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Effects of Simulated Acid Rain on Histology, Water Status and Growth of *Pinus densiflora*

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인공산성빗물이 소나무의 조직, 수분수지 및 생장에 미치는 영향

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ABSTRACT

To clarify the effects of acid precipitates on histological damage, water status, and growth of *Pinus densiflora* green house experiment applying simulated acid rain was carried out.

Contact angle of water droplet on needles of *P. densiflora* seedlings treated with simulated acid rain of different pHs decreased with the increase of acidity and the amount of simulated acid rain. The lower pH of simulated acid rain was, the more rapid transpiration was. Leaf water potential after water withdrawal was also reduced rapidly in proportion to acidity of simulated acid rain. Height growth of *P. densiflora* seedlings treated with simulated acid rain of pH 2 decreased, while growth of seedlings treated with that of pH 3 and 4 increased comparing with that treated with normal rain of pH 5.6. pH of cultivated soil in pH 2 plot was acidified with the amount of simulated acid rain applied but that in pH 3 and 4 plots did not show any directional change. From those results, it could be interpreted that decrease of height growth in pH 2 plot was originated from multiple effects of water deficit from rapid transpiration and soil acidification. On the other hand, increase of height growth in pH 3 and 4 plots would be originated from the supply of N and S included in simulated acid rain.

Key words: *Pinus densiflora*, Histological damage, Water status, Simulated acid rain, Soil acidification.

INTRODUCTION

Forest decline syndromes have increased dramatically throughout the world since early 1980s (Krause *et al.* 1986, Nilsson and Duinker 1987). Hypotheses postulating several causes as causal factor of forest decline have been suggested (Freedman 1986, Smith 1990). Major cause of forest decline was not clearly known yet, but acid precipitates has been thought as one of important causal factor of forest decline (Zottle *et al.* 1989, Ulrich 1990). Especially plants with cuticle damaged by acid precipitates are affected in their resistance against water stress during drought seasons (Mengel *et al.* 1989). Moreover increased Al in acidified soil aggravate water status of plants through inhibition of fine root and abnormal distribution of root system ((Truman *et al.* 1986, Bengtsson *et al.* 1988, Roy *et al.* 1988, Cronan *et al.* 1989, Rhyu and Kim 1994d, Lee 1997). In this way acid precipitates could be involved directly or indirectly in forest decline.

In an investigation on *P. densiflora* grown in the Metropolitan area of Seoul epicuticular degradation on needle leaves, rapid transpiration of needles and growth inhibition of annual ring were reported (Lee *et al.* 1998). Also structural change, such as retrogression of succession might be induced by decline in vitality of *P. densiflora* and functional one like soil acidification were investigated in that area (Lee 1996, Lee *et al.* 1998). Soil acidification and histological damage of needle leaves would be caused by continuous falling of acid precipitates. On the other hand, growth inhibition and decline in vitality of *P. densiflora* were suspected to the water deficit by 1) rapid transpiration through epicuticular injury by acid precipitates, and 2) deterioration of water absorption capacity through growth inhibition or abnormal distribution of fine roots in acidic soil (Rhyu 1994, Lee 1997).

The purpose of the present study is to prove experimentally the results obtained in field investigation (Lee *et al.* 1998). Simulated acid rain (Lee and Weber 1979, Riding and Percy 1985, Rinall *et al.* 1986, Rhyu and Kim 1993a,b, 1994c,d) and acid fog (Mengel *et al.* 1989, Kim and Um 1996) have been generally applied in experimental studies assumed environmental conditions associated with forest decline. Acids in precipitates cause injury on leaf surface of plants (Evans *et al.* 1977, Mengel *et al.* 1989) and acidification of soil. It is believed that damage of epicuticular layer originated from those acid precipitates should have an influence on the holding of water and the growth of plants. Thus the present study focused on the questions as to whether the water holding capacity and the height growth of Pinus densiflora seedlings would be affected after they had received a treatment with simulated acid rain. During the experiment, contact angle, transpiration, water potential, height growth, and pH of cultivated soil were, therefore, measured.

METHODS

Simulated acid rain was prepared by mixing H_2SO_4 and HNO_3 in a 3:1 equivalant ratio to the distilled water. pH of simulated acid rain was adjusted into 2, 3 and 4. Normal rain of pH 5.6 was adjusted by dissolving CO_2 in distilled water. Each treatment plot consisted of 5 replicates with 9 seedlings per pot. Seeds of *P. densiflora* were obtained from Forest Genetics Korean Institute for Breeding of trees. Current year seedlings were used as sample plants. Cultivation of sample plants was continued for 2 months in the greenhouse experiment. Simulated acid rain was sprayed everyday by 50 ml during cultivation. Contact angle of water droplet dropped by microsyringe on the needle leaf was measured by graduator installed in dissecting microscope (Boyce and Berlyn 1988). Amount of water used to measure contact angle was 1 μl. Cuticular transpiration was determined by measuring the water loss that occurred from excised shoots in incubator condition of 25℃ (Mengel *et al.* 1989). Shoots were sampled from 5 seedlings. Shoot was cut off just below cotyledons and cut surface was immediately covered with parafilm to prevent water loss from this surface. Shoots were weighed with a chemical balance and loss of weight was regarded as water loss. Water potential was determined by Shardakov method (Barrs 1968).

Height of seedlings was determined by the length from the soil surface to apical part of seedling. Soil pH was analysed at 1 week interval for 6 weeks from the 4th week after treatment of simulated acid rain. Surface soil in the cultivation pot was used as sample soil. pH was measured by pH meter after shaking soil mixed with distilled water by ratio of 1:5 for 1 hour.

RESULTS AND DISCUSSION

Histological damage on needle leaves

Contact angle on needles of *P. densiflora* seedlings was measured after spray of simulated acid rain of 750 ml, 3,000 ml and 6,000 ml (Fig. 1). Contact angle prior to treatment of simulated acid rain was $79 \pm 2.0°$ in all the plot. Contact angles in pH 2 plots were $70 \pm 2.0°$ $56 \pm 8.0°$ and $45 \pm 4.0°$ after treatment of simulated acid rains of 750 ml, 3,000 ml, and 6, 000 ml, respectively and those in pH 3 plots showed $72 \pm 2.5°$ $59 \pm 8.0°$ and $50 \pm 3.0°$ and those in pH 4 plots showed $75 \pm 3.2°$ $64 \pm 8.0°$ and $58 \pm 3.2°$ respectively. Differences between previous contact angle to treatment of simulated acid rain and those after

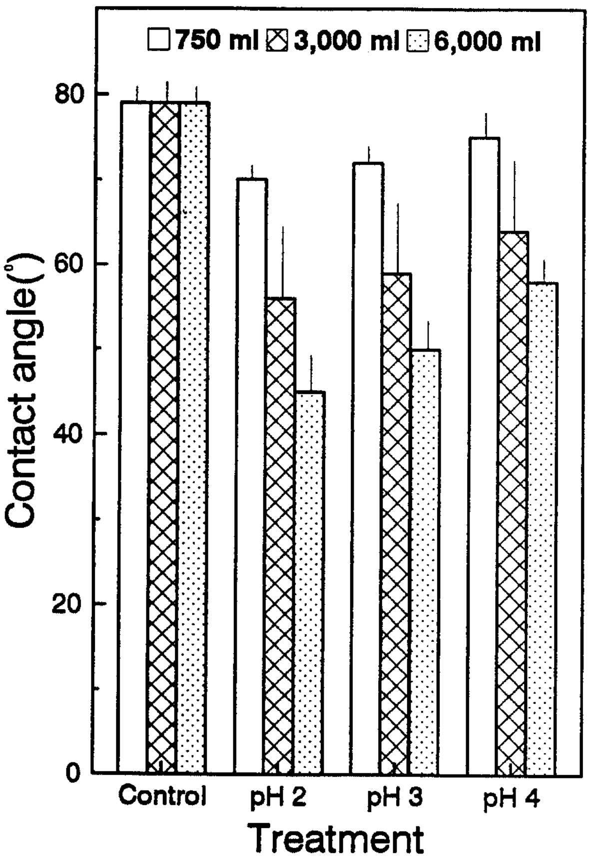

Fig. 1. Contact angle of water droplet on the surface of needle leaves of *Pinus densiflora* seedling treated with simulated acid rain different in pH and amount.

treatment were significant at 1% level. Differences among treatment plots different in the amounts of simulated acid rain were also significant at 1% level. But differences among treatment plots different in pH of simulated acid rain was not significant. Contact angle of water droplet on plant leaf is an index generally used to detect initial symptom of air pollution damage as degree of wettability on epicuticular wax layer (Boyce and Berlyn 1988, Barnes and brown 1990). Steubing and Fangmeier (1991) showed that contact angle on plant leaves exposed to mixed gas of SOx, O3 and NOx was lower than that exposed to single state of above mentioned gases. Kim *et al.* (1994) clarified that contact angle was proportional to visible damage and inversely to the wax content of needles by analysing the relationships between contact angle and plant damage in study areas different in distance and topography from the pollution source in Daesan industrial complex. Lee *et al.* (1998) confirmed that contact angle on needles of *P. densiflora* grown in polluted area around the Metropolitan area of Seoul was lower than that in unpolluted area.

Transpiration rate

Regression coefficients in equations between the lapsed time after excision of shoot and water loss showed −0.29, −0.28, and −0.23 in pH 2, pH 3 and pH 4 plots, respectively and that in control plot was −0.17 (Fig. 2). That is, the lower pH of simulated acid rain was, the more rapid transpiration was. Mengel *et al.* (1989) showed that transpiration rate of plant leaves exposed to acid fog was more rapid than that of control plot. Rhyu and Kim (1994d) reported that transpiration rate of pitch pine treated with simulated acid rain was more rapid than that treated with normal rain. These results suggest that transpiration rate is closely related to the degree of pollution damage.

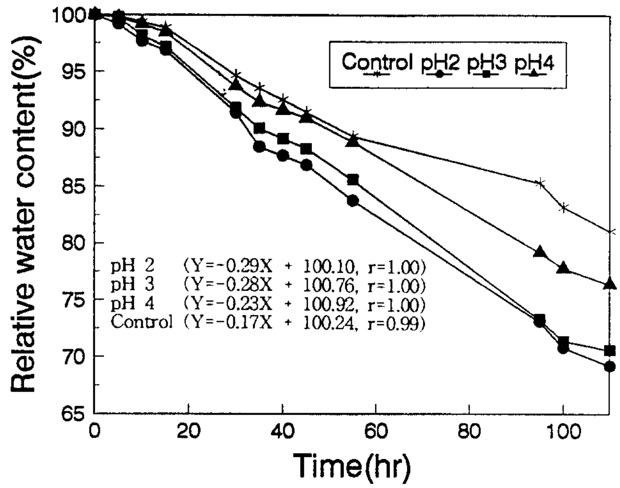

Fig. 2. Changes of relative water content of shoot with the lapse of time after excisement in *Pinus densiflora* seedling treated with simulated acid rain of different pH.

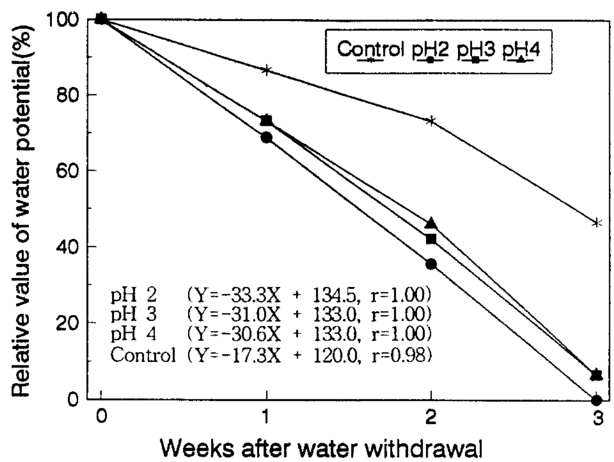

Fig. 3. Changes of relative value of water potential of needle leaves since water withheld in *Pinus densiflora* seedling treated with simulated acid rain of different pH.

Response on water stress

The response on water stress of *P. densiflora* seedlings treated with simulated acid rain of different pH was measured by changes of leaf water potential after water withdrawal (Fig. 3). Water potential was shown in percentage between maximum water potential and minimum one measured during experimental periods. Water potential percentage in pH 2 plot showed 100, 69, 36 and 0% before water withdrawal, in 1, 2, and 3 weeks after water withdrawal, respectively. Those in pH 3, pH 4 and control plots showed 100, 73, 42 and 7%, 100, 73, 46 and 7% and 100, 87, 73 and 47%, respectively. Regression coefficients from equations showing the reduction of water potential after water withdrawal was −33.3, −31.0, −30.6, and −17.3 in pH 2, pH 3, pH 4 and control plots, respectively. That is, the lower pH of simulated acid rain was, the more rapid decrease of water potential percentage after water withdrawal was.

These results suggest that acid rain can induce water stress in plants during the drought season. In fact, Mengel *et al.* (1989), Lee *et al.* (1990a,b), and Rhyu and Kim (1994d) reported that acid precipitates imposed severer water stress on sample plants. These results are also closely related to results on leaf tissue injury measured by contact angle and transpiration rate above mentioned. That is, acid rain made plant leaf injure and injury in leaf tissue induces rapid transpiration and decline of water potential. Water potential is closely related to plant growth (Lee and Kim 1987, Salisbury and Ross 1992) and decrease of water potential become a crucial factor inducing forest decline in the area of polluted air (Omasa *et al.* 1985, Pierre and Queiroz 1988, Mengel *et al.* 1989, Rhyu and Kim 1994d).

Plant growth

Heights of 1 year-old *P. densiflora* seedlings of exposed to simulated acid rains for 60days were 5.5 ± 0.5, 7.0 ± 0.3, and 6.1 ± 0.3cm in pH 2, pH 3, and pH 4 plots, respectively and that of control plot was 5.8 ± 0.3 cm (Fig. 4). Percentages of growth in each experimental plot to that in control plot were 95%, 121%, and 105% in pH 2, pH 3, and pH 4 plots, respectively. Comparing with control plot any other experimental plots excluding pH 3 plot did not show significant differences in height growth of *P. densifl-*

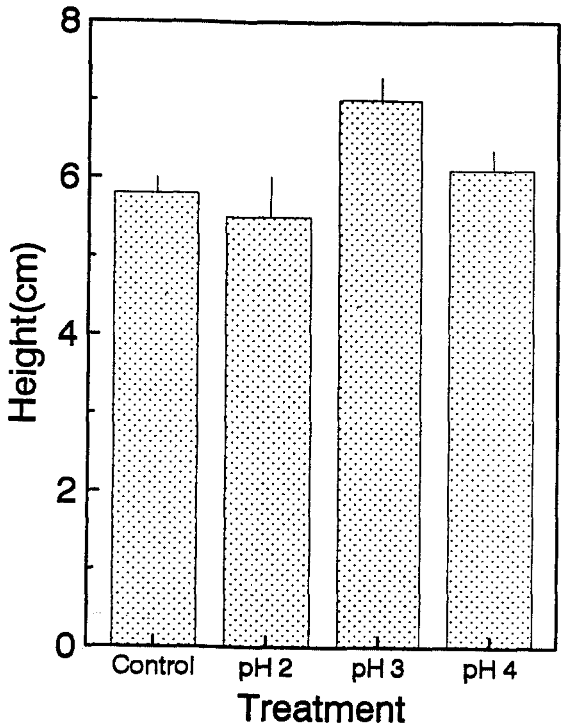

Fig. 4. Height of *Pinus densiflora* seedlings treated with simulated acid rain of different pH.

ora seedlings. Even though so growth of seedlings in all experimental plots excluding pH 2 plot was higher than that in control plot. These results might occur from that N and S included in simulated acid accelerated seedling growth. In fact, short-term treatment of simulated acid rain accelerated plant growth and those results reported by many scientists (Freedman 1986). On the other hand, height of seedlings grown in plot treated with simulated acid rain of pH 2 was lower than that in control plot. Those growth inhibition would be interpreted as synergic effect occured from combination of direct effect of acid precipitates and indirect effects related to soil acidification (Rhyu and Kim 1994a,b,c,d, Lee 1997).

pH of cultivated soil

Changes of soil pH measured at 1 week interval from the 4th week after treatment of simulated acid rain were shown in Fig. 5. Soil pHs in pH 2, pH 3,

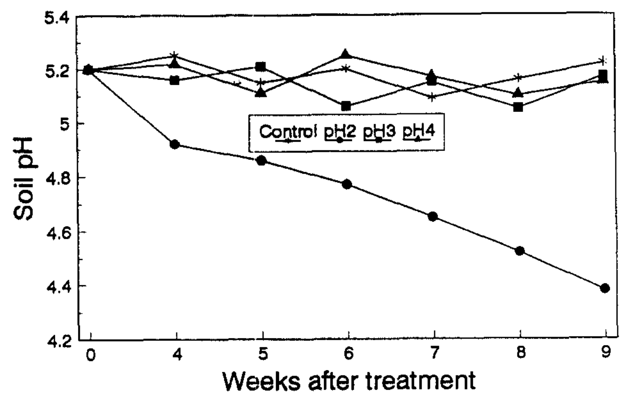

Fig. 5. Changes of pH in pot soil that *Pinus densiflora* seedlings were cultivated with the lapse of time after treatment with simulated acid rain.

pH 4 and control plots maintained equilibrium state with some fluctuation but that in pH 2 plot decreased from pH 5.2 before treatment of simulated acid rain to pH 4.4 after treatment.

Studies through resampling of soil in the same location after a widely spaced time interval and a comparison of areas receiving a large or a small depositions of acidifying substances showed that soil acidification occurred from acidic deposition (Tamm and Hallbacken 1988). On the other hand, as a result of application of acidic solution in the short term, a notable acidifying effect in soil only occurred when highly acidic solution below pH 3 was used (Freedman 1986). In acidified soil like this, phenomena such as, deficiency of basic cations, Al toxicity, ion imbalance, and so on known as causing forest decline appeared (Freedman 1986, Smith 1990) From those results, growth inhibition in pH 2 plot could be interpreted as synergic effect occured from combination of direct effect of acid precipitates inducing water deficit and indirect effects related to soil acidification (Rhyu and Kim 1994a,b,c,d, Lee 1997).

CONCLUSION

Results obtained from this study were summarized in Fig. 6. As was shown in Fig. 6, acid precipitates

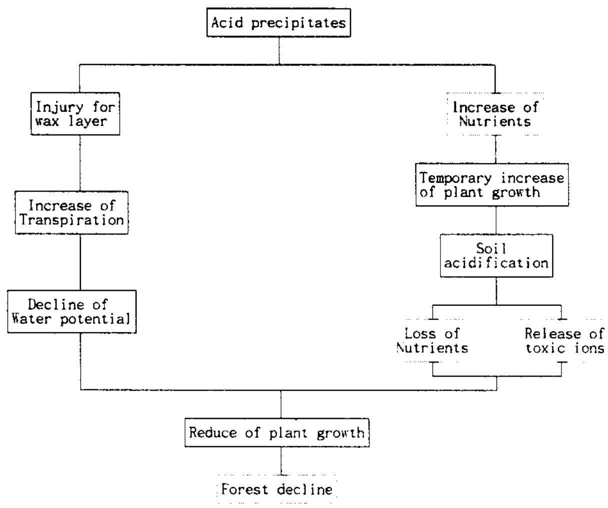

Fig. 6. Diagram showing the mechanism of forest decline caused by acid precipitates. Solid boxes indicate measured results and dotted boxes show predicted ones.

can affect both plants and soil. Their effects on plants result in injury for wax layer. In this study we confirmed that contact angle on needles of *P. densiflora* seedlings in plot treated by simulated acid rain was lower than that on needles before treatment. Reduction of contact angle tended to proportional to the amount or pH of simulated acid rain sprayed. On the other hand, evidence on wax layer injury was also found in water status. For example, transpiration and reduction in water potential of needles after water withdrawal in needles treated with simulated acid rain was more rapid than those in needles treated by normal rain.

Similar results were also obtained in SO_2 exposure experiment (Lee and Bae 1991). Decrease of water potential by these serial processes might be operated to inhibit growth considering that plant growth is closely related to water potential (Lee and Kim 1987, Salisbury and Ross 1992). Acid precipitates operate as an inhibition factor in plant growth as above mentioned, though plant growth in the present experimental study treated by simulated acid rain of pHs 3 and 4 was better than that in control plot, which normal rain was applied. These results might be originated from the effects of N and S included in simulated acid rain (Freedman 1986). But plant growth in plot treated with simulated acid rain of pH 2 was lower than that in control plot. The cause of this difference can be found in change of soil properties considering that deficiency of basic cations, Al toxicity, ion imbalance and so on appear in acidified soils (Freedman 1986, Smith 1990). In fact, pH of cultivated soil in pH 3 and pH 4 plots maintained equilibrium state without directional change but that in pH 2 was more acidified after treatment of simulated acid rain. Such a growth inhibition can be, therefore, interpreted as synergic effect occurred from combination of direct effect of acid precipitates and indirect effects related to soil acidification (Rhyu and Kim 1994a,b,c,d, Lee 1997).

적 요

온실조건에서 소나무 유식물을 대상으로 인공산성빗물을 처리한 실험을 통하여 산성강하물이 식물에 미치는 영향을 밝혔다. 침엽상에서 물방울의 접촉각은 처리한 인공산성빗물의 pH가 낮고 처리량이 많을수록 낮았다. 증산속도와 수분공급 차단 후 수분포텐셜의 감소속도는 처리한 인공산성빗물의 양과 pH에 비례하는 경향이었다. pH 2의 인공산성빗물을 처리한 유식물의 높이생장은 대조구와 비교하여 감소하였으나 pH 3과 4의 인공산성빗물을 처리한 것은 오히려 증가하였다. 전자의 경우 재배토양의 pH는 크게 낮아졌으나 후자의 것은 뚜렷한 변화를 나타내지 않았다. 이러한 결과로부터 전자의 구에서 생장감소는 수분결핍과 토양산성화의 복합효과에 기인하고, 후자의 구에서 생장증가는 산성빗물에 포함된 질소와 황의 영향으로 해석되었다.

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