Source–sink coupling in young barley plants and control of phloem loading

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Abstract

Phloem loading of carbohydrate within a mature exporting leaf of a barley seedling is shown to respond quickly to a change in the temperature of the root and the shoot meristem. This is interpreted as a close coupling between source supply and sink demand for carbohydrate, through the hydrostatic pressure gradient linking source and sink generated by the solute concentration within the sieve tubes. This interpretation was tested by using anoxia to alter solute concentration within the sieve tubes of one region of a leaf while observing phloem loading in an adjacent region. Responses to anoxia could not be explained by the above model, suggesting that either this model is incorrect or other signalling pathways are involved. There is evidence in the literature for coarse control of phloem loading but no evidence was found of fine control by solute content of the loaded sieve elements.

Key words: Anoxia, barley, carbohydrate, leaves, phloem loading, temperature.

Introduction

Mass balance demands that a sustained carbon flux out of a source leaf must equal the capacity of the sinks to utilize it. If utilization of assimilate by the sinks is altered, then this must be followed by a matching change in export from the source leaves. Only small, short-term imbalances between source and sinks can be buffered by temporary storage and remobilization within the leaf, the transport pathway and the sink (Farrar et al., 2000).

The phloem literature has many examples of source and sink coupling both in the long-term (days) and short-term (minutes–hours). Those over the short term provide most information on the mechanisms of existing metabolic and transport processes and their controls, whilst those over the longer term can be a consequence of adaptation. For example, Ho (1976) showed that during the day, carbohydrate export from a source leaf of tomato was at least partially independent of the photosynthetic rate. Warming and cooling of fruits can result in changes in carbon flux out of the source leaf (Moorby and Jarman, 1975; Walker and Ho, 1977), although such responses are not always found (Geiger and Fondy, 1985). Reducing import into seeds of peas resulted in changes in source leaf carbon flux (van Oene et al., 1992a, b). Areas of source leaf function and carbohydrate metabolism that may be affected are discussed in detail by Stitt (Stitt, 1996) and Farrar (Farrar, 1999). Differences in the degree of source–sink coupling can probably be attributed to the amount of buffering capacity available within the source, sink and the linking phloem pathway.

Little is known about the signals linking sources and sinks that provide the necessary coupling. The general belief is that a change in either source supply or sink demand results in local changes in sieve tube solute concentration which alters the hydrostatic pressure gradient linking source and sink, resulting in changes in flow. Both the solute concentration and hydrostatic pressure have the potential of acting as a signal. Smith and Milburn (1980) found that phloem loading responds to changes in sieve tube turgor, whilst Daie and Wyse (1985) suggested that enhanced sucrose uptake in response to low turgor was mediated through the plasmalemma ATPases and most likely occurred at the site of loading. Chiou and Bush (1998) demonstrated that sucrose fed to beet leaves via the xylem over a period of 24 h reduced sucrose transport activity and, therefore, the capability of active phloem loading.

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Source to sink coupling was investigated by using temperature to alter sink demand for carbohydrate quickly, while observing phloem loading of recently fixed carbon and its subsequent transport to the sink. The question was asked: is there evidence for rapid coupling between source and sink that is detectable within minutes? The next question was whether the mechanism of this coupling involves solute-regulated phloem loading, using anoxia to alter phloem loading and hence solute concentration within the sieve tubes, both upstream and downstream of the region where loading was measured.

Materials and methods

Most of the methods used have been fully described elsewhere, so will only be summarized here.

Plants

Barley (Hordeum distichum L. Lam cv. Triumph, provided by Crop and Food Research, NZ) was grown hydroponically for experiments using temperature treatments, so the roots of these plants could be rapidly cooled or warmed. Plants were used at 13–18-d-old when the blades of leaves 1 and 2 were fully expanded. For anoxia treatments, plants were grown in potting mix; the entire sink was at a fixed temperature determined by the constant environment. Development times for both growth methods were similar.

The evening before an experiment, a single plant was transferred into the experimental cabinet maintained at the same conditions as for growth. For the temperature treatments, hydroponic plants were used with the lower 50 mm of the leaf sheath enclosing the shoot meristem embedded in lanolin within a heat exchanger connected in series with a second heat exchanger within the root bathing solution. Temperature control was achieved by pumping water from a temperature-controlled bath through the heat exchangers, so that the roots, shoot meristem and growing leaves were held at the same temperature. The combined shoot and root sinks were referred to as the entire sink.

For tracer labelling, a 6 mm long Perspex leaf chamber was placed at about the mid-point of the 2nd leaf blade (which was approximately 200 mm long). For the anoxia experiments the distal or proximal 100 mm of the leaf blade was enclosed in a second independent chamber. Initially, air was bubbled through water and continuously passed through the second chamber at 1.0 l min⁻¹ and, during an experiment, gas was supplied from another bubbler connected to a special gas mix, leaving the dew point unchanged. The small labelling chamber was connected to the gas loop associated with labelling. This loop was maintained at the current ambient of 370±2 ppm CO₂, dew point 12 °C with a flow rate of 0.5 1 min⁻¹. The remainder of the leaf blade was enclosed in an open chamber, to act as a positron shield. A two-channel infrared gas analyser (Binos) was used to measure the CO₂ concentration for control purposes, as well as the CO₂ differential due to photosynthesis in the 6 mm central leaf chamber.

Every 2 h about 1 GBq of ¹¹C⁰₂ was produced (More and Troughton, 1973) and used to replenish a reservoir connected to the closed gas loop. ¹¹C⁰₂ was bled into the gas loop as required, beginning 5 h into the photoperiod.

Experimental treatments

Temperature treatments consisted of switching the source of temperature-controlled water in the heat exchangers to give rapid temperature changes to the entire sink while movement of ¹¹C-labelled photosynthate was followed. In the case of a temperature rise, the entire sink of the experimental plant was precooled the night before the experiment.

Anoxia experiments similarly involved a sudden switch between gas sources supplying the chamber surrounding the leaf blade distal or proximal to the labelled segment as appropriate. Anoxia was provided in two ways: either as nitrogen, or as nitrogen plus 360 ppm CO₂ (‘oxygen-free air’).

Radiation monitoring

Scintillation detectors were positioned within radiation shielding to be uniformly sensitive to well-defined segments of the plant and, after corrections for background, dead-time, and their different sensitivities to equal amounts of tracer (Thorpe and Minchin, 1991), combined to give the following:

- (1) whole plant, including the labelling chamber, but not the gas within this chamber (the gas component having been subtracted out),
- (2) exported tracer, that is all the plant excluding the labelled segment,
- (3) entire sink (roots, shoot meristem, growing leaves),
- (4) segment of leaf blade distal to the labelled segment.

The distance between the labelled segment of leaf and the temperature-controlled ‘entire sink’ (roots plus shoot) consisted of 100 mm of leaf blade plus 20 mm of the upper sheath, that is a total distance of 120 mm.

Strips of clear Perspex were placed around the plant not already enclosed within Perspex chambers, to ensure that the β⁺-radiation escaping from the plants was annihilated near to its source. This is necessary to ensure good spatial resolution, as the β⁺ particles emitted by ¹¹C have a maximum path length in air of about 1 m.

Data analysis

Input-output analysis of these tracer profiles was carried out to obtain the transfer function description of tracer movement through the load-zone (whole plant to mobilized) and through the leaf base towards the major sink (mobilized to entire sink) (Minchin and Grusak, 1988). From the transfer function description of tracer movement, the system gain and average transit times were calculated. For the load-zone, the system gain is reported as the efflux fraction, that is, the fraction of the fixed tracer that is eventually loaded into the phloem and transported out of the labelled zone. The average time between tracing being fixed and subsequently transported out of the labelled segment of leaf was calculated, and is reported as the leaf transit time. For long-distance phloem transport down the leaf blade towards the sink the pathway loss was reported, which is 1.0 minus the estimated gain. Pathway loss is an estimate of the fraction of labelled photosynthate that is leaked from the phloem pathway and retained within this leaf segment. Also the pathway transit time was calculated, that is the average time taken for tracer to move the 120 mm from the labelled zone to the sink.

In the authors’ experience, estimates of these transport parameters when repeated for a specific plant are similar, but are often quite different in magnitude between plants. Hence, comparisons between the values estimated have never been used, but instead consistency of the direction and relative magnitude of changes induced by treatments have been used as indicative of treatment responses. Hence the directions and qualitative magnitudes of the observed changes in these transport properties are reported. Most of the experiments were carried out at several times, except for a small number done to confirm the interpretation of the key experiments.

It is important to realize that: the estimates of gain, transit time and other parameters (e.g. loss), are based on the ¹¹C-tracer profiles and
so reflect what is happening to the recently fixed photosynthate. The short half-life of the tracer means that any tracer that takes a pathway with a transit time longer than about 150 min is effectively lost, which is not the case for unlabelled photosynthate.

**Results**

Movement of tracer through the labelled segment of leaf was followed by comparing the $^{11}$C profiles for the whole plant and exported tracer, whilst movement through the phloem pathway along the leaf blade was followed by comparison between the profiles of exported tracer and of that entering the entire sink. The treatment responses observed for both transport systems are presented in the Table 1. After the treatment in some experiments tracer moved distally, towards the tip of the leaf, as noted in the text.

**Controls**

There was a continuous but slow change in tracer transport through the labelled zone into the phloem pathway, seen as a slow fall in export fraction and slow rise in leaf transit time. Transport through the phloem from the labelled segment to the major carbohydrate sinks changed very little during the 600 min of observation, reflected by a very stable pathway transit time and a small rise in pathway loss. This behaviour was seen in all the experiments prior to application of experimental treatments (data not shown).

### Table 1. Responses to various treatments for movement of labelled photosynthate through the 6 mm labelled zone and subsequent long-distance phloem transport in the leaf blade

The direction of response is indicated by arrows (↓ or ↑), with two arrows for a very strong response, and a dash (−) if there was no response. Each row summarizes the observations made in the same experiment.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pre phloem-loading</th>
<th>Long-distance phloem transport</th>
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<tbody>
<tr>
<td></td>
<td>Export fraction</td>
<td>Leaf transit time</td>
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<tr>
<td>Sink cooling</td>
<td>↓↓</td>
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<td></td>
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<td></td>
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<td>Sink warming</td>
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<td></td>
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<tr>
<td>Upstream anoxia</td>
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<tr>
<td>Upstream nitrogen</td>
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<td>−</td>
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<tr>
<td>Upstream anoxia plus shade</td>
<td>↓</td>
<td>−</td>
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<tr>
<td>Downstream anoxia</td>
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</table>

**Cooled sink**

Towards the end of the second tracer pulse moving through the plant, the entire sink (see Materials and methods) was cooled from 24 °C to 10 °C. On cooling there was a drop in export fraction and a marked increased in leaf transit time.

Sink cooling causing a slight but perceptible rise in pathway transit time and no observable effect on pathway loss.

**Warmed sink**

In these experiments the entire sink was cooled to 11 °C the previous day and held at this temperature until the sink was warmed while tracer movement was being followed. With the initially lower temperature, the export fraction was lower than for the control, and the leaf transit time considerably higher, though both were drifting in a similar manner to the control. On rapidly warming the entire sink from 11 °C to 24 °C the export fraction rose markedly in one experiment and was unchanged in two experiments, while the leaf transit time declined in all cases.

Pathway transit time declined abruptly on warming the sink while the pathway loss fell (to zero).
Various anoxia treatments upstream of the labelled region

On applying oxygen-free air to the 100 mm long leaf segment distal to the labelled segment there was an increase in leaf transit time, while the export fraction remained unaffected. Pathway transit time along the leaf blade increased slightly after application of the upstream anoxia, but there was no change in pathway loss. When the distal segment of the leaf was made anoxic by applying nitrogen (no carbon dioxide) there was no effect on leaf transit time and a decline in export fraction. Pathway loss and transit time both showed a marked increase. Also, with nitrogen gas producing anoxia upstream, there was a measurable, though small, movement of tracer into the treated distal region of the leaf which did not occur when oxygen-free air was used (data not shown).

Anoxia based upon oxygen-free air (containing carbon dioxide) plus complete darkening of the tip segment of the leaf gave the same responses as nitrogen gas. The responses seen both for the labelled zone and for tracer transport through the proximal leaf blade were similar with both nitrogen and oxygen-free air, but nitrogen resulted in a stronger effect.

Anoxia downstream of the labelled region

Oxygen-free air applied to 100 mm of the leaf blade downstream from the labelled segment resulted in a somewhat variable response. Where affected, export was much smaller and sometimes faster. Long-distance transport (through the anoxic region) occurred with both more and less loss, and little effect on transit time.

Photosynthetic rate

The net carbon exchange of the labelled region did not change with any of the treatments.

Discussion

The export fraction, estimated by comparison of the whole plant and mobilized tracer profiles, is an in vivo measure of phloem loading of recently fixed photosynthate. This is because the only routes for movement of labelled photosynthate out of the labelled zone are by leaf respiration or phloem transport. Respiratory loss has been shown to be small (Thorpe and Minchin, 1991). Once loaded into the sieve tubes of the phloem, tracer is moved by bulk flow out of the labelled segment of the leaf into the field of view of the ‘mobilized’ detector (see Materials and methods). Based upon the speed of phloem transport seen in the lower leaf blade, tracer would remain a maximum of 20 s in the sieve tubes of the 6 mm length of the labelled region after transfer from the mesophyll. This is very small compared to the leaf transit time for movement of tracer after fixation until exit from the labelled region, typically at least 40 min (data not shown).

These observations of responses to root temperature are interpreted as responses to changes in sink demand, where sink demand includes all the attributes of a sink which affect the inflow of carbon. The temperature of a sink affects its metabolic rate and hence its capacity to utilize carbohydrate (Farrar, 1988). The carbon inflow is also affected by the demand of other sinks and the ability of the sources to supply. (In one mechanistic model of partitioning sink demand is quantified by the kinetics of unloading, and the resistance of the flow pathway associated with that sink: Minchin et al., 1993).

The rapidity of the temperature responses clearly indicates a very close coupling between sink demand and phloem loading within barley seedlings. When the sink was warmed phloem loading increased within minutes and, conversely, when the sink was cooled loading decreased within minutes. The authors’ interpretation is that a change in demand resulted in a change in sieve tube turgor in the sink, which was rapidly propagated throughout the phloem (Watson, 1976), giving a change in pressure difference from source to sink, a change in pathway transit time (which was seen: Table 1), and a change in sieve element turgor in the source. A change in sink demand will eventually lead to altered photosynthetic rate, but this did not occur within the time-scale of these experiments. In the short term, an increase in demand can be met by an increase in export fraction (i.e. less temporary storage of tracer in the leaf) and a reduced leaf transit time (less time for temporary storage). These were the responses found, except in two of the three sink-warming experiments where no significant increase in loading was observed, even though, as expected, the leaf transit time decreased. With sink warming there was a large decline in pathway loss, suggesting that some of the increased sink demand was met by a reduction in unloading in the pathway. The overall conclusion is that sink-source coupling can be very close in barley. Nevertheless, a response may not always occur in every source, since there are obviously circumstances when a source cannot respond, or when there may be sufficient buffering in the pathway to delay the propagation of the turgor change to it for some time.

These experiments, where root temperature was changed, have demonstrated that the carbohydrate requirements of a sink and the dynamic properties of a source can be closely coupled, presumably by means of the hydraulic coupling through sieve tubes. Therefore, it is necessary to see if treatments designed to affect sieve tube solutes in the source can also affect phloem loading. Such a treatment is leaf anoxia, which rapidly inhibits phloem loading. With anoxia as oxygen-free air (nitrogen/carbon dioxide) some phloem loading continues in the light due to the oxygen released by photosynthesis, but anoxia in darkness or with no carbon dioxide inhibits phloem loading completely.
(Thorpe et al., 1979). As a more direct means of altering sugar concentration in the sieve tubes of the source anoxia treatments were applied (with and without carbon dioxide) to the immediately adjacent regions of the leaf segment where tracer loading was observed. With reduced loading the concomitant osmotic inflow of water would also decrease, but assimilate concentration within the sieve tubes must fall unless there is a parallel reduction in sink utilization.

Using oxygen-free air, anoxia upstream of the labelled region had little effect on export fraction, and leaf transit time increased very significantly. Using nitrogen for upstream anoxia (a similar but more severe treatment), export fraction decreased and pathway transit time again increased. These treatments would have reduced carbohydrate concentration and turgor of sieve tubes both in the upstream and labelled regions. Warming the root was expected to increase root demand for carbohydrate, as discussed above, giving rise to an increased export fraction and reduced leaf transit time. This opposite response means either that the treatment did not depress sieve tube turgor and carbohydrate concentration, or that loading of recent photoassimilate is not sensitive to those parameters of the local sieve element. The treatment did slow down tracer transport in the leaf blade proximal to the labelled region, showing that there was a reduction in turgor difference from labelled region to the base of the leaf, disproving the first explanation. Hence phloem loading of recently fixed photoassimilate does not respond to changes in local hydrostatic pressure. The effect of upstream anoxia on speed was rather small: pathway transit time though the leaf blade proximal to the labelled region increase by only 10%, when about half the leaf distal to the labelled region was treated. One might have expected a large increase in pathway transit time, given that a large fraction of the leaf blade was treated. Any change in transit time for tracer moving downstream in the proximal region reflects a change in turgor gradient. The rather small change in transit time suggests that more solute was being loaded elsewhere to maintain the gradient, but it was seen that recent photoassimilate was not loaded faster, so we must conclude that some other solute (perhaps unlabelled sugar or potassium) entered to help compensate for the effects of anoxia upstream. There is no doubt that the pressure gradient distal from load-zone was affected, because where the more severe anoxia treatment was used (i.e. in shade or with no carbon dioxide) tracer transport was observed towards the leaf tip.

The effect of downstream anoxia on export fraction from the labelled region confirms the unexpected conclusion that phloem loading did not increase when sugar concentration or turgor was decreased within the sieve elements of the labelled region. The response was variable, and the leaf export fraction reduced or was unaffected; it certainly did not increase.

It appears that phloem loading can be affected by local solute concentration within the sieve tubes, but it is not controlled in that way. If we were controlled by solute concentration, then it would be expected that an increase in loading when the sieve tube concentration was reduced would be observed, and vice versa. Kehr et al. (1998) have suggested that ‘the concentration of sucrose present in the phloem is directly related to, and may be controlled, by the availability of sucrose in the source leaves’. This interpretation is supported by Grusak et al.’s (1990) observation that some phloem loading continued after heat-girdling the petiole of a mature Vicia faba leaf, where the girdle would have stopped phloem export. Grusak et al. (1990) also found a change in allocation of recently fixed photosynthate into the insoluble fraction (mainly starch) relative to the ethanol-soluble fraction, within 12 min of applying the girdle. This would result in less of the recently fixed photosynthate being available for phloem loading.

The sucrose transporter involved with active phloem loading has been shown to be diurnally regulated, with SUT1 mRNA and protein levels decreasing after 4 h in the presence of cyloheximide (Kuhn et al., 1997), suggesting that this transporter is continuously turned over with a time-scale of hours. Control of the expression of sucrose transporters at the transcriptional, post-transcriptional and post-translational (by phosphorylation) levels has been demonstrated (Chiou and Bush, 1998; Robin et al., 1998; Delrot et al., 2000). Apoplastic sucrose concentration has been shown to have no direct effect on plasma membrane H⁺-ATPase activity and the sucrose-dependent decrease in transport activity was shown not to be caused by change in osmotic pressure (Chiou and Bush, 1998). This then offers a mechanism of coarse control of phloem loading.

In the situation where the sink was warmed or cooled, it was concluded that changes in solute concentration within the sieve tubes resulting in change in the hydrostatic pressure gradient between source and sink are able to account for the observed effects on tracer movement. However, this mechanism of linking sink usage with source supply does not explain changes in either phloem loading or in long-distance phloem transport when the sieve tube solute concentrations were changed by application of anoxia to a source leaf. There appears to be a different signalling process involved for the two experimental treatments. Some evidence for coarse control of phloem loading, via the number of phloem loaders, has been reviewed, but no evidence was found for fine control.

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