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CITATION:

Kaida, Rumi ...[et al]. Intake of Radionuclides in the Trees of Fukushima Forests 5. Earthquake Could Have Caused an Increase in Xyloglucan in Trees. Forests 2020, 11(9): 966.

ISSUE DATE:

2020-09

URL:

http://hdl.handle.net/2433/260709

forests

MDPI

Communication

Intake of Radionuclides in the Trees of Fukushima Forests 5. Earthquake Could Have Caused an Increase in Xyloglucan in Trees †

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† This paper is dedicated to Peter Albersheim who died on 23 July 2017.

Received: 11 June 2020; Accepted: 31 August 2020; Published: 4 September 2020

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Abstract: A megathrust earthquake caused the Fukushima–Daiichi nuclear power plant accident, which dispersed abundant radioiodines, causing them to be bound to xyloglucan into forest trees. Nevertheless, targeted xyloglucan was found in increased quantities in the annual rings of forest trees affected by the earthquake. We propose that trees could acclimate rapidly to shaking stress through an increase in xyloglucan deposition as a plant response under natural phenomena.

Keywords: Fukushima disaster; megathrust earthquake; oak; xyloglucan deposition

1. Introduction

The earthquake and tsunami on 11 March 2011 led to a meltdown followed by a hydrogen explosion at the Fukushima–Daiichi nuclear power plant in Japan, causing the dispersal of abundant radionuclides into forests and local residences by gaseous or aerosol forms [1–4]. Radioiodine and radiocesium were likely incorporated and fixed in forest trees. In tree bodies, xyloglucan serves as a main absorber of radioiodine gas, while radiocesium, likely in the form of a potassium ion, moves into the growing regions and the heartwood via pit membranes [1–3]. Therefore, growing cells and young tissues could be targets for the deposition of radionuclides in trees. Xyloglucan is one of the major factors involved in wall loosening during plant cell elongation [5,6]; by this means, it reinforces wall structure during growth. Therefore, this polysaccharide is believed to occur as a primary constituent of primary cell walls [7–9], where it binds to cellulose microfibrils through hydrogen [10–12] and hydrophobic [13] bonds and also to pectin RG-1 (rhamnogalacturonan I) via galactan through covalent bonds [14,15]. We expect that shaking stress might trigger the re-organization of cell walls in the growing regions, whether xyloglucan signals can be detected in the growth rings of forest trees following megathrust earthquakes. This paper describes the effect of seismic stress on xyloglucan signals in forest trees.

Japan's islands are subject to numerous small earthquakes daily, as well as larger megathrust earthquakes occurring every 30 to 50 years, each of which is followed by numerous aftershocks. These earthquakes are due to the movement of the earth's tectonic plates, through which oceanic plates are subducting beneath continental plates just along Japan islands [16–18]. The Eurasian plate causes the

Pacific plate to be subducting along the east-side coast of Japan islands and the Philippine Sea plate to be descending along the southwest coast of Japan islands. This is why large inter-plate earthquakes occur along the plate boundaries off of the Pacific coast of the Japan islands. Furthermore, intraplate earthquakes within the continental plate take place in the upper crust beneath the Japan islands. Such contacts cause their ruptures, followed by great underthrust earthquakes. Since 90% of northeastern Japan is forested, trees in Japan must be able to withstand the seismic stress associated with earthquakes in addition to other external mechanical stresses such as wind and rain.

The 2011 earthquake off the Pacific coast of Tohoku, which struck northeastern Japan on 11 March 2011, had a magnitude of 9.0 and a JMA (Japan Meteorological Agency) seismic intensity of 7 (a modified Mercalli intensity of 9). It was followed within 15 days by more than 400 aftershocks of magnitude greater than 5.0 [19,20]. This megathrust earthquake was followed by a tsunami and a subsequent nuclear power plant accident. We are studying the effects of this cluster of disasters on forest trees, including the intake of radionuclides by forest trees. This unique opportunity may enable us to discover novel findings about plant science. Our present findings, for example, may cause a paradigm shift in our understanding of radioiodine-targeted xyloglucan.

2. Materials and Methods

2.1. Sampling Trees

A total of nine straight-standing oak (*Quercus aliena* L.) trees were cut in 2016 at the localities shown in Figure 1. Three trees sampled in Soma were obtained at 37°45′50″N/140°50′26″E and 37°45′51″N/140°50′23″E. Three trees sampled in Minamisoma were obtained at 37°38′14″N/140°54′20″E. Three trees sampled in Tokyo was obtained at 35°38′26″ N/139°37′53″E.

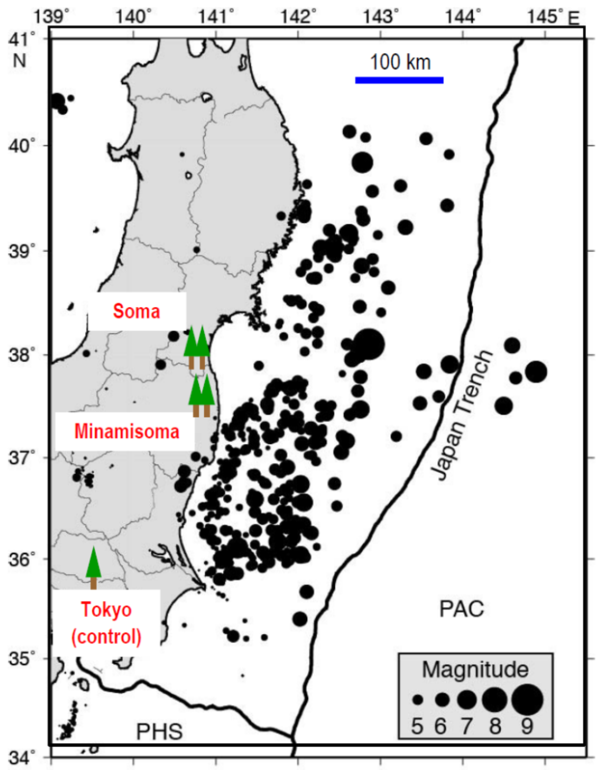

Figure 1. Three sites of tree sampling in Japan. Soma and Minamisoma were selected close to the hypocenter location of the mainshock and aftershocks exceeding a magnitude of 5 (black circles) within 24 h of the 11 March 2011 megathrust earthquake in northeast Japan. Tokyo was used as a control.

The oak trees in Soma and Tokyo were between 20 and 30 m tall (about 40 years old), whereas the oak trees sampled in Minamisoma were 15 to 21 m tall (about 15 years old). After falling, we obtained an approximately 2 cm thick cross section by cutting the tree transversely at mid-height. Analyses revealed that annual growth layers were formed in all trees without any obvious biases related to damage or climate; this observation held true for all radii and all sampling heights. Each cross section was transversely cut in two radial directions at right angles to obtain four radial sections from pith to cambium (1 cm thick), in which annual growth rings were clearly visible as parallel lines. Ring widths were measured for the period of 2000–2015, and were transformed into the average values of four sections by taking the mean of the radial section values.

The seismic intensity is given in the form of JMAII (Japan Meteorological Agency instrumental intensity) because it is hard to convert seismic intensity from JMAII values to MMI (modified Mercalli intensity) values. According to Kunugi [21], however, JMAII values can be roughly converted to MMI values using the TriNet method developed by Wald et al. [22] for California earthquakes.

2.2. Immuno-Staining of Stem Sections

Approximately 1 cm wide radial sections of oak xylem were sliced to obtain 2 mm thick cross-sections, which were soaked in 90% (*v*/*v*) aqueous acetone for 12 h. The defatted sections were bleached three times with 8% $NaClO_2$ in 1.5% acetic acid at 38 °C for 12 h and washed with water. The delignified sections were further digested with xylanase M6 (Megazyme) at 40 °C for 12 h and soaked with 2% KOH containing 0.1% $NaBH_4$ for 12 h. The sections were neutralized with acetic acid and washed with water. The thin sections were then incubated with a 20-fold dilution of the anti-xyloglucan antibody LM15 (Plant Probes) [23], followed by a second antibody anti-Rat immunoglobulin G attaching a high-sensitivity polymer with peroxidase (Vector), which was stained with peroxidase substrate (Vector).

3. Results and Discussion

3.1. Megathrust Earthquake and Aftershocks in Fukushima

To examine the effects of mechanical stresses on the xylem of trees in the field, we sampled trees in Soma and Minamisoma after the 2011 Tohoku megathrust earthquake; each location was a known distance from the earthquake's epicenter (Figure 1). The seismic intensities achieved by the megathrust earthquake were markedly high in Soma and Minamisoma compared with the lower intensities recorded in Tokyo (Table 1). Although the sampling sites in Soma and Minamisoma are only 20 km apart, they differed not only in the number of earthquakes experienced but also in the seismic intensity. In addition, the Minamisoma forest was also affected by the dispersal of high levels of radionuclides due to the accident at the Fukushima nuclear power plant, whereas the Soma forest was affected at a lower level by this accident. As shown in Table S1, the megathrust earthquake struck Soma in the form of one foreshock at a maximum JMA seismic intensity of more than 3 on March 9, the mainshock on March 11 with a maximum JMA seismic intensity of 6 for 6 min, and 42 aftershocks with a maximum JMA seismic intensity of more than 3 over 7 days. The earthquake struck Minamisoma in the form of three foreshocks with a maximum JMA seismic intensity of more than 3 on March 9 and 10, the main shock on March 11 with a maximum JMA seismic intensity of 6 for 6 min, and 44 aftershocks with a maximum JMA seismic intensity of more than 3 over 7 days. In Tokyo, in contrast, the main shock on March 11 had a maximum JMA seismic intensity of 5 for 6 min, and 4 aftershocks had a maximum JMA seismic intensity of more than 3 over 7 days.

Table 1. Annual earthquakes (number), mean wind force (m/s), and annual rainfall (mm) during 2007–2015.

Year	Annual Earthquake § (Number)			Mean Wind Force ‡ (m/s)			Annual Rainfall ‡ (mm)		
	Soma	Minamisoma	Tokyo	Soma	Minamisoma	Tokyo	Soma	Minamisoma	Tokyo
2007	1	3	0	2.3	0.9	3.4	1394	1706	1332
2008	8	9	0	2.2	0.9	2.8	1656	1653	1858
2009	5	5	1	2.3	0.9	2.9	1516	1468	1802
2010	5	4	0	2.1	1.2	2.9	1437	1720	1680
2011	102	98	8	2.3	1.3	2.9	1316	1274	1480
2012	21	21	7	2.3	1.4	3.0	1406	1482	1570
2013	14	16	5	2.3	1.5	3.1	1135	1260	1614
2014	10	13	6	2.4	1.4	2.9	1561	1780	1808
2015	9	9	2	2.3	1.3	2.8	1671	1775	1782

Each location and environment of oak tree was obtained due to the mean wind force and annual rainfall close to the epicenter of megathrust earthquake (Figure 1). § The numbers of earthquakes for a maximum JMA seismic intensity of more than 3 at each location were obtained from the records of JMAII (http://www.data.jma.go.jp/svd/eqdb/data/shindo/index.php). JMAII devices identify earthquakes and measure their intensity. The specific devices that gathered the data shown here were selected because, among the 4378 device locations in Japan, these were the closest to the epicenter of the megathrust earthquake (1.2 to 8.6 km). ‡ Mean wind force (m/s) and annual rainfall (mm) in the locations of trees were obtained from the Japan Meteorological Agency records (http://www.jma.go.jp/jma/menu/menureport.html).

3.2. Xyloglucan Signals in the Annual Rings of Oak Trees

Oak trees were chosen for analysis of the annual rings in the stems. Three oak trees were obtained from the Soma forest, three were obtained from the Minamisoma forest in Fukushima, and three were obtained from Tokyo as controls. The oak trunks from the two localities in northeastern Japan exhibited little if any noticeable reduction in annual growth rings starting in 2011 compared with the control oaks in Tokyo (Figure 2). Immuno-staining with LM15, an antibody against xylosyl-glucosyl residue [23], followed by a second antibody attaching a high-sensitivity polymer with peroxidase, revealed marked increases in xyloglucan signals starting near the 2011 annual ring, with increases of varying degrees among the rings from various years in both trees from Soma (on a mountaintop) and trees from Minamisoma (in a basin). The latter trees showed some decreases in growth trends, compared with those from Soma and Tokyo [24]. Although annual ring patterns were not similar between 14-year-old tree trunks and 40-year-old tree trunks, the increases in xyloglucan signals could be observed in the three trunks from Minamisoma as well as in those from Soma. Particularly strong xyloglucan signals were observed in the 2011 annual rings, especially in trees from the basin in Minamisoma, although signals also remained strong after 2011, especially in trees from the mountaintop in Soma. Increased xyloglucan signals were also observed in other annual rings near the 2011 ring. Given that only faint increases were observed in all rings in the control oaks from Tokyo, we consider it likely that trees from all locations had endured a certain level of seismic intensity and had adapted to seismic stress throughout their lives, not just since 2011. It should be noted that these signals could have been caused not only by varying levels of seismic stress but also by other external mechanical stresses in the environment such as strong wind (Table 1).

We predict that such increase will continue for a few years and that some xyloglucan formed in ray parenchymal cells could have been transported by a process similar to that seen for lignin between cell walls in mature xylem [25,26]. Nevertheless, the distinguishable increases around 2011 and other annual rings could have been caused not only by the high seismic stresses, but also by other external mechanical stresses in their environment (Table 1). The concern in question is the function of xyloglucan, specifically whether an increase in xyloglucan could enhance acclimation to seismic stress in woody tissue.

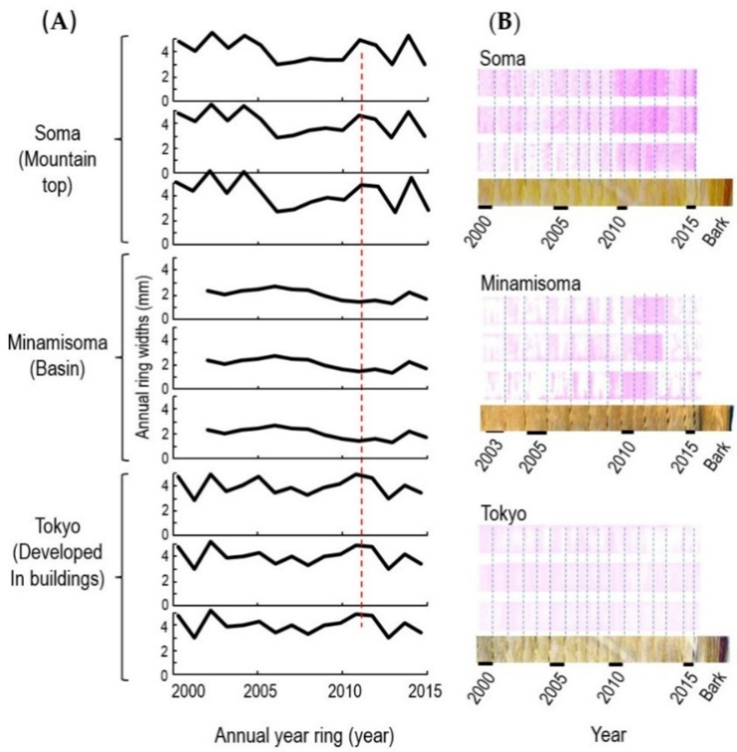

Figure 2. Annual rings of oak trees. (**A**) Annual growth-ring widths as measured in oak tree samples (Figure 1 and Table 1). Width values represent the mean of four sections of trunks. The dotted red line shows the year 2011. The oak trees sampled in Minamisoma were 15 years old, although others were more than 40 years old. (**B**) Immuno-staining of xyloglucan in the annual rings of oak trunks from different locations and environments. Tree-ring sections were stained with antibody LM15 followed by a second antibody attaching a polymer with peroxidase.

4. Conclusions

Here, we showed that radioiodine-targeted xyloglucan was elevated in the annual rings of forest trees after a megathrust earthquake (Figure 2). Therefore, we conclude that the increased xyloglucan signals that we observed in the growth rings of forest tree trunks in the wild were likely caused, at least in part, by earthquakes, different from the intake of radionuclide in the trees due to the accident involving a nuclear power plant [1–4]. The 2011 megathrust earthquake not only triggered a nuclear power plant accident that dispersed radioiodine, which then became bound to xyloglucan in trees as an artificial event due to a human error, but also increased xyloglucan signaling in annual rings of forest trees as a plant response against seismic stress under natural phenomena. These opposite findings require a paradigm shift in our understanding of xyloglucan, as our previous study of the uptake of radionuclides by trees in Fukushima forests suggested. Further studies are required to determine whether shaking stress induces the re-organization of cell walls in response to the increase in xyloglucan in trees. This idea sheds light on the development of xyloglucan in the cell walls of trees against external mechanical stresses.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/9/966/s1, Table S1: Times, locations, magnitude, and JMAII (Japan Meteorological Agency instrumental intensity) seismic intensities of the Tohoku megathrust earthquake in Soma, Minamisoma, and Tokyo.

Author Contributions: Y.S. (Yuya Sasaki), K.O., and T.H. performed forest tree samplings; T.M. performed tree-ring analysis; R.K., Y.S. (Yuya Sasaki), K.O., H.O., and K.B. performed immuno-staining; and R.K., T.T., Y.S. (Yoichi Sakata), and T.H. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported through a grant from the Tokyo University of Agriculture for the Eastern Japan Reconstruction Support Project after the Fukushima Disaster, as well as the the MEXT (Ministry of Education, Culture, Sports, Science and Technology in Japan)-supported Program for the Strategic Research Foundation at Private Universities (S1311017).

Acknowledgments: We would like to thank J. Takezawa and Somachiho (Forest Owners' Cooperative, Soma City, Japan) for providing sampled trees; N. Uchida and T. Matsuzawa for providing the information in Figure 1; and T. Matsuzawa, T. Shibutani, and T. Kunugi for discussions about earthquakes in Japan. Thanks also to L. Lu for the preparation of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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