Mechanism of Inhibition of Translocation by Localized Chilling

R. T. Giaquinta and D. R. Geiger
Department of Biology, University of Dayton, Dayton, Ohio 45469

ABSTRACT
Arrhenius plots of translocation velocity as a function of petiole temperature show a marked increase in temperature dependence below 10 C in bean (a chilling-sensitive species) but not in sugar beet (chilling-resistant). The increased temperature dependence below 10 C was not observed for cytoplasmic streaming or oxygen uptake in bean. Bean petioles were severed to release pressure in order to determine whether sieve tubes are obstructed in cold-treated petioles. The resulting pressure release caused serious displacement of the crystalline protein bodies in the sieve tubes of petioles at 25 C, but in those locally cooled to 0 C for 30 minutes little displacement occurred, indicating obstruction in the latter. An ultrastructural study of sieve tubes in tissue frozen rapidly in situ and dehydrated by freeze substitution revealed that treatment at 0 C for 30 minutes caused structural alteration and displacement of the cytoplasmic material lining the sieve tube wall resulting in occlusion of sieve plates. The sieve plates of the control petioles at 25 C were generally clear of obstructions. The results indicate that inhibition of translocation by chilling in chilling-sensitive plants results from physical blockage of sieve plates rather than from direct inhibition of a metabolic process which drives translocation.

Since the early work of Child and Bellamy (1) there has been considerable interest in the stoppage of translocation caused by chilling a short portion of the path. Aside from its agronomic importance, this phenomenon is of interest because it is cited to support the view that translocation is driven by energy supplied along the translocation path, for example, by electroosmosis, micropoeptisalis, or transcellular strand streaming. Recent work has shown that drastic inhibition does not occur above 0 C in chilling-resistant plants such as sugar beet and willow (6-8, 20, 21). Evidence from previous studies suggests that the cause of the inhibition may be physical obstruction due to chilling damage rather than direct inhibition of the force driving translocation (7, 22).

The experiments reported here were conducted to determine whether the severe inhibition of translocation noted in chilling-sensitive plants such as bean and squash is caused by physical damage to the conducting system. The effect of cooling a zone of the petiole on translocation was studied by measuring velocity and mass transfer of translocation in coordination with light and electron microscope observation of the treated tissue. The results show that in bean, a chilling-sensitive species, the inhibition of translocation observed below 10 C is produced by obstruction of sieve plates caused by chilling-induced cytoplasmic changes. There was no evidence of transcellular strands or other cytoplasmic structures traversing the sieve pores in actively translocating petioles. The slight temperature dependence observed in bean above 10 C and in sugar beet above 0 C appeared to be the result of viscosity changes in the sieve tube sap.

MATERIALS AND METHODS
Sugar beet (Beta vulgaris L., Klein E type monogerm hybrid) and bean (Phaseolus vulgaris L., cv. Black Valentine) plants were grown according to procedures described previously (9). Plants were trimmed to a simplified source-path-sink system. In bean plants arrival of translocate was monitored in the terminal leaflet of the first trifoliate leaf while one of the primary leaves served as the source leaf. Apparent translocation velocity was measured by pulse labeling the source leaf with 40 to 50 µC of 14CO2 for 5 to 7 min and monitoring the initial arrival of 14C-translocate in a young sink leaf using a thin window G-M tube (8, 9). The proportion of the 14C pulse translocated from the source leaf during the first 45 min for sugar beet and first 30 min for bean was used as a measure of mass transfer rate as previously described (8). A 2-cm cold block along the source petiole, thermostatically held at the treatment temperature, was used to inhibit translocation. Sugar beet petioles were cooled for 10 min or 90 min prior to labeling while in bean a 10-min cooling period preceded labeling. The amount of 14C translocated was measured with an ion chamber electrometer following conversion of the plant tissue to CO2 (9).

Oxygen consumption rates were determined as a function of temperature for sugar beet and bean petiole tissue slices using a model 53 YSI oxygen electrode. Temperature was maintained by a thermostat-controlled circulating bath.

Velocity of cytoplasmic streaming in bean leaf hair cells was measured with a Nomarski microscope, equipped with a cooling stage. An ocular reticle and stopwatch were used to measure the time required for organelles to travel a known distance. Data were evaluated by analysis of covariance to determine whether a marked change in temperature dependence occurs.

Displacement of protein crystals in sieve tubes was brought about by the pressure released when the petiole was severed quickly. The degree of displacement was compared in tissue at 0 C and 25 C by treating paired primary leaves. Cuvettes containing an ice-water mixture and 25 C water, respectively, were attached to the opposite petioles. After a 30-min treatment, the opposite petioles were severed rapidly and simultaneously with a razor blade at their midpoints. The water was immediately replaced with buffered glutaraldehyde-parafomaldehyde, and the petiole segments on either side of each initial cut were slit longitudinally. The tissue was thus fixed for 45 min in 4% glutaraldehyde-4% paraformaldehyde in 0.1 M
phosphate buffer at pH 7.2 before further cutting. Petiole segments were collected and fixed for an additional 2 hr in the aldehyde fixative, postfixed in 2% (w/v) OsO₄ in 0.1 m phosphate buffer, pH 7.2, and processed for light microscopy. Paraffin sections of 4 to 5 μm thickness were cut, viewed, and scored as to the degree of crystal displacement.

Because sieve tube exudate cannot be collected readily from bean plants, exudate was collected from the chilling-sensitive castor bean (Ricinus communis, L.) by stem incision (17). Fluidity (reciprocal of viscosity) was measured as a function of temperature by a falling-sphere microviscometer enclosed in a temperature-controlled cooling jacket.

Tissue from bean petioles cooled to 0 C for 30 min, and from 25 C-treated petioles was processed for electron microscopy by conventional techniques and by freeze-substitution (5). For chemical fixation, paired primary leaf petioles at 25 and 0 C were severed rapidly while immersed in phosphate-buffered 4% (w/v) paraformaldehyde-4% (v/v) glutaraldehyde mixture at the appropriate temperature. The tissue was cut in the manner described above. Data from the pressure release experiments indicated that sieve plate blockage near the sieve of temperature 

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RESULTS AND DISCUSSION

Previous studies have shown that chilling-resistant plants, such as sugar beet and willow, show neither severe nor lasting inhibition of solute translocation as a result of path chilling (6–8, 20, 21). On the other hand, chilling-sensitive plants such as bean show a marked inhibition of mass transfer rate when a short region of stem or petiole is cooled (7, 19, 22). In this study temperature dependence curves for translocation velocity and mass transfer were determined by pulse labeling a source leaf while a 2 cm region of the petiole was held at a given temperature (8). Following the rationale of Lyons and Raison (16) Arrhenius plots of log translocation rate versus the inverse of absolute temperature were constructed. Velocity of ¹³C-labeled translocate is shown as a function of petiole temperature for sugar beet and bean in Figure 1. Arrhenius plots for mass transfer rate are very similar to the velocity plots and are not shown. A distinct increase in temperature dependence is observed below 10 C in bean and below −1 C in sugar beet. It is difficult to apply statistical analysis to the sugar beet data to determine if a break in the curve occurs in the 0 to −2.5 C range, but it appears that severe inhibition occurs below −1 C (Fig. 1A). The velocities in Figure 1 are measured after 10 min of petiole cooling before feedback adjustments can occur. If the velocity is measured after 90 min of petiole chilling in sugar beet, both velocity and mass transfer rate adjust to the precooling rate (Fig. 2). The time course of this adjustment transient has been shown previously for sugar beet (8, 20). The subzero portion of the curve for sugar beet (Fig. 2) does not show marked recovery within the 90-min period and in this respect resembles the portion of the curve for bean below 10 C. The effect produced by localized cooling at temperatures above 10 C in bean or above −1 C in sugar beet can be explained by changes in fluidity of the sieve sap (Table I and Fig. 3). The Qin values in the upper temperature ranges for translocation velocity and mass transfer are close to the values for change in the fluidity of sieve tube exudate and 10% sucrose. Poiseuille's Law states that flux (cm²/sec·cm) and hence velocity (cm/sec) are directly related to sieve tube sap fluidity. The effect of increased resistance to flow appears to be overcome by 90 min of cooling, probably by virtue of an increased pressure gradient.

Our attention was directed to the more drastic and long lasting effect of chilling below 10 C in bean. Petioles could be chilled to −2.5 to −3 C with only occasional freezing. In the latter case a rapid temperature rise was observed, apparently the result of the freezing of the xylem sap or perhaps the water in the entire petiole. The complexity of the freezing pattern in plants has been discussed by Havis (12). Although the narrow working range between −1 and −3 C made it impractical to make a detailed study at these temperatures, it appears that there is a marked reduction in translocation of sugar beet even in the absence of freezing. When the Arrhenius plots for bean and sugar beet are compared (Fig. 1), the pattern resembles that for respiration of isolated mitochondria in chilling-sensitive and chilling-resistant plants measured by Lyons and Raison (16). Temperature dependence of petiole tissue respiration and of cytoplasmic streaming in leaf hair cells was analyzed by Arrhenius plots but did not show a marked break in the slope (Ref. 10 and Table I). From these results it was inferred that the large increase in temperature dependence for translocation velocity and mass transfer rate is not a result of the onset of severe inhibition of respiration or of cytoplasmic streaming at temperatures below 10 C in bean.

Results of earlier studies (7–9, 22) suggest that some type of damage to the sieve tubes might cause the marked inhibition of translocation observed in chilling-sensitive plants such as bean. The abruptness of the slope change (Fig. 1) suggests that below a threshold temperature of about 10 C a marked change occurs which interferes with movement of translocate. To determine whether this event might be a physical blockage of the sieve tubes resulting from localized chilling of the petiole, displacement of protein crystals during the sudden pressure release caused by rapid severing of the petiole was measured in tissue prepared for light microscope viewing. Crystal displacement was scored on a scale of 1 (severe displacement) to 4 (intact). When a petiole held at 25 C is severed quickly, the pressure release sweeps the protein crystals toward the sieve plates for a distance of over 1 cm from the cut (Fig. 4). Average scores for the control tissue indicate that the crystals are loosely to tightly appressed against the sieve plates as pressure...
escapect toward the cut. At 0 C pressure release displaces the crystals only in the first several millimeters, and beyond 4 mm from the cut there is little or no displacement (Fig. 4). The inability of these crystals to respond to the pressure release suggests that chilling has physically blocked the sieve tubes, perhaps by gelling of the sieve sap or by obstruction of the sieve plates.

To test whether sieve sap gels below some critical temperature, the viscosity of sieve tube exudate from castor bean, a chilling-sensitive plant, was measured between 0 and 30 C. Sieve tube exudate was collected by stem incision (17) and immediately sealed in a capillary along with a small sphere. The effect of cooling on the fluidity of sieve tube exudate as measured by a falling-sphere microviscometer is shown in Figure 3. The temperature curve, which is nearly identical to that for 10% (w/v) sucrose, does not show any abrupt changes in fluidity. The Q as for the rate of fall of the sphere (a measure of fluidity), is 1.3 for both 10% (w/v) sucrose solution and sieve sap (Table I). The change in fluidity can account for the gentle slope in the translocation rate between 25 and 10 C but not the abrupt change in velocity below 10 C (Table I and Figs. 1B and 3). These data do not rule out the possibility that the sieve tube exudate may be lacking some component normally present in the sieve sap of the intact plant which gels at about 10 C, although the data which follow make this less likely.

Recently Fisher (5) used rapid freezing and freeze-substitution to demonstrate reasonably undenatured and intact sieve tubes. This method was used in the present study to determine if some structural change in the sieve tubes might cause the blockage observed in chilled bean petioles. Adjacent regions of a petiole, or paired petioles, which were dissected to one vascular bundle, served as treated and control tissue. After being at the treatment temperature for 30 min, they were rapidly frozen and prepared for electron microscopy by freeze-substitution in methanol (5). Representative electron micrographs from tissue at 25 C and at 0 C are shown in Figure 5.

In the petiole at 25 C, the sieve pores are generally open (Fig. 5, A to D). Cytoplasmic material is sometimes associated with the sieve plates but is usually absent immediately over the pore (Fig. 5, B and D). Callose is generally minimal, and organelles and cytoplasm line the walls of the sieve tubes (Fig. 5, C and D). Protein crystals and some sieve tube plastids are present in the sieve sap. Generally the lumens are relatively free of fibrillar or membranous material, and no transcellular strands or other material is found passing from cell to cell in the control tissue. In petioles frozen rapidly after 30 min at 0 C, the pores are generally plugged with cytoplasmic material which is often continuous with the material lining the walls (Fig. 5, E to H). The severity of the plugging varies, but plates generally show most or all pores plugged. Some plates show the effect of pressure exerted on the plugging material (Fig. 5,
Table 1. Comparison of Q₁₀ Values for Translocation, Respiration, Cytoplasmic Streaming, and Viscosity over Selected Temperature Ranges

<table>
<thead>
<tr>
<th>Process Measured</th>
<th>Q₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td></td>
</tr>
<tr>
<td>Translocation velocity, no adjustment period</td>
<td>1.35</td>
</tr>
<tr>
<td>Translocation velocity, with adjustment period</td>
<td>0.98</td>
</tr>
<tr>
<td>Mass transfer rate, no adjustment period</td>
<td>1.5</td>
</tr>
<tr>
<td>Mass transfer rate, with adjustment period</td>
<td>0.96</td>
</tr>
<tr>
<td>Bean</td>
<td></td>
</tr>
<tr>
<td>Translocation velocity</td>
<td>1.10</td>
</tr>
<tr>
<td>Mass transfer rate</td>
<td>1.33</td>
</tr>
<tr>
<td>Petiole respiration</td>
<td>3.85</td>
</tr>
<tr>
<td>Streaming velocity</td>
<td>1.58</td>
</tr>
<tr>
<td>Castor bean</td>
<td></td>
</tr>
<tr>
<td>Sieve tube exudate, fluidity (reciprocal of viscosity)</td>
<td>1.32</td>
</tr>
<tr>
<td>10% sucrose solution</td>
<td></td>
</tr>
<tr>
<td>Fluidity</td>
<td>1.34</td>
</tr>
</tbody>
</table>

1 Statistical analysis did not give a clear indication of a marked increase in temperature dependence in sugar beet below 0 C because of the uncertainty of the slopes for the very limited temperature range from 0 to -2.5 C. In all cases the slope gave a Q₁₀ in excess of 4.

F and H). In some sieve tube elements, possibly those ahead of the trailing edge of the chilled zone, the plugging material is more loosely arrayed (Fig. 5E). The walls of cold-treated sieve tubes seem generally to contain less material (Fig. 5I), suggesting that chilling has disrupted the cytoplasmic material lining the walls, causing it to be swept into the pores. Flow, apparently, causes streamers to protrude through many of the plates (Fig. 5F). The absence of plugging of this type in the 25 C control tissue demonstrates that we are not observing an artifact of tissue preparation techniques.

Chemically fixed material shows some aggregation of material around the sieve plate in both the warm- (Fig. 5D) and the cold-treated (Fig. 5H) petioles. However, the organization of the sieve tube reticulum as seen in the 25 C glutaraldehyde-fixed material appears disorganized and is replaced by vesicles in the 0 C material prepared by chemical fixation. Part of the material present around the plates of chemically fixed tissue appears to be the result of injury caused by fixation itself (13) and partially masks the difference between control and chilled tissue. Consequently, it is difficult to determine with certainty the presence of chilling damage from chemically fixed material alone.

Membrane damage can be expected to result from cooling chilling-sensitive plants below 10 C (14–16). The onset of severe inhibition of translocation in the present study coincides with the point of marked inhibition of respiration of isolated mitochondria in chilling-sensitive plants (16). The membranes of chilling-sensitive plants (15) and of homeotherms (15) generally have a lower content of unsaturated lipids and consequently have a higher crystallization point for the membrane lipids. The marked break in the Arrhenius plots for certain processes in chilling-sensitive plants and homeotherms is thought to be the result of this temperature-induced phase change in the membrane lipids (15, 16). Luzzati and Husson (14) noted that under physiological conditions the lipoprotein complex is not far from a phase transition from a liquid-crystalline state to a gel. When the temperature is lowered, the hydrocarbon chains crystallize, disrupting the physiological activity of the lipid. Lyons and Raison reported that this transition temperature is close to body temperature in hemootherm animals (22 to 24 C) and is approximately 10 C in chilling-sensitive plants (15). They noted no transition for chilling-resistant species above 0 C as judged from Arrhenius plots.

The slope of the temperature dependence curves for various processes can be used to determine the nature of the temperature effect (11). The values for Q₁₀ of several processes are given in Table I. The slope for the translocation velocity and mass transfer rates above the critical temperature are between 1.1 and 1.5, indicating a physical process. The change in

Fig. 3. Arrhenius plot showing the effect of temperature on the fluidity of sieve tube exudate from castor bean. The rate of fall of the sphere is directly proportional to the fluidity of the fluid.

Fig. 4. Degree of displacement of p-protein crystals along the petiole as a result of sudden pressure release in bean petioles at 25 and 0 C. Representative graph obtained by scoring crystals in 20 sections of petioles from three plants.
CHILLING INHIBITION OF TRANSLOCATION

The inhibition of translocation by localized chilling results not from direct inhibition of the force driving translocation but rather from physical obstruction of the sieve pores. The variability and the paradoxical results that mark the literature of localized chilling and translocation appear to result from varying chilling sensitivities of different species and ecotypes.

LITERATURE CITED


Fluoride as a function of temperature has a Q10 in the same range (1.3). After an adjustment period the effect of temperature disappears in sugar beet, as shown by a Q10 of approximately 1.0. Below the critical transition temperature the steepened slope reflects a Q10 greater than 4, usually between 6 and 60 (Table I). The short range of temperatures makes exact calculation difficult, especially for sugar beet. These high Q10 values characterize processes such as denaturation or coagulation of protein rather than metabolic inhibition and are consistent with obstruction of pores as a result of cytoplasmic damage.

Chilling-associated changes in the cytoplasm noted in the present study include increased vesiculation of the sieve tube cytoplasm (Fig. 5, E, F, and H), disruption of the sieve tube reticulum (Fig. 5H), and the appearance of extraneous masses of cytoplasm inside vacuoles (Fig. 5I). Low temperature treatment has been found to cause cytoplasmic changes in a variety of organisms. Podbielskowa and Kaczperska-Palace (18) observed formation of membranous structures and vacuolization in the cytoplasm of onion root cells chilled to 5 C for 2 hr. The structural changes were reversed upon return to room temperature. Hamster intestinal cells showed membrane changes and severe vesiculation of cytoplasm after a 1-hr treatment at 0 C (4). Cooling tobacco cells causes formation of numerous small vacuoles or vesicles in the cytoplasm (3). Upon rewarming, the cytoplasm forms globular masses that gradually reform cytoplasmic strands. It seems likely that many, if not all, of the above changes are the result of membrane changes caused by chilling.

From the correlation of physiological and cytological data observed in this study we conclude that inhibition of translocation brought about by localized chilling is the result of cytoplasmic changes which result in occlusion of the sieve pores. It appears that chilling causes the lipid portion of the plasma membrane or perhaps the sieve tube reticulum to undergo a phase change. This phase change limits the fluidity of the membrane and may lead to vesicle formation and detachment from the wall. The collapse of the material lining the wall would allow flow to carry the cytoplasm, organelles, p-protein, and membranes into the sieve plate. The reduction in membrane fluidity could conceivably dislodge the plasma membrane along with the p-protein and various organelles from the wall of the sieve tube. In the chilling-sensitive bean this damage has a temperature threshold of about 10 C and a high Q10. The chilling-resistant sugar beet has a lower threshold for damage, perhaps -1 C. Adaptation or recovery occurs in sugar beet within 90 min even near 0 C. Below the threshold for cytoplasmic damage, recovery may be due to a gradual reversal of the changes by displacement or dissolution of the obstructions. The physical basis for low temperature inhibition and the low Q10 above the damage threshold are in line with the recent findings of Coulson, Christy, Cataldo, and Swanson (2). These workers noted no stoichiometric relationship among petiolar sucrose transport, petiolar respiration, and calculated ATP turnover rates when petioles of sugar beet plants were cooled.

FIG. 5. Electron micrographs of control bean petioles at 25 C (A-D) or petioles treated at 0 C for 30 min (E-I). Tissue prepared either by freeze-substitution (A-C, E, G, and I) or by chemical fixation (D, H). A: Sieve plate of control petiole showing open pores with minimal callose deposition. × 25,000. B: Oblique section through sieve plate of control petiole showing open pores and cytoplasmic material on plate but absent from pore openings. × 11,000. C: Sieve tube of control petiole showing open sieve pores, cytoplasmic material on the lateral walls and the absence of structures within the lumen. × 14,000. D: Chemically fixed, control petiole showing organized sieve tube reticulum on the lateral walls. × 10,500. E: Sieve tube of cold-treated petiole showing dispersed plasids and cytoplasmic material in lumen with loose plugging of sieve pores. × 13,000. F: Cold-treated petiole showing sieve pores occluded with cytoplasmic material and protrusions extending from pores. × 19,000. G: Cold-treated petiole showing continuity of cytoplasmic material in pores with that on the lateral wall. × 19,000. H: Chemically fixed cold-treated petiole showing extensive blockage of pores and vesiculation of cytoplasmic material. × 16,000. I: Cold-treated petiole showing vesiculation of cytoplasm into vacuole of a companion cell adjacent to sieve tube and reduced amount of cytoplasmic material along sieve tube walls. × 18,000.