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The influence of temperature and light intensity on the leaf growth and chlorophyll synthesis

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The influence of temperature and light intensity on the leaf growth has been the subject of many investigations (see Milthorpe, 1956). The optimum temperature for the growth of a cucumber leaf was established for about 24°C (Milthorpe 1959). Moreover Milthorpe has also established that the influence of this factor is a very complicated process. The optimum for a leaf to attain maximum area, and that for new leaves to grow differ from each other; the latter one lying in somewhat higher temperatures.

The optimum temperature for the chlorophyll accumulations is between 26° and 30° C (Lubimenko and Hubbenet 1932; Friend 1960). Friend (1960, 1961) found that the chlorophyll content per unit of fresh weight or leaf area is higher at 30° that at 25°C. It means that in higher temperatures the rate of fresh weight and of leaf area formation is more reduced than the rate of chlorophyll synthesis.

Many authors have also reported the fact that in dicotyledonous plants light is an indispensible factor of leaf growth (e. g. Sinnott 1960). The growth of leaves of many plants is reduced at light intensities lower than 20 per cent of a full day light intensity (Shirley 1929; Crocker 1948), whereas, higher light intensities either remain without any action on the growth or even may abate it slightly. Therefore, sun leaves are often smaller in size than shadow leaves (e. g. Talbert and Holch 1957).

The influence of temperature is often modified by the intensity of light and *vice versa;* e. g. in the range of 20—25°C the influence of temperature on the growth rate of *Lemna minor* is higher in light of 700 f. c. than of 180 f. c. (Templeton, cit. Blackman 1956). Friend (1960) compared the influence of temperature and light intensity on the chlorophyll content in wheat leaves and established that in the range 20°—30°C the chlorophyll concentration increases concomitantly with the increase of light intensity from 200 f. c. to 2000 f. c.; whereas for temperatures below 20° C the optimum light intensity for chlorophyll accumulation is about 1000 f. c.

This paper is a closer study of the correlation between the growth of the leaf (fresh weight) and the chlorophyll synthesis in dependence on temperature and light intensity.

MATERIAL AND METHODS

a. Plant culture

The experiments were carried out on the two primary leaves of beans *(Phaseolus vulgaris* var. *Bronowicka)* grown in a light thermostat on a liquid medium (Knop), in controlled conditions of light and temperature. The method growing the plants was described in previous paper (Więckowski 1959).

Seeds were soaked in darkness in tap water at 24°C for 24 hours and then in the same conditions germinated for the following 48 hours. Since the moment the germinated seeds were placed over the nutrient solution the conditions of different temperatures (19 ± 0,5° C, 24 ± 0,5°C, 29 ± 0,5°C) and light intensities (690, 1360, 4950 lx) were applied.

In some experiments, seeds germinated in the above given conditions, were kept in darkness at the temperature of 29 ± 0,5°C for the following 48 hours and then exposed to the action of the examined factors (this refers to experiments the results of which are presented in Tables 1 and 2).

Eight white fluorescent tubes (Telam 25 W) placed immediately above the glass plate of the thermostat, provided the light source. In order to reduce the light intensity neutral filters (blackened metallic nets) were used.

b. Chlorophyll determination

Pigments were determined by means of a previously described spectrophotometric method (Więckowski 1960) at wave lengths 660 and 642,5 mμ. The spectrophotometer (Uvispek Hilger) was equipped with a glass prism and 1 cm thick cells.

RESULTS

a. Influence of the temperature

The curve representing the growht of leaf has a sigmoid shape which is independent on the temperature (in the range 19—29°C) in which the plants were grown (Fig. 1). However, the lower the temperature, the more pronounced sigmoid shape of the curve. At the lower temperature (19°C) the leaves atteined their final size 4 days

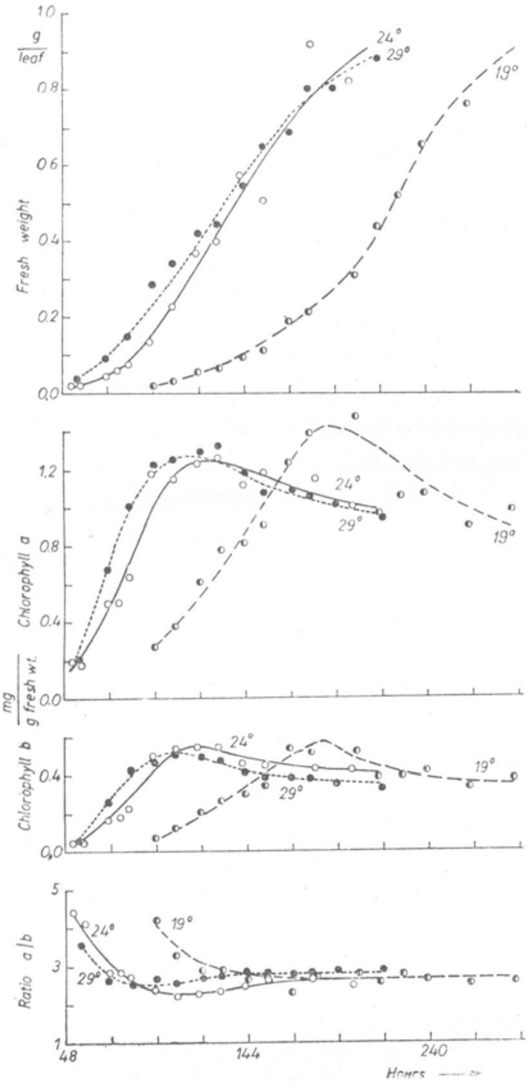

Fig. 1. The increase of fresh weight, changes of chlorophyll concentration and changes of the ratio of chlorophyll *a* to chlorophyll b in leaves growing in different temperatures. Continuous illumination (4950 lx). Each point — mean of 4—8 leaves. Abscissae: Time elapsing from the moment of placing germinated seeds over the nutrient solution

later. It results also from the graph that the delay in growth is chiefly caused by a lower rate of growth in the initial phase; and so, at 19°C primary leaves appear by about 24 hours later and their initial phase of growth is longer than in plants grown in 29°C. Within the examined range of temperature minimum differences were observed in the phase of intensive growth. The final sizes of the leaves (fresh weight) attained similar values.

Changes in chlorophyll concentration (Fig. 1) during the leaf growth proceed in a similar way in all examined temperatures. Thus, an intensive increase of chlorophyll content per unit of fresh weight is observed in the initial phase. This increase is followed by a drop in the later stage. This drop is the result of a certain kind of „dilution" of chlorophyll. This phenomenon has been already discussed in detail in previous papers (Więckowski 1959, 1960 a, b). At temperature of 29°C, however, the rate of changes of chlorophyll concentration per unit of fresh weight is quicker than in plants grown in lower temperatures and specially in 19°C. The higher the temperature the sooner the phase of maximum chlorophyll concentration is reached; e. g. at 29°C maximum concentration of chlorophyll occurs about the third day after the leaves have appeared and at 19°C it occurs by four days later. At 24°C this delay amounted to a few hours only.

As it results from Fig. 1 the discussed changes concern as well chlorophyll *a* as chlorophyll *b*.

The changes of the ratio of chlorophyll *a* to *b* (*a*/*b*) are also illustrated in Fig. 1. It results from this fig. that at 29°C the ratio *a*/*b* attains a constant value within the shortest period of time e. g. already after the first day of taking measurements; whereas, at a lower temperature it occurs a little later. It has been established in all the three cases that the ratio *a*/*b* attains a constant value when the concentrations of chlorophyll *a* and *b* are 1 mg and 0.4 mg respectively per 1 g fresh weight of the leaf. It may be also seen that the high value of the ratio *a*/*b* observed in the beginning of the experiment decreases to a certain minimum and then slightly increase until it attains a constant value. However, no special significance is attributed to this kind of depression, because it did not occur in many experiments.

Table 1

Influence of temperature on the values of the ratio *a*/*b* in leaves of plants exposed to light for 12 and 24 hours. Light intensity 4950 lx. Means of 14 leaves. Other details in the text

Temperatures (°C)	Ratio *a*/*b*	
	12h	24h
19	7.64	4.20
24	3.78	2.87
29	3.48	2.73
34	2.77	2.62

The influence of temperature on the stabilisation rate of the ratio *a*/*b* in the first 24 hours after exposing to light, is also shown in Tab. 1. In this experiment the different temperatures were applied from the moment the primary leaves had appeared. Until this moment the seedlings were grown in darkness in the same temperature (29°C). Just before exposition to light, the seedling in the same stage of development were selected, and their leaves were delicately drawn aside so that their whole surface might be exposed to light.

It was observed that, in this case as well, the lower the temperature the later the stabilisation of the ratio of chlorophyll *a* to *b*. At 34°C this stabilisation proceeds at a higher rate than at 29°C. It is caused by higher rates of leaf growth and chlorophyll synthesis in this temperature. This acceleration takes place only in the initial phase of leaf growth. After a few days the rate of growth is considerably reduced; the leaves look anormally, they are dark green and curled up.

Figure 2 shows the influence of temperature on the increase of chlorophyll content in the whole leaf (in the initial phase). At 29°C chlorophyll synthesis is proportional to time almost from the moment

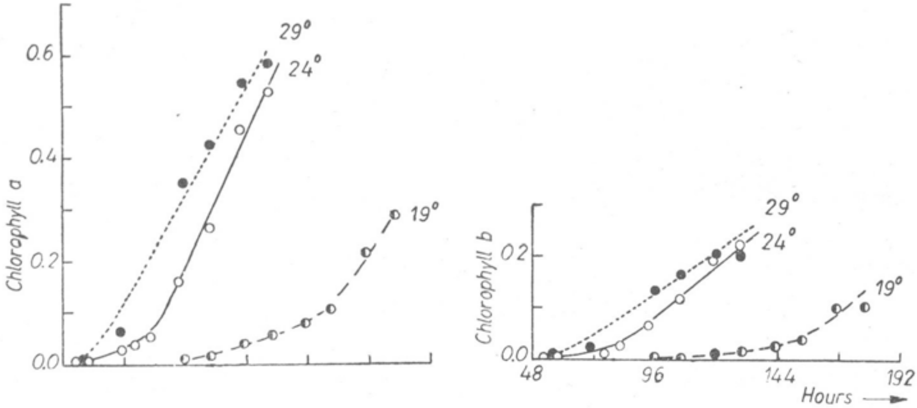

Fig. 2. Increase of chlorophyll content in one leaf (mg/leaf) in different temperatures Other details as in Fig. 1

the measurements started. In lower temperatures (24° and 19°C) two phases may be distinguished: a phase of slow rate of chlorophyll accumulation and a phase of high rate of chlorophyll accumulation. The lower the temperature the longer is the first phase.

b. Influence of light intensity

The highest light intensity (4950 lx) did not limit the leaf growth in a remarkable way (Fig. 3). The leaf blades were often even larger than those grown in natural conditions, whereas lower light intensities (1360 and 690 lx) considerably limited the growth. Fresh weight of a leaf from maximum light attained the value of about 0.9 g, whereas in light intensities of 1360 lx and 690 lx these values did not exceed 0.19 g and 0.15 g respectively. In lower light intensities and in the initial phase the rate of increase of fresh weight is lower and the leaves finish their prowth earlier; e. g. in light intensity of 4950 lx the growth of a leaf is accomplished by about 8 days; and in the lowest intensity (690 lx) by 3 days already.

An inhibition of chlorophyll synthesis is the result of an inhibition of the leaf growth caused by a weaker illumination. The chlorophyll concentration increases as long as the leaf grows and subsequently

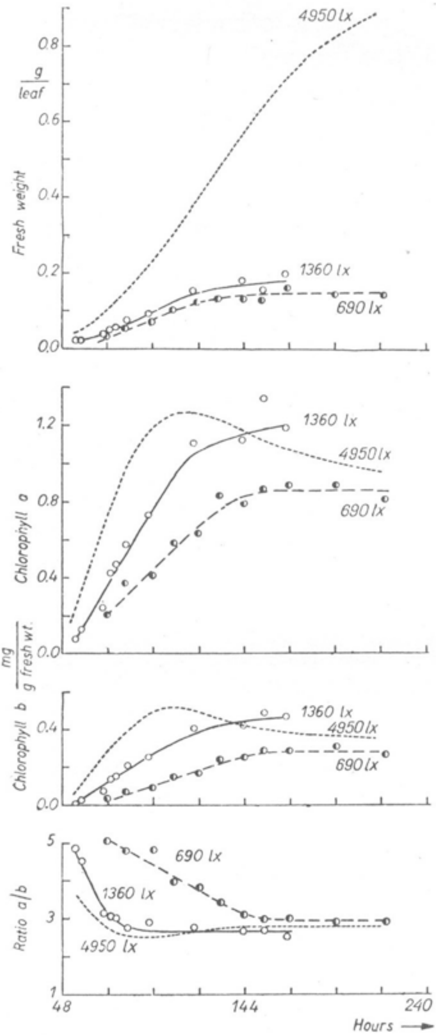

Fig. 3. Increase of fresh weight, changes of chlorophyll concentration and changes of the ratio of chlorophyll *a* to *b* in leaves growing in different light intensities. Temperature 29°C. Curves for 4950 lx as in Fig. 1. For other details see Fig. 1

it remains on a more or less constant level (Fig. 3). In leaves illuminated with light of 1360 lx the concentrations of chlorophyll *a* and *b* attain about 1.2 mg and 0.5 respectively per 1 g of fresh weight. These values are higher than those found in fully grown leaves of plants exposed

Table 2
Influence of light intensity on the values of the ratio a/b in leaves of plants exposed to light for 12 and 24 hours. Temperature 29°C. Means of 14 leaves. Other details in the text

Light intensities (lx)	Ratio a/b	
	12^h	24^h
690	4.51	4.30
1360	3.58	3.41
4950	3.31	2.86

to the higher light intensity. In 690 lx light intensity the chlorophyll concentration attains a lower value than in plants from maximum light intensity. In leaves of plants illuminated with lower light intensities no decrease of chlorophyll concentration in the later phase of leaf growth is observed. The maximum concentration of chlorophyll is lower than the maximum concentration of this pigment in leaves of plants exposed to full light.

The delayed chlorophyll synthesis in lower light intensities brings about a retardation of the stabilisation of the ratio a/b. The decrease of light intensity from 4950 lx to 1360 lx caused only a slight delay in the stabilisation. On the contrary the further decrease of light intensity to 690 lx resulted in a 3 days long retardation.

A similar influence of lower light intensities on the rate of ratio a/b stabilisation was observed in leaves of plants which were kept in

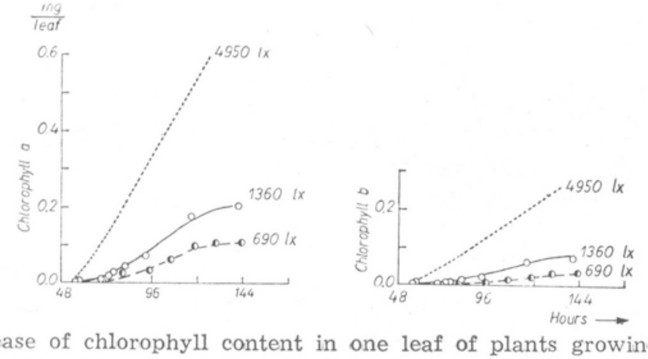

Fig. 4. Increase of chlorophyll content in one leaf of plants growing in different light intensities. For other details see Figs. 1 and 3

darkness before being exposed to light (Tab. 2). The other growth conditions were the same as in experiments the results of which are presented in Table 1.

The changes of chlorophyll content in one leaf (in its initial developmental stage) are shown in Fig. 4. The lower the light intensity the lower is the rate of chlorophyll synthesis. In lower light intensities two phases may be distinguished: a phase of slow increase of chlorophyll content followed earlier or later by a phase of high rate of synthesis.

DISCUSSION

The above presented results were obtained on plants grown in specific conditions (very low light intensity, continuous light) sometime very different from conditions applied by other authors. For this reason the discussion of these results requires a great dose of criticism.

The sigmoid shape of the curve representing the leaf growth is independent of the temperature (19°—29°C). In all cases three parts can be distinguished on the curve corresponding to the initial (slow) phase of growth, the phase of intensive growth and the final phase when growth gradually decreases. The lower the temperature the longer is the initial phase; on the contrary the influence of temperature on the second phase is much weaker.

The difference in the rate of the leaf growth between temperatures of 29°C and 24°C is much smaller than the corresponding difference between temperatures of 24°C and 19°C. The same may be said about the changes in the concentrations of chlorophyll. These results are in conformity with the well known fact that effects produced by intensity changes of a factor are the less the nearer the optimum value is the intensity of the factor in question.

The growth of a leaf is a very complicated process and the final size of a leaf depends on: *a.* the number of primordial cells, *b.* the rate of cell division, c. the duration of the phase of cell division, d. the final size of the mature cells (Gregory 1959). It is generaly assumed that the cell division is predominant in the initial phase of the leaf growth and cell elongation in the later one. According to Sunderland's statement (1960) no exact time separation between these two phases can be drawn in leaf as these processes occur concomitantly for a longer period of time. In the case of sunflower leaves the cell division ceases when the leaf has attained 3/4 of its final size. On the other hand cell divisions only are observed in tobacco leaves which are still in a bud (Avery 1933).

It seems, however, that the above mentioned facts are not incompatible with the statement that the process of cell division is predominant in the first phase (a higher percentage of dividing cells) and the elongation process in later stages (a higher percentage of elongating cells). It should be expected that the temperature will modify these processes in various ways. The decrease of temperature from 29°C to 19°C causes, first of all, a decrease of cell division rate.

In the same range of temperature the inhibition, if any, of the cell elongation is considerably reduced.

The weaker growth of a leaf in the initial phase caused by a drop of temperature may be compared to a certain extent with the results obtained by Templeton (cit. after Blackman 1959) in his study on the growth of the leaf of *Salvinia natans*. He established that the increase of temperature from 20 to 30°C causes an increase of the number of leaves, whereas the sizes of individual leaves attain the maximum value at the temperature of about 24°C.

This phenomenon may be regarded as a certain kind of ecological adaptation. Lower temperatures in spring are a factor limiting the development of buds. In this way they are protected from an early development and in consequence from freezing during a possible return of lower temperatures. A drop of temperature in later stages when the leaves are more developed will have a smaller effect on their growth. It should be expected, however, that a considerable drop of temperature below the examined range will inhibit completely or partially the growth of an even well developed leaf.

Light is another indispensable factor for normal growth of leaves of dicotyledonous plants. According to Shirley (1929) it becomes limiting factor when its intensity sinks below 20 per cent of full day light. Light modifies the processes of cell division and elongation (see: Sinnott 1960). Marton and Watson (1948) established that in leaves of sugar beet the cells are considerably less numerous if the plant developed in darkness for a longer time in its early stage. Their cells, however, do not differ in size from cells of leaves of plants growing in continuous light.

In our experiments, light of 4950 lx intensity was not a factor limiting the leaf growth. The leaf blades attained even greater sizes than in natural conditions. Lower light intensities, however, considerably reduced their growth. During the first three days the leaves continued to grow but at a lower rate. In the next period of time a complete cessation of growth was observed. Light seems to play negligible part in the early developmental stages only i. e. before the leaves emerge from the seed.

The chlorophyll concentration per unit of fresh weight undergoes similar changes in various temperatures: firstly an increase of chlorophyll concentration is observed which at a later stage decreases. In this kind of changes are involved both chlorophyll *a* and *b*. These changes in concentration have been discussed in previous papers (Więckowski 1959, 1960 a, b). However, at 29°C the changes of chlorophyll concentration proceed at a higher rate. This is in agreement with the results obtained by Friend (1960) who found that the optimum temperature for the rate of chlorophyll accumulation in wheat leaves is at about 30°C. In our case the rate of chlorophyll synthesis was the highest at 34°C, but only in young leaves. Its further decrease in this temperature is most probably connected with a generally lower rate of leaf growth in its later stages. In lower light intensities changes in chlorophyll concentration proceed in another way i. e. the concentration increases to a certain value and then remains on a constant level. The characteristic phenomenon of chlorophyll „dilution" is not observed in later stages. It is a consequence resulting from a complete cessation of leaf growth in low light intensities. Besides, it has been established that the lower the light intensity the lower the rate of chlorophyll formation. The chlorophyll concentration increases as long as the leaf grows. Thus, the cessation of growth shown by leaves exposed to low light intensities causes a concomitant inhibition of chlorophyll synthesis.

A drop of temperature and light intensity causes a delay in the stabilisation rate of the ratio of chlorophyll *a* to *b*; nevertheless, the values attained after the stabilisation show only small differences and do not exceed 2.5—2.8.

It is known that the ratio *a*/*b*, is as a rule, genetically fixed. To a certain extent, however, the environmental conditions (specially light conditions) can modify it (see: Egle 1960). In our experiments the intensities of light were much lower than full day light, and these may be the cause why the changes in the ratio *a*/*b* were relatively small.

SUMMARY

1. The influence of temperature (19°, 24° and 29°C) and light intensity (690, 1360 and 4950 lx) on the synthesis od chlorophyll and the growth of the primary bean leaves has been examined.

2. At 19°C the leaf accomplished its growth 4 days later than at 29°C. This delay is caused by a slower growth in the first developmental phase.

3. In the examined range, variations of temperature are almost without influence on the final size of the leaves.

4. In the same range (19—29°C) the rate of chlorophyll synthesis increases with the rise of temperature.

5. In all temperatures characteristic changes of chlorophyll content per unit of fresh weight have been observed: the concentration increases in the initial growth phase and begins to drop after a few days. This decrease of concentration is caused by a certain „dilution" of chlorophyll in the tissue of the leaf. In higher temperatures these changes proceed at a higher rate.

6. In lower temperatures the stabilisation of the ratio of chlorophyll *a* to chlorophyll *b* is a little delayed. In the examined range, the

temperature, however, exerts only a small influence on the final ratio a/b (the differences do not exceed the range 2.5—2.8).

7. Illumination with light of 4950 lx intensity is not limiting the leaf growth, whereas, in lower light intensities (1360 and 690 lx) the leaves attain very small sizes and their growth ceases much earlier.

8. Accumulation of chlorophyll takes place only as long as the leaf grows.

9. In lower light intensities the chlorophyll concentration increases initially and then maintains on a more or less constant level. No characteristic drop of chlorophyll concentration is observed in the later phase of leaf growth.

10. The stabilisation of the ratio of chlorophyll a to chlorophyll b is delayed in lower light intensities. However, the differences between the final values of this ratio are insignificant.

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