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ABSTRACT

The first research on the transport of metastable-technetium-99 (^{99m}Tc) in the form of pertechnetate ($^{99m}TcO_4^-$) within plants suggested that $^{99m}TcO_4^-$ may be mobile in the phloem. In contrast, more recent evidence indicates the anion is transported in the xylem. Here we demonstrate that observations of ^{99m}Tc transport in the test subject of these initial investigations, the moonflower (*Ipomoea alba* L.), are incompatible with phloem flow. Rather, the presence of only minute amounts of ^{99m}Tc in typical sinks for phloem solutes and ^{99m}Tc transport out of labeled leaves when shaded but not when illuminated strongly suggest that the radionuclide is transported in the xylem. The study increases confidence in the identification of $^{99m}TcO_4^-$ as a xylem mobile compound whose distribution in plants can be visualized using nuclear medicine scintigraphic imaging techniques.

Keywords: radiotracer, gamma, scintigraphy, vessel, apoplast, Convolvulaceae

1. Introduction

Research on technetium (Tc) in plants has focused on the bioaccumulation of the radionuclide in the context of radioactive pollution that results primarily from nuclear weapons and power generation (Luykk, 1984). In contrast to clinical, pre-clinical and veterinary nuclear medicine research, in the plant sciences there has been very little exploration of the potential for metastable technetium-99 (^{99m}Tc) to serve as an radiotracer for the study of physiological processes (Desmet, 1984). One impediment in this area of research is the lack of a clear understanding of how common forms of Tc, such as pertechnetate (TcO_4^-) and Tc-complexes, are transported in the xylem and phloem of plants.

The focus of this research is ^{99m}Tc transport in the moonflower, *Ipomoea alba* L. This species was chosen because earlier research suggested that it may transport Tc, introduced to the plant in the form of $^{99m}TcO_4^-$, in the phloem. Thirty-five years ago Pickard and Hill (1975) published the first and one of the few papers to explore the usefulness of ^{99m}Tc as a radiotracer in plants. These authors suggested that ^{99m}Tc transport was not driven by the mechanisms responsible for coherent flow in the xylem (hydrostatic pressure driven by processes such as transpiration and osmosis). Rather, they cautiously interpreted their results based on the assumption that the radionuclide was transported in the phloem but could be easily transferred to the apoplast (cell walls and intercellular spaces including xylem vessels and tracheids). This interpretation contrasts with subsequent evidence for the presence of $^{99m}TcO_4^-$ in the xylem sap of soybean (Cataldo et al., 1978) and tomato (Krijger et al., 1999a) plants. The interpretation also contrasts with the inability of $^{99m}TcO_4^-$ to accumulate in grape berries during ripening

(Currie et al., 2010, Fig. 1), one of many reproductive organs whose development includes a phase dominated by vascular inputs via the phloem (Wang et al., 2000; Rogiers et al., 2006). The present work therefore presents the opportunity to assess whether Tc transport in the moonflower is atypical. If this is not the case then observations of Tc transport in the moonflower will consolidate our understanding of the vascular transport of this radionuclide in plants.

Given the equivocal evidence for the phloem mobility of Tc in *Ipomoea* the aim of this paper is to improve understanding of the long-distance transport of ^{99m}Tc, introduced as $^{99m}TcO_4^-$, in this particular species. Our research is focused on ^{99m}Tc, which emits gamma radiation with a six hour half-life, but is equally applicable to radionuclides of Tc with longer half-lives such as the pollutant, ^{99}Tc. Because gamma rays have an extremely short wavelength they are highly penetrating, especially with respect to thin plant tissues such as leaves. As demonstrated by Krijger *et al.* (1999b) with experiments on tomato plants, these properties make it possible to quantitatively detect ^{99m}Tc distributions and concentrations in plant tissues, despite their complex geometry, using scintigraphic techniques. Our scintigraphic images of *Ipomoea* plants demonstrate that ^{99m}Tc transport from labeled leaves takes place when these leaves are shaded and identify the sinks for this translocation as other mature leaves. These observations and the presence of trace amounts of ^{99m}Tc in developing pods and immature leaves provide strong evidence that the radionuclide is xylem mobile in *Ipomoea*.

2. Materials and methods

2.1. Radionuclide

$^{99m}TcO_4^-$, eluted from a molybdenum-99 generator using 15 mmol L^{-1} NaCl, was provided by a local medical imaging clinic. Doses of the desired radioactivity were obtained by assaying the labeled saline solution in a dose calibrator (Atomlab 100, Biodex Medical Systems, New York).

2.2. Moonflower plants

Plants used in these experiments were *I. alba* grown from directly sown seeds (The Digger's Club, Dromana, Victoria, Australia). Plants were raised in a commercial potting mix in a 2.6 L pot, drip irrigated three times daily and grew as a single shoot trained up a vertical cane placed in the potting mix at the time of seed germination. The plants were maintained in a glasshouse (25/23 °C day/night) under natural lighting (decreasing night length) and transferred to the laboratory for labeling and imaging the day prior to experimentation. Plants were approximately 75 – 100 days old at the time of imaging and many bore reproductive organs at developmental stages ranging from immature floral buds to developing seed capsules.

2.3. Labeling of individual leaves

Six individual, fully-expanded *Ipomoea* leaves (one leaf per plant) were labeled with $^{99m}TcO_4^-$ by lightly abrading both sides of a leaf with sandpaper, immersing the leaf in a Petri dish containing 1 GBq of $^{99m}TcO_4^-$ for 60 min and air-drying the leaf for 60 min. Leaf abrasion was chosen as a treatment because it allows entry

of phloem-mobile solutes into leaves (Grignon et al., 1989). Each plant was then illuminated with a 100W lamp for six hours. Three of these labeled leaves were covered in aluminium foil and three were left uncovered. The experiment was repeated with another six leaves (three covered and uncovered) omitting the leaf abrasion.

2.4. Labeling the soil of potted plants

The soil of eight potted *Ipomoea* plants was labeled with 1 GBq of $^{99m}TcO_4^-$. The plants were then illuminated with 100 W lamps for four to six hours. The pots were shielded with lead prior to scintigraphic imaging.

2.5. Labeling the stem

The following methods are adapted with minor modifications from Pickard and Hill (1975). Two days prior to labeling, leaves and fruit were removed from the bottom 50 cm of *Ipomoea* shoots that were approximately 80 cm long. To label with $^{99m}TcO_4^-$, the shoot was laid prostrate and, while immersing a portion in deionised water in a Petri dish, a longitudinal slit approximately 25 mm long was cut either in the internode immediately below the lowest remaining leaf or the internode between two remnant leaves. 500 MBq of $^{99m}TcO_4^-$was then added to the deionised water and the labeled shoot was illuminated with three 100W lamps for 60 min. Plants were then positioned on the imaging window of a gamma camera and scintigraphic imaging of the plant approximately 15 cm below the loading point began either while these lights were on (n = 2) or immediately after they were turned off (n = 4). Lead shielding was used to minimise scatter from the

labeling pool to the camera head. After approximately 20 min of imaging in darkness dry ice was applied to the stem acropetal of the labeling point. A further 20 min later dry ice was applied to the stem basipetal of the labeling point and scintigraphic imaging was terminated 20 min later.

2.6. Scintigraphic image acquisition and processing

Scintigraphic images were acquired using a gamma camera (Philips Prism 1000, Picker, Cleveland) with high resolution parallel hole collimation (photopeak centred on 140 keV ± 15%, 1 min acquisition and 128 × 128 pixel matrix for the prostrate shoot experiment and a 5 min acquisition and 256 × 256 pixel matrix for all other images). Images displaying total acquisition counts were processed using a computer (Philips Odyssey VP, Picker, Cleveland) by truncating the count range to span that typically observed in labeled leaves (up to ca. 500 counts). The greyscale range of the image was adjusted to enhance the contrast between labeled plant parts and the image background. Truncated images of suitable contrast were exported after applying a colour palette and areas of radionuclide localisation were confirmed by digitally merging (Merge ver 2.0, www.graphicutils.com) these with a digital photograph taken of the plant during scintigraphic acquisition. Count data reported correspond to images prior to truncation.

3. Results

3.1. Translocation resulting from labeling of individual leaves

All individual leaves directly labeled with $^{99m}TcO_4^-$ exhibited high concentrations of activity when imaged scintigraphically. Stems and other leaves did not appear

in the scintigraphic images when the abrasion treatment of the labeled leaf was not implemented (Fig. 1). Sap exuded from these leaves using a Scholander pressure chamber and collected on absorbent paper did not exhibit counts distinguishable from background when imaged scintigraphically (results not shown). This suggests the radionuclide was not present in the xylem sap of non-abraded leaves. With one exception counts indiscernible from background were present in shoots whose abraded leaf remained uncovered after labeling. The exception was a plant whose labeled leaf wilted, presumably due to damage caused by the leaf abrasion treatment or by heat generated by the artificial light sources. Of the plants with abraded and foil-covered labeled leaves, the first to be imaged exhibited radioactivity extending to the proximal end of its petiole. The shoots of the next two plants exhibited extensive labeling acropetal of the labeled leaf, as well as minor labelling basipetal (Fig. 2). In these experiments the sinks for the translocated ^{99m}Tc were primarily other fully-expanded leaves but young leaves on shoot tips were also labeled by translocated radionuclide. ^{99m}Tc was not present in developing flowers.

3.2. Pertechnetate uptake from labeled soil

When $^{99m}TcO_4^-$ was added to the soil of potted *Ipomoea* plants nearly all above-ground plant parts were labeled with ^{99m}Tc (e.g. Fig. 3). The relative intensity of ^{99m}Tc labeling in plant organs generally conformed to the trend: well-lit leaves > other leaves > corollas and mature floral buds > immature floral buds and seed capsules (Table 1).

3.3. Translocation resulting from labeling the stem

Of our six attempts to replicate the typical trend reported by Pickard and Hill (1975, their Fig. 1) we observed basipetal transport of radiolabel on three occasions. Two of these plants, both of which were imaged in darkness, exhibited radioactivity basipetal of the loading point when imaging began and either a decrease or no change in counts in this region during imaging. In these plants the basipetal transport stopped abruptly at or 2 cm below the node of an excised leaf, respectively and we conclude that the basipetal transport occurred while the shoots were illuminated, prior to scintigraphic imaging. A third plant was imaged in the light, then darkness, and showed no basipetal transport of activity until the acropetal dry ice treatment was applied. This treatment resulted in the immediate and rapid basipetal transport of activity that was halted with the application of the basipetal dry ice treatment (Fig. 4).

4. Discussion

As observed in other plant species (e.g. Cataldo et al., 1984; Myttenaere et al., 1984), ^{99m}Tc from $^{99m}TcO_4^-$ labeled soil was rapidly transported to the leaves of *Ipomoea*. This indicates that $^{99m}TcO_4^-$ is highly bioavailable for uptake by *Ipomoea* roots in well-drained soil substrates such as commercial potting mix. This observation is in agreement with the high bioavailability of TcO_4^- in field soils under aerobic conditions (Yanagisawa and Muramatsu, 1995).

In this study, translocation of ^{99m}Tc from labeled leaves was highly sensitive to covering leaves with foil. This treatment is expected to reduce transpiration by removing light cues for opening stomatal guard cells (Sharkey and Raschke,

1981) and increasing the resistance of the leaf boundary layer to water vapour diffusion (Monteith, 1965). Because transpiration is the primary mechanism for generating tension in the xylem of plants (Canny, 1995), the covering is expected to have the effect of reducing hydrostatic tension generated by the labeled leaf. The presence of technetium in other leaves suggests their transpiration is sufficient to generate the tension necessary for xylem flow out of and away from the labeled leaf. Uncovered leaves also transported ^{99m}Tc to other plant parts but only when the labeled leaf wilted. We suggest that the absence of turgid cells makes the leaf water (and solutes dissolved in it) susceptible to backflow through the xylem. Under both conditions the ^{99m}Tc is redistributed to other sinks for xylem sap, particularly transpiring leaves.

The major sinks for ^{99m}Tc translocated from labeled *Ipomoea* leaves were other fully-expanded leaves. This observation is not consistent with typical models of long-distance phloem transport in which rapidly growing, immature organs are terminal sites in the transport pathway (Canny, 1973). Pickard *et al.* (1978) demonstrated that the phloem export of recently acquired photosynthate from illuminated *Ipomoea* leaves should be detectable within minutes after labeling. These authors also demonstrated that shading reduces the phloem export of carbon from source leaves and the transport of carbon to the growing tip (Pickard et al., 1978). The sinks for ^{99m}Tc transported from labeled leaves identified here are therefore incompatible with phloem transport. Conversely, they are consistent with ^{99m}Tc being transported according to hydrostatic pressure gradients in the xylem.

The study of dynamic ^{99m}Tc transport in *Ipomoea* shoots lead Pickard and Hill (1975) to equivocally conclude that this species transported the radionuclide in the phloem. Following their methods, upon the application of dry ice we were able to reproduce the basipetal transport typical of their experiments (Fig. 4). We suggest that interpreting the results of such experiments in terms of normal vascular transport processes is erroneous. This is because the dry ice effectively isolates the labeling point from major sources and sinks for translocated solutes. Instead, the observations provide insights into how xylem transport processes respond to drastic perturbations. Following the evidence for xylem transport of $^{99m}TcO_4^-$ reported here, we suggest that basipetal transport such as that depicted in Figure 4 reflects the loss of tension in xylem vessels (or tracheids). These conduits transported ^{99m}Tc acropetally until the dry ice froze the xylem sap, eliminating the hydrostatic connection between the acropetal and basipetal portions of the plant. With the hydrostatic tension reduced, the transpiration stream is able to retract basipetally until it is halted by the application of dry ice between the labeling point and detector field. Thus, despite the atypical conditions, such observations are nonetheless explicable in terms of the transport of ^{99m}Tc as a solute in the xylem of *Ipomoea*.

The present research demonstrates that Tc introduced to leaves as $^{99m}TcO_4^-$ can be translocated in the xylem from leaves to other organs. This is in agreement with research that indicates that TcO_4^- remains highly soluble and complexes with few metabolites (and therefore remains highly mobile) within the first 24 hours of leaf labeling (Myttenaere et al., 1980; Lembrechts and Desmet, 1984; Neel and Onasch, 1989). When combined with the versatility with which plant organs can be labeled with ^{99m}Tc (Currie et al., 2010), this rapid transport and the limited

biological fixation within the first four half-lives of the radionuclide make the scintigraphic detection of ^{99m}Tc a promising method for tracing pathways of xylem transport within plants.

5. Conclusions

Our observations of ^{99m}Tc transport in *Ipomoea* plants are typical: the patterns of (a) the ^{99m}Tc uptake and accumulation from $^{99m}TcO_4^-$ labeled soil and (b) translocation from $^{99m}TcO_4^-$ labeled leaves are consistent with xylem transport of this radionuclide. In particular, ^{99m}Tc primarily accumulated in fully expanded leaves and was not exported from labeled leaves unless they were shaded or wilted. These observations increase confidence in the use of ^{99m}Tc in the form of TcO_4^- as a radionuclide for tracing vascular solute transport in plants. The research also implies that Tc should be considered a xylem-mobile element in radionuclide bioaccumulation models.

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Figure legends

Fig. 1. Scintigraphic (a) and photographic (b) images of an *Ipomoea* plant after labeling an unabraded leaf with $^{99m}TcO_4^-$. The labeled leaf is indicated in (b). Prior to imaging the leaf was wrapped in aluminum foil and the plant was illuminated with an artificial light source for six hours. The star-shaped distribution of apparent counts surrounding the labeled leaf in (a) is a common scintigraphy artifact resulting from a localized area of high counts and truncation of the count range displayed.

Fig. 2. Scintigraphic (a) and photographic (b) images of an *Ipomoea* plant after labeling an abraded leaf with $^{99m}TcO_4^-$. The labeled leaf is indicated in (b). Prior to imaging the leaf was wrapped in aluminum foil and the plant was illuminated with an artificial light source for six hours. The numbers in (a) indicate counts per pixel per acquisition. Trace amounts of radioactivity were discernible in the leaf immediately below the labeled leaf and the stem and immature leaves at the shoot apex.

Fig. 3. Scintigraphic (a) and photographic (b) images of an *Ipomoea* plant labeled with ^{99m}Tc after the addition of $^{99m}TcO_4^-$ to the potting mix of the plant. Numbers in (a) indicate counts per pixel per acquisition. The number in the top right corner of (a) corresponds to the two overlapping leaves seen in (b). The arrow and associated ratio in (a) refers to the seed capsule apparent in (b).

Fig. 4. Temporal trend in the accumulation of ^{99m}Tc in the stem of an *Ipomoea* plant basipetal of a loading point following the methods of Pickard and Hill (1975). Counts were acquired in 1 minute intervals. As indicated by the labels, data acquisition commenced while the plant was illuminated, the lights were then turned off, 22 minutes later a dry ice freezing block was applied acropetal of the loading point and a further 23 minutes later an additional freezing block was applied basipetal of the loading point.