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The effect of local cooling on growth and water content of plants

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Summary

Local treatment at low temperature induces in maize a decrease or complete inhibition of leaf elongation. The cause of this decrease is dependent on the place of cooling.

a. Low root temperature, e.g. 5° C, decreases leaf elongation, caused by water deficiency which retards cell extension.

The plant adjusts by increasing the osmotic potential, followed by an increase in the water potential and recovery of leaf elongation.

At extremely low temperatures recovery of growth is not complete, the older leaves dying. This results in a loss of photosynthetic capacity inducing a shortage of carbohydrates. This does not occur at gradual lowering of the temperature, which probably allows the plant sufficient time to adjust. The response to extremely low temperature is considerably less in older plants than in younger ones.

b. Decreased growth at low meristematic temperature is caused by the effect of low temperature on the biochemical processes in the meristematic tissues, decreasing or completely stopping cell division and cell extension.

There is no water shortage in the plant and no carbohydrate shortage in the meristematic region. There is no recovery.

c. Low temperature locally above the meristematic region temporarily retards growth by inhibition of the carbohydrate translocation, which is gradually recovered by an increasing concentration difference. The temporary growth retardation is the result of a complete or partial inhibition of cell extension. Cell division is probably only affected slightly. There is no water shortage in the plant.

Inhibition in the carbohydrate translocation could be clearly demonstrated by applying $^{14}CO_2$ to parts above the cooled region.

Introduction

Since temperature affects almost all the processes in plants, temperature effects on plant growth are rather complicated. In former papers we have demonstrated that cooling of the root medium primarily reduced the water potential of maize plants by its effect on the water permeability of the root tissue. As a consequence the elongation rate of the leaves was also reduced. The reduction in growth rate was closely correlated with the degree of water deficit. In all cases growth reduction was much less pronounced during the dark period than in the light. During prolonged cooling of the roots only a gradual recovery of both water content and leaf elongation rate was observed. This recovery was ascribed to an increase in osmotic potential, a rise in the level of soluble sugars being measured.

Cooling of the meristematic region of the shoots resulted in comparable growth reductions. In contrast with root cooling this treatment did not affect leaf water content and we never observed the slightest recovery during prolonged treatment. We assumed, therefore, that in this case biochemical processes leading to cell division and cell extension were directly affected.

Although evidence was presented in favour of these explanations a number of additional suppositions cannot be excluded completely. Cooling might have affected the flow of many other growth essentials to the growing regions. In this paper the relative importance of the carbohydrate supply will be considered.

Materials and methods

Maize seedlings (variety 'Pioneer 395') were grown on Hoagland solution in a climate room at 20° C and about 85 % r.h. During this pre-treatment the plants received an 18-hour light period per 24 hours and a light intensity of about 70,000 erg cm^{-2} sec^{-1} (HPL).

Unless otherwise reported the plants were transferred at the 8-leaf stage (8th leaf just emerging from the surrounding sheaths) to the experimental room, where root temperature, meristem temperature or the temperature locally above the meristem could be varied independently.

During the experimental period the culture solution was aerated. Daylength, light intensity and relative air humidity were the same as in the pre-treatment period, except where daylength or relative air humidity was used as experimental treatments.

Leaf extension was continuously measured and recorded (Kleinendorst and Brouwer, 1970). The water content was determined qualitatively and continuously recorded by using a beta-gauge (Mederski, 1961; Kleinendorst and Brouwer, 1970). The water content is recorded as the relative number of counts per second, which are transmitted through the leaf. The number of transmitted counts per second in the middle of the dark period during the control day is put at 100 (plants guttating, full turgor, water potential about 0).

During the experimental period the geometry of source-leaf-detector remained constant (Takechi, 1970).

Results and discussion

Influence of the root temperature on leaf elongation and water content

In earlier experiments (Kleinendorst and Brouwer, 1970) it was found that when the root temperature is lowered from 20° C to 5° C, leaf elongation decreased and nearly stopped. The water content paralleled leaf elongation rather closely. Growth rate and

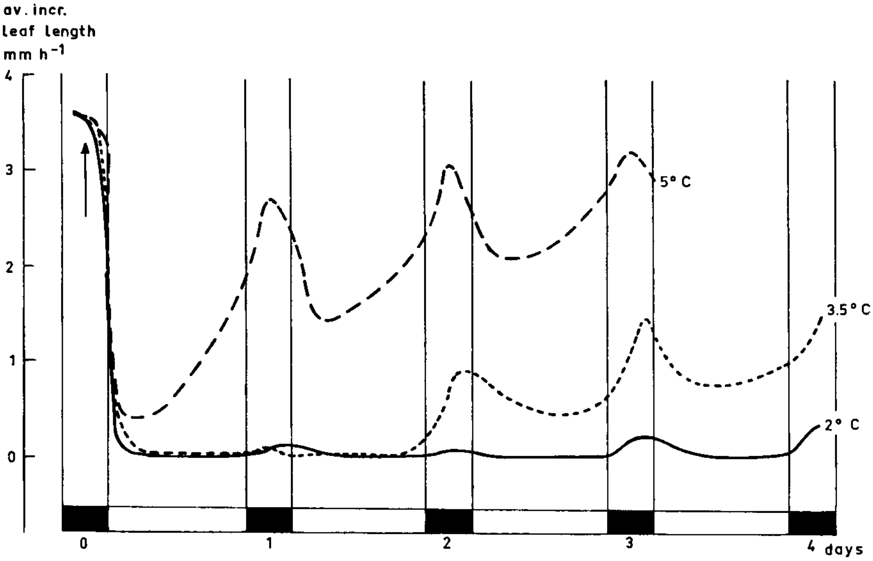

Fig. 1. Response of leaf elongation (8th leaf) to a change in root temperature from 20° C to 5°, 3.5° or 2° C (start indicated by arrow).

water content recovered gradually during the treatment (Fig. 1).

At $3^1/_2$° C (Fig. 1) there is only a small recovery in leaf elongation after 2 days, in contrast with 5° C, where during the end of the first day there was already a clear recovery in leaf elongation. When the root temperature is lowered more severely (to 2° C) there is no recovery (Fig. 1).

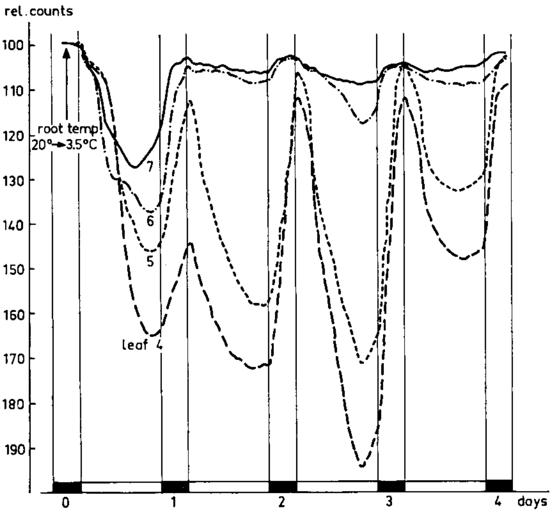

Fig. 2. Response of water content (rel. counts per second) of the 4th, 5th, 6th and 7th leaf to a change in root temperature from 20° C to 3.5° C (100 counts = full turgor, water potential about 0).

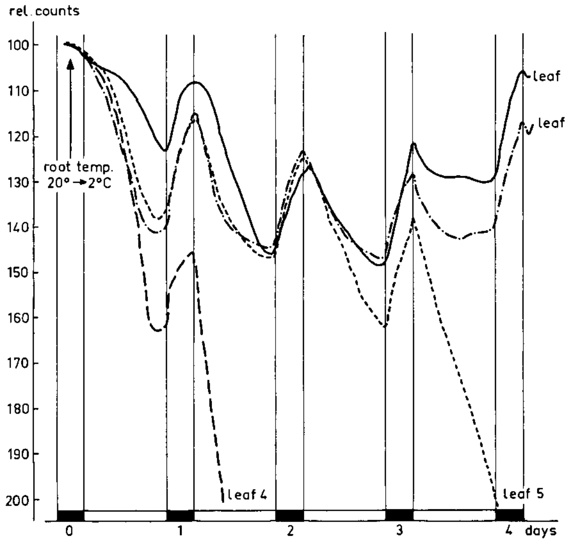

Fig. 3. Response of water content to a change in root temperature from 20° C to 2° C.

Fig. 2 and 3 show the water content measured on 4 various leaves on one single plant at root temperatures of 3½° C and 2° C, respectively. At 3½° C recovery of the water content is much slower than at 5° C. At 5° C there was already a nearly complete recovery after 2 days (Kleinendorst and Brouwer, 1970). The water deficit increases, if root temperature decreases. This is demonstrated most clearly in the older leaves. Only during the dark period the leaves are nearly turgescent. After 4 days at a root temperature of 3½° C the recovery of leaf 7 is not yet complete.

At a lower root temperature a higher osmotic concentration has to be built up to regain full turgor. Evidently, during the light period turgor pressure is still inadequate for optimum growth.

In Fig. 3 the root temperature is lowered to 2° C. At this root temperature water content decreases more than at 3½° C. During the first days water deficits still increase during the subsequent light periods. In the 4th light period there is a little recovery of the water content and in the 4th dark period the water content of the 7th leaf has nearly reached the level of complete recovery. By reason of this it could be expected that the rate of leaf elongation would have recovered as well. However this was not the case (Fig. 1).

Fig. 3 shows that at a root temperature of 2° C the 4th and 5th leaves are wilting to death (this also applies to the first, second and third leaves). Hence growth reduction at a root temperature of 2° C at first is due to the decreased water potential; however recovery of the water content after severe wilting does not lead to a recovery of growth rate probably, because a large fraction of the leaf area is irreversibly lost which may lead to a carbohydrate shortage. Fig. 4 shows the same, but over a prolonged period at a root temperature of 2½° C. Also in this experiment there is no recovery of elongation, although the 7th leaf regains almost full turgor. After 8 days already 6 leaves are dead, hence a shortage in carbohydrates is likely to be involved. It may be concluded from these experiments that recovery of the elongation growth proceeds the more slowly the lower the root temperature, and recovery does not occur at all at extremely low

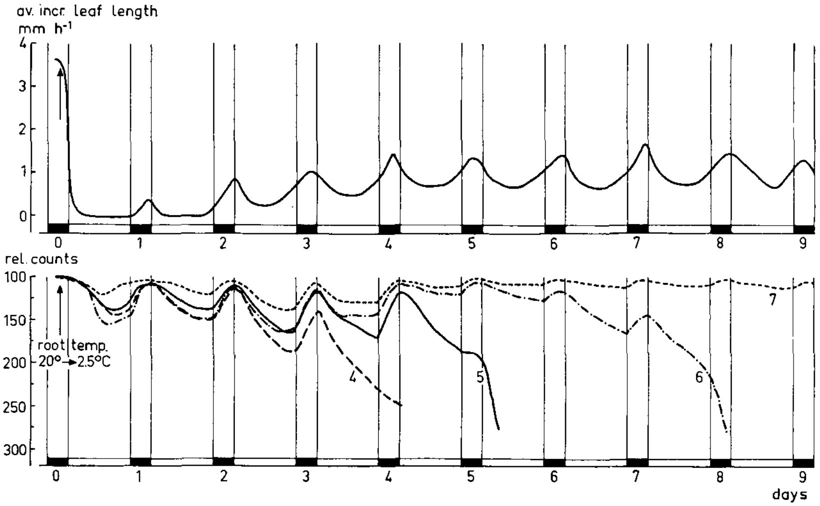

Fig. 4. Response of leaf elongation and water content to a change in root temperature from 20° C to 2.5° C, during 9 days.

root temperatures, when applied at once. However, a more gradual lowering of the root temperature to the extremely low temperature of 2° C results in less damage to the lower leaves (Fig. 5). Stepwise osmotic adjustment is supposed to be responsible for this rather moderate effect. Moreover older plants (11-leaf stage) appeared to be less

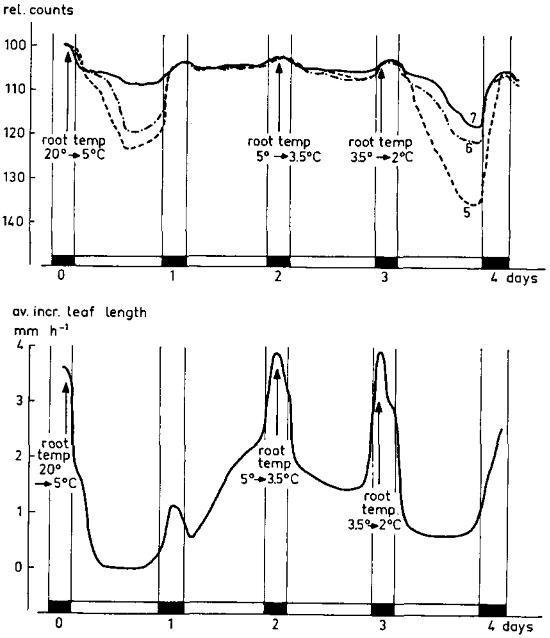

Fig. 5. Response of leaf elongation and water content to a gradual change in root temperature from 20° C to 2° C.

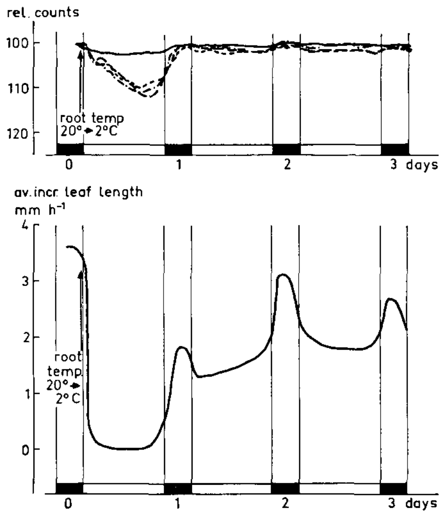

Fig. 6. Response of leaf elongation and water content to a change in root temperature from 20° C to 2° C. The water content is measured in the 7th, 8th, 9th and 10th leaf. The plant was in the 11th leaf stage (leaf elongation was measured on the 11th leaf).

sensitive to a deep cooling of the roots (Fig. 6) even when applied at once.

A similar differential response of young and older wheat plants to drought (lowered osmotic potential of the nutrient solution) has been described by Lawlor (1971). His conclusion that older plants are less sensitive to drought conditions may also apply to this type of drought simulation by a low root temperature. The latter effect, however, could also be expected when the passive water permeability (not controlled by the living system) increases with age. Both suggestions, however, are a description rather than an explanation. Future research will have to show what actually is involved.

The effect of low root temperature on the water content of various leaves on one single plant and on the level of total soluble carbohydrates

Since all our treatments started in the middle of the dark period, so at rather low transpiration, there was not much of a reaction immediately after application. After illumination started, however, the increased transpiration combined with the low water permeability in the root system obviously have resulted in a water deficit in the main pathway of water, xylem vessels and leaf cell walls (Weatherley, 1965). This did not result in a sudden decrease in water content, since the water reservoirs along the water tracks will only gradually equilibrate with the forces in the tracks itself. In the meanwhile a continuous difference between transpiration and absorption will lead to a continuously increasing water deficit in the leaves. Obviously at the beginning the younger leaves are loosing more of their original water content (Fig. 7) which may be due to the higher light intensities they are exposed to (light from above).

Since growth is considerably reduced and certainly more than photosynthetic carbohydrate production, sugars will accumulate in the leaves (Fig. 8) (Boyer, 1970; Wardlaw, 1969; Acevedo et al., 1971; Grobbelaar, 1963). The resulting enhanced osmotic potentials may facilitate water absorption from the water under tension in the cell walls. As a result water content of the leaves will be able to increase even at a constant level of total water potential in the leaf cells ($\Psi_1 = \pi_1 - P_1$ and $\Psi_1 = \pi_2 - P_2 \rightarrow$

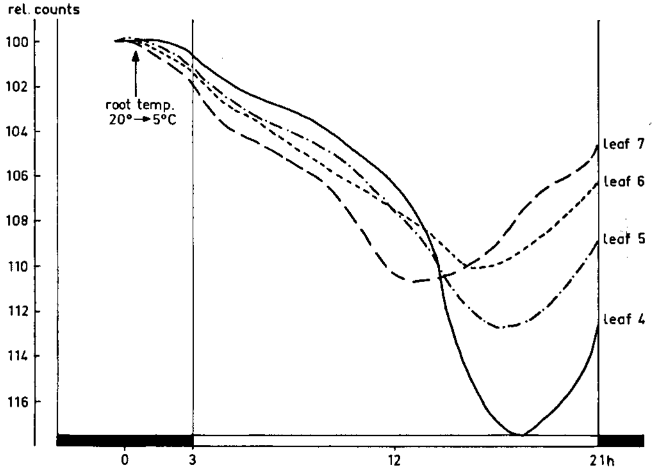

Fig. 7. Response of water content to a change in root temperature from 20° C to 5° C. The water content was measured in the 4th, 5th, 6th and 7th leaf.

$\pi_2 - \pi_1 = P_2 - P_1$; Ψ = water potential, π = osmotic potential, P = turgor potential), because P can only increase after increase of the vacuole content. The younger leaves are not only loosing water faster than the older ones, they are also accumulating sugars more readily. This results in rehydration of the younger leaves in advance of the older leaves (Fig. 7 and 8). We do not know, however, whether this is due to differen-

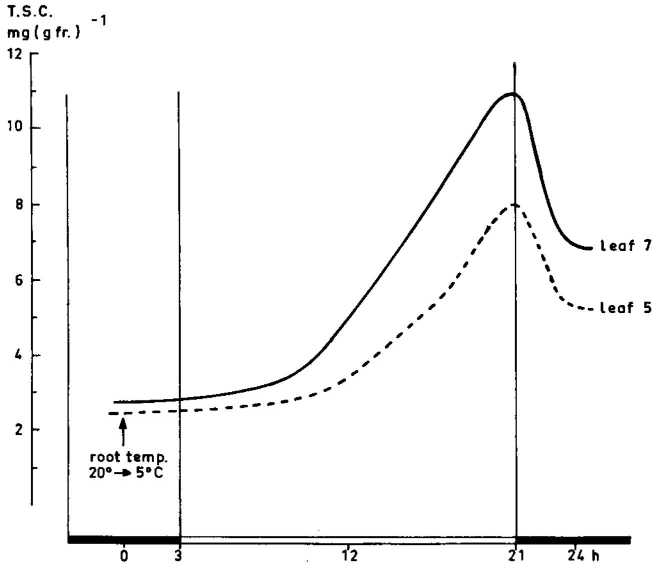

Fig. 8. Changes in total soluble carbohydrates (mg per g fresh weight) in the 5th and 7th leaf after changing the root temperature from 20° C to 5° C (during the dark period).

ces in intrinsic properties or a matter of spherical distribution in relation to the light source in our experiments.

It is particularly interesting to notice that the increase of soluble sugar content and the recovery of the water content with this light intensity only start 6-9 hours after the beginning of illumination with a slight difference in favour of the younger leaves. This may mean that differences in the duration of the light period may have important consequences for the relation between root temperature and growth and water content.

Influence of the relative air humidity and daylength on leaf elongation at low root or low meristem temperature

The effect of differences in the length of the light period on elongation growth are difficult to predict. On one hand the light period will affect the total amount of carbo-

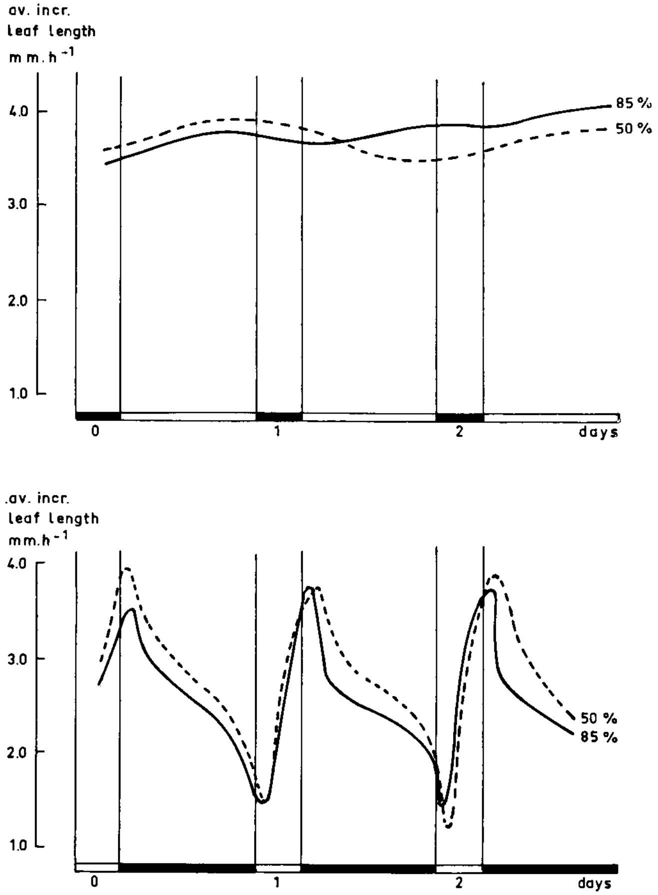

Fig. 9. Effect of relative air humidity (85 % and 50 %) and daylength on leaf elongation (8th leaf) at 20° C; top: daylength 18 hours; bottom: daylength 6 hours.

hydrates produced, on the other hand, especially when low root temperatures are used, it will influence the duration of the period of severe stress in the light relative to the duration of the period of moderate stress in the dark. Moreover the duration of the light period may influence the elongation rate via intrinsic photoperiodically determined factors (Ryle, 1966).

The transition from dark to light and vice versa did not affect elongation rates when root temperatures were near optimum and light dark cycles were used of 18 h – 6 h, respectively. The elongation rate, therefore, did not seem to be directly dependent on the simultaneous production of carbohydrates (photosynthesis).

In addition, the minor effect of light intensity on leaf length (Bensink, 1971, Friend et al., 1962) coinciding with a stimulation of width growth by enhancing the light intensity might indicate that the carbohydrate supply does not influence elongation rate too much. We found however that shortening the light period to 6 hours definitely reduced elongation growth (Fig. 9). Obviously there is a discrepancy between short time effects and prolonged treatments. We may assume here that during early stages of leaf growth at a low light intensity narrow leaf initials are produced which are able to maintain rapid elongation even at a low level of carbohydrate production. In contrast our plants initiated wider leaves during the pre-treatment which continued growth during the experiment. Now the rate of elongation of many cells gets short of carbohydrates much more readyly. It can be seen that in this case the direct production leads to a gradually increasing elongation rate during the 6 hours light. As a consequence of a lag period due to distance between source and sink this increase continued during the first hours of darkness but then reversed into a decrease due to exhaustion.

As soon as water stress is induced by applying low root temperature the relative humidity of the air becomes important as well as the duration of the light period. By reducing total transpiration over a 24-hour period either by enhanced air humidity or

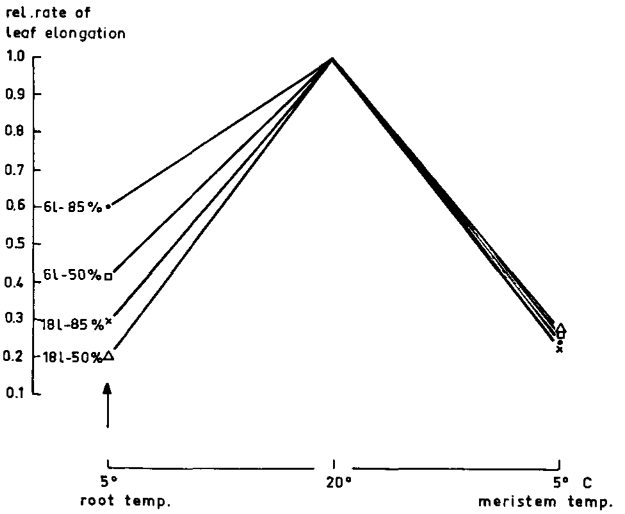

Fig. 10. Relative changes in rate of leaf elongation at 5° C root temperature or 5° C meristem temperature compared with 20° C root and meristem temperature at a low and high relative air humidity (85 % and 50 %) in daylengths of 6 and 18 hours (during 3 days).

by shortening the light period the average water stress at a root temperature of 5° C is reduced. From Fig. 10 (⋏) it can be seen that this has a favourable effect on daily elongation rates.

It can be seen (Fig. 10) that the same treatments are ineffective on leaf elongation when the meristem is cooled, since the latter treatment affects growth rate directly by reducing the rate of processes leading to growth and not indirectly via the water content (Kleinendorst and Brouwer, 1970).

Comparison of meristem cooling with local stem cooling above the meristem

Local cooling of the leaf sheaths above the meristem to 5° C (between 4th and 5th leaf) results in a considerable decrease in the rate of leaf elongation (Fig. 11). In contrast to meristem cooling the effect rather decreases than increases with time (cf. Kleinendorst and Brouwer, 1970; Fig. 7). Similar to meristem cooling leaf water content is not affected. This reduction in elongation rate probably indates an inhibition of the translocation of substances from the leaves to the meristematic region (Swanson and Whitney, 1953; Mortimer, 1961; Whitehead, 1962; Thrower, 1965; Webb and Gorham, 1965; Webb, 1967; Swanson and Geiger, 1967; Webb, 1971).

Several workers (Esau et al., 1957; Bohning et al., 1952; Thrower, 1965; Ford and Peel, 1967) have pointed out that low-temperature inhibition of translocation could be either the result of a physical effect (e.g. increased viscosity of protoplasm or of sieve-tube cell sap), or the result of a direct effect on respiratory reactions supplying the energy for the translocation process.

In our case the reduction of translocation was demonstrated in an experiment with $^{14}CO_2$ application to the most active leaf (5th leaf) which had just finished elongation (Hofstra and Nelson, 1969; Doodson et al., 1964; Thrower, 1967) with and without local cooling to 5° C at the base of the leaf blade. Fig. 12 shows the translocation after about 1½ hour. At that time the control plants contained ^{14}C in all growing regions. Cooling the base of the leaf blade supplied with $^{14}CO_2$ resulted in a considerable reduc-

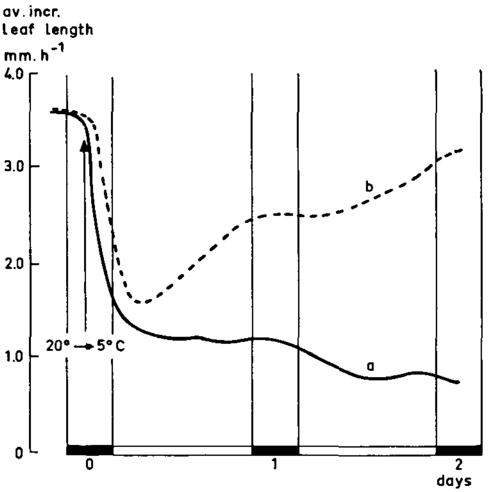

Fig. 11. Effect of meristem (a) cooling (5° C) or local cooling above the meristem (b) on leaf elongation (8th leaf).

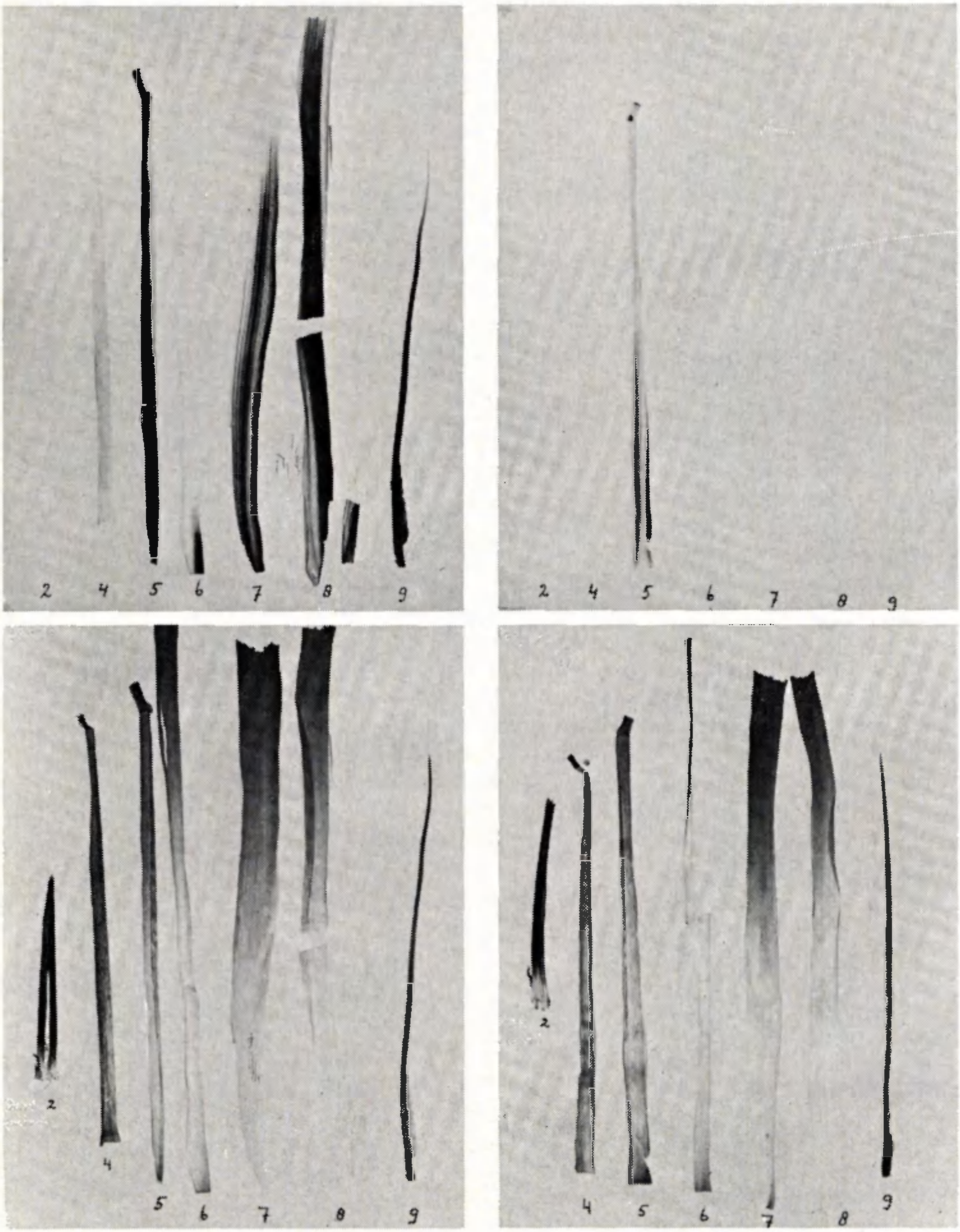

Fig. 12. Bottom: photos of the leaf sheaths (2nd - 9th leaf)
Top: autoradiographs of the same 1½ h after applying $^{14}CO_2$ to the top of the 5th leaf
Left: control
Right: plant cooled locally at the 5th leaf (just above the change of leaf sheath to leaf lamina).

Table 1. Percentage of total soluble carbohydrates in the leaves, part of the stem above the meristem and part of the stem at the meristematic region at a low temperature of 5° C locally above or at the meristem (after 2 days).

	Control	Temperature 5° C	
		local above meristem	meristem region
leaves	<0.88	2.1	2.2
stem (above meristem)	3.4	6.9	5.9
stem (mer. region)	3.3	4.4	11.6

tion of the translocation of ^{14}C which in fact appeared to be restricted to the treated leaf.

In the control plants carbohydrates move from the leaves to the meristem and are consumed there. Cooling of the meristem region reduces growth and the rate of carbohydrate consumption. Obviously the translocation from the leaves continues at first, so that an accumulation of carbohydrates in or near the meristem is to be found (Table 1). Cooling of the translocation track above the meristem reduces translocation rate. Since at first the consumption of carbohydrates and growth continue, a rather low concentration (of the same order or somewhat lower than that of the controls) of carbohydrates is induced. After some time the rate of elongation decreases, because the concentration of carbohydrates is limiting. However, since the reduction of translocation rate as a consequence of local cooling is expected to be only temporary, the supply of the meristem would be almost normal after a few hours of inhibition (Swanson and Geiger, 1967).

This would mean that the increased concentration gradient completely compensates the changes in viscosity induced by low temperature treatment.

A comparable recovery at continuous local low temperature treatment was observed by Zurzycki (1951) in *Elodea densa* Casp., Geiger (1969) in sugar-beet, Swanson and

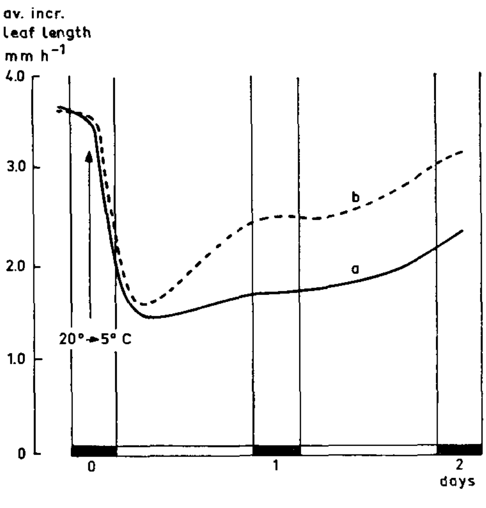

Fig. 13. Comparison of the influence of the first 4 leaves removed (a) or no leaves removed (b) in leaf elongation (8th leaf), at a temperature locally above the meristem of 5° C (just above 4th leaf).

Bohning (1951) in *Phaseolus vulgaris,* Swanson and Geiger (1967) in sugar-beet, Bowling (1968) in *Helianthus,* and Webb (1971).

Geiger 1969) considered the recovery due to a reversal of structural deviations rather than to an increasing concentration gradient. Whatever the mechanism, a gradual recovery of the elongation rate during local cooling of the translocation pathway (Fig. 11) indicates that translocation also recovers gradually.

When cooling is applied above the insertion of the four oldest leaves the presence of these leaves interacts with the cooling effects. Obviously, substances produced in these leaves induce the youngest leaf to grow a little better than without this supply. Since it is assumed usually that the oldest leaves primarily serve the supply of the root system, it may be expected that it will take some time before the growth of the young leaves profits of this supply (Fig. 13). It stands to reason that the presence of these older leaves does not interact with leaf growth, when the meristematic region is cooled.

Although these experiments clearly indicate that a reduction in phloem translocation reduces leaf elongation rate, it is still uncertain whether this reduction results from shortage of carbohydrates or from a shortage of some other essential growth substances. In this respect a number of possible essentials can be considered such as phloem translocated minerals and hormones. As one of the possible candidates, we examined the effect of indolacetic acid placed on the cut surfaces where leaf blades were removed. It was found that growth rate profitted somewhat by this treatment. Since, however, the elongation rate also increased when intact leaves were supplied with I.A.A., growth being stimulated to the same extent when intact or cut plants were treated (Fig. 14) we tentatively concluded that the observed growth reduction after leaf cutting and therefore also after cooling phloem translocation was due to inhibition of carbohydrate supply to the meristem.

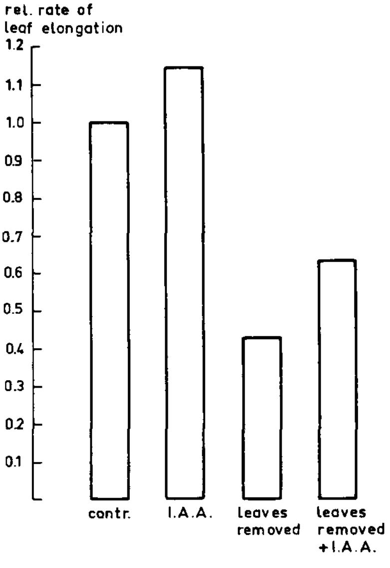

Fig. 14. Effect of applying indol-acetic-acid to intact plants and to plants of which all the leaves had been removed, on the rate of leaf elongation (8th leaf) during a 5-day experiment.

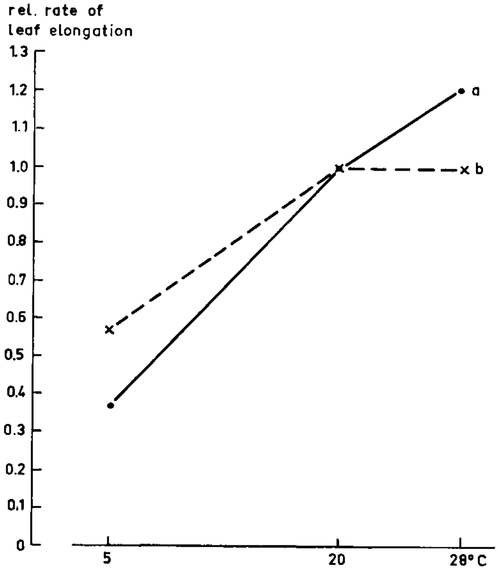

Fig. 15. Effect of meristem temperature (a) and of the temperature of the transport track (b) on leaf elongation.

From the results presented above it is clear that changes in temperature of the meristem itself are more effective than changes in the temperature of the pathway of assimilation products. Plotting the effect of both processes against temperature (Fig. 15) shows that increasing the temperature of the translocation pathway above 20° C is not effective, whereas an increase in temperature of the meristem from 20° C to 28° C resulted in growth stimulation. This means that the temperature of the translocating tissues is not limiting growth, when kept around 20° C.

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