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# Prechilling of *Xanthium strumarium* L. Reduces Net Photosynthesis and, Independently, Stomatal Conductance, While Sensitizing the Stomata to $CO_2$[1]

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## ABSTRACT

**Greenhouse-grown plants of *Xanthium strumarium* L. were exposed in a growth cabinet to 10 C during days and 5 C during nights for periods of up to 120 hours. Subsequently, $CO_2$ exchange, transpiration, and leaf temperature were measured on attached leaves and in leaf sections at 25 or 30 C, 19 C dew point of the air, 61 milliwatts per square centimeter irradiance, and $CO_2$ concentrations between 0 and 1000 microliters per liter ambient air. Net photosynthesis and stomatal conductance decreased and dark respiration increased with increasing duration of prechilling. The reduction in net photosynthesis was not a consequence of decreased stomatal conductance because the intercellular $CO_2$ concentration in prechilled leaves was equal to or greater than that in greenhouse-grown controls. The intercellular $CO_2$ concentration at which one-half maximum net photosynthesis occurred remained the same in prechilled leaves and controls (175 to 190 microliters per liter). Stomata of the control plants responded to changes in the $CO_2$ concentration of the air only slightly. Prechilling for 24 hours or more sensitized stomata to $CO_2$; they responded to changes in $CO_2$ concentration in the range from 100 to 1000 microliters per liter.**

Exposure of plants to low temperatures reduces net photosynthesis (4, 9, 10, 13, 14). The effect persists for at least 1 day after chilled plants are returned to a warmer environment (10). Prechilling also causes a reduction in stomatal aperture (1, 14) but it is not clear whether this is due to a direct effect on the stomata (as proposed by Tschäpe [14]) or a response to an increased intercellular $CO_2$ concentration, resulting from an impairment of the photosynthetic apparatus. It is also possible that reduced stomatal apertures in chilled plants are a response to a lowered water permeability of the roots and thus decreased water potential in the leaves.

We wanted to separate the effects of prechilling on the stomata from effects of prechilling on the photosynthetic apparatus. We thought this task could be accomplished by relating stomatal conductances as well as rates of net $CO_2$ uptake separately to the intercellular $CO_2$ concentrations. If, for instance, the intercellular $CO_2$ concentration were lower in prechilled plants than in the controls, all other conditions remaining equal, then a low stomatal conductance would have been a cause and not a consequence of diminished net photosynthesis. We conducted our experiments at several levels of $CO_2$ concentration in the air in order to cover a broad range of intercellular $CO_2$ concentrations.

We used *Xanthium strumarium* L. for our experiments because we knew that the stomata in this species were less widely open in chilled plants than in plants kept at 25 C (1). We made measurements on attached leaves, as well as on leaf sections well supplied with water, in order to see whether a reduced water supply from the root was responsible for the reduction in stomatal aperture after chilling.

## MATERIALS AND METHODS

Plants of *Xanthium strumarium* L. were grown in potting mixture in a greenhouse at 25 C during the day and 21 C during the night. The relative humidity was about 75%. The natural photoperiod was extended by supplementary illumination (30 $\mu$w cm$^{-2}$) with Sylvania Gro-lux lamps (F40/GRO/WS) from 4:30 to sunrise and from sunset to 24:00; the plants did not flower. The plants were pruned to carry five or six leaves.

For chilling, the plants were transferred to a growth cabinet with 10 C, 50% relative humidity during the day and 5 C, 80% relative humidity during the night. The photoperiod was 14 hr of light (10.2 mw cm$^{-2}$) from General Electric cool white fluorescent lamps (FR/T12/CW/1500) supplemented by light from incandescent bulbs. Another growth cabinet with 23 C, 70% relative humidity during the day, 20 C, 80% relative humidity during the night and the same light conditions as in the low-temperature cabinet served as a second control environment besides the greenhouse. However, since rates of net photosynthesis and stomatal conductances measured at 300 $\mu$l l$^{-1}$ $CO_2$ did not differ in leaves from plants grown in the warm chamber or in greenhouse-grown plants, we used greenhouse plants almost exclusively as controls during our investigation.

For the measurement of net photosynthesis and stomatal conductance, assimilation chambers were clamped to the upper and lower surfaces of fully expanded attached leaves or to leaf sections. Leaf temperature was controlled by submerging chambers and leaves in a water bath. (Leaf parts not covered by the chambers were in contact with stirred water.) The temperature of the bath was 25 C or 30 C. The dew point of

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the air supplied to the chambers was 19 C. In some experiments, the concentration of $CO_2$ was constant at 300 (±5) $\mu$l $l^{-1}$ but in others it was changed stepwise in the range from 0 to 1000 $\mu$l $l^{-1}$. Light was supplied by a 6 kw xenon arc lamp. An infrared-absorbing filter (C.S. 1–69, Corning 4600) was placed between the lamp and the submerged Plexiglas chambers. The irradiance was 61 mw $cm^{-2}$ of photosynthetically usable light, corresponding to a quantum flux density of 270 nE $cm^{-2}$ $sec^{-1}$. Solenoid valves periodically connected the gas lines coming from the chambers to infrared gas analyzers for the determination of changes in the $CO_2$ and water vapor content of the air. Voltages from the gas analyzers, along with those from thermocouples measuring leaf temperatures and the dew point of the air supplied to the chambers, were recorded on magnetic tape. Results were computed for each leaf surface separately.

Measurements were completed 3 hr after removal of the plants from the low temperature treatment. Measurements took up to 6 hr if the experiments included changes in the $CO_2$ concentration of the air.

Intercellular $CO_2$ concentrations were computed from determinations of transpiration, water vapor pressure difference between leaf and air, $CO_2$ concentration in the ambient air, and net photosynthesis, on the assumption that the water vapor pressure within the leaf is equal to that of free water (11). However, this assumption may be invalid (5). The water vapor pressure at the surfaces of the mesophyll cells may be lower than assumed and this depression appears to depend on the intercellular $CO_2$ concentration (3). Therefore, all conductances and intercellular $CO_2$ concentrations reported by us are only apparent. Nevertheless, comparisons between rates of net photosynthesis and stomatal conductances and their relationships to intercellular $CO_2$ concentrations will be little affected because the same errors in the determination of the absolute intercellular $CO_2$ concentration would apply to both relationships and the possible underestimation of stomatal conductances does not change the qualitative conclusions we drew from the results of our experiments.

The conductances we present include the conductance of the boundary layer. Since the conductance for $CO_2$ of the boundary layer was kept constant at 2.6 cm $sec^{-1}$ on either surface of the leaf and the highest epidermal conductance was approximately 1 cm $sec^{-1}$, observed differences of conductances between treatments and controls indicate changes in the epidermis.

We determined epidermal conductances but shall call them stomatal conductances because gas flux through the stomata was estimated to be 10 to 30 times greater than through the cuticle.

Spectral reflectivity and transmissivity of leaves were measured at normal incidence of light with an integrating sphere attached to a Zeiss PMQ II spectrophotometer.

## RESULTS

**Decreases in Net Photosynthesis and Stomatal Conductance in Relation to the Duration of Prechilling.** Net photosynthesis and stomatal conductance as measured at 30 C and ambient $CO_2$ concentration between 295 and 310 $\mu$l $l^{-1}$ declined with lengthening exposure of the plants to low temperatures. After 24 hr chilling, dark respiration increased but did not match the decrease in photosynthesis (Fig. 1). Intercellular $CO_2$ concentration (as measured at 30 C) was little affected by prechilling treatments lasting up to 48 hr; it increased only after longer exposures to low temperatures. After 96 hr or more of prechilling, the intercellular $CO_2$ concentration was almost as high as in the ambient air.

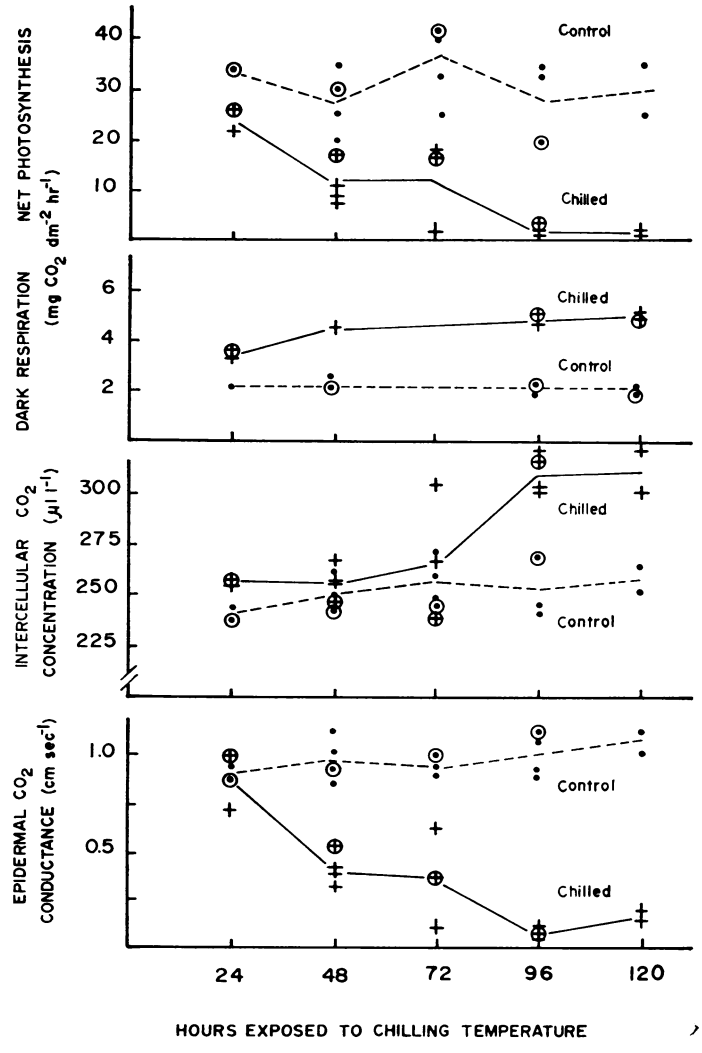


FIG. 1. Rate of net photosynthesis, dark respiration, intercellular $CO_2$ concentration during photosynthesis, and epidermal $CO_2$ conductance (abaxial and adaxial) in prechilled plants (+) and controls (●) as related to the duration of chilling. Chilling was begun at the start of a light period and measurements were made at 30 C after the end of a dark period. Circled data are from attached leaves; the other data are from leaf sections. Irradiance: 61 mw $cm^{-2}$; dew point of the air: 19 C.

The results were virtually the same whether measurements were made on attached leaves or leaf sections (Fig. 1). Some reversal of the effect of prechilling occurred while the measurements were made. Rates of photosynthesis and stomatal conductances measured at about 300 $\mu$l $l^{-1}$ $CO_2$ were 10 to 15% higher at the end of a 6-hr exposure to 25 C than 1 or 2 hr after transfer of the plants from the chilling to the measuring condition.

**Net Photosynthesis in Relation to the Intercellular $CO_2$ Concentration.** Net photosynthesis was measured at 25 C in attached leaves and leaf sections at different $CO_2$ concentrations in the air supplied to the assimilation chambers. Intercellular $CO_2$ concentrations were computed and the measured values of net photosynthesis were related to them. The results obtained with attached leaves are shown in Figure 2. Measurements on leaf sections gave very similar results, except that the magnitude of net photosynthesis was approximately 60 to 90% of that in attached leaves. Apparently, the duration of prechilling did not affect the $CO_2$ compensation point nor the

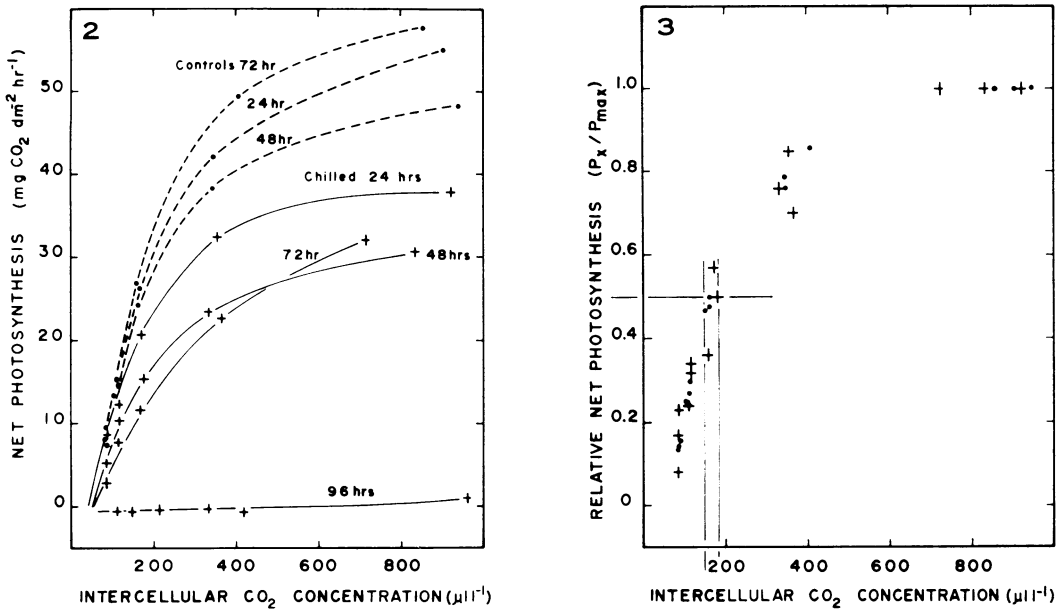


FIG. 2. Rate of net photosynthesis in prechilled plants and in controls as related to the intercellular $CO_2$ concentration. Data are for attached leaves only. Temperature: 25 C; irradiance: 61 mw $cm^{-2}$; dew point of the air: 19 C.

FIG. 3. Relative rates of net photosynthesis computed as the ratios between net photosynthesis at a measured intercellular $CO_2$ concentration ($P_x$) and net photosynthesis at the maximal intercellular $CO_2$ concentration used ($P_{max}$). Data are from Figure 2, omitting data points for 96 hr chilling. +: prechilled; ●: nonchilled (control) plants. The lines enclosed indicate the range of intercellular $CO_2$ concentrations in which the rate of net photosynthesis appears to have been half-saturated with respect to $CO_2$.

pattern of the relationship between intercellular $CO_2$ concentration and net photosynthesis; only a parameter determining the amplitude of the curves was affected. This became even more evident when the curves were normalized with respect to the highest value of net photosynthesis measured in each treatment which always occurred at the highest concentration of intercellular $CO_2$. The relationships between net photosynthesis and intercellular $CO_2$ concentration were similar (Fig. 3). Similar results were obtained when the measured rates of dark respiration were added to the values of net photosynthesis. (Rates of dark respiration were unaffected by the intercellular $CO_2$ concentration.)

The intercellular $CO_2$ concentration at which one-half the maximum rate of net $CO_2$ uptake occurred was nearly the same for all treatments; it was between 175 and 190 $\mu l\ l^{-1}$.

**Stomatal Conductance in Relation to the Intercellular $CO_2$ Concentration.** In the control leaves, stomata hardly responded to changes in ambient and intercellular $CO_2$ concentration. Stomatal conductances measured at an intercellular concentration of approximately 900 $\mu l\ l^{-1}$ were on the average 85% of those measured at approximately 100 $\mu l\ l^{-1}$ (Fig. 4). Prechilling made the stomata sensitive to $CO_2$; the largest responses were those to changes in intercellular $CO_2$ concentration between 150 and 400 $\mu l\ l^{-1}$. Maximal stomatal conductances did not occur at the lowest intercellular $CO_2$ concentrations achieved but at somewhat higher values, around 150 $\mu l\ l^{-1}$.

At any one intercellular $CO_2$ concentration, stomatal conductance decreased with increasing exposure to chilling conditions prior to the measurement. An exception to this rule was observed only after 24 hr of chilling, when stomata exposed to low intercellular $CO_2$ concentrations opened wider than in the controls (Fig. 4). After 96 hr of chilling, the stomata hardly opened at all.

Stomatal conductances of the upper (adaxial) epidermis were virtually always lower than the ones of the lower epidermis. The ratio of conductances was always larger than would have been expected from the ratio of stomatal densities (between 1.2 and 1.3, lower:upper).

**Absorbance Changes during Chilling.** Chilled plants looked yellowish. The absorbance of their leaves was, however, only slightly lower than in the control plants. This reduction occurred during the first 24 hr in the cold chamber and hardly developed further during the following 96 hr. After 120 hr exposure of the plants in the cold chamber we determined the absorbances given in Table I. We conclude that the Chl content of the leaves did not drop substantially during chilling.

## DISCUSSION

Figure 1 shows that in normal air, with the $CO_2$ concentration between 295 and 310 $\mu l\ l^{-1}$, the intercellular $CO_2$ concentration of prechilled leaves was only a few $\mu l\ l^{-1}$ (maximally 50 $\mu l\ l^{-1}$) below the $CO_2$ concentration in the ambient air, indicating that the stomata did not represent the major resistance to the movement of $CO_2$ from the atmosphere to the sites of carboxylation. After 96 hr of chilling the intercellular $CO_2$ concentration was close to that in the air outside the leaf. An inspection of the relationship between net photosynthesis and the intercellular $CO_2$ concentration (Fig. 2) also leads to the conclusion that the reductions in net photosynthesis which occurred in prechilled plants were primarily caused by a direct effect of chilling on the photosynthetic apparatus and not by effects upon the stomata.

Reduction of net uptake of $CO_2$ by prechilling can only partially be ascribed to increased rates of $CO_2$ evolution, if at all. If prechilling indeed caused an increase in the liberation of $CO_2$ in the light as large as that measured in the dark, about 19% of the reduction in net photosynthesis would have been explained. (The same fraction of 19% was found after all durations of chilling when net photosynthesis was determined at an intercellular $CO_2$ concentration of 250 $\mu l\ l^{-1}$). Changes in photorespiration cannot explain the reduction in net photo-

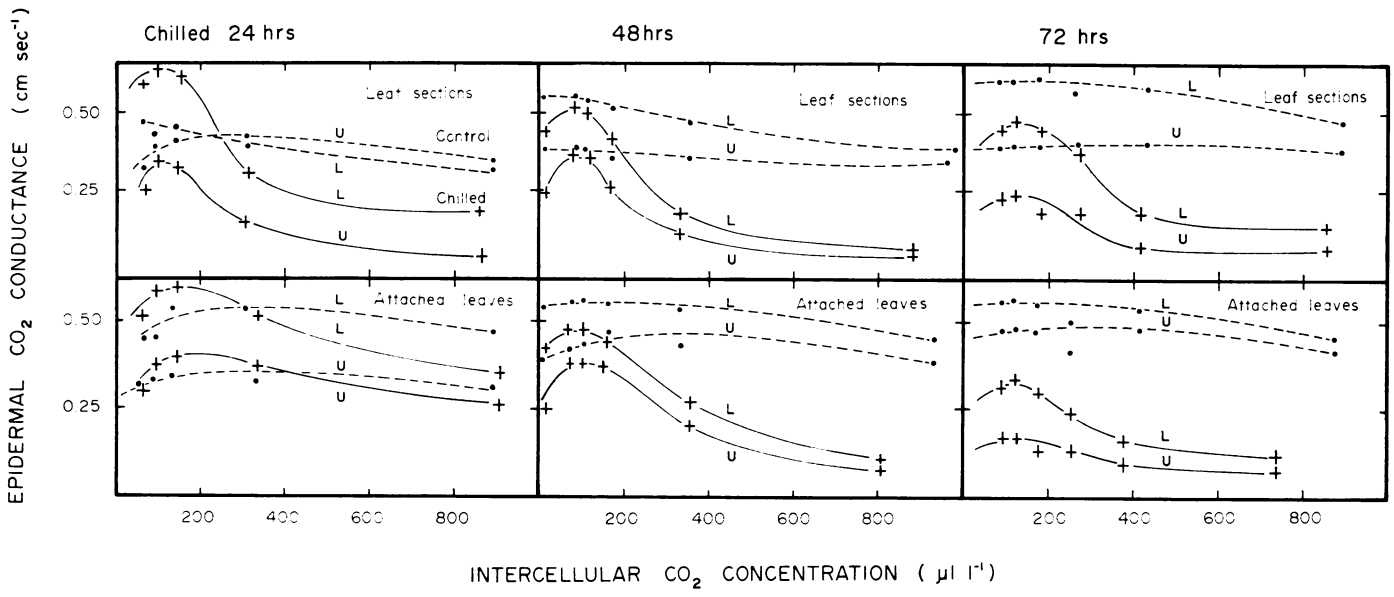


FIG. 4. Epidermal $CO_2$ conductances of upper (U) and lower (L) leaf surfaces in prechilled (+) and control plants (●) as related to the intercellular $CO_2$ concentration. Temperature: 25 C; irradiance: 61 mw $cm^{-2}$; dew point of the air: 19 C. After 96 hr of chilling, total conductances of attached leaves were 0.14 cm $sec^{-1}$ at an intercellular $CO_2$ concentration of 10 $\mu l\ l^{-1}$, 0.08 cm $sec^{-1}$ at 300 $\mu l\ l^{-1}$, and 0.07 cm $sec^{-1}$ at 970 $\mu l\ l^{-1}$.

synthesis either since the $CO_2$ compensation points were similar in all plants, prechilled ones and controls. (Compensation points were determined by extrapolation of the "saturation curves" of net photosynthesis—Fig. 2).

The results of our determinations of leaf absorbances make it unlikely that the depression of photosynthesis by prechilling was mediated by a reduction in the Chl content of the leaves.

The observed reduced activity of the photosynthetic apparatus could have been the result of a cold-induced defect in the photosynthetic electron transport mechanism which has been found in chloroplasts isolated from chilled leaves (7, 8). However, RuDP-carboxylase could also have been affected (6).

The relationship between net photosynthesis and intercellular $CO_2$ concentration did not follow exact saturation kinetics, nor was this to be expected. Nevertheless, the intercellular concentrations at which one-half the maximum rate of net photosynthesis was attained were the same in prechilled plants and in controls. We conclude, therefore, that prechilling affected the maximal rates of net photosynthesis, but not the apparent affinity of the assimilatory apparatus for $CO_2$. The intercellular $CO_2$ concentration at which net photosynthesis proceeded at half the maximal rate was surprisingly low, if compared for instance, with values reported for *Nicotiana* and *Zea* (16).

The increase in dark respiration after chilling reminds us of similar observations made on cucumber fruits (2) whose $CO_2$ production increased when they were transferred to 25 C after periods of chilling of various lengths. Our data are insufficient to explain the increase in $CO_2$ evolution in the dark following chilling.

Although the reduction in net photosynthesis by prechilling was not due to an effect on the stomata, chilling did also affect the stomata. The most remarkable effect was a sensitization to $CO_2$. Stomata in the controls did not close in response to increases in intercellular $CO_2$ concentration (reminiscent of Zelitch's report on stomatal insensitivity to $CO_2$ in leaf sections of tobacco floating on water [15]). Chilling produced a pattern of stomatal response to intercellular $CO_2$ concentration

Table I. *Absorbances of Leaves of Xanthium strumarium*

Absorbance = log (1-reflectivity-transmissivity). Chilled leaves were in the cold chamber for 120 hr.

| Wavelength | Absorbance | | $\Delta A$ |
|---|---|---|---|
| | Control | Chilled | |
| *nm* | | | |
| 450 | 0.959 | 0.954 | 0.005 |
| 550 | 0.858 | 0.810 | 0.048 |
| 670 | 0.967 | 0.964 | 0.003 |

characterized by a maximum conductance at about 150 $\mu l\ l^{-1}$ $CO_2$ and a steep decline in conductance as the intercellular $CO_2$ was raised above this level. The stomata of the upper and the lower leaf epidermis were sensitized to $CO_2$ to similar extents (Fig. 4). We suspect that abscisic acid was produced in leaves of *Xanthium strumarium* during chilling. We base this assumption on the finding that an application of this hormone to leaves of this species sensitized stomata to $CO_2$ (12); (experiments confirming this hypothesis have since been concluded in the second author's laboratory).

In addition to acquiring a sensitivity to $CO_2$ during chilling stomata did not open in prechilled plants as widely as in the greenhouse-grown controls. Figure 4 shows that at any intercellular $CO_2$ concentration (except below 200 $\mu l\ l^{-1}$ in leaves prechilled for 24 hr) stomatal conductance decreased with increasing duration of the chilling treatment. Drake and Salisbury (1) showed that stomata in prechilled leaves did not open as wide at any subsequent temperature between 5 and 45 C as did stomata in unchilled leaves. Tschäpe (14) observed that stomata responded sluggishly after chilling. She also showed that the ability of stomata to open in epidermal strips floating on solutions of KCl was impaired if the strips were taken from chilled plants. However, differences between treatments and controls became apparent only at high concentrations of $K^+$. One would have expected a damage to the ion transport system to become most evident at low concentrations of $K^+$.

It remains in question, therefore, which part of the stomatal mechanism was affected by chilling.

The effects of prechilling on net photosynthesis and on the stomata were the same in attached leaves and in leaf sections. This rules out the possibility that reduced water uptake by the root caused stomatal closure. Chilling might have affected some other property of the root than its water permeability, and triggered the transmission of a signal to the leaves causing the photosynthetic apparatus and the stomata to respond in the observed manner. This is, however, unlikely because in experiments of Tschäpe (14) in which the roots of plants of *Cucumis sativus, Phaseolus vulgaris, Glycine max* and *Zea mays* were kept at 20 C while the tops were chilled, similar inhibitions of stomatal opening and $CO_2$ uptake occurred, as were observed earlier when whole plants were subjected to low temperatures.

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