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Growth Response to Acid Rain, Mg Deficiency and Al Surplus, and Amelioration of Al Toxicity by Humic Substances in Pitch Pine Seedlings

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The individual and combined effects of acidic rain, Mg deficiency (−Mg) and Al surplus (+Al) on the growth of shoots and roots of pitch pine seedlings and the effect of humic substances (Lit) on Al toxicity were investigated. The growth of height and dry matter were not significantly less for pitch pine seedlings sprayed with simulated acid rain (SAR) of pH 3.5 than for those sprayed with SAR of pH 5.6. But treatments of Al and +Al−Mg in soil solution reduced the growth of seedlings in terms of height of shoots, and dry matter of shoots or roots. Effect of Mg deficiency on the growth of seedlings was apparent only when Al was treated simutaneously. The growth of seedlings, regardless of rain pH, decreased in the following order: control= −Mg>Lit+Al> +Al> +Al−Mg. Treatments of Al and +Al−Mg in soil solution reduced the total length of secondary and tertiary roots of seedlings regardless of rain pH, and decreased in the following order: the primary root<the secondary root<the tertiary root. Total length of tertiary roots were the lowest in treatment of +Al−Mg. Total length of tertiary roots treated with Al was 47% of those of control. But total length of tertiary roots treated with Lit+Al was 90% of control. Decrease in the growth of seedlings by Al toxicity was significantly ameliorated by addition of humic substances such as litter extract.

Keywords: pitch pine, Al toxicity, Mg deficiency, simulated acid rain, amelioration of Al toxicity

During recent decades acidification has been accelerated in forest soils by acid precipitation (Rhyu, 1994). The soil acidification is accompanied by a loss of basic cations from the exchange pool (Rhyu and Kim, 1993a). Trees grown in acidic soil with low availability of basic cations may develop deficiencies as a result of reduced uptake and increased soil leaching (Rhyu and Kim, 1994a). As such, low Mg may be both a result of and a contributor to growth decline of pitch pine in metropolitan area of Seoul (Rhyu and Kim, 1994b). When pH in the soil solution falls below 4.5, in general, the concentration of Al increases exponentially. Ulrich (1980) hypothesized that forest decline was due to increasing levels of soluble aluminium in acidic soil. Acid precipitation may also impose a significant stress on plant nutrient relationships in a direct manner by increasing the rate of foliar leaching, which could results in reduced foliar nutrient concentrations (Rhyu and Kim, 1993b).

In metropolitan area of Seoul, it was reported that rains of strong acidity have been precipitating for a long time (Rhyu, 1994), and the soil acidification caused by acidic deposition, therefore, could lead to the growth decline of pitch pines (Rhyu *et al.*, 1994). Among factors of soil properties, low Mg contents and high Al contents in soils were selected as the primary and the secondary factors, respectively, causing recent decrease of annual ring widths of a pitch pine in Seoul metropolitan areas (Rhyu and Kim, 1994b).

The purpose of this study was to determine the individual and combined effects of acidic rain, Mg deficiency and Al surplus on the growth of shoots and roots of pitch pine seedlings, and to investigate

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the effect of humic substances on Al toxicity to plant growth.

MATERIALS AND METHODS

Pitch pine (*Pinus rigida* Mill.) cones were collected at Mt. Kwanak, Seoul, Korea, in the fall of 1991 and seeds were germinated in vermiculite in early April, 1992. Nine seedlings were transferred from vermiculite to acid washed sand in a pot (15 cm×15 cm×15 cm) in early May and then grown in greenhouse *in situ*. All seedling roots were cut at 1 cm from the end of tap root to stimulate lateral root production before transplantation. They were sufficiently watered with a nutrient solution (control) containing: 1.5 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 0.5 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 50 μM KCl, 25 μM H_3BO_4, 2 μM $MnCl_2 \cdot 4H_2O$, 2 μM $ZnCl_2$, 0.5 μM $CoCl_2 \cdot 6H_2O$ and 20 μM FeNa-EDTA (Cumming and Weistein, 1990), and treatment solutions which consisted of 100 μM Al (+Al), Al and Mg deficiency (+Al−Mg), Al+litter extract (Lit+Al) and Mg deficiency (−Mg) (Rhyu, 1994). One hundred μM Al is a solution containing 100 μM Al as Al_2SO_4 added to control nutrient solution which was outlined above except with $NH_4H_2PO_4$ at 0.05 mM, KNO_3 at 1.0 mM, and NH_4NO_3 at 0.5 mM to reduce precipitation reactions between Al and P. Mg deficiency is a solution containing an amount of Mg of a twentieth part compared with control nutrient solution. Al and Mg deficiency is a solution containing 100 μM Al and amount of Mg of a twentieth part compared with control nutrient solution. Al+litter extract (humic substance) is a solution mixing ½ control solution containing 100 μM Al with ½ litter extract which was filtered after placing at 28℃ for 10 d after mixing 500 g of air-dried pine litter with 10 L of distilled water. All solutions were adjusted to pH 4.1 with H_2SO_4 (Cumming and Weistein, 1990). Watering was carried out at every 2 d until late September, 1992.

The pH of simulated acid rain (SAR) was adjusted to pH 3.5 by adding H_2SO_4 and HNO_3 in a 3 : 1 equivalent ratio to distilled water. The pH of control rain was adjusted to pH 5.6 by dissolving CO_2 to distilled water.

Seedlings were soaked completely with SAR by hand sprayer for 30 min at 9 AM. These procedures were carried out every 2 d from May to September. Total design comprised 10 treatments (five soil treatments×two rain treatments). Each treatment consisted of 36 seedlings.

The heights of seedling shoots were measured at an interval of 20 d from May to September, 1992. In late September when the growth rate decreases, seedlings were harvested carefully in order not to be injured, and sands stuck to roots were removed completely by tap water. Roots were measured by a vernier caliper after dividing them into three groups: 1) the tap root as the primary root, 2) roots sprouting from the primary (tap) root as the secondary root, 3) roots sprouting from the secondary roots as the tertiary root. Then length of roots over 1 cm at each group was summed up. The roots below 1 cm among roots sprouting from the secondary root were measured after regarding as the root tip. Then shoots and roots were weighed after drying in a 80℃ dry oven for 48 h.

RESULTS

Height growth of seedlings

At the end of growing season, height growth in control soil was not significantly less for pitch pine seedlings sprayed with SAR of pH 3.5 than for those sprayed with SAR of pH 5.6 (Fig. 1).

Height growth in soil treated with +Al−Mg, however, was reduced a little for seedlings sprayed with SAR of pH 3.5 compared with those sprayed with SAR of pH 5.6, but the difference was not statistically significant. Acidity of rain had a little direct effect on the decrease of height growth of pitch pine seedlings. But treatments of Al and +Al−Mg in soil solution reduced the height growth of seedlings (Fig. 1). Although Mg deficiency treatment did not reduce the height growth of seedlings, effect of Mg deficiency treatment on the seedlings was apparent when Al was treated simultaneously, *i.e.* the height of seedlings measured was the lowest in treatment of +Al−Mg at the end of growing season. The height growth of seedlings regardless of treated rain pH decreased in the following order: control=−Mg>Lit+Al>+Al>+Al−Mg. Decrease of the height growth of seedlings by Al toxicity was significantly ameliorated by the addition of humic substances such as litter extract.

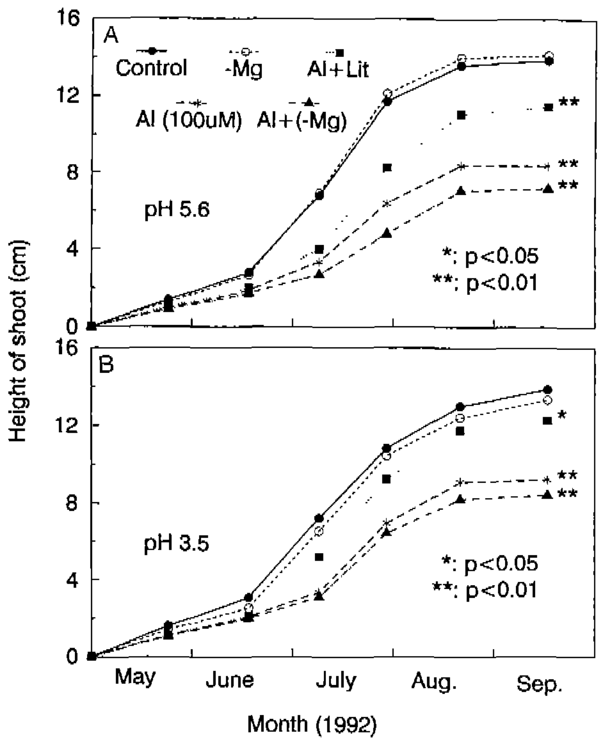

Fig. 1. Seasonal changes in shoot height of pitch pine seedlings sprayed with SAR of pH 5.6 (A) or pH 3.5 (B) for 5 months in sand culture with varying treatments in greenhouse. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al +Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

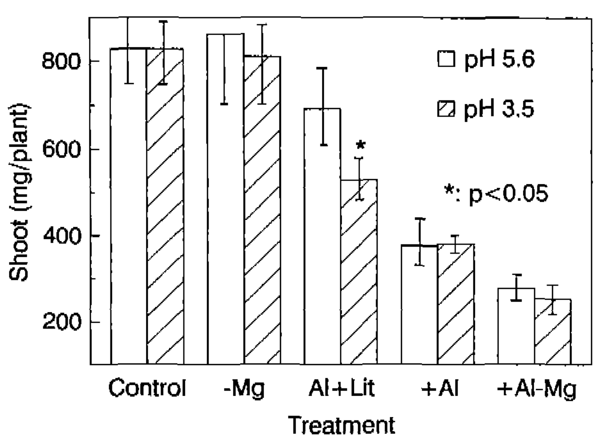

Fig. 2. Comparison of shoot dry matter of pitch pine seedlings grown by spraying with SAR of pH 5.6 or pH 3.5 for 5 months in sand culture with varying treatments. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

Dry matter of shoots

The dry matter of shoots in −Mg, Lit+Al and −Mg+Al soil were less for pitch pine seedlings sprayed with SAR of pH 3.5 than for those sprayed with SAR of pH 5.6 (Fig. 2). Treatments of Al and +Al−Mg in soil solution significantly reduced the dry matter of shoots (Fig. 2, Table 1).

However, effects of Mg deficiency on decrease of dry matter were apparent only when Al was treated

Table 1. Matrix of probabilites in ANOVA among shoot dry matter with various treatments. (A) is a result of treatment of pH 5.6 SAR and (B) is a result of treatment of pH 3.5 SAR

(A)

	Spraying with pH 5.6 SAR				
	Control	−Mg	Al+Lit	+Al	+Al−Mg
Control	1.000				
−Mg	0.994	1.000			
Al+Lit	0.231	0.104	1.000		
+Al	0.000	0.000	0.000	1.000	
+Al−Mg	0.000	0.000	0.000	0.553	1.000

(B)

	Spraying with pH 3.5 SAR				
	Control	−Mg	Al+Lit	+Al	+Al−Mg
Control	1.000				
−Mg	1.000	1.000			
Al+Lit	0.000	0.000	1.000		
+Al	0.000	0.000	0.000	1.000	
+Al−Mg	0.000	0.000	0.000	0.253	1.000

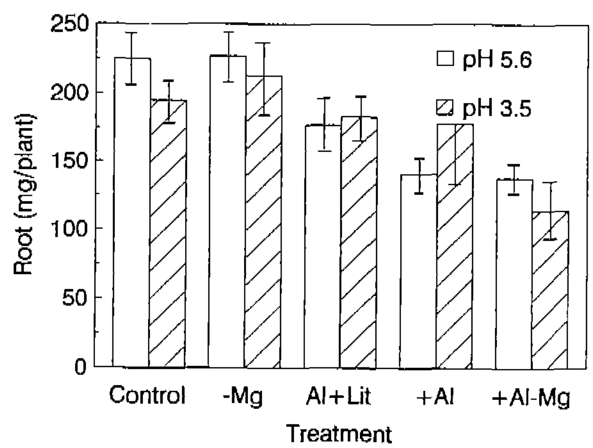

Fig. 3. Comparison of root dry matter of pitch pine seedlings grown with spraying of SAR of pH 5.6 or pH 3.5 of SAR for 5 months in sand culture with varying treatments. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

Table 2. Matrix of probabilites in ANOVA among root dry matter with various treatments. (A) is a result of treatment of pH 5.6 SAR, and (B) is a result of treatment of pH 3.5 SAR

(A)

	Spraying with pH 5.6 SAR				
	Control	−Mg	Al+Lit	+Al	+Al−Mg
Control	1.000				
−Mg	1.000	1.000			
Al+Lit	0.173	0.121	1.000		
Al	0.003	0.002	0.458	1.000	
Al+(−Mg)	0.002	0.001	0.400	1.000	1.000

(B)

	Spraying with pH 3.5 SAR				
	Control	−Mg	Al+Lit	+Al	+Al−Mg
Control	1.000				
−Mg	1.000	1.000			
Al+Lit	0.803	0.713	1.000		
Al	0.657	0.555	0.999	1.000	
Al+(−Mg)	0.002	0.001	0.028	0.051	1.000

−Mg, Mg deficiency; Al+Lit, 100 μM Al plus litter extract; +Al, 100 μM Al; +Al−Mg, 100 μM Al plus Mg deficiency.

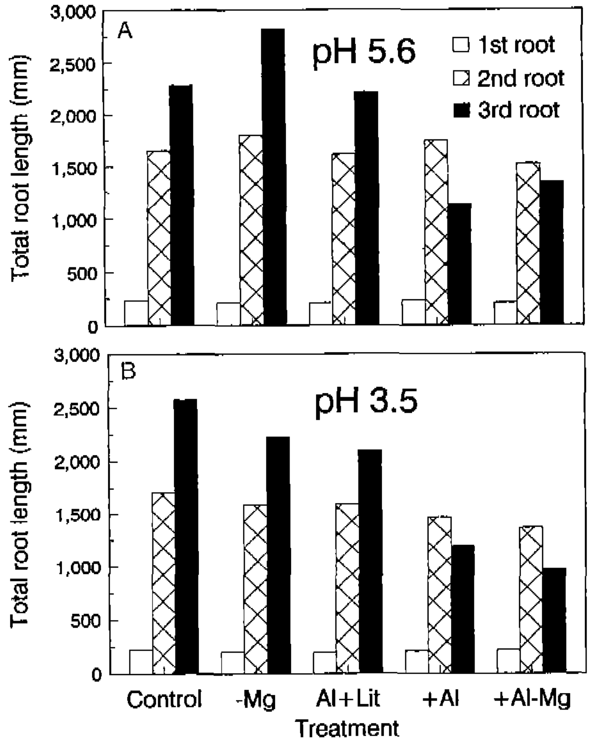

Fig. 4. Comparison of total length of the primary, secondary and tertiary roots of pitch pine seedlings grown with spraying of SAR of pH 5.6 (A) and pH 3.5 (B) for 5 months in sand culture with varying treatments. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

simultaneously. Dry matter of shoots regardless of treated rain pH decreased in the following order: control= −Mg>Lit+Al≫Al> +Al−Mg. Decrease of dry matter of shoots by Al toxicity was significantly ameliorated by addition of humic substances (Table 1).

Dry matter of roots

There was no significant difference in dry matter of roots of seedlings grown in control soils treated with SAR of pH 5.6 and pH 3.5 (Fig. 3). Acidity of rain had no significant effect on dry matter of roots at all ionic treatments. But treatments of Al and +Al−Mg in soil solution significantly reduced the dry matter of roots (Fig. 3, Table 2).

Dry matter of roots regardless of rain pH decreased in the following order: control= −Mg>Lit+Al > +Al≫ +Al−Mg. Dry matter of roots in treatment of +Al−Mg was especially low. Decrease of dry matter of roots by Al toxicity was significantly ameliorated by the addition of humic substances (Table 2).

Total length of roots

Acidity of rain had no significant effect on total length of roots (Fig. 4). Treatments of Al and +Al−Mg in soil solution, however, reduced the total length of the secondary and the tertiary roots of seedlings regardless of rain pH, and decreased in the following order: the primary root<the secondary root<the tertiary root (Fig. 4).

Total length of the tertiary roots was the lowest in treatment of +Al−Mg. Total length of the tertiary roots treated with Al were 49 and 46% at conditions of pH 5.6 and pH 3.5, respectively, compared with those of control. But total length of the tertiary roots treated with Lit+Al were 97 and 82% of controls, respectively. Ratio of the secondary roots to the tertiary roots regardless of rain pH decreased in the following order: control= −Mg>Lit+Al≫ +Al= +Al−Mg (Fig. 5).

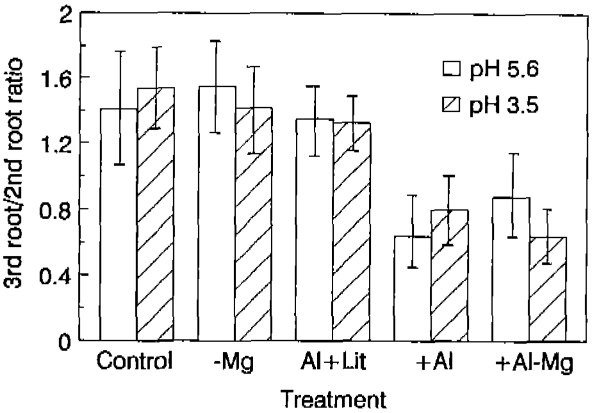

Fig. 5. Comparison of ratios of secondary root length to tertiary root length of pitch pine seedlings grown with spraying of SAR of pH 5.6 and pH 3.5 for 5 months in sand culture with varying treatments. Seedlings were grown under conditions with control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

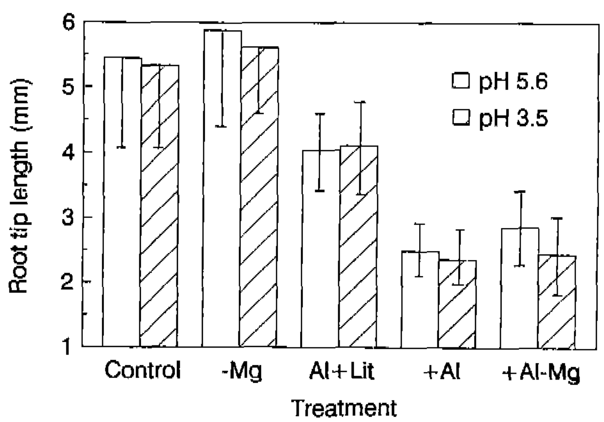

Fig. 6. Comparison of root tip length of pitch pine seedlings grown with spraying of SAR of pH 5.6 and pH 3.5 for 5 months in sand culture with varying treatments. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

Length of root tips

SAR had no effect on the growth of length of root tips at all ionic treatments (Fig. 6). But treatments of Al and +Al−Mg in soil solution significantly reduced the length of root tips (Fig. 6). Length of root tips regardless of rain pH decreas-ed in the following order: control = −Mg > Lit+Al ≫ +Al = +Al−Mg. The length of root tips decreased by Al was significantly ameliorated by the addition of humic substances.

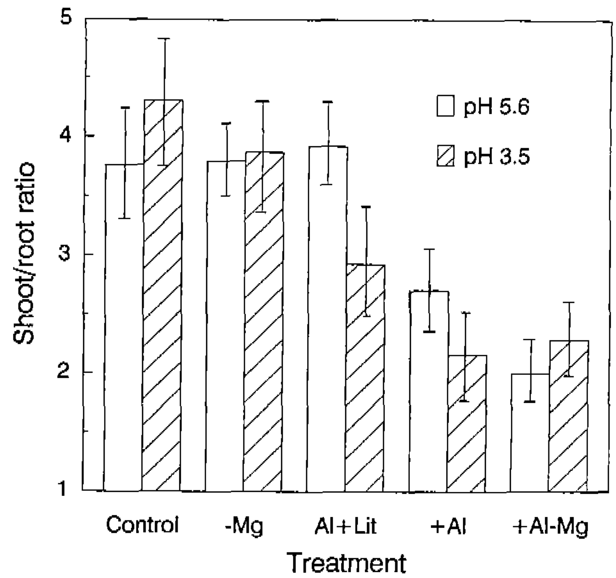

Fig. 7. Comparison of shoot/root ratios in dry matter of pitch pine seedlings grown with spraying of SAR of pH 5.6 or pH 3.5 for 5 months in sand culture with varying treatments. Seedlings were grown under conditions of control, Mg deficiency (−Mg), 100 μM Al plus litter extract (Al+Lit), 100 μM Al (Al) and 100 μM Al plus Mg deficiency (Al−Mg).

Shoot to root ratio

High acidity of rain reduced shoot/root ratio in seedlings grown at soil solutions containing Lit+Al, Al and +Al−Mg (Fig. 7). High acidity of rain reduced the growth of shoots more than that of roots, and reduced the growth of seedlings in infertile soil more than in fertile soil. Treatments of Al and +Al−Mg in soil solution reduced the ratio of shoot to root in seedlings at pH 5.6 (Fig. 7). Decrease of shoot/root ratio in seedlings by Al toxicity was significantly ameliorated by the addition of humic substances.

DISCUSSION

Direct effect of aicd rain on plant growth

The growth of pitch pine seedlings grown at complete nutrient solution was not significantly decreased by the treatment with SAR of pH 3.5 for 5 months (Figs. 1 and 2). The fact that there was no direct effect of acid rain on plant growth was in accordance with the result by Edwards *et al.* (1991). On the contrary, the increase of growth rate in *Picea abies* and *Pinus taeda* by treatment of acid rain were reported by Lee *et al.* (1990) and Wright *et al.* (1990/1991). The increase of growth rate in plants by treatment of acid rain was thought to be due to the increase of inorganic nutrients, especially, nitrogen through rain, and by the activation of insoluble ions through a soil acidification (Aber *et al.*, 1982; Tveite *et al.*, 1990/1991). Plant growth, therefore, might be promoted early in fertile soil by acid rain. Although plants were damaged directly by acid rain, the damage of plants can be compensated through an increase of ion absorption from fine roots in soil. The growth of plant growing in soil treated with +Al−Mg, however, was reduced a little by treatment of acid rain of pH 3.5 (Fig. 2). The direct effect of acid rain on plant growth was evident when plants did not absorb sufficient nutrients in infertile soil. As soil is acidified by acid precipitation, basic cations decrease, while Al simultaneously increases (Ulrich, 1980; Rhyu and Kim, 1993a). Therefore, the growth of plants in infertile soil caused by acid precipitation will be still worse by the direct effect of acid rain. The direct effects of acid rain on plant growth were reported as the ion leaching through leaves (Rhyu and Kim, 1993b), the increase of transpiration through epicuticular degradation (Mengel *et al.*, 1989; Rhyu, 1994) and the decrease of photosynthetic rate (Sigal and Johnston, 1986), and these effects can be amplified by treating with ozone simultaneously (Reich and Amundson, 1984; Lee *et al.*, 1990).

Al Toxicity and Mg deficiency to plant growth

The height growth, dry matter and root length of pitch pine seedlings were significantly decreased by the treatment of 100 μM Al (Figs. 1-4) (Hutchinson *et al.*, 1986). The 100 μM Al used in this experiment was the average concentration measured in soil solution of pitch pine forests at Mt. Kwanak from July, 1991 to April, 1993 by Rhyu (1994). The growth reduction by Al toxicity appeared in *Pinus rigida* over 75 μM Al (Cumming and Weistein, 1990), in *Pinus taeda* between 150-1500 μM Al, in *Picea rubens* between 200-250 μM Al (Cronan *et al.*, 1989), in *Picea abies* over 37-74 μM Al (Balsberg Pahlsson, 1990). On the whole, the plant growth was reduced over 50 μM Al, which existed naturally in soil solution of pitch pine forests in metropolitan area of Seoul. Therefore, pitch pines in polluted Seoul and its vicinity must be suffered by Al toxicity. Metabolic aspects of Al toxicity were reported in detail by Clarkson (1969) and Cronan *et al.*(1990).

Contrary to Al, pitch pine seedlings treated with Mg deficiency exhibited no growth reduction, and no Mg deficiency symptom was observed during growing season. Mg deficiency treatment used in this study was probably not low enough to induce a Mg deficiency symptom in the seedlings. The same result as ours was reported by Edwards *et al.* (1991). Although seedlings treated with Mg deficiency during growing seasons showed no growh reduction, they showed low Mg content in tissues of plants (Edwards and Mays, 1992). The effect of Mg deficiency on plant growth was exhibited by simultaneous Al treatment, *i.e.* Al toxicity to pitch pine seedlings was significantly amplified by treatment of Mg deficiency. Therefore, the cause of growth decline of pitch pines in metropolitan area of Seoul was proved to be the deficiency of Mg content and the increase of soluble Al by soil acidification.

Recovery from Al toxicity

Fine roots of pitch pines were reported more in litter layer but less in soil layer in acidic soil of metropolitan areas of Seoul as compared with fine roots at unpolluted rural areas by Rhyu *et al.* (1994). The abnormal vertical distribution of fine roots in stands of forest decline was repoted by Mazner *et al.* (1986), Ulrich (1990) and Schneider and Zech (1990/1991). The little amount of fine roots in acidic soil layer was thought to be caused by Al toxicity, and the higher amount of fine roots in litter layer might be due to the reduction of Al toxicity by humic substances. This assumption was verified by the result that the growth reduction of fine root by Al toxicity was almost recovered by the treatment of litter extract containing humic substances (Figs. 3, 4, 6). This result was in accordance with the result of Suthipradit *et al.* (1990), reporting that treatment of fulvic acid reduced Al toxicity to soybean and cowpeas. Kretzeschmar *et al.* (1991) also reported that the soluble Al content of soil was decreased by the treatment of millet litter both in the field and in the laboratory. The mechanism of resistance to Al toxicity is related to a variety of factors including the production of varying amounts of humic substances, changes in root surface pH, excretion of surface mucilage or differential effects on membrane and transport processes (Horst *et al.*, 1990).

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酸性 빗물, Mg 缺乏, Al 過剩에 대한 리기다소나무 幼植物의 生長 反應과 腐植酸에 의한 Al 毒性의 緩和

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적 요

빗물의 산도, Mg 결핍, Al 과잉이 리기다소나무 유식물의 생장에 미치는 영향과 낙엽 추출액에 의한 리기다소나무 유식물에 미치는 Al 독성의 완화에 대하여 조사하였다. pH 3.5의 빗물을 5개월 동안 맞은 리기다소나무 유식물은 키와 건물량(지상부와 지하부)에서 정상 빗물을 맞은 것에 비해 작은 경향이 있었으나 통계적으로 유의하지 않았다. 그러나 영양용액에 100 μM Al 처리구와 100 μM Al+Mg 결핍 처리구에서 유식물의 생장이 급격히 감소하였다. Mg 결핍구에서는 유식물의 생장이 대조구의 것에 비해 감소되지 않았으나, 100 μM Al+Mg 결핍구에서 유식물의 생장은 100 μM Al 처리구에 비해 억제되었다. 즉 유식물의 생장은 여러 무기이온 처리에 대해 대조구=Mg 결핍구>낙엽추출액+100 μM Al 처리구>100 μM Al 처리구>100 μM Al+Mg 결핍 처리구 순으로 억제되었다. 빗물의 산도에 관계없이 100 μM Al 처리구와 100 μM Al+Mg 결핍 처리구에서 유식물의 2차근과 3차근의 생장은 크게 감소하였으며, 특히 유식물의 3차근이 크게 억제되었다. 100 μM Al 처리구의 3차근의 총 길이는 대조구의 것에 비해 47%이었으나, 낙엽추출액+100 μM Al 처리구에서는 90%까지 잔뿌리의 생장이 회복되었다. 즉 Al에 의한 리기다소나무 유식물의 생장 억제는 낙엽 추출액에 의해 지상부 및 지하부 모두에서 크게 완화되었다.

주요어 : 리기다소나무, Al 독성, Mg 결핍, 인공 산성 빗물, Al 독성의 완화

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