Responses of Root Production in Japanese Red Cedar (*Cryptomeria japonica* D. Don) Saplings to Duration of Treatment with Acidic Solutions

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The effects of treatment with acidic solutions for various periods on root system biomass and nutritional status were examined in sand cultures of Japanese red cedar (*Cryptomeria japonica* D. Don) saplings. A mixture of H_2SO_4 and HNO_3 solution at a molar ratio of 2 : 1 (pH 2.0) was applied for 4 to 12 weeks to both above- and below-ground parts of saplings grown in Yahagi sand. Deionized water was used as the reference treatment (control). At the 4th week, white roots were already injured and the biomass allocation to white roots was significantly lower than in the control. At the 12th week, values of the root surface area index, which were represented as the weight of $Ca(NO_3)_2$ attached to the root surface per unit dry weight of the roots, differed significantly between the pH 2 and control treatments, although they were not significantly different at weeks 4 and 8. The concentrations of Al in the white roots at pH 2 were consistently higher than those in the controls at weeks 4, 8 and 12. These results suggest that the effects of pH 2 solution on the root systems of Japanese red cedar saplings in Yahagi sand appear from an early stage, by the end of the 4th week of treatment, and that white root development is affected both indirectly through above-ground parts, and directly through low pH and excess Al in the media solution.

Key words: acidic solution, *Cryptomeria japonica*, root surface area index, treatment duration, white root

Introduction

Approaches for evaluating the ecophysiological status of forest trees using below-ground as well as above-ground parts are of great importance for assessing the vigor of such trees (Clemensson-Lindell and Persson 1995). In Japan, there have been several experimental attempts to detect the effects of acid precipitation on the Japanese red cedar (*Cryptomeria japonica* D. Don) (Izuta *et al.* 1990a; Yagi *et al.* 1990; Matsumoto *et al.* 1992; Miwa *et al.* 1993; Kohno *et al.* 1995; Hirano *et al.* 1997). Miwa *et al.* (1993) and Hirano *et al.* (1997) have revealed that the threshold pH at which visible injury occurs on the foliage of Japanese red cedar saplings treated with acidic solutions falls within a pH range of 2.0-2.5. Moreover, Izuta *et al.* (1990a) have reported that visible injury begins to appear on foliage within 10 days in the case of pH 2.0 treatment.

Although visible symptoms on the above-ground parts of plants can be readily traced soon after their appearance, it is more difficult to detect the effects of acidity on below-ground parts without harvesting. Some experimental studies have shown that artificial acidic precipitation of pH 2.0-2.5 leads to reductions in the root biomass of Japanese red cedar saplings (Izuta *et al.* 1990a; Yagi *et al.* 1990; Matsumoto *et al.* 1992; Miwa *et al.* 1993; Kohno *et al.* 1995; Hirano and Yokota 1996; Hirano *et al.* 1997). These results imply that an acidic solution can function as a stressor to root systems. In these studies, the duration of treatment with the acidic solutions varied greatly from 30 days (Yagi *et al.* 1990) to 23 months (Kohno *et al.* 1995), but the symptoms due to acidity such as reductions in root biomass were observed only at final harvesting after the treatment. Therefore, it is necessary to determine when and how symptoms appear in the root system in order to clarify the process of reduction in root biomass caused by acidity.

In the present study, an attempt was made to do this using Japanese red cedar saplings treated with acidic solution at pH 2.0 by harvesting the saplings every 4 weeks, noting particularly changes in the root biomass and the nutritional status of the roots in relation to treatment duration.

Materials and Methods

Experimental design

Two-year-old Japanese red cedar (*Cryptomeria japonica* D. Don) saplings (*ca* 30 cm in height) reared in

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the nursery of the Aichi Prefectural Forest Experimental Station were used as plant materials. On June 24, 1994, the saplings were planted individually in plastic pots (Wagner pot, 1/5000a) containing Yahagi sand. Yahagi sand is river gravel with a low nutrient content, and was used to eliminate the complex effects of soil nutrients on the growth of the saplings. The experiment was performed in a greenhouse to prevent natural precipitation. The pots were shaded at 60% of full light to promote their root growth. Average temperature in the greenhouse was 28.2°C during the experimental period.

A mixture of H_2SO_4 and HNO_3 solution in a molar ratio of 2 : 1 at pH 2.0 (pH 2 treatment) was applied to both the below-ground and above-ground parts of the saplings. Deionized water was used as the reference treatment (control). Each treatment was applied to 12 saplings.

About 300 ml of acidic solution (pH 2 treatment) or deionized water (control) was applied as a fine mist to the above-ground parts of the saplings with an automatic sprayer. Only at the time of the treatment, the surface of the potting medium was covered with a vinyl sheet to eliminate drops of acidic dew. About 300 ml of acidic solution or deionized water was applied to the below-ground parts in a watering pot. These treatments were carried out in the evening twice a week for 12 weeks from August 4 to October 27, 1994. Because of the low water holding capacity of Yahagi sand, about two thirds of the applied solution leached out of the pots. The total amount of precipitation in each pot during the acidic solution treatment (above-ground and below-ground parts) corresponded to 12 weeks of annual precipitation of about 1560 mm. Throughout the treatment period, all the experimental pots were irrigated daily with deionized water and supplied with liquid fertilizer (N : P : K=15-30-15 mg pot^{-1} $week^{-1}$) at weekly intervals.

Sample analysis

The saplings were scrutinized to detect visible symptoms on the foliage. Every four weeks (at weeks 4, 8 and 12), 4 saplings in each treatment group were carefully harvested. The saplings were separated into foliage, stem, white roots (newly grown roots) and other roots. White roots were defined as roots that were clearly white, and thus distinguishable from other roots (Karizumi 1979; Hirano and Yokota 1996; Hirano *et al.* 1997). The samples were oven-dried at 80°C for 48 h to measure their dry weight. Biomass allocation was defined as the ratio of each organ to the entire biomass of the sapling on a dry weight basis. Root surface area index (g $Ca(NO_3)_2$/g root dw) was estimated according to a modified gravimetric method (Carley and Watson 1966). The root surface area index was represented as the weight of $Ca(NO_3)_2$ attached to the root surface per unit dry weight of the roots.

Dried samples of current-year foliage and white roots were finely ground for nutrient analysis. After digestion with a mixture of sulfuric acid and hydrogen peroxide, concentrations of Ca and Mg were determined by atomic absorption spectrophotometry, and concentrations of K were determined using a flame photometer. Concentrations of P and Al were determined colorimetrically by the molybdenum-blue method and the aluminon method, respectively.

Every four weeks, four samples of the Yahagi sand in each treatment were analyzed to determine the concentrations of water-soluble cations. A 10-g sample of medium was added 50 ml of distilled water, and the medium suspension was stirred for 1 h and filtered through quantitative filter paper. Concentrations of Ca and Mg in the filtrate were determined by atomic absorption spectrophotometry, and concentrations of K and Na were determined by flame photometry.

Statistical analysis

After arcsine transformation of percentage data (nutrient concentrations in current-year foliage, those in white roots and biomass allocation) to angles, Student's *t*-test was used to examine differences between the pH 2 and the control treatments.

Results and Discussion

With pH 2 treatment, visible injury in the form of small red spots appeared on the foliage about 3 weeks after the start of the acidic treatment. By the end of the 4th week of treatment, the white roots had clearly changed morphologically to a shorter and thicker form (Fig. 1). The same trend was also observed for the white roots of saplings harvested at weeks 8 and 12. However, this experiment could not indicate which part of the saplings was first affected by the acidic treatment, because sapling harvesting was not sequential.

Although both biomass allocation to the entire roots (white roots+other roots) and other roots did not differ significantly between the pH 2 and control treatments over the entire experimental period (*t*-test, $p>0.05$), the biomass allocations to white roots treated at pH 2 at weeks 4, 8 and 12 were all significantly lower than that

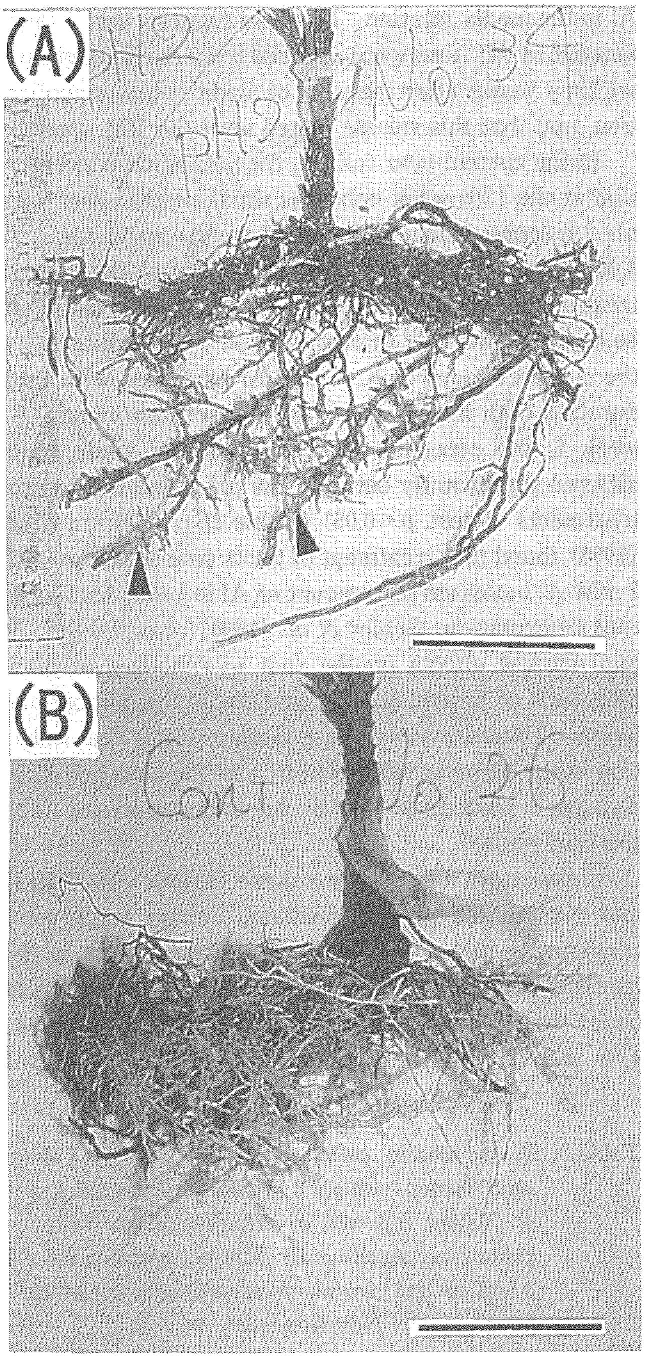

Fig. 1. Root systems of Japanese red cedar saplings with pH 2 treatment (A) and with control treatment (B) at week 12. Arrows indicate white roots characterized by thicker and shorter roots. Bar=50 mm.

of the control (t-test, $p<0.05$) (Table 1). There are two possible reasons for the reduction in biomass allocation to white roots. One is a direct effect of the acidic solution on the above-ground parts of saplings which resulted in a successive decrease in photosynthate allocation to the root system (Izuta *et al.* 1990a; Hirano *et al.* 1997). The other is the effect of acidity on the rhizosphere in the pots. Kohno *et al.* (1996) suggested that the effects of soil acidification on forest trees are hypothesized to effects of both low soil pH and phytotoxic metals such as Al dissolved in the aqueous component of the soil. The reduction in biomass allocation to white roots by the end of the 4th week of treatment, may therefore be due to the above-ground and/or below-ground component (Hirano *et al.* 1997).

The root surface area index for the pH 2 treatment tended to be lower than that for the control treatment

Table 1. Biomass allocation to the entire roots (white roots+other roots), white roots and other roots of Japanese red cedar saplings at weeks 4, 8 and 12 (mean±S.D.). Values followed by different letters within a column are significantly different between the pH 2 and control treatments according to t-test ($p<0.05$).

	Biomass allocation (%)		
	Entire roots	White roots	Other roots
4th week			
pH2	27.9 ± 2.4^{a}	1.6 ± 0.5^{a}	26.6 ± 2.5^{a}
Control	26.3 ± 3.2^{a}	4.1 ± 1.9^{b}	22.2 ± 2.8^{a}
8th week			
pH2	27.3 ± 4.0^{a}	1.2 ± 0.5^{a}	26.1 ± 3.7^{a}
Control	30.2 ± 2.6^{a}	5.0 ± 1.1^{b}	25.2 ± 1.9^{a}
12th week			
pH2	25.8 ± 2.9^{a}	1.4 ± 1.0^{a}	24.4 ± 2.0^{a}
Control	29.9 ± 2.0^{a}	4.3 ± 0.4^{b}	25.6 ± 1.9^{a}

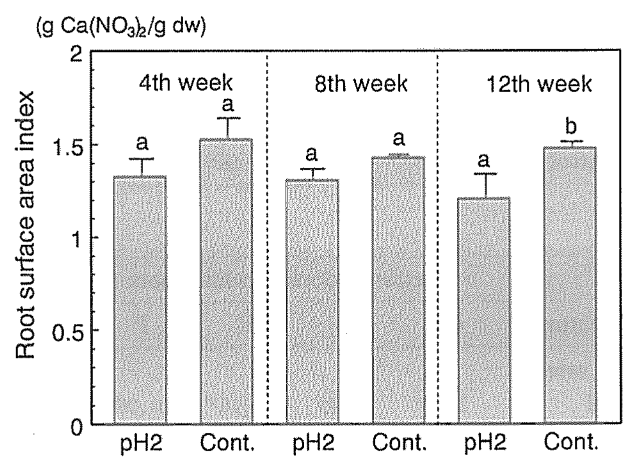

Fig. 2. Root surface area index of Japanese red cedar saplings at weeks 4, 8 and 12. Each value is given as the mean of 4 saplings. Vertical lines show the standard error of the mean. Bars with different letters in each of the 4th, 8th and 12th week are significantly different between the pH 2 and control treatments (t-test, $p<0.05$).

over the entire experimental period, and the difference between the pH 2 and the control treatments was significant at the 12th week (t-test, $p<0.05$) (Fig. 2). The index for pH 2 treatment at week 12 was smaller than those at weeks 4 and 8, suggesting that the roots after 12 weeks of pH 2 treatment were relatively thicker than those given the other treatments. It is well documented that new roots of Al-treated woody plants such as pitch pine, Scots pine, red spruce and other conifers are significantly thicker and shorter than roots without Al (Schaedle *et al.* 1989; McQuattie and Schier 1992; Oleksyn *et al.* 1996). Therefore, the changes in root morphology with pH 2 treatment may be due to excess Al in the media solution. This also suggests that a large amount of Al^{3+} ions were released from the Yahagi sand within 4 weeks after the start of acidic solution application, and that this release lasted until the 12th week.

In the current-year foliage, the potassium concentration at the 12th week only was significantly lower with pH 2 treatment than with control treatment (t-test, $p<0.05$) (Table 2A). For both the pH 2 and the control treatments, the concentrations of Ca, K and P tended to be higher at week 12 than at week 4. In the white roots, the concentrations of K, P and Al increased with time duration both the pH 2 and the control treatments. At week 8, the concentrations of Al in the white roots differed significantly between the pH 2 and the control treatments (t-test, $p<0.05$) (Table 2B). Oleksyn *et al.* (1996) found that treatment of Scots pine seedlings with 2 mM Al increased the amount of Al in roots, leading to root deformation. Schier *et al.* (1990) reported that Al had marked effects on the root morphology of pitch pine, such as browning and reduction in the number and length of lateral roots. These findings imply that reduction in the biomass allocation to, and the morphological changes in white roots may be due to the effects of Al on the root system.

Concentrations of water-soluble cations (Ca, Mg, K and Na) in the growth medium, Yahagi sand, were consistently higher in the pH 2 treatment than in the controls (Table 3). In particular, the concentrations of Ca at weeks 4 and 8, the concentrations of K at weeks 4, 8 and 12, and the concentration of Na at week 4

Table 2. Concentrations of elements in current-year foliage (A) and white roots (B) of Japanese red cedar saplings treated with pH 2 solution (mean values, $n=4$). Values followed by different letters within a column are significantly different between the pH 2 and control treatments according to t-test ($p<0.05$). N. D.: Not detected.

(A)

	Concentrations in current year foliage (%)				
Treatment	Ca	Mg	K	P	Al
4th week					
pH2	0.37[a]	0.20[a]	1.26[a]	0.15[a]	0.03
Control	0.45[a]	0.20[a]	1.26[a]	0.16[a]	N. D.
8th week					
pH2	0.30[a]	0.17[a]	1.42[a]	0.16[a]	0.03
Control	0.38[a]	0.22[a]	1.40[a]	0.22[a]	N. D.
12th week					
pH2	0.49[a]	0.18[a]	1.32[a]	0.30[a]	0.03[a]
Control	0.62[a]	0.21[a]	1.56[b]	0.29[a]	0.03[a]

(B)

	Concentrations in white roots (%)				
Treatment	Ca	Mg	K	P	Al
4th week					
pH2	2.29[a]	0.06[a]	0.16[a]	0.08[a]	0.16
Control	1.89[a]	0.04[a]	0.07[a]	0.09[a]	N. D.
8th week					
pH2	2.52[a]	0.04[a]	0.29[a]	0.21[a]	0.61[a]
Control	1.82[a]	0.06[b]	0.44[a]	0.20[a]	0.06[b]
12th week					
pH2	1.62[a]	0.05[a]	0.71[a]	0.26[a]	0.50[a]
Control	1.40[a]	0.07[a]	0.66[a]	0.31[a]	0.16[a]

Table 3. Water-soluble cation concentrations in Yahagi sand treated with pH 2 solution (mean values, $n=4$). Values followed by different letters within a column are significantly different between the pH 2 and control treatments according to t-test ($p<0.05$). N. D.: Not detected.

	Concentrations (meq/100g dry soil)			
Treatment	Ca	Mg	K	Na
4th week				
pH2	0.008[a]	0.002	0.009[a]	0.010[a]
Control	0.004[b]	N. D.	0.002[b]	0.002[b]
8th week				
pH2	0.016[a]	0.006	0.007[a]	0.009[a]
Control	0.004[b]	N. D.	0.005[b]	0.003[a]
12th week				
pH2	0.011	0.003	0.005[a]	0.003[a]
Control	N. D.	N. D.	0.002[b]	0.003[a]

differed significantly between the pH 2 and the control treatments (t-test, $p<0.05$). These results lend support to the notion that the concentrations of water-soluble cations in the medium increased with increasing acidity in the treatment solutions, as reported by Izuta *et al.* (1990b) and Miwa *et al.* (1994). The mean pH(H_2O) values for Yahagi sand after treatment of only the below-ground parts with about 300 ml of pH 2.0 solution and with deionized water twice a week for 15 weeks were 3.9 and 5.4, respectively (Y. Hirano, unpublished data). On the other hand, the mean pH(H_2O) values for brown forest soil treated similarly were 3.4 and 4.3, respectively (Y. Hirano, unpublished data). In the above experiment using Yahagi sand, as well as in the present study, about two thirds of the applied solution leached out of the pots. These results indicate that the change in pH(H_2O) value of Yahagi sand was larger than that of brown forest soil, and suggest that Yahagi sand has much lower acid-neutralizing capacity than brown forest soil. Therefore, it can be assumed that Yahagi sand itself was responsible for the effects of low pH and/or Al on the root systems of the saplings. The low acid-neutralizing capacity of Yahagi sand may have led to the acute reduction in the root systems observed at the 4th week.

The present study has indicated that the effects of pH 2 solution application, indirect effects through above-ground parts, and effects of low pH and excess Al in the media solution on the root systems of Japanese red cedar saplings appear even at an early stage, by the end of 4th week of treatment, of white root development. In forest soil which has a high acid-neutralizing capacity, however, detrimental effects are likely to appear 4 weeks later than those in the case of Yahagi sand.

Our study has also demonstrated that morphological changes, as well as biomass reduction, occur in white roots by the 4th week. As the mechanism responsible for the reduction in root biomass remains unclarified, further experimental studies will be necessary to reveal the processes involved in the detrimental effects of acid treatment on root systems.

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酸性溶液の処理期間がスギ苗の根系生産に与える影響

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pH 2.0 酸性溶液処理におけるスギ苗の根系衰退が，どのくらいの処理期間でどのように起きるかを明らかにするために，2 年生スギ苗を矢作砂を培地として砂耕栽培し，4 週間ごとの掘り取りによる根系調査を 12 週間行った。酸性溶液は，苗木の地上部と地下部の双方に暴露した。その結果，4 週間の酸性溶液処理において，pH 2 区における白根の乾重比は，対照区に比べすでに有意に低下しており，白根が太くかつ短くなる傾向が認められた。pH 2 区における根全体の表面積は，4 週間，8 週間の酸性溶液処理においては，対照区と有意差が認められなかったものの，12 週間の酸性溶液処理においては，対照区に比べて有意に減少した。本実験の結果から，pH 2.0 酸性溶液処理は，矢作砂の酸中和能力の低さのために根圏における低 pH や Al の影響を導き，白根の発育初期段階にさえ負の影響を与えることが明らかとなった。さらに酸性溶液処理期間が長くなるに伴って，その影響は根全体へと及ぶことが示唆された。

キーワード：酸性溶液，スギ，根表面積，処理期間，白根