Review Article

Plant sink-source relationships and carbon isotopic labeling techniques

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Abstract

The concept of source and sink strength is presently well-recognized and accepted by the scientific community as a pertinent approach describing the mechanisms of carbohydrate partitioning into the different and competing organs at a whole plant or canopy scales. Sink–source relationships have a clear role in the size of sink organs. Besides the effect on organ size, sink/source ratio might also affect photosynthesis. Crop biomass productivity is closely related to source and sink capacities and the balance between them. Determination of the carbon balance of a whole plant and relation between source and sink were studied by carbon isotopic labeling techniques. Techniques using ^{14}C or ^{13}C have been used for many years to study the allocation of carbohydrates produced by photosynthesis between different parts of the plant. This method also allows calculation of the proportion of the total carbon accumulated in each sink that was supplied by all source leaves, as well as the growth contribution from each source leaf to the main plant sinks. These data are vital to determine the productivity of each source leaf.

Keywords: carbon balance, photosynthesis, source leaves, ^{13}C, ^{14}C.

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Introduction

In the process of plant growth and development, assimilated carbon is redistributed between plant tissues for usage and storage. This process depends largely upon the partitioning of assimilated carbon between photosynthetic sources such as mature leaves, and photosynthetically less active or inactive sink tissues such as flowers, roots and fruit (Farrar and Williams, 1991). Regulation of carbon partitioning between source and sink tissues is important because it has a vast influence on both plant growth and development. The regulation of carbon partitioning at the whole plant level is directly linked to the cellular pathways of

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assimilate transport and the metabolism and allocation of sugars, mainly sucrose and hexoses in source leaves, and sink organs such as roots and fruit (Osorio et al., 2014).

Plant structures can be divided into two broad groups on the basis of these processes (Sonnewald and Willmitzer, 1992). Functionally, a plant can be divided into source and sink. Within a plant, the sources are the parts where net fixation of carbon dioxide occurs, or a photosynthesizing tissue and organ with export of carbon skeletons. Source organs are the photosynthetically active portions such as mature leaves. The sinks are the sites where assimilates are stored or used, or the ones requiring import of carbon (Long, 2005). Sink regions that draw assimilate to them are net importers of fixed carbon for vegetative and reproductive structural growth and maintenance (Long, 2005). Through the course of plant growth, organ structures will vary in assimilate metabolizing behavior. For example, a newly expanding leaf is a net importer of fixed carbon before maturing into a net exporter (Hay and Walker, 1992). These sources are usually other mature leaves or photosynthetic organs on the plant, or in the case of a seedling, the cotyledons (Roberts et al., 1998). As the lamina expands and the leaf matures, levels of photosynthesis increase until the leaf can support itself. When the amount of carbon accumulated by photosynthesis is greater than the requirement of respiration and growth, a positive carbon balance is achieved by that leaf. The leaf can then become an exporter of carbon to sink organs (Roberts et al., 1998). Sink strength can be defined as the competitive ability of an organ to import photoassimilates (Blanke, 2009). The sink strength is the capacity of a tissue or organ to import and store further compounds from the sources (Blanke, 2009).

The activities of source and sink organs are regulated in different ways, so that crop growth and yield can be either source or sink limited (Ho, 1988). Movement of assimilated carbon into a particular plant organ is determined by its sink strength, and by the capacity of the plant for photoassimilate production (Ho, 1988).
The growth and yield of a crop plant is limited by the size and activity of either assimilate sources or sinks (Ho et al., 1989). The source-sink system is coordinated and considered "source-limited" i.e., plant growth and development are normally limited by photosynthetic resources. Leaf photosynthesis is normally down-regulated by the sink and the presence of fruit sinks retard leaf senescence. After fruit harvest, the roots become the dominant sink (Blanke, 2009).

In source-limited plants, the number of sinks is often reduced through flower or fruit abortion (e.g. Bertin, 1995). In sink-limited plants, the development and/or photosynthetic activity of leaves can be altered (El-Keblawy and Lovett-Doust, 1996). In cantaloupe, fruits constitute large sinks relative to the whole plant, which may significantly affect the source-sink balance at fruiting (Schaffer et al., 1996). Hence, this species is a suitable model to study source-sink interactions. When sink organs e.g. fruits are removed from a plant, photosynthesis rate may decrease through a negative feedback loop.
Sink organs depend on the delivery of sucrose (or other forms of carbohydrates) by the phloem for their growth and development. A plant may be regarded as a series of sources and sinks with an overall carbon fixation capacity and several sinks “competing” for the available photoassimilates. This creates a priority system among sinks. Roots and young leaves are major sinks during the early developmental stages, whereas tubers, fruit and seeds become major sinks during the reproductive stages (Wardlaw, 1990).

In order for plants to reach a balanced development and optimize their reproductive fitness, priority for access to photoassimilates needs to be established between sinks. Changes in carbon partitioning and switches between the apoplastic and symplastic pathways occur throughout development or as a response to the environment (Roitsch, 1999; Godt and Roitsch, 2006).

Sink–source relationships have a clear role in the size of sink organs. Besides the effect on organ size, sink/source ratio might also affect photosynthesis (Stitt, 1991). As a result of diminished export of carbohydrates from photosynthetic tissues due to diminished sink demand, starch accumulates in the chloroplasts, and CO_2 fixation decreases as a result of negative feedback control producing a down regulation of photosynthesis (Stitt, 1991; Goldschmidt and

Huber, 1992). These alterations in leaf carbohydrate metabolism occur while the photosynthate production, i.e. source activity, far outstrips the capacity of utilization by the sink organs, i.e. sink activity (Van Gestel et al., 2005). Thus, under conditions of non-limiting assimilate availability, accumulation of starch is expected, and under these conditions the potential size of sink organs can be determined. Studies on the metabolic process of photoassimilates and enhancement of sugar accumulation into fruit are important in fruit crop production. Several techniques are available to assess carbon allocation in plants. The labeling techniques using isotopic carbon are promising approaches to monitor translocation of photoassimilates in plants and are discussed in this article.

^{13}C and ^{14}C isotope studies

The word "isotope" derives from the Greek words "isos" and "topos", which refer to occupation of the same place" in the periodic table of elements (De Groot 2004, 2008). Central carbon metabolism has been studied for many decades with the help of both radioactive (^{11}C and ^{14}C) and stable (^{13}C) carbon isotopes. Each isotope offers its own advantages and disadvantages. The ^{11}C isotope has a very short half-life of 20.334 minutes but emits high energy positrons upon decay. Therefore, it is well suited for in vivo studies of phloem transport, partitioning of carbon between alternative sinks, such as roots, leaves and seeds and to calculate the rates of carbon transport and leakage (Gould et al., 2012). Furthermore, the short half-life means that the same biological material can be labeled sequentially (Schwachtje et al., 2006; Babst et al., 2013). Labeling techniques using ^{14}C or ^{13}C have been used for many years to study the allocation of carbohydrates produced by photosynthesis between different parts of the plant, especially on herbaceous species grown in controlled environmental conditions or in natural abundance conditions (IAEA, 2001).

Principles of isotopic tracer methodologies

"Tracing carbon fluxes" means tracking carbon atoms in chemical reactions or during displacement. Isotopes are ideally suited for this purpose. Isotope techniques were proved useful to partition photosynthesis and respiration to quantify carbon allocation to different compartments and partitioning into different biochemical compounds at various scales, from the cell to the globe (Gamnitzer et al., 2009; Grams et al., 2011).

There are two principal ways by which isotopes can produce traceable signals in study objects. Either the signal is created artificially by exposure to isotopically altered substrate, or it arises naturally in metabolism or transport processes. Artificial tracer approaches have made use of the radioactive short-lived ^{11}C (half-life 20.5 min) and long-lived ^{14}C (5,760 years) as well as the stable ^{13}C. Methods of label provision include exposure to isotopically altered CO_2 (Haupt-Herting et al., 2001; Gamnitzer et al., 2009) or feeding with uniformly or position-labeled organic substrates, such as sugars and amino acids (Libourel and Shachar-Hill, 2008; Schwender, 2009; Kruger and Ratcliffe, 2009). There are two popular methods of applying labeled CO_2 and monitoring the propagation of the tracer: pulse (chase) labeling and dynamic (long-term) labeling.

^{13}C stable isotope labeling techniques

Stable isotopes are nonradioactive atoms whose nucleus contains the same number of protons but a different number of neutrons. The major elements used in environmental studies include carbon, hydrogen, oxygen, nitrogen and sulfur (IAEA, 2001). The ^{13}C stable isotope analysis is widely used to study physiological, ecological, and biogeochemical processes related to ecosystems (Dawson et al., 2002).

At natural abundance level stable isotopes are used mainly as natural tracers and integrators, allowing ecologists to evaluate the net result of processes, without disrupting the natural flow of the elements (Högberg, 1997). The isotopic composition of carbon can provide information at different temporal and spatial scales (Miller et al., 2003). For example, ecosystem photosynthesis and respiration can be

separated, because these two processes have contrasting effects on the isotopic ratio of ^{13}C (Flanagan and Ehleringer, 1998). Thus, isotopic measurements can reveal climate change by variation of temperature, soil moisture and all other factors that influence photosynthesis and respiration (Flanagan et al., 1997).

Recently, ^{13}C labeling has been used to analyze central carbon metabolism. In metabolic flux analysis, heterotrophic or mixothrophic cells are fed with positionally ^{13}C labeled sugars (e.g. [6-^{13}C]-glucose) until a steady state is reached. Determination of the isotope pattern in several pathway-relevant metabolites allows metabolic fluxes to be calculated, based on an existing metabolic model (Schwender et al., 2004; Williams et al., 2008).

Natural abundance of C isotopes

The ratio of ^{13}C to ^{12}C in the atmosphere can vary with different physiographic parameters such as altitude, latitude, and temperature as well as some biological processes (Lefroy et al., 1993). Atmospheric CO_2 contains the naturally occurring carbon isotopes consisting of 98.892% ^{12}C and 1.108% ^{13}C (Dawson et al., 2002). Plants contain less ^{13}C relative to ^{12}C than atmospheric CO_2 in their tissues. In other words, plants discriminate against the heavier isotope of carbon, and they have smaller ratios of ^{13}C to ^{12}C than are found in atmospheric CO_2 (Farquhar, 1983). When plants fix carbon during photosynthesis there is a degree of discrimination between the amount of ^{13}C and ^{12}C. This is due to the faster diffusion of $^{12}CO_2$ into the leaves and the faster reaction rate of Rubisco with ^{12}C during photosynthesis (Farquhar et al., 1982; Flanagan and Ehleringer, 1998). Discrimination occurs during the carboxylation step in photosynthesis, with the greater discrimination against ^{13}C in C_3 (Calvin cycle) plants than in C_4 plants, due to the greater discrimination in the primary carboxylation step of C_3 plants. This primary carboxylation step is catalyzed by the enzyme ribulase biphosphate carboxylase (RuBP) resulting in a lower ^{13}C:^{12}C ratio in C_3 plants than in C_4. CAM plants (crassulacean acid metabolism) plants show variable discrimination, but it is more often similar to C_4 plants (IAEA, 2001).

The ^{13}C:^{12}C ratio is generally measured as $\delta^{13}C$. A C_4 species such as maize will have a $\delta^{13}C$ value of approximately -12‰ whereas in a C_3 species such as wheat or rice it will be approximately -26‰ (Schwartz et al., 1986).

^{13}Carbon isotope calculation

Isotopic signatures for ^{13}C are commonly noted with delta ($\delta^{13}C$) notation, which is defined by the equation: where R is the molar ratio of $^{13}C/^{12}C$ and VPDB is the Vienna Pee Dee Belemnite international standard (based on carbonate from the Pee Dee formation). Delta values are commonly given in “per mil” (‰) due to the small fractional differences in natural abundance (Dawson et al., 2002).

Negative values stand for depletion versus the standard and positive values for enrichment in the heavy isotope. It has been extensively studied and robust models have been developed for both plant types (Farquhar et al., 1982; Farquhar, 1983).

Carbon isotope composition ($\delta^{13}C$) was calculated as deviation of the carbon isotope ratio ($^{13}C/^{12}C$ called R) from the reference international standard (VPDB, Vienna Pee Dee Belemnite): $\delta^{13}C = [(R_{sample} - R_{standard}) / R_{standard}] * 1000$.

Design of a single leaf labeling chamber and control experiment

To study the source-sink relationship a method has been developed for labeling plants with $^{13}CO_2$ (Barzegar et al., 2011). They carried out labeling experiment (with the heavy stable isotope of carbon, ^{13}C) using a labeling chamber coupled to the Licor-6400 gas exchange system (Fig. I and III). The chamber was 350 cm^2 made from poly carbonate and PVC pipes (Fig. II). Mercury vapor lamps were used to provide additional lighting in cloudy days above the chamber. The temperature (24±1°C) and humidity (65±3%) were both regulated by a cooling circulator with a heat exchanger inside the chamber.

For each plant, a single leaf was placed in a specially designed labeling chamber and fed with $^{13}CO_2$ for 4 hours under natural light conditions in the greenhouse. A 99% ^{13}C-labeled CO_2 source (Eurisotop, Saint Aubin, France) was used for labeling experiments without any dilution. Since the gas analyzer system cannot detect the heavy carbon isotope, the CO_2 concentration parameters of the Licor were fixed before labeling using non-labeled CO_2 to get 400 µmol mol^{-1} in the labeling chamber during the Labeling period (for supporting information and a review see Barzegar et al., 2013). This method is ideal for measuring carbon export into sink regions, because feeding individual leaves of intact plants with labeled carbon allow tracing carbon transport and carbon partitioning simultaneously as well as determining assimilated carbon transported form source leaves to sink organs such as the fruits, shoots, and root in parallel.

^{14}C stable isotope labeling techniques

Labeling with $^{14}CO_2$ has been used extensively in the last decades to study the allocation of photo assimilated carbon in different species such as crops (Farrar and Farrar, 1985), trees (Pumpanen et al., 2009) and grasses (Atkinson and Farrar, 1983; DaCosta et al., 2006). In most of these approaches, a setup containing polyethylene bags or chambers mounted over single leaves yielded valuable information of carbon allocation.

Single leaf labeling with $^{14}CO2$ isotopes

Kölling et al. (2013) described the design of a system for supplying isotopically labeled CO_2 to single leaves of *Arabidopsis thaliana* (Fig. IV). Labeling experiments on single leaves were performed using the same light conditions specified above. By acidification of sodium ^{14}C bicarbonate (Hartmann Analytic GmbH, Germany) supplied with a specific activity of 59.5 mCi/m), $^{14}CO2$ was released in the sealed reservoir chamber. This increased the CO_2 content in the chamber by only 5–10 ppm. The leaf cuvette was clamped onto an individual mature leaf of the plant. After a 'pulse' period (labeling times of 5 to 10 min were typically

Fig. I. Labeling chamber coupled to the Licor-6400 gas exchange system (Barzegar, 2011).

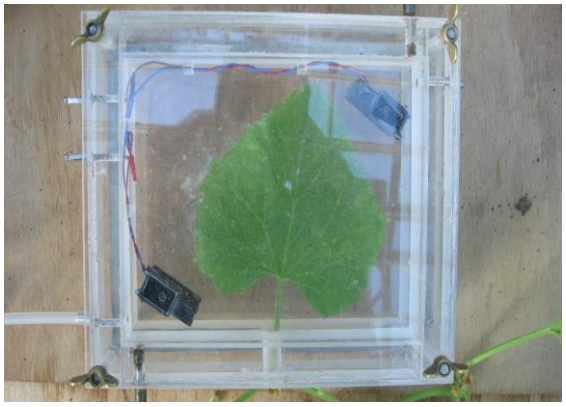

Fig.II. Single leaf labeling chamber with approximately 350 cm2 size (Barzegar, 2011).

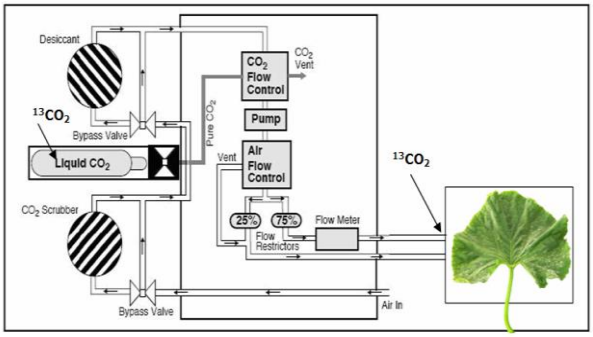

Fig. III. LI-6400 open system of photosynthetic gas exchange (IRGA); the incoming air stream can be conditioned for CO_2 concentration, humidity, and temperature. There are chemical tubes for scrubbing CO_2 and H_2O. The air was diverted through these tubes and $^{12}CO_2$ from the incoming air was removed, and $^{13}CO_2$ source injected to system.

used), the cuvette was isolated from the reservoir and opened. The leaf was removed and the plant was kept in normal air for a chase period (as specified, but typically 60 min). They showed the design and functionality of a system for single-

leaf isotopic labeling in Arabidopsis suitable for both $^{13}CO_2$ and $^{14}CO_2$.

Whole plant labeling with $^{14}CO2$ isotopes

Sanchez et al. (2000) described a simple method for uniform labeling of plant material using a pulse labeling technique (Fig. V). This chamber measures 2.5 m long, 1.3 m wide and 1 m high that made from aluminum frame and PVC pipes and the sides from clear, gas proof ethyl-vinyl alcohol film. A commercial air-conditioning unit is attached on one side to circulate the air inside the chamber and at the same time regulates the temperature to about 25 °C. If additional lighting is required e.g. cloudy weather or short daylight hours, lights such as mercury vapor lamps should be used above the chamber. Labeling the $^{14}CO_2$ can be generated from of Na_2 $^{14}CO_2$ with lactic acid injected through a thin plastic tube that runs through the side of the labeling chamber. Before the labeling CO_2 pulse, the CO_2 concentration inside the chamber is allowed to drop from 350 ppm to 300 ppm. The chamber should be closed overnight to contain any labeled CO_2 released during respiration and prevent any labeled CO_2 leaking into the atmosphere. The plants were pulse-labeled with $^{14}CO_2$ fifteen times during the growing season and the frequency of labeling increased from once a week to four times a week as the plant biomass increased (for supporting information see Sanchez et al., 2000).

The use of ^{14}C and/or ^{13}C in plant assimilation studies

Previous studies on the relationship between source-sink by carbon isotopes technique has confirmed that source leaves supply adjustment organs. On the other hands, every sink organs receive their carbon from nearby leaves, so the top leaves send a greater carbon to the apical bud and young growing leaves and the basal leaves supply carbon to the lower stem and roots and the fruits are dominant sinks (Palit, 1980; Barzegar et al., 2013). To measure the carbon allocation from the leaf numbers 20-22 to the developing cucumbers on the plant, Dieleman et al. (2006) fed leaves by

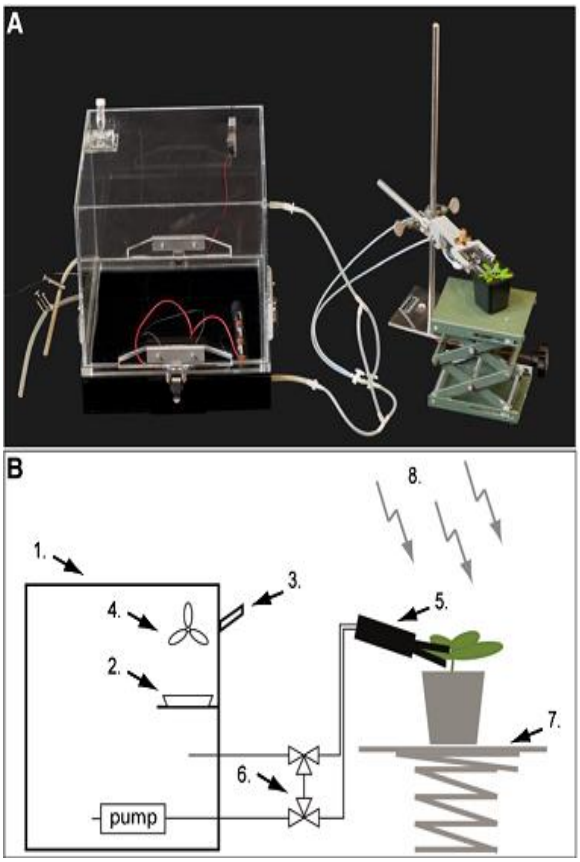

Fig. IV. Chamber for isotopic labeling of individual leaves; (A) Picture of the single leaf labeling chamber. The labeling system comprises of a reservoir chamber (1) in which the isotopically labeled sodium-bicarbonate is placed within a petri dish (2). The bicarbonate is acidified by injecting an excess of lactic acid through a rubber seal (3) into the petri dish, releasing labeled CO_2, which is homogenously dispersed within the chamber with a fan (4). The labeled air is pumped through a tubing system to the single leaf cuvette (5) or through a bypass (6) when exchanging leaves are to labeled. An adjustable table (7) is used to position the plants in relation to the external light source (8). (B) Scheme of the single leaf labeling chamber in use (Kölling et al., 2013).

pulse-labeled $^{13}CO_2$, then harvested leaves and cucumbers 24 hours after application of $^{13}CO_2$. Labeling was performed at several stages of crop development under typical production conditions in a greenhouse. Results showed that after 24 hours, between 45-64 % of the applied $^{13}CO_2$ was exported from the labeled leaves. Most of the labeled exported ^{13}C-sugars were allocated to the developing cucumbers (initially to the upper cucumbers on the main stem, later to the cucumbers on the lateral shoot. As long as these leaves are green, photosynthesis takes place and assimilates are allocated towards developing

fruits (Dieleman et al., 2006). Labeling experiments using $^{13}CO_2$ were conducted to study the photoassimilate import from leaves to fruits in relation with leaf and fruit positions. Results clearly showed that the labeling level in retained fruits is higher when the adjacent leaf is labeled and that the fruits retained in the middle part of the branch receive the highest labeled photoassimilates (Barzegar et al., 2013). Nogues et al. (2006) used $^{13}C/^{12}C$ isotope labeling technique to study the allocation of recently assimilated C by photosynthesis at short term (over 2 days) and further night respiration after the photoperiod in adult trees of Fagus sylvatica. They found that night-respired CO_2 was not completely labeled, only 27% of new carbon found in respired CO_2 immediately after the labeling and the labeling level progressively disappeared during the next day.

The $^{14}CO_2$ feeding technique was used to analyze photoassimilates in cucumber plants' vascular system. The radioactivity of ^{14}C-stachyose was as high as that of ^{14}C-sucrose in the vascular bundles of petiole and internode just below the $^{14}CO_2$-fed leaf as well as in the midrib, although in the vascular bundles of the peduncle, the ratios of the radioactivity of ^{14}C-stachyose and ^{14}C-raffinose were lower, and the ratio of the radioactivity of ^{14}C-sucrose was higher. Therefore, it seemed that ^{14}C-stachyose and ^{14}C-raffinose were hydrolyzed to ^{14}C-sucrose in the peduncle (Ohkawa et al., 2010).

Qi et al. (2006) labeled tomato plants by $^{14}CO_2$ feeding to determinate the composition and specific activity of carbohydrate at different sites and transport of assimilates from source leaves to sinks. The results indicated that almost all of the photosynthates resulting from 1 h of photosynthesis could be transported out of the leaf within 72 h and more than 85% of them were exported within 24 h. The greatest amount of sucrose transport occurred in the internode pedicel vascular bundle 8 h after the start of photosynthesis. Some of the sucrose in the fruit came directly through the phloem at an early stage of fruit development, not through the synthesis of glucose and fructose.

To quantify the origin of assimilates that translocate to fruit sinks, Hughes et al. (1983) fed $^{14}CO2$ to melon leaves and reported that leaves

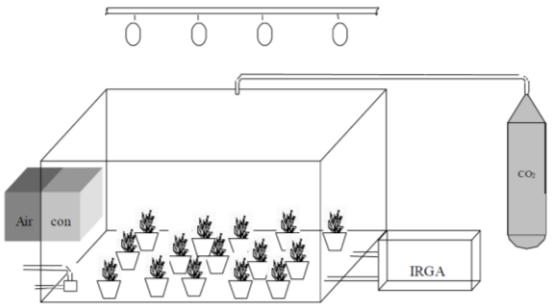

Fig. V. Set-up of the labeling chamber for isotopic labeling of whole plants (Sanchez et al, 2000)

which were 3 nodes acropetal to fruit exported 65% of the label in 6 h, while those further from the fruit retained the label longer. Liu (1997) traced the distribution of ^{14}C-photoassimilate for 120 h beginning immediately after labeling and observed that photoassimilates were mostly exported out of source leaves within 48 h after carbon fixation both in water stressed and well-irrigated peach or apple trees.

To investigate the effect of water stress on allocation of carbon assimilates among sink organs, shoots of peach were fed with $^{14}CO_2$ twice, either during fruit pit hardening stage or the final rapid fruit growth stage. The results showed that to regulate deficit irrigation (RDI) and stage to half-root stress (HRS) induced an altered allocation pattern of ^{14}C-assimilates so that the import to shoot apices was reduced. It is concluded that RDI and HRS resulted in a decreased sink activity in the shoot and a change of carbon allocation toward stressed roots and seeds without negative effects on fruit growth (Yuan et al., 2009).

Shishido et al. (1999) studied the carbon balance of a whole plant by the $^{14}CO2$ steady-state feeding method and found that the source-sink relationship in tomato plants is independently formed between each source leaf and all sinks, or between each sink and all source leaves and sink strength varies due to its distance from and relative position to the source leaves.

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