Journal of Environmental Radioactivity 103 (2012) 54–58

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Long-distance transport of pertechnetate in the moonflower (*Ipomoea alba*)

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ARTICLE INFO

Article history:
Received 19 January 2011
Received in revised form
17 August 2011
Accepted 17 August 2011
Available online 25 September 2011

Keywords:
Radiotracer
Gamma
Scintigraphy
Vessel
Apoplast
Convolvulaceae

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.

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 a 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 6 h 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

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doi:10.1016/j.jenvrad.2011.08.012

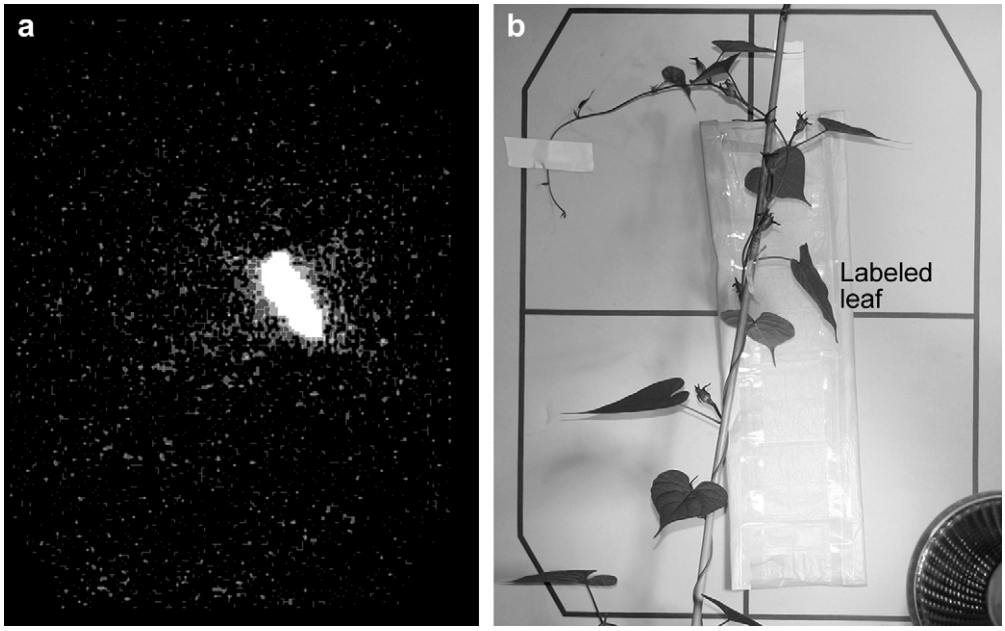

Fig. 1. Scintigraphic (a) and photographic (b) images of an *Ipomoea* plant after labeling an unabraded leaf with $^{99m}TcO_4^-$. The labeled leaf is indicated in (b). Prior to imaging the leaf was wrapped in aluminum foil and the plant was illuminated with an artificial light source for 6 h. The star-shaped 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.

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 100 W lamp for 6 h. Three of these labeled leaves were covered in aluminum 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 6 h. 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 deionized 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 deionized water and the labeled shoot was illuminated with three 100 W 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 minimize scatter from the