The Transient Inhibition of Phloem Translocation in *Phaseolus vulgaris* by Abrupt Temperature Drops, Vibration, and Electric Shock

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ABSTRACT

Bean plants (*Phaseolus vulgaris* L. cv. Fardenlosa Shiny) approximately 3 weeks old were labelled with carbon-11 via their most basal compound leaves; and the transient inhibitions to stem translocation caused by (i) abrupt drops in temperature, (ii) vibration, or (iii) electric shock were studied. The duration of the inhibition caused by abrupt drops in temperature was found to decrease steadily with increasing temperature and to be absent above 40 °C, and the same was true of inhibitions caused by electric shock; however, translocation seemed relatively insensitive to temperatures as high as 55 °C. This inhibition, which could be observed in both intact stems and those with the epidermis removed, was abolished in the latter by presoaking in lanthanum-containing solutions although it was not sensibly affected by EGTA solutions or by calcium ionophores. Certain combinations of closely spaced stimuli (e.g. temperature—temperature or electroshock—vibration) caused the response to fatigue while others (e.g. electroshock—temperature) seemed not to. More detailed investigation of the electric shock inhibition showed (i) that it varied only slightly with electrode separation, (ii) that its duration was independent of shock polarity, but increased with shock duration to a plateau which was achieved at about 2 s, and (iii) that its duration increased steadily from a threshold at a shock intensity of about 4 V mm⁻¹.

Key words: Phloem translocation, cold shock, vibration, electric shock, calcium, calcium channels.

INTRODUCTION

It was noted some years ago (Pickard, Minchin, and Troughton, 1978a) that a small, abrupt drop in temperature (i.e. a negative-going step) applied to a translocating stem could produce a momentary cessation of translocation even though both endpoints of the step were well within the normal physiological operating range of the stem; abrupt rises in temperature had no obvious effect. This observation has since been extended and confirmed by Minchin and Thorpe (1983), and collateral data have been provided by Faucher, Bonnemain, and Doffin (1982) and Goeschl, Magnuson, Fares, Jaeger, Nelson, and Strain (1984). More recently, this effect and other fast cooling responses in plants have been hypothesized to be associated with increases in cytosolic free calcium (Minorsky, 1989).

In a collateral development, it has long been observed by workers who measure translocation with on-line isotopic techniques (Pickard, Minchin, and Troughton, 1978b) that, immediately after loading into the experimental cabinet, a plant will sometimes fail to translocate vigorously (or at all) whereas the same plant left to recover from handling for a few hours seems to translocate in a normal manner. This finding was examined in greater detail by Jaeger, Goeschl, Magnuson, Fares, and Strain

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Abbreviations: CPS—Counts per second; EGTA—ethylenebis(oxyethylenenitriilo)tetraacetic acid; HEPES—N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulphonic acid]; PIPES—1,4-piperazinediethanesulphonic acid; SR—Slope Ratio (decreases as inhibition increases); STS—Standard Tray Solution; TT—Tangent—Tangent interval (measures length of inhibition).

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(1988) who showed that even mild mechanical stimulation could effect a transient disturbance of normal translocation.

Suppose, in accordance with the Minorsky (1989) hypothesis, that fast cooling did have, as one link of the chain leading to inhibition of translocation, an increase of free calcium somewhere in the phloem-associated cytoplasmic matrix. Such an increase could well be the result of influx of calcium through normally closed calcium channels gated momentarily open as a result of the temperature drop; the origin of the calcium might be internal (e.g. vacuolar) or external (i.e. apoplastic). In turn, the existence of mechanical inhibition could be hypothesized to result from the opening of mechanosensitive calcium channels (Morris, 1990); and these channels might even be identical to those opened by temperature drops. Moreover, the off/on kinetics of most channels show at least some voltage sensitivity, so that it might be predicted that electric shock would also lead to increased cytosolic free calcium and to a transient interruption of translocation qualitatively similar to that produced by abrupt drops in temperature and by vibration. These speculations motivated experimentation intended (i) to assess the importance of calcium in the temperature-drop inhibition phenomenon, (ii) to determine whether translocation is sensitive to electric shock, and (iii) to explore the relationships among the inhibitions caused by abrupt temperature drops, vibration, and (prospectively) electric shock.

MATERIALS AND METHODS

Plant material

Plants of *Phaseolus vulgaris* L. cv. Fardenlosa Shiny (a bean of climbing habit) were grown in environmental cabinets in individual containers filled with potting soil and continuously moistened from below by a capillary watering system. The cabinets were maintained at a relative humidity of 50%, with a daily cycle of 16 h light at 28 °C and 8 h dark at 20 °C. Irradiance was 600 μmol m⁻² s⁻¹ 25 cm above the cabinet floor in the absence of plants. Normally, plants were used when about 3-weeks-old and 75–100 cm long; such plants had well-developed primary leaves and an exporting but still growing first trifolium (most basal pinnate trifoliate compound leaf). A few experiments with older plants gave results similar to those with standard plants.

For experiments requiring pharmacological challenge to phloem tissue, the epidermis was delicately peeled from a roughly 50 mm length of stem (Minchin and Thorpe, 1987); and the stem was then lanolin-sealed into a Perspex trough (see below). This technique seemed to give better penetration of bathing solution than the split stem technique occasionally employed (Pickard and Hill, 1975; Pickard, Minchin, and Troughton, 1978c).

All experiments were carried out in an environmental cabinet with conditions roughly comparable to those in the plant growth cabinets and a nominal temperature of 25 °C. A suitable plant (still rooted in its pot) was laid on its side in the cabinet and fastened with masking tape to a platform of lead bricks; the precise orientation of the plant will be described below. At two locations between the first and second trifolia, acrylic ‘challenge chambers’ (see below) were affixed to the stem for convenience in administering treatments intended to affect translocation. The first trifolium (sometimes trimmed to its central leaflet) was sealed with CaCO₃-loaded lanolin into a leaf chamber into which cabinet air was continuously drawn by a pump and from which it was voided outside the cabinet. The apical stem, which might extend several tens of centimetres beyond the second challenge chamber, was coiled loosely and taped down. Finally, to assure apical translocation, several centimetres of stem were steam-gridled between the first trifolium and the primary leaves. The plant was ordinarily allowed to equilibrate for at least 60 min before an experiment was commenced since data on vibration-induced inhibition indicated that this usually sufficed for complete recovery; however, plants with epidermal peels seemed to have impaired source export unless allowed to recover overnight.

Solutions

The basic medium, called Standard Tray Solution (STS), made up as 10× stock solution and diluted as needed, was composed of: KCl, 1-0 mol m⁻³; NaCl, 1-0 mol m⁻³; PIPES, 2-5 mol m⁻³; HEPES, 2-5 mol m⁻³; NaOH at 2-0 kmol m⁻³, was added as required for the desired pH. In the process of dilution, the STS stock solution could be mixed with additional chemicals to yield a variety of test solutions:

- **STS6-5-Ca.** STS at pH 6-5 and enriched to 1-0 mol m⁻³ with CaCl₂.
- **STS6-0-La.** STS at pH 6-0 and enriched to 10 mol m⁻³ with LaCl₃.
- **STS6-5-EGTA.** STS at pH 6-5 and enriched to 10 mol m⁻³ with EGTA.
- **STS6-5-A23187.** STS at pH 6-5 and enriched to 1-0 mol m⁻³ with CaCl₂ and to 10 mmol m⁻³ with the calcium ionophore A23187 (diluted from ethanolic solution).
- **STS8-0-ionomycin.** STS at pH 8-0 and enriched to 1-0 mol m⁻³ with CaCl₂ and to 10 mmol m⁻³ with the calcium ionophore ionomycin (diluted from ethanolic solution).
- **STS6-5-procaine.** STS at pH 6-5 and enriched to 10 mol m⁻³ with CaCl₂ and to 200 mol m⁻³ with procaine.

Application of isotope and detection of radiation

Carbon-11 (half-life 204 min) was prepared as CO₂ by the method of More and Troughton (1973) and used to label the photoassimilate and trace its movement. To load carbon-11 into a plant, air flow through the leaf chamber was stopped, approximately 0-7 GBq of label injected into the leaf chamber, photosynthesis allowed to proceed for 5 min, and normal leaf chamber ventilation restarted; uptake of at least 75% of the tracer was usual.

Three narrow slits (5-0 mm) separated by 10 cm were milled through the lead platform upon which the plant rested, giving slit collimation to scintillation detectors placed below. Also scintillation detectors were placed within lead shielding on top of this platform to view:

(i) the loaded trifolium and its petiole with adjacent stem;
(ii) the entire stem apical to the most basal challenge chamber (see Fig. 1 and below);
(iii) the entire stem apical to the most apical challenge chamber (see Fig. 1 and below).

These three broad-field detectors were sufficiently distant from the regions monitored to possess a fairly uniform sensitivity to tracer within their fields of view. The overall arrangement is
shown in Fig. 1. A 30 s counting period was employed. All reported data have been background, dead time, and decay corrected.

**Challenge chambers and challenges**

A variety of Perspex structures, called 'challenge chambers', were employed to facilitate the application of stimuli to the stem. These normally extended over 60–75 mm of stem and provided an isolated region of stem up to 50 mm long.

Temperature steps were applied using either (a) a closed chamber into which the stem was sealed with lanolin/CaCO$_3$ paste and through which temperature controlled water was continuously pumped or (b) an open trough into which the stem was sealed using lanolin and from which the bathing solution could be sucked rapidly to be replaced with solution at a different temperature. The measured rise time (10–90%) of the external thermal transient was less than 1 s, by contrast, the rise time for heat diffusion into a typical bean stem is roughly 3 s (Carslaw and Jaeger, 1959).

Vibratory stimuli were administered in a manner crudely analogous to that described by Jaeger et al. (1988): a metal rod, thickly sheathed by a length of rubber tubing and shaken by an engraving tool, was rubbed back and forth along a 30 mm segment of stem for roughly 5 s.

Electrical stimuli were given by applying precisely controlled voltages between two sewing needles which had been driven through the stem while the plant was being emplaced. However, in three experiments impalement took place after loading, and transient inhibitions of translocation lasting 10 min or less were observed.

**General experimental procedure**

The experimental observations of interest were the sequential 'counts per 30 s' data collected by the six detectors and, subsequently, background and half-life corrected by the data analysis system. An experiment consisted of challenging the translocating stem and observing the change (if any) in the trend displayed by the sequence of decay-corrected data points associated with a suitable detector.

As the experimental programme unfolded, it became apparent that a challenged segment of stem could become fatigued so that the inhibitions of translocation produced by successive challenges were progressively less pronounced. Thus, if the responses were to be comparable, it was imperative to allow considerable time between successive challenges to a given segment of stem. The canonical experiment which ultimately evolved allowed 50 min to elapse between challenges to a particular segment of stem, unless fatigue itself was being examined. In such a canonical experiment, translocation profiles were allowed to develop until detector-2 was running at perhaps 100 counts per second uncorrected; then a challenge applied at the more basal challenge chamber and the response at detector-4 studied. Twenty five minutes later an adequate counting rate had usually translocated to detector-3 and detector-6; if so, a challenge was applied at the more apical challenge chamber and the response at detector-6 studied. Twenty five additional minutes later the stem was again challenged at the more basal chamber; and finally, another 25 minutes later, the stem was challenged again at the apical chamber. Thus, challenges at the more basal challenge chamber were interleaved with challenges at the more apical one. This protocol was not followed rigidly, occasional variations being made in response to unusual experimental conditions; but it describes how data were normally taken.

**Data analysis**

Generally, the outputs of detector-4 and detector-6 were used for analysis since they gave an indication of the total label in apical portions of two different sizes and their temporal variations unambiguously indicated total transport into the apex (Minchin and Thorpe, 1987). Figure 2 shows a typical profile of activity arriving within the plant apex. At 63 min, the region between the apical and basal challenge chambers (Fig. 1) was vibrated for 5 s, which gave rise to a transient inhibition of tracer input. The observed count rate displayed an approximately linear increase before the inhibition and a lesser (but also linear) rate of increase during the inhibition. It seems natural, therefore, to define the strength of the inhibition as the ratio of the slope just after inhibition to that just before inhibition: in Fig. 2 this Slope Ratio is \( SR = \tan 70°/\tan 330° = 0.19 \). Following a challenge, import eventually recovered and the count rate began to rise at close to its pre-inhibition linear pace. A duration
FIG. 2. Response of translocation to vigorous vibration. At the time mark near 63 min, the stem was stimulated over the region between the basal and apical challenge chambers, producing a transient but incomplete blockade of translocation into the apex. The momentary drop in CPS at the time of vibration is an artifact caused by the momentarily emplaced vibrator. The tangent lines were drawn by eye and signify an inhibition of roughly 81% which lasted roughly 3-7 min [Exp. 950].

of inhibition could, therefore, be defined as the time interval between the two intercepts of the pre- and post-inhibition tangent lines with the tangent line during inhibition: in Fig. 2 this Tangent-Tangent interval is $TT = 3-7$ min.

Where quantitative measures of inhibition are presented, they will be given in terms of: the duration of the inhibition, the Tangent-Tangent interval $TT$; and the strength of the inhibition, the Slope Ratio $SR$. Note that $SR$ falls with increasing strength of inhibition. The uncertainty in $TT$ is estimated to be ±0.3 min; that of $SR$ is estimated to be less than ±0.13 for arbitrary $SR$ and less than ±0.05 for small $SR$.

RESULTS

Temperature variation of rapid chilling response

If in fact the rapid chilling response was due to the transient, chill-associated opening of calcium channels, then this opening could be presumed to be temperature sensitive. Further, if the inhibition was the result of a momentary gellation of the phloem sap, then one might also expect a temperature dependence.

To test these predictions, abrupt temperature drops were applied to the stem and the resulting Tangent-Tangent intervals determined. The results are presented in Fig. 3.

The anticipated temperature dependence of the duration of inhibition was observed: for $10^\circ C$ drops, the duration of inhibition rose linearly from a threshold of initial temperature somewhere in the low forties to a Tangent-Tangent interval of roughly 10 min for initial temperatures in the mid-teens; still lower initial temperature led to sustained inhibition of translocation, possibly because auxiliary processes came into play at temperatures near zero.

A seemingly unlikely explanation of this temperature dependence (and also the similar dependence seen for electric shock) is that high temperature itself significantly modifies the process of translocation. This probably can not be the case because the import of label into the apex through a heated region was affected only slightly by elevated temperatures. For example, Fig. 4 illustrates the general finding for seven experiments that, at least in the short-term, translocation in pathway seemed relatively insensitive to stem temperatures in the high forties to low fifties.

Response to lanthanum and other pharmacological agents

To test the hypothesis that the inhibition following a sudden chill is calcium-dependent, peeled stems were

FIG. 3. Variation of the duration of inhibition ($TT$) for an abrupt temperature drop of $10^\circ C \pm 0.5^\circ C$ and a variety of initial temperatures. Key: •, standard challenge, being the first (i.e. unfatigued) challenge given the stem; •, same as standard challenge, except region mildly fatigued due to only 20 min since previous temperature drop; ○, same as standard challenge but with plant subjected to STS60-La treatment at a peel or split apical to the region of temperature challenge; △, a typical lanthanum challenged plant, except a temperature drop of $11.2^\circ C$; ○, lanthanum case, except only 12 min rest allowed since previous treatment; ▽, standard case except stem split below steam-girdle and immersed in tap water; ◊, standard case except stem peeled at apical challenge chamber and soaked in STS6-5-EGTA.

FIG. 4. Accumulation of apical activity despite elevated pathway temperature. At the first time mark the basal challenge chamber was raised abruptly from $35.5^\circ C$ to $50.3^\circ C$. At succeeding time marks it was reset to $52.4, 53.3, 54.6, 55.9$, and, finally, $56.8^\circ C$ [Exp. 940].
soaked overnight in four different test solutions and then tested for responsiveness to abrupt temperature drops:

STS6-0-La abolished the inhibition normally observed after a 10–15 °C temperature drop from a nominal 25 °C (four standard trials and several preliminary confirmatory trials). In two instances, the soaked region was also electroshocked and shown to be without normal electrosensitivity.

STS6-5-EGTA was without effect on the temperature shock phenomenon (two trials).

STS6-5-A23187 was without obvious effect on the temperature shock phenomenon (two trials).

STS8-0-ionomycin was without obvious effect on the temperature shock phenomenon (two trials).

In two related trials, STS6-5-procaine was found to abolish translocation totally if left on overnight and to weaken it markedly if applied just before loading the carbon-11; however, in the trial where translocation was only weakened, the temperature shock phenomenon was not abolished, even though the Tangent–Tangent interval was greatly increased.

Verification of vibrational and electrical sensitivity

It was reported by Jaeger et al. (1988) and confirmed by ourselves (see Fig. 2) that vibratory stimuli are inhibitors of translocation.

Figure 5 shows that the predicted electrical sensitivity was also present.

Electrical sensitivity: variation with electrode spacing

The effect of electrode spacing was studied in a set of three experiments. Three stem-piercing electrodes were placed in a row with an inter-electrode spacing of 40 ± 1 mm. For a particular treatment, only two were used, the third being left unconnected; this gave a spacing between the active electrodes of 40 or 80 mm. The duration of the interruption produced by a voltage pulse 5 s long was measured as a Tangent–Tangent interval. The results of three paired trials with pulses 50 min apart are given in Table 1.

Increasing the electrode spacing produced a small increase in the duration of inhibition. This behaviour is reminiscent of that reported for rapid cooling of various lengths of stem (Minchin and Thorpe, 1983).

Electrical sensitivity: variation with electric field

Nine experiments which bear upon the variation of inhibition with shock magnitude were carried out. In each, the length of the stimulus pulses was 5 s.

First, there was a threshold. Sufficiently low electric field strengths evoked no response in translocation to the apex. As the electric field was progressively increased beyond a threshold, progressively greater inhibitions were observed. This is illustrated in Fig. 5. Table 2 shows the rise of the Tangent–Tangent interval and the fall of the Slope Ratio for yet another experiment; it does not, however, pinpoint the threshold field.

Second, even for stimuli so large that the Slope Ratio was negligible (<0.10), increasing the electric field of the pulse increased the Tangent–Tangent interval. For example, Table 3 gives the results of a series of four experiments.

<table>
<thead>
<tr>
<th>Experiment #997</th>
<th>#1000</th>
<th>#1006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field strength [V mm⁻¹]</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Electrode spacing [mm]</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>TT [min]</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>SR</td>
<td>0.76</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Fig. 5. Graded response of translocation into the apex to a sequence of 5 s shocks of gradually increasing strength; the data are half-life and background corrected. The first time mark corresponds to a subthreshold shock of 0.45 V mm⁻¹, the second to 0.91 V mm⁻¹, and the third to 1.82 V mm⁻¹. Casual visual observation reveals that the second (intermediate-strength) shock partially abolished import of label to the apex (SR c. 0.4) for a short time (TT c. 3 min) while the third (strong) shock totally abolished it (SR = 0.0) for a much longer period (TT c. 6 min) [Exp. 941].
Cross-reactivities of the three modalities

It was reported by Minchin and Thorpe (1983) that 'If the treated region was returned to its original temperature as soon as transport resumed, and if only 1–2 min was allowed at the higher temperature, a second cold shock had little or no effect'. That is, the capacity of translocation to be inhibited by an abrupt temperature drop can fatigue. But if the inhibitions produced by vibratory or electrical stimuli share some common pathway with that produced by rapid temperature drops, then they too might display fatigue; and, more intriguing, crossing modalities (e.g. electric shock followed by vibration, \(E \times V\)) might also produce fatigue of the second inhibitory response. This raises nine possibilities for pairing stimulus types to produce fatigue. It was technically inconvenient to cross temperature with vibration (\(T \times V\)) or vibration with temperature (\(V \times T\)), but the remaining seven were tried with care being taken to assure that the second stimulus was unambiguously within the region exposed to the first and powerful enough to induce complete stoppage:

\(T \times T\): Clear fatigue response (3 trials). This is a sort of paradigm for the fatigue phenomenon which seemed always to work reliably.

\(T \times E\): No fatigue response (5 trials). In four of the five trials, the inhibition induced by the electric shock was conspicuously less than that induced by the temperature drop. However, in only one was the electric inhibition markedly diminished from that normally expected. Unequivocal evidence of fatigue would require near total suppression of the second response as was usually the case with \(T \times T\) or \(V \times V\).

\(V \times E\): No fatigue response (8 trials). The mean Tangent–Tangent interval following the shock of \(1-75 \pm 0-10\) V mm\(^{-1}\) was \(TT = 5-3 \pm 0-6\) min, a value within the normally expected range.

\(E \times T\): No fatigue response (6 trials). In those cases where Tangent–Tangent intervals could be com-

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**Table 3. The variation of Tangent-Tangent interval (TT) with electric field strength (E)**

Each experiment compares the results of two 5 s shocks spaced 50 min apart, and the results are presented in the order the shocks were given. Where a Slope Ratio could be defined, it was 005 or less.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>#995</th>
<th>#998</th>
<th>#999</th>
<th>#1001*</th>
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<tr>
<td>E [V mm(^{-1})]</td>
<td>0-90</td>
<td>1-79</td>
<td>1-75</td>
<td>0-88</td>
</tr>
<tr>
<td>TT [min]</td>
<td>40</td>
<td>102</td>
<td>70</td>
<td>3-3</td>
</tr>
</tbody>
</table>

*In Experiment \#1001, the shock at 1-76 V mm\(^{-1}\) was given only 37 min after a series of four subthreshold shocks.

**Electrical sensitivity: variation with pulse polarity and pulse length**

Preliminary experiments, in which the Tangent–Tangent interval was studied, gave evidence for the following patterns of behaviour: (i) the variation from pulse to pulse for identical widely-spaced pulses was small (± 10%); (ii) the effect of pulse polarity was apparently small (< 20%); and (iii) as the pulse duration was lengthened, the inhibition lengthened.

More detailed experiments were carried out on the same region of stem using paired pulses 50 min apart and 1-75 ± 0-10 V mm\(^{-1}\) high. Two experiments with paired 5 s pulses confirmed pattern (i): \(TT = 3-9\) min, \(TT = 4-7\); \(TT = 5-9\); \(TT = 6-3\). Two experiments with paired 5 s pulses likewise confirmed pattern (ii): \(TT_{\text{ap}} = 5-7\); \(TT_{\text{neg}} = 5-3\); \(TT_{\text{neg}} = 5-2\); \(TT_{\text{pos}} = 4-3\). To test (iii), a series of six experiments examined the effects of paired pulses of different lengths; the results, presented in Table 4, confirm the expected pattern and suggest that the curve of \(TT\) versus pulse length approaches an asymptote for pulses of the order of 2–5 s long.

**Electrical sensitivity: variation with temperature**

Three preliminary experiments suggested that raising the ambient air temperature of the shocked region of stem might lower the duration of inhibition as represented by the Tangent–Tangent interval.

Therefore, a set of seven experiments was undertaken using paired pulses 5 s long, 1-75 ± 0-10 V mm\(^{-1}\) high, and 50 min apart. The data are given in Table 5 and show that, in all seven paired trials, raising the stem temperature lowered the duration of the electroshock-induced inhibition. In particular, the inhibition was negligible above 40 °C.

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**Table 4. The variation of Tangent-Tangent interval (TT) and Slope Ratio (SR) with duration (D) of an electric shock**

Each experiment compares the results of two 1-75 ± 0-10 V mm\(^{-1}\) shocks spaced 50 min apart, and the results are presented in the order the shocks were given.

<table>
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<tr>
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<th>#983</th>
<th>#986</th>
<th>#987</th>
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<tr>
<td>D [s]</td>
<td>5-0</td>
<td>2-0</td>
<td>5-0</td>
<td>10</td>
<td>0-5</td>
<td>10</td>
</tr>
<tr>
<td>TT [min]</td>
<td>5-3</td>
<td>5-3</td>
<td>9-4</td>
<td>8-0</td>
<td>3-3</td>
<td>6-8</td>
</tr>
<tr>
<td>SR</td>
<td>0-00</td>
<td>0-09</td>
<td>0-00</td>
<td>0-00</td>
<td>0-00</td>
<td>0-07</td>
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Table 5. The variation of Tangent-Tangent interval (TT) and Slope Ratio (SR) with temperature (T) of an electrically shocked stem

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<th>#993</th>
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<th>#996</th>
<th>#997</th>
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<tr>
<td>T [°C]</td>
<td>0-36</td>
<td>0-36</td>
<td>0-36</td>
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<td>0-36</td>
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</tr>
<tr>
<td>TT [min]</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>SR</td>
<td>0-36</td>
<td>0-36</td>
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The actual inhibitory process is more likely confined to the sieve element interior and is presumed to be the result of a local disturbance of pathway. Our records of activity at detectors far from the putative site of stimula-

Discussion
The calcium hypothesis

An initial motivator of this experimental programme was a desire to examine the role of calcium in the temperature drop inhibition of translocation. A number of lines of evidence made calcium involvement seem probable. First, it has been implicated in a number of temperature sensing phenomena of plants (Minorsky, 1989). Second, at least one of these is known to be inhibited by lanthanum (Minorsky and Spanswick, 1989), a reputed calcium channel blocker (Takata, Pickard, Lettvin, and Moore, 1966 and references therein). Third, sensory transduction is frequently mediated by ion channels; and calcium-modulated ion channels occur in plants (Hedrich and Schroeder, 1989). Fourth, the role of calcium in callose formation, a potentially blockading process, is commonly accepted (Kauss, 1987).

Our experiments to date have yielded mixed results. The blockade of both the temperature drop and the electroshock inhibitions by lanthanum does support the hypothesis of a central role for calcium in the inhibition response. But the lack of effect of the calcium chelator EGTA argues that, if calcium is involved, it is not apoplastic calcium. And the lack of effect of the calcium ionophores A23187 and ionomycin can not be clearly interpreted: only a positive result would have been unambiguous.

Cross-reactivity

The experiments thus far carried out have shown fatigue in the T x T and V x V and the E x V combinations and have at least adduced prima facie evidence for its absence in the T x E, the V x E, the E x T, and the E x E combinations. These data do not accord with a supposition of one receptor and one mechanism of blockade.

The initial transduction of the stimulus could be confined to either phloem-associated cytoplasm or phloem-associated membranes. The latter appears somewhat more likely since ion channels (or membrane bound proteins) are most commonly the transductive elements in a sensory system and since both voltage-activated (Hille, 1984) and mechanosensitive (Sachs, 1987; Morris, 1990) ion channels are well known. Because there are many different types of ion channels in general (Hille, 1984) and calcium channels in particular (Hosey and Lazdunski, 1988), it would not be surprising if more than one species of receptor existed.

The actual inhibitory process is more likely confined to the sieve element interior and is presumed to be the result of a local disturbance of pathway. Our records of activity at detectors far from the putative site of stimula-

Figure 6. Apparent fatigue of inhibition response seen with an electric shock followed closely by vibration. At the first time mark, 1-70 V mm⁻¹ was applied for 5 s between electrodes located outside the apical challenge chamber; at the second time mark, the stem was vibrated within the chamber [Exp. 985].
tion often revealed a less complete disruption of label movement such as would be consistent with a mass flow model and a localized blockade rather than a massive inhibition of either source or sink. In accordance with the basics of creeping flow, such a blockade could be due either to an increase of sap viscosity (e.g. gellation) or an unfavourable alteration of pathway geometry (e.g. pore clogging). The phloem sap of many species does possess an ability to gel (Walker, 1972; Read and Northcote, 1983) which is associated with the P-protein (Kollmann, 1980). And at least one form of mechanical stimulation (geotropic) is known to cause the rapid deposition of callose in both pea and corn (Jaffe and Leopold, 1984).

The prospective existence of several different receptor mechanisms and as many as two blockade mechanisms could easily suffice to explain the fatigue phenomena observed thus far. The obvious preconception—that all three kinds of stimulus access a common mechanism in a common-way—is not supported by the present data.

Temperature phenomena

The fall-off with increasing ambient temperature of both the temperature drop and the electroshock inhibi-
tions and their blockade by lanthanum could be construed as evidence for at least some common steps in the two responses. But there is no clear evidence which might serve to localize the source of this temperature variation: is it (i) in the sieve element sap or companion cell cytoplasm, or (ii) in some phloem-associated membrane, or (iii) both?

However, it is probable that the transducers of the temperature stimulus reside in phloem-associated mem-
branes. Were temperature the only effective stimulus, it might appear superficially attractive to argue for a bio-
chemical sensing mechanism which originated in the temperature variation of one or another biochemical process within the cytoplasmic matrix of the sieve element. But, in fact, such a mechanism would not necessarily suffice because biochemical reactions are normally ima-
gined to depend upon the local temperature and not upon how that temperature was achieved. Therefore, since only fast drops are effective temperature stimuli, a simple cytoplasmic biochemical origin becomes a somewhat remote possibility.

Electrical phenomena

For several explicitly electrophysiological reasons, the electrical sensitivity of the pathway was not surprising. First, the resting potential of the sieve element is so negative that it almost surely has an electrogenic compon-
ent (Wright and Fisher, 1981); and electrogenicity fre-
quently is associated with electrical excitability, for example in the giant cells of characean algae or in Mimosa (Sibaoka, 1962). Second, in all cells, both plant and animal, examined to date the electrical properties of the plasmalemma are intimately related to ion channels; and ion channels in general (Hille, 1984) and calcium channels in particular (Hosey and Lazdunski, 1988) are frequently voltage-sensitive. Third, rapid chilling of stem can quickly produce a prolonged increase of electrical activity (Pick-
ard, 1984). Fourth, plants are well known to generate a variety of electrical signals (Pickard, 1973) and to respond electrically to a variety of stimuli (Van Sambeek, Pickard, and Ulbright, 1976; Williams and Pickard, 1979). Fifth, it was recently reported by Vreugdenhil and Spanwick (1988) that Ricinus displays wounding potentials reminiscent of those reported in nineteenth century studies of injury current in nerve and muscle (Biedermann, 1896); this suggests the possible presence of voltage-sensitive ion channels. Hence the electrical sensitivity of the phloem might have been expected.

However, since saturation of inhibition duration with increasing applied field was not observed, even at fields strong enough to produce complete inhibition (SR = 0), it seems likely that the response of the phloem to the field is graded rather than all-or-none; in particular, it seems questionable that propagating action potentials are being elicited by the shocks since these would presumably gate most electrically responsive transducers and drive the inhibition duration to saturation.

In common with compound peripheral nerve, the phloem is a heterogeneous tissue containing several cell types and offering a variety of complex intra- and extracel-
ular current paths. Thus it might be anticipated that the behaviour of the inhibitory response would show analogies to that of the compound action potential (Erlanger and Gasser, 1937). For example, it could show threshold phenomena (observed), strength-duration phenomena (observed), and recruitment (observed if the decline of the Slope Ratio with increasing electric field is taken as evidence for involvement of an increasing fraction of the phloem in the response). What would not be predicted is the continuing increase in the length (TT) of the inhibition as the electric field is increased beyond values needed to achieve maximal strength (SR = 0) of inhibition.

Concluding comments

The data presented describe an, as yet, poorly under-
stood phenomenon of translocation and address in no obvious way the important biological question: Of what is this response diagnostic? Is it merely a collateral mani-
festation of physiological processes largely unrelated to the stimuli sensed? Does it signal the existence of a defense mechanism (possibly analogous to the clotting of blood) by which the plant attempts to minimize the impact of predation? Might it be related to pathway-based mechan-
isms for the allocation of photosynthe (Grusak and Lucas, 1986)? To conclude, why is this phenomenon present in the first place and what benefits does the plant
derive from the processes which give rise to it?
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LITERATURE CITED


