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Review

Plant-PET Scans: *In Vivo* Mapping of Xylem and Phloem Functioning

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Medical imaging techniques are rapidly expanding in the field of plant sciences. Positron emission tomography (PET) is advancing as a powerful functional imaging technique to decipher *in vivo* the function of xylem water flow (with ^{15}O or ^{18}F), phloem sugar flow (with ^{11}C or ^{18}F), and the importance of their strong coupling. However, much remains to be learned about how water flow and sugar distribution are coordinated in intact plants, both under present and future climate regimes. We propose to use PET analysis of plants (plant-PET) to visualize and generate these missing data about integrated xylem and phloem transport. These insights are crucial to understanding how a given environment will affect plant physiological processes and growth.

PET Scanners Are Ready to Bridge the Gap Between Medical and Plant Sciences

Positron emission **tomography** (PET) scanners have been developed to give the medical world a tool to measure the activity of organs, cell aggregates, or brain zones. With the positron-emitting radioactive molecule fluorodeoxyglucose (^{18}FDG), a proxy for sugar, cancerous lesions can now be readily located because of their higher metabolic activity, and brain functioning can be mapped by imaging active and passive brain zones [1,2]. Whereas ^{18}FDG is currently the most widely used radiopharmaceutical in PET, many other positron-emitting radionuclides are available for clinical use [3]. Despite the wide use of PET in the medical field, only a few plant-PET experiments have been undertaken. There is, however, no reason why PET could not be widely used in plant science, because humans and plants are similar at cellular and biochemical level [4], which makes the use of PET scanners in plant research straightforward. In addition to mapping spots with higher or lower metabolic activity, the dynamic information that can be derived from plant-PET is of particular interest. Where does water and sugar flow to and at what speed, how are axial and lateral movements coordinated, and what is the importance of xylem–phloem interactions? In the same way as medical PET scans led to major breakthroughs in explaining organ function and detecting health issues in humans, PET has the potential to rapidly advance our understanding of xylem and phloem function, the dynamic interactions between xylem and phloem, and the extent to which these two long-distance transport pathways aid or hamper each other's function in a given environment [5–11].

Water and Sugar as the Alpha and Omega of Plants

For centuries, scientists have been fascinated by water and sugar flow in plants because of their vital importance for plant growth and life. If we aspire to explain plant growth or to increase productivity of agriculture or forestry, it is crucial that we understand the dynamics and the controls of water and sugar flow, as well as their interactions. This is essential to unravel how plants function, how they cope with stress, and which features determine whether a plant thrives or dies. Water is transported in the xylem from the roots to the leaves where it is transpired.

Trends

There is evidence that PET can be successfully used in plant science, but the few plant-PET studies to date have been limited to short-term qualitative research, whereas routinely quantitative research should be aimed for.

The function and coordination of xylem and phloem transport is currently under intense debate, and plant-PET, either alone or in combination with MRI and SPECT, may assist by visualizing these pathways and their interactions *in vivo* in intact plants.

Compartmental analysis can be used to provide estimates of axial transport velocity and lateral exchange rates, which are important to advance our knowledge on xylem and phloem coupling.

Plant-PET can be used to study carbon-assisted embolism repair, to unravel carbon storage regulation, and to quantify the contribution of woody tissue photosynthesis to the overall carbon budget of the plant.

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During this transport, water and dissolved nutrients are supplied to all living plant cells [12–14]. Because only well-watered cells grow and function properly [12], water transport is essential, and any malfunction may result in some degree of drought stress [8,12]. The other vascular system, the phloem, is responsible for the distribution of sugars throughout the plant, ensuring that all cells are supplied with fuel for maintenance and growth [11,13,15]. If the supply of sugar is reduced, a plant will have fewer resources to grow and maintain its living cells [11,15,16]. However, these two long-distance transport pathways are also highly interlinked. For instance, phloem transport will only operate if there are both sufficient water and sugars. Sugars are loaded into the phloem, at sites termed sources, and exert an osmotic potential which draws water into the phloem [15]. This water flow generates a positive turgor pressure, pushing the solution through the sieve tubes along a pressure gradient, which is at the sink sites controlled by the release or unloading of sugars [15].

The importance of highly interconnected xylem and phloem also emerges in drought-related plant studies. It is well known that drought hampers photosynthesis [17–19]. This drought-related reduction in photosynthesis also results in reduced water flow from xylem to phloem at the sources owing to a decrease in phloem loading [11]. Furthermore, it has been speculated that drought may lead to lower cell wall permeability, which may result in a reduced water supply to the phloem, as well as increased viscosity because of the decreased dilution of sugars [10]. A too-syrupy fluid and a weak pressure gradient in the sieve tubes will hamper the proper function of phloem transport and sugar distribution [10], and may greatly weaken the plant [8]. These examples illustrate that, without a proper functioning of the xylem, phloem functioning will also be constrained, but these interactions are currently understudied because methodological approaches have not advanced sufficiently to routinely study these interactions. Many open questions remain about xylem–phloem interactions in a changing environment. Will the reduction in sugar or rather the reduction in water have the largest impact [20]? Will reduced turgor pressure cause problems for phloem transport or will it be instead the increased viscosity of the sugar sap [10]? Will sources and sinks behave and compete differently under a changing climate regime [21]? To what extent will plants be able to adapt or avoid these above-mentioned problems? Plant-PET scans and, by extension, all functional medical imaging techniques, may help in answering these vital questions to further our knowledge of drought tolerance and resilience, growth, reproduction, and yield (Outstanding Questions).

Measurements of Water and Sugar Flow with PET

PET scanners make use of high-energy decay of short-lived radioactive isotopes to visualize their distribution (Box 1 and Figure 1A for the physical principles behind PET). PET can be used to visualize water flow in intact plants with $H_2{}^{15}O$ or ^{18}F (Figure 2) [22–28]. The fluorine atom is used as an **apoplastic** (see Glossary) proxy for water because the size of fluorine and hydrogen are comparable and thus both behave and bond similarly [3]. Because of its higher **half-life** (respectively, 109.8 min versus 2.03 min; Table 1), ^{18}F is preferred over ^{15}O in experiments where water transport needs to be studied over a longer period [25,27,28]. With $H_2{}^{15}O$, Ohya *et al.* [26] recently demonstrated the occurrence of lateral water exchange during water transport towards the leaves. This lateral water supply is not only important for sugar transport when it is directed from xylem to phloem [10,15], but it also plays a crucial role when water is released from internal phloem water reserves because it helps to buffer abrupt changes in xylem water potential [14,29]. In addition, aquaporins are expected to play an important role in this lateral water movement [14,26,30]. To quantify lateral exchange parameters from PET scans, the use of compartmental models is required (as discussed below).

Whereas many alternatives exist to quantify water flow *in vivo*, ranging from the use of heat as a tracer [31] to magnetic resonance imaging [32–34] and neutron imaging [35], the options are much more limited for *in vivo*, non-invasive carbon quantification. This is where the isotope ^{11}C

Glossary

Apoplast: continuum of extracellular spaces and cell walls.
Annihilation: collision between a positron (β^+) and an electron (e^-), resulting in the antiparallel (180°) emission of two γ-rays.
Autoradiography: distribution of γ-rays or β particles (β^+ or β^-) on an image plate. It is not possible to image in real-time or without disturbing the plant, and exposure to dark conditions is required, limiting the usefulness to monitor in real-time physiological plant mechanisms. However, its spatial resolution is much higher than for PET and uncertainty related to annihilation is eliminated.
Collimator: physical configuration to restrict the directions for which the detector is sensitive. In case of positron emitters, lead or tungsten shielding are most commonly used to reject rays coming from other areas.
Half-life ($t_{1/2}$): the time taken for the amount of radionuclides to decay to 50% of the initial amount. This property is different for each radioactive isotope.
Photomultiplier: a vacuum tube that increases the strength of a signal. When light hits the photomultiplier tube an electron is produced and a chain reaction is initiated which produces a very high number of electrons. Eventually the output of a photomultiplier is a relatively strong electrical signal, proportional to the energy deposited on the scintillator, which can easily be detected, transmitted, and processed.
Scintillator: detector that is used for PET and SPECT, which, when hit by a high-energy particle, is excited and sends out its energy as light.
Tomography: imaging technique that generates 2D cross-sections of a 3D object in a non-destructive way. The object is visualized by means of a penetrating wave.
Voxel: the 3D counterpart of a pixel.

Box 1. A Brief Introduction to PET

PET is a non-invasive and *in vivo* functional imaging technique that, when applied with plants, produces a 3D image of transport and distribution in the vascular tissues [25,39,80]. PET is based on coincident detection of antiparallel γ-rays (180°) with an energy of 511 keV, produced by the **annihilation** of an electron (e^-) and a positron (β^+), the latter being emitted by a short-lived radionuclide tracer upon decay (Figure 1A) [21,81]. The most important short-lived radionuclides used in biological studies are ^{11}C, ^{13}N, ^{15}O, and ^{18}F (Table 1) [21,25,80]. These isotopes have a short half-life ($t_{1/2}$), which makes them ideal for studying short-term processes (minutes to hours) because of their high signal output during the short time-period they exist. The downside is that positrons travel only a small distance before they annihilate (Table 1 and Figure 1A), which fundamentally limits the maximum attainable resolution of PET [25,39,49,82,83].

Scintillators detect γ-rays, formed during annihilation, and the signal is enhanced by **photomultipliers** (Figure 1A). Typically, a trade-off between resolution (~1 mm) and sensitivity (~1%) is set by specifying the acceptance angle for opposite scintillators and defining the time-interval in which coincident events may occur, typically <20 ns [39,80,84]. A smaller acceptance angle or shorter time-interval increases resolution but decreases sensitivity.

In leaves, many decay events occur close to the surface, which results in a large fraction (up to 60%) that escapes into the atmosphere before annihilating [85]. This undetected fraction can be strongly reduced by placing radiation shields made of plastic or aluminium foil, but interference with plant physiology (e.g., transpiration or light interception) should be accounted for [85].

To generate an accurate image, data handling is required – including corrections for scatter and random events, attenuation, detector efficiency, geometry, and system dead-time [80,86]. Furthermore, the exponentially decreasing tracer activity needs to be decay-corrected, which increases the noise with time, and limits scanning time to about 7–9 half-lives (Table 1) [25,50]. A PET scanner only detects a line of response (connecting opposing scintillators) where the annihilation took place, and during data handling the signal is spread with a software-based probability distribution over several pixels. New developments are underway that will further improve the resolution (to sub-mm resolution) and quantitative accuracy [83,87].

may contribute significantly [25,36–43] (Figure 2). This short-lived isotope can be widely used because of the presence of carbon in many plant molecules. The most straightforward way to make use of ^{11}C is to feed gaseous $^{11}CO_2$ to a leaf. Uptake of this primary substrate for photosynthesis will be incorporated into photoassimilates, and the distribution of the ^{11}C-labeled sugars can be imaged with PET for several hours (Box 1). PET scanning has been used to examine the dynamics of carbon distribution in roots and fruits. Roots, that are otherwise difficult to investigate, can be imaged with PET [41], and this permitted clear analysis of active root tips [44]. In storage organs, including roots and fruits, sectoriality has been observed, with specific leaves supplying assimilates to specific parts of the organ [38,39]. Consecutive PET measurements on these storage organs can map the active zones as well as the distribution of assimilates, which is imperative for us to better understand the growth and development of such important structures [38–40]. Carbon transport and its sectoriality can also be studied in stems with PET [42]. Blockage and subsequent changes in the phloem transport pathway throughout the entire tree following girdling were visualized with PET, demonstrating that, already after 1 day, new paths were used to bypass the interrupted phloem vessels [42].

As an alternative to $^{11}CO_2$, sugar transport can also be traced with ^{18}FDG (Figure 2). Similarly to its use in the medical world, where ^{18}FDG serves as a surrogate for glucose to identify cancer cells, it might help in visualizing plant sugar metabolism using PET. Autoradiographic imaging (**autoradiography**) has shown that plants can uptake ^{18}FDG. For administration of this tracer, it is necessary to damage the plant surface (Figure 2), after which it is transported and incorporated into secondary metabolites. Because intact ^{18}FDG is transported, questions arise whether it really mimics actual sugar transport [45], and this might explain why ^{18}FDG has not yet found wide application in plant research [46].

^{11}C Has Already Proven Its Importance in Plant Sciences

Phloem vessels are known to be notoriously difficult to investigate because they operate under high pressures [47]. They are highly sensitive to any perturbation, resulting in immediate blockage when they are damaged [7,47]. An important merit of ^{11}C studies is the ability to

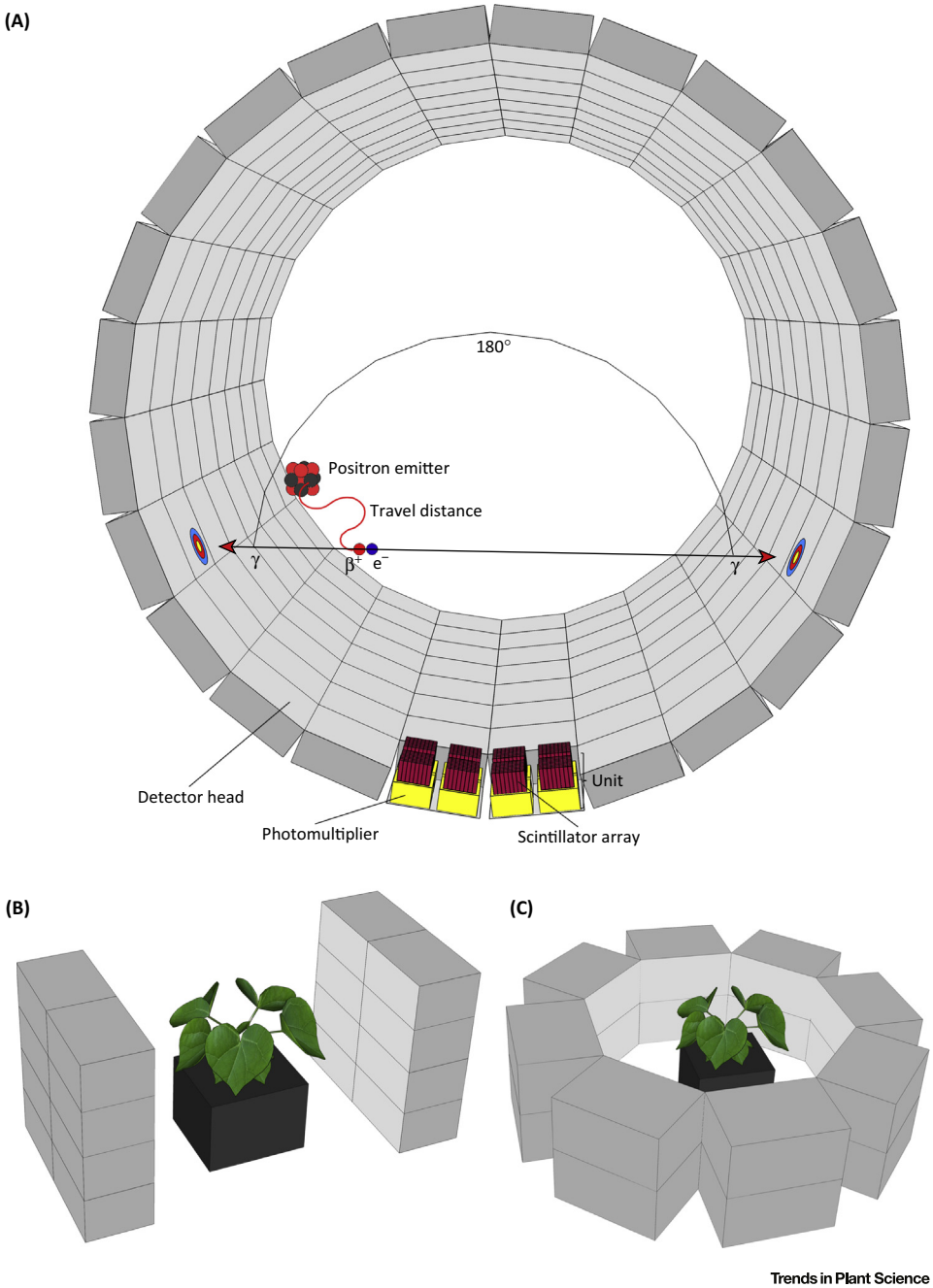

Figure 1. Positron Emission Tomography (PET) Detection Systems. (A) The most commonly used clinical PET scanner configuration. A detection ring consists of many detectors with several units. Each unit has an array of scintillators connected to a photomultiplier. Inside the cylinder annihilation takes place. A decaying radionuclide emits a positron (β^+), which meets an electron (e^-) within a short travel distance, leading to annihilation that results in the back-to-back (180°) emission of two γ-rays with an energy of 511 keV. Both γ-rays can be detected by scintillators within a specified time-interval, and are registered as a coincident signal. Non-coincident signals are rejected, making the use of collimators redundant. Other arrangements of PET modules are possible, and (B) planar and (C) circular configurations represent the most promising developments for plant-PET applications [43].

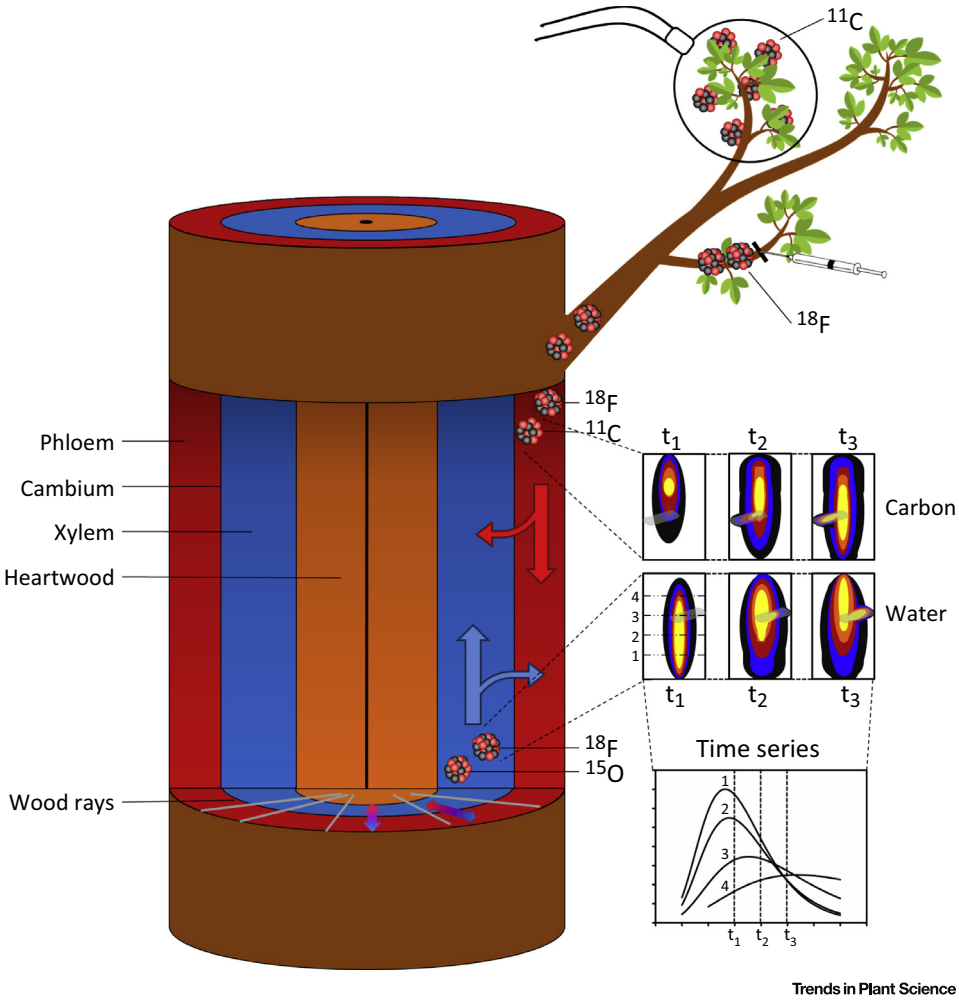

Figure 2. Schematic of Transport Processes in Xylem and Phloem Which Can Be Mapped with Positron Emission Tomography (PET). In general, carbon is transported downward in the phloem and water is transported upward in the xylem. Lateral exchanges of both water and carbon take place between phloem and xylem. Transport of water can be imaged by supplying a solution of water containing ^{15}O ($H_2{}^{15}O$) or ^{18}F. Carbon transport can be visualized by exposing leaves to airborne ^{11}C ($^{11}CO_2$) or by administering a solution with ^{18}F-fluorodeoxyglucose (^{18}FDG) through an incision. Subsequent transport can be monitored with PET, generating dynamic tracer profiles from which time-series can be derived and transport characteristics determined. Grey zones in the tracer profiles represent wood rays.

visualize sugar flow *in vivo* without damaging the phloem or altering its characteristics, which is essential to unravel phloem transport in living plants. In the past, Minchin and coworkers have extensively contributed to the current understanding of phloem transport by their ^{11}C experiments [21,25]. Using collimated detectors, their most pioneering discovery was the leakage and retrieval of carbohydrates along the phloem pathway [21,25,48]. This leakage–retrieval mechanism is now considered to be of great importance for plants in supplying intermediate cells between sources and sinks with sugars, and for maintaining the pressure gradient driving phloem transport [15].

Although many interesting phloem features have been discovered with collimated detectors, PET might further our knowledge as a more complete picture of phloem transport and its characteristics is acquired. The major breakthrough of PET technology was the use of coincidence detection (Box 1), which made **collimators** redundant and enabled 3D imaging.

Table 1. Overview of the Most Commonly Used Short-Lived Positron-Emitting Radionuclides in Biological Research [82]

Radionuclide	$t_{1/2}$ (min)[a]	Mean travel distance in water (mm)[b]
^{11}C	20.4	1.1
^{13}N	9.96	1.5
^{15}O	2.03	2.5
^{18}F	109.8	0.6

[a]Half-life of the radionuclides.
[b]Distance which the emitted positron travels on average in water before annihilating with an electron. This is an important factor in determining the resolution of PET.

Whereas a collimated detector dynamically registers single radiation intensities from an entire field of view (FOV), PET generates a complete 3D image with **voxel**-based intensities across the FOV, which can be a leaf, a fruit, a root, or a stem segment. Furthermore, the flexibility offered by PET to define regions of interest or to place virtual detectors (similar to collimated detectors, but software-based) in retrospect makes it possible to analyze the data with respect to how the tracer has been transported throughout the plant, which in turn feeds into model calculations of phloem transport velocities, exchange, and unloading rates [36–39,42,49].

Quantitative data from PET measurements can be obtained by applying one of several mathematical frameworks [49–51]. Initially, ^{11}C tracer profiles obtained with several collimated detectors were analyzed with the input–output framework developed by Minchin and coworkers [21,25,48,50,52]. Although this framework has been widely and successfully used, including for PET data [36], it is not entirely uncontested and has been criticized because the analysis is considered to be data-driven, and does not restrict model outcomes with physical boundaries or pose realistic ranges of solute transport characteristics [49,51]. To tackle some of these issues, mechanistic compartmental models have been developed to analyze PET tracer profiles [49,51]. These models simulate long-distance transport and exchange of solutes within a chosen number of compartments, which may include phloem conduits, tissue where solute from the conduit is exchanged, and tissue where solute is immobilized and stored [49,51]. By optimizing the parameters, these models output translocation velocities and tracer exchange between compartments, and their modular design makes it possible to readily merge with other existing mechanistic plant models [29,53]. When merged, data on sugar transport velocity and lateral exchange rates can be linked to other data, such as measured stem diameter changes and sap flux densities, to develop integrative water and carbon relationships [11,14,53–55], which will advance our understanding of xylem–phloem interactions.

An important advantage of working with short-lived isotopes is the ability to pulse repeatedly the same individual, for which the plant then serves as its own control. Because of the short half-life (Table 1), tracer levels are reduced swiftly, enabling multiple measurements per day. Because short-lived isotopes do not need destructive harvesting of the plant, and there is no build-up of tracer from previous pulses [21], including no radioactive waste, a sequence of independent pulses can be used to investigate *in vivo* the effects of a given treatment in the same individual, significantly reducing the number of replicates needed and improving experimental consistency. With this feature, variability in phloem transport within a single individual, between individuals, and between species can be thoroughly examined with plant-PET both on a spatial and a temporal scale.

Combining Imaging Techniques To See the Whole Picture

MRI (magnetic resonance imaging) scans provide a high-resolution (up to 30 μm^3 per voxel) anatomical and structural background, and allow quantification of water flow in both xylem and

phloem. However, the low phloem water flow is difficult to detect and, in contrast to PET, it does not represent the amount of sugar that is transported with the water [32,34,39]. With the combination of ^{11}C-PET and MRI, carbon flow can be displayed on a highly detailed structural background, while imaging the water flows [39,42], which will assist in unraveling the contribution of water and carbon to both xylem and phloem flow, as well as their interaction [32]. The hypothesis of carbon-assisted embolism repair could, for instance, be tested in a set-up where cavitation in xylem vessels is scanned with MRI [34,56,57], carbon transport from inner bark to sapwood is shown by PET, and the subsequent water refilling of embolized vessels is again displayed by MRI.

SPECT (single photon emission computed tomography) might be another interesting option to visualize xylem and phloem functioning in intact plants. While its technical applicability has been proven in a few plant studies with ^{99m}Tc as a proxy for water transport [58,59], no plant physiological insights have yet been derived from SPECT because most biological molecules of interest are covered by PET (Box 1) whereas SPECT employs non-biological tracers [58–60]. Nevertheless, SPECT has potential in plant research because, by using heavier γ-ray emitting atoms, there is no loss of resolution resulting from positron travel distance as there is in PET (Box 1) [60]. However, the overall sensitivity of SPECT is lower than for PET (two to three orders of magnitude) because shielded collimators are used instead of coincidence detection [60]. Today, higher resolutions than PET can be achieved with new collimators such as multi-pinhole SPECT [61] or clustered-pinhole SPECT [62]. The energy levels in SPECT are lower than for PET (140 keV for ^{99m}Tc compared to 511 keV for positron emitters), but both are sufficiently high to penetrate thick wood samples [63]. Recent developments also make it possible to use positron emitters (for instance ^{11}C) with SPECT detectors, resulting in improved resolution for a limited FOV [62]. While it is technically possible to Image ^{99}Tc-water and ^{11}C-carbon flow in plants with SPECT, the technique is still in its infancy, but further developments in this area are to be expected in the future.

Opportunities in Plant Research Remain To Be Seized

Phloem function and transport have been, and still are [9], an important subject of debate, and while ongoing research provides new insights on phloem traits and its operating principles, it lacks visualization of the fate of carbon inside plants (e.g., [7,10,15,21,53,55,64–67]). To improve our knowledge about the ecological implications of xylem and phloem function, as well as their interactions, several missing links need to be deciphered. Plant-PET may play an important role in this quest, in particular when quantitative analysis of PET data is pursued. To date, PET scanners are not readily available for plant scientists, but plant researchers can profit from the advances that have been made in medical imaging, and can use, as temporary solution, clinical PET scanners [68]. Access to custom-built, plant-dedicated PET scanners, with a configuration that is better suited for plants than the horizontal orientated medical scanners, is expected to rapidly develop (Figure 1B,C).

To quantify the overall carbon budget of plants, a better understanding of both short- and long-term carbon mechanisms is needed. Longer-term dispersal of carbon within plants can be determined with the long-lived radioactive isotope of carbon, ^{14}C ($t_{1/2}$ = 5730 years), or with the stable isotope ^{13}C [16]. Both methods give information on carbon sinks, fractionation, or remobilization of carbon reserves, but they require destructive harvesting of the plant (where ^{11}C-PET does not), and build-up of the tracer prevents independent repeated measurements on the same subject [21]. The short-lived radioactive isotope ^{11}C does not have these issues, making this tracer preferable over its longer-lived counterparts to unravel short-term carbon mechanisms. For instance, a recent study on the fate of xylem-transported CO_2 at leaf level showed a more detailed spatial distribution of CO_2 within a single tissue, which facilitated more accurate quantification of differences between different leaf tissues when using

^{11}C- compared with ^{13}C-labeled CO_2 [69]. In addition, compartmental analysis of PET data enables quantification of the allocation of carbon to different storage compartments (Figure 2). Carbon allocation to storage is currently the focus of an intense debate because open questions remain about whether it is a passive overflow system or is actively regulated, and how it relates to tree mortality [13,70,71]. It is hypothesized that stored carbohydrates are not solely used as reserves, but that they also play a role in maintaining plant hydraulic functioning during drought, which may create an incentive to actively regulate carbohydrate storage in a given environment [13]. Another challenge in understanding carbon allocation is to quantify the contribution of woody tissue photosynthesis to stem growth [11,72]. Lateral phloem solute leakage, which can be quantified from PET data, might differ between stems with and without woody tissue photosynthesis. Stems with a reduced or absent local production of sugars might have an increased carbon supply from the leaves through the phloem. In addition, the function of wood rays and their role in xylem–phloem coupling [73] could be assessed using plant-PET because radial transport through these rays occurs over distances which can be directly visualized by PET (Figure 2).

Driven by medical impetus, ongoing technical advances continue to improve the versatility, compactness, and resolution of PET scanners [74–77], of which plant-PET could fruitfully make use in future applications. Plant-PET scanners with modules allowing different configurations (Figure 1B,C) are a first step in making the scanners more mobile and applicable to measure plants, even when they have larger dimensions (e.g., [42,43]). Medical mobile PET systems, that have been configured to fit around the head of a small laboratory animal, could easily be mounted on a branch or small stem segment [74], and portable MRI systems, dedicated to plant research, have recently been developed [78,79]. These are all advances that bring us a step closer to measuring plants in their natural environment.

Concluding Remarks and Future Perspectives

PET technology and other medical imaging techniques present promising avenues to decipher a broad range of unknown or poorly understood vital plant mechanisms and functions. Despite its huge potential, there have been few plant-PET studies to date. Nevertheless, studies using clinical PET scanners or custom-made devices have clearly demonstrated their applicability and usefulness, but have mainly been restricted to short-term qualitative analyses. Quantitative analysis of plant-PET scans may reinforce integrated studies that link water and carbon dynamics, and their interplay with growth. Simultaneous observation, visualization, and analysis of the dynamic behavior of water and sugar movement in intact plants is one of the greatest challenges in modern plant science, and represent an untapped resource that will deepen our current and future fundamental understanding of the coordination of water and sugar flow in plants, and its impact on plant development, environmental suitability, and yield.

Outstanding Questions

Can PET help us to understand the coordination between xylem and phloem flow?

Could PET and ^{11}C advance our understanding of the fate of xylem-transported CO_2 in plants growing in a given environment?

Can PET be applied to study climate change-related effects (elevated CO_2, elevated temperature, drought) on phloem loading, phloem flow, and cavitation repair?

Can plant-dedicated PET scanners find their way out of the laboratory into growth chambers where plant responses can be studied under more natural conditions?

How quickly can compartmental models be developed to facilitate analysis of plant-PET data?

Is the plant science community ready to embrace PET as functional imaging technique in a wide field of applications, including high-throughput systems? PET can be used to study not only xylem–phloem functioning, but also topics related to herbivory, plant hormone function, and phenotyping.

Will PET be used principally as a separate technique or will it be used in combination with other technologies such as MRI or SPECT?

The development of smaller and more-mobile medical imaging systems is rapidly advancing, but will efforts also be directed towards systems for application in plant science?

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