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# Intake of Radionuclides in the Trees of Fukushima Forests 4. Binding of Radioiodine to Xyloglucan

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**Abstract:** The 1,4-linked glucans such as xyloglucan and amylose are known to form a complex with iodine/iodide ions and to also be precipitated with $CaCl_2$ in the presence of iodine. Here, we show that iodine gas could be specifically incorporated into xyloglucan. Furthermore, we show that $[^{125}I]I_2$ gas is, over time, incorporated at high levels into the entire outer surface of poplar seedlings but that spraying seedlings with abscisic acid to close stomata decreases the incorporation of the gas. There was less incorporation of the gas in a transgenic poplar overexpressing xyloglucanase at the early stages when compared with a wild type. This shows that xyloglucan serves as a key absorber of iodine gas into a plant body. After individual leaves of cultured seedlings were exposed to the gas for 30 min, no radioiodine was emitted from those leaves over the following two weeks, indicating that no turnover occurs in radioiodine once it is bound to the polysaccharides in plant tissues. We conclude that forest trees could serve as one of the largest enormous capture systems for the radioiodine fallout following the nuclear power plant accident in Fukushima.

**Keywords:** radioiodine gas; forest function; 1,4-linked glucans; poplar seedlings; xyloglucan

## 1. Introduction

The Fukushima Daiichi nuclear disaster on March 2011 caused the dispersal of high levels of radionuclides into the atmosphere, resulting in major environmental damage [1]. Among the major aerosol and volatile fission radionuclides, the most dangerous fallout could be that from the radioiodines, as exposure of the internal thyroid to them causes a thyroid cancer risk in children. Iodine-131, iodine-132, and iodine-133 have been estimated at $1.6 \times 10^{17}$ Bq, $2.2 \times 10^{14}$ Bq, and $6.8 \times 10^{14}$ Bq, respectively, and their half-lives are 8.02 days, 2.295 h, and 20.8 h, respectively [2]. Since the radioiodines have short half-lives, the period shortly after nuclear power plant accidents is the most dangerous.

The radioiodines associated with Fukushima were mainly dispersed in a gaseous form, which has a slightly different deposition pattern from the aerosol form of radiocesium [3,4]. Nothing is known about radioiodine infiltration into forest trees, even though more than 90% of the rural land of Fukushima is forested. This paper reports the potential function of forest trees, based on the binding properties of radioiodine gas to xyloglucan.

Iodine is known to display a blue/purple color in the presence of starch and a green/blue color in the presence of an amyloid xyloglucan found in tamarind seeds [5,6]. Iodine can also display a blue/purple color in the presence of other polysaccharides such as carboxymethylcellulose. In the microscopic visualization of plant cell walls, a dichroic dye composed of chlor-zinc-iodine is used to reveal the arrangement of cellulose microfibrils, which are coaligned from layer to layer [7]. The iodine can bind to the surface, as well as to amorphous regions of cellulose microfibrils. Nevertheless, this staining could be enhanced in the presence of xyloglucan between microfibrils [8]. An analysis of the xyloglucan/iodine complex through small-angle X-ray scattering data showed that the iodine/iodide ions may be incorporated between the xyloglucan chains [9]. The polyiodide species may be arranged in a uniaxial manner along the chains. On the other hand, a complex composed of amylose and iodine shares the presence of $I_3^-$ species, and its X-ray structure shows a cyclomaltohexaicosaose triiodide inclusion complex [10]. Atomic force microscopy also shows networks, chains, and superhelical structures in a starch/iodine complex [11,12]. Since these interactions between the glucans and iodine are enhanced in the presence of a strong $CaCl_2$ solution, Gaillard [13] proposed the precipitation of the 1,4-linked glucans with iodine and $CaCl_2$. The aim of this study is to reveal the interaction between radioiodine gas and 1,4-linked glucans in poplar seedlings.

Albersheim et al. proposed that xyloglucan functions as a bridge-like component between the cellulose and the matrix in the macromolecular complex of the primary cell wall [14–17] because its 1,4-β-glucan backbone binds to cellulose microfibrils through hydrogen [18–20] and hydrophobic [21] bonds, and the other part covalently links to the galactan moiety of RG-1 [22,23]. This paper attempts to show the significance of xyloglucan in forest trees following a nuclear power plant accident.

When a meltdown followed by a hydrogen explosion occurred gradually from units 1 to 5 in operation at the Fukushima Daiichi nuclear power plant on March 12 to 15, everything (traffic transport, radiation leaking information, governmental leadership, etc.) was stopped in Fukushima for one week. However, people in Fukushima remembered the Chernobyl disaster, after which many children were diagnosed with thyroid cancer. Parents, thus, took their children and left by car, determined to find a safer place. Even the Japanese government was unable, for more than one week, to say how radioiodine was moving within the area. A similar confusion occurred for more than one month after the Chernobyl disaster, and, unfortunately, the Soviet Union failed to save all of the people who were living in the area.

Here, we propose a proxy through experiments in a laboratory. We do not have any results related to how much atmosphere passes through the plants and disperses in relation to radioiodines in Fukushima forests because the radionuclides have short half-lives. This would be difficult to measure, even in the future, because we cannot predict future accidents at nuclear power plants.

## 2. Materials and Methods

### 2.1. Materials

The sample leaves and stems (Japanese cedar, cypress, and nanten) were taken at a height of 100 to 200 cm from the trees in Minamisoma and Soma. [$^{125}$I]NaI (37 kBq/pmol, 59.4 days of half-life) was obtained from Nordin (Ottawa, Canada). Potato amylose, carboxymethylcellulose (medium viscosity), dahlia inulin, and beechwood xylan came from Sigma (St. Louis, MI, USA). Galacturonan and lichenan were purchased from MP Biochemicals (Illkirch, France). Potato galactan and barley mixed-linkage glucan were obtained from Megazyme (Wicklow, Ireland), tamarind seed xyloglucan from DSP Gokyo Food & Chemical (Osaka, Japan), cellohexaose from Seikagaku (Tokyo, Japan), and maltohexaose from

Hayashibara (Okayama, Japan). Xyloglucan heptasaccharide (XXXG) came from Tokyo Chemical Industry (Tokyo, Japan). Xyloglucans were also prepared from pea (*Pisum sativum*) [24] and poplar (*Populus alba*) [25]. Galactosyl glucomannan was taken from Norway spruce (*Picea abies*) [26].

### 2.2. Transgenic Poplars

Transgenic white poplars (*P. alba*) overexpressing xyloglucanase (AaXEG2, AY160774) [27] and transgenic poplars (*P. tremula*) overexpressing endo-cellulase (AtCel1, X98544) [28] under control of the CaMV35S promoter have been previously described [8].

### 2.3. Staining and Precipitation with Iodine

0.25 mL of a solution of 0.5% $I_2$ in 1% KI and 2.5 mL of 20% $Na_2SO_4$ were added to 0.5 mL of a solution containing 150 µg of polysaccharide. The mixture was vortexed, and the absorption spectra were measured after the tube had been allowed to stand for 1 h at 4 °C in the dark [5].

400 µL of a solution of $CaCl_2$ (specific gravity 1.3) and 1 µL of 2% $I_2$ in 2% KI were added to 100 µLof a solution containing 150 µg of polysaccharide. The mixture was vortexed, and the tube was allowed to stand for 1 h at 4 °C in the dark [13]. The insoluble complex was precipitated after centrifugation at 15,000 rpm at 4 °C for 20 min. The amount of precipitated glycan was recalculated after the sugar content in the supernatant was measured according to the phenol/sulfuric acid method [29]. The decreased amount of sugar in the supernatant was shown as the amount of precipitated glycan.

### 2.4. Radioiodine Gas Experiments

We used I-125 instead of I-131, I-132, and I-133, because the properties of iodine gas are the same, with the exception that the half-life of iodine-125 is 59.4 days. Since we imported it from Canada and used it in Japan, it was better for us to use it, as the short half-life ones were difficult to use. Radioiodine gas ($^{125}I$) was produced from 0.1 pmol of [$^{125}I$]NaI (37 kBq/pmol) in the presence of hydrogen peroxide and blown through a glass case (0.5 m × 0.5 m × 0.5 m). [$^{125}I$]NaI (0.1 pmol) and hydrogen peroxide were placed in the bottle by a small film barricade. When the glass case was shaken, [$^{125}I$]NaI and hydrogen peroxide could be mixed and begin to produce radioiodine gas.

Radioiodine gas is one of the most dangerous chemicals. To ensure safety, first, the gas was used in a fume hood. The fume hood (approximately 2 $m^2$) was located in the corner of the lab. The iodine gas was used in the glass case (0.5 m × 0.5 m × 0.5 m) in the fume hood. Second, all workers took an iodine tablet every day in order for their entire body to be saturated with non-radioactive iodine. Since taking these tablets for too long causes serious damage to the human body, we alternated between working with a tablet every day for one week and not working and not taking a tablet for one week.

### 2.5. Assay for Adsorption of Radioiodine to Polysaccharides

Plant polysaccharides were tested as a solution in about 17 mg glass-fiber filters (12 × 12 × 1 mm). Glass-fiber filter samples contained 2.5 mg of polysaccharide in 100 µL of 10 mM potassium phosphate buffer (pH 7.5). In the case of insoluble cellulose, 2.5 mg of cellulose was placed on 50 mg of glass fibers and moistened by the addition of 200 µL of the buffer. Following this, the glass-fiber filter was placed on top of a bottle by a filter attachment, and this was all in the glass case. [$^{125}I$]NaI (0.1 pmol) and hydrogen peroxide were placed in the bottle by a small film barricade. When the glass case was shaken, [$^{125}I$]NaI and hydrogen peroxide could be mixed and begin to produce radioiodine gas. Then, the gas went through the glass-fiber filter in the glass case and also through the holes of the case to outside. The radioiodine gas that was formed completely disappeared in 30 min. The gas could bind to the polysaccharide solution only through the glass-fiber filter during gas formation. The incorporation of iodine gas into the filter was directly determined with an Aloka AccuFlex γ7001 scintillation counter (Aloka, Tokyo, Japan).

### *2.6. Radioiodine Incorporation into Poplar Seedlings*

Potted poplar seedlings, approximately 18 cm in height, were placed in the glass case. The exposure of individual leaves to radiation was carried out as follows: one of the leaves on each poplar seedling was enclosed in a soft plastic bag (0.1 m × 0.1 m × 0.3 m), which was sealed with a rubber band at the petiole, and the gas (0.1 pmol of [$^{125}$I]NaI, 37 kBq/pmol) was blown through this bag. After this leaf had been exposed to the gas for 30 min, the seedlings were kept under culture without further radiation exposure in the sealed glass case (0.5 m × 0.5 m × 0.5 m). All the experiments were conducted under a fume hood. Poplar seedlings were subjected to autoradiography with an image analyzer (Fujifilm, Tokyo, Japan) for 3 h. Each experiment was carried out three times with independent samples of seedlings.

### *2.7. Wall Analysis*

Fresh leaves and stems were freeze-dried to determine their dry weight. The leaves were then washed three times with acetone and extracted three times with 50 mM sodium phosphate buffer (pH 6.2) at room temperature to obtain cell wall preparations [30]. The preparations were then extracted six times with 4% KOH/0.1% $NaBH_4$ in an ultrasonic bath below 30 °C for 3 h to remove much of the polymeric material while retaining most of the xyloglucan and cellulose. The wall residue was further extracted six times with 24% KOH/0.1% $NaBH_4$ in an ultrasonic bath below 50 °C for 3 h in order to solubilize hemicelluloses between 1,4-β-glucans. This wall residue was neutralized with 2 M acetic acid, washed with water, and is referred to here as the "24% KOH-insoluble residue (cellulose fraction)".

The amount of xyloglucan was determined by methylation analysis after treatment with α-amylase [30]. The amount of 4,6-linked glucose was attributed to xyloglucan. The 24% KOH-insoluble wall residue (cellulose fraction) was extracted with acetic/nitric reagent (80% acetic acid/concentrated nitric acid, 10:1) in a boiling water bath for 30 min [31]. The resulting insoluble material was washed in water, freeze-dried, and weighed to determine the amount of cellulose.

## 3. Results

### *3.1. Radioiodine in the Trees of Fukushima*

The half-lives of iodine-131, 132, and 133 are 8.02 days, 2.295 h, and 20.8 h, respectively—even the longer one is eight days. Given this, it was impossible for us to work in the forests of Fukushima after the accident and to measure the level of radioactivity in the various trees. After two months, we were able to enter a limited forest area because the government controlled the entrance to the forests of Fukushima.

Low levels of iodine-131 were found in the leaves and stems of forest trees in Minamisoma in May 2011, two months after the Fukushima Daiichi nuclear power plant accident (Table 1), probably because Minamisoma was a highly contaminated area rather than Date forest. On August 2011, five months after the accident, it was difficult to detect the radioiodine in any of them. Although a high level of radioiodine was dispersed into the forest fields in Fukushima in March [32,33], its associated radiation level dropped quickly. Iodine-131 has a half-life of 8.04 days, which is the longest half-life of all the radioiodines.

### *3.2. Interaction between Iodine and Glycan*

Figure S1 shows the absorption spectra of iodine with soluble 1,4-linked glucans between 400 and 900 nm. There were no differences in the spectra of xyloglucans obtained from elongated pea stems, poplar xylem, and tamarind seeds (Figure S1B). Barley β-glucan has a slightly higher absorption, at 640 nm, than lichenan (mixed linkage 1,3:1,4-β-glucan from lichen) has (Figure S1C), which is potentially due to the higher levels of consecutive 1,4-linked glucose units in the β-glucan [34]. Galactan (1,4-β-galactan), galacturonan (1,4-α-polygalacturonic acid), xylan (1,4-β-xylan), and inulin

(1,2-β-fructan) have very low but still measurable absorptions at 640 nm (Figure S1D). A carboxymethyl derivative of cellulose showed a measurable level of absorption at 640 nm (Figure S1E), but glucan fragments such as cellohexaose, maltohexaose, and xyloglucan heptasaccharide (XXXG) had lower absorption levels, at 640 nm, than their polymers did.

**Table 1.** Fallout iodine-131 specific activities in leaves with stems in Fukushima forests.

| Location and Tree Species | Iodine-131 Bq/kg Dry Weight | |
|---|---|---|
| | 15 May 2011 | 3 August 2011 |
| Minamisoma at 37°38′11″ N/140°54′18″ E | | |
| Japanese cedar (*Cryptomeria japonica*) | 107 | ND |
| Cypress (*Chamaecyparis obtusa*) | 126 | ND |
| Nanten (*Nandinadomestica*) | 114 | ND |
| Date at 37°45′19″ N/140°41′37″ E | | |
| Japanese cedar | ND | ND |
| Cypress | ND | ND |

ND = not detected. Each data point represents the mean of three independent samples of each tree, with individual values varying from the mean by <12.5%.

The staining that occurred was uniformly green in the presence of xyloglucan, purple in the presence of amylose, red in the presence of β-glucomannan and β-glucan, and orange to yellow in the presence of pectin (galacturonan), galactan, xylan, and inulin (Figure S2A). Weak purple staining also occurred in the presence of carboxymethylcellulose, but not in the presence of the glucan fragments (Figure S2B). As shown in Figure S2C, glycans in $CaCl_2$ solution were also precipitated, to varying degrees, by the addition of iodine: 100% of xyloglucan, about 81% of amylose, about 61% of carboxymethylcellulose, and about 10% of galactosyl glucomannans and mixed-linkage glucan were precipitated (Table S1). It is hard to determine the amounts of galacturonan, galactans, and xylan precipitated with iodine because these polysaccharides became insoluble in the $CaCl_2$ solution.

We conclude that xyloglucans formed a higher absorption complex at 640 nm with iodine/iodide ions and were also precipitated at their largest amounts with $CaCl_2$ in the presence of iodine. It appears that xyloglucan could be the most active substance to bind to iodine in plant components.

### *3.3. Adsorption of Radioiodine to Xyloglucan*

After many trials, an assay method was developed for the adsorption of $[^{125}I]I_2$ to plant polysaccharides, in which the polysaccharides were tested as a dry powder, a gel, and a solution in a glass-fiber filter (12 × 12 × 1 mm) in the presence of radioiodine gas. By passing radioiodine gas through the filter containing each polysaccharide solution, the gas could be incorporated into the solution. In this experiment, xyloglucan showed a higher adsorption level compared to the other glucans (Table 2), although less than 1% of radioiodine was incorporated into the control containing 100 μL of potassium phosphate buffer alone. The levels of iodine captured by the glycans correlated with the levels of glucan precipitated by the addition of iodine, except for those of amylose and carboxymethylcellulose (Table S1).

### *3.4. Incorporation of Radioiodine into Poplar Seedlings*

In the gaseous form, 5 kBq $[^{125}I]I_2$ (37 kBq/pmol) was, over time, incorporated at high levels into the entire outer surface of poplar seedlings growing in a glass case (Figure 1A,B). When the seedlings were sprayed with 10 μM abscisic acid to close the stomata [35], however, the incorporation level after 30 min was markedly decreased. We had two types of mutants: one had less xyloglucan content in the wall through the overexpression of xyloglucanase (AaXEG2), and the other had less binding to cellulose microfibrils in the wall through the overexpression of endo-cellulase (AtCel1). Low levels of radioiodine were observed in transgenic poplars containing less xyloglucan (Table S2). In transgenic poplars overexpressing endo-cellulase, which contained weakly wall-bound xyloglucan

(4% KOH-soluble), incorporation after 30 min appeared to be greater than that in the wild type. It should be noted that transgenic poplars overexpressing xyloglucanase did not close their stomata after being sprayed with abscisic acid, probably because xyloglucan is required for the stomata to close.

**Table 2.** Proportions of captured radioiodine gas.

| Polysaccharide | Relative Capture Level % |
|---|---|
| Poplar xyloglucan | 100 |
| Pea xyloglucan | 100 |
| Tamarind xyloglucan | 97.3 ± 0.3 |
| Amylose | 45.1 ± 3.5 |
| Carboxymethylcellulose | 40.2 ± 2.8 |
| Cellulose | 26.7 ± 2.6 |
| Galactosyl glucomannan | 12.7 ± 1.7 |
| Mixed-linkage glucan | 12.2 ± 1.5 |
| Galactan | 4.0 ± 1.1 |
| Xylan | 3.3 ± 1.0 |
| Galacturonan | 3.0 ± 0.5 |
| Lichenan | 1.0 ± 0.8 |
| Inulin | 1.0 ± 0.5 |
| Control | 0.7 ± 0.3 |

Each data point represents the mean of three independent measurements of one polysaccharide line. The relative capture levels of glycans are shown as the level based on poplar xyloglucan at 100%.

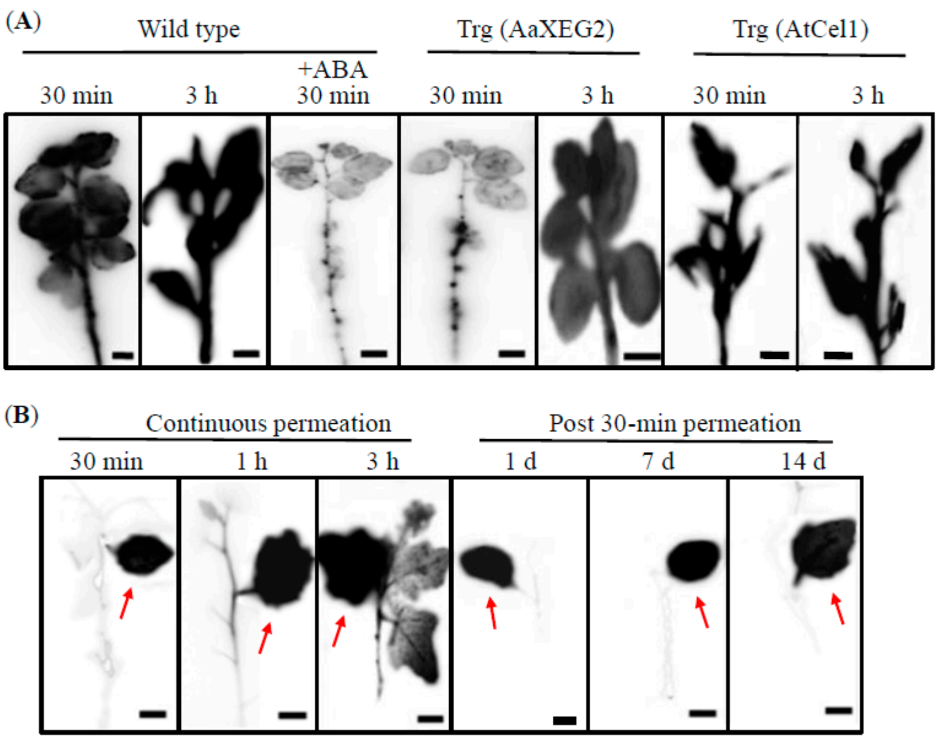


**Figure 1.** Incorporation of radioiodine gas into poplar seedlings. (**A**) Autoradiographs of a $[^{125}I]I_2$-gas-labeled wild-type poplar seedling at 30 min and 3 h; the third seedling was sprayed with 10 μM abscisic acid (ABA) before being treated with radioiodine gas for 30 min. Autoradiographs show $[^{125}I]I_2$-labeled transgenic poplars overexpressing xyloglucanase [Trg (AaXEG2)] and those overexpressing endo-cellulase [Trg (AtCel1)] at 30 min and 3 h. (**B**) Autoradiographs showing $[^{125}I]I_2$-gas labeling of one leaf at 30 min, 1 h, and 3 h. Time course showing poplar seedlings cultured for one day, seven days, and 14 days after one leaf was permeated with radioiodine for 30 min (The exposure times used to image the plates varied). The red arrows indicate the exposed leaves. Bars = 2 cm.

When only one leaf in a poplar seedling was continuously exposed to $[^{125}I]I_2$ gas (Figure 1B), the radioiodine spread to the stem within 1 h and to other leaves at lower levels within 3 h. It is likely that radioiodine delivered in this form can spread throughout the plant, potentially through the apoplast. In seedlings cultured after one leaf had been exposed to the gas for 30 min, the radioiodine neither moved to other tissues over time nor left the leaf in gaseous form for two weeks. No radioiodine was detected in other components such as active charcoal traps placed beneath the seedlings in the sealed glass case (Table 3).

**Table 3.** Distribution of radioiodine during 14 days of cultivation in a sealed glass case after a 30-min exposure of one leaf of each wild-type poplar seedling to $[^{125}I]I_2$ gas.

| Cultivation | kBq | | |
|---|---|---|---|
| | **1 day** | **7 days** | **14 days** |
| Poplar seedling | 3.13 ± 0.55 | 3.02 ± 0.66 | 2.88 ± 0.61 |
| Soil | ND | ND | ND |
| Water | ND | ND | ND |
| Pot | ND | ND | ND |
| Tray | ND | ND | ND |
| Charcoal | ND | ND | ND |

ND = not detected. Each data point represents the mean of three independent samples of trees for each line.

## 4. Discussion

Here, we show that xyloglucan serves as a key absorber involved in the response of forest trees to extreme exposure to radioiodine gas. In fact, a greater proportion of radioiodine was absorbed by xyloglucan than by any other wall component or starch (Table 2). As shown in Figure 1A, compared with that in the wild type, the level of incorporation was lower in a transgenic poplar overexpressing xyloglucanase [27], which had lower xyloglucan levels, and higher in a poplar overexpressing endo-cellulase [28] at its early stages, which had higher levels of weakly wall-bound xyloglucan. The increased incorporation of radioiodine could be due to an increase in non intercalated xyloglucan via the hydrolysis of the amorphous regions of cellulose microfibrils. This is in agreement with previous findings [36,37] showing that the overexpression of plant cellulase resulted in increased soluble xyloglucan and cello-oligosaccharides in the leaves of *Arabidopsis* and sengon.

The existence of an interaction between glucan and iodine was confirmed by the very close correlation between the levels of iodine gas captured by the glucans (Table 2) and the levels of glucans precipitated by the addition of iodine (Table S1). The levels in both cases might be derived from the content of consecutive 1,4-linked glucose moieties, due not only to the higher levels of amylose and carboxymethylcellulose compared to glucomannan and mixed-linkage glucan [34]. Since radioiodine was incorporated at a lower level into cellulose than into carboxymethylcellulose, iodine could be mainly bound to the amorphous regions of cellulose microfibrils.

Based on the half-life of iodine-131, iodine-131 could have been incorporated into trees (about 20,000 Bq per kg body) at the Minamisoma forest field (Table 1). As shown by the direct incorporation of the radionuclide into wild-type poplars (Figure 1), radioiodine fallout could be likewise incorporated and fixed in the forest trees after the nuclear power plant accident. No turnover probably occurs in the incorporated radioiodine once it is bound to the body, in which radioiodine could be captured predominantly by xyloglucan (Figure 1B and Table 3). Leaves and growing cells could be a target for the deposition of radionuclide in trees because xyloglucan exists largely in the primary wall [38].

The radioiodines were dispersed throughout the rural areas of Fukushima, more than 90% of which is forested, a greater percentage than the circa 50% that existed around Chernobyl. Forest trees could serve as one of the largest enormous capture systems for the radioiodine fallout that followed the nuclear power plant accident in Fukushima. That is why the thyroid equivalent doses assessed in evacuees may be much lower than the mean thyroid dose after the Chernobyl disaster [39]. The forests play an important role in reducing the harmful impact of this radionuclide on the residents of the rural areas in Fukushima.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1999-4907/11/9/957/s1, Figure S1. Adsorption spectra of iodine with plant carbohydrates. (A) Poplar xyloglucan (blue), amylose (brown), galactosyl glucomannan (green), and barley β-glucan (purple). (B) Pea xyloglucan (blue), poplar xyloglucan (brown), and tamarind xyloglucan (green). (C) Mixed-linkage glucan (red) and lichenan (blue). (D) Galactan (blue), galacturonan (green), xylan (brown), and inulin (purple). (E) Carboxymethylcellulose (blue), cellohexaose (brown), maltohexaose (purple), and xyloglucan heptasaccharide XXXG (green). Each reaction contains 150 μg of carbohydrate, except tamarind xyloglucan (100 μg); Figure S2. Iodine-staining colors and iodine precipitation with plant carbohydrates. (A) Iodine-staining colors for natural carbohydrates: (a) poplar xyloglucan, (b) amylose, (c) galactosyl glucomannan, (d) mixed-linkage glucan, (e) galacturonan, (f) galactan, (g) xylan, (h) inulin, and (i) control water. (B) Iodine-staining colors for artificial carbohydrates: (a) carboxymethylcellulose, (b) cellohexaose, (c) maltohexaose, (d) xyloglucan heptasaccharide XXXG, and (e) control water. (C) Iodine precipitations: (a) xyloglucan, (b) amylase, (c) carboxymethylcellulose, (d) galactosyl glucomannan, (e) mixed-linkage glucan, (f) lichenan, (g) inulin, and (h) control water. Each reaction contains 150 μg of carbohydrate.; Table S1. Amounts of glycans precipitated by the addition of iodine; Table S2. The amount of xyloglucan and cellulose in the 4% KOH- and 24% KOH-soluble fractions of poplar leaves and stems.

**Author Contributions:** M.N., C.Y., S.A., M.I., K.B., R.K., and T.H. performed iodine gas experiments and S.W., P.C., and T.H. performed iodine-staining experiments. O.S. performed the transgenic poplars overexpressing endo-cellulase experiment and Y.S. did the abscisic acid experiment. M.T. and T.H. sampled in Fukushima. K.B., R.K., T.T., and T.H. drafted the manuscript. All authors have read and agree to the published version of the manuscript.

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