Leaf Export and Partitioning Changes Induced by Short-Term Inhibition of Phloem Transport

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ABSTRACT
Both electric shock and cold shock applied to the phloem pathway, and known to induce temporary inhibition to long-distance phloem transport, are shown to have an immediate and sustained effect upon both export of carbon from the leaf and its partitioning between alternative sinks. The changes in export occurred by an unknown mechanism, while the changes in partitioning are interpreted as responses to changed export.

Key words: Carbon partitioning.

INTRODUCTION
It is becoming increasingly clear that to understand phloem transport within plants requires that the entire system, consisting of phloem loading, long-distance transport and associated unloading/reloading processes, and unloading at the sink, must be studied as an integrated whole. Numerous interactions between these subsystems make the results of detailed studies on individual subsystems difficult if not impossible to integrate. Hence whole plant studies are essential to obtaining an understanding of source–sink interactions. Carbon partitioning has been described in terms of sink strength (Wareing and Patrick, 1975), implying that it is the sinks that determine partitioning patterns. However, Grusak and Lucas (1985) demonstrated that localized slow cooling of a sugar beet petiole can induce a spontaneous change in the distribution between the sinks for the photoassimilate being transported through the cooled petiole. Grusak and Minchin (1989) subsequently showed that phloem transport through the petiole was unchanged by the slow cooling. An osmotic shock applied to the apoplast of a petiole or a rapid cooling/warming cycle also induced repartitioning of photoassimilate passing through the treated petiole (Grusak and Lucas, 1986; Grusak and Minchin, 1989). Hence, a perturbation to a localized segment of the phloem transport pathway common to a number of sinks can alter partitioning of photoassimilate among these sinks. Grusak and Lucas (1986) suggested that the mechanism of this partitioning change might involve the transmission of a rapid physical signal, generated by the localized stimulus, being rapidly transmitted along the phloem pathway to the site of pathway bifurcation where pathway connections are usually complex and involve numerous anastomoses.

It is now possible to make continuous in vivo measurements of the export of recently fixed photosynthate from a leaf while also measuring the partitioning of this mobilized photosynthate between it or all of the sinks (Thorpe and Minchin, 1991; Minchin and Thorpe, 1992). In this paper, we present data which demonstrate that a localized electroshock or cold shock, applied to a short segment of the phloem pathway, affects export from a leaf and also induces a change in partitioning of photosynthate between alternative sinks. Also, the change in partitioning is probably brought about by the change in the amount of photosynthate exported from the source leaf.

MATERIALS AND METHODS
Garden pea (Pisum sativum L. cv. Tere) plants were grown singly in 700 cm³ pots filled with potting mix and continuously supplied with water from a capillary mat. The environment cabinets were maintained at a relative humidity of 50%, with a photoperiod of 16 h at 25 °C and 8 h dark period at 18 °C. The

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light level, 25 cm above the cabinet floor and in the absence of plants, was 600 \mu mol m^{-2} s^{-1} PAR.

Flowering began 30 d after planting and the fruits used in these experiments were about 10 d post-anthesis, at which stage pod elongation was complete and seed fill had just begun. Most fruit were in pairs, and only paired pods were used in this work.

The evening before experimental use, a plant was laid horizontally on a floor of lead within an environmental cabinet maintained at the same conditions as for growth. Each pod of a pair was carefully positioned over holes within the lead floor, each of these holes being at the top of a 20 cm high lead chimney with a scintillation detector at the chimney's base. Further lead shielding was positioned to enable the labelled leaf only to be seen by a scintillation detector. Additionally, the entire mobilized tracer (i.e. tracer within the whole plant excluding the load leaf) was monitored by another detector; and positron annihilations were forced to occur within this detector's field of view by enclosing this region of the plant within clear 5 mm thick perspex. All of the scintillation detectors were positioned far enough from their regions of view to ensure uniform sensitivity to tracer within their view. To this end, we reduced the extent of mobilized tracer distribution within the plant by steam girdling the main stem at approximately 1 cm above and below the node associated with the source leaf and the subtended peduncle/fruit system. This operation restricted label movement to a single leaf-peduncle unit. Because of the lead needed around the leaf chamber for \gamma-radiation shielding, an auxiliary spot light was used to illuminate the source leaf at an intensity equal to the rest of the plant. A further radiation detector observed label within the gas loop. These procedures gave us detectors that were uniformly sensitive to label within (i) either pod, (ii) all tracer exported from the labelled leaf, the mobilized label, (iii) the labelled leaf, and (iv) labeled within the circulating gas.

The leaf chamber formed part of a closed loop system through which air was circulated at 1000 cm^3 min^{-1}, with the dew point controlled to 10°C and carbon dioxide concentration held at 320 parts 10^{-6} (±5%). Approximately 1 GBq of \(^{14}\)CO\(_2\) was produced every 2 h and used to replenish a reservoir connected to the closed loop gas system. \(^{14}\)CO\(_2\) was bled from this reservoir into the circulating gas stream as required.

To determine the relative sensitivities of all the radiation detectors, two linked procedures were used. First, with a plant in place, the load leaf was darkened and an aliquot of label bled into the gas loop. Once this aliquot was well mixed with the circulating gas, indicated by a constant half-life corrected count rate seen by the gas-detector, count rates seen by the gas and load-zone detectors were noted, the load leaf reillumimated, and data collection commenced. Secondly, at the end of the day while label still remained in the load leaf, the load leaf was left in the leaf chamber while the rest of the plant was removed, the load leaf count rate was taken after flushing all labelled gas from the leaf chamber. Then this labelled leaf, still in the leaf chamber, was positioned where the shoot had been and the count rate seen by the 'mobilized' detector recorded. Similarly, using the leaf in the leaf chamber positioned over each pod detector, the pod detector counts were recorded. Allowing for decay, this gave us count rates at all detectors for the same amount of tracer within the respective fields of view. It was important that the labelled leaf was left in the leaf chamber, the leaf chamber acting as a positron annihilator, because removing the chamber resulted in a change in detector sensitivity. This second procedure gave us relative counting sensitivities for each plant detector, while the first procedure gave relative sensitivities of the load-zone and gas-detectors. For analysis, the contribution to the total count rate seen by the load-zone detector due to \(^{14}\)CO\(_2\), within the leaf chamber was calculated as a proportion of that seen by the gas-detector, and this subtracted from the observed count rate, leaving the contribution from fixed label. Also, the total plant activity was calculated as the sum of the mobilized and the gas-corrected load-zone count rates, each suitably corrected for their differing sensitivities. All of the reported tracer profiles have been corrected for dead time, background and differences in relative sensitivities between detectors.

The method of data analysis has been fully described elsewhere (Minchin and Grusak, 1988; Minchin and Thorpe, 1989; Thorpe and Minchin, 1991). The source leaf export fraction at a specified time is defined as the fraction of the carbon-11 labelled photoassimilate fixed at that time which is eventually exported from the source leaf; this labelled exported photoassimilate is referred to as 'mobilized'. The sink partitioning fraction of this mobilized photoassimilate is defined as the fraction of photoassimilate exported from the source leaf at a specified time which eventually arrives at a particular sink.

Treatments to the common pathway between source leaf and fruits were imposed in two ways: electric shock and cold shock. In setting up the plant for electroshock, the peduncle was impaled by a pair of needles several centimetres from the node. For treatment, an electric field of about 2 V mm^{-1}, giving a current of about 0.5 mA, was applied between the needles for 5 s. With this treatment, phloem transport is inhibited for about 5 min (Pickard and Minchin, 1990). Alternatively, for cold shock, a chamber was placed around a similar part of the peduncle and filled with water at cabinet temperature (21°C) before labelling started. For treatment, the water was replaced with water at about 10°C, and left to regain ambient temperature (about 15 min). With this treatment, phloem transport also stops briefly (Pickard et al., 1978).

In one series of experiments, the labelled leaf was deprived of oxygen for about 10 min by flowing nitrogen containing 300 parts 10^{-6} carbon dioxide through the leaf chamber. Treatments were carried out at least three times, except for leaf anoxia which was only done twice.

**RESULTS**

**Controls**

The fraction of labelled photosynthate exported from a leaf, the export fraction, remained constant over a period of 400 min (Fig. 1a). The fraction of mobilized photosynthate which eventually arrived at each pod, the partitioning fraction, likewise remained almost constant, sometimes with a slight tendency to fall monotonically (Fig. 1b).

**Electroshock**

Immediately after application of an electroshock, the export fraction of labelled photosynthate was markedly reduced and gradually recovered, but to a lower value than prior to the treatment (Fig. 2a). A subsequent electroshock once more immediately reduced the export fraction, which began to recover and was still increasing at the termination of the experiment. The electroshock caused an immediate reduction in partitioning to both pods (Fig. 2b); partitioning to the pods began to recover within a few minutes of the shock, and stabilized after about 100 min. In the case of the experiment illustrated...
in Fig. 2, partitioning to one pod stabilized to a similar value to that before the shock, while the other pod stabilized to a higher value. In all of four such experiments electroshock to the peduncle resulted initially in a large reduction in both the export fraction and pod partitioning, but the level to which these parameters recovered was unpredictable.

In general, the steady-state partitioning to each pod responded unpredictably to the electroshock; we saw increases and decreases as well as no change to pod partitioning. But the steady-state export fraction always changed markedly and rapidly, and in all cases but one to a lower value.

**Cold shock**

In the experiment illustrated in Fig. 3, application of the first cold shock to the peduncle produced an immediate reduction in the export fraction from the source leaf which stabilized to a level below the pretreatment level. A second cold shock, applied about 100 min after the first, induced a further brief reduction in export followed by a slow increase back to the stable level established after the first cold shock. The first cold shock induced a marked and sustained fall in partitioning to pod 1 and the second cold shock produced a further fall which slowly recovered back to the level before the second
treatment. The first cold shock had little immediate effect on pod 2 and then possibly induced a slow increase in partitioning to this pod. The second cold shock quickly reversed this trend, with partitioning to pod 2 returning to a similar level at the end of the measurement to that at the beginning. Partitioning to the two pods behaved quite differently to the treatments. At the beginning of our measurements pod 1 was receiving a larger fraction of the available photosynthate than pod 2 (Fig. 3b), and at the end of the measurement each pod was receiving a similar fraction.

Source leaf anoxia

Anoxia, applied to the source leaf for 11 min, induced an almost immediate and sustained reduction in export fraction (Fig. 4a). A second period of anoxia caused a further sustained reduction in export fraction. Both of these anoxic treatments produced a marked parallel reduction in partitioning to both pods followed by a parallel recovery (Fig. 4b). After the first treatment partitioning to both pods recovered to levels above the pretreatment levels.

DISCUSSION

In the control data illustrated in Fig. 1 the two pods were receiving quite different fractions of the mobilized photosynthate. In some control data (not shown) these fractions were similar, and in all the other figures the two pods were receiving similar fractions of the mobilized photosynthate. The sum of the partitioning fraction for each pod did not equal unity, because not all of the mobilized photosynthate was transported into the two pods. Photosynthate would have been unloaded from the phloem pathway within the peduncle for use in both the growth and maintenance requirements of the peduncle. Also, pools within the peduncle buffering short-term changes in supply and demand would be exchanging photosynthate with the pathway, and as carbon-11 has a short half-life compared with the time-scale of these flows there will appear to be a net loss of labelled photosynthate from the transport pathway (Minchin and Thorpe, 1987).

Both electroshock and cold shock of a pea peduncle had a major effect upon (a) the export of recently fixed photosynthate from the leaf supplying this peduncle with photosynthate, and a smaller effect on (b) partitioning of recently fixed photosynthate to each of the pods.

Because there was sometimes a slow drift downward, but never upward, in the partitioning fraction associated with each pod over a period of 500 min, we cannot be certain that the small long-term (>300 min) changes in partitioning seen after a treatment were a consequence of the treatment or the natural drift downwards. We can be sure that the short-term, and large changes are a direct consequence of the treatments and any longer-term upward drifts are certainly treatment responses.

It has been previously reported that an electroshock applied to a short segment of a bean stem temporarily inhibited phloem transport through the electroshocked region, but source and sink responses were not measured (Pickard and Minchin, 1990). Cold blockage of the phloem pathway has been shown to have no major immediate effect on tracer movement out of a source leaf several centimetres away or into sinks several centimetres away (Minchin et al., 1983) because of buffering of short-term changes in sieve tube contents by tissue adjacent to the phloem pathway. This qualitative work could not have shown the small change in export seen in Fig. 3. Blockage of the phloem pathway by heat girdling of a petiole has been shown to affect phloem loading within the leaf of *Vicia* in 10–15 min (Ntsika and Delrot, 1986; Grusak et al., 1990) and photosynthetic rate within several hours in *Cucumis*, *Gossypium* and *Raphanus*, but not in *Capsicum*, *Solanum*, *Phaseolus* or *Ricinus*, which was shown not to be associated with stomatal closure (Mayoral et al., 1985; Plaut et al., 1987). We have demonstrated that both cold shock and electroshock applied to the phloem pathway of *Pisum* causes an immediate reduction in leaf export and changed the partitioning of mobilized photosynthate to the two pods. It has been shown previously that the inhibition of the phloem transport induced by either electroshock (Pickard and Minchin, 1990) or cold shock (Minchin and Thorpe, 1983) is short-lived, lasting for several minutes, while the
change in both export and partitioning lasted for at least several hours.

These data shed no light upon the mechanism affecting export from the leaf when phloem transport is inhibited. The signalling of phloem blockage back to the source is probably more than a simple build-up of hydrostatic pressure in the source due to blockage of the pathway as the inhibition of export was significantly greater for electroshock than for cold shock. It is interesting that the reduction of export from the leaf persisted for a much longer period than the reduction of pathway translocation through the peduncle. There may be an ecological advantage in this, since when the pathway was damaged, it could lessen carbohydrate loss while repair was underway.

From these pathway treatments it is uncertain whether the observed responses at the source leaf and at the sinks were independent of one another, or whether the response induced at one end of the source–sink system in turn gave rise to the response at the other end. We suggest that it was the reduction in export which affected partitioning since, when the export fraction was reduced by anoxia, we saw an immediate reduction in partitioning to both pea pods with recovery to a higher level in both pods (Fig. 4). Hence changes in export fraction can induce changes in partitioning at the sinks, and a reduced export fraction can give rise to an increased proportion of subsequently exported photoassimilate being partitioned to a specific sink. Changes in source strength have been shown to result in immediate changes in source–sink patterns (Borchers-Zampini et al. 1980; Fondy and Geiger, 1980), so it seems reasonable to attribute the sustained changes in partitioning of recently fixed photosynthate to a pair of alternative sinks, induced by either an electric shock or a cold block to a short segment of peduncle, to changes induced at the source.

A simple mechanistic model based upon pressure driven flow along the phloem pathway, and saturatable unloading kinetics in each sink, predicts partitioning changes with changes either in source supply or in the resistance of the pathway from the source which is common to all of the sinks (Minchin et al., 1993). It is unlikely that there was a sustained increase in pathway resistance, since translocation through a segment of bean stem was inhibited by an electric shock for a time measured in minutes (Pickard and Minchin, 1990) while source export and partitioning to the sinks was affected for times measured in hundreds of minutes (Figs 2, 3). Similarly, recovery of translocation from cold shock is measured in minutes (Minchin and Thorpe, 1983). A build-up in source to sink pressure gradient to overcome an increase in pathway resistance, induced by the electroshock or cold shock, is unable to account for the observed flows before, during, and after the induced flow inhibitions, as non-physiological pressure increases would be necessary. Source export was changed for 50 min or more by both of these pathway treatments, so the change in export appears to be the most likely cause of the sustained change in sink partitioning.

Grusak and Lucas (1986) suggested that pressure waves travelling within the phloem could signal to either the source or sink regions. These waves could be induced by some event occurring within the treatment region and travel to either end of the phloem system at the speed of sound within water. Alternatively, an electrical signal could be induced by the pathway treatments. Electrical signalling is involved in turgor changes in response to mechanical stimulation within Mimosa and Dionaea; and it has been shown that a wide range of plants are able to transmit action potentials and variational potentials in response to a wide variety of shock (Pickard, 1973, 1974; Davies, 1987). Also, it has been suggested that electrical signalling can be used to induce biochemical responses elsewhere within a plant (Wildon et al., 1992).

In summary, we have demonstrated that a treatment to the phloem pathway between source and sinks which causes temporary blockage of the pathway can induce a sustained reduction in the fraction of recently fixed photosynthate exported from the source leaf, as well as a change in partitioning of the mobilized photosynthate between sinks. A change in export from a leaf is known to result in a change in partitioning of photosynthate, so a pathway treatment which induces a change in leaf export can be expected also to change the photosynthate partitioning from this leaf. We cannot rule out the possibility of a long-term change in pathway resistance, with an increase in driving force (hydrostatic pressure) between source and sinks to maintain flows, but without further information we prefer to apply Occam’s razor and take the simpler explanation. The mechanism for the change in export is unknown.

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