Available online at www.sciencedirect.com

ScienceDirect

Palaeoworld 25 (2016) 287–291

Palaeoworld

www.elsevier.com/locate/palwor

Plant cytoplasm preservation in a baked root of *Abies*

Hao-Xuan Shen [a,b], Kai-He Du [c], Xin Wang [a,b,*]

[a] *State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*
[c] *Jiangsu Key Laboratory for Supramolecular Medicinal Materials and Applications, College of Life Sciences, Nanjing Normal University, Nanjing 210046, China*

Received 12 June 2014; received in revised form 27 November 2014; accepted 10 November 2015
Available online 8 December 2015

Abstract

Plant cytoplasm was thought impossible to be preserved in fossils. This stereotype is now undermined by increasing reports of plant cytoplasm in fossils. Previous simulations of high temperature preservation for plant cytoplasm were performed at short time scales, leaving the effectiveness and durability of such preservation an open question. Here we attempt to investigate the long time effect of high temperature on plant cytoplasm preservation in a root of *Abies concolor* that was baked in the wild at least 8 years ago. Light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) indicate that cytoplasm and possible organelles such as mitochondria can be preserved in the baked tissue. The high frequency of wildfire in nature suggests that the potential for plant cytoplasm preservation in fossils is greater than commonly assumed. The good preservation of plant cytoplasm in the studied material after 8 years indicates that plant cytoplasm indeed can be preserved longer than previously proven.

Keywords: Cytoplasm; Fossilization; Fire; Mitochondria

1. Introduction

Cytoplasm of plant is commonly thought to be labile, since the hydrolysis starts destroying the cells after death (Hara-Nishimura and Hatsugai, 2011). Thus people tend to believe that cytoplasm cannot be preserved in fossils. However, research in the last decades has been undermining this assumption (Niklas et al., 1978; Niklas and Brown, 1981; Niklas, 1983; Niklas et al., 1985; Schoenhut et al., 2004; Wang, 2004, 2006, 2007; Wang and Cui, 2007; Wang et al., 2007, 2008, 2011). Recent palaeobotanical research indicates that high temperature may preserve cytoplasm in fossils, and some simulations have been performed to elucidate the preservation mechanism in laboratories (Edwards and Axe, 2004; Wang, 2007; Li et al., 2013). However, these experiments were done in man-made condition and completed in relatively short times. It remains to be an open question how long the preservation can last. To demonstrate the potential of cytoplasm preservation in the fossil record in a more natural circumstance, we studied the tissues in a root of *Abies concolor* that was baked in the wild at least eight years ago. Light microscopy, scanning electron microscopy, and transmission electron microscopy indicate that, at least 8 years after the baking, cytoplasm is still recognizable in plant tissue and some of its organelle like mitochondrion can be preserved as well. It is hoped that the results reported here could further expel the mystery about fossil cytoplasm and its ultrastructures, and demonstrate a greater potential for related studies.

2. Materials and methods

The studied material was collected from a tree of *Abies concolor* at La Porte (31°41′N, 120°59′W, California, USA) during a BSA (Botanical Society of America) post-conference field trip in August, 2006. The tree was in good health except its root was partially exposed due to road cutting. This exposed root became partially baked into dark color, probably due to a camping fire, and the material studied here was collected from the exposed

* Corresponding author at: State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China. Tel.: +86 25 83282266.
E-mail address: xinwang@nigpas.ac.cn (X. Wang).

http://dx.doi.org/10.1016/j.palwor.2015.11.007
1871-174X/

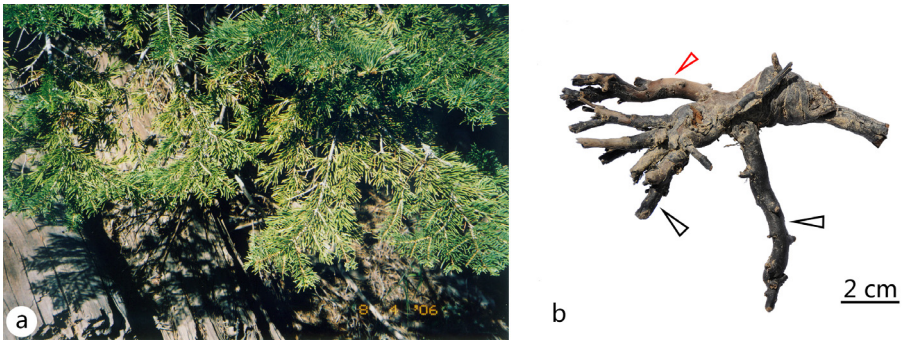

Fig. 1. *Abies concolor* and its partially baked root. (a) The tree of *Abies concolor*, picture taken in August, 2006. (b) General view of the collected material; note the baked part appears dark (black arrow), whereas the other part is brown (red arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

baked root. The specimen is housed in the Nanjing Institute of Geology and Palaeontology, CAS, Nanjing, China. Specimen number is PB22112.

The tree was photographed using an Olympus C765UZ digital camera (Fig. 1a). The sample has been kept intact in a newspaper package since 2006 and was not processed until this study. The sample was photographed by a Nikon D300S digital camera in May, 2014 (Fig. 1b). We sampled the baked epidermis and a rootlet. The materials were observed and photographed in detail using a Nikon SMZ1500 microscope with a DS-Fi1 camera (Figs. 2a, 3a). A piece of material was removed using a knife, purged using ethyl alcohol three times and each time for 10 min. The material was broken into fragments to expose anatomical details and coated with gold before SEM observation. SEM observation was performed using a Leo 1530 VP SEM at Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, China. The TEM sample was embedded in Epon 812 for ultrathin sections at Nanjing Normal University, Nanjing, China, according to the following procedure. The recipe for the 20-ml resin solution was 10.28-ml Epon 812, 1.24-ml DDSA, 8.48-ml MNA, and 0.34-ml DMP-30. The sample was immersed in 100% acetone for 3 min, in 50% and 100% resin solutions in acetone each for 1 h. Next, the sample was embedded in fresh, pure resin in a container and cured in a progressively warmer oven set at 30 °C, 45 °C, and 60 °C, each for 24 h. Then the cured block was trimmed and sectioned using a Leica Ultracut R ultramicrotome set at a 70-nm interval using a diamond knife. The ultrathin sections were stained with lead citrate at a concentration 1/60 of the standard solution. Observation was performed using a Hitachi-7650 TEM at Nanjing Normal University, Nanjing, China.

All results were recorded as digital images, saved in JPEG or TIFF format, processed, and then organized for publication using Photoshop CS6.

3. Results

The root of *Abies concolor* was exposed due to road cutting, and became partially baked, probably due to a camping fire. The collected root was 11 cm × 8 cm × 3 cm in size, with individual rootlets ranging from 0.2 to 2 cm in diameter (Fig. 1b). Some of the root surface was baked dark in color (black arrow in Fig. 1b), the other part appeared brown (red arrow in Fig. 1b). Preservation of cytoplasm varies in different tissues (Figs. 2c–d, 3b–f).

The baked rootlet was black to naked eye, about 3 mm long and 0.8 mm in diameter (Fig. 2a). Only the peripheral portion of the root was baked to dark color and cracked, whereas the inner part was intact and brown in color (Fig. 3a). When sampled, the tissue broke along the cambium, and the cortex and epidermis of the rootlet were removed for further electron microscope observation.

Under SEM, the epidermis and the cortex of the root were well preserved (Figs. 2b–d, 3b–c). The epidermal cells are visible from the surface (Fig. 2c–d) while the fusiform initials and ray initials were seen on inner face of the sample (Fig. 3b–c). The cells were round rectangular or polygonal in shape, up to about 40 μm long and about 16 μm wide. The cell wall in epidermis was about 0.7 μm thick, locally sandwiched between straps of cytoplasm that occupied the peripheral of the cell lumina (Fig. 2d). The cell wall of cortical parenchyma was about 3 μm thick, and they framed the lumina, in which the cytoplasm either occupied the whole volume or just the peripheral portion (Fig. 3b–c). The cell remnants in the cortex may fully fill the cell lumina (Fig. 2c). Sometimes anatomical structure like bordered pits may be seen on the cell wall of the tracheid in the cortex (Fig. 2c). The cell remnants in ray initials may be preserved but in a slightly shrunken state (Fig. 3b–c).

Under TEM, the cells in the cortical parenchyma were well preserved (Fig. 3d). The cells were polygonal in shape, up to about 33 μm long and about 14 μm wide (Fig. 3d). These cells in the same section might appear heterogeneous (Fig. 3d). Cell remnants appeared spongy and dark-stained, separated each other by about 3 μm thick light-colored cell walls (Fig. 3d–e). The cytoplasm only occupied the peripheral of the cell lumina and stuck to the cell wall that was about 0.8 μm thick in some cells, with empty central area suggestive of the presence of a vacuole (1–5 in Fig. 3d). But in other cells, the cytoplasm may fill up the cell lumina (6, 7 in Fig. 3d). Organelles similar to mitochondria were seen in some cells. The mitochondrion is enclosed by two layers of membranes, and there are numerous cristae delimited by membranes inside, either connected to the inner enclosing membrane of the mitochondrion or appearing isolated within the mitochondrion matrix (Fig. 3f).