THE EFFECT OF LOW TEMPERATURE ON DRY MATTER PRODUCTION, CHLOROPHYLL CONCENTRATION AND PHOTOSYNTHESIS OF MAIZE PLANTS OF DIFFERENT AGES*

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SUMMARY

Young seedlings of *Zea mays* L. scarcely grow at temperatures of 15 °C or lower. The leaves are yellowish, the chlorophyll concentration being lower than 10 μg (a+b) per cm^2, and the rate of photosynthesis is negligible. When a period of low temperature is given to older plants, which already have some green leaf surface, the reduction in relative growth rate and photosynthesis is less severe. Only those leaf parts that elongate during the cold treatment are yellow.

It can be shown that the day temperature is the important factor in influencing the chlorophyll concentration in growing leaf parts. Plants subjected to low night temperatures remain fully green and their growth rate is virtually unaffected. The data suggest that photosynthesis rate is reduced at concentrations of chlorophyll (a+b) below 40μg per cm^{-2}. When plants are brought back to temperatures of 20° or higher both photosynthesis rate and growth rate soon reach values normal for this temperature.

INTRODUCTION

It is a well known fact that maize, which is usually sown at the end of April, often grows slowly, if it grows at all, when there is a spell of rather low temperatures after emergence. The young leaves are so yellow that hardly any photosynthesis seems to be possible. At the end of the growing season, at comparable temperatures, no such features are observed. The plants remain fully green and there is still a considerable gain in weight.

To gain more insight into this problem some effects of temperature on growth, chlorophyll formation and photosynthesis were studied.

METHODS

Seeds of *Zea mays* L., variety C.I.V. 7, were germinated in moist perlite at room temperature. After approximately one week the young seedlings were transplanted into one litre black plastic pots by fixing them in a hole in the lid with foam-plastic strips. The pots were filled with a Hoagland solution of half strength. Iron was added as 1 ml of a 0.3 molar solution of ferrous sulphate in 1 N sulphuric acid, which brought the pH of the solution to approximately 6.

* Dedicated to Professor Dr. W. H. Arisz.

At the beginning the culture solution was renewed twice a week, but as the plants grew the frequency was increased and eventually the renewal took place daily. In addition to this a fortnight after transplanting the one litre pots were replaced by five litre ones.

All growth experiments were carried out in growth rooms with artificial illumination given by 400 Watt high pressure mercury vapour lamps (1600 Watt. m^{-2}) and a relative humidity of $\pm$ 80%. The light intensity at the level of the pots was 0.08 cal.cm^{-2}. min^{-1} and the daylength was 17 hours. The temperature régimes were different and will be described separately for each experiment.

When harvesting the plants the roots were blotted with filter paper and separated from the shoots. The visible leaf blades were cut off at the ligule or – for the young leaves – where they emerged from the preceding leaf. The rest, consisting of a short stem and leaf sheaths, as well as the growing point and the very young leaves, was designated as stem. Fresh and dry weights of these three plant portions were determined. When measuring the chlorophyll concentration of a leaf, discs of 1.9 cm^2 were punched out, usually about half way along its length. The discs were weighed fresh and their chlorophyll (a + b) content was determined using the method given by ARNON (1949). In a few cases, when the leaf was too narrow, sections of a leaf or even complete leaves were taken after the determination of leaf area and fresh weight.

Measurements of the rate of photosynthesis were carried out either in whole-plant chambers or in leaf chambers. The CO_2 uptake was calculated from the air speed through the chamber and the CO_2 concentration of the in- and outgoing air, which was determined with a Beckman infra-red gas analyser. For a detailed description see LOUWERSE & VAN OORSCHOT (in press).

In the whole plant chamber one or more young plants were fixed with the shoot base in holes in the bottom of the chamber, the roots remaining outside in bottles with nutrient solution. The fully emerged leaf blades were fixed in a horizontal position by means of thin nylon threads. After the CO_2-exchange measurements in light and darkness the horizontal leaf parts were cut off and their area was measured. The photosynthesis of the remaining stump, consisting of "stem" and the undeveloped top leaves, was then measured again as before. The rate of gross photosynthesis per cm^2 of horizontal leaf was calculated from the difference between the two measurements and the area of the cut leaf blades (see *table 2*).

With older plants, which were too big for the whole-shoot chambers, leaf chambers were used consisting of two perspex halves, between which a leaf was fixed by means of foam-rubber strips. Both the top and the bottom "window" of the chamber consisted of a double wall with flowing tap water in between, to keep the temperature at a reasonable value. The leaf in the chamber was kept attached to the plant.

Light in the photosynthesis chambers was again provided by 400 Watt mercury vapour lamps, giving a maximum light intensity at the level of the measured leaf of approx. 0.35 cal.cm^{-2}.min^{-1} in the whole-shoot chamber and 0.45 cal.cm^{-2}. min^{-1} in the leaf chamber. Reductions in light intensity were

obtained by means of metal screens with holes of different size, placed between the light source and the chamber. Four small matched and calibrated silicon photocells were placed in the vicinity of the leaves and connected parallel to a galvanometer, so that a mean output value could be recorded.

RESULTS

In the first experiment young seedlings were planted upon nutrient solution in a growth room at 20 °C. Another group was placed in a similar growth room at 10°. At the same time a number of these seedlings was taken for the determination of the fresh and dry weight of the different plant portions. One week later a number of plants from both groups was harvested and the remaining plants of the 10° treatment were transferred to 20°. At the same time a number of plants that had been at 20° was placed in a growth room at 10° for one week; part of it was harvested one week later and the remaining plants were then put back at 20°, and so on. Thus the rate of growth was recorded for plants growing at 20° throughout and for plants that were transferred to 10° for one week at a different age and brought back to 20° thereafter.

The results are presented in *fig. 1*. The growth rate (RGR) at 20° was rather small (0.05 $g.g^{-1}.day^{-1}$) during the first week and increased thereafter to a more or less constant value of 0.16 $g.g^{-1}.day^{-1}$. The RGR at 10° was negligible when the plants were placed there immediately after planting. It increased when the 10° period was given at a later stage, to reach a value of 0.11 $g.g^{-1}.day^{-1}$ when the 10° period started three weeks after planting. When the plants were trans-

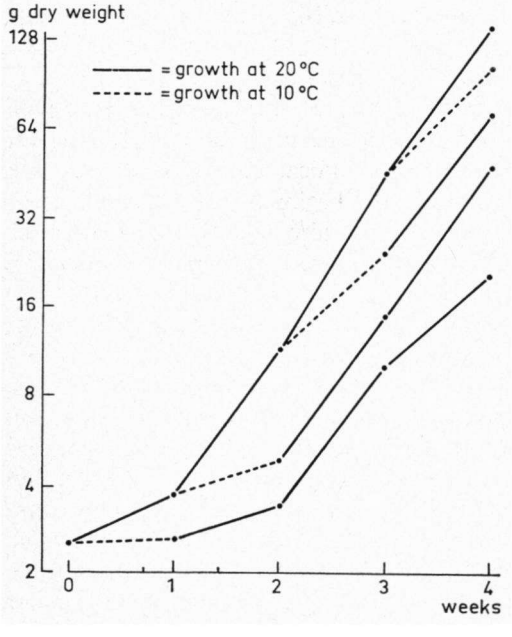

Fig. 1. Dry matter production of maize plants transferred from 20° to 10°C for one week at different stages of development.

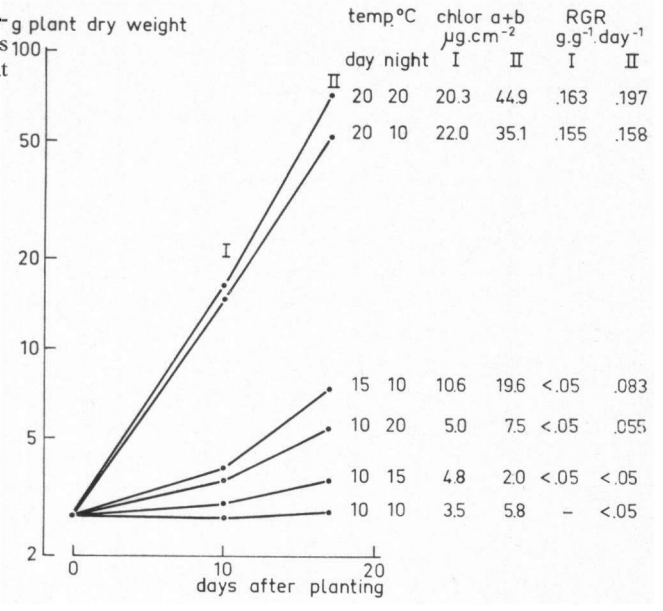

Fig. 2. Dry matter production of maize plants at different day and night temperatures.

ferred back to 20° the RGR came almost instantaneously to the normal value for that temperature, except in the week after the first 10° period when the RGR was much lower. A repetition of this experiment gave approximately the same results. In these experiments no chlorophyll concentrations were determined but it could be seen that young seedlings placed at 10° were almost completely yellow after a week, whereas in plants placed at 10° at a later stage only those leaves and leaf parts that were formed at 10° were yellow.

In order to determine more precisely the influence of temperature on growth and chlorophyll concentration, young maize seedlings were placed at six different day and night temperature régimes, as indicated in *fig. 2*, for a growth period of 17 days. Harvests were taken at the beginning and the end of the growth period and an intermediate harvest was also taken 10 days after the beginning. The data show that the day temperature is the major influence in bringing about the reductions in RGR and chlorophyll concentration. The growth rate at 20° day- and night temperature was about 0.18 $g.g^{-1}.day^{-1}$ over the whole growth period, a value comparable with that of the preceding experiment, except that in this instance it was more or less constant from the beginning. Lowering the night temperature to 10° caused only a small reduction in RGR to 0.16 $g.g^{-1}.day^{-1}$, and a negligible effect on the chlorophyll concentration. However, when the day temperature was lowered both the RGR and the chlorophyll concentration dropped considerably. At a 15° day temperature the RGR and chlorophyll content were reduced to at least about half the value at 20°. At 10° during day and night the dry matter production was negligible although the chlorophyll concentration was not substantially below that of

higher night temperatures. The RGR increased somewhat with increasing night temperatures but remained at relatively low values. In all treatments the chlorophyll concentrations at the end of the experiment were about twice as high as those half- way through; at all day temperatures below 20° the RGR during the second growth period was distinctly higher than that during the first, except at 10° throughout, when there was no growth at all.

To check whether the low day temperature affects only the chlorophyll concentration of the leaves formed at that temperature, and not the concentration in the leaves already full grown at the time of transference, a further experiment was carried out. Seedlings were planted upon nutrient solution and kept at 20° until the fourth leaf was fully emerged and the sixth just visible, when the plant were divided into two groups, one group being kept at 20° throughout (20/20), and the other at 10° during the day and at 20° during the night (10/20). After a week the plants at 20/20 had seven more or less fullyemerged leaves; those at 10/20 had six. The chlorophyll concentrations in the leaves at the end of the experiment are given in *table 1*, which shows that there was no large difference in chlorophyll concentration between the older leaves of both groups. However, with the 10/20 group there was a decrease in chlorophyll concentration from the fourth leaf onwards, whereas with the 20/20 plants a drop could only be observed at the seventh leaf. This shows that a change in day temperature from 20° to 10° has no influence on the chlorophyll concentration of the fully emerged leaves, but causes a decrease in the content of the elongating parts of the developing leaves.

Table 1. Influence of day temperature on the chlorophyll (a + b) content (μg. cm^{-2}) of successive leaves (see text).

Leaf number	1	2	3	4	5	6	7
Day temp. treatment							
Constant 20°	23.5	43.2	48.9	48.3	54.8	45.3	36.6
Shift from 20° to 10°	30.8	44.4	41.4	33.1	12.6	12.9	–

The direct influence of a period with low temperatures on the rate of photosynthesis, has been studied in the following two experiments.

In the first experiment two groups of seedlings were placed on nutrient solution, one at 20°, the other one at 10°. Eight days later the rate of photosynthesis was measured in the whole plant chamber. A further batch of seedlings was first placed at 20° foi a week before being divided into two groups, one being left at 20° while the other was transferred to 10°. Here again the rate of photosynthesis was measured eight days after the commencement of the temperature treatment. The temperature of the leaves in the photosynthesis apparatus during the light period was 22–23°, and during the dark period 18–19°. The rate of gross photosynthesis at a light intensity of approximately 0.35 cal. cm^{-2}. min^{-1} is given in *table 2*, both per chamber before and after cutting off the horizontal fully emerged leaves and per cm^2 horizontal leaf.

Table 2. Influence on photosynthesis of a period of low temperature (10°C), given at different times during the development.

Pretreatment	Respiration per chamber before cutting	Gross photosynthesis per chamber		Leaf area	Gross photosynthesis per unit area
		before cutting	after cutting	cm^2	
	$\mu l\ CO_2\ h^{-1}$	$\mu l\ CO_2\ h^{-1}$	$\mu l\ CO_2\ h^{-1}$		$\mu l\ CO_2\ cm^{-2}\ h^{-1}$
8 days 10°	1556	707	*	79	9
8 days 20°	2337	58137	16769	190	218
1 week 20° + 8 days 10°	2352	14396	659	186	74
1 week 20° + 8 days 20°	4732	83232	21261	264	235

* = not measurable

The plants kept at 10° for 8 days directly after planting had a negliglibe rate of photosynthesis as compared with plants kept at 20° for the same period. From the rate of respiration of the former plants it can be concluded that the compensation point was not even reached. When the transfer to 10° took place one week after transplanting the rate of photosynthesis of the 10° plants was considerably improved, although it was still about one third of that of the 20° plants, in which the rate was much the same as after the first week.

In a second photosynthesis experiment plants were grown from the seedling stage at 20° until the fourth leaf was fully emerged. From that time on one group was kept at this temperature (20/20), while the other was transferred to a day temperature of 10°, the night temperature remaining at 20° (10/20). After a fortnight some of the 10/20 plants were brought back to the 20° growth room, and the day after a further batch of plants was transferred, giving in all four groups of plants: 20°, 10° + 2 days 20°, 10° + 1 day 20°, and 10° (all day temperatures). The next day the rate of photosynthesis of the fourth and sixth leaves was measured at light intensities ranging from darkness to 0.45 $cal.cm^{-2}.min^{-1}$, with leaf temperatures varying between 19° and 23°. The fourth leaf was already fully emerged at the beginning of the cold treatment and at the time of measurement it looked green over its whole length in all groups of plants. The sixth leaf, which was still elongating when the different treatments began, was green with the control plants at 20° but was yellow at the base with all plants that had undergone cold treatment, although in the plants that were put back at 20° for one or two days it was beginning to turn green. Measurements of photosynthesis in the leaf chambers and determinations of chlorophyll content in these leaves are presented in *fig. 2* and *table 3*.

The light intensity – photosynthesis curves demonstrate that there were no big differences between the groups in the performance of the fourth leaf. That of the 10/20 plants was only slightly below that of the 20/20 plants and those

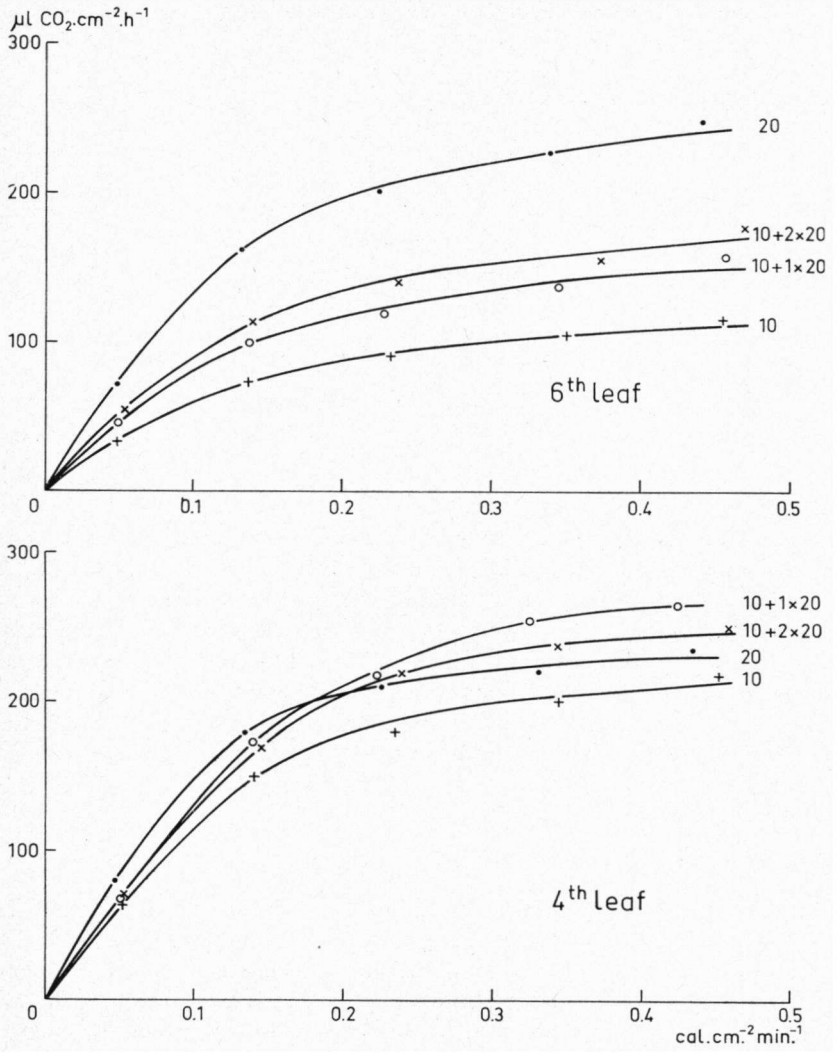

Fig. 3. Relation between light intensity and rate of gross photosynthesis of leaves of different age from plants grown at different day temperatures.

plants that were recovering from the 10° treatment had even a slightly higher rate of photosynthesis at higher light intensities. With the sixth leaf the differences were much greater. The light saturation value of the 10/20 plants was less than half the value of the 20/20 plants and those of the recovering plants were in between, 2 days recovery being slightly higher than 1 day.

The second column of *table 3* shows the chlorophyll concentration per unit area of leaf discs, punched out of the leaf segments that had been in the photo-

Table 3. Influence of different day temperature treatments on photosynthesis and chlorophyll concentration.

Pretreatment	Chlorophyll concentration per unit leaf area $\mu g\ cm^{-2}$	Leaf weight per unit area $g\ cm^{-2}$	Chlorophyll concentration per unit leaf weight $mg\ g^{-1}$
6th leaf			
20°	31.79	15.07	2.11
10° + 2 d 20°	11.78	15.04	0.78
10° + 1 d 20°	14.35	17.96	0.80
10°	9.33	17.25	0.54
4th leaf			
20°	32.73	19.30	1.70
10° + 2 d 20°	52.76	25.79	2.04
10° + 1 d 20°	59.39	25.79	2.30
10°	51.92	22.22	2.34

synthesis chamber. In the sixth leaf at 10° the concentration was very low; where these leaves had been back at 20° for one or two days the values were higher but still less than half the concentration in the 20° leaves. The concentration in the fourth leaf of the control plants had about the same value but, unexpectedly, the cold treated leaves had a much higher chlorophyll content, with or without a recovery period. The third column of figures shows that this is correlated with a rather high fresh weight per unit area and, consequently, the chlorophyll figures are less extreme when expressed per unit fresh weight (fourth column).

DISCUSSION

The influence of temperature on dry matter production is rather complicated, since it has an effect on photosynthesis, respiration, dry matter distribution, life span and probably on other factors. With maize its influence is even more complicated in that it affects the chlorophyll content at a level where most other plants cultivated in temperate zones remain fully green. Although this effect is very conspicuous in maize fields early in the season, it has not received much attention from plant physiologists, and literature on the subject is scarce. In text books on photosynthesis little or no attention is paid to the influence of temperature on chlorophyll formation and maize is not mentioned as an example where this influence is very marked. FRIEND (1961) shows that with wheat there is an increase in chlorophyll concentration with temperature, but his value of 26 $\mu g.cm^{-2}$ chlorophyll (a + b) at 10 °C is substantially higher than that found in young maize seedlings at 20° *(fig. 2)*. For maize plants, however, FRIEND (1966) states that the minimum temperature for growth is 15 °C, since below that value chlorophyll concentration is negligible. WENT (1957) mentions the importance of a low photo- temperature in inhibiting chlorophyll formation.

The present experiments show the effects of temperature on relative growth rate, chlorophyll concentration and photosynthesis but do not contribute to an insight into the influence of temperature on chlorophyll formation nor on the differences between plant species in this respect. As shown by WENT (1957) it is exclusively the temperature during the light period that exerts an influence on chlorophyll formation. An influence of the night temperature could not be demonstrated. In some experiments there was actually a negative correlation between night temperature and chlorophyll content (see below).

Even a 15° day temperature has such an adverse influence on chlorophyll concentration that the RGR of young seedlings is lower than 0.05 $g.g^{-1}.day^{-1}$, while at 10° the young seedlings may lose weight, or at the least show no measurable increase over a period of more than two weeks *(fig. 2)*.

At 10° the chlorophyll formation does not come to a complete standstill, since its content in the leaves of the second harvest was always greater than that of the first *(fig. 2)*. Whether this process of chlorophyll formation is influenced by night temperature cannot be definitely decided at present, since although the data shown here give an indication of a higher chlorophyll concentration with higher night temperatures – the very low content at 10/15, second harvest, being an exception – a repetition of this experiment showed just the opposite trend. The chlorophyll content at different day and night temperature combinations in this second experiment was as follows:

Day/night temperature:	10/20	10/15	10/10 °C
Chlor. (a + b) content:	13.9	8.2	2.7 $\mu g.cm^{-2}$

Despite this uncertainty about the effect of night temperature on chlorophyll concentration, dry matter production was always stimulated by higher night temperatures in the range from 10 tot 20°. This is contrary to the findings of many others and to the general statement made by WENT (1957) that a lower night temperature always has a beneficial influence on dry matter production through a decreasing rate of respiration. It is, however, evident that the influence of temperature is more complicated than was thought. The increase in weight with higher night temperature found in maize is correlated with an increase in the percentage leaf weight *(fig. 2)* and in leaf size. Apparently the higher rate of respiration is counteracted by a higher rate of photosynthesis through a greater leaf area per plant. Possibly there is also an influence on the transport of carbohydrates from the seed to the young plant, but this cannot be established from the present experiments, since the seeds were not weighed separately.

The increase of RGR with time *(fig. 2)* may well be the result of the gradual increase of the chlorophyll concentration in the leaves at low day temperatures, since all the concentrations of the first harvest and nearly all of the second harvest lie in a range in which, according to GABRIELSEN (1960), rate of photosynthesis is influenced. Further experiments on the relation between chlorophyll content and rate of photosynthesis are in progress, but from the data obtained here the impression is gained that up to 45 $\mu g.cm^{-2}$ chlorophyll concentration still has an influence (see below).

Although it is usual to find that the growth rate of young seedlings at 20° is lower during the first week *(fig. 1)* this is not always the case as can be seen from *fig. 2*, where there is hardly any difference in RGR of the 20/20 plants between the first and the second growth period, although the chlorophyll concentration increased from 20.3 to 44.9 $\mu g.cm^{-2}$. It is, therefore, not yet certain what is the cause of the slow start so often observed in young maize seedlings.

When the RGR at 10° is determined after some time at 20°, the values increase with increasing length of the 20° period as *fig. 1* shows. This is undoubtedly caused by the fact that the leaves which have attained a certain chlorophyll concentration at 20° keep this value when transferred to 10° *(table 1* and *3)*. The later in the growth period this transfer occurs, the more green leaf tissue is present and the higher the RGR at 10°. After the transfer from 10 to 20° there must be a rapid recovery of the rate of photosynthesis, for the growth rate at 20° directly after a period at 10° hardly differs from that of plants without a cold treatment.

Fig. 3 shows furthermore that there is practically no after effect on the rate of photosynthesis when fully green leaves are transferred from 10° to 20°. The difference in the photosynthesis is only small and leaves that had been moved to 20° one or two days before the measurements were made had in fact a higher saturation value than the control plants.

The recorded saturation level of these leaves reached a maximum around 265 μl $CO_2.cm^{-2}.h^{-1}$, which is rather low for maize. In other experiments values as high as 300 μl were found, so there is clearly a considerable degree of variation for some yet unknown reason. The range of values found in the literature is of the same order of magnitude at least (MOSS 1964, 1968; MURAMOTO *c.s.* 1965).

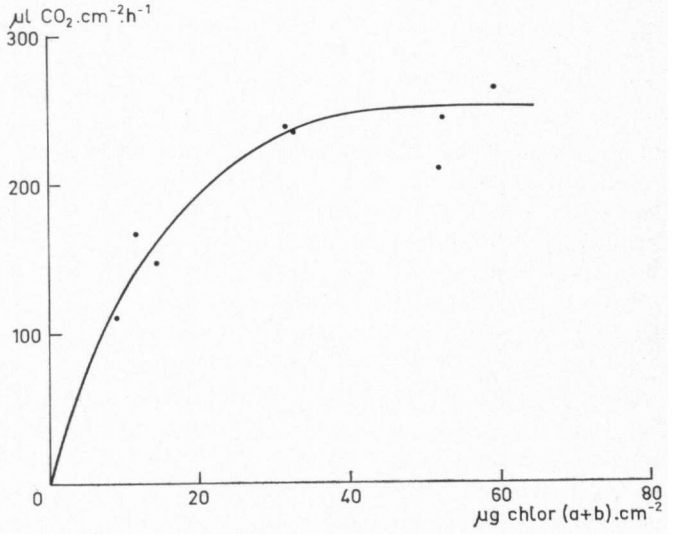

Fig. 4. Relation between chlorophyll (a +b) concentration and saturation rate of photosynthesis.

When the youngest part of the sixth leaf was measured the differences were much greater than with the fourth leaf. The saturation value of the 10/20 plants was less than half of the 20/20 plants (240 against 110 μl $CO_2.cm^{-2}.h^{-1}$). Saturation values of the leaves transferred from 10° tot 20° one and two days before the measurement were 150 and 170 μl $CO_2.cm^{-2}.h^{-1}$ respectively. These differences were correlated with differences in chlorophyll content, whether expressed per unit area or per unit weight. *Fig. 4* shows the relationship between chlorophyll (a + b) concentration per unit area and the saturation value of the photosynthesis rate. Approximately the same relationship is found when the chlorophyll concentration is plotted against the photosynthesis rate at low light intensity. GABRIELSEN (1960) found a similar curve with low light intensity photosynthesis and suggested a causal relationship between photosynthesis and chlorophyll concentration. According to him this relationship would not be evident at the light saturation level where dark processes are limiting, although his own results demonstrate great differences at high light intensities. One cannot, therefore, be sure at present whether the differences in photosynthesis are directly related to chlorophyll concentration, or whether there are other differences between the leaves grown at different temperatures which affect the rate of photosynthesis.

Further experiments on the effect of temperature on photosynthesis and chlorophyll formation are in progress.

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