished in each curve. The leaf length at the transition of the curvilinear to the adjoining linear part is about two third of the final length of the leaf. The length of the leaves at the point of intersection of the two linear segments increases with time, but this increase levels off at later harvests. To avoid any further confusion in terms, the leaves along the first linear segment are
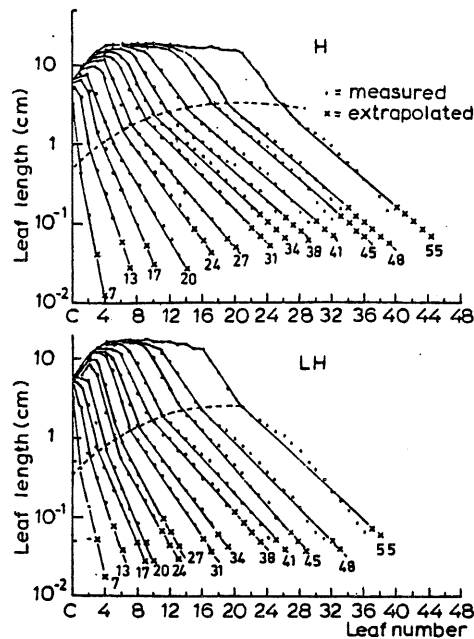
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Fig. 12. Leaf pattern curves for leaf length: the relation between the logarithm of leaf length and serial leaf number of cucumber plants at successive harvests. Examples are given for the High (H) and Low-High (LH) light treatment. The numbers refer to the day of harvest after sowing. Further details in the text.

called primordial leaves and along the second linear segment expanding leaves. The lines formed by the primordial and expanding leaves are referred to as line A and B, respectively. Similar relations as for leaf length occur for leaf area and leaf weight, as is illustrated in Fig. 13 for the H-treatment and the harvest on the 31st day. It appears that the 5th leaf is at the inflection point between the curvilinear and the linear parts and the 9th leaf is at the inflection point of the two linear parts.

It was inferred from the observations that the inflection between the linear parts occurred at the moment that the leaf unfolded or disengaged itself from the primordial leaves stacked at the apex. It is also seen in the figures that at first the slope of the lines A and B decreases and the length of the leaf at unfolding increases with increasing age of the plants. Older plants reach a stationary stage where the slope A and B and the length of the leaf at unfolding remain constant and the number of primordial leaves does not increase further.

The linear relations are maintained under varying conditions of temperature and light as is shown in Fig. 14 which concerns the cucumber variety Ohgen, grown in Tokyo (Japan) practically in the open. The relation between the length of the unfolding leaf and the number of leaf primordia accumulated at the apex is also the same as for the plants
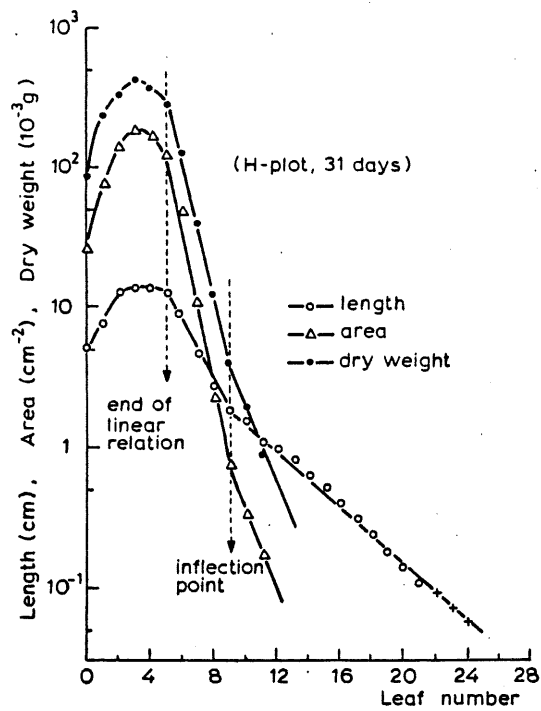
Fig. 13. The relation between the logarithm of leaf length, leaf area and leaf dry weight and leaf number as determined at day 31 after sowing for a plant grown at the High (H) light treatment.
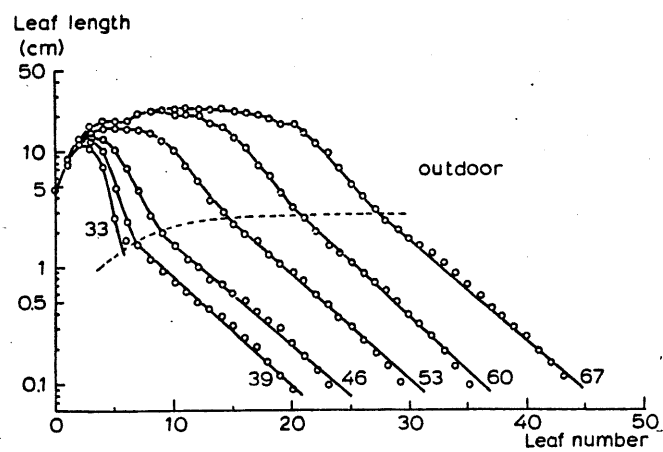

Fig. 14. Leaf pattern curves for leaf length: the relation between the logarithm of leaf length and serial leaf number at successive harvests. Data of cucumber plants grown in the open in Japan. Numbers refer to the day of harvest after sowing.
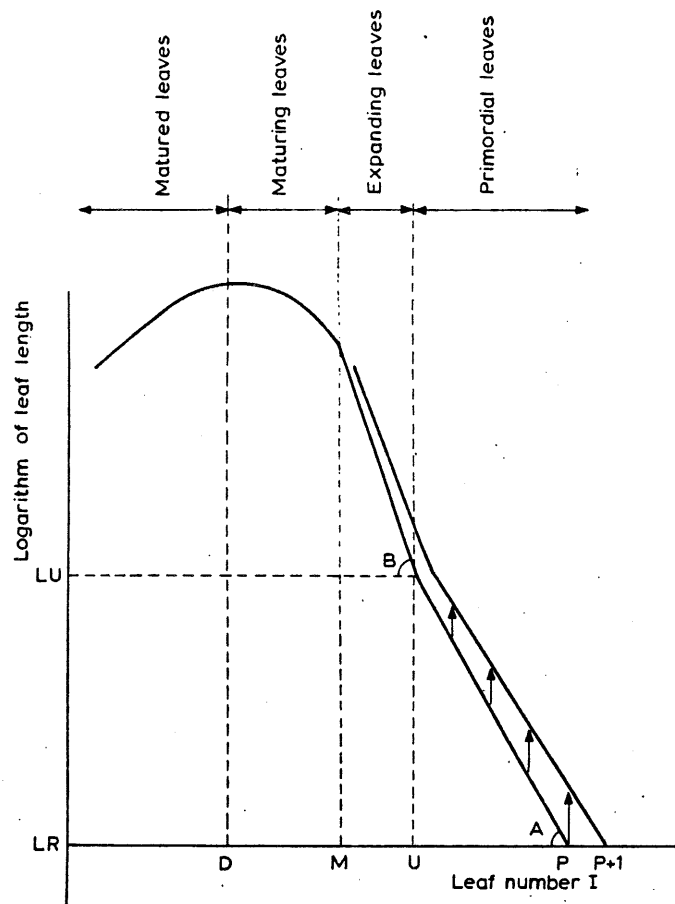
Fig. 15. A leaf pattern curve: the relation between the logarithm of leaf length and serial leaf number, schematically drawn for a plant of plastochron age P, and partly for a plant of plastochron age P+1. The reference length LR is used as the base line. The leaves I are numbered stem upwards from left to right. Those with number $U < I < P$ are considered as primordial leaves, those with leaf number $M < I < U$ as expanding leaves, those with leaf number $D < I < M$ as maturing leaves, and those with $I < D$ as matured leaves. The vertical distances between the two A-lines, drawn one plastochron apart, represent the relative growth in length of the successive primordial leaves during that plastochron.

grown in the phytotron. However, the final leaf length is considerably larger.

A leaf pattern curve for a plant at plastochron age P is schematically presented in Fig. 15 with the reference length LR as the base line. This figure defines the four phases of leaf growth that are distinguished. The leaf with leaf number U passes from the primordial to the expanding phase, the one with leaf number M from the expanding to the maturing phase and with leaf number D from the maturing to the mature phase. The latter two boundaries are more arbitrarily chosen than the first, as

will be discussed later. $D < M < U < P$, since the leaves are numbered stem upwards. The length of the i-th leaf from a plant with plastochron age P is presented by the symbol $L_{p,i}$, i being smaller than P.

The following equation can now be written for the linear relation between the logarithm of the length of the leaves in the primordial phase and their number:

(4.7) $\ln L_{p,i} = \ln LR + A (P - I)$ for $U \leqslant I \leqslant P$

it being supposed that the leaves are initiated at a reference length LR and a positive sign being allotted to the slope A.

The defining equation for the relative length growth rate on time basis of the i-th leaf on a plant with plastochron age P is

(4.8) $RT_{p,i} = \dfrac{1}{L_{p,i}} \dfrac{dL_{p,i}}{dt} = \dfrac{1}{L_{p,i}} \dfrac{dL_{p,i}}{dP} \cdot \dfrac{dP}{dt} = \dfrac{d\ln L_{p,i}}{dP} \cdot k$

The symbol k stands for the leaf production rate $\dfrac{dP}{dt}$.

Taking into account that the derivative of $(P - I)$ with respect to P equals 1, it is found upon substitution of eq. 4.7 in 4.8 and differentiating that

(4.9a) $RT_{p,i} = kRP_{p,i}$

(4.9b) $RP_{p,i} = A + \dfrac{dA}{dp} \cdot (P - I)$ for $U \leqslant I \leqslant P$.

Since the value of A decreases with increasing plastochron age $(dA/dP \leqslant 0)$ the relative length growth of the leaves on basis of plastochrons decreases linearly with the leaf number. This is also schematically shown in Fig. 15, where two A-lines one plastochron apart are drawn and the growth of the successive leaves during one plastochron is presented by the vertical arrows between these lines. As has been said, the slope of A approaches a stationary value, A_s. Then, dA_p/dp is zero and equation 4.9b reduces to

(4.10) $RP_{p,i} = A_s$

which means that the relative length growth rates on basis of plastochrons of all leaves in the primordial phase are the same and do not change with time. Only then, do all the leaves grow with the same constant relative growth rate on basis of real time, provided that the leaf production rate k is constant.

It may be seen also in Fig. 15 that the logarithm of the leaf length

in the expanding phase equals:

(4.11) $\ln L_{p,i} = \ln LR + A(P-U) + B(U-I)$ for $M \leqslant I < U$

in which B and A are the two slopes and U the number of the leaf which is just unfolding. This expression yields upon differentiating:

(4.12) $RP_{p,i} = A + \dfrac{dA}{dP}(P-U) + \dfrac{dB}{dP}(U-I) + (B-A)\dfrac{dU}{dP}$

The additional term $(B-A)\,dU/dP$ signifies a sudden increase in growth rate upon unfolding. For older plants dU/dP equals one and then this sudden increase is equal to $(B-A)$. In this stationary state dA/dP and dB/dP approach zero so that then the relative growth rate on plastochron basis of the unfolding leaves equals $RP_{p,i} = B_s$.

B_s being the stationary value of B.

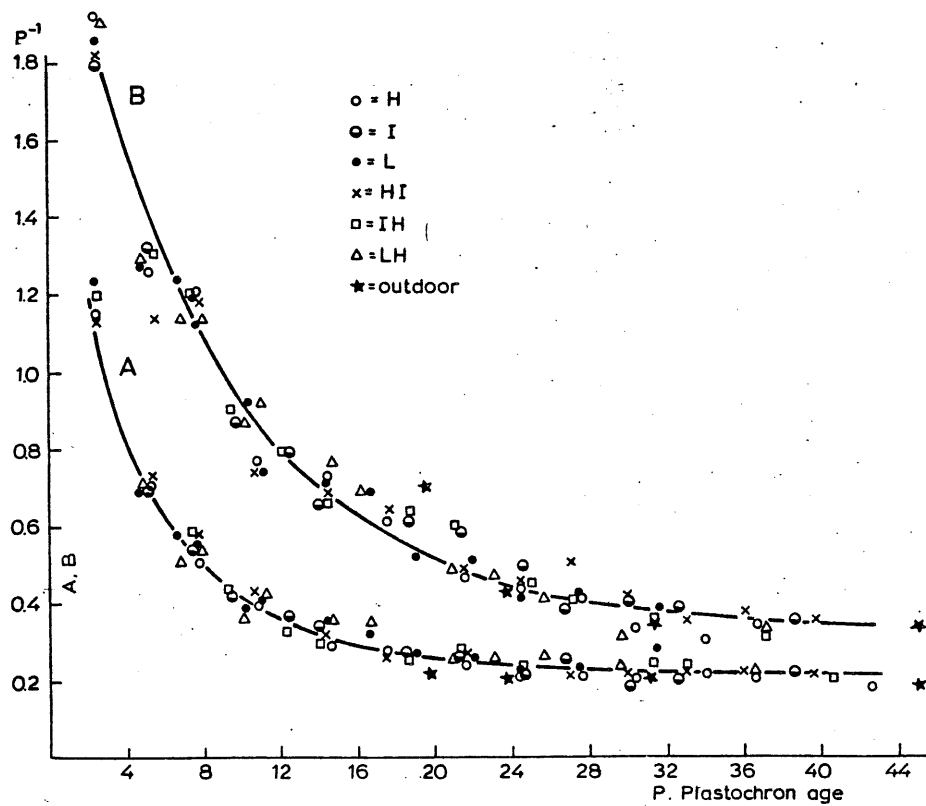For younger plants, the number of primordial leaves increases with

Fig. 16. A and B (see Fig. 15) as a function of the plastochron age P of the plant, for cucumber plants grown under different light treatments indoor and in the open field in Japan. Symbols used as in Fig. 1.
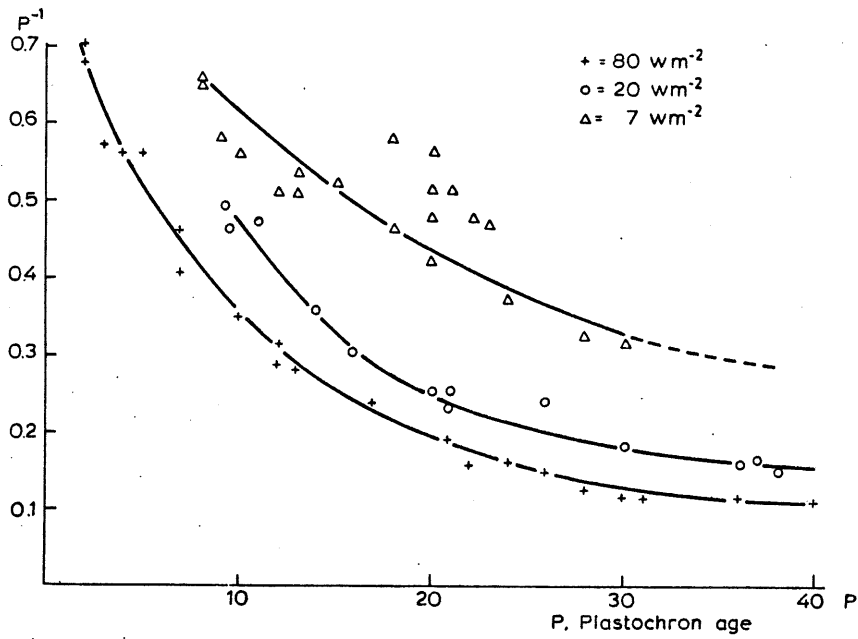
Fig. 17a. A as a function of the plastochron age P of the plant, as determined for lettuce plants grown at three light intensities at one temperature (17a), and five different temperatures at one light intensity (17b). (Partly unpublished data of Bensink, 1971).
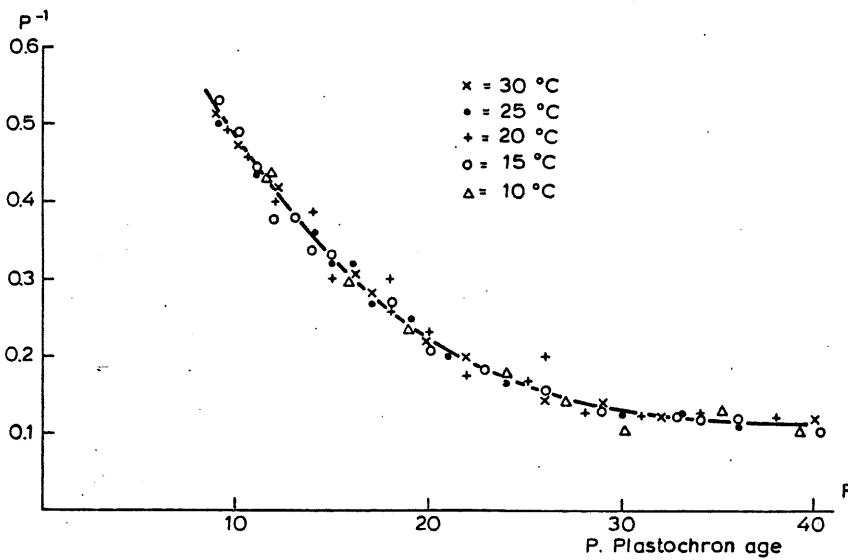

Fig. 17b. A as a function of the plastochron age P of the plant, as determined for lettuce plants grown at three light intensities at one temperature (17a), and five different temperatures at one light intensity (17b). (Partly unpublished data of Bensink, 1971).

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age, so that dU/dP is smaller than one and the sudden increase in growth rate upon unfolding accordingly is smaller than (B −A).

The value of the two variables A and B are presented in Fig. 16 as a function of the plastochron age of the plants grown under different radiation treatments in the phytotron and in the field in Japan. It appears that neither A nor B are affected by the growth conditions. In other words, at the same plastochron age, the slopes of the straight segments of the leaf pattern curves in Fig. 12 are the same, irrespective of radiation.

Experiments at different temperatures were not done, but the reasonable agreement of the results of the experiments in the field where the temperature varied and in the phytotron where the temperature was kept constant, suggests strongly that these two basic parameters are also not affected by temperature. This suggestion is supported by experimental results of Hofstra, Hesketh and Myhre (1977), obtained with soybeans under different temperature regimes. They found that the rate of leaf expansion determined at the linear phase of growth is very much dependent on temperature when expressed in time units, but independent of temperature when expressed on basis of the leaf production rate. They found also that the relative growth rate of the leaf length is proportional with the leaf production rate. In terms of the present interpretation, these results can only be understood by assuming that the values of A and B are independent of temperature.

Reconsidering the partly unpublished observations with lettuce of Bensink (1971), it was found that a similar decrease of the slope of the leaf pattern curve in the primordial phase was independent of the temperature in the measured range between 10 and 30 °C. However, it was affected by light intensity, as illustrated in Fig. 17 a and b.

It appears in Fig. 16 that both A and B decrease approximately according to a negative exponential function to constant values. The decrease can be described by the functions:

(4.13) $A = A_s + A_d \exp(-P/\lambda_a)$

and

$$B = B_s + B_d \exp(-P/\lambda_b)$$

A_s and B_s in these equations are the stationary values of A_p and B_p for older plants ($P = \infty$) and ($A_s + A_d$) and ($B_s + B_d$) are the values of A and B at P equal to zero. The parameters λ_a and λ_b are the plastochron constants of adjustment.

Regression analyses resulted in the following estimates:

$A_s = 0.22$ $A_d = 1.17$
$B_s = 0.34$ $B_d = 2.07$
$\lambda_a = 5.7$ $\lambda_b = 8.0$

The weight of the leaves was only determined in the expanding phase

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(Fig. 10). It appears that the leaf pattern curve in this phase is also straight and that B-curves for the three light intensities are also the same although the scattering is larger, as illustrated in Fig. 18.

To describe the leaf pattern curve in the primordial and expanding phase fully, it is necessary to characterize the position of the A and B slopes with respect to each other at any plastochron age. This may be done by means of the size of the successive unfolding leaves, which could have been measured during the experiment. This was not done because there is considerably ambiguity in the determination of the moment of unfolding and the importance of the measurement was not fully grasped. Therefore the length of each leaf at the moment of unfolding (LU) had to be estimated after the event from the position of the intersects of the A- and B-slopes (see Fig. 12), so that the results are confounded by the estimates of the slopes themselves. They are also inaccurate for the leaves of younger plants, because then the slopes are steep and based on only a few observational points, as may be seen by inspection of Fig. 12. In fact, it appears that below a plastochron age of 8, the readings are too much effected by errors of judgement to be useful. Therefore, only the result of the exercise for plants older than 8 plastochrons is presented in Fig. 19 as the logarithm of the length of the unfolding leaf, normalized
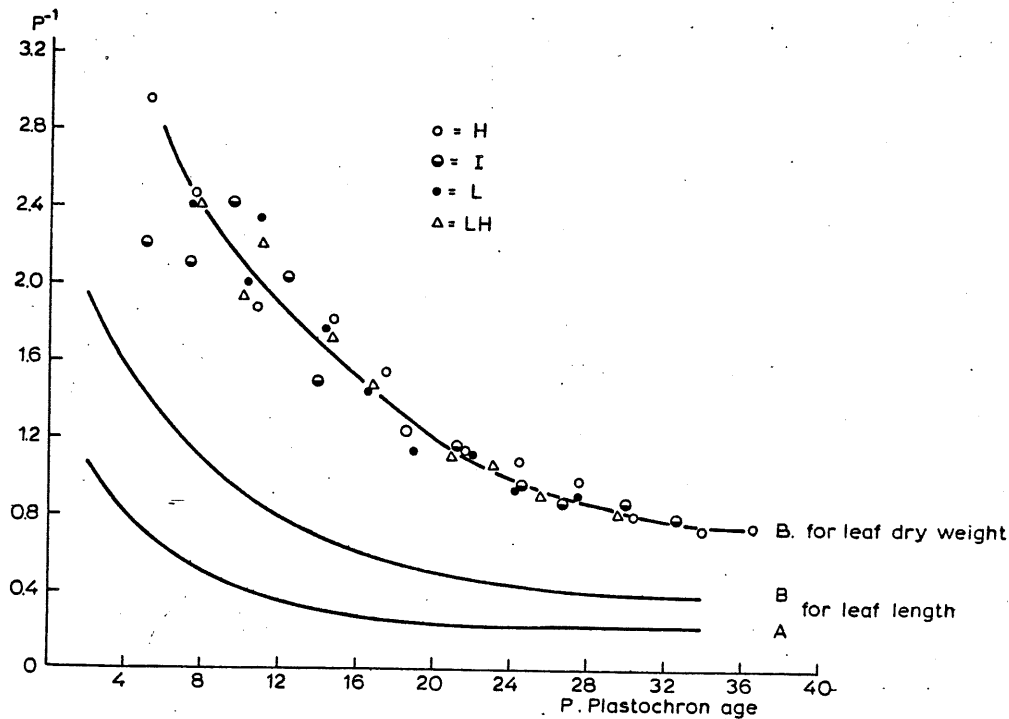

Fig. 18. B for dry weight presented as a function of the plastochron age of the plant, for cucumber plants grown under High (H), Intermediate (I), and Low (L) irradiance. The A and B curves presented in Fig. 16 are also given.
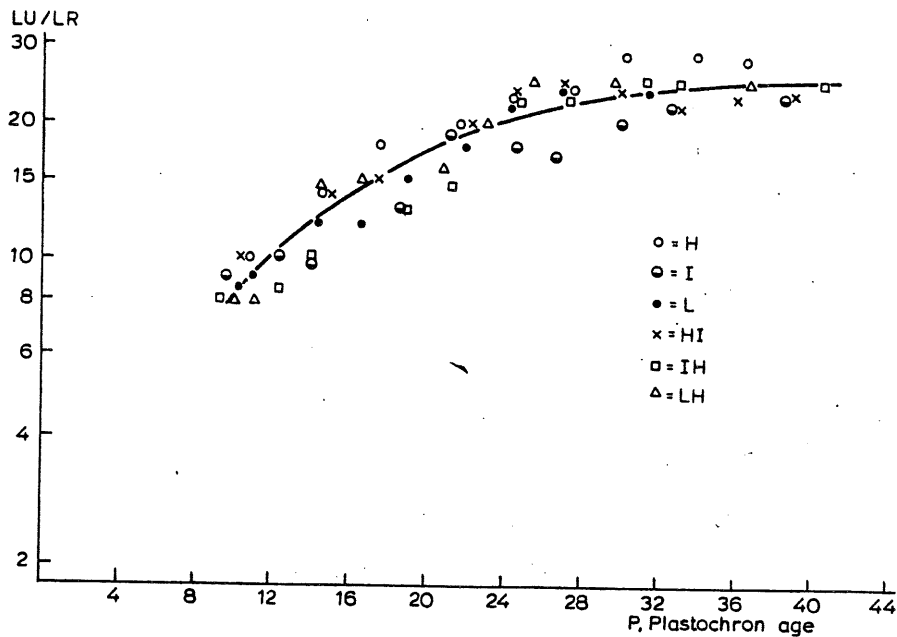
Fig. 19. The length of the unfolding leaf (LU), normalized with respect to the reference leaf length (LR), that is as LU/LR, presented on a logarithmic scale as a function of the plastochron age of the plant (P), for cucumber plants grown at different light treatments. Data are derived from the intersects of the two linear lines as is shown for the "H" and "LH" light treatment in Fig. 12. Only values of plants older than 8 plastochrons are given. Symbols used as in Fig. 1.

with respect to the reference leaf, that is as ln(LU/LR). The scattering is larger than for the A- and B-slopes in Fig. 16, but again the conclusion is justified that the curve is not affected by the radiation treatment.

The position of the A- and B-slopes with respect to each other may be also characterized by the serial number of the unfolding leaf (U) in relation to the plastochron age, as is done in Fig. 20. The readings below plastochron age 8 are again omitted for the reason already explained. Indeed, the P, U function and the P, ln(LU/LR) function in Fig. 19 and 20 are related through eq. 4.7 which may be rewritten as:

(4.14) $U = P - \ln(LU/LR)/A$

so that both functions cannot be determined independently of each other. The leaf length function can be presented by the equation

(4.15) $\ln(LU/LR) = C_s - C_d \exp(-P/\lambda_c)$

C_s is the stationary value of $\ln(LU/LR)$, $C_s - C_d$ the value of $\ln(LU/LR)$ at P is zero and λ_c the plastochron constant of adaptation.
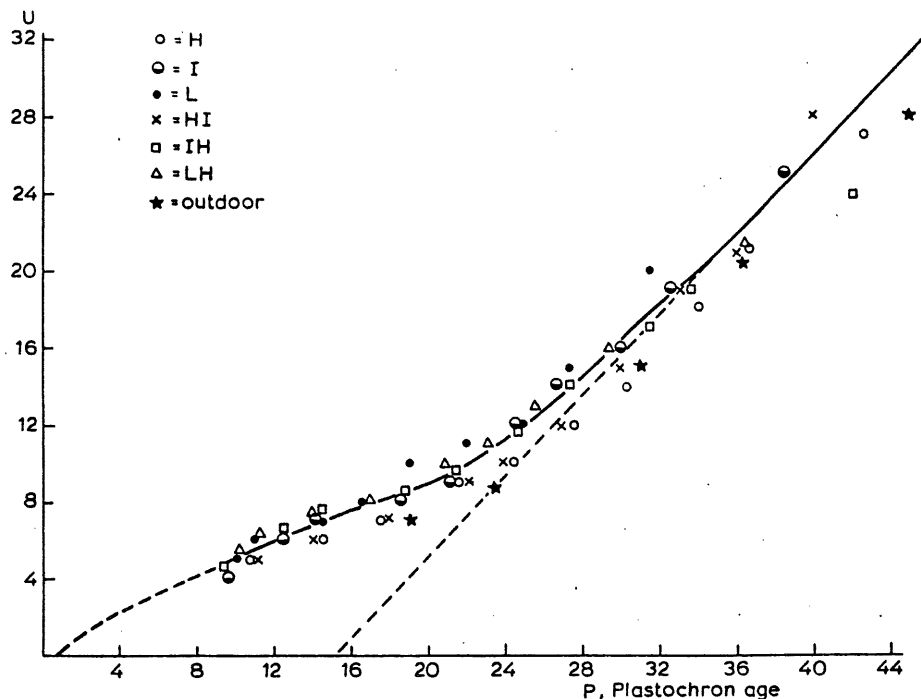
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Fig. 20. The serial number (U) of the unfolding leaf as function of the plastochron age of the plant, for cucumber plants grown at different light treatments. Data derived from curves as presented in Fig. 12, only for plants older than 8 plastochrons. Symbols used as in Fig. 1.

The P, U function is then presented by

$$(4.16) \quad U = P - (C_s - C_d \exp(-P/\lambda_c))/(A_s + A_d \exp(-P/\lambda_a))$$

The difference between P and U approaches to the constant value C_s/A_s with increasing P and it is directly seen from the position of the 45° line in Fig. 20 that this value amounts to about 15. With $A_s = 0.22$, this means that the value of C_s equals 3.2 and LU/LR 25, in agreement with the maximum value that is approached with increasing P in Fig. 19.

The value of the plastochron constant λ_c can be directly estimated from the curvature in Fig. 19, whereas C_d is estimated from the position of the curve. With some iterations and comparison with the data in Fig. 20 it was found that $C_s = 3.30$, $C_d = 3.30$ and $\lambda_c = 10$.

The resulting curves are presented in Fig. 19 and 20. It should be noted that the P, U curve does not pass through the origin; at P equal to zero, U equals $-(C_s - C_d)/(A_s + A_d)$ and is thus negative. The apparent irregular shape of this curve is due to the circumstance that the transformed time constants of the two determining functions in eq. 4.16 are different. Finally, it is to be noted that the position of the observations for outdoor plants deviates from the calculated relation between U and P.

The functions for ln(LU/LR) and U can now be used to calculate the logarithm of the length of each leaf at the moment of unfolding, again normalized as ln(LU/LR), in dependence of its leaf number. The result is presented in Fig. 21 by the discrete series of latin crosses. It concerns here a property of the leaf and not of the plant, so that leaf number and not plastochron age is given along the horizontal axis.
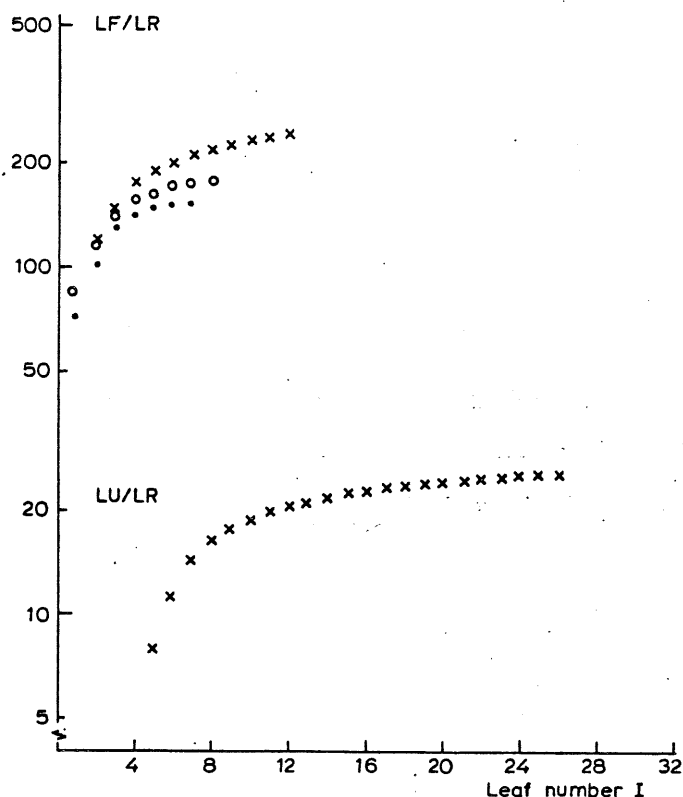

Fig. 21. The length of each leaf (L_l) at the moment of unfolding, normalized with respect to the reference length LR=1 mm (LU/LR) as calculated by eq. 4.15 and 4.16, and the experimentally determined final leaf length, again normalized as LF/LR, for three different light conditions; "L", "H" and "outdoor".

The other main characteristic of the leaf pattern curve is the final leaf length, i.e. the envelope of the family of curves in Fig. 12. The logarithm of this final leaf length, normalized as ln(LF/LR) is also presented in Fig. 21 for the L, H, and outdoor conditions. Here two distinct effects of the environmental conditions are apparent. The lengths of the leaves of the plants grown outdoor are distinctly above and of the leaves of the plants grown at low light distinctly below the average. Otherwise it is noted that the scattering in Fig. 12 for the observations of the final leaf size

is relatively small compared with the scattering in Fig. 19 for the unfolding leaves and this suggests that the latter must be described for the greater part to observational errors.

5. CELL DIVISION AND CELL EXPANSION

5.1. *Final leaf size and epidermal cell number*

The average final size of epidermal leaf cells of plants well supplied with water and nutrients is to a large extent independent of the light level and assimilation. Exceptions are reported, but Pieters (1974) has shown that these can often be attributed to adverse growing conditions, especially water stress induced by high radiation levels.

Final leaf size depends therefore on the epidermal cell number and this number is determined in the earlier phases of growth. This was shown very clearly by Bensink (1971) who related the width of lettuce leaves during growth with the average cross section of the epidermal cells, his results being presented in Fig. 22 for 12th lettuce leaves grown at different light levels. At first the leaf width increases, but the crossection of the epidermal cells remains the same. Later on leaf width and crossection increase strictly proportionally. It is also seen that the size of the dividing cells and of the fully expanded cells is independent of the light level. The ratio of the square root of the area is about 4 to 5 in all cases and this ratio was also observed for cucumber (Wilson, 1966) and Xanthium (Maksymowych, 1973).
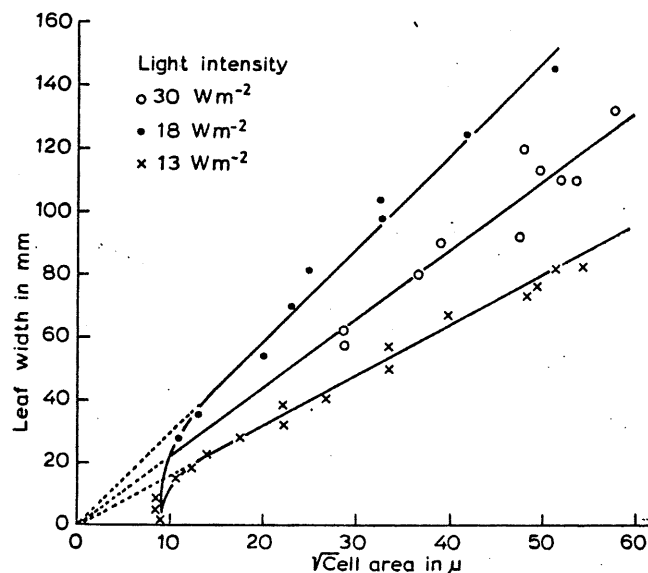

Fig. 22. Relation between leaf width and epidermal cell size for the twelfth leaf of lettuce plants during their growth at three light intensities. (Bensink, 1971).

Bensink (1971) observed no discontinuity in the leaf pattern curve during the transition from the dividing to the expanding phase, Fig. 23, which implies a continuity in growth rates (eq. 4.12). It is therefore not correct to state that a leaf grows first by cell division and then by cell elongation. Instead, it should be stated that leaves grow in length and that this growth goes at first together with cell division but not so later on (Haber and Foard, 1964).
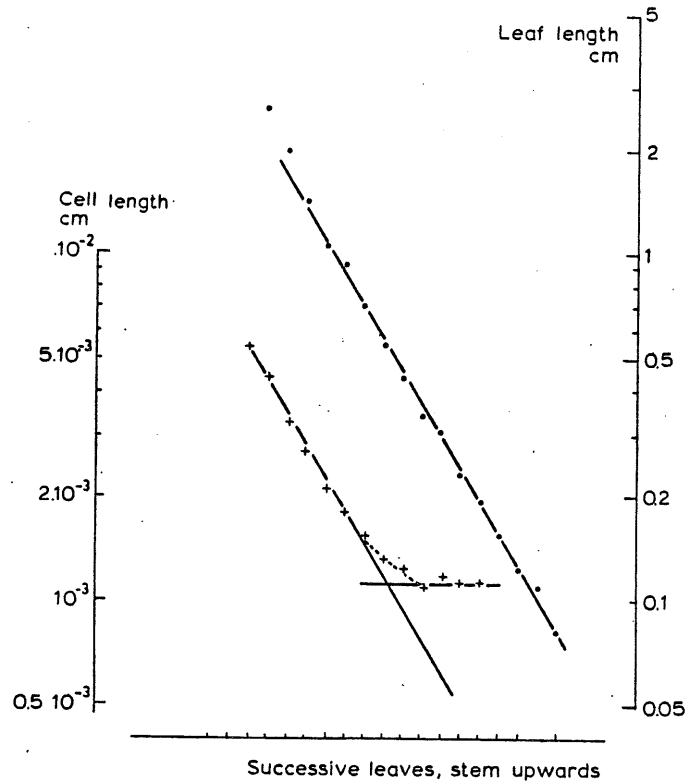

Fig. 23. The length (.) of successive primordial leaves (stem upwards) of a lettuce plant and the corresponding average epidermal cell length (+) on the midrib presented on logarithmic scales (Bensink, 1971).

The same phenomena have been firmly established for cucumber. Wilson (1966) observed that the diameter of dividing cells is constant (roughly 8.10^{-4} cm) and data from Milthorpe and Newton (1963) show that the fully expanded cells are 4 times larger. Newton (1963) and Wilson (1966) found that the final cell size of cucumber is hardly, while the number of cells greatly affected by light intensity. Milthorpe and Newton (1963) showed that nutritional deficiency leads to smaller final cell size and this emphasizes again that it is prudent to restrict the analyses at first to situations where water and nutrient stresses are absent.

5.2. Relative cell division rate and leaf production rate

The above analysis implies that the relative cell division rate of the epidermal cells along the midrib is the same as the relative length growth rate in the stage of cell division. And since the relative length growth rate is proportional to the leaf production rate, this implies also proportionality between leaf production rate and relative cell division rate of the leaves, irrespective of the growing conditions.

This is illustrated by a rearrangement of data on cucumber from Newton (1963), Milthorpe and Newton (1963) and Wilson (1966). They cultivated plants of the same cultivar at 24 °C under the same nutritional conditions, but at different light levels. The dependency of the rate of leaf production on the radiation was according to the quadratic eq. 4.6 of Newton (1963). Milthorpe and Newton (1963) and Wilson (1966) determined the mean rate of relative cell division of the third leaf during the primordial stage, again as a function of the irradiance level. The results shown in Fig. 24 were obtained by combining both data sets and this confirms that a proportionality exists between leaf production rate and relative cell division rate. Now it may be observed in Fig. 25 that the weight is proportional to the length to the third power around the time of unfolding. This indicates a random cleavage in the primordial phase, so that the relative cell division rate in one direction should be one-third of the rate determined by total maceration. For the plants of Fig. 24, this amounts to one-third of 1.0–1.1 day^{-1} or about 0.35 day^{-1}. This value is in good agreement with the mean relative rate of length growth of the
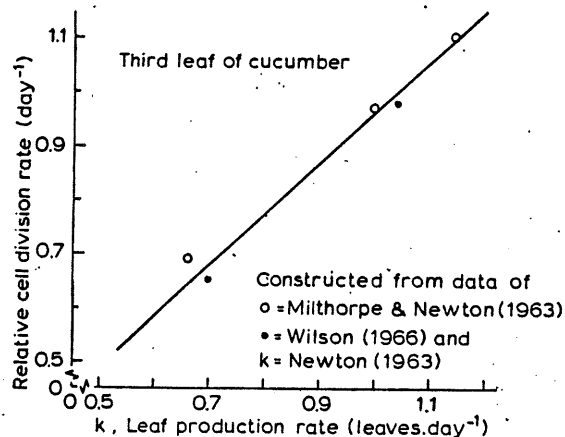

Fig. 24. The linear relationship between the relative cell division rate of epidermal cells along the midrib of the third leaf in the primordial stage and the rate of leaf production as shown by a rearrangement of data on cucumber plants of Newton (1963), Milthorpe and Newton (1963) and Wilson (1966).
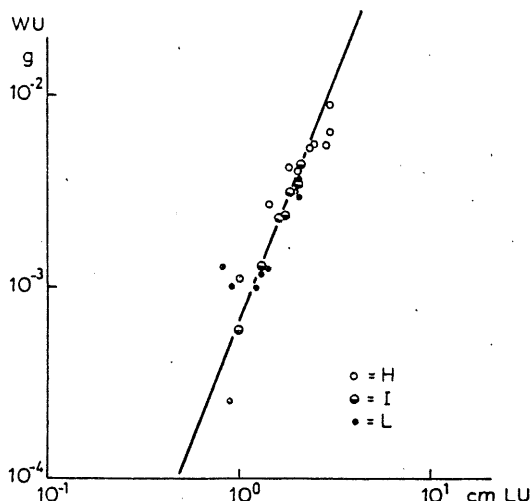
Fig. 25. The relation between the logarithm of the fresh weight (WU) and of the length (LU) of the leaves at the time of unfolding for three light treatments. The line through the observations has a slope 1 : 3.

third leaf of cucumber plants in the primordial phase, calculated by means of equation 4.8. These observations show again the consistency of the data sets for cucumber, irrespective of the experimental situation.

5.3. *Termination of cell division*

The length of the period of cell division is of crucial importance, since the final size of the leaves is determined by the number of cells.

It was already observed by Sharman (1942) that maturation of maize leaves proceeds from the top downwards after they unfold from the stock of sheathes of the preceding leaves. The appearance of the ligule thus marks the end of all meristematic activity of the leaf.

It is also firmly established for cucumber (Milthorpe and Newton, 1963; Wilson, 1966) that cell division begins to decline upon unfolding, although 90 percent of the cells may be formed after that time (Sunderland, 1960). Hence the question remains how rapidly cell division decreases. Milthorpe and Newton (1963) showed that this may be formally described by assuming that the relative rate of cell division of the actively dividing cells is the same before and after unfolding, but that the fraction of dividing cells decreases. They assumed a decay according to a negative logistic function. Dale (1970), however, preferred a negative exponential function. The difference between both functions is large. However, the number of cells after unfolding is reasonably described by taking a middle course. This is illustrated in Fig. 26 for Dale's data with beans, it being assumed that the number of dividing cells decreases linearly from 100% at day 2 to 0% at day 9.

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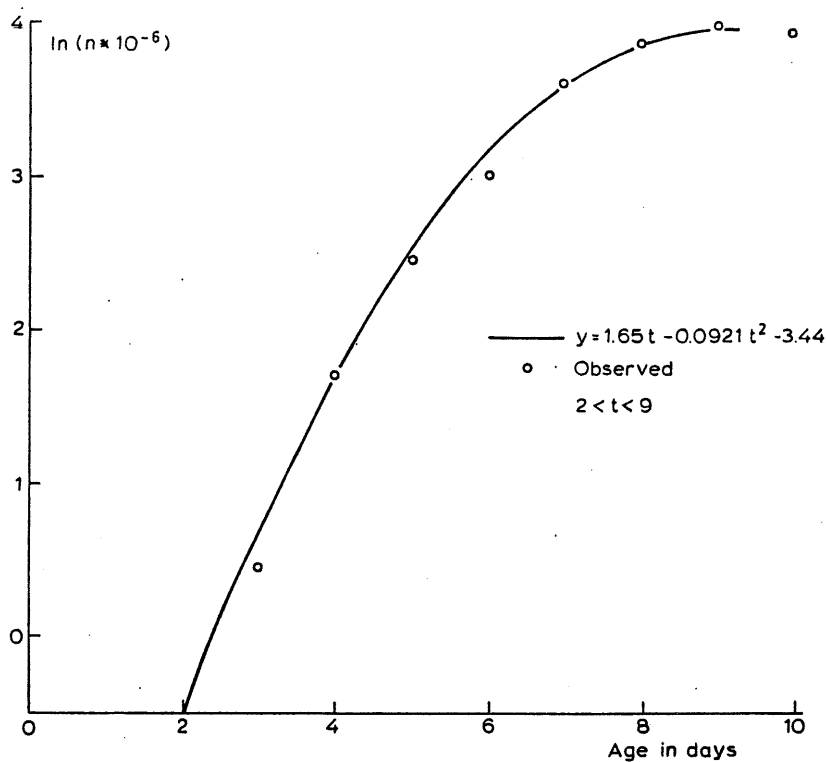
Fig. 26. The increase of cell number with time for the primary leaves of Phaseolus vulgaris, assuming a linear decrease of the fraction of dividing cells. Data of Dale (1969) are used as given in his table and Figure 1.

The decay time for cucumber appears to be in the order of 6 days, but a sufficiently reliable estimate for the present purpose cannot be made on basis of the data of Milthorpe and Newton for two reasons. Cell numbers were determined by maceration and concern thus the total number of cells and this number depends also to a large extent on the growth in thickness during the expanding and maturing phase. Moreover, the onset of unfolding of the leaves was in their analyses arbitrarily set at the moment that a surface of 1 cm^2 was reached.

(To be continued)

A formal template for the development of cucumber in its vegetative stage. III

by T. Horie[1,2], C. T. de Wit,[1,3], J. Goudrian[1] and J. Bensink[4]

1. *Department of Theoretical Production Ecology, Agricultural University, Wageningen.*
2. *Natinal Institute of Agricultural Sciences, Nishigahara, Tokyo, Janpan.*
3. *Contre for Agrobiological Research (CABO), Wageningen.*
4. *Department of Plant Physiological Research. Agricultural University, Wageningen.*

6. FURTHER ANALYSES

6.1. *Another formulation of the regularity of the leaf pattern curve*

The value of $RP_{p,i}$ in eq. 4.9 is geometrically presented in relation to the A versus P function, the latter being redrawn in Fig. 27 for this purpose. According to eq. 4.9, the value of $RP_{p,p}$ that is the relative growth rate of plastochron basis for the leaf at reference length is given by curve A itself.

The value of $RP_{p,i}$ for the older primordial leaves at plastochron age P of the plant, is found by drawing the tangent to the curve at P and reading its value at the position $(P+(P-I)$ at the abscissa. For $I=P-1$ this means reading at $P+1$, for $I=P-2$ at $P+2$ and for $I=I$ at $2P-I$, in conformity with eq. 4.7.

Hence the tangent is used at point x. This point seems close to point y on the curve itself. Indeed, the differences between both assumptions are very small, as is shown in Table 2, where the relative growth rates on plastochron base for the 4th, 8th and 12th leaf are compared for both cases.

This implies that the A-curve may be used directly to determine $RP_{p,i}$, its value for leaf I at plastochron age P being obtained by reading the horizontal axis at $(2P-I)$. Hence, at any moment the value of $RP_{p,p-m}$ for the leaf which was at its reference length m plastochrons in the past,
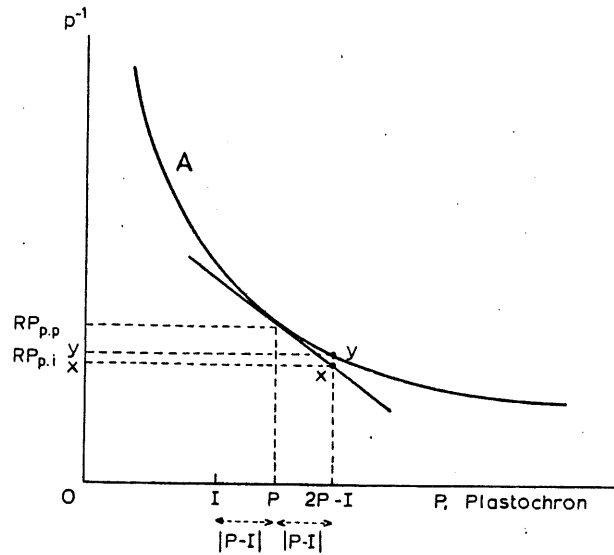
Fig. 27. Two methods to calculate $RP_{p,i}$ on basis of the A-function: X, along the tangent at point P according to equation 4–9b; Y, along the A-function itself.

Table 2. The ratio of the relative growth rates of leaf 4, 8 and 12 in the primordial phase, at different plastochron ages, as calculated for the "X" and "Y" assumption in Fig. 26.

leaf number	plastochron age	Ratio RGR ("X"/"Y")
4	4	1.
	8	.95
8	8	1.
	12	.96
	16	.91
12	12	1.
	16	.97
	20	.95
	24	.93

is the same as the value of $RP_{p+m,p+m}$ for a leaf which will be at its reference length m plastochrons in the future.

These equality relations give an alternative formulation of the regularity of the leaf growth pattern, but do of course not offer an explanation of this regularity itself.

6.2. *The apical unit*

According to the definition of Sunderland and Brown (1956, 1976) an apical unit consists of the primordial leaves and their internodes implanted above the reference leaf plus the apical dome. Older plants are in a

stationary state, characterized by the same relative length growth rate on plastochron basis for primordial leaves. The relative volume growth rate for all primordial leaves on plastochron basis is then also constant and equal to

$$(6.1) \quad RWP = g \cdot A_s$$

As shown in section 5.2, the geometrical factor, g, is about 3. The relative volume growth rate of the primordial laves on a real time basis varies then with the leaf production rate k according to

$$(6.2) \quad \overline{RWT} = \overline{k} \cdot g \cdot A_s$$

$\overline{RWT}$ and $\overline{k}$ being averages per plastochron.

It stands to reason that the relative volume growth rate of the apical unit is the same in the stationary state and this implies a relative increase in weight during one plastochron of exp (gA_s).

The apical unit does not increase in size when the plant is in the stationary stage, so that this volume increase during each plastochron is dissociated from the apical unit through leaf and internode of the newly emerging primordial leaf at the end of each plastochron. This relative increase in volume during one plastochron is thus also equal to

$$(NA + NI + NL)/NA$$

NA is here the number of cells in the apical unit at the beginning of a plastochron and NL and NI are the number of cells dissociated with the reference leaf from the apical unit and its internode at the end of each plastochron. The size of the reference leaf, and with this, NL is per definition constant and so are NA and NI, since the plants are in a stationary state. Obviously, the growth of the apical unit per plastochron is large if the apical unit is small and slow if this unit is large. Combining both expressions gives

$$(6.3) \quad \frac{NA + NI + NL}{NA} = \exp (g\ A_s)$$

or

$$(6.4) \quad NA/(NI + NL) = 1/(\exp (gA_s) - 1)$$

The equilibrium value of A is 0.22 for cucumber and with g = 3, the ratio (NI+NL)/NA equals 0.93, therefore during each plastochron the number of cells within the apical unit practically doubles, the produced amount being dissociated at the end of each plastochron with the new leaf and its internode.

For cucumber, the equilibrium value of A is independent of radiation and temperature. This means that neither the relative size of the apex,

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nor the relative size of the internode is affected by those environmental factors, presumably because both the relative weight growth rate on real time basis and the leaf production rate k are influenced in exactly the same way. However, in case of lettuce, A_s is independent of temperature, but increases with decreasing radiation (Fig. 17). In some experiments of Bensink (1971) it was observed that lettuce at low light intensity showed a marked stem elongation or etiolation. Although cell numbers of internodes were not determined, this etiolation suggests that the relative amount of cells dissociated with the internodes increases with decreasing light intensity, so that less cells come available to the leaf. It takes then more time to reach the reference leaf length (per definition constant), and this leads to a larger decrease in the leaf production rate, k, than in the relative growth rate, so that A is increased. It is therefore concluded that the relative distribution of cells over reference leaf and its internode. is not affected by temperature, but increases in favour of the internode with decreasing light for lettuce, but not for cucumber.

Eq. 6.3 for the stationary phase suggests that the decrease of A in the earlier, transient phase is mainly associated with the increase of the size of the apical unit. This increase was already inferred from the accumulation of leaf primordia between the reference lengths of 10 and 1 mm (Fig. 7), which suggested an accumulation of primordia below 1 mm.

To calculate the size of the apical unit, it is assumed that the slope of the leaf pattern curve for the primordial leaves may be extrapolated beyond the reference length. Hence, the relative length growth rate of the leaves within the apical unit increases linearly with the leaf number, but their size decreases exponentially so that summation of all lengths and volumes may be conveniently done over an infinite number of primordia. According to eq. 4.7

(6.5) $\quad \ln L_i = \ln LR - iA \text{ or } L_i = LR \exp(-iA)$

when $(P - I)$ is replaced by i.

The total length of the leaves in the apical unit is then

(6.6) $\quad \sum_{i=1}^{\infty} L_i = LR \sum \exp(-iA) = LR/(\exp(A) - 1)$

and the total volume of the leaves relative to the volume of the reference leaf is

(6.7) $\quad \sum_{i=1}^{\infty} (L_i/LR)^g = 1/(\exp(gA) - 1)$

With $g = 3$, and by means of the A-curve (Fig. 17), this relative total volume of the primordial leaves in the apical unit is calculated as a function of the plastochron age and presented in Fig. 28. Eq. 6.7 for the relative weight does not contain the reference length. However, the relation between A and plastochron age depends on the size of the reference
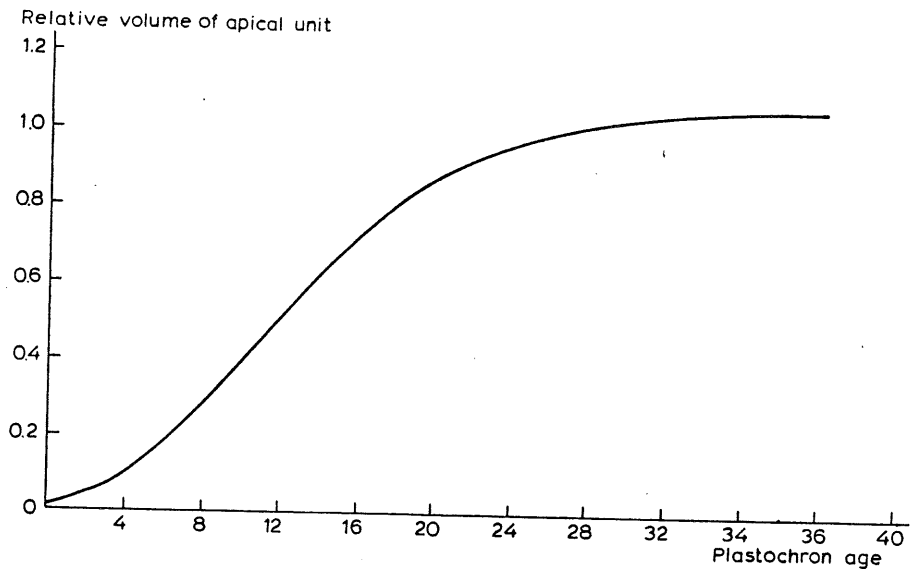
Fig. 28. The volume of the apical unit, relative to the volume of the reference leaf LR, presented as a function of the plastochron age of the plant.

leaf, so that this does not imply at all that the relative increase in size of the apical unit is independent of the length of the reference leaf.

The same calculation could be done for the internode weight, but the data are not available. However, it stands to reason to assume that the relative increase in total weight of the apical unit does not differ much from the increase for the leaf weight only. In that case, eq. 6.4 and eq. 6.7 are the same. The only difference is that for the derivation in the stationary state, it was not necessary to assume that the linearity between the logarithm of leaf size and leaf number also holds for the leaves in the apical unit, as is done with eq. 6.5.

6.3. Final cell number and leaf length

From Dale's measurements (Fig. 26) it may be inferred that from the moment of unfolding onwards, the fraction of dividing cells decreases linearly with time, until all cell division stops. The final leaf length is attained soon thereafter. The time span during which cell division still proceeds after unfolding may change slowly with leaf number and thus as a function of the development stage of the plant. For leaf number 4 it proceeds 2 to 3 times as long as for leaf number 20, since the size at unfolding is 26 times smaller than its final size for leaf number 4 compared to only 6.5 times for leaf number 20 (at the low light intensity). The rate of leaf unfolding on the other hand is also 2 to 3 times slower when leaf 4 opens than it is when leaf 20 opens (Fig. 7).

As a consequence, the number of unfolded leaves in which cell division still occurs is almost constant as the plastochron age proceeds. Thus, the

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fraction of dividing cells in the leaf decreases linearly with the number of leaves that unfold after the leaf in question or:

(6.8a) $ND_{p,i} = NT_{p,i}(1 - (U - I)/\nu)$ $0 < U - I < \nu$

and

(6.8b) $ND_{p,i} = 0$ $U - I > \nu$

In this equation $NT_{p,i}$ is the total number of epidermal cells and $ND_{p,i}$ the number of dividing epidermal cells, both along the midrib of leaf I. U is the leaf number of the presently unfolding leaf. The timespan of linear decrease ν is expressed in a number of leaves.

The rate of increase of the total number of cells is

(6.9) $\dfrac{dNT}{dP} = RDP_{p,i} \cdot NT_{p,i} \cdot (1 - (U - I)/\nu)$

in which RDP is the relative division rate of the dividing cells on plastochron basis. In section 5.1 it was shown that the relative length growth rate of a leaf is not affected by the absence or presence of cell division and that the average size of the dividing cells does not change with time. Therefore the relative division rate is equal to the relative length growth rate. If the final cell size is 4 times as large as the size of the dividing cells, the final leaf size is also 4 times larger than the final cell number times the size of the dividing cells. The final cell number can be found by integration of eq. 6.9. The final length of the leaf and its length upon unfolding are known so that ν can be found by an iterative procedure.

The CSMP program used for this purpose is given in Table 3. It appears that ν is virtually independent of the plastochron age of the plant (Fig. 29a). Only the growing conditions exert some influence: under high light the fraction of dividing cells decreases at a slower rate, so that the final leaf size is larger. In Fig. 28b the value of ν is plotted against the daily light total, it being remarked that the outdoor light is an average over the whole growing period. The value of ν may be a function of temperature but information on that point is lacking at present.

During the first phase of leaf expansion the relative length growth rate on plastochron basis is independent of light intensity, only the fraction of dividing cells drops faster under low light conditions.

Under variable light conditions the fraction of dividing cells F decreases at a variable rate:

$$\frac{dF}{dU} = -\frac{1}{\nu}$$

in which ν depends on the environmental conditions. Expressed on plastochron basis this equation becomes

(6.10) $\dfrac{dF}{dP} = -\dfrac{dU}{dP} \cdot \dfrac{1}{\nu}$

Table 3.

```
TITLE FINAL CELL NUMBER IN CUCUMBER
TITLE ITERATIVE SIMULATION TO FIND THE VALUE OF NU
INITIAL
INCON NU=1.
*    IMPLICIT LOOP DETERMINES THE VALUE OF PLASTOCHRON AGE P
*    AT WHICH LEAF I UNFOLDS, E.G. I=U
       STARTP=IMPL(IP,0.001,P1)
       IA=.22+1.17*EXP(-STARTP/5.7)
       IB=.34+2.07*EXP(-STARTP/8.)
       IC=3.3*(1.-EXP(-0.1*STARTP))
       P1=I+IC/IA
INCON IP=0.
PARAM I=(2.,10*2.)
*    SERIES OF LEAF NUMBERS

PARAM LFM=5.08
*    LFM IS THE LOGARITHM OF THE FINAL LEAF LENGTH OF LEAVES WITH A HIGH
*    LEAF NUMBER , AT LOW LIGHT
       LNCM=LFM-1.1+1.1*(1.-EXP(-I*0.5/1.1))-ALOG(4.)
*    LNCM IS THE LOGARITHM OF THE FINAL CELL NUMBER OF LEAF I
*    ALOG(4.) ACCOUNTS FOR THE MATURE CELLS BEING FOUR TIMES AS LONG
*    AS THE DIVIDING CELLS
DYNAMIC
       P=TIME+STARTP
*    IC IS THE LOGARITHM OF THE CELL NUMBER AT UNFOLDING
       LNC=INTGRL(IC,RPC)
*    LNC IS THE LOGARITHM OF THE CELL NUMBER
*    RPC IS THE RELATIVE GROWTH RATE OF THE CELL NUMBER
       RPC=RP*FRDCEL
       FRDCEL=INTGRL(1.,INSW(U-I,0.,-DUDP/NU))
*    FRDCEL IS THE FRACTION OF DIVIDING CELLS
*    U IS THE NUMBER OF THE UNFOLDING LEAF
       U=P-C/A
       DUDP=1.-(A*DCDP-C*DADP)/(A*A)
*    DUDP IS THE DERIVATIVE OF U WITH RESPECT TO P
*    THIS EXPRESSION FOR DUDP FOLLOWS ALGEBRAICALLY FROM THE ONE FOR U
       DADP=(0.22-A)/5.7
       DBDP=(0.34-B)/8.
       DCDP=(3.3- C)*0.1
*    A,B, AND C APPROACH THEIR EQUILIBRIUM VALUES
*    AS AN ASYMPTOTIC EXPONENTIAL WITH P
       A=INTGRL(IA,DADP)
       B=INTGRL(IB,DBDP)
       C=INTGRL(IC,DCDP)
       RP=A+DADP*(P-U)+DBDP*(U-I)+(B-A)*DUDP
TIMER FINTIM=100.,OUTDEL=0.2,PRDEL=1.
FINISH FRDCEL=0.001
PRINT NU,LNC,P,A,B,C,U,RP,FRDCEL
*    IN THE TERMINAL THE ITERATION IS CONTROLLED
TERMINAL
       IF(ABS(LNC-LNCM).LT.0.001) GO TO 10
       NU=NU+(LNCM-LNC)*3.
       CALL RERUN
    10 CONTINUE
       IF(ABS(LNC-LNCM).LT.0.001) TYPE 800,NU,I,STARTP,LFM
  800 FORMAT('  NU ,I,STARTP AND LFM ARE :',4F10.2)
END
PARAM LFM=5.24
*    LFM FOR HIGH LIGHT
END
PARAM LFM=5.5
*    LFM FOR OUTDOOR LIGHT CONDITIONS
END
STOP
ENDJOB
```
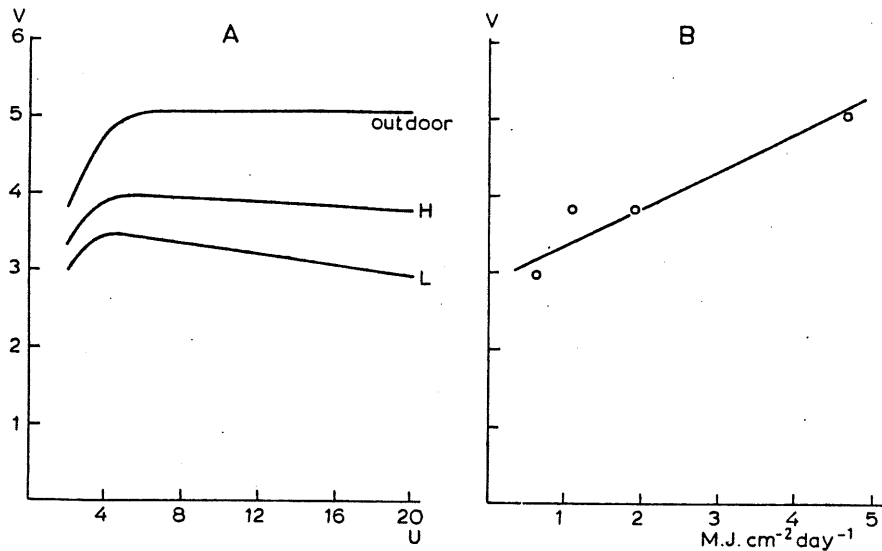
Fig. 29. A: The value of v (that is the number of unfolded leaves in which cell division decreases linearly) as a function of the serial number of the unfolding leaf U, under different light conditions (L, H, and outdoor). B: The stationary value of v as a function of total daily radiation.

By the use of this expression and the dependence of v on light conditions, it is possible to compute the final length of a leaf when grown under varying light intensity.

There is some doubt whether the adjustment under varying light is better described with the assumption that the fraction of dividing cells is instantaneously adjusted according to eq. 6.9 or its rate of decrease with eq. 6.10. Further experiments are necessary to discriminate between the two possibilities.

6.4. Some final remarks

A general CSMP-program to simulate the time dependence of cell number, leaf length and leaf area in dependence of time under varying light intensity is given in Table 4. This program is used for generation of the following figures.

The cell number of the 4th and 20th leaf, at low (L) and high (H) light are given in Fig. 30a and b as a function of the plastochron age of the plant. The relative growth rates of cell number are given in the same graph also expressed on a plastochron basis.

The relative growth rate on plastochron basis of the young, 4th leaf decreases until the sudden increase at the moment of unfolding and continues then to decrease. The relative growth rates are independent of light intensity except at the end where the length growth of the leaf at

high light continues for a longer time because more cells are formed during the period of expansion. The growth of the leaves stops rather suddenly when the final length is reached because it is assumed that the relative growth rate continues as dictated by the B-curves. The observed more smooth transition in the maturing phase (Fig. 9) may be mimicked by multiplying the growth rates with a reduction factor equal to $1.1(1 - L/LF)^{\frac{1}{4}}$ in which L is the length at any given moment and LF the total number of cells at that moment, multiplied by the diameter of the mature cells and the value of 3 is chosen rather arbitrary. This multiplication factor

Table 4

```
TITLE SIMULATION LEAF LENGTH IN CUCUMBER AGAINST TIME
STORAGE LL(20)
INITIAL
FUNCTION NUTB=(0.,.64),(2.,.84),(4.,1.),(20.,.97),(40.,.97)
        STARTP=1.
PARAM EXPO=0.333333
        F=(1.-1./4.)**(-EXPO)
        IA=.22+1.17*EXP(-STARTP/5.7)
        IB=.34+2.07*EXP(-STARTP/8.)
        IC=3.3*(1.-EXP(-0.1*STARTP))
NOSORT
        DO 1 I=1,20
        FDCELI(I)=1.
        LENI(I)=0.1
      1 CONTINUE
DYNAMIC
        P=INTGRL(STARTP,K)
        KS=AFGEN(KSTB,LIGHT)
FUNCTION KSTB=(0.,0.584),(1.,0.8),(2.,0.95),(4.,1.06),(10.,1.06)
        K=KS*(1.-EXP(-P/3.))
        LF=2.9+0.49*LIGHT
*  LIGHT MUST BE EXPRESSED IN MJ VISIBLE  M-2 DAY-1
        LIGHT=AFGEN(LTB,TIME)
FUNCTION LTB=(0.,1.87),(100.,1.87)
        U=P-C/A
        DUDP=1.-(A*DCDP-C*DADP)/(A*A)
        DADP=(0.22-A)/5.7
        DBDP=(0.34-B)/8.
        DCDP=(3.3- C)*0.1
        A=INTGRL(IA,K*DADP)
        B=INTGRL(IB,K*DBDP)
        C=INTGRL(IC,K*DCDP)
NOSORT
        AREA=0.
        DO 3 I=1,20
        RPB=A+DADP*(P-U)+DBDP*(U-I)+(B-A)*DUDP
        RP=INSW(P-I,0.,INSW(U-I,A+DADP*(P-I),RPB))*
        DF(I)=INSW(U-I,0.,-K*DUDP/(LF*AFGEN(NUTB,I)))
        RPC(I)=K*RP*AMAX1(FRDCEL(I),0.)
        RNC=EXP(LNC(I))
        REDF=F*AMAX1(0.,1.-10.*LENGTH(I)/(RNC*4.))**EXPO
        DLEN(I)=K*LENGTH(I)*RP*REDF
        LL(I)=ALOG(10*LENGTH(I))
        AREA=AREA+0.39*LENGTH(I)*(LENGTH(I)-0.5)
      3 CONTINUE
        FRDCEL=INTGRL(FDCELI,DF,20)
        LENGTH=INTGRL(LENI,DLEN,20)
        LNC=INTGRL(0.,RPC,20)
TIMER TIME=5.,DELT=0.1,FINTIM=55.,OUTDEL=5.,PRDEL=5.
```

Table 4 (continued)

```
METHOD RKSFX
PRINT P,U,A,B,C,AREA
       PRT=IMPULS(0.,PRDEL)
       IF(PRT+KEEP.EQ.0.) GO TO 5
       WRITE(1,800)TIME
   800 FORMAT(' TIME IS ',F10.2)
       WRITE(1,801)
   801 FORMAT(' LNC(1-50) IS ')
       WRITE(1,802)LNC
   802 FORMAT(10F6.2)
       WRITE(1,803)
   803 FORMAT(' LENGTH(1-50) IS ')
       WRITE(1,802)LENGTH
     5 CONTINUE
END
FUNCTION LTB=(0.,0.53,),(100.,0.53)
END
FUNCTION LTB=(0.,0.53),(34.9,0.53),(35.1,1.87),(100.,1.87)
END
STOP
ENDJOB
```

LIST OF SYMBOLS
================

| NAME	DESCRIPTION	UNIT
A	SLOPE OF LN LEAF LENGTH VERSUS LEAF NUMBER IN PRIMORDIAL PHASE	
AREA	AREA OF ALL LEAVES OF A PLANT	CM 2
B	SEE A, FOR LEAVES IN EXPANDING PHASE	
C	LOGARITHM LEAF LENGTH AT UNFOLDING	
DADP	DERIVATIVE OF A TO P	P-1
DBDP	DERIVATIVE OF B TO P	P-1
DCDP	DERIVATIVE OF C TO P	P-1
DF	DERIVATIVE OF FRDCEL TO P	P-1
DLEN	DERIVATIVE LENGTH TO P	CM P-1
DUDP	DERIVATIVE OF U TO P	P-1
EXPO	EXPONENT OF THE COSMETIC TREATMENT	
F	FACTOR TO OBTAIN EQUAL RGR FOR LENGTH AND CELL NUMBER AT THE ONSET OF THE EXPANDING PHASE	
FDCELI	INITIAL FRACTION OF DIVIDING CELLS (1)	
FRDCEL	FRACTION OF DIVIDING CELLS IN EXPANDING PHASE	
I	LEAF SEQUENTIAL NUMBER	
K	LEAF PRODUCTION RATE	P DAY-1
KS	EQUILIBRIUM K UNDER PREVAILING LIGHT REGIME	P DAY-1
LENGTH	LENGTH OF THE LEAVES	CM
LENI	INITIAL LENGTH OF THE LEAVES (0.1 CM)	CM
LF	MULTIPLICATION FACTOR FOR INFLUENCE OF LIGHT ON NU	
LIGHT	LIGHT (VISIBLE) LEVEL	MJ M-2 DAY-1
LL	LOGARITHM OF LENGTH	
LNC	LOGARITHM OF CELL NUMBER	
LTB	LIGHT AS A FUNCTION OF TIME	
P	PLASTOCHRON INDEX	P
REDF	REDUCTION FACTOR FOR THE RELATIVE LENGTH GROWTH RATE	
RNC	NUMBER OF CELLS	
RP	RELATIVE DIVISION RATE OF DIVIDING CELLS	DAY-1
RPB	RP IN THE EXPANDING PHASE	DAY-1
RPC	RELATIVE GROWTH RATE OF THE CELL NUMBER IN THE EXPANDING PHASE	DAY-1
STARTP	INITIAL VALUE OF P	P
U	LEAF NUMBER OF UNFOLDING LEAF	
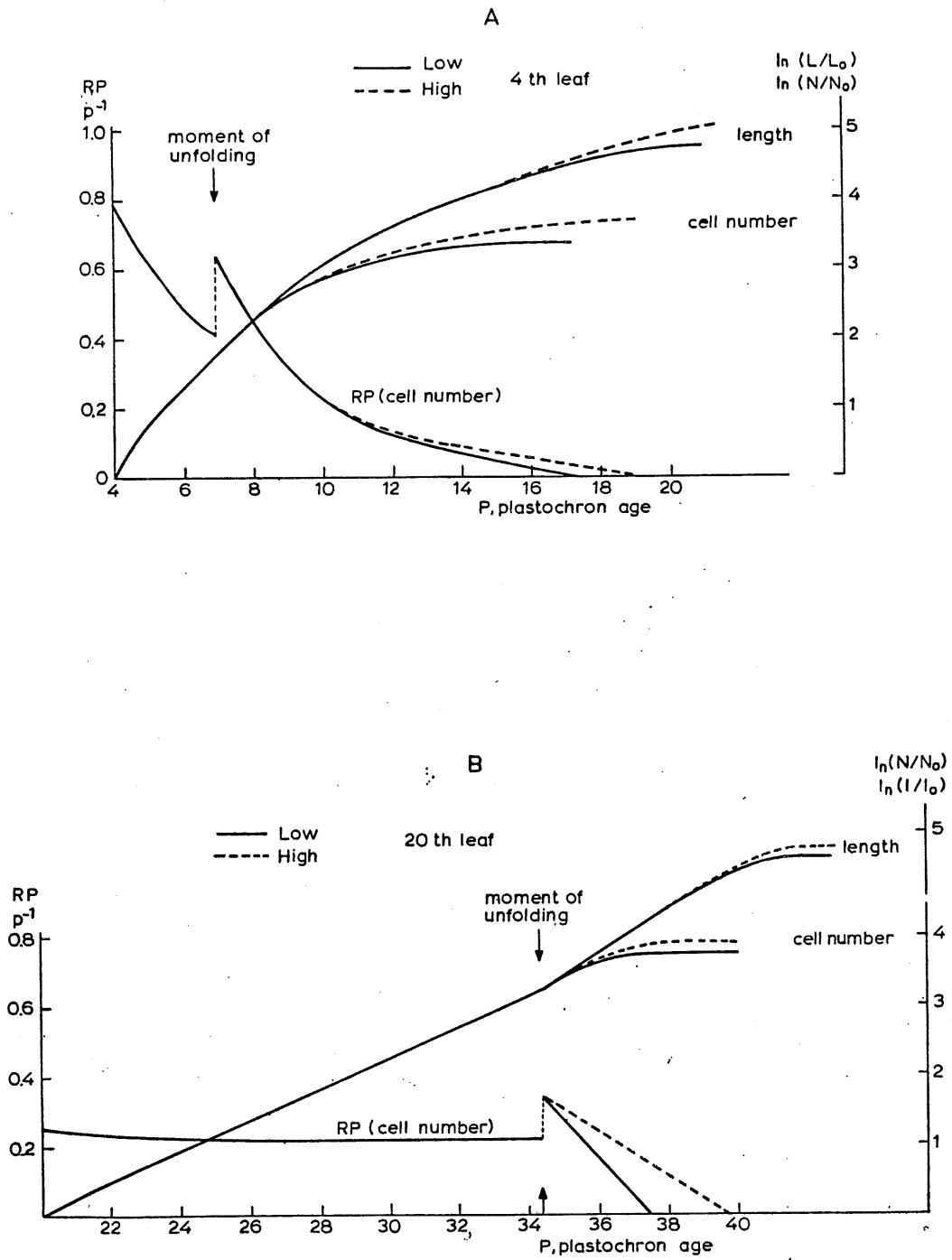
Fig. 30. The length, number of cells and relative growth rate of cell number (A) calculated for the 4th leaf for the Low (L) and High (H) light treatment presented as a function of the plastochron age of the plant. The same curves calculated for the 20th leaf (B).
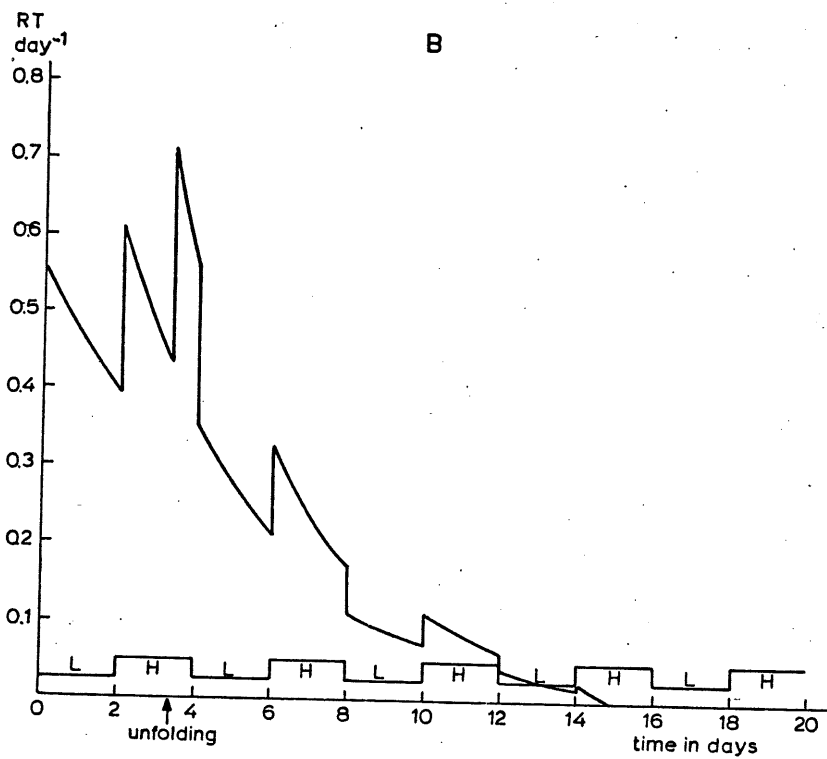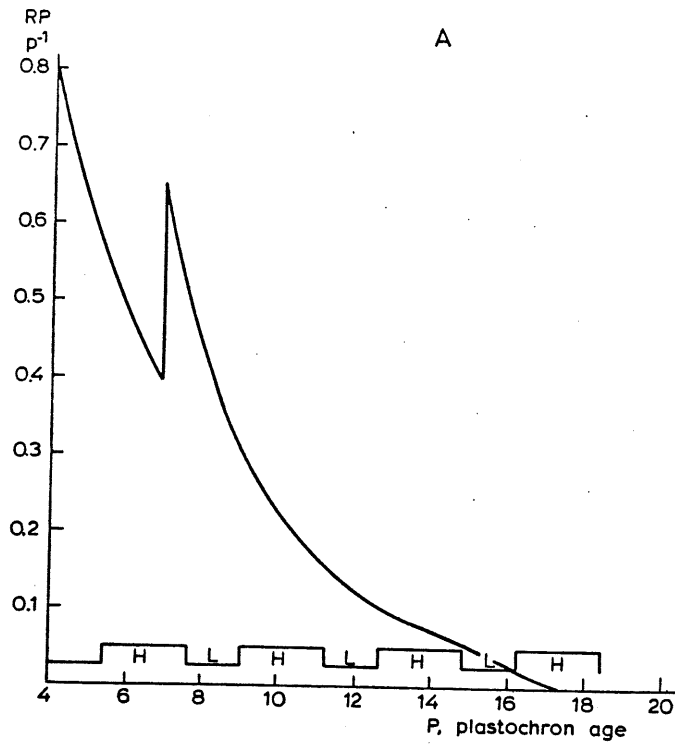
Fig. 31. Relative growth rates of leaf length, expressed on plastochron basis (RP), calculated for the 4th leaf growing under *changing* light conditions: High (H) and Low (L) light condition alternating every other day (A). The same curves expressed on real time basis (B).

equals $1.1(1-1/4)^{\frac{1}{2}}=1$ at the moment of unfolding and only exerts its influence when the length L approaches the final length LF. This "cosmetic" treatment is used in the graphs presented in Fig. 29.

For the 4th leaf, the relative growth rates are calculated also for changing light conditions: from high and low light intensity alternating every other day. As might be expected hardly any light effects are apparent as long as growth is expressed on plastochron basis, as presented in Fig. 31a. However, when growth is expressed on time basis, the light intensity effects are evident, as shown in Fig. 31b. Transformation of the relative growth rates on plastochron basis to relative growth rates on time basis is done by means of eq. 4.9a, k being taken as 0.7 for the low and 1.1 for the high light condition.

However complicated the course of the growth under varying environmental conditions, the principles are extremely simple. Until the moment of unfolding, the growth pattern on plastochron basis is fully independent of the growing conditions. In fact the growth pattern before the moment of unfolding does not matter at all, since the number of the unfolding leaf and its length is completely determined by the plastochron age of the plant. Light intensity and temperature exert their influence only through their influence on the rate of leaf production. Hence, the working hypothesis that morphogenesis and weight growth can be treated independently of each other is fully confirmed up to this stage. After unfolding, the same holds in first instance for the length growth rate, but light and perhaps temperature effect in addition the number of cells, so that from then on the leaves develop differently, even when expressed on plastochron basis.

For the length growth rate, or the number of epidermal cells along the midrib, this influence could be expressed by means of the linear decrease of cell division. Although this is in reasonable agreement with the observations of Milthorpe, Newton and Dale, this aspect of leaf development still needs further study.

At the moment of unfolding there exists a one to three relation between leaf length and leaf weight, as illustrated in Fig. 13. Hence, the leaf weight at that time is also fully determined. The growth of the leaf weight after unfolding is in first instance also only dependent on the plastochron age of the plant, as appears from the observation that the B-curve for leaf weight is independent of the light intensity, as is illustrated in Fig. 18. Although there is some ontogenetic drift with plastochron age, the ratio between the B-values for weight and length is about 2 instead of 3. This means that after unfolding the relative growth rate in specific leaf weight is smaller than in leaf length or leaf surface. It would be possible to treat the growth of thickness of the leaves in number of cells in the same way as the growth in length. However, only specific leaf dry weights are available and these may be so strongly affected by accumulation of carbohydrates and thickness of cell walls, that they cannot be used as a

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substitute for number of cells. Here again, further experimental analysis is necessary.

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