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Effects of Simulated Acid Rain on the Growth of *Pinus rigida*×*taeda* Seedlings Inoculated with Ectomycorrhizal Fungi, *Pisolithus tinctorius* and *Suillus luteus*[1]

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人工酸性雨가 모래밭버섯과 비단그물버섯 菌根菌으로 接種한 리기테다소나무 苗木의 生長에 미치는 效果[1]

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ABSTRACT

The purposes of this study were to evaluate the effects of acid rain on tree growth and on the mycorrhizal formation and the effects of mycorrhizae on the host tolerance to acid rain. Simulated acid rain was applied for five months to *Pinus rigida*×*taeda* seedlings in pots inoculated with *Pisolithus tinctorius* (Pt) and *Suillus luteus* (Sl). Mycelial inocula of Pt and Sl were either mixed with entire pot soil (Mix) or casted as a band (Band) after soil sterilization. Three pH levels of acid rain (pH 3.0, 4.5 and 6.4 adjusted by 3 : 1 mixture of of sulfuric and nitric acids) were tested.

Pt-Mix was most effective in growth stimulation and resulted in 45-90% increase in tree height in sandy loam. Pt-Band was less effective than Pt-Mix in growth stimulation and mycorrhizal formation. Simulated acid rain at pH 4.5 stimulated height growth by 10-55%, while acid rain at pH 3.0 did not significantly affect the height growth. The top/root ratio was increased by pH 4.5 treatment, while pH 3.0 treatment reduced it. Mycorrhizal infection rate was not affected by acid rain. Pt inoculation reduced acid-induced leaf injury by 28-58% in both pH 3.0 and 4.5 compared with un-inoculated plants. Sl was also effective in growth enhancement, but was less effective than Pt in both mycorrhizal infection and reducing leaf injury.

Key words : *ectomycorrhizae* ; *simulated acid rain* ; *leaf injury* ; *mycorrhizal infection* ; *Pinus rigida*×*taeda* ; *Pisolithus tinctorius* ; *Suillus luteus*.

要 約

本 研究는 酸性雨가 樹木의 生長과 菌根의 形成에 미치는 影響, 그리고 菌根形成이 寄主植物의 酸性雨에 對한 抵抗性에 미치는 影響을 調査하기 위하여 遂行하였다. 리기테다소나무의 種子를 土壤消毒한 花盆에 播種하고, 모래밭버섯균(Pt)과 비단그물버섯균(Sl)의 菌糸로 接種한 후, pH 3.0, 4.5, 6.4의 人工酸性雨(黃酸과 窒酸의 3 : 1 稀釋)로 灌水하면서 5個月間 野外에서 生育시켜서 生長量, 菌根形成 및 葉被

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害率 등을 調査하였다.

Pt菌을 花盆全體에 混合시켜 接種한 境遇, 苗木의 苗高生長이 接種하지 않은 比較區보다 45-90% 增加하였으며, Pt菌을 band 形態로 土壤에 섞어 接種한 境遇에는 效果가 減少하였다. pH 4.5 酸性雨를 砂質土壤에 處理한 境遇, 苗高生長을 10-55% 促進시킨 反面에, pH 3.0 酸性雨는 苗高生長에 큰 影響을 주지 않았다. pH 4.5 處理는 T/R率을 增加시키고, pH 3.0은 T/R率을 減少시켰다. 人工酸性雨는 菌根形成率에 影響을 주지 않았으며, Pt菌 接種은 pH 3.0과 4.5의 酸性雨에 依한 葉被害率을 28-58% 減少시켰다. Sl菌도 苗高生長을 促進시키고 葉被害率을 減少시켰으나, 그 效果는 Pt菌보다 낮았다.

INTRODUCTION

Mycorrhizae are a common feature of most forest tree species of economic importance throughout the world, and have been known for their important role in nutrient absorption and cycling in forest ecosystem (Harley and Smith, 1983). Particularly in pines, feeder roots function properly in nutrient uptake only if they are infected by ectomycorrhizal fungi. Among the known benefits of mycorrhizae, increased tolerance of host plants to various environmental extremes have been emphasized. For example, mycorrhizal roots tolerate high temperature (Marx and Bryan, 1971), drought conditions (Dixon *et al*., 1980), soil toxins (Marx, 1972), and extremes of soil pH (Marx and Artman, 1979).

Recently acid rain on the forests has received a special attention due to its potential hazard to various components of forest ecosystem (Johnson and Siccama, 1983 ; Kozlowski and Constantinidou, 1986a, 1986b). Particularly activities of soil microorganisms are seriously affected by acid precipitation (Bitton and Boylan, 1985). A few studies have been reported on the effect of acid rain on ectomycorrhizae of forest trees (Reich *et al*., 1985 ; Stroo and Alexander, 1985). Soil acidification and subsequent alteration of rhizosphere condition might affect the growth, survival, and infectivity of mycorrhizal fungi (Hung and Trappe, 1983). On the other hand, slightly acidic rain temporarily stimulated plant growth through fertilizer effect of nitrogenous compounds in the simulated acid rain (Kim, 1987).

The purposes of present study were 1) to evaluate the effects of simulated acid rain on the mycorrhizal formation and subsequent growth of the treated plants in relation to different soil texture, 2) to examine the effects of mycorrhizae on the tolerance of host plants to the treated acid rain.

MATERIALS AND METHODS

Fungal Culture :

Pisolithus tinctorius #1392 (abbreviation as Pt) was introduced as mycelium in test tube slants from Institute for Mycorrhizal Research and Development, USDA Forest Service in Athens, Georgia, through Department of Biology, Virginia Polytech. Inst., Blacksburg, Virginia, U.S.A. *Suillus luteus* was isolated from fruit bodies collected under 28-year-old *Pinus densiflora* stand in Yangyang-gun, Kangwon Province. These fungi were initially cultured on agar containing Modified Melin-Norkrans' nutrient medium (MMN) and further mass cultured for 4 months in 1 *l*-glass bottles (Ringer's Solution bottle) containing vermiculite and peatmoss (77 : 3 in volume) moistened with MMN solution as described by Koo *et al*. (1982).

Preparation of Plant Material and Pot Soils :

Pinus rigida$\times$*taeda* seeds were collected from a clone bank at 20 years of age at Institute of Forest Genetics, Suwon. The seeds were surface-sterilized with sodium hypochlorite before sowing on agar plate and transferred to 550ml-plastic pot. Three kinds of soil texture were prepared by mixing nursery soil with river sand in this experiment : sandy loam, loam, and silty clay. The chemical properties of these soils are shown

Table 1. The chemical properties of the three types of soil used as an initial pot medium.

Soil Texture	pH (1 : 5 N-KCl)	Org. Mat. (%)	Total N (%)	Avail. P_2O_5 (ppm)	K (ppm)	Exch. Al (me/100g)	So_4 (ppm)
Sandy loam	5.7	0.03	0.036	15	0.22	trace	13
Loam	5.1	0.64	0.040	10	0.21	123	34
Silty clay	4.9	2.11	0.152	5	0.22	190	55

in Table 1.

Preparation of acid rain and fungal inoculation :

Acid rain in general contains sulfate and nitrate (McColl and Firestone, 1980). The simulated acid rain was prepared by adding sulfuric acid and nitric acid (3 : 1 by volume) to tap water to adjust pH to 3.0 and 4.5. As a control tap water with pH 6.4 was used. The simulated acid rain was applied 5mm each time on the plants twice a week to supply adequate moisture during the five months of experimental period.

For mycorrhizal infection, two inoculation methods were tested. Fifty milliliters of mycelial inoculum in vermiculite and peatmoss were added to each pot either by mixing the inoculum with the entire pot soil (Pt-mix), or by casting the inoculum as a band (Pt-band) in the middle of the pot soil column. Inoculated pots were arranged on styrofoam bed and roofed at 1.2 m in height with semitransparent plastic slate to block natural precipitation. Each treatment was replicated ten times in individual pots.

Growth Measurement :

The plants were grown for five months and harvested for following measurements : seedling height, fresh weight, root collar diameter, dry weight, top/root ratio, mycorrhizal infection rate (percent of mycorrhizal roots over total number of feeder roots), and leaf injury rate (percent of length of shoot with injured leaves over length of shoot bearing leaves). For calculation of mycorrhizal infection, six out of ten seedlings were randomly sampled and their roots were stained for about 5 minutes in a 10% glacial acetic acid solution containing 0.1% Ponceau S as described by Daughtridge *et al*. (1986). Mycorrhizal roots were stained red, while non-mycorrhizal roots remained unstained.

RESULTS AND DISCUSSION

Height Growth :

Figure 1 shows monthly height growth of *Pinus*

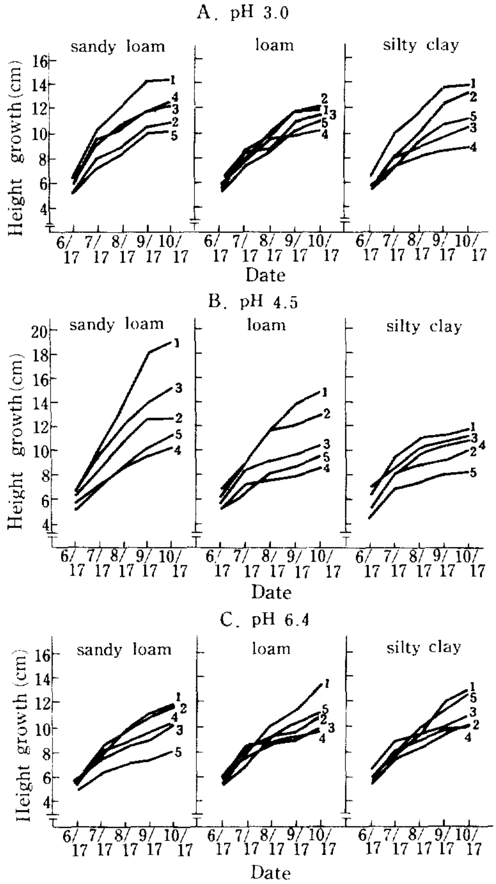

Fig. 1. Monthly height growth of one-year-old *Pinus rigida* × *P. taeda* seedlings inoculated with *Pisolithus tinctorius* (Pt) and *Suillus luteus* (Sl) at three kinds of soil in three pH levels of simulated acid rain. (1. Pt-mix, 2. Pt-band, 3. Sl-mix, 4. Sterilization only, 5. Unsterilized)

rigida × *taeda* seedlings five months after the weekly application with simulated acid rain. Plants in sandy loam performed better than those in loam and silty clay, even though organic matter and total nitrogen contents were higher in silty clay than sandy loam as shown in Table 1. Plants treated with acid rain grew as fast as control plants treated with pH 6.4. Particularly plants treated with acid rain at pH 4.5 was 10-55% taller than those treated with pH 3.0 and 6.4(significant at 1% level), suggesting possible fertilizer effect of simulated acid rain at pH 4.5.

When mycorrhizal effect was compared, Pt treatment with entire soil mixing (Pt-mix) was most effective in plant growth stimulation and resulted in 45-90% increase in tree height in sandy loam. Pt applied to soil by band(Pt-band) was not much effective as Pt-mix in growth stimulation. The superior effect of Pt-mix was shown in all the treatment combinations. *Suillus* inoculation was also effective in most of the combinations, even though it is inferior to Pt.

The harmful effect of pH 3.0 acid rain on plant growth was not shown in this figure, probably because the acid rain was added to soil only for five months of relatively short period. Instead, the simulated acid rain at pH 3.0 might have added significant amount of nitrogen to the tested soil, and the mycorrhizal roots absorbed extra nitrogen faster than un-inoculated plants. Alexander and Fairly(1983) suggested this mechanism in spruce forests, while Mitchell *et al*.(1984) emphasized the importance of various isolates of ectomycorrhizal fungi in efficiency of nutrient uptake.

Top-Root Ratio :

Table 2 shows top/root(T/R) ratio of treated plants based on oven dry weight. The T/R ratio increased in pH 4.5 treatment, while pH 3.0 treatment reduced it. The increased T/R ratio in pH 4.5 resulted from stimulation of height growth of the treated seedlings. When inoculation type was compared, Pt-mix significantly increaed T/R ratio of seedlings in pH 4.5 through enhancement

Table 2. Top/Root ratio (dry weight basis) of one-year-old seedlings of *Pinus rigida* × *taeda* treated with simulated acid rain and inoculated with two kinds of ectomycorrhizal fungi after soil sterilization.

Inoculation Type	pH of Simulated Acid Rain 3.0	4.5	6.4
Pt-mix	2.24	2.90	2.57
Pt-band	2.35	2.47	2.41
Sl-mix	1.97	2.87	2.42
Sterilized	2.16	1.99	2.08
Unsterilized	2.45	2.49	2.47

Pt : *Pisolithus tinctorius*
mix : inoculum mixed with entire soil
Sl : *Suillus luteus*, band : inoculum casted as a band

of height growth. Seedlings in sterilized treatment without mycorrhizal inoculation had lower T/R ratio than other treatments. Lee(1984) reported that Pt treatment to *Pinus rigida* × *taeda* seedlings did not result in significant change in top to root ratio, while Marx *et al*.(1976) reported reduction in top to root ratio by Pt inoculation.

Mycorrhizal Infection :

Mycorrhizal infection rate is shown in Figure 2. Pt-mix(inoculum mixed with entire pot soil) treatment was most effective in mycorrhizal formation, which resulted in over 80% infection rate. Pt-band and *Suillus* were slightly less effective than Pt-mix. Plants grown in initially sterilized soil without inoculation also formed

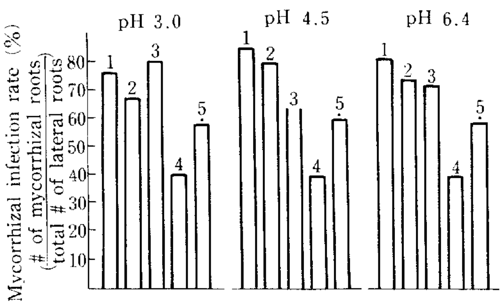

Fig. 2. Mycorrhizal infection rate of *Pinus rigida* × *taeda* seedlings inoculated with *Pisolithus tinctorius* (Pt) and *Suillus luteus* (Sl) at three levels of simulated acid rain. (1. Pt-mix, 2. Pt-band, 3. Sl-mix, 4. Sterilized, 5. Unsterilized, See also Table 2 for abbreviation)

mycorrhizae at the end of the experiment indicating natural contamination of the sterilized soil with air-born spores of ectomycorrhizal fungi. However, the level of infection in sterilized soil and unsterilized control treatment was significantly lower than infection by Pt and Sl. The low infection rate in these two treatments is considered to be a main reason for the slow growth of the plants. The mycorrhizal infection rate is reported to be closely related to the growth rate of the host plants(Marx, 1980), and over 80% of feeder roots should be infected by Pt to exert a stimulatory effect on host growth(Marx *et al*., 1982). Present study confirmed their report, and simulated acid rain at pH 3.0 did not reduce the mycorrhizal infection rate. However, Reich *et al*. (1985) reported decreased mycorrhizal infection in *Quercus rubra* by acid rain. Gobl(1986) also observed deformation of spruce mycorrhiza and 50% reduction in mycorrhizal formation by simulated acid rain.

Leaf Injury :

Table 3 shows leaf injury rates of *Pinus rigida* × *taeda* seedlings treated with simulated acid rain. Three months after beginning of irrigation with pH 3.0 acid rain, the treated seedlings started to show leaf injury. The seedlings inoculated with mycorrhizal fungi showed significantly lower level of leaf injury than un-inoculated control plants, suggesting beneficial effect of mycorrhizae on protection of host plants from high acidity in soil. Particularly *Pisolithus tinctorius*(Pt) was more effective in reduction of leaf injury than *Suillus luteus* and natural populations of mycorrhizal fungi. Kim(1987) and Cheong (1987) working with *Ginkgo biloba* and *Pinus koraiensis*, respectively, found harmful effect of acid rain at pH 3.0 on chlorophyll content and histological deformation. However, their results are difficult to compare with present study, because they did not specifically consider the effect of mycorrhiza on host tolerance to acid rain.

Table 3. Leaf injury rate of *Pinus rigida* × *taeda* seedlings inoculated with *Pisolithus tinctorius* (Pt) and *Suillus luteus* (Sl) at three pH levels of simulated acid rain. See Table 2 for the abbreviation.

Unit : %

Soil Condition	Inoculation Type	pH of Simulated Acid Rain 3.0	4.5	6.4
Sterilized	Pt-mix	56	27	41
	Pt-band	58	61	69
	Sl-mix	73	6	62
	Control	78	65	72
Unsterilized	Pt-mix	36	37	34
	Pt-band	33	27	30
	Sl-mix	52	24	39
	Control	43	45	44

It should be mentioned that experimental period in this study extended only five months during which simulated acid rain was applied twice a week. Significant effect of pH 3.0 acid rain on tree growth would be expressed, if acid rain is treated much longer period than five months.

Another interesting observation in Table 3 was reduced leaf injury in unsterilized soil, regardless of pH treatments and inoculation types. The reason of the reduced leaf injury could not be fully explained. It is likely, however, that unsterilized soil possibly contained unknown groups of microorganisms which might help to resist change in soil pH. Another possible reason is altered chemical properties of sterilized soil, buffering capacity of which would be reduced. Further study may be necessary to confirm these speculations on reasons of less injury in unsterilized soil.

Table 4 summarized overall effect of mycorrhizal inoculation on host growth and leaf injury, when three pH levels of acid rain were summed. In sterilized soil Pt-mix treatment stimulated, when compared with control, height growth by 33%, increased dry weight by 71%, increased T/R by 20%, and reduced leaf injury by 39%. Similar effect of mycorrhizae was also shown in un-sterilized soil, even though the degree of growth stimulation was slightly less than that in sterilized soil.

It is concluded from this experiment that

Table 4. Effect of mycorrhizal inoculation on host growth and leaf injury, when three pH levels of simulated acid rain were summed. (See Table 2 for pH levels).

Soil Condition	Inoculation Type	Seedling height (cm)	Dry weight (g)	T/R ratio	Mycorrhizal infection (%)	Leaf injury (%)
Sterilized	Pt-mix	13.8	0.36	2.54	84	44
	Pt-band	11.6	0.23	2.24	78	69
	Sl-mix	11.4	0.26	2.21	74	64
	Control	10.4	0.21	2.12	43	72
Unsterilized	Pt-mix	13.3	0.37	2.47	80	28
	Pt-band	11.3	0.27	2.36	74	50
	Sl-mix	13.6	0.32	2.42	77	34
	Control	10.4	0.30	2.31	65	38

mycorrhizal inoculation of *Pinus rigida* × *taeda* with specific strains of ectomycorrhizal fungi, such as *Pisolithus tinctorius* #1392, was highly effective in growth stimulation of host plant regardless of pH of simulated acid rain and soil texture. The Pt was also effective in reduction of leaf injury caused by acid rain. Therefore, it may be necessary in the future to re-evaluate or re-emphasize the importance of ectomycorrhizal fungi in the present forests with declining productivity due to acid deposition. Further study may be necessary to select best strains of ectomycorrhizal fungi which have adapted to acidic forest soils continuously exposed to air pollutants and acidic precipitation.

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