



American Journal of
Plant Physiology

ISSN 1557-4539



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A Modular Supply and Load-balancing Mechanism Is a Prerequisite for Export in Compound Leaves of Pea Plants

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Abstract: The functionality of a well-developed cross-connected phloem supply between paired leaflets in peas and the subsequent connection to the petiole and the stem vascular supply was determined using the phloem mobile fluorophore 5,6-Carboxyfluorescein (5,6-CF). The fluorophore was applied routinely to one of a pair of leaflets. After incorporation within the mesophyll cells, 5,6-CF was first transported to the leaflet opposite the fed leaflet, irrespective of the leaflet pair being in a source, sink or sink to source transition state. In sink leaflets, the fluorochrome was transported between leaflet pairs across the cross connected nodal supply, without any visible transfer out of the compound leaf. In transition and source leaflets, the fluorochrome was transported first to the opposite leaflet of the pair before export via the axis took place. Present data shows strong evidence that transport across the vascular connections between leaflets is independent of the relationship between the fed leaf and other leaves within the phyllotaxy; or of the source to sink gradient. Our experiments further suggest and support the concept of a modular transport process, which ensures that re-allocation and balancing of assimilates occur between leaflets of the same physiological age, photosynthetic and transport status, thereby load balancing their local transport system, before exporting any available assimilate to other younger (sink) regions.

Key words: Opposite leaflet, vascular connections, modular transport, sink, source, transition

Introduction

According to Patrick *et al.* (2001), most actively growing regions, as well as storage organs (sinks) of higher plants, import assimilates in solution by bulk flow through the phloem, where movement of assimilate is driven by differences in hydrostatic pressure along the source to sink phloem transport pathway. Furthermore, Patrick *et al.* (2001) stated that osmotic water movement generates the required pressure gradient between the extremities within the phloem transport pathway. There is considerable evidence for leaves becoming exporters once the leaves become fully matured, after which phloem loading and the subsequent export from source leaves is largely irreversible (Turgeon, 1989). At maturity, leaves function as the source of minor vein-loaded photoassimilate and assimilate is transported towards the base of the leaf, where it enters the long-distance transport compartment of the phloem. Heterotrophic tissues (that is, sink leaves) receive nutrients either by symplasmic delivery via plasmodesmata, or through the apoplast via sugar transport systems located on the plasma membranes of the Companion-Cell-Sieve-Element (CC-SE) complex and surrounding meristematic or receiver cells (Ruiz-Medrano *et al.*, 2001). The relationship between phloem

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transport and the maturation of the phloem transport pathway in leaflet pairs in a hypothetical plant is shown in Fig. 1. Export from pairs of mature source leaves is, we believe, very similar, given that these leaflets are formed at the same time. Phloem translocation and the subsequent delivery of assimilates will be directed acropetally towards the strong sink region which is represented by the sink leaflets. Transition leaflets will progressively lose their sink strength and assimilate import will decline as the leaflets undergo transition from a sink to a source state and little if any import of assimilate would occur under these conditions. It is logical therefore to expect that in plants with compound leaves, opposite leaflet pairs would have approximately near-identical structural and functional states. It is also expected therefore that leaflets of the same age would transit through sink to source states at approximately the same time as well. The hypothesis is therefore, that a degree of balancing of imports into sink leaflets would be necessary to ensure that leaf expansion and maturation proceeded in synchrony. In order to be able to test the hypothesis experimentally, transport of the phloem-mobile fluorophore 5,6-carboxyfluorescein (5,6-CF) was studied by loading one of the pair of leaflets and examining the subsequent transport state of the leaflets.

The aim of the experiments was thus to determine if assimilates are transported between paired leaflets of the compound leaves of pea plants.

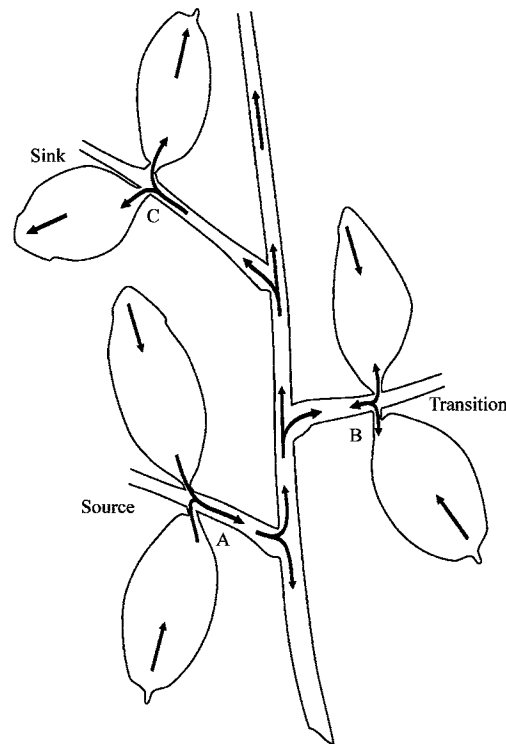


Fig. 1: Shows the hypothetical phloem stream functioning within a plant with compound leaves. The paired leaflets are assumed to be of same age and therefore at the same sink/source state, with similar assimilate flow pattern. Assimilates move from source leaflets (A), into the main assimilation stream within the stem and thereafter are taken up by importing regions and out of exporting regions of transition leaflets (B); movement is inwards in sink leaflets (C)

Materials and Methods

Pisum sativum var. Green feast was grown from seed under controlled environment in growth chambers (Conviron Model S10H, Controlled Environments Ltd, Winnipeg, Canada) under 25/18°C day/night at 16 h photoperiod with CO₂ maintained at 360 µmol mol⁻¹ with fluctuations within ± 15 µmol mol⁻¹. CO₂ was monitored using the integrated computer-controlled Horiba APBA-250 indoor CO₂ monitor (Horiba Ltd, Japan). Plants were illuminated using a combination of fluorescent tubes (F48T12.CW/VHO1500, Sylvania, USA) and frosted incandescent 60W bulbs (Philips, Eindhoven, The Netherlands). Photosynthetic active radiation (PAR at 400 -700 nm) was measured at about 250 µmol m⁻² s⁻¹, 20 cm above soil level (Olivier and Annandale, 1998), with a Li-85A Quantum sensor (Li-Cor Inc, Nebraska, USA). Pot positions were changed every day in a matrix pattern, to avoid any chamber position effect. Plants were allowed to grow for at least two weeks before experiments were carried out. Unless otherwise stated, experiments were repeated six times and the micrograph are thus representatives of the treatment regimen described.

Plastochron Analysis

Plastochron Index of plants (PI) per day was calculated for each plant based on the Erickson and Michelini (1957)'s formula, as revised by Ade-Ademilua and Botha (2005) where the reference length is 20 mm:

$$PI = n + \frac{\log L_n - \log 20}{\log L_n - \log L_{n+1}} \quad (1)$$

Where,

n is the serial number (counting from the shoot base) of that leaf which just exceeds 20 mm;

- $\log L_n$ is the natural logarithm of the length of the leaf n and
- $\log L_{n+1}$ is the natural logarithm of the next or subsequent leaf with a length that is less than 20 mm.
- Consequently, the Leaf Plastochron Index (LPI) is calculated as

$$LPI_n = PI - n \quad (2)$$

This is derived by simply subtracting the leaf's serial number from the plant's plastochron age (Erickson and Michelini, 1957).

Surface Loading of Phloem Transport Tracer

All experiments were carried out using intact plants. The leaf loading protocol used to load 5,6-CF was adapted from that of Grignon *et al.* (1989) and Roberts *et al.* (1997). Experiments were carried out by applying, 5,6-carboxyfluorescein diacetate (5,6-CFDA) to source leaflets and monitoring the transport of the fluorophore in leaflets above the fed leaflet. Another set of experiments was carried using leaflets of all LPI groups, in which the transport of the fluorophore into the opposite leaflet (paired to the fed leaflet) and petiole between the paired leaflets was monitored. Experiments involving the application of 5,6-CFDA were undertaken 7 h after the beginning of the photoperiod. In all cases, the adaxial surface of an attached leaflet was gently abraded with fine sandpaper and rinsed with distilled water. Lamina zones lacking large veins were chosen as abrasion sites. After abrasion, 100 µL of working strength 5,6-carboxyfluorescein diacetate (5,6-CFDA) was applied directly to the abraded area to the leaflet surface and covered with transparent polythene film (Housebrand, Brackenfell, South

Africa) to prevent evaporation. At the end of the leaf loading experiments, the leaflets and petioles of interest, were detached 30 min to 3 h after application of 5,6-CFDA and observed for 5,6-CF distribution, using an Olympus BX-61 (Tokyo, Japan) epifluorescence microscope fitted with a U-YFP filter set (10C/Topaz 41028, Chroma Technologies, Battleboro USA). Several montages of all regions of each leaflet were taken using an Olympus PlanApo 2x objective (Tokyo, Japan) and the whole leaflet images were reconstructed using the Multiple Image Alignment (MIA) routine in analSiS 3.2 (Soft Imaging System GmbH, Münster, Germany).

Effects of Illumination

Experiments were also undertaken by feeding 5,6-CFDA to a predarkened leaflet and the resultant movement of the cleavage product, 5,6-CF across the internode into the illuminated opposite leaflet of the pair was examined 30 min to 3 h after application of the fluorophore. Conversely, the illuminated leaflet was offered 5,6-CFDA and the transport of the cleavage product, 5,6-CF into the darkened leaflet was studied. Experiments were repeated six to eight times and the micrographs are thus representative of the treatment regimen described above.

Preparation of Working Strength 5,6-CFDA

5,6-CFDA (C-195) was purchased from Molecular Probes, Eugene, Oregon USA in 100 mg units, to which 1 mL of 0.2% dimethylsulphuroxide (DMSO) was added. This stock solution was foil-wrapped and stored at -4°C until needed. Working strength 5,6-CFDA was prepared by taking a 1 µL aliquot of the stock solution and adding to 1 mL of distilled water. Working strength 5,6-CFDA was foil-wrapped and stored at -4°C until needed. This resultant mixture was applied directly to abraded leaflet surfaces. Once loaded the acetate moiety is cleaved and the resultant 5,6-CF is transported within the phloem.

Results

With the results we reported in Ade-Ademilua and Botha (2006), on the source to sink transition characteristic of the Green feast variety, a series of experiments were set up in order to investigate transport of the tracer in the phloem from a leaflet within the first pair of leaflets of sink, transition and source leaves to the opposite leaflet. The transport characteristics of the fluorophore were examined in the leaflet opposite the fed leaflet, 30 min to 3 h after application, by examining the whole leaflets from their abaxial surface using the Olympus BX-61 epifluorescence microscope. Images of leaflets from our previous report in Ade-Ademilua and Botha (2006) in which 5,6-CF was loaded from a distant source leaflet (Fig. 2A-C), were compared to those at similar LPI used in the present study (Fig. 2D-F).

In sink leaflets, 5,6-CF was transported across the node and unloaded via the primary vein to the class II and class III veins in the leaflet. Unloading from the class III veins to the mesophyll was routinely observed. No export out of the leaf axis was observed (Fig. 2D). In transition leaflets, 5,6-CF first transported across the node into the primary vein and into lower (i.e., first and some (more basal) class III veins (Fig. 2E). Unloading was observed to be dependent on the state of transition of the leaflet pair, in other words, the plastochron age of these leaflets. The fluorophore was thereafter exported out of the transition leaflet via the petiole. Where a source leaflet was offered 5,6-CFDA, the 5,6-CF first transported across the node interlinking the leaflet pair, to the opposite leaflet. Distribution of the fluorophore always occurred via the primary vein, to the class II vein up to or near the tip of the leaflets and the leaflet margins, via the class II veins (Fig. 2F). No unloading was observed in the leaflet mesophyll. The fluorophore exited the leaflets afterwards.

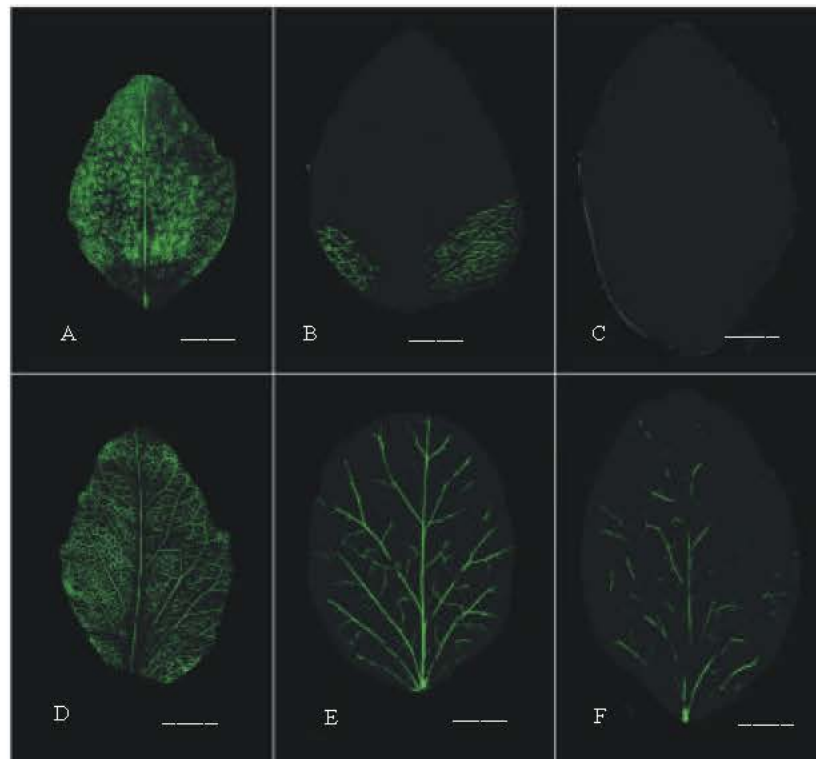


Fig. 2: Epifluorescence images showing the transport and distribution of 5,6-CF via the phloem in intact leaflets of *P. sativum*, 3 h after application of the fluorophore from distant (source) and local (opposite) leaflets. Each leaflet was reconstructed from montages of overlapping regions of the leaf surface. Bars in A-F = 4 mm.

- A: A typical sink leaflet in which the 5,6-CF imported from the fed distant source leaflets was distributed throughout the lamina with high labelling of the class III veins.
- B: A typical transition leaflet in which the class III veins in the immature basal portion of the lamina are still functional as an import pathway for the 5,6-CF from the fed (distant source) leaflets while the apical region has ceased import, thus no 5,6-CF is evident in this region.
- C: A typical mature source leaflet in which no 5,6-CF is evident. No import of 5,6-CF from the fed distant source leaflet occurred.
- D: The distribution of the 5,6-CF within an attached sink leaflet paired to the opposite sink leaflet to which 5,6-CFDA was applied. The fluorophore is evident throughout the lamina with high labelling of the class III veins, in a pattern similar to that in Fig. 2A.
- E: The distribution of the 5,6-CF within an attached transition leaflet paired to the opposite transition leaflet to which 5,6-CFDA was applied. In contrast to the pattern in Fig. 2B, the fluorophore was transported up to the extremities of the major veins and into the class III veins in the basal, less mature regions of the leaflet. Thereafter, the fluorophore was exported via the petiole.
- F: The distribution of the 5,6-CF within an attached source leaflet paired to (opposite) a fed source leaflet. The fluorophore was transported by and restricted to, the major veins of the leaflet before exiting via the petiole; in contrast to Fig. 2C where no evidence of the fluorophore was observed.

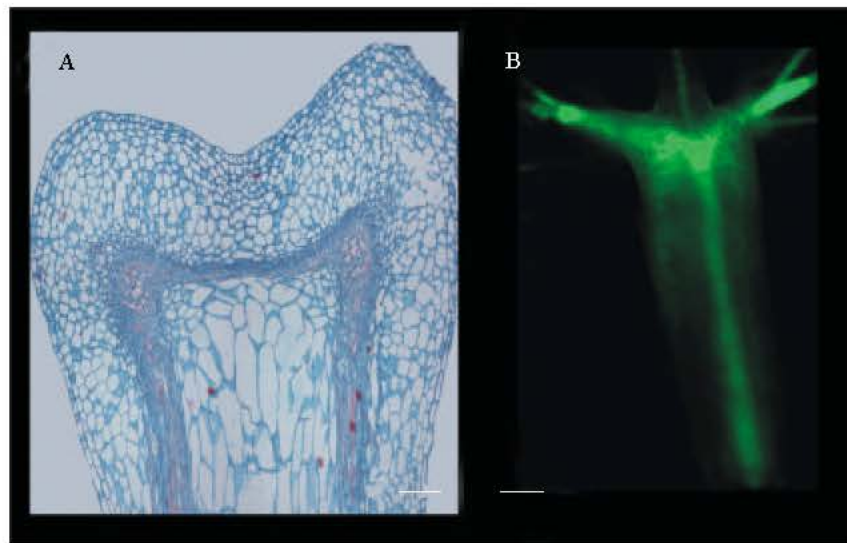


Fig. 3: Details of the anatomy (A) and transport of 5,6-CF (B) across the petiole between paired leaflets in longitudinal section.

A: The petiole traces to a leaflet pair in longitudinal section. Note that the axial traces are cross-connected via a strongly developed lateral vascular trace. Bar = 1.0 mm

B: 5,6-CF moved from the site of application (to the right) and crossed to the opposite leaf trace via the cross-connection. Some evidence for axial transport is evident. Bar = 0.5 mm

When distant source leaves were offered 5,6-CFDA as discussed in our reports in Ade-Ademilua and Botha (2006), accumulation of 5,6-CF in paired sink or transition leaflets were typified by results shown in Fig. 2A and 2B for sink and transition leaflets i.e., 5,6-CF entered both leaflets.

Experiments carried out by applying 5,6-CFDA to predarkened leaflets gave the same results as those undertaken with leaflets that had been exposed to continuous light. In these experiments, 5,6-CF first moved from the predarkened fed leaflet into the opposite leaflet (which was maintained in continuous light) and thereafter exited the leaflets via the petiole in source and transition leaflets.

Figure 3A shows a nodal complex between mature source leaflets. The cross-connection of the axial phloem strands via a well-developed vascular bridge is evident in this micrograph. Fig. 3B is a longitudinal section of a nodal complex which shows strong 5,6-CF-associated fluorescence within the phloem strands which interconnect a source leaflet pair. The leaflet to which 5,6-CFDA was applied (the fed leaflet) was attached to the right. In this example as in all other experiments, 5,6-CF first moved via the interconnecting phloem bridge to the opposite leaflet, before exiting the leaf via the axial phloem strands.

Discussion

The experiments using connected, attached leaflets of the pea plant convincingly demonstrate that the fluorophore 5,6-CF, was first transported to the opposite leaflet before any export occurred. Results reported in Ade-Ademilua and Botha (2006) have demonstrated that 5,6-CFDA is taken up by the leaf and that the cleavage product 5,6-CF is transported to and is subsequently taken up by the phloem tissue in pea leaves and thereafter, that it is transported via the phloem, following classical source to sink transport rules. The phloem-mobile fluorophore was transported along a source to sink

pathway, common with any substance which can (a) enter the phloem and (b) which can be transported passively within the symplasmic translocation pathway. Of greater significance is that the data offer convincing evidence for the existence of a load-balancing mechanism between leaflet pairs in compound leaves such as in *P. sativum*.

Functional cross-connections exist within the nodal complex at the base of leaflet pairs in compound leaf of peas and that this nodal complex is in turn, directly connected to the axial phloem system within the petiole itself. These cross-connections appear therefore to provide the phloem infrastructure necessary for an operative load balancing mechanism. Connectivity across the petiole via subsidiary traces may thus provide a functional channel through which assimilate (or phloem sap) concentrations in oppositely-positioned leaflets are maintained at equal, or near equal sap concentration, during import to, or export from leaflets via the axial traces.

The data demonstrate unequivocally that local source 5,6-CF uptake and its subsequent translocation into sink leaflets, as well as its uptake into transition and source leaflets, is consistently preceded by movement first into the opposite leaflet before any axial transport takes place. Of interest is the appearance of two distinct phloem transport pathways into transition and source leaflets. If the source of assimilates in transition leaflets is local (i.e., the opposite leaflet of the pair), then import (as evidenced by the passively-transported 5,6-CF) is via the major veins (primary and class II) veins to the extremities of the veins, as well as in class III veins in the basal, less mature regions of the transition leaflets, which is quite evident in Fig. 2E. In contrast, Fig. 2B shows the phloem transport pathway of 5,6-CF, where the source of 5,6-CF is distant. Here the 5,6-CF enters the opposite leaflet and is confined to the lateral veins (class II and class III vein network) in the basal less mature region of this transition leaflet. When one of a pair of source leaflets was offered 5,6-CFDA, the 5,6-CF was transported to the opposite leaflet via the major veins. Note however, that no evidence of 5,6-CF appeared in same leaflets where 5,6-CFDA was applied at a more distant source leaf. Clearly, local application of 5,6-CFDA to sink, transition and source leaflets gave consistently different results which we interpret as a local balancing, local transport system which ensures near equal concentration of assimilate in leaflet pair.

The results find support in the earlier results obtained by Isebrands and Larson (1977) who conclusively demonstrated that subsidiary vascular bundles provided vascular continuity between the stem and specific portions of the leaf lamina in Eastern cottonwood. However, as tempting as it may be, to speculate that a bi-directional modality exists within the vein in leaflets, it is important to stress that this has, as yet, not been demonstrated to be the case for a single file of sieve tubes (Turgeon, 1989; Henton *et al.*, 2002). The differences in the pattern of distribution of 5,6-CF transported from distant sources into sink, transition and source leaflets (Fig. 2A-C) compared to that seen in local sources in leaflets of same physiological state (Fig. 2D-F), show that the vascular link between the stem and the lamina is different from the vascular crosslink between the paired leaflets. The strong and consistent lateral movement of the fluorochrome out of fed leaflets and its subsequent appearance in the opposite leaflets, irrespective of the sink/source state of the cross-connected leaflets, is strong evidence that transport across vascular connection between leaflets is autonomous and independent of the relationship between the leaflet pair in question and other compound leaves along the phyllotaxy or also independent of the source to sink gradient.

Plants have previously been described as modular organisms, consisting not only of morphological and of physiological subunits, which act autonomously in part and are thus termed functional modules or integrated physiological units (IPUs) that have their own carbon source or supply (Watson and Casper, 1984; Watson, 1986). A modular structure has important consequences for the control of internal resource translocation and distribution in plants and therefore in the regulation, by partitioning, of differential growth of plant parts. Indeed, some plant parts may act as partially autonomous functional modules with their own resource supply and localized control of growth

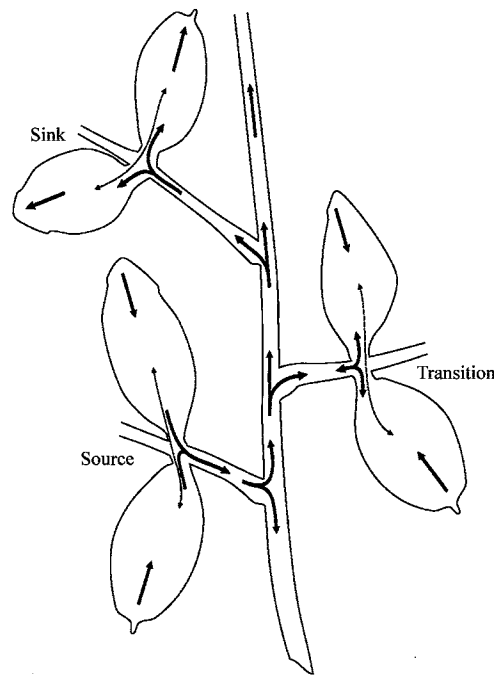


Fig. 4: Illustration of the assimilate pathway through the phloem of a plant with compound leaves, as interpreted from the results. Accumulation patterns of 5,6-CF suggests that phloem transport takes place between paired leaflets (dotted lines), irrespective of the sink/source state of the leaflets; and it is independent of the movement along the source to sink gradient (bold lines)

(Kaitaniemi and Honkanen, 1996). Yang and Midmore (2005) have proposed a model in which the transport process is included as a significant component of the modular nature of growth. Yang and Midmore (2005) argue that because of the distance and complex structure and function of the transport pathway, each foliage branch takes (first) priority for the allocation of the carbon that it fixes, thereby satisfying its own requirement for assimilate despite fluctuation in supply, before any excess assimilate is transported to other parts of the plant.

In conclusion, the data offer strong support to the concept of modular transport and the evidence which is presented here for reallocation of and balancing of assimilate across leaflet pairs takes place to ensure that leaflets of the same physiological age, maintain similar or near-identical transport status, thereby utilizing assimilates first, before any export can or will take place. We argue that load balancing of the local transport system before exporting is thus a prerequisite before export to other younger (sink) regions can be take place. We argue further that the model for describing phloem transport into and out of plants bearing simple leaves is inappropriate to the situation in compound leaves such as *P. sativum* (Fig. 1). The interpretation of phloem transport across paired leaflets (Fig. 4) suggests that phloem transport between leaflet pairs is independent of the sink/source state of the leaflets, or of movement along the source to sink gradient.

Acknowledgments

On Ade-Ademilua acknowledges the International Center for Scientific Culture (ICSC) World Laboratory, Switzerland and NRF, Pretoria for support. The NRF, Pretoria and Rhodes University JRC are acknowledged for their continued support of CEJB.

References

- Ade-Ademilua, O.E. and C.E.J. Botha, 2006. Sink to source transition of *Pisum sativum* in relation to leaf plastochron index. *Am. J. Plant Physiol.*, (Accepted for Publication).
- Erickson, R.O. and F.J. Michelini, 1957. The plastochron index. *Am. J. Bot.*, 44: 297-305.
- Grignon, N., B. Touraine and M. Durand, 1989. 6(5) Carboxyfluorescein as a tracer of phloem sap translocation. *Am. J. Bot.*, 76: 871-877.
- Henton, S.M., A.J. Greaves, G.J. Piller and P.E.H. Minchin, 2002. Revisiting the Münch pressure-flow hypothesis for long-distance transport of carbohydrates: Modelling the dynamics of solute transport inside a semipermeable tube. *J. Exp. Bot.*, 53: 1411-1419.
- Isebrands, J.G. and P.R. Larson, 1977. Organization and ontogeny of the vascular system in the petiole of eastern cottonwood. *Am. J. Bot.*, 64: 65-77.
- Kaitaniemi, P. and T. Honkanen, 1996. Simulating source-sink control of carbon and nutrient translocation in a modular plant. *Ecol. Model.*, 88: 227-240.
- Olivier, F.C. and J.G. Annandale, 1998. Thermal time requirement for the development of green peas (*Pisum sativum* L.). *Field Crop. Res.*, 56: 301-307.
- Patrick, J.W., W. Zhang, S.D. Tyerman, C.E. Offler and N.A. Walker, 2001. Role of membrane transport in phloem translocation of assimilates and water. *Funct Plant Biol.*, 28: 697-709.
- Roberts, A.G., S. Santa Cruz, I.M. Roberts, D.A.M. Prior, R. Turgeon and K.J. Oparka, 1997. Phloem unloading in sink leaves of *Nicotiana benthamiana*: Comparison of a fluorescent solute with a fluorescent virus. *Plant Cell*, 9: 1381-1396.
- Ruiz-Medrano, R., B. Xoconostle-Cázares and W.J. Lucas, 2001. The phloem as a conduit for inter-organ communication. *Curr. Opin Plant Biol.*, 4: 202-209.
- Turgeon, R., 1989. The sink-source transition in leaves. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 40: 119-138.
- Watson, M.A. and B.B. Casper, 1984. Morphogenetic constraints on patterns of carbon distribution in plants. *Annu. Rev. Ecol Syst.*, 10: 109-145.
- Watson, M.A., 1986. Integrated physiological units in plants. *Trends Ecol. Evolut.*, 1: 119-123.
- Yang, Z.J. and D.J. Midmore, 2005. Modeling plant resource allocation and growth partitioning in response to environmental heterogeneity. *Ecol. Model.*, 181: 59-77.