



<b>Title</b>	Ecophysiological Study of the Growth of Conifers in Korea in Acidified Soil with Elevated CO <sub>2</sub> : the Role of Ectomycorrhizal Infection
<b>Author(s)</b>	Choi, DongSu
<b>Citation</b>	Eurasian Journal of Forest Research, 11(1), 1-39
<b>Issue Date</b>	2008-03
<b>Doc URL</b>	<a href="http://hdl.handle.net/2115/33063">http://hdl.handle.net/2115/33063</a>
<b>Type</b>	bulletin (article)
<b>Note</b>	Article
<b>File Information</b>	01-Choi.pdf



[Instructions for use](#)

## Ecophysiological Study of the Growth of Conifers in Korea in Acidified Soil with Elevated CO<sub>2</sub>: the Role of Ectomycorrhizal Infection

CHOI DongSu\*

Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology,  
Fuchu, Tokyo 183-8509, Japan

### Abstract

Several Pinaceae species (*Pinus* and *Larix* sp.) in northeastern Asia, especially Korea, show symptoms of decline due to soil acidification and air pollution. In the industrial area of northwest Korean, growth and physiological activities of *Pinus koraiensis* and *P. rigida* are adversely influenced by increased deposition of fluorine (F), chlorine (Cl) and Manganese (Mn), involving both wet and dry deposition; these also have harmful effects on the ectomycorrhizal status. The ectomycorrhizal infection level at the damaged site was 30% less than at a control site. To determine the role of ectomycorrhizal symbiotic association with seedlings of *P. densiflora*, *P. koraiensis*, *P. rigida* and *Larix kaempferi* during progressive soil acidification, the relation between relative total dry mass (TDM) and the molar ratio of base cation to aluminum (BC/Al) was examined. The TDM of *P. koraiensis* and *L. kaempferi* seedlings was approximately 40% at a BC/Al ratio of 1.0, but that of *P. rigida* and *P. densiflora* was approximately 50% at the same BC/Al ratio. With increasing amount of H<sup>+</sup> added to the soil, the water-soluble concentrations of Ca, Mg, K, Al and Mn increased, especially below a soil pH of 3.8. Development of ectomycorrhizae, i.e. *Pisolithus tinctorius* (*Pt*), Ectodrench (*EC*) or *Cenococcum geophilum* (*Cg*) in all four test species was significantly greater in the 10 and 30 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments than in control treatments. Ectomycorrhizal development in the 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments was significantly lower than the other treatments.

Atmospheric CO<sub>2</sub> increases yearly that may affect mycorrhizal growth and diversity in forest ecosystems through host plant physiology. The ectomycorrhizal development of each species was significantly higher under elevated CO<sub>2</sub> concentration (720 μmol·mol<sup>-1</sup>) than at ambient levels (360 μmol·mol<sup>-1</sup>) in the present study. The P concentration in needles and roots following inoculation with *Pt*, *EC* or *Cg* was significantly higher in all species than without inoculation, at both CO<sub>2</sub> concentrations. Furthermore, no photosynthetic down-regulation was found in any species when inoculated with *Pt*, *EC* or *Cg*, and photosynthesis was significantly higher than in non-inoculated seedlings at both CO<sub>2</sub> concentrations. Stomatal limitation in each species at each CO<sub>2</sub> concentration was less than in non-inoculated seedlings.

**Key words:** Elevated CO<sub>2</sub>, soil acidification, fluoride, chloride, ectomycorrhiza, photosynthetic rate, pine species

### Chapter 1 GENERAL INTRODUCTION

#### 1.1 Forest ecosystem in a changing environment

Environmental change, in particular global warming following increasing atmospheric CO<sub>2</sub> and atmospheric acid deposition, is now an important environmental issue. In the last 100 years huge amounts of air pollutants have been released from Europe, North America and Japan, including sulphur dioxide (SO<sub>2</sub>), nitrogen oxides (NO<sub>x</sub>), carbon dioxide (CO<sub>2</sub>), fluorides (HF), from industrial activities and fossil fuel burning. Moreover, gaseous air pollution has increased rapidly in recent decades because of economic growth, industrial development and urbanization, with consequent increases in energy demand in Asia, Africa and Latin America. Therefore, problem of 'climate change' is now worldwide.

Air pollution can impair vegetation by wet and dry

deposition, i.e. by damaging canopy foliage and reducing the rate of photosynthesis. Acid deposition reduces the pH of soil by both direct and indirect mechanisms (Ulrich 1990, Okita 1996). Wet and dry depositions of acid substances may gradually lead to acidification of forest soil through increased leaching of base cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>) from the rhizosphere soil (Ulrich 1983, Grodziński *et al.* 1990). Furthermore, dissolution of phytotoxic metals such as aluminum (Al) and manganese (Mn) into the soil accelerates soil acidification (Ulrich *et al.* 1980, Hallbacken and Tamm 1986). Moderate atmospheric deposition of nitrogen is reportedly beneficial for plants, but excess atmospheric deposition loading onto the forest floor can do long-term harm to forest ecosystems (Nilsson 1987, Moore 1995). Adverse effects include soil acidification (Lamerdorf and Mayer 1993), nutrient imbalance (Havranek *et al.* 1990, Okochi *et al.* 1995, Dueck *et al.* 1998, Grulke *et al.* 1998), nonoptimal carbohydrate

content (Barnes *et al.* 1990, Paynter *et al.* 1991) and poor growth (Thornton *et al.* 1990). Trees growing in forest soils acidified by acid deposition are probably affected adversely not only by low soil pH, but also by phytotoxic metals dissolved in the soil solution (Ulrich 1989).

The atmospheric carbon dioxide concentration (CO<sub>2</sub>) has continued to rise in recent decades (Houghton *et al.* 1990, Wyman 1991, Houghton 1995). This trend has indirect influences through global climatic greenhouse effects, and strong direct influences on ecosystems through increased availability of CO<sub>2</sub> for photosynthesis. At the physiological level, elevated CO<sub>2</sub> generally enhances photosynthesis, growth and water-use efficiency (WUE), and reduces the transpiration rate (Bazzaz 1990, Eamus 1991, Poorter 1993, Rogers *et al.* 1994, Field *et al.* 1995) and the partitioning of below-ground carbon (Stulen and den Ertog 1993, Rogers *et al.* 1996). Photosynthesis down-regulation or accumulation occurs with nutrient restriction, however, especially of P or N (Conroy *et al.* 1986, Coleman *et al.* 1993, Jach and Ceulemans 2000, Koike *et al.* 2000, Choi *et al.* 2005b), and also reduced sink strength (Stitt 1991, Rogers *et al.* 1998, Griffin *et al.* 2000).

### 1.2 Pine species

Pine is a diverse and important tree species for forest rehabilitation and planting of man-made forests in relatively poor soil conditions. There are approximately 100 species of pine, and most are significant for high-quality timber and high biomass productivity (Burns and Honkala 1990). Despite their taxonomic variety and wide distribution in the Northern Hemisphere, all pines have much in common ecologically in the world's forest ecosystems (Barnes *et al.* 1998).

The pine has a deep main root, and the growing root tips require large soil interstices to permit penetration. Because of the deep main root and poor penetrative ability of the roots, pines do not grow well in poorly drained soils. Consequently, they often have a strong symbiotic relationship with ectomycorrhizae. They are pioneer species in succession, and invade areas where mineral soil has been exposed by major disturbances such as forest fire or degradation by pollutants. Some pine species have high tolerance against drought, poor nutrition and pollutants including acid agents (Choi *et al.* 2005a, 2006). In Korea, *Pinus koraiensis* and *P. densiflora* are the main afforestation species. These pine species are planted in and close to large cities and industrial areas as shade trees. To rehabilitate degraded forested areas, *P. rigida* and *Larix kaempferi* were introduced at the start of the 20th century from North America and Japan (Kim 1999).

### 1.3 Role of ectomycorrhizae in a changing climate

Most tree species are symbiotic with ectomycorrhizae (Brundrett 1991, Smith and Read 1997). Ectomycorrhizae improve a plant's uptake of water and nutrients, particularly nitrogen and phosphorus from the soil solution. This is because a

greater soil volume can be exploited via the widely distributed hyphae (Smith and Read 1997, Lambers *et al.* 1998). Ectomycorrhizal association can also reduce the toxicity of metals such as Al, Mn, Zn, Cu and Ni; the effect depends on the mycorrhizal fungi and plant host species involved (Wilkens 1991, Jentschke and Godbold 2000). Aluminium toxicity has been associated with decreased uptake of base cations (BC) (Ca, Mg and K) (Schaedle *et al.* 1989, Jentschke and Godbold 2000). These base cations can be taken up by the mycorrhizal mycelium (Finlay 1992), even in plants exposed to high Al concentrations (Finlay 1995). Ectomycorrhizal plants therefore increase the uptake of water and nutrients and protect roots from toxic metals by decreasing the uptake of toxic ions (Kayama *et al.* 2005). These characteristics of ectomycorrhizae are expected to enhance physiological activity and growth of forest plants under climate change that includes soil acidification due to atmospheric deposition and elevated atmospheric CO<sub>2</sub>.

### 1.4 Objectives and outline of this study

This study consists broadly of two parts; i.e. environmental pollution and its effects on the decline of pines in an industrial region are treated in Chapter 2, and the growth and physiological responses of pines in a changing environment are considered in Chapters 3 and 4. The main concerns of this study are:

What is the most significant factor in the decline of pines in an industrial region?

What is the response of growth and physiological activities in pine trees facing soil acidification and elevated CO<sub>2</sub>, especially in regard to their symbiosis with ectomycorrhizae?

Growth and physiological responses of pine forests in both damaged and undamaged areas were examined, and the main factor responsible for the decline of pine forests at the damaged site was identified (Chapter 2). The effect of soil acidification on the growth and physiological responses of pine, and the role of ectomycorrhizae, were investigated (Chapter 3). Elevated CO<sub>2</sub> influences the growth and photosynthetic characteristics of pines; the function of ectomycorrhizae is discussed in Chapter 4. Finally, the results are summarized and the possibility of regeneration under disturbance and changing environment is discussed (Chapter 5).

The following research approach will be used to meet the designed objectives.

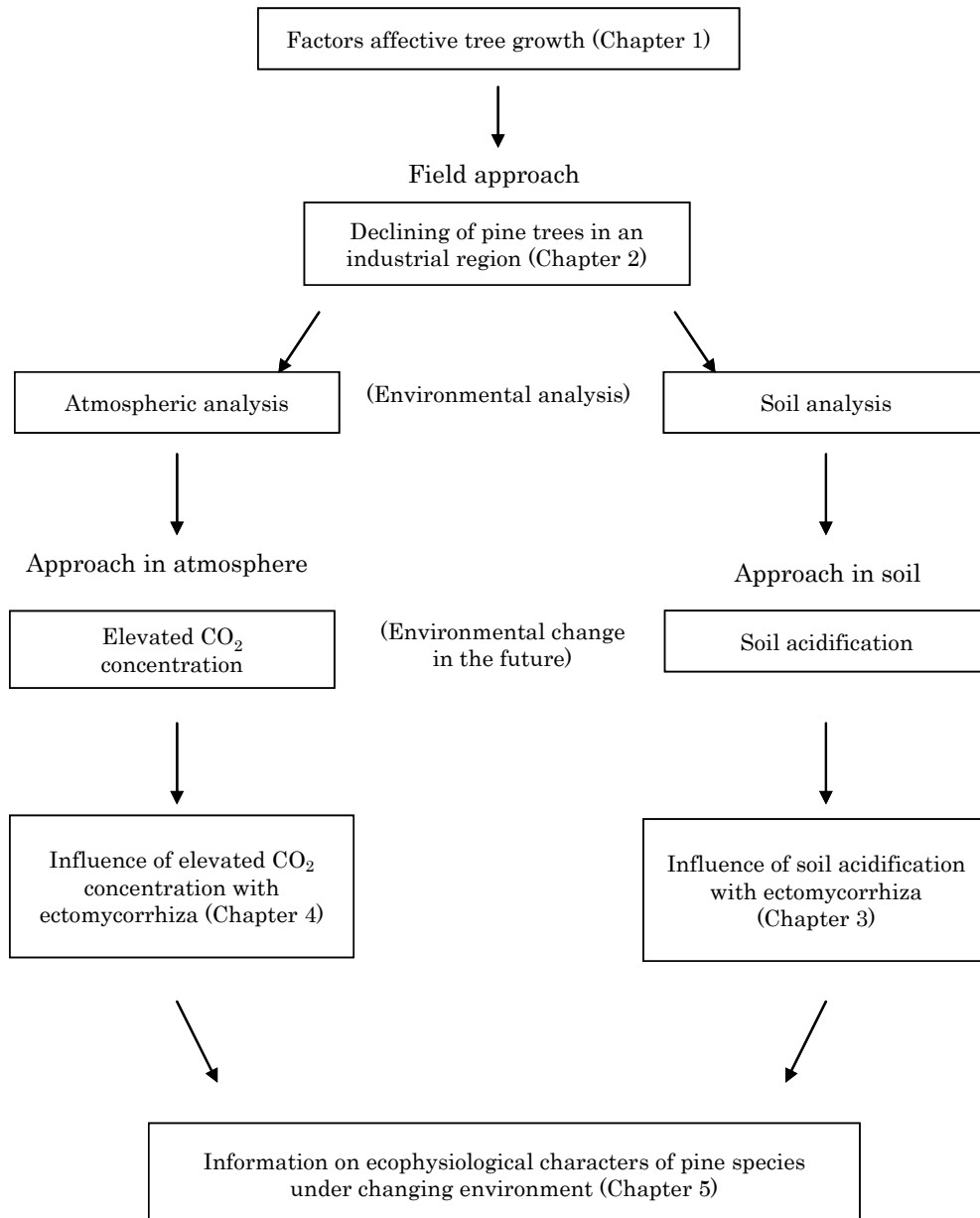


Fig. 1.1. Structure of present study.

## Chapter 2 Growth and physiological response of pine species planted in a polluted industrial region

### 2.1 Introduction

Pine trees are the most popular species for shade tree in cities, roadsides and rehabilitating degraded regions in Korea (Kim 1999). Therefore, Korean pine (*Pinus koraiensis* Sieb. et Zucc.) and Pitch pine (*Pinus rigida* Miller) are the most important afforestation tree species in Korean forests, since they can grow in infertile and barren soil, including shallow soil and sandy or gravelly soil. To rehabilitate degraded forested areas, *P. rigida* was introduced from North America in 1906 (Kim 1999). This species has high resistance to O<sub>3</sub> and soil

acidification (Burns and Honkala 1990). In recent years, however, pine forests have been in decline near industrial areas and large cities (Lee *et al.* 1996, Choi *et al.* 2003, 2006, Kim *et al.* 2003).

In most Korean forests the soil is gravelly and not particularly fertile, and is easily acidified because it derives from granite (Lee *et al.* 1998). Even though large amounts of chemicals such as lime or nitrogen fertilizers have been administered to reduce or reverse soil degradation, the decline is continuing (Yoo *et al.* 1998, Choi 2003).

What are the reasons for the decline in pine forests near industrial areas in Korea? Environmental pollutants (such as SO<sub>2</sub>, O<sub>3</sub>, NO<sub>x</sub>) usually reduce plant growth (Sucoff 1975, Matyssek *et al.* 1995a,

Sandermann *et al.* 1997, Shindo 2002a, 2002b, Fornasiero 2003, Kayama *et al.* 2003) through their negative effects on photosynthetic function (Hinrichsen 1986, Darral 1989, Furukawa 1991, Weber *et al.* 1994, Heber *et al.* 1995, Matyssek *et al.* 1995b, Lambers *et al.* 1998, Mansfield 1998, Larcher 2003), on leaf stomatal conductance (Winner 1981), on chlorophyll content and leaf longevity (Reich 1983, Matyssek *et al.* 1993a, 1993b, Reich *et al.* 1995) and through wet and dry deposition (Izuta 1998, Izuta *et al.* 2001, Choi *et al.* 2006). Moreover, these pollutants deposit to soil and increase soil acidification. Soil acidification increases the soluble Al, Mn or other phytotoxic metals and inhibits the uptake of essential elements and water for pine growth and the association roots with ectomycorrhiza, as a result of poor development of root system.

Preliminary surveys of this study found symptoms of shoot blight of pines (e.g. needle burn from the tip and margin or earlier shedding of needles) near an industrial area (Choi *et al.* 2003, Kayama *et al.* 2004). There are many industrial plants, including glassworks, steelworks, brickworks, ceramics plants, dye works and fuel coal combustion near the damaged site. These plants emit usually NO<sub>x</sub>, SO<sub>x</sub>, as well as fluoride (F) and chloride (Cl) (Supharungsun and Wainwright 1982). Fluoride seems to be the most toxic of these pollutants (Statens forurensningstilsyn 1992, Aluminiumindustriens Miljøsekretariat 1993). Forest ecosystems in Korea have faced heavy industrial pollution, mainly SO<sub>2</sub>, NO<sub>x</sub>, and acid rain, for about half a century. In recent years the level of these toxic pollutants emitted into the atmosphere has fallen. The similar levels of SO<sub>2</sub>, NO<sub>x</sub>, and acid rain have been observed between near an industrial area and in university forests, where they had air clean condition (Ministry of Environment 2002), but pine species grown near industrial areas have been still been in decline. What kinds of pollutants are most harmful to pine forests?

According to preliminary surveys, photosynthetic rates at light saturation with ambient CO<sub>2</sub> of pine saplings (*Pinus koraiensis* and *P. rigida*) in polluted industrial areas are significantly lower than that in clean University Forests (Choi *et al.* 2003, Kayama *et al.* 2004). In polluted areas the soil pH is lower and plants usually contain Mn (Kitao *et al.* 1997a). Recently, Choi *et al.* (2005a) found that *P. koraiensis* is more tolerant to soil acidification, based on the BC/Al ratio, than *P. densiflora*. I therefore hypothesize that reduced growth and photosynthetic capacity of pines at the damaged site is due to excess Mn accumulation in pine needles and to the direct effect of F (and other air pollutants). Which pine species are more tolerant to soil acidification and air pollutants?

To answer these questions, I measured the growth performance and the photosynthetic activity *in situ* of pine saplings planted in polluted and clean areas, considering climatic factors.

## 2.2 Materials and Methods

### 2.2.1 Study sites

To compare the effect of pollutants on growth and physiological changes, two plantation sites of Korean pine (*Pinus koraiensis* Sieb. et Zucc.) and Pitch pine (*Pinus rigida* Miller) were selected. The control site is located in southeast of Seoul city in Korea, and the damaged site is located in the southwest part of Seoul city. The control sites of *P. koraiensis* and *P. rigida* trees were in the Toi-chon Experimental Forest of Kyunghee University (37.2N, 127.1E), where the air is relatively clean (Kwangju, Kyunggido, Korea). The damaged sites were located in the Ansan industrial area (37.2N, 126.4E) (Ansan, Kyunggido, Korea). This industrial area was established in 1977, and comprises mainly machinery, ceramics, glass, chemicals and dye works industries (Fig. 2.1).

There were three study plots (10 m×10 m) for the control site, and six for the damaged site. Three of the plots at the damaged site were on two slopes (a

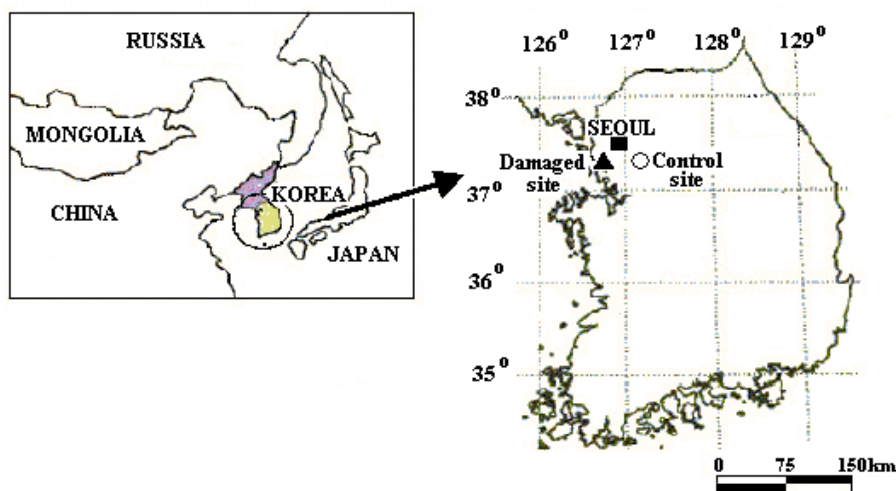


Fig. 2.1. The two field study sites located in Korea. The opened circle and closed triangle represent the control site at the Toi-chon Experimental Forest of Kyunghee University and damaged site at the Ansan industrial complex in Korea, respectively.

west-facing slope and a south-facing slope). There was no difference in soil properties between the control and damaged sites; both consist of granite-derived brown forest soil. There was almost no difference in total solar radiation, between the control and damaged sites during the study period (Korea Meteorological Administration 2002a). The altitude of the control site was 90 m and that of the damaged site was 50 m. The annual mean precipitation at the control and damaged sites was 1,468 and 1,204 mm, respectively, and the mean air temperature during the growing season (April to October) was 18.9 and 19.2°C, respectively (Korea Meteorological Administration 2002b). The rainy season in Korea is from July to September, and more than 60% of the annual precipitation falls in these three months (Korea Meteorological Administration 2002b, 2003).

### 2.2.2 Plant materials

Needles of *Pinus koraiensis* and *P. rigida* were collected from plantations at the control and damaged sites for chemical analysis. Sample needles were taken randomly from sunny branches of five trees per species in early September 2002, and were divided into current-year needles and older needles. The distance between sample trees was approximately 10m. Both sites were reforested 10 years ago, and the trees of each species were 13 years old at the time of sampling (they were planted at three years old). The tree height for each species varied between 1.6 m and 2.0 m.

### 2.2.3 Analysis of soil chemistry

The soil pH, and the concentrations of nitrogen (N), available phosphorus (P), base cations (Ca, Mg, K), Al and heavy metal (Mn) in soil were measured. The soil pH was determined by a pH meter (CH-8603, Mettler-Toledo, Greifensee, Switzerland) from a soil suspension. The suspension was made by mixing 20 g fresh soil and 50 ml distilled water in a 100 ml beaker, and was stirred by a glass stick every 20 minutes for one hour. The same soil samples were dried at 105°C for 24 h and the N concentration was determined by a CHNS/O analyzer (PE 2400 Series II, Perkin-Elmer, Norwalk, CT). P was extracted with 0.5 M sodium bicarbonate solution (pH = 8.5) (Olsen and Sommers 1982), and exchangeable Ca, Mg and K were extracted from 2.5 g dry soil with 50 ml of 1 M ammonium acetate solution (pH = 7.0) after shaking for 1 h. Al and soluble Mn were extracted from 5.0 g of dry soil with 0.1 N HCl after shaking for 1 h at 30°C. P, Ca, Mg, K, Al and Mn extracted from soil were all measured from these prepared solutions by an ICP (IRIS, Jarrel Ash, Franklin, MA).

### 2.2.4 Assessments of shoot growth, needle survival ratio and root infection rate

To evaluate shoot growth, the length of leader shoots for 20 samples (4 branches from each of 5 trees) of *P. koraiensis* and *P. rigida* were measured at the control and damaged sites in September 2002, once the leader shoot was observed to be fully grown. And, needle longevity was measured on four branches of five

individual trees of each pine species at each study site. The branches were selected from a sunny crown of trees on the forest edge at a height of about 1.2-1.5m. The main branch was divided up according to shoot age. Twelve shoots of each age from the five trees per species were dried for 4 days at 80°C. After drying, the actual number of needles and the number of needle scars were determined for each shoot. The survival measure of the needles (SN) was calculated from the following formula: SN (%) = RN/TN × 100, where RN and TN respectively denote the number of retained and total needles (estimated from multiplying the number of needles borne in bundle by the number of scars) (Kayama *et al.* 2002).

It dug out 0.07-0.1 m of the tip portion of the roots of both pines at about 0.1 m depth at the control and damaged sites to look for infection by ectomycorrhiza. The root tips were observed and counted the infected and non-infected roots (Quoreshi 2003). The infection rate of ectomycorrhiza (IRE) was determined as IRE (%) = ER/(ER+NR) × 100, where ER and NR respectively denote the number of ectomycorrhizal and non-ectomycorrhizal roots.

### 2.2.5 Measurement of photosynthesis

The photosynthetic capacities of five trees of *P. koraiensis* and of *P. rigida* were measured by two methods in September 2002 at the control and damaged sites. In the first method, the photosynthetic light response curve of current year needles was examined in sunny crown trees at the edge of the forest at a height of around 1.3 m. For these measurements, a portable gas analyzer (H4A, ADC BioScientific, Hoddesdon, U.K.) was used; the ambient temperature was 23-25°C and the ambient CO<sub>2</sub> concentration was 355-360 μmol·mol<sup>-1</sup>. Between 15 and 20 needles of two pines were covered with a conifer chamber (137 cm<sup>3</sup>, ADC BioScientific, Hoddesdon, U.K.), and the photosynthetic rate was measured. Supplementary light was provided by a halogen lamp (WALZ, Effeltrich, Germany). The photosynthetic photon flux density (PPFD) was changed from high to low to dark (2000, 1000, 500, 100 and 0 μmol·m<sup>-2</sup>·s<sup>-1</sup>) using cloth shades (Krary, Osaka, Japan). After determining the photosynthetic light curve, the width and length of needles were measured using vernier calipers, and then calculated the net photosynthetic rate per unit area. From the resulting data, photosynthetic light response curves were drawn as described by Thornley (1976) using the formula:

$$P = \frac{\phi I + P_{\max} - \sqrt{(\phi I + P_{\max})^2 - 4\theta\phi I P_{\max}}}{2\theta} - r_d$$

where  $P$  is the net photosynthetic rate,  $\phi$  is the initial slope of the curve,  $I$  is the incident PPFD,  $P_{\max}$  is the light-saturated rate of gross photosynthesis,  $\theta$  is the convexity factor of the curve, and  $r_d$  is the dark respiration rate. The light saturation point was taken to be the PPFD value when  $P_n$  reaches 95% of  $A_{\max}$  of the non-linear function.

The second method of measuring photosynthesis

capacity looked at the  $A/C_i$  ( $A$ =photosynthetic rate,  $C_i$ =intercellular  $CO_2$  concentration) curves for the same current-year needles, using an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA). The change in the net photosynthetic rate was measured at light saturation, under a photosynthetic photon flux density (PPFD) of 1,000-1,200  $\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$  provided by a cool halogen lamp (WALZ, Effeltrich, Germany). The leaf temperature was 25°C and the relative humidity was 50-70%. Leaves were allowed to acclimatize to their surroundings for 10 minutes before measurement began, after which I began the determination with 150-1500  $\mu\text{mol}\cdot\text{mol}^{-1}$   $CO_2$  concentrations.

Distinct processes regulate the rate of  $CO_2$  assimilation at low and high  $C_i$  concentrations (Farquhar and Sharkey 1982, Sharkey 1985, Matyssek *et al.* 1993b). The initial slope of the  $A/C_i$  curve is proportional to the carboxylation activity of Rubisco (i.e., the carboxylation efficiency). The net photosynthetic rate at high  $CO_2$  concentrations is limited by the regeneration rate of ribulose-1, 5-bisphosphate (RuBP).

### 2.2.6 Analysis of chlorophyll and chemical elements in needles

Chlorophylls were extracted with dimethyl sulfoxide (DMSO) and were measured spectrophotometrically (Type 100-50, Hitachi, Tokyo, Japan) to determine the concentration of chlorophyll (a and b) from three branches of five trees of each pine species at each study site (Barnes *et al.* 1992, Shinano *et al.* 1996). The amount of chlorophyll b increases when the needles grow in shady conditions (Larcher 2003).

The remaining needle samples were dried at 60 °C for one week. The dried samples were then ground to a fine powder in a vibrating sample mill (Wonder Blender, Osaka Chemical Co., Osaka, Japan). To determine the concentration of mineral nutrients and heavy metals (Ca, K, Mg, Na, P and Mn), the samples were digested by a microwave digestion system (O-I analytical, College Station, TX) and then underwent ICP analysis. The fluoride (F) content of the needles was determined by the La Alizarin Complexon method, and the chloride content by the Mohr method (Greenhalgh and Riley 1961, Alvarez 1995, Shindo 2002a).

### 2.2.7 Absorption of fluoride and chlorine in the atmosphere

This study used the Lime Treated Filter Paper Technique (LTP) to absorb F in the atmosphere (Miller *et al.* 1953, Choi *et al.* 2003). Toyo NO.5B (185 mm) filter paper of size 5×15 cm was dipped in 1% lime suspension, hung on a glass rack, and dried in an oven at 50-60°C for about 6 hours. Three sets of the papers at each measurement point were established at the control and damaged sites, under shelter but with good ventilation, and were exposed to the wind for one month.

### 2.2.8 Analysis of fluoride and chlorine in precipitation and soil water

Precipitation and soil water samples were collected from each site, making three replications. Precipitation was collected in a polyethylene bucket (26 cm diameter, 30 cm deep) from April to November 2003. The bucket was positioned 1.5 m above the ground. Soil water was collected from depths of 0.1, 0.3 and 0.6 m using by ceramic porous cup tension lysimeters (DIK-8390, Dai-Ki, Tokyo) from September to October 2003, when damage was most clearly visible. Anions from the samples were analyzed by ion chromatography (DX500, Dionex, CA, USA). The total amount of precipitation was almost the same at the control and damaged areas, so that it is valid to compare the dose of pollutants from the observed values.

### 2.2.9 Statistical analysis

Mean values of shoot growth, survival of needles, photosynthetic rate, chlorophyll and concentrations of elements in needles, air, precipitation, soil water and soil were examined between species and sites by the *t*-test (Li 1964) using the Stat View 5.0 software (SAS Institute, Cary, NC, USA). Relations between  $P_{\text{sat}}$  and each element were estimated using (S)MATR (Falster *et al.* 2003).

## 2.3 Results

### 2.3.1 Concentration of chemical elements in atmosphere, precipitation, soil and soil water

Table 2.1 shows the concentrations of Cl and F in the atmosphere at the control site and the damaged site. No difference was found in the Cl and F concentrations between the two slopes at the damaged site. The concentration of F in the air at the damaged site was about three times higher than at the control site (Table 2.1); this difference is statistically significant ( $p<0.05$ ). The concentration of Cl in the atmosphere behaved similarly ( $p<0.01$ ).

The concentration of Cl and F in the precipitation was significantly higher at the damaged site than at the control site, at least in the growing season ( $p<0.05$ ) (Fig. 2.2). The amount of trapped Cl and F scarcely differed between the two slopes in the damaged area. However, between July and September, there was no significant difference in Cl and F concentrations in the precipitation between the control and damaged sites.

Table 2.2 shows the soil condition at the control and damaged sites. Soil was more acidified at the damaged site than at the control site, however, there was no statistical difference in Al concentration between control and the damaged site. The concentration of Mn in the soil at the damaged site was significantly higher than at the control site ( $P<0.01$ ). No statistical difference in soil conditions was observed between the two slopes at the damaged site. The two slopes as representing the damaged site were averaged. Moreover, exchangeable base cations (Mg, K) and P at the damaged site were significantly lower than at the control site ( $P<0.05$ ). Concentrations of N and Ca were higher at the damaged site than at the control site, however.

Concentrations of Cl and F in soil water showed similar trends to concentrations in precipitation, and

Table 2.1. The concentration of fluorine in the air from the control site and damaged site (Unit= $\mu\text{g}\cdot\text{m}^{-3}\cdot\text{LTP}/\text{month}$ , mean $\pm$ S.D.), and the concentration of chlorine. (Unit=ppm, mean $\pm$ S.D., \* $P<0.05$ , \*\* $P<0.01$ ) (力 No sample was collected)

		June	September	October	November
F	Control	29.5 $\pm$ 2.41	36.8 $\pm$ 6.99	-	-
	Damaged	97.0 $\pm$ 28.43 **	104 $\pm$ 41.52 *	125 $\pm$ 22.06	69.2 $\pm$ 19.56
Cl	Control	0.28 $\pm$ 0.02	0.09 $\pm$ 0.03	-	-
	Damaged	0.45 $\pm$ 0.05 **	0.87 $\pm$ 0.30 **	1.13 $\pm$ 0.68	-

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

Table 2.2. The soil pH and concentration of soil chemical elements of A and B horizons at the control and damaged site. (\*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ ) (Unit of C and N =  $\text{mg}\cdot 100\text{mg}^{-1}$ ; Ca, Mg, K, Al and Mn =  $\text{mg}\cdot 100\text{g}^{-1}$ )

	Control		Damaged	
	A	B	A	B
pH	5.03 $\pm$ 2.20	5.25 $\pm$ 2.36	4.69 $\pm$ 0.17	4.64 $\pm$ 0.07
N	0.14 $\pm$ 0.04	0.06 $\pm$ 0.02	0.24 $\pm$ 0.12	0.12 $\pm$ 0.03 *
Ca	5.39 $\pm$ 0.64	2.78 $\pm$ 0.48	6.10 $\pm$ 1.84	2.89 $\pm$ 1.18
Mg	6.20 $\pm$ 0.51	3.84 $\pm$ 0.49	3.73 $\pm$ 0.15 ***	2.71 $\pm$ 0.38 *
K	40.20 $\pm$ 8.25	17.47 $\pm$ 3.47	17.40 $\pm$ 4.73 **	24.55 $\pm$ 7.27
P	2.42 $\pm$ 0.25	2.35 $\pm$ 0.21	1.29 $\pm$ 0.88 *	0.20 $\pm$ 0.11 ***
Al	20.45 $\pm$ 0.76	19.74 $\pm$ 2.81	18.69 $\pm$ 1.25	20.65 $\pm$ 1.26
Mn	3.16 $\pm$ 0.66	1.59 $\pm$ 0.44	10.56 $\pm$ 3.86 **	13.29 $\pm$ 1.55 ***

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

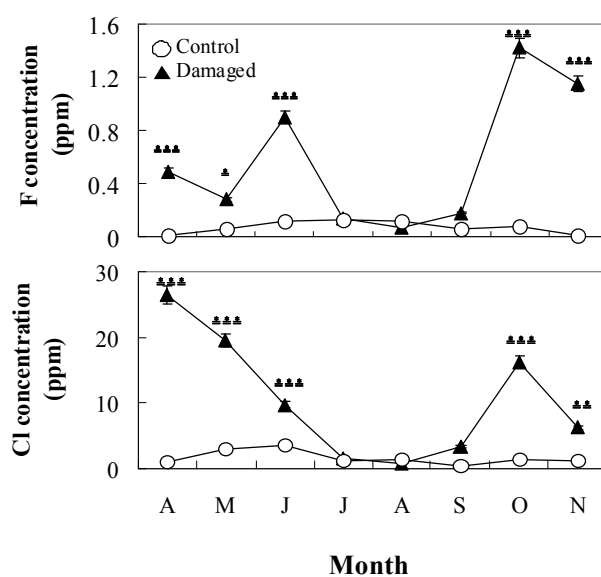


Fig.2.2. The concentration of F and Cl in the precipitation from April to November 2003 at the control and damaged site.

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)



were significantly higher at the damaged site than at the control site ( $p < 0.05$ ) (Fig. 2.3). The concentration of Cl and F in soil water fell with increasing soil depth; this tendency was clearer at the damaged site than at the control site. The F concentration in soil water at depths of 0.1 and 0.3 m from the surface at the damaged site was significantly higher than at the control site, but at 0.6 m depth there was no significant difference between the sites. For Cl, the concentration in soil water at 0.1, 0.3 and 0.6 m down at the damaged site was significantly higher than at the control site ( $P < 0.001$ ).

**2.3.2 Shoot length, survival of needles and root infection rate**

Shoots were shorter at the damaged site than at the control site, being 0.14-0.22 m (damaged site) and 0.26-0.34 m (control site) for *P. koraiensis* and 0.14-0.19 m and 0.23-0.28 m for *P. rigida* (Fig. 2.4). These figures correspond to reductions of 24-47% for *P. koraiensis* and 26-48% for *P. rigida*, and attained statistical significance ( $p < 0.01$ ).

Survival of needles of *P. koraiensis* and *P. rigida* is shown in Figure 2.5. *P. koraiensis* and *P. rigida* both shed their needles earlier at the damaged site than at the control site. *P. koraiensis* retained more than 60% needles of up to 5 years of age at the control site, but at the damaged site it had shed more than 80% of its needles of 3 years of age. Survival of needles of *P. koraiensis* at the damaged site was significantly worse than at the control site ( $p < 0.05$ ). Also, most aged needles of *P. rigida*, more than 3 years old, were shed at both sites.

No infection with ectomycorrhiza was observed in newly formed roots of either pine at the damaged site (data not shown).

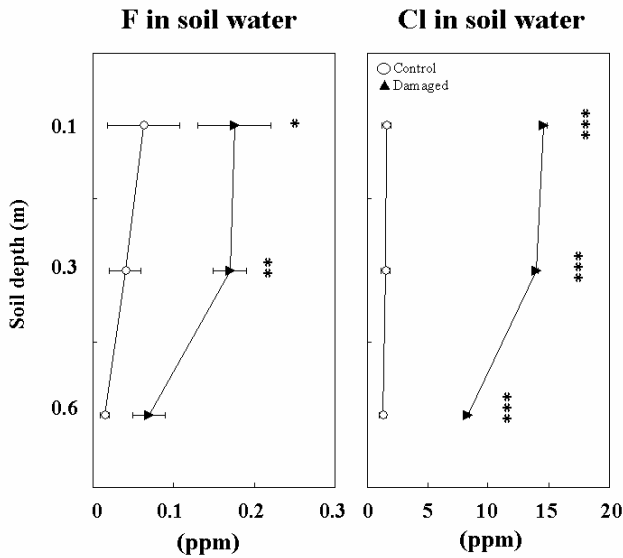


Fig. 2.3. Example of concentration of F and Cl in the soil water at the control and damaged site. (mean ± S.D., n=20, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) (Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

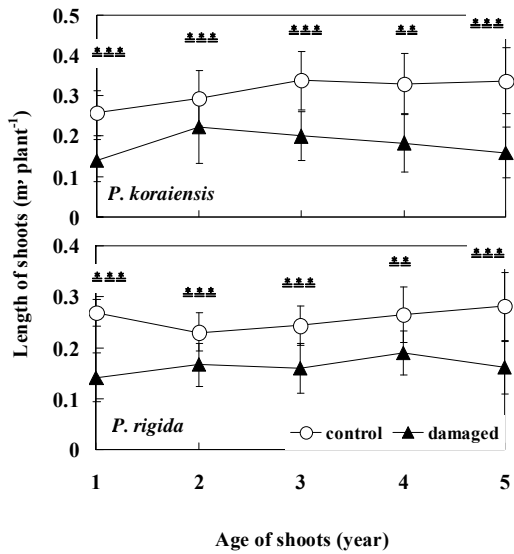


Fig. 2.4. Length of shoots at different age classes for *P. koraiensis* and *P. rigida* at the control and damaged sites. (mean ± S.D., n=20, \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) (Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

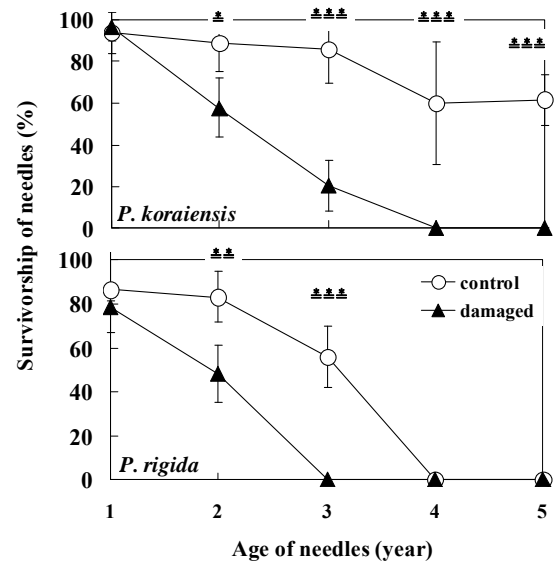


Fig. 2.5. Survivorship of needles at different age classes for *P. koraiensis* and *P. rigida* at the control and damaged sites. (mean ± S.D., n=20, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) (Adopted from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

### 2.3.3 Concentrations of elements in needles

Table 2.3 shows the concentrations of elements in needles of *P. koraiensis* and *P. rigida* at the control and damaged sites. The concentrations of magnesium (Mg), potassium (K) and phosphorus (P) in 1 to 2-year-old needles of *P. rigida* at the damaged site and in 2 or 3-year-old needles of *P. koraiensis* were significantly lower than the corresponding values at the control site ( $p < 0.05$ ). The K and P concentration in needles at both sites fell with needle age in both species. Calcium (Ca) accumulated in needles with aging of the needles, especially at the damaged site.

The concentration of manganese (Mn) in needles was higher at the damaged site than at the control site for both species, but especially for *P. koraiensis* ( $p < 0.05$ ). The concentration of Mn increased with needle age in both species at both sites. Concentrations of Cl and F in needles of both species at the damaged site were

significantly higher than values at the control site ( $p < 0.05$ ). The Cl and F content of needles increased with needle age in both species, especially at the damaged site.

### 2.3.4 Chlorophyll concentration in needles

Chlorophyll (a+b) and chlorophyll b concentration in 1 to 3-year-old needles of *P. koraiensis* at the control site showed a similar level of ca.  $1.5 \mu\text{mol}\cdot\text{g}^{-1}$  (a+b) and ca.  $0.25 \mu\text{mol}\cdot\text{g}^{-1}$  (b), but concentrations at the damaged site fell rapidly from 1.60 to  $0.11 \mu\text{mol}\cdot\text{g}^{-1}$  (a+b) and 0.23 to  $0.04 \mu\text{mol}\cdot\text{g}^{-1}$  (b) with needle age (see Fig. 2.6). Chlorophyll (a+b) and b concentrations were significantly lower at the damaged site for *P. koraiensis* than at the control site ( $p < 0.01$ ), except in 1-year-old needles.

Chlorophyll (a+b) and b concentrations in 1 to 3-year-old needles of *P. rigida* at the control site

Table 2.3. Concentration of elements in every age of needles of *P. koraiensis* and *P. rigida*. (Unit =  $\text{mg}\cdot\text{g}^{-1}$  and  $\mu\text{g}\cdot\text{g}^{-1}$  \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) (C : control site, D : Damaged site) (—) No needle was collected)

Needle age	<i>P. koraiensis</i>						<i>P. rigida</i>		
		1	2	3	1	2	3		
Ca ( $\text{mg}\cdot\text{g}^{-1}$ )	C	2.93 <sup>2</sup> 0.83	3.55 <sup>2</sup> 0.89	3.28 <sup>2</sup> 0.27	2.14 <sup>2</sup> 0.37	3.00 <sup>2</sup> 0.71	4.09 <sup>2</sup> 1.12		
	D	3.21 <sup>2</sup> 0.27	5.20 <sup>2</sup> 0.33	7.05 <sup>2</sup> 0.98 **	1.73 <sup>2</sup> 0.10	3.03 <sup>2</sup> 0.26	-		
Mg ( $\text{mg}\cdot\text{g}^{-1}$ )	C	1.03 <sup>2</sup> 0.13	1.07 <sup>2</sup> 0.16	1.05 <sup>2</sup> 0.10	1.16 <sup>2</sup> 0.13	1.02 <sup>2</sup> 0.01	1.12 <sup>2</sup> 0.07		
	D	0.88 <sup>2</sup> 0.05	0.82 <sup>2</sup> 0.10	0.81 <sup>2</sup> 0.09 *	0.61 <sup>2</sup> 0.04 ***	0.63 <sup>2</sup> 0.10 **	-		
K ( $\text{mg}\cdot\text{g}^{-1}$ )	C	5.49 <sup>2</sup> 0.52	4.92 <sup>2</sup> 0.39*	5.25 <sup>2</sup> 1.41	4.36 <sup>2</sup> 0.26	3.75 <sup>2</sup> 0.51	3.45 <sup>2</sup> 0.95		
	D	4.32 <sup>2</sup> 1.01	3.83 <sup>2</sup> 0.47	3.08 <sup>2</sup> 0.49 *	3.44 <sup>2</sup> 0.42 *	1.67 <sup>2</sup> 0.22 **	-		
P ( $\text{mg}\cdot\text{g}^{-1}$ )	C	3.22 <sup>2</sup> 0.46	2.61 <sup>2</sup> 0.38	2.61 <sup>2</sup> 0.19	2.44 <sup>2</sup> 0.28	2.36 <sup>2</sup> 0.33	2.08 <sup>2</sup> 0.33		
	D	2.63 <sup>2</sup> 0.19	2.28 <sup>2</sup> 0.39	1.74 <sup>2</sup> 0.10 **	1.83 <sup>2</sup> 0.09 **	1.49 <sup>2</sup> 0.07 **	-		
Mn ( $\text{mg}\cdot\text{g}^{-1}$ )	C	0.38 <sup>2</sup> 0.11	0.38 <sup>2</sup> 0.17	0.44 <sup>2</sup> 0.14	0.34 <sup>2</sup> 0.06	0.62 <sup>2</sup> 0.16	0.73 <sup>2</sup> 0.11		
	D	1.98 <sup>2</sup> 0.69 *	2.55 <sup>2</sup> 0.93 *	2.24 <sup>2</sup> 0.71 **	0.44 <sup>2</sup> 0.04	0.91 <sup>2</sup> 0.20	-		
Cl ( $\mu\text{g}\cdot\text{g}^{-1}$ )	C	0.57 <sup>2</sup> 0.35	0.56 <sup>2</sup> 0.20	0.70 <sup>2</sup> 0.25	0.85 <sup>2</sup> 0.36	0.71 <sup>2</sup> 0.18	0.83 <sup>2</sup> 0.50		
	D	1.43 <sup>2</sup> 0.30 **	2.39 <sup>2</sup> 0.36 ***	3.84 <sup>2</sup> 0.92 ***	1.77 <sup>2</sup> 0.42 *	3.26 <sup>2</sup> 0.86 **	-		
F ( $\mu\text{g}\cdot\text{g}^{-1}$ )	C	13.6 <sup>2</sup> 5.91	20.2 <sup>2</sup> 15.86	20.6 <sup>2</sup> 1.46	11.3 <sup>2</sup> 1.86	24.1 <sup>2</sup> 1.75	-		
	D	31.6 <sup>2</sup> 2.01 **	73.0 <sup>2</sup> 11.23 **	94.3 <sup>2</sup> 21.80 **	37.8 <sup>2</sup> 4.05 ***	102 <sup>2</sup> 24.40 **	-		

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

Table 2.4. Parameter estimates as functions of the  $A/C_i$  curves and light curves.  $P_{\text{sat}}$  is light-saturated net photosynthesis ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ),  $\Phi$  is apparent quantum yield ( $\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{Pa}^{-1}\cdot\text{CO}_2\cdot\text{s}^{-1}$ ), CE is carboxylation efficiency ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\text{Pa}$ ), and RuBP regeneration is the rate of RuBP regeneration ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

		$P_{\text{sat}}$	$\Phi$	CE	RuBP regeneration
<i>P. koraiensis</i>	Control	3.97 <sup>2</sup> 0.16	0.02	0.20	35.91 <sup>2</sup> 5.00
	Damaged	2.55 <sup>2</sup> 0.05 ***	0.012 **	0.19	23.71 <sup>2</sup> 5.30
<i>P. rigida</i>	Control	3.29 <sup>2</sup> 0.36	0.011	0.14	37.16 <sup>2</sup> 2.90
	Damaged	1.38 <sup>2</sup> 0.51 **	0.007 **	0.06 ***	32.34 <sup>2</sup> 0.01

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

increased slightly with needle age, but concentrations at the damaged site fell rapidly with needle age, falling to zero in 3-year-old needles. As a result, the chlorophyll (a+b) and b concentrations in *P. rigida* were significantly lower at the damaged site than at the control site ( $p < 0.05$ ).

### 2.3.5 Photosynthetic responses

The net photosynthetic rate of 1-year-old needles saturated at approximately  $1300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD for both *P. koraiensis* and *P. rigida* at the control site; at the damaged site saturation was reached at approximately  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD for *P. koraiensis* and  $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD for *P. rigida* (Fig. 2.7). The

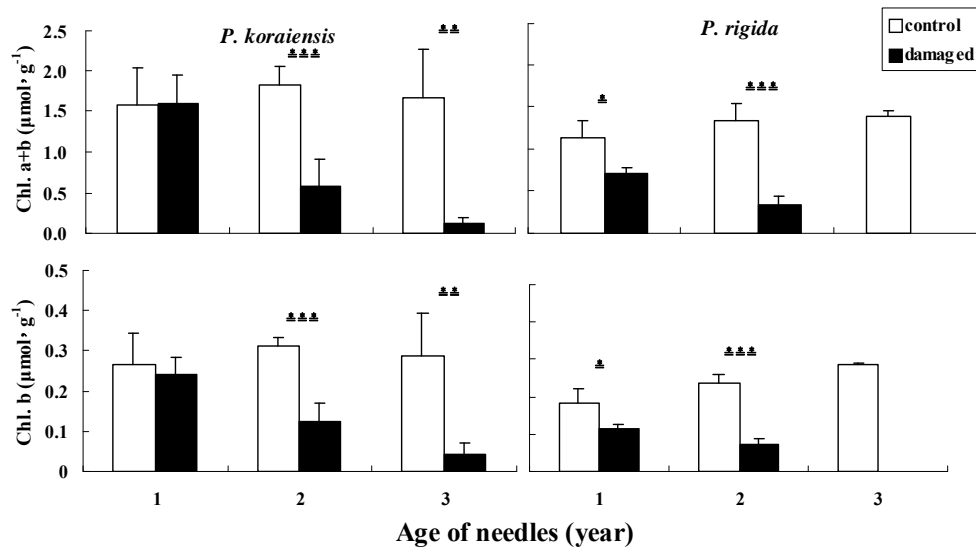


Fig. 2.6. Concentration of chlorophyll (a+b) and b in needles of different age classes at the control and damaged sites for *P. koraiensis* and *P. rigida* (mean±S.D., n=15, FM = fresh mass). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

(Cited from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

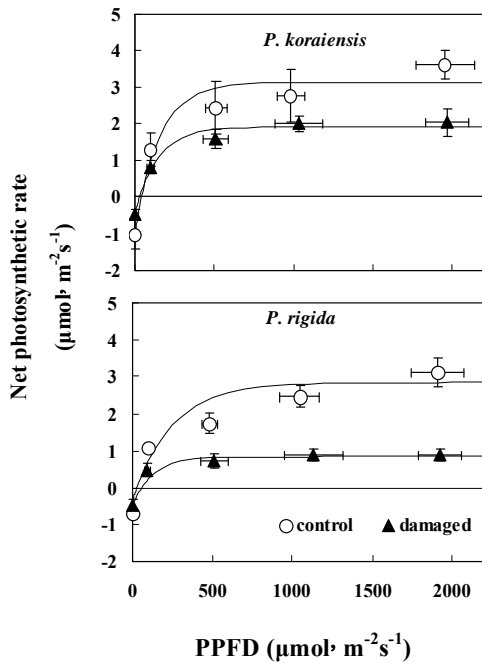


Fig. 2.7. Photosynthetic light response curves of 1-year-old needles at the control and damaged site for *P. koraiensis* and *P. rigida*.

(Adopted from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

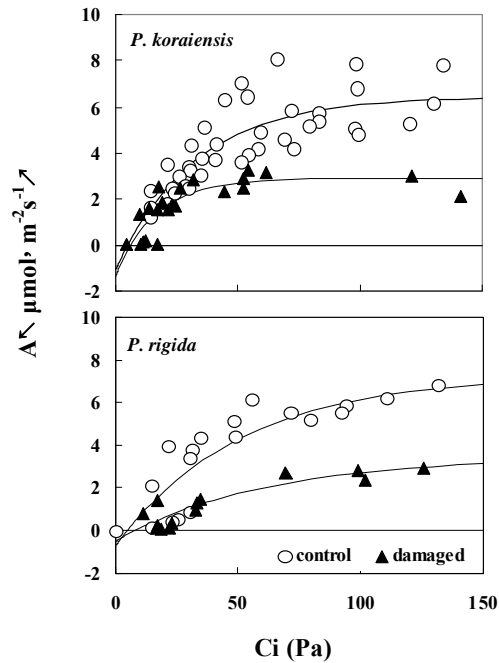


Fig. 2.8. The  $\text{CO}_2$  assimilation ( $A_n$ ) response curve to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) of current year needles at the control and damaged sites for *P. koraiensis* and *P. rigida*.

(Adopted from Choi *et al.* 2006. Environmental Pollution Elsevier with permission)

photosynthetic rate at light saturation ( $P_{\text{sat}}$ ) was significantly lower for both species at the damaged site than at the control site (*P. koraiensis*, 3.97 to 2.55  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ; *P. rigida* 3.29 to 1.38  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ;  $p < 0.01$ ). Moreover, the apparent quantum yield ( $\Phi$ ) was significantly lower at the damaged site than at the control site (i.e. 0.02 to 0.012  $\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{Pa}^{-1}\cdot\text{CO}_2\text{s}^{-1}$  for *P. koraiensis*, and 0.011 to 0.007  $\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{Pa}^{-1}\cdot\text{CO}_2\text{s}^{-1}$  for *P. rigida*; see Table 2.4).

Figure 2.8 shows the photosynthetic dependence of intercellular  $\text{CO}_2$  ( $C_i$ ) at light saturation, ( $A/C_i$ ), for *P. koraiensis* and *P. rigida* growing at the control and damaged sites. The net photosynthetic rates ( $A$ ) of *P. koraiensis* and *P. rigida* were clearly lower at the damaged site than at the control site, being reduced from 6  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to 3  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Also, the carboxylation efficiency (CE) of the  $A/C_i$  curve for *P. rigida* at the damaged site is significantly smaller than at the control site (0.06  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\text{Pa}$  and 0.14

$\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\text{Pa}$ ;  $p < 0.001$ ; see Table 2.4). However, any clear decrease in the CE of the  $A/C_i$  curve of *P. koraiensis* at the damaged site was not found relative to the control site. The CE was suppressed more in *P. rigida* than in *P. koraiensis*. Also, the RuBP regeneration rates of *P. koraiensis* and *P. rigida* at the damaged site were less than at the control site (Table 2.4). From the  $A/C_i$  curve, the reduction in each parameter was smaller in *P. koraiensis* than in *P. rigida*.

### 2.3.6 Relation between photosynthesis and Mn, Cl and F concentrations

$P_{\text{sat}}$  decreased with increasing Mn concentration in needles in both species (Fig. 2.9). The gradient of the plot of  $P_{\text{sat}}$  versus F and  $P_{\text{sat}}$  versus Cl also showed a similar tendency. In all cases the regression lines fell more sharply for *P. rigida*. In particular, the gradient of the plot of  $P_{\text{sat}}$  versus Mn differed significantly between *P. koraiensis* and *P. rigida* ( $P < 0.01$ ).

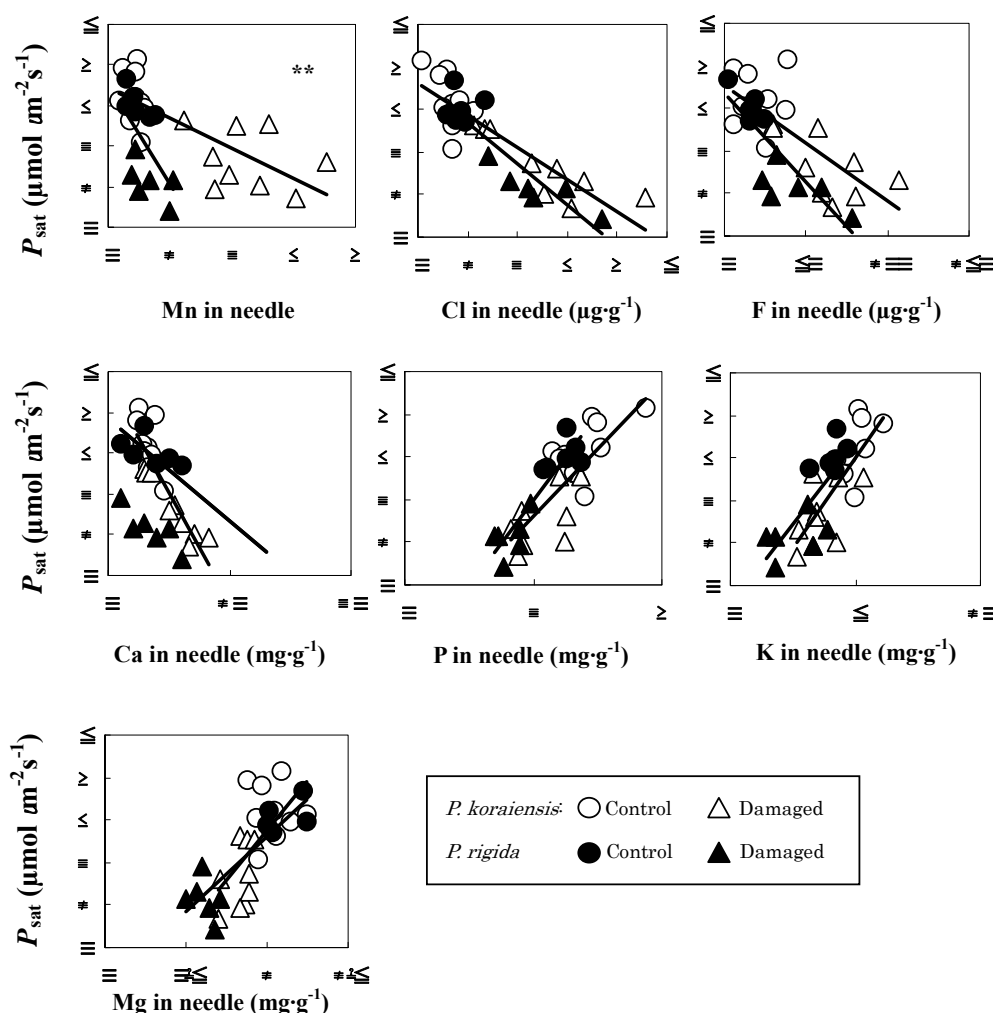


Fig. 2.9. The relationship between concentration of Mn, F, Cl, Ca, P, K or Mg in needle and photosynthetic rate at light saturation ( $P_{\text{sat}}$ ) of *P. koraiensis* and *P. rigida* at the control and damaged sites.

## 2.4 Discussion

There were no significant difference in soil pH and Al concentration in soils between the control and the damaged sites (Table 2.2). Therefore, this study focused on the factors affecting photosynthetic function (Table 2.4).  $P_{\text{sat}}$  decreased with Mn, Cl or F accumulation in the needles of both species. The gradient of the regression lines for *P. rigida* were steeper than for *P. koraiensis* (Fig. 2.9), although *P. koraiensis* accumulated more of these elements in needles than *P. rigida*. Fluoride is absorbed via the stomata, is transported by transpirational flow in the apoplast, and can accumulate up to toxic levels in the tips and margins of the leaves since there is no removal mechanism. Moreover, fluoride dissolved in water on the leaf surface can be absorbed by diffusion through the cuticle, leading to chlorosis and necrosis of leaf tips and margins (Treshow and Anderson 1989). Chloride also causes damage to plants from tip and margin burn, necroses, and suppression of physiological activity (Kayama *et al.* 2003, Larcher 2003). It also reduces the rate of mycorrhizal colonization (Duke *et al.*, 1986) as it was observed in the pine species at the damaged site.

The concentrations of Cl and F in the atmosphere, in precipitation and in soil water were significantly higher at the damaged site than at the control site ( $p < 0.05$ ), but did not differ between the sites in precipitation from June to September (Fig. 2.2). This is the rainy season in Korea (Korea Meteorological Administration 2002b, 2003), and concentrations of Cl and F in the precipitation were therefore similar at the control and damaged sites even though the damaged site is quite near to the sea. It is interesting to note that the concentration of Cl and F in precipitation between the control and damaged sites were similar, in spite of that in the atmosphere was significantly higher at the damaged site compared to the control site in September. The concentration of Cl and F in the atmosphere was analyzed after hang up during one month under shelter but with good ventilation, moreover, the concentration of Cl and F in the precipitation was analyzed immediately after rain. Therefore, it is thought that the concentration of Cl and F in the precipitation was diluted since a great quantity of precipitation from June to September. The Cl concentration at the damaged site in the air, precipitation and soil water did not differ between the two slopes, implying that the damaged site is scarcely influenced by Cl from the sea. An increase in air pollutants (i.e. Cl and F) invariably raises the concentration of harmful elements in precipitation and soil water. Enhanced Cl accumulation in needles reduces tree growth; for example, spruce (*Picea abies* and *P. glehnii*) growing along the roadside is damaged by Cl in deicing chemicals (Kayama *et al.* 2003). The photosynthetic function is also sensitive to chloride air pollutant in leaves of both native and introduced pine species (Gratani *et al.* 2000, Zhang *et al.* 2001). It is well known that F restrains enzyme activity (it is often used as an enzyme restrainer), accelerates genetic damage and disrupts the immune system. Accumulation of these harmful elements in plants directly reduces photosynthesis.

It has also been reported that Mn is a co-factor of photosynthesis (Marschner 1995); however, excess Mn in foliage organs usually reduces photosynthetic function at PSII (Nable *et al.* 1988, Kitao *et al.* 1997a, 1997b). Enhanced accumulation of Mn, Cl and/or F in needles therefore reduces the carboxylation efficiency in Rubisco, the RuBP regeneration rate, and also  $P_{\text{sat}}$ , especially in *P. rigida*.

Concentrations of base cations (Mg and K), P ( $p < 0.01$ ) and chlorophyll (a+b) and b ( $p < 0.05$ ) in needles of both pine species were significantly less at the damaged site than at the control site, except for Ca in *P. koraiensis* (Table 2.3, Fig. 2.6).

Symbiosis with mycorrhizae increases the uptake of nutrients (particularly P) and water, and the resulting vigorous physiological response and increase in growth of host plants enlarges the absorptive surface of the root (Smith and Read 1997, Lambers *et al.* 1998). At the damaged site, the development of mycorrhizae is liable to be restricted by environmental pollution (Allen 1996), inhibiting the uptake of nutrients (especially P) and water. The reduction of chlorophyll concentration in the needles influences the capture, absorption and conversion of solar energy in photosynthesis; usually, P deficiency in chloroplasts affects photosynthesis through RuBP regeneration (Brooks 1986, Fredeen *et al.* 1990, Kirschbaum and Tompkins 1990, Jacob and Lawlor 1991, Lewis *et al.* 1994), and reduces the peak carboxylation velocity or peak capacity of electron transport (Conroy *et al.* 1986, Lauer *et al.* 1989, Harley and Sharkey 1991). Nutrient deficiency in plants is accompanied by a reduction in the rate of CO<sub>2</sub> assimilation, which in turn reduces shoot growth and accelerates needle loss (Fig. 2.4, 5) (e.g. Field and Mooney 1983). This reduction in shoot length and needle lifespan in both pines at the damaged site is the result of photosynthetic suppression through environmental stressors such as Mn, Cl and F. Needle loss and premature senescence due to air pollutants such as SO<sub>2</sub>, O<sub>3</sub>, NO<sub>x</sub>, or acid deposition, have been reported in pine, birch and poplar (Reich 1983, Matyssek *et al.* 1993a, 1993b, Reich *et al.* 1995, Sandermann *et al.* 1997).

The toxicity of these pollutants is mostly due to their interference with respiration and photosynthetic function (Heber *et al.* 1995, Matyssek *et al.* 1995a, 1995b, Sandermann *et al.* 1997, Lambers *et al.* 1998, Pukacki 2000). In consequence, the energy status of damaged trees may be reduced (Ernst 1976, Ernst and Joosse-van Damme 1983) by reduction in the uptake of mineral nutrients (Adams 1981, Larcher 2003).

It is concluded that reduction in growth of both pine species at the damaged site is due to various physiological stresses induced by air pollutants. In particular, Cl and F discharged from nearby industrial regions suppressed growth of *P. koraiensis* and *P. rigida*. Moreover, *P. rigida* is less tolerant to pollutants and excess Mn in needles than *P. koraiensis*, and needle loss in *P. rigida* at the damaged site was accelerated more than for *P. koraiensis* at the damaged site.

## Chapter 3

### Effect of soil acidification on growth and physiological activities of four pine species seedlings inoculated with ectomycorrhiza

#### 3.1 Introduction

In recent years, growth of Pitch pine (*Pinus rigida*), Korean pine (*Pinus koraiensis*), Red pine (*Pinus densiflora*) and Japanese larch (*Larix kaempferi*), which are the representative pine species for making plantation in Korea, has been suppressed near industrial areas and large cities with inhibition of ectomycorrhiza development (Lee *et al.* 1996, Choi *et al.* 2003, 2006). These declining symptom are partly related to the edaphic condition of Korean forests where soils are originated from granite. Pine species are dominant in the granite derived soils. Many studies show that excess acid precipitation accelerate soil acidification, as a result forest ecosystem has declined (Van Breemen *et al.* 1982, Rollwagen and Zasoski 1988, Larcher 2003). Moreover, rehabilitation practices with pine species are employed near industrial regions, although the initial mortality of planted seedlings is high; this appears to be due to physiological imbalances of plantation seedlings or harsh environmental conditions, especially the soil environment. Excess Mn can prevent photosynthetic activity by reducing Rubisco activity and/or PS II (Kitao *et al.* 1997a, 1997b). However, little is known about the effect of Al and Mn on the development, the nutrient status and the physiological functions of pine species (Hecht-Buchholz *et al.* 1987, Miyake *et al.* 1991, Izuta *et al.* 1995, 1996a, 1996b, Kohno *et al.* 1995, Choi *et al.* 2005a, 2006).

Soil acidification due to dry or wet deposition is generally accompanied by increased leaching of base cations (BC) such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$  ions from the soil to the ground water, and an increase in the solubility of phytotoxic metals such as Al and Mn in the rhizosphere (Ulrich *et al.* 1980, Yoshida and Kawahata 1988). Aluminum dissolved in the soil can inhibit the uptake of mineral elements essential for plant growth and absorption of water from the soil, as a result of poor differentiation of root meristems (Foy *et al.* 1978, Rengel 1992). This would cause a disturbance of the nutrient status of forest trees. Tree vitality is strongly influenced by nutrient deficiency, an imbalance of cations, excess accumulation of Al and Mn under high N loading, etc. (Ulrich *et al.* 1980, Van Breemen *et al.* 1982, Ulrich 1989). Declining tree vitality and health are believed to lead poor development of the root system, fungal growth and symbiotic micro-organisms (Dighton and Skeffington 1987, Nakaji 2002).

Infection with ectomycorrhizal fungi can increase the ability of plants to tolerant environmental stresses, especially infertile condition, desiccation, etc. (Brown and Wilkins 1985, Godbold *et al.* 1998, Kayama *et al.* 2005). Ectomycorrhizal association can reduce toxicity of metals such as Al, Mn, Zn, Cu and Ni, which has analysis of the interaction between the mycorrhizal fungi and host plant species (Wilkins 1991, Jentschke and Godbold 2000, Ahonen-Jonnarth *et al.* 2003). Al and/or Mn, increased soil acidity caused by acid

precipitation, have been affecting mycorrhizal fungi and uptake of nutrient elements (Thompson and Medve 1984, Jentschke and Godbold 2000). However, symbiosis with ectomycorrhizae that enhances tolerance against heavy metals, such as Al and Mn, should result in an increase in physiological activities, growth and survival of ectomycorrhizal plants on acidified soil (Cumming and Wenstein 1990, Ahonen-Jonnarth *et al.* 2003).

The objective of this chapter is to evaluate the possible role of ectomycorrhizae in modifying the effects of acidified forest soil in nutrient uptake, physiological activity and growth of pine species. For accessing this purpose, three ectomycorrhiza species on four pine species were examined, for estimating sensitivity to soil acidification of four pine species and the tolerance of ectomycorrhizal seedlings. The findings in this study should be useful in improvement in the regeneration and conservation of acid-damaged forest ecosystems.

#### 3.2 Materials and Methods

##### 3.2.1 Plant materials

Seeds of Pitch pine (*Pinus rigida* Miller), Korean pine (*Pinus koraiensis* Sieb. et Zucc.), Red pine (*Pinus densiflora* Sieb. et Zucc.) and Japanese larch (*Larix kaempferi* Sarg.) were collected at the Yongmun Experimental Field of the Korea Forest Service (37.3°N, 127.2°E). After seeds were put in 4°C for 10-day cold treatment, they were surface sterilized with 30%  $\text{H}_2\text{O}_2$  for 20 min and rinsed 4-5 times with sterile deionized water, and were then germinated on sterilized media, black sand : vermiculate : peat moss = 3:2:1 (volume), in a glasshouse at day/night temperatures 25/20°C with a 16-h photoperiod.

##### 3.2.2 Ectomycorrhiza

Ectomycorrhiza fungi, *Pisolithus tinctorius* (Pers.) Coker et Couch (*Pt*) and the Diehard Ecto drench (EC) - (*Pt* + *Rhizopogon* spp. + *Laccaria* spp. + *Scleroderma* spp.), were obtained from Horticultural Alliance, Inc., USA. *Cenococcum geophilum* Fr. (*Cg*) was isolated from root of *Picea glehnii* (Fr. Schm.) collected from a mixed larch and spruce stand located at the experimental forests of Forestry and Forest Products Research Institute (FFPRI), Sapporo, Japan. The isolated fungal culture and the culture obtained from other laboratory were maintained on modified MMN (Modified Melin-Norkrans) agar medium, and mycorrhizal synthesis was followed further inoculation study (Qu *et al.* 2003). The *Pt* and EC spores were suspended in distilled water. The *Pt*, EC or *Cg* were inoculated directly to roots of each species seedlings and mixed with sterilized brown forest soil of each acidic treatment. The seedlings inoculated with each ectomycorrhiza were naturalized in plastic pots (upper diameter 10.5 cm, height 8.5 cm, lower diameter 7.5 cm) filled with sterilized brown forest soil of each acidic soil treatment.

##### 3.2.3 Soil treatments

Brown forest soil originating from granite was

collected from the organic layer 0-10 cm deep removed from under the stands of pine trees in the town of Shintoku (Hokkaido, Japan). The soil was sieved through a 5-mm mesh immediately after collection to homogenize it. Because of common soil condition in Korea, I used this type of granite soil for this experiment.

An acid solution having anions in the mol ratio as  $\text{SO}_4^{2-} : \text{NO}_3^- : \text{Cl}^- = 5 : 3 : 2$ , prepared from  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{HCl}$ , was applied to the soil. This is the anion ratio occurring in precipitation during the late 20th century near Seoul in Korea (Jin *et al.* 1999). Prepared mother liquid (1 N  $\text{H}^+$  - acid solution) was mixed 0, 10, 30, 60 and 90 ml with per 1 kg of brown forest soil. The concentration of  $\text{H}^+$  ions in the soil was 0, 10, 30, 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$ . Soil used as a control was not supplemented with  $\text{H}^+$ . One month after acidification of the soil, seedlings of each pine species were planted individually in plastic pots to simulate rehabilitation practice. In a well-ventilated greenhouse, dry deposition must have occurred. The seedlings were irrigated with distilled water during the growth period for 180 days.

### 3.2.4 Analysis of elements in soil and soil pH

The soil pH was determined using a pH meter (CH-8603, Mettler-Toledo AG) and a soil suspension left undisturbed for one hour. The suspension was made by mixing 20 g of fresh soil and 50 ml of distilled water in a 100-ml beaker with stirring using a glass stick every 20 minutes for 1 hour.

The rest of the soil sample was sieved through a 1-mm mesh after drying in room conditions, and was used for element analysis. The concentration of elements (Ca, Mg, K, Al and Mn) was determined by ICP analysis (IRIS, Jarrel Ash, Franklin, MA, USA) after mixing 20 g of soil with 100ml of distilled water in a 300-ml Erlenmeyer flask, which was then shaken for 1 hour at 25°C in the shaking incubator.

### 3.2.5 Infection rate with ectomycorrhiza

It was observed that the root tips and counted both infected and non-infected roots (Quoreshi 2003). The inoculation rate of ectomycorrhiza (IRE) was determined according to the following formula :  $\text{IRE} (\%) = \text{ER}/(\text{ER}+\text{NR})\times 100$ , where ER and NR respectively denote the number of ectomycorrhizal and non-ectomycorrhizal roots.

### 3.2.6 Measurement of element concentrations in plants

The dried plant tissues were ground to a fine powder using a vibrating sample mill (Wonder Blender, Osaka Chemical Co., Osaka, Japan). To determine the concentrations of elements (P, Al and Mn), dried samples were digested by the microwave digestion system (O-I Analytical, College Station, TX, USA) prior to ICP analysis (IRIS, Jarrel Ash, Franklin, MA, USA). The nitrogen concentration in the shoots and roots was determined with a N/C analyzer (Yanagimoto MT 500W, Tokyo, Japan).

### 3.2.7 Gas exchange characteristics of needles

Net photosynthetic rate of each species was examined using an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) between 09:00 and 15:00 local time. From 4 to 5 cm of above ground part was covered with a conifer chamber. The change in net photosynthetic rate was measured at PAR saturation, corresponding to a photosynthetic photon flux density (PPFD) of 1000-1200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  which was provided by a cool halogen lamp (Walz, Effeltrich, Germany). The leaf temperature was 25°C and the relative humidity was 50-70%.

### 3.2.8 Plant growth analysis

To determine the growth rate of the seedlings, five seedlings were randomly sampled from each species and each treatment to determine the total dry mass (TDM) of each organ of the seedlings (shoot, root). The sampled seedlings were washed with distilled water and separated into shoots and roots. All plant organs were dried at 60°C for 1 week and weighed using an electronic balance (HR-202, A&D, Japan).

The relative total dry mass (TDM) of the seedlings was calculated as follows :  $\text{relative TDM}(\%) = (\text{average TDM of each seedling grown at each soil acidified and each ectomycorrhiza treatment}) / (\text{average TDM of the seedlings grown in the control soil})\times 100$ .

### 3.2.9 Statistical analysis

The plants were arrayed in a randomized complete block design involving all five treatments (control, 10, 30, 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$ ), with 6 single plant replications of each ectomycorrhiza treatment and each species. Analyses were performed using the Mixed and Genmod procedures of the SAS system (SAS Institute, Inc., 1998). Tukey's Studentized Range (HSD) test was used to separate means at  $p < 0.05$ .

## 3.3 Results

### 3.3.1 Soil condition

Table 3.1 shows the pH and concentration of water-soluble elements in the soil solution. Unsurprisingly, the pH of the soil solution decreased with increasing  $\text{H}^+$  load. The concentration of Al and Mn in water-soluble form increased with decreasing soil pH, with an abrupt increase in Al at soil pH ( $\text{H}_2\text{O}$ ) = 3.77, 60  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatment. Also, the concentrations of base cations (Ca, Mg, K) in water-soluble increased with decreasing soil pH. The concentration of Ca increased abruptly at soil pH ( $\text{H}_2\text{O}$ ) 3.77; the increase for K and Mg were less than for Ca.

### 3.3.2 Mycorrhizal development

Fig. 3.1 shows the inoculation rate of each ectomycorrhiza (*Pt*, *EC* or *Cg*) for *P. rifida*, *P. koraiensis*, *P. densiflora* and *L. kaempferi* after 180 days grown at each soil treatment. Each ectomycorrhizal development of each pine species was significantly increased at 10 and 30  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments compared with control (0  $\text{mmolH}^+\cdot\text{kg}^{-1}$ ) treatment ( $P < 0.05$ ). However, at the 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments significantly decreased than

that of control treatment. Moreover, I did not find that any other ectomycorrhiza development in the non-inoculated each seedling grown at each soil treatment.

### 3.3.3 Condition of chemical elements in plant organs

Fig. 3.2 shows the concentration of nitrogen in needles for each species and each soil treatment. The concentration of N in needle tended to increase with progressive addition of mixed acid solution ( $H_2SO_4$ ,  $HNO_3$  and  $HCl$ ). In particular, at the 60 and 90  $mmolH^+ \cdot kg^{-1}$  treatments at each species and each ectomycorrhiza treatment was significantly greater than in controls ( $P < 0.05$ ).

For ectomycorrhizal seedlings grown with *Pt*, *EC* or *Cg* in acidified soils, the concentration of Al in roots and Mn in needles significantly lower than non-ectomycorrhizal (NE) seedlings, at each species and each soil treatment ( $P < 0.05$ ) (Fig. 3.3, 3.4). And, the concentration of Al in roots and Mn in needles increased with increasing add  $H^+$ , especially 60 and 90  $mmolH^+ \cdot kg^{-1}$  treatments relative to controls. Addition of solution containing  $H^+$  to the soil increased the concentration of N and Mn in the needles and Al in the roots. And, I did not find that the difference of Mn concentration in needle for *L. kaempferi* seedlings grown at 90  $mmolH^+ \cdot kg^{-1}$  treatment.

Table 3.1. Initial pH and concentration of elements in soil immediately before transplantation planted with each seedling for a growth period of 180 days. Mean of 5 pots  $\pm$  SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each item.

Soil treatment	pH ( $H_2O$ )	Element concentration ( $\mu g \cdot g^{-1}$ )					(Ca+Mg+K)/Al ( $mol \cdot mol^{-1}$ )
		Ca	Mg	K	Al	Mn	
control	4.91 $\pm$ 0.03 a	66.2 $\pm$ 3.31 e	2.08 $\pm$ 0.02 e	10.4 $\pm$ 0.53 e	1.4 $\pm$ 0.07 d	0.83 $\pm$ 0.06 e	37.29
10 $mmolH^+ \cdot kg^{-1}$	4.45 $\pm$ 0.03 b	92.9 $\pm$ 4.67 d	6.66 $\pm$ 0.02 d	15.4 $\pm$ 0.77 d	9.9 $\pm$ 0.15 cd	3.33 $\pm$ 0.38 d	8.14
30 $mmolH^+ \cdot kg^{-1}$	3.85 $\pm$ 0.03 b	157.0 $\pm$ 7.90c	14.58 $\pm$ 0.02 c	22.1 $\pm$ 1.10 c	19.0 $\pm$ 0.95 c	15.83 $\pm$ 0.82 c	7.22
60 $mmolH^+ \cdot kg^{-1}$	3.77 $\pm$ 0.02 b	231.2 $\pm$ 11.56 b	17.91 $\pm$ 0.01 b	27.9 $\pm$ 1.40 b	98.6 $\pm$ 4.8 b	28.32 $\pm$ 0.9 b	1.98
90 $mmolH^+ \cdot kg^{-1}$	3.18 $\pm$ 0.03 c	263.6 $\pm$ 13.18 a	21.24 $\pm$ 0.01 a	32.5 $\pm$ 1.63 a	229.8 $\pm$ 11.49 a	44.57 $\pm$ 1.67 a	0.97

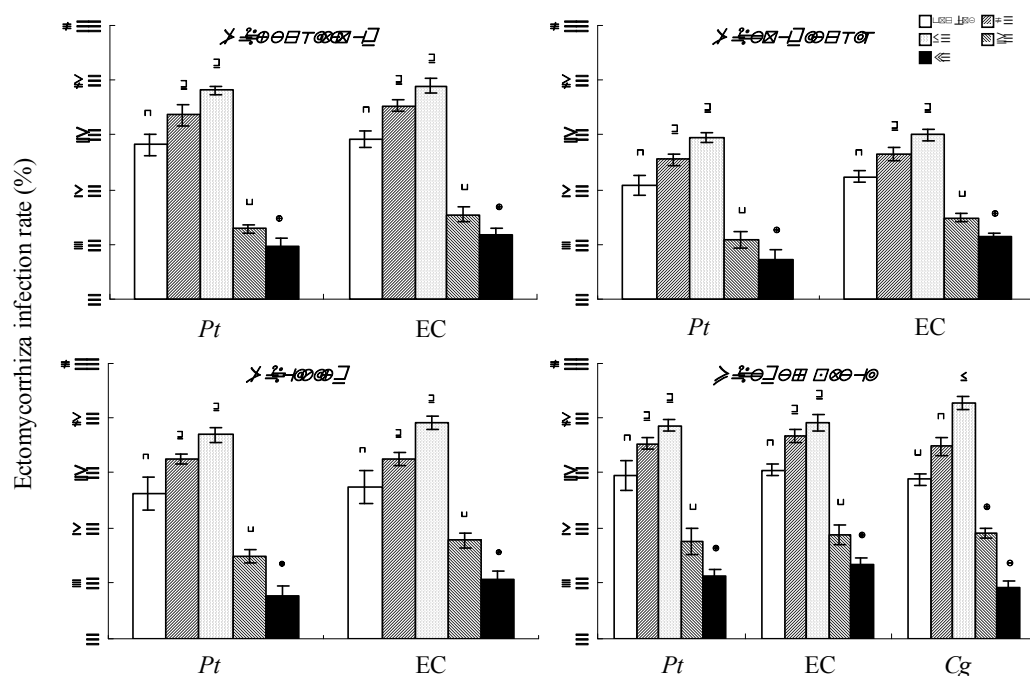


Fig. 3.1. The ectomycorrhiza infection rate of each species grown at control (0), 10, 30, 60 and 90  $mmolH^+ \cdot kg^{-1}$  treatments after 180 days. Mean of 5 seedlings  $\pm$  SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tinctorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.



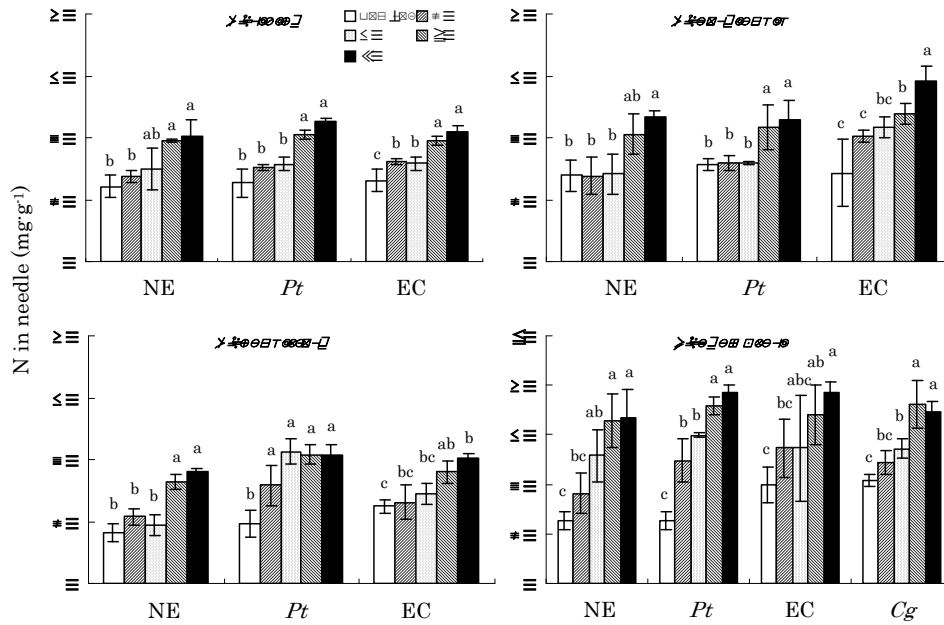


Fig. 3.2. The concentration of N in needle of each seedling at soil treatment of control (0), 10, 30, 60 and 90 mmolH<sup>+</sup>·Kg<sup>-1</sup> grown with each ectomycorrhiza at 180 days. Mean of 5 seedlings ± SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. NE represented non-ectomycorrhizal seedlings and *Pt*, EC and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, Ectodrench or *Cenococcum geophilum*, respectively.

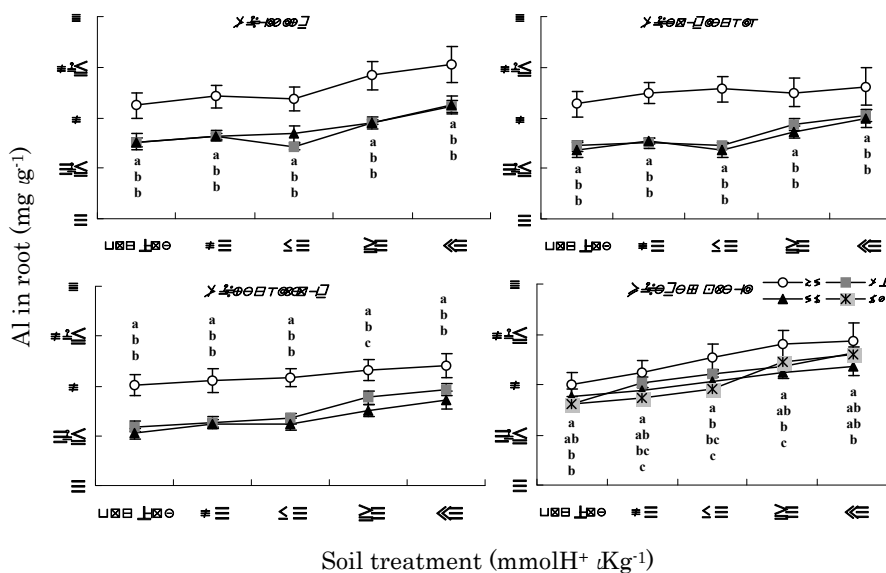


Fig. 3.3. The concentration of Al in roots of each seedling at soil treatment of control (0), 10, 30, 60 and 90 mmolH<sup>+</sup>·Kg<sup>-1</sup> grown with each ectomycorrhiza at 180 days. Mean of 5 seedlings ± SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. NE represented non-ectomycorrhizal seedlings and *Pt*, EC and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, Ectodrench or *Cenococcum geophilum*, respectively.

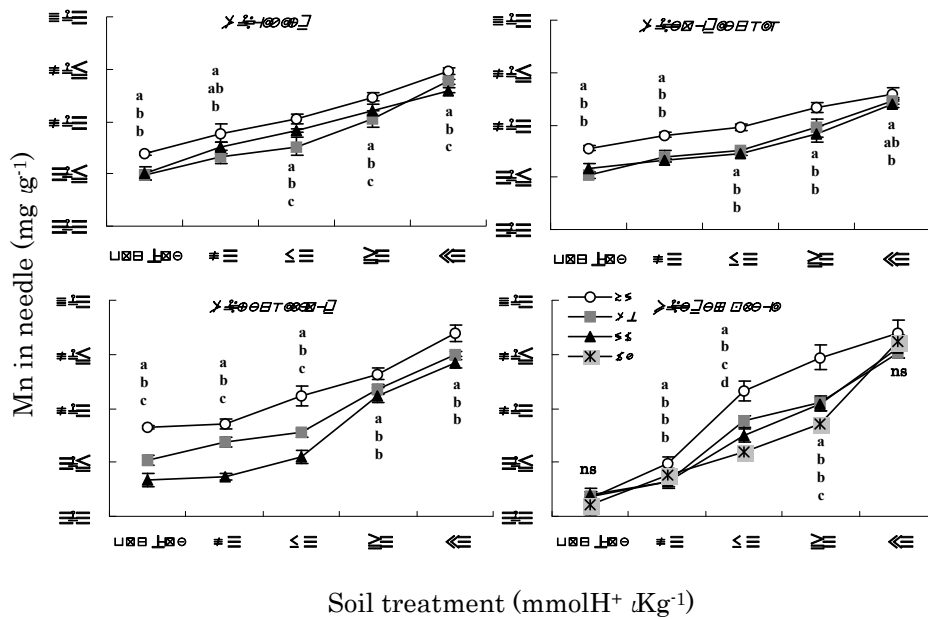


Fig. 3.4. The concentration of Mn in needle of each seedling at soil treatment of control (0), 10, 30, 60 and 90 mmolH<sup>+</sup>·Kg<sup>-1</sup> grown with each ectomycorrhiza at 180 days. Mean of 5 seedlings ± SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. NE represented non-ectomycorrhizal seedlings and *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

### 3.3.4 Photosynthetic response

Fig. 3.5 shows the maximum net photosynthetic rate at saturated CO<sub>2</sub> concentration ( $P_{max}$ ) of each seedling at soil treatment of control (0), 10, 30, 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> grown with each ectomycorrhiza at 180 days. The value of  $P_{max}$  of NE seedling of each species increased at 10 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments than that of control treatment, and decreased over 30 mmolH<sup>+</sup>·kg<sup>-1</sup>, in particular 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments markedly decreased.  $P_{max}$  of each species inoculated with *Pt*, *EC* or *Cg* was significantly higher than for non-ectomycorrhizal seedlings of each species ( $P < 0.05$ ). And,  $P_{max}$  of each ectomycorrhizal seedling of each species increased to 30 mmolH<sup>+</sup>·kg<sup>-1</sup> and then decreased 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatment.

### 3.3.5 Plant responses to deliberate soil acidification

The dry mass of the whole plant of NE, *Pt*, *EC* or *Cg* at each species increased to 10 or 30 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments, but I did not find significant difference with control treatment, but *L. kaempferi* seedlings grown with *Pt* or *EC* show significant difference (Fig. 3.6). However, the TDM of each species and each ectomycorrhiza treatment decreased significantly for the 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments, relative to the control and the 10 and 30 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments, even if that of ectomycorrhizal seedlings of each species increased, which was less for NE seedlings.

## 3.4 Discussion

Most possible factors limiting the growth and nutrient status of plants grown in acid soil are the high

soil acidity itself, phyto-toxic metals such as Al or Mn dissolved in the soil solution, and reduced availability of certain elements essential for plant growth (Adams 1981, Ulrich 1989, Kochian 1995). A high concentrations of Al or Mn in nutrient solutions or in acidic soils reduces the uptake of elements essential for growth and the uptake of water through the roots, and reduces the net photosynthetic rate, transpiration rate and leaf chlorophyll concentration of several plants (Suresh *et al.* 1987, Nable *et al.* 1988, Macfie and Taylor 1992, Kitao *et al.* 1997a, 1997b).

The activity of the meristems of the root tip is inhibited by Al (Kelly *et al.* 1990) or by deficiencies of Ca and Mg induced by antagonism at the root surface (Ericsson *et al.* 1995), which reduces root development. Moreover, even though Mn is a co-factor of photosynthesis (Marschner 1995), excess Mn in foliage organs usually reduces photosynthetic function at PS II (Kitao *et al.* 1997a, 1997b). These combined effects of Al in roots and Mn in needles may reduce the photosynthetic rate and growth of *P. rigida*, *P. koraiensis*, *P. densiflora* and *L. kaempferi*. Similar tendencies have been observed in the red pine (Lee *et al.* 1999) and Japanese cedar (*Cryptomeria japonica*) seedlings treated with H<sub>2</sub>SO<sub>4</sub> (Miwa *et al.* 1994) and AlCl<sub>3</sub> (Izuta *et al.* 1996b).

The concentrations of elements essential for growth, such as Ca, Mg, K, and P are reduced in conifers grown in a nutrient solution having a pH below 4.0 or in acidic soil (Izuta and Totsuka 1996, Yang *et al.* 1996). However, in the present study, the nutrient status of each species and each mycorrhizal treatment were not

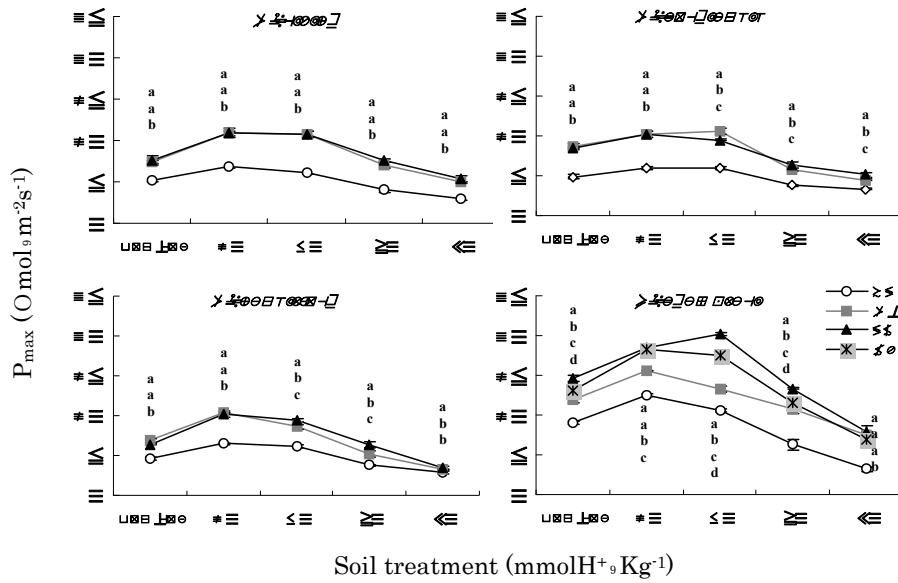


Fig. 3.5. Maximum net photosynthetic rate at saturated  $CO_2$  concentration ( $P_{max}$ ) of each seedling at soil treatment of control (0), 10, 30, 60 and 90  $mmol\ H^+ \cdot Kg^{-1}$  grown with each ectomycorrhiza at 180 days. Mean of 5 seedlings  $\pm$  SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. NE represented non-ectomycorrhizal seedlings and *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

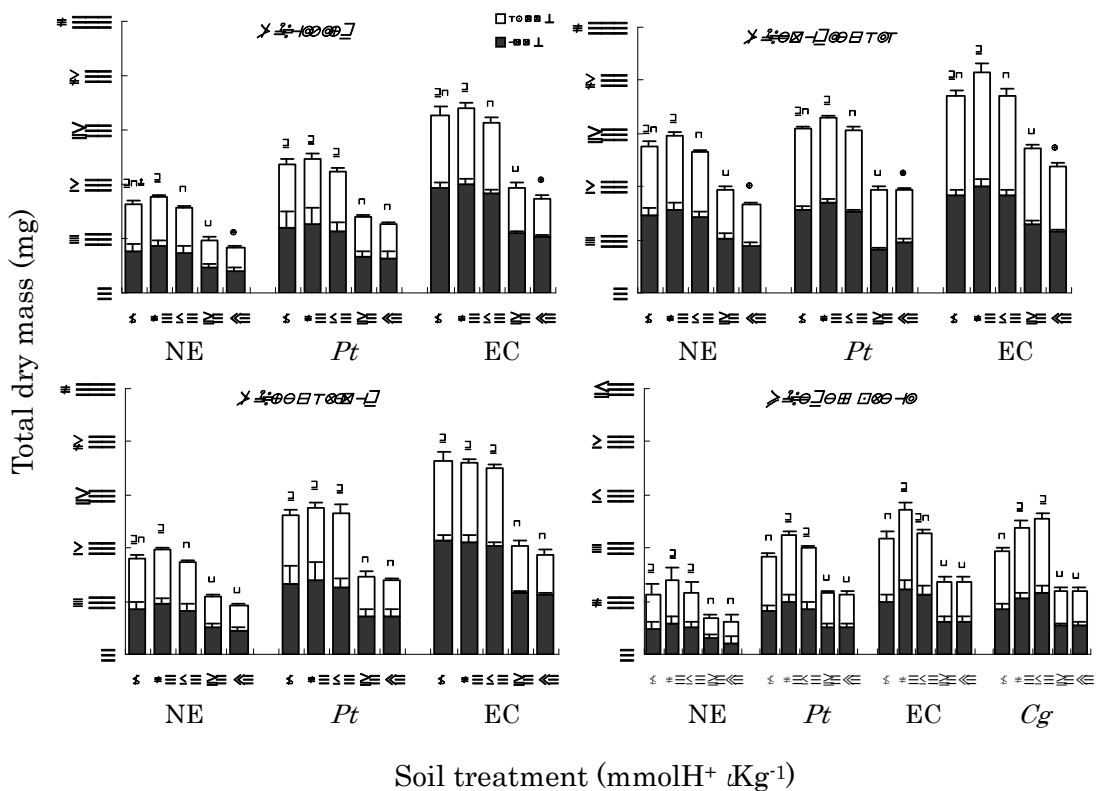


Fig. 3.6. Effect of soil acidification on dry mass of *Pinus rigida*, *P. koraiensis*, *P. densiflora* and *Larix kaempferi* seedlings. NE represented non-ectomycorrhizal seedlings and *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively. Mean of 5 seedlings  $\pm$  SE. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species.

adversely affected by extremely acidified forest soil (data not shown). Similar tendencies have been observed in plant organs grown in low pH solution (Hutchinson 1980, Kim 1986, Izuta *et al.* 1996b, Choi *et al.* 2005a). This shows that the ability to take up essential nutrients via the roots of each species was not affected by extremely acidified forest soil. Moreover, the concentration of N in needle at each species was significantly greater than in controls, especially in the 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments. Why did the N content in each organ increase significantly with added acid solution compared to the control treatment? The reason is as follows : nitrogen can be absorbed by plants in three distinct forms: nitrate, ammonium, and amino acids (Lamber *et al.* 1998).

Therefore, it was believed that the seedlings grown in the 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments can absorb more nitrogen than controls, because I administered an acid solution comprising H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, and HCl. Nitrogen and phosphorus are found to limit tree growth by reducing photosynthetic activity more frequently than any other nutrient (Fisher and Binkley 2000). The present results suggest that the main reason for the growth reduction and photosynthesis depression of each species grown in extremely acidified forest soil (e.g. 60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup> treatments) is accumulation of Al and Mn in root or needle not due to nutrient deficiency.

The molar ratios of cations such as Ca, Mg, and K to Al in soil or nutrient solutions are important factors limiting the growth and nutrient status of woody plants (Hecht-Buchholz *et al.* 1987, Kruger and Sucoff 1989, Sverdrup *et al.* 1994, Cronan and Grigal 1995). This study found a strong positive correlation between the

relative TDM of each seedling and the molar water-soluble (Ca+Mg+K)/Al (i.e. BC/Al) molar ratio in the soil (Fig. 3.7).

Sverdrup *et al.* (1990) proposed critical limits for growth: forest soil solution pH lower than 4.4 for the B horizon; labile aluminum higher than 2.0 mg L<sup>-1</sup>; and BC/Al molar ratio lower than 1.0. Similar relations between plant growth and BC/Al in spruce or pine native to Europe and America have been reported (Rengel 1992, Sverdrup *et al.* 1994, Cronan and Grigal 1995), and this reduction in growth of pine seedlings grown on acidified soil is due to the effect of nutrient imbalance between Al and (Ca + Mg + K) in the soil.

This study found the reduction of the relative TDM of *P. koraiensis* and *L. kaempferi* seedlings of approximately 40% at a BC/Al ratio of 1.0, but that of *P. rigida* and *P. densiflora* of approximately 50% at a BC/Al ratio of 1.0 (Fig. 3.7). And, also relative TDM of ectomycorrhizal seedlings of each species decreased at a BC/Al ratio of 1.0 compared with their control treatment (i.e. control treatment of each ectomycorrhizal seedling), approximately the same ratio compared with each non-ectomycorrhizal species. However, ectomycorrhizal seedlings of each species increased relative TDM by 20-100% at a BC/Al ratio of 1.0 than NE seedlings of each species, even if those also reduced. My results indicate that *P. koraiensis* and *L. kaempferi* tended more tolerant to BC/Al than *P. rigida* and *P. densiflora*. Moreover, ectomycorrhizal infection significantly increased nutrients uptake and significantly protected plant growth against Al and Mn (Wilkins 1991, Jentschke and Godbold 2000, Ahonen-Jonnarth *et al.* 2003) compared with

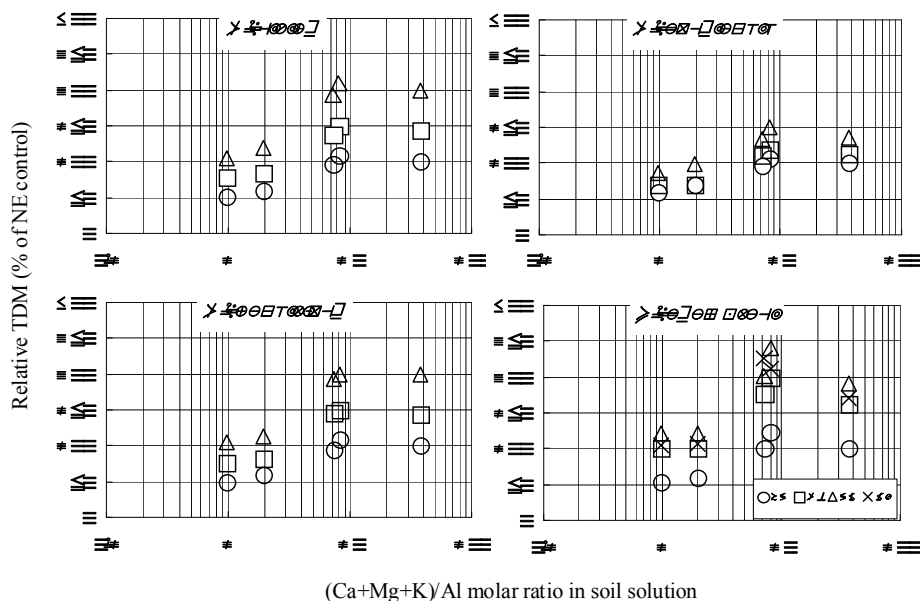


Fig. 3.7. The relationships between (Ca+Mg+K)/Al molar ratio in soil solution and relative total dry mass (TDM) of *Pinus rigida*, *P. koraiensis*, *P. densiflora* and *Larix kaempferi* seedlings. NE represented non-ectomycorrhizal seedlings and *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tinctorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

non-ectomycorrhizal seedlings of each species. As a result, the growth decline at the acidified forest soil was moderated as compared with non-ectomycorrhizal seedlings.

Further studies will be need to clarify the physiological function and status of pine species with ectomycorrhizae under severe soil acidification, i.e. BC/Al ratio of 0.01-1.0.

## Chapter 4

### Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of three pine species seedlings grown under elevated CO<sub>2</sub> concentrations

#### 4.1 Introduction

Pine species (*Pinus rigida*, *P. koraiensis*, *P. densiflora* and *Larix kaempferi*) is the dominant conifer in infertile granite regions in the Korean peninsula. These species can survive and grow on the nutrient-poor ridges of mountains after disturbances. These species usually has a symbiotic interaction with ectomycorrhizae allowing for vigorous growth. When there have been several disturbances, i.e. forest decline, fire, typhoon etc., we have been planted pine species as pioneer for forest rehabilitation practices in nutrient-poor soils (e.g. Moroto *et al.* 1987), because of their fast growth drought tolerant traits. In particular after forest fires, charcoal in soil increases germination rate and surviving living seedlings for increases the aperture of soil, holds the soil moisture and raises soil temperature (Debano *et al.* 1998).

The pattern and amount of precipitation at middle and high latitudes have recently been predicted to undergo great changes with the increase in atmospheric CO<sub>2</sub> concentration [CO<sub>2</sub>] (IPCC 1996). Precipitation could decrease in middle latitude. Moreover, photosynthetic adjustment in down-wards is frequently observed in plants grown under high [CO<sub>2</sub>] because of dilution effects of nutrient reduction of enzyme activities, enhanced accumulation of photosynthates in photosynthetic organs and sink strength (Wardlaw 1990, Farrar and Williams 1991, Berntson and Bazzaz 1996).

Symbiotic micro-organisms such as ectomycorrhiza usually act as an efficient root system for absorbing water and essential nutrients (nitrogen, phosphate) (Bolan 1991, Smith and Read 1997, Quoreshi *et al.* 2003). The activity of the host plants should be enhanced at high [CO<sub>2</sub>] because symbiotic ectomycorrhiza play an essential role in absorbing water and important elements such as nitrogen and phosphate, and also act as a large carbon sink (Ceulemans and Mousseau 1994, Smith and Read 1997). Therefore, no down-regulation of photosynthesis is predicted following pine species inoculation with ectomycorrhiza at high [CO<sub>2</sub>] as observed in *Pinus densiflora* (Choi *et al.* 2005b).

Several ectomycorrhizae form symbiotic interactions between pine species (Smith and Read 1997). Of these, *Pisolithus tinctorius* (Pers.) Coker et Couch (*Pt*) and *Cenococcum geophilum* Fr. (*Cg*) are the most common species in pine forests world-wide (Marx *et al.* 1984, Trappe 1988, Allen 1996). I study here the effect of

ectomycorrhizae, e.g. *Pt*, *Cg* and mixed mycorrhiza (EC) on the physiological responses of three pine tree seedlings under high [CO<sub>2</sub>]. My aim is to estimate the effect of ectomycorrhiza association and development on the down-regulation of photosynthesis responses and the growth of pine species at increased [CO<sub>2</sub>] through stomatal limitation and P concentration in needles.

## 4.2 Materials and methods

### 4.2.1. Experiment site and design

This experiment with *Pinus koraiensis*, *P. densiflora* and *Larix kaempferi* was conducted at the Hokkaido Research Center, Forestry and Forest Products Research Institute (FFPRI), Sapporo (Japan) in 2002. The seedlings were grown in a FFPRI phytotron with a natural sunlight, day/night temperature range of 26/16 °C and humidity range of 55-75% during the study period lasting 18 weeks. Six cabinets were used for cultivation, with two differing [CO<sub>2</sub>]. The rhizo-boxes were allocated at random such that half of the seedlings experienced ambient [CO<sub>2</sub>] (360 μmol·mol<sup>-1</sup>) and the other half experienced elevated [CO<sub>2</sub>] (720 μmol·mol<sup>-1</sup>) (Koike 1995). At each CO<sub>2</sub> concentration (three cabinets of phytotron), the each seedling was inoculated with ectomycorrhiza fungus (*Pt*, EC or *Cg*) or non-ectomycorrhizal seedlings (NE). The seedlings number of each treatment in a cabinet was sixteen. Each treatment was repeated in three cabinets, and the subgroups are denoted as follows: (1) 360 μmol·mol<sup>-1</sup>, NE, (2) 360 μmol·mol<sup>-1</sup>, inoculated (*Pt*, EC or *Cg*), (3) 720 μmol·mol<sup>-1</sup>, NE, (4) 720 μmol·mol<sup>-1</sup>, inoculated (*Pt*, EC or *Cg*).

### 4.2.2 Plant materials

Seeds of Korean pine (*Pinus koraiensis* Sieb. et Zucc.), Red pine (*Pinus densiflora* Sieb. et Zucc.) and Japanese larch (*Larix kaempferi* Sarg.) were collected at the Yongmun Experimental Field of the Korea Forest Service (37.3°N, 127.2°E). After seeds were put in 4°C for 10-day cold treatment, they were surface sterilized with 30% H<sub>2</sub>O<sub>2</sub> for 20 min and rinsed 4-5 times with sterile deionized water, and were then germinated on sterilized media, black sand : vermiculate : peat moss = 3:2:1 (volume), in a glasshouse at day/night temperatures 25/20°C with a 16-h photoperiod.

### 4.2.3 Ectomycorrhizae

Ectomycorrhiza fungi, *Pisolithus tinctorius* (Pers.) Coker et Couch (*Pt*) and Diehard Ecto drench (EC) - (*Pt* + *Rhizopogon* spp. + *Laccaria* spp. + *Scleroderma* spp.), were obtained from Horticultural Alliance, Inc., USA. *Cenococcum geophilum* Fr. (*Cg*) was isolated from root of *Picea glehnii* (Fr. Schm.) collected from a mixed larch and spruce stand located at the experimental forests of Forestry and Forest Products Research Institute (FFPRI), Sapporo, Japan. The isolated fungal culture and the culture obtained from the laboratory of Forest Resource Biology, Hokkaido University (offered by Dr. Y. Tamai) were maintained on modified MMN agar medium and mycorrhizal synthesis was followed further inoculation study (Qu *et al.* 2003). The *Pt* and EC spores were suspended in

distilled water. The *Pt*, *EC* or *Cg* were inoculated directly to roots of each species seedlings and mixed with prepared soil media (see below). The seedlings inoculated with each ectomycorrhiza were naturalized in a rhizo-box (100×230×18 mm) filled with sterilized medium consisting of vermiculate : black sand : peat moss = 2:2:1 (in volume).

#### 4.2.4 Measurement of photosynthesis

The  $A/C_i$  ( $A$ =net photosynthetic rate,  $C_i$ =intercellular  $CO_2$  concentration) curves were examined using an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) between 09:00 and 15:00 local time. 5 - 7 cm of above ground part was covered with a conifer chamber. The change in  $A$  was measured at PAR (photosynthetic active radiation) saturation, corresponding to a photosynthetic photon flux density (PPFD) of 1000-1200  $\mu mol \cdot m^{-2} \cdot s^{-1}$  which was provided by a cool halogen lamp (Walz, Effeltrich, Germany). The leaf temperature was 25°C and the relative humidity was 50-70%. Foliages were allowed to acclimatize to their surroundings for 10 min prior to measurement, and then determined with 150-1500  $\mu mol \cdot mol^{-1} CO_2$  concentrations.

The initial slope of the  $A/C_i$  curve is proportional to the carboxylation activity of Rubisco [i.e., the carboxylation efficiency (CE,  $\mu mol \cdot Pa^{-1}$ )]. CE was calculated from the increment in intercellular  $CO_2$  partial pressure ( $C_i$ ) and the net photosynthetic rate of the initial slope of the  $A/C_i$  curve (Terashima 1992).

The maximum net photosynthetic rate at  $CO_2$  saturation concentration ( $P_{max}$ ,  $\mu mol \cdot m^{-2} \cdot s^{-1}$ ) was determined of 1200  $\mu mol \cdot mol^{-1} CO_2$ . The relative stomatal limitation of photosynthesis ( $l_s$ , %) was calculated as follows, based on the  $A$  to variation in  $C_i$  ( $A/C_i$  curves) :  $l_s = (1 - A_i/A_a) \times 100$ , where  $A_i$  is the actual net photosynthetic rate and  $A_a$  is the net photosynthetic rate when stomatal resistance to  $CO_2$  diffusion is zero (Sharkey 1985a). After the gas exchange measurement, the needle projection area was measured with an image scanner (FB636U, Canon, Japan), and then calculated the net photosynthetic rate per unit needle area.

#### 4.2.5 Infection rate of ectomycorrhizae

The root tips was observed and counted both infected and non-infected roots (Quoreshi 2003). The inoculation rate of ectomycorrhizae (IRE) was determined according to the following formula:

$$IRE (\%) = ER / (ER + NR) \times 100,$$

where ER and NR respectively denote the number of ectomycorrhizal and non-ectomycorrhizal roots.

#### 4.2.6 Growth rate

Shoot and root dry mass of each seedling was measured after dried at 60°C for 1 week using an electronic balance (HR-202, A&D, Japan).

#### 4.2.7 Phosphorus analysis

The dried samples were then ground to a fine powder in a vibrating sample mill (Wonder Blender, Osaka Chemical Co., Osaka, Japan). To determine the

concentration of P, the samples were digested by a microwave digestion system (O-I analytical, College Station, TX) and underwent ICP analysis.

#### 4.2.8 Germination with charcoal

The germination rate of *P. densiflora*, *P. rigida* and *L. kaempferi* seeds was examined on sterilized brown forest soil (Chapter 3.2.3) or brown forest soil mixed charcoal. The cold treatment and surface sterilization are follows like up (Chapter 4.2.2). The prepared seeds were germinated in a FFPRI phytotron under 360  $\mu mol \cdot mol^{-1}$  and 720  $\mu mol \cdot mol^{-1}$  (Chapter 4.2.1). The germinated seedlings tested inoculation rate under the same condition with *EC* of several ectomycorrhizae (Chapter 4.2.3).

#### 4.2.9 Statistical analysis

Mean values of the inoculation rate, the P concentration in needles,  $P_{max}$ ,  $l_s$ , CE, RuBP regeneration rate and the shoot and root growth were examined by the t-test (Li 1964) using the Stat View 5.0 software (SAS Institute, Cary, NC, USA)

### 4.3 Results

#### 4.3.1 Shoot and root growth

Different ectomycorrhizal inoculation induced large differences in dry mass between non-ectomycorrhizal seedlings at each species (Fig. 4.1). In *P. koraiensis*, *Pt* or *EC* inoculation significantly increased the shoot and root dry mass relatively to non-ectomycorrhizal seedlings grown at 720  $\mu mol \cdot mol^{-1} [CO_2]$  ( $P < 0.05$ ), but I did not find that significantly difference between those grown at 360  $\mu mol \cdot mol^{-1} [CO_2]$ . In *P. densiflora*, the shoot and root dry mass of ectomycorrhizal seedlings (*Pt*, *EC* or *Cg*) significantly increased relatively to non-ectomycorrhizal seedlings grown at 360  $\mu mol \cdot mol^{-1} [CO_2]$  and 720  $\mu mol \cdot mol^{-1} [CO_2]$ , with the exception of *Pt* at 720  $\mu mol \cdot mol^{-1} [CO_2]$ . In particular, the seedling inoculated with *EC* was greater than that inoculated with other ectomycorrhizae (e.g. *Pt* and *Cg*). Also, dry mass of *L. kaempferi* seedlings grown with ectomycorrhiza significantly increased compared with non-ectomycorrhizal seedlings grown at 360  $\mu mol \cdot mol^{-1} [CO_2]$  and 720  $\mu mol \cdot mol^{-1} [CO_2]$  ( $P < 0.05$ ). Ectomycorrhizal inoculation increased dry mass of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings at both  $CO_2$  concentration and especially *EC* inoculation more increased dry mass of each species relatively to *Pt* or *Cg* inoculation.

#### 4.3.2 Mycorrhizal development and P concentration in needles

Fig. 4.2 shows the infection rate of ectomycorrhiza (*Pt*, *EC* and *Cg*) after 18 weeks growth at both ambient and elevated  $CO_2$  concentrations. Ectomycorrhizal development of *EC* and *Cg* for *P. koraiensis*, *P. densiflora* and *L. kaempferi* was significantly affected by the  $CO_2$  concentration ( $P < 0.05$ ). Moreover, *Pt* development with *P. koraiensis* was significantly increased grown at 720  $\mu mol \cdot mol^{-1} [CO_2]$  than that at 360  $\mu mol \cdot mol^{-1} [CO_2]$ , however, that of *P. densiflora* and *L. kaempferi* was not significantly increased than

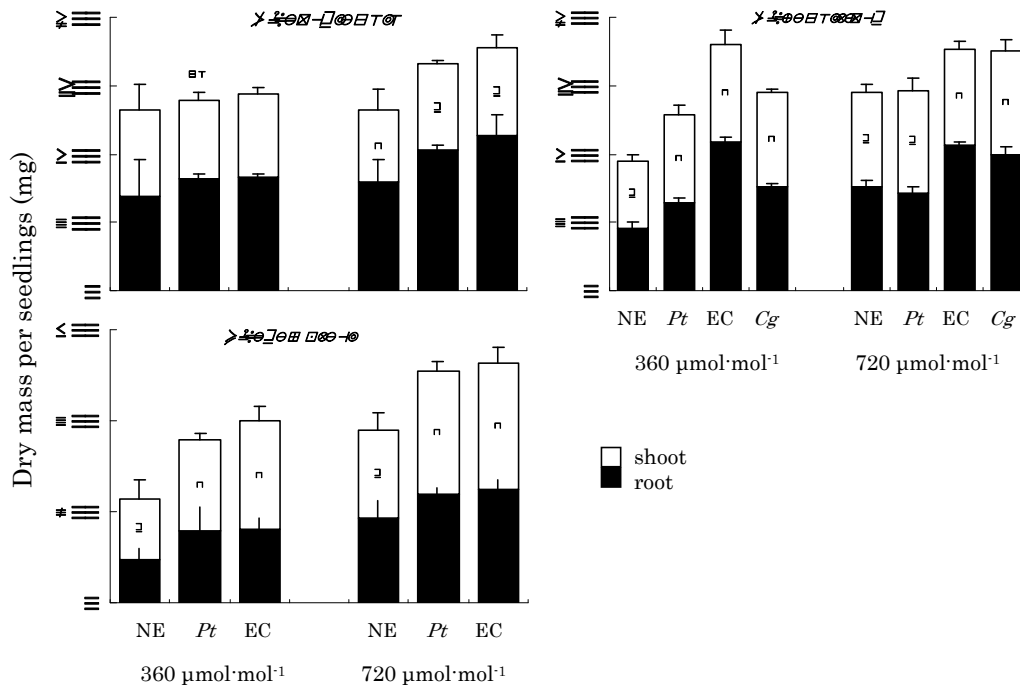


Fig. 4.1. Effect of ectomycorrhizal inoculation by *Pt*, *EC* or *Cg* on the shoot and root dry mass of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings and non-inoculated (NE) seedlings grown at  $360 \mu\text{mol}\cdot\text{mol}^{-1}$  or  $720 \mu\text{mol}\cdot\text{mol}^{-1}$ . Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

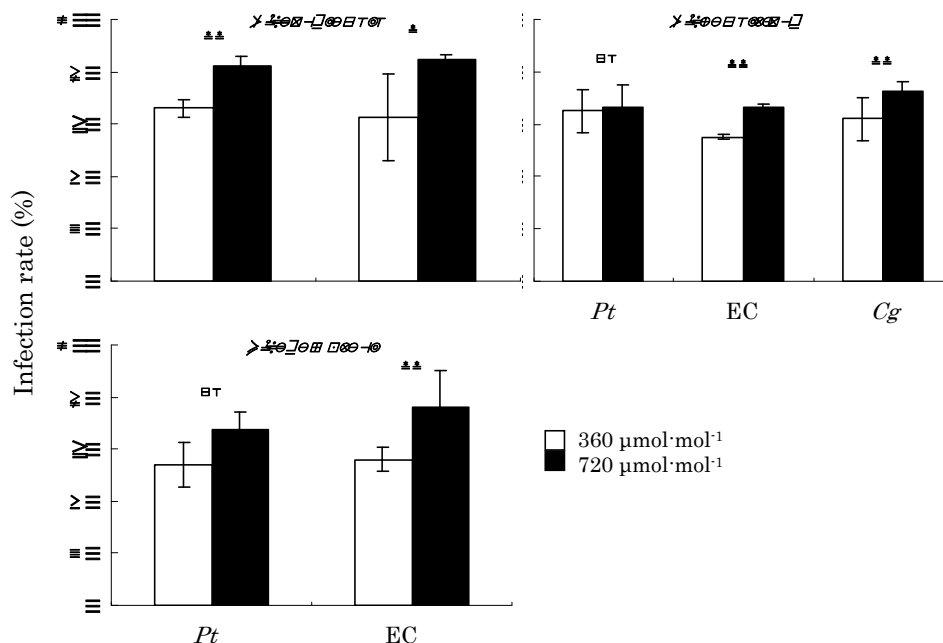


Fig. 4.2. Ectomycorrhizal infection rate of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings inoculated *Pt*, *EC* or *Cg* seedlings grown at  $360 \mu\text{mol}\cdot\text{mol}^{-1}$  or  $720 \mu\text{mol}\cdot\text{mol}^{-1}$ . Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>], even if increased.

The P concentration in needles and roots inoculated with *Pt*, *EC* or *Cg* of each species was significantly higher than without inoculation at both CO<sub>2</sub> concentrations. ( $P<0.05$ ) (Fig. 4.3). Especially, *EC* or *Cg* inoculation in *P. densiflora* was significantly increased P concentration in needles and roots than non-ectomycorrhizal seedlings and *Pt* inoculation seedlings.

#### 4.3.3 Photosynthetic response

Fig. 4.4 shows the photosynthetic response to intercellular CO<sub>2</sub> (C<sub>i</sub>) at PAR saturation i.e. (A/C<sub>i</sub>) for *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] and 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>]. Values of A of *P. koraiensis*, *P. densiflora* and *L. kaempferi* inoculated with *Pt*, *EC* or *Cg* were significantly higher than those of control seedlings in both [CO<sub>2</sub>] treatments. The maximum net photosynthetic rate at saturated CO<sub>2</sub> concentration (P<sub>max</sub>) of each seedling inoculated with *Pt*, *EC* or *Cg* was higher than for controls ( $P<0.05$ ) (Table 4.1). Moreover, the carboxylation efficiency (CE) and RuBP regeneration rate of the A/C<sub>i</sub> curve for each species inoculated with *Pt*, *EC* or *Cg* were significantly higher than for non-inoculated seedlings at both CO<sub>2</sub> concentrations, and especially at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] except for *P. koraiensis* at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] (Table 4.1 and Fig. 4.5,  $P<0.05$ ). And, these of

non-ectomycorrhizal seedlings of each species show photosynthetic down-regulation, i.e. carboxylation efficiency and RuBP regeneration rate decreased at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] than 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>], but I did not find the tendency at the inoculated seedlings.

#### 4.3.4 Stomatal limitation

Stomatal limitation (*ls*) of *P. koraiensis* seedlings at each CO<sub>2</sub> concentration and *L. kaempferi* at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  inoculated with *Pt* or *EC* was lower than that of non-inoculated seedlings, but I did not find significant difference (Fig. 4.6). On the other hand, *ls* of *P. densiflora* at each CO<sub>2</sub> concentration and *L. kaempferi* at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  inoculated with *EC* or *Cg* was significantly lower than that of non-inoculated each seedling ( $P<0.05$ )

#### 4.3.5 Germination rate and ectomycorrhizal infection rate in soil with mixing charcoal

Germination rate of *P. rigida*, *P. densiflora* and *L. kaempferi* sow seeds in a forest soil mixed charcoal after 21 days was significantly higher than that sow seeds in a forest soil grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  and 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] ( $P<0.05$ ) (Fig. 4.7). However, the ectomycorrhizal infection rate of *P. rigida*, *P. densiflora* and *L. kaempferi* seedlings grown at forest soil mixed charcoal decreased significantly as compared with at forest soil at each CO<sub>2</sub> treatment ( $P<0.01$ ) (Fig. 4.8).

Table 4.1. Parameter estimates as functions of the A/C<sub>i</sub> curve. Maximum net photosynthetic rate at saturated CO<sub>2</sub> concentration (P<sub>max</sub>) and RuBP regeneration rate of photosynthesis in the needles of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings. Data within a series followed by the same small letter do not differ significantly ( $P<0.05$ ) in each species.

	CO <sub>2</sub> treatment ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	Inoculation	RuBP Regeneration ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	P <sub>max</sub> ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
<i>P. koraiensis</i>	360	NE	41.12 ± 3.40 b	4.30 ± 0.32 b
		<i>Pt</i>	52.73 ± 6.59 a	5.00 ± 0.10 a
		<i>EC</i>	50.25 ± 2.52 a	6.57 ± 0.26 a
	720	NE	33.50 ± 3.64 b	3.55 ± 0.23 b
		<i>Pt</i>	54.03 ± 5.94 a	6.00 ± 0.15 a
		<i>EC</i>	67.82 ± 10.05 a	6.51 ± 0.10 a
<i>P. densiflora</i>	360	NE	19.72 ± 8.10 b	8.58 ± 0.11 c
		<i>Pt</i>	51.24 ± 5.90 a	11.84 ± 0.71 b
		<i>EC</i>	45.99 ± 7.82 a	15.36 ± 1.03 a
	720	<i>Cg</i>	45.16 ± 9.01 a	17.01 ± 0.76 a
		NE	18.66 ± 5.02 b	7.10 ± 0.22 c
		<i>Pt</i>	62.63 ± 3.48 a	13.73 ± 2.02 ab
<i>L. kaempferi</i>	360	<i>EC</i>	62.31 ± 0.52 a	16.02 ± 0.11 a
		<i>Cg</i>	67.02 ± 1.85 a	13.36 ± 0.09 b
		NE	42.14 ± 13.88 b	5.81 ± 0.51 b
	720	<i>Pt</i>	52.78 ± 3.53 a	9.14 ± 0.82 a
		<i>EC</i>	86.84 ± 8.32 a	10.53 ± 1.36 a
		NE	41.79 ± 0.08 b	6.99 ± 0.52 b
720	<i>Pt</i>	56.56 ± 0.03 ab	11.13 ± 0.24 a	
	<i>EC</i>	103.64 ± 31.35 a	11.24 ± 0.63 a	

\*: NE represented non-inoculated seedlings. *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.



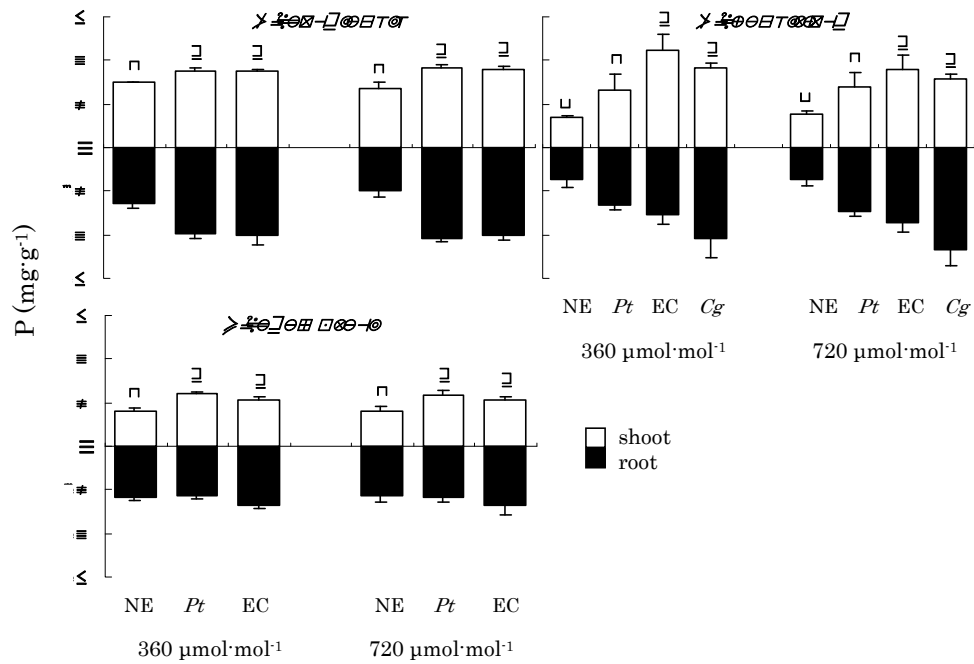


Fig. 4.3. Phosphorus concentration of *P. koraiensis*, *P. densiflora* and *L. kaempferi* in shoot and root inoculated *Pt*, *EC* or *Cg* and non-inoculated (*NE*) seedlings grown at  $360 \mu\text{mol}\cdot\text{mol}^{-1}$  or  $720 \mu\text{mol}\cdot\text{mol}^{-1}$ . Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species. *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

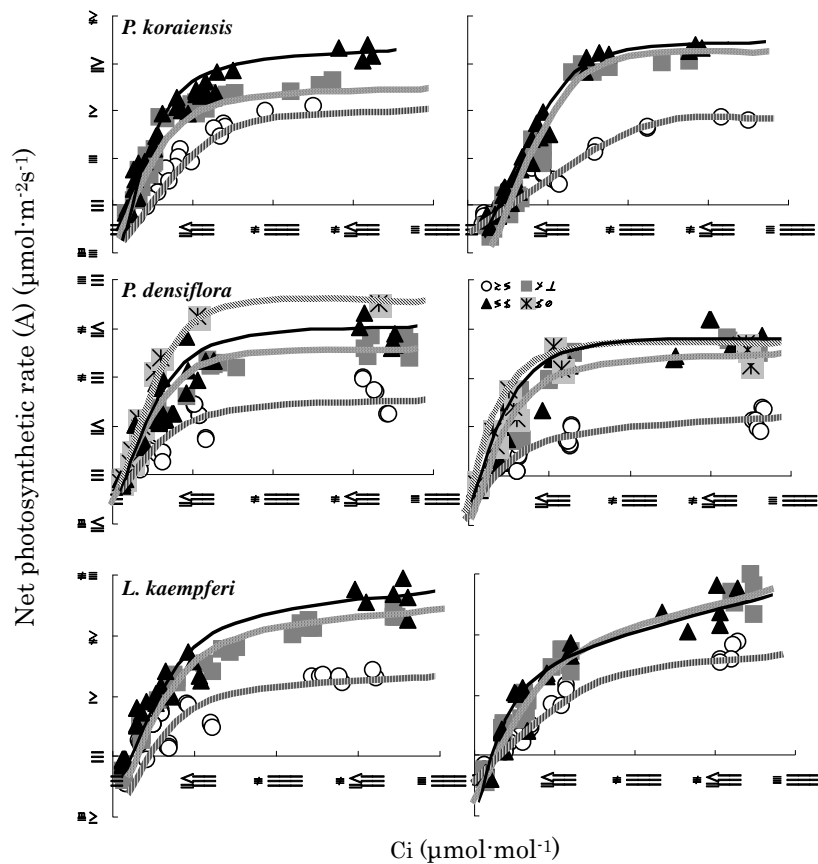


Fig. 4.4. Relationship between net photosynthetic rate (*A*) and intercellular  $\text{CO}_2$  concentration (*C<sub>i</sub>*) in *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings inoculated *Pt*, *EC* or *Cg* and non-inoculated (*NE*) seedlings grown at  $360 \mu\text{mol}\cdot\text{mol}^{-1}$  or  $720 \mu\text{mol}\cdot\text{mol}^{-1}$ . *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively.

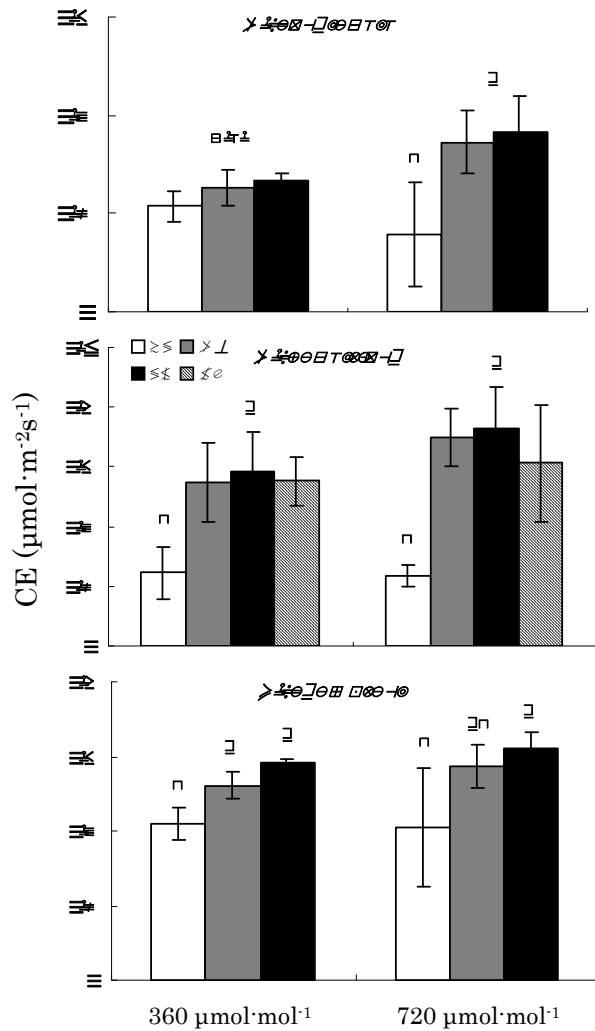


Fig. 4.5. The carboxylation efficiency (CE) in *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings inoculated *Pt*, *EC* or *Cg* and non-inoculated (NE) seedlings grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  or 720  $\mu\text{mol}\cdot\text{mol}^{-1}$ . *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively. Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species.

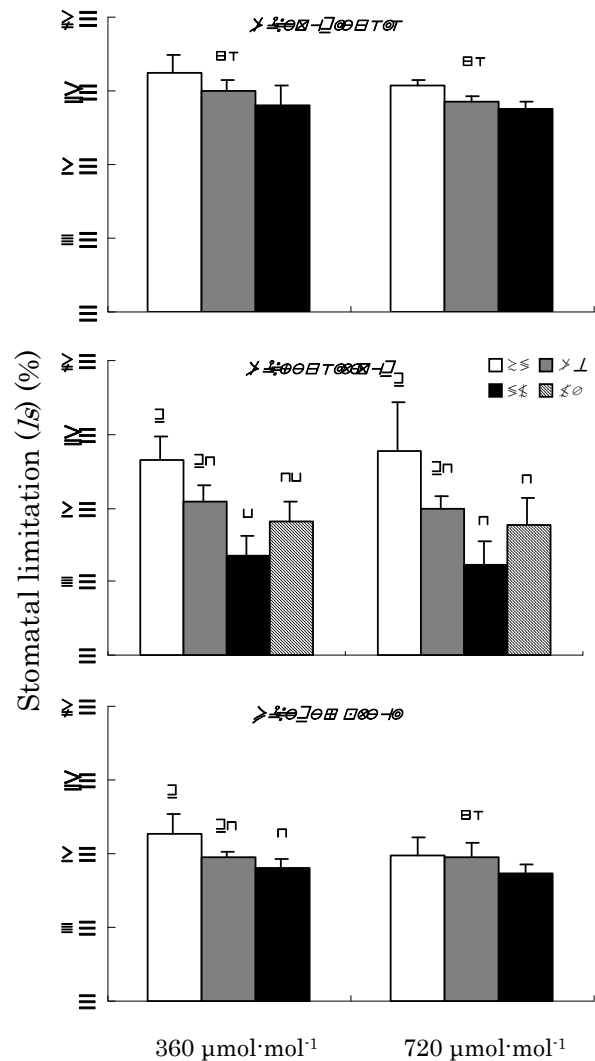


Fig. 4.6. Stomatal limitation ( $l_s$ ) in *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings inoculated *Pt*, *EC* or *Cg* and non-inoculated (NE) seedlings grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  or 720  $\mu\text{mol}\cdot\text{mol}^{-1}$ . *Pt*, *EC* and *Cg* represented inoculated seedlings by *Pisolithus tintorius*, *Ectodrench* or *Cenococcum geophilum*, respectively. Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). Data within a series followed by the same small letter do not differ significantly ( $P < 0.05$ ) in each species.

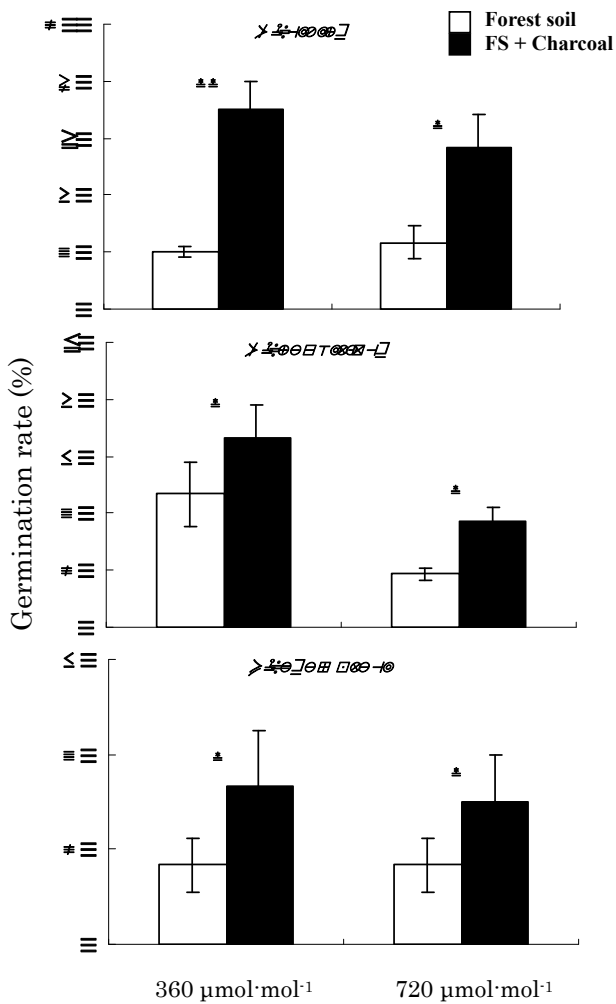


Fig. 4.7 Germination rate (%) of *P. rigida*, *P. densiflora* and *L. kaempferi* sow seeds in forest soil or forest soil mixed charcoal after 21 days grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  or 720  $\mu\text{mol}\cdot\text{mol}^{-1}$ . Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). (\*  $P<0.05$ , \*\*  $P<0.01$ )

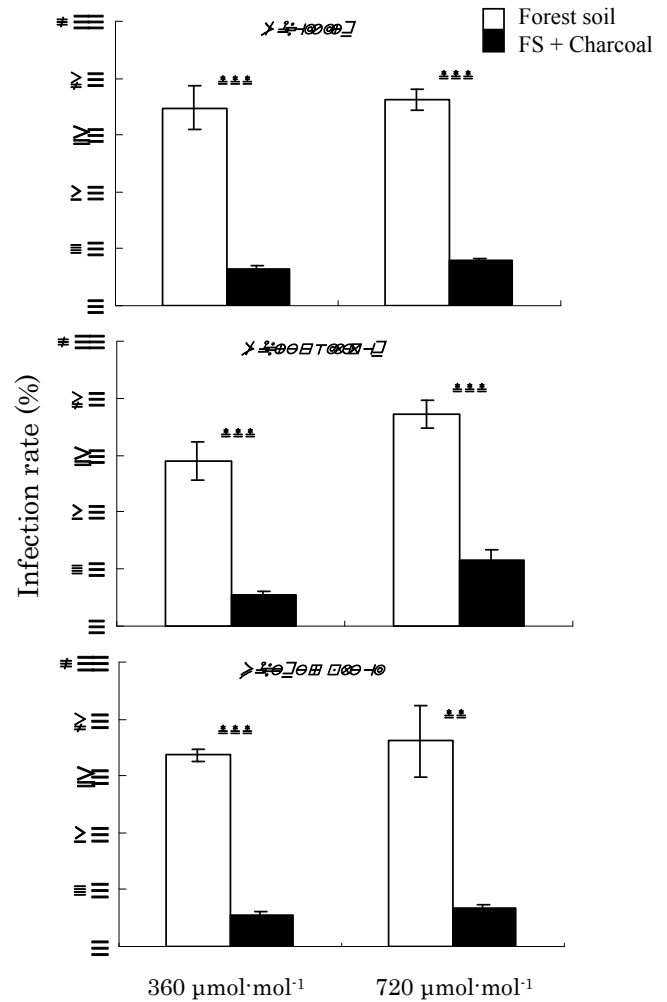


Fig. 4.8 Infection rate (%) of *P. rigida*, *P. densiflora* and *L. kaempferi* seedlings in forest soil or forest soil mixed charcoal after 18 weeks grown at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  or 720  $\mu\text{mol}\cdot\text{mol}^{-1}$ . Vertical bars represent the standard error of its mean ( $\pm\text{SE}$ ). (\*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ )

#### 4.4 Discussion

Inoculation with ectomycorrhizae significantly increased the dry mass of seedlings grown at both CO<sub>2</sub> concentrations after 18 weeks (Figs. 4.1). Previous research on the effect of ectomycorrhiza and elevated CO<sub>2</sub> concentration on the biomass growth of forest tree seedlings reveals a varied response (Tissue *et al.* 1996, Gavito *et al.* 2000). Growth improvement in ectomycorrhizal plants has been found in several species (Allen 1992, Mousseau *et al.* 1996, Norby *et al.* 1996, Tissue *et al.* 1996, Smith and Read 1997, Rouhier and Read 1998, 1999).

The infection rate of EC or Cg in *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings grown at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] was significantly higher than at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>]. The infection rate of *Pt* in *P. densiflora* and *L. kaempferi* seedlings grown at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] increased more than that at 360  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>], but these were not significant. The significantly high P concentration in needles of *Pt*, EC or Cg inoculated seedlings is therefore due to the development of ectomycorrhiza (Fig. 4.3). Previous studies have also found that ectomycorrhizal development in seedlings of several tree species at elevated [CO<sub>2</sub>] was greater than at ambient [CO<sub>2</sub>] (Seegmüller and Rennenberg 1994, Ineichen *et al.* 1995, Rey and Jarvis 1997, Runion *et al.* 1997, Rouhier and Read 1998). Ectomycorrhizal development enlarges the absorptive surface of the root, with widely ramified hyphae allowing the release of phosphatase, which enhance the availability of organic phosphate and exude organic acids. These interactions between host plant and ectomycorrhiza increase the use efficiency of limited soluble phosphate and organic N in soil (Smith and Read 1997, Lambers *et al.* 1998). The A, CE, RuBP regeneration rate and P<sub>max</sub> of *Pt*, EC or Cg inoculated *P. koraiensis* seedlings grown at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  [CO<sub>2</sub>] and *P. densiflora* and *L. kaempferi* seedlings grown at both CO<sub>2</sub> concentrations were significantly higher than those of non-inoculated seedlings.

The P concentration in needles was also significantly greater in inoculated seedlings (see Table 4.1, Figs. 4.3, 4, 5). These photosynthetic changes are regulated by the capacity of Rubisco to fix CO<sub>2</sub> and the capacity to regenerate ribulose-1,5-bisphosphate (RuBP) (Farquhar *et al.* 1980, Farquhar and Sharkey 1982). Under high and CO<sub>2</sub> saturation, phosphate (Pi) may limit photosynthesis if Pi utilization during CO<sub>2</sub> assimilation and RuBP regeneration exceed the capacity for Pi release during starch and sucrose synthesis (Sharkey 1985b, Lambers *et al.* 1998).

Ectomycorrhizal plants enhance the rate of photosynthesis over non-mycorrhizal plants as a result of improved plant nutritional status, including N and P (Reid *et al.* 1983, Bolan 1991, Finlay 1992, Smith and Read 1997, Qureshi 2003, Qureshi *et al.* 2003, Choi *et al.* 2005c), even if the P concentration is the same in needles (Rousseau and Reid 1990). Moreover, ectomycorrhizal improve *ls* (Guehl *et al.* 1990, Duñabeitia *et al.* 2004) by enlarging the soil volume exploited, via widely distributed hyphae (Smith and Read 1997, Lambers *et al.* 1998). In general, the P

concentration in needles affects photosynthesis through RuBP regeneration (Sharkey 1985a, Fredeen *et al.* 1990, Kirschbaum and Tompkins 1990, Lewis *et al.* 1994), through thylakoid (Conroy *et al.* 1986, Lambers *et al.* 1998) and through the peak carboxylation velocity or peak capacity of electron transport (Harley and Sharkey 1991). It was concluded that ectomycorrhiza development in inoculated seedlings and the increased P concentration in needles enhance the photosynthetic responses and reduce stomatal limitation relative to non-inoculated seedlings (Smith and Read 1997, Hensen *et al.* 1998) (Table 4.1, Figs. 4.3, 4, 5, 6).

Net photosynthetic rate of non-inoculated seedlings grown at 720  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub> for 18 weeks tends to be down-regulated, i.e. the seedlings have low CE, RuBP regeneration rate, P<sub>max</sub> and A/Ci curve; in contrast, ectomycorrhizae inoculated seedlings show no down-regulation at elevated [CO<sub>2</sub>]. This phenomenon has been reported in other species raised adequate soil N fertility (Idso and Kimball 1991), or with symbiotic N<sub>2</sub> fixing species even when grown in poor soils (Vogen and Curtis 1995), or with mycorrhizal plants (Staddon *et al.* 1999). Phosphorus or nitrogen limitation (Conroy *et al.* 1986, Jach and Ceulemans 2000, Koike *et al.* 2000, Eguchi *et al.* 2004) and reduced sink strength (e.g. Rogers *et al.* 1998, Stitt 1991, Griffin *et al.* 2000) have both been proposed as mechanisms inducing down-regulation, via reduction of RuBP regeneration capacity and reduced Rubisco activity (Sharkey *et al.* 1994, Adam *et al.* 2004).

Therefore, I suggest that *Pt*, EC or Cg inoculated seedlings have increased the uptake of nutrient and water, which leading to improvement of plant nutritional status and giving rise to more vigorous physiological response, in particular photosynthetic activity; and that these responses delay down-regulation at elevated [CO<sub>2</sub>], especially seedlings inoculated with EC.

The present results indicate that ectomycorrhiza inoculation increases uptake of nutrients and water, which the resulted in vigorous physiological response significantly increases the dry mass of each seedlings grown at ambient and elevated concentrations of CO<sub>2</sub>. Moreover, ectomycorrhizal seedlings moderated down-regulation at elevated CO<sub>2</sub> concentrations. Ectomycorrhiza therefore appear to have strong positive effects on *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings at both CO<sub>2</sub> concentrations. Especially, EC, four species ectomycorrhiza mixed, more strongly affected on three pine species for their physiological activity and growth. Moreover, charcoal in soil increases the aperture of soil, as a result holds the soil moisture and raises soil temperature (DeBano *et al.* 1998). Therefore significantly increase germination rate (Fig. 4.7) and can increase surviving living seedlings. However, these practices of charcoal, lifting up the soil pH and hold the soil moisture, and inhibit the ectomycorrhizal development ( $P < 0.01$ ) (Fig. 4.8). It is still question, how to increase ectomycorrhizal development in forest soil mixed charcoal for increase the survival and establishment rate of seedlings.

## Chapter 5

### GENERAL DISCUSSIONS

#### 5.1 Effects of air pollutants and soil acidification on pine growth

Pine species are one of the important conifers for forest rehabilitation in Korea, since pine species can grow in low-nutrient and barren soil. These species are characterized by their fast growth rate and tolerance to environmental pollution, including air pollution and soil acidification (Burns and Honkala, 1990). For rehabilitating degraded forest areas, *P. rigida* and *L. kaempferi* were introduced at the start of the 20<sup>th</sup> century, from the USA and Japan respectively (Kim 1999). These pine species have declined near large cities and industrial complexes (Lee *et al.* 1996, Choi *et al.* 2003, 2005, Kim *et al.* 2003, Kayama *et al.* 2004). A continuing increase in soil acidification and atmospheric CO<sub>2</sub> concentration is expected in view of the increasing emission of air pollutants. How do pine trees respond to these environmental conditions?

Decline symptoms of pine forests in industrial complexes were observed. The length of shoots and the survival of needles of *P. koraiensis* and *P. rigida* were significantly less at the damaged site than at the control site. Moreover, the physiological responses, specifically the net photosynthetic rate (light and CO<sub>2</sub> response), P<sub>sat</sub>, Φ, CE and RuBP regeneration rate of *P. koraiensis* and *P. rigida* at the damaged site were significantly less than at the control site. The concentrations of chlorophyll (a+b) and chlorophyll b in needles at the damaged site were significantly less than at the control site. There is a reduction in physiological activities of *P. koraiensis* and *P. rigida* at the damaged site due to air pollutants, in particular F, Cl and excess Mn in acidified soil following acid deposition. The rise in air pollutants directly damaged the plant physiological responses as a result of increased concentrations of F, Cl in the atmosphere and Mn in the soil and soil water, which finally accumulates in needles.

It is well known that pine species are symbiotic with ectomycorrhizae. Increased harmful elements in soil inhibit the development of ectomycorrhizae at the damaged site. It therefore appears that reduced physiological responses and reduced ectomycorrhizal development due to harmful elements hinder the growth of *P. koraiensis* and *P. rigida*. Physiological damage and reduced growth at the damaged site were more evident for *P. rigida* than for *P. koraiensis*.

Atmospheric air pollutants gradually cause an increase in soil acidity. Acidified soil increases the leaching of base cations, such as Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> ions, from soil to the ground water, and increases the solubility of phytotoxic metals such as Al and Mn in the rhizosphere (Ulrich *et al.*, 1980). Acidified soil may therefore harm tree growth not only because of low soil pH, but also through nutrient deficiency and Al and/or Mn in the soil solution. Adequate nutrient and water is vital for tree growth, and to prevent phytotoxic metals from entering acidified soil. It is possible that trees would be helped by symbiosis with ectomycorrhizae in acidified soil.

The concentration of Al and Mn in water increased with decreasing soil pH in the range 5.0 - 4.0, i.e. with progressive soil acidification. Increased Mn and Al in water-soluble form accumulated in tree roots and needles of *P. rigida*, *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings. This phenomenon is clearly related to progressive soil acidification. Most pine species are symbiotic with ectomycorrhizae. Moreover, soil acidification affects ectomycorrhizal development in the rhizosphere. Forest soil that had been acidified artificially (10 and 30 mmolH<sup>+</sup>·kg<sup>-1</sup>) significantly increased ectomycorrhizal development relative to controls, whereas more strongly acidified forest soil (60 and 90 mmolH<sup>+</sup>·kg<sup>-1</sup>) significantly reduced ectomycorrhizal development.

Ectomycorrhizal development significantly reduced the concentration of Al in roots and Mn in needles in each species, in a comparison with non-ectomycorrhizal seedlings. Mycorrhizal infection in host plants usually prevents the absorption of heavy metals, such as Ni and Mg (Kayama *et al.* 2003). The concentration of N in needles increased slightly with progressive soil acidification around the ectomycorrhizal and non-ectomycorrhizal seedlings, since the acid added included HNO<sub>3</sub>. It is believed that the reduced photosynthetic activity of *P. rigida*, *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings was a consequence of the accumulation of Mn in needles and Al in roots, rather than nutrient or nitrogen deficiency. Suppression of the rate of photosynthesis affects the growth rate of *P. rigida*, *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings. Moreover, the relative total dry mass (TDM) of *P. koraiensis* and *L. kaempferi* seedlings is approximately 40% at a BC/Al ratio (water-soluble) of 1.0, but the value for *P. rigida* and *P. densiflora* is approximately 50% at the same BC/Al ratio. Ectomycorrhizal seedlings of each species nevertheless had higher relative TDM value at a BC/Al (water-soluble) of 1.0 than NE seedlings of each species, by 20-100%.

Atmospheric pollutants and soil acidification inhibit physiological responses and plant growth, but ectomycorrhizal infection increases nutrient and water uptake and protects against phytotoxic metals such as Al and Mn. As a result, the decline in growth of pine seedlings in the presence of atmospheric pollutants and soil acidification was less than for non-ectomycorrhizal seedlings. *P. koraiensis* and *L. kaempferi* were more tolerant to atmospheric pollutants and/or soil acidification than *P. rigida* or *P. densiflora*. The effect of ectomycorrhizal infection on the seedlings of *P. koraiensis* was small, however. *P. koraiensis* has few root hairs, so that it suffers less from soil acidification and gains less benefit from ectomycorrhizal infection. *L. kaempferi* has greater resistance against soil acidification upon infection of ectomycorrhizal than *P. rigida* or *P. densiflora*. Mixed ectomycorrhizae gave better results.

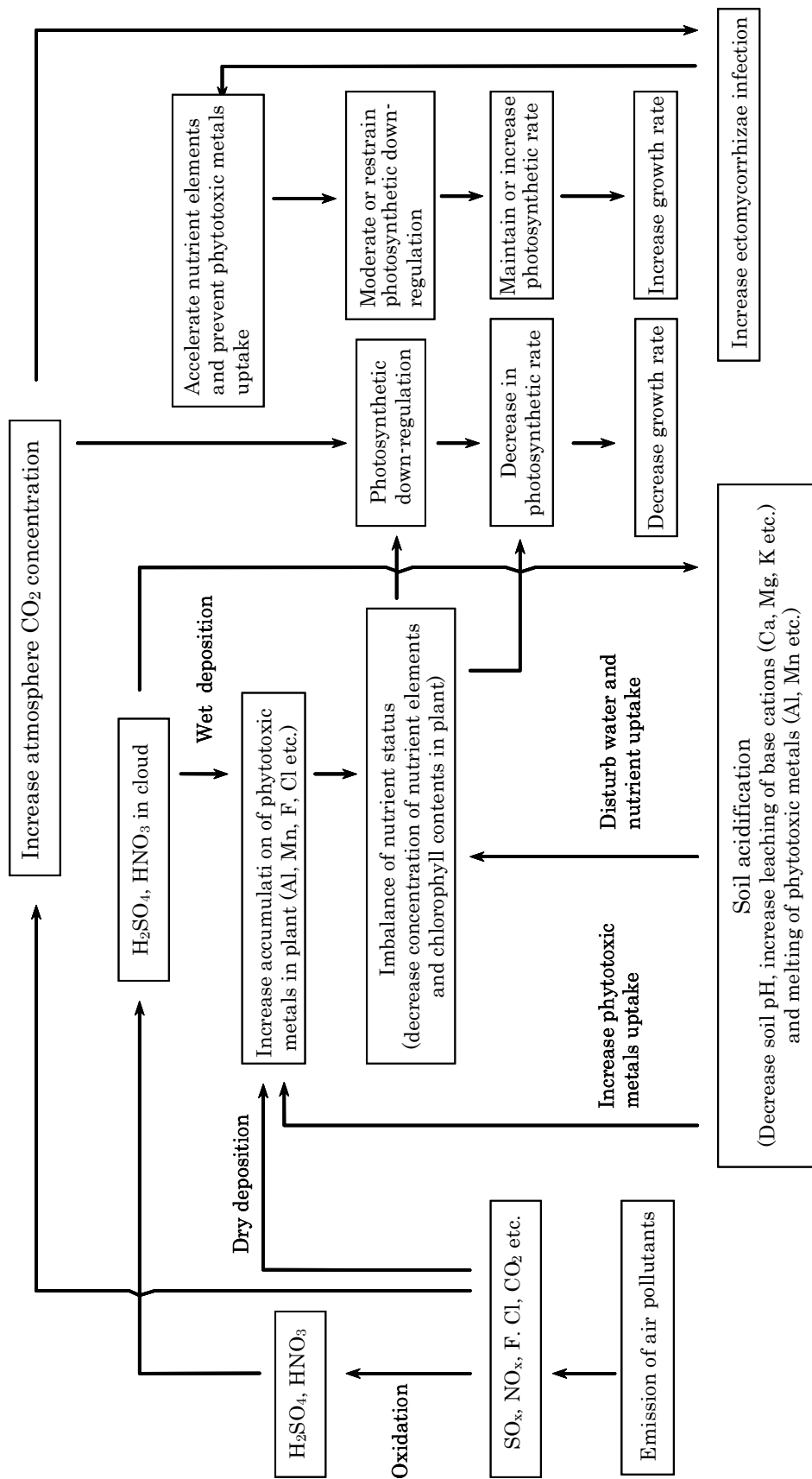


Fig. 5.1. The schematic presentation of climate changes and ecophysiological responses.

### 5.2 Effects of elevated CO<sub>2</sub> concentration

Elevated CO<sub>2</sub> concentration significantly increased the infection rate of ectomycorrhiza in seedlings of *P. koraiensis*, *P. densiflora* and *L. kaempferi*. Ectomycorrhizal development at each CO<sub>2</sub> concentration increased the P concentration in needles of each species, with greater photosynthetic activity than non-ectomycorrhizal seedlings of each species. Moreover, ectomycorrhizae help to overcome stomatal limitation (*Is*) by enlarging the surface area of the root system available to soil, via broadly distributed hyphae. Ectomycorrhizal inoculation of seedlings also improves physiological activity (Smith and Read 1997, Lambers *et al.* 1998).

Photosynthetic activities were down-regulated, specifically the CE, RuBP regeneration rate, P<sub>max</sub> and net photosynthetic rate of non-ectomycorrhizal seedlings grown at elevated CO<sub>2</sub> concentrations. Ectomycorrhiza-inoculated seedlings of each species showed no down-regulation at elevated CO<sub>2</sub> concentrations, however. It is believed that ectomycorrhizal infection of seedlings increases the uptake of nutrients and water, generating improved plant nutritional status and a more vigorous physiological response, including photosynthetic activity. These responses will reduce the down-regulation of photosynthesis at elevated CO<sub>2</sub> concentrations, especially with mixed ectomycorrhizae EC.

### 5.3 Forest rehabilitation after disturbance

The most important factor governing natural regeneration in forests is germination and rooting after natural and/or artificial disturbance. After germination, seedlings 'plug into' the network of compatible ectomycorrhizal fungi supported by surviving living trees, gaining the opportunity for early ectomycorrhizal formation and establishment (Amaranthus 1994).

Germination is strongly related to temperature, water and oxygen (Lambers *et al.* 1998). Most Korean forest soil is granite-derived brown forest soil, generally of low fertility and rapid permeability and easy to compact. Precipitation in the winter-to-spring season is also very low (Korea Meteorological Administration 2002b, 2003). These conditions are not suitable for the germination and rooting of seedlings and ectomycorrhizal development.

Charcoal added to the soil improves the moisture, temperature and physical properties (DeBano *et al.* 1998), significantly increasing the germination rate (Fig. 4.7) and increasing the survival of living seedlings. Moreover, charcoal can reduce tree damage by soil acidification, by increasing the soil pH of acidified forest soil. Unfortunately, charcoal addition usually inhibits ectomycorrhizal development (Fig. 4.8). The survival and establishment rates of seedlings depend on the rate at which they become ectomycorrhizal (Janos 1980, 1996). If trees become connected to a mycelial network larger than that which they could maintain on their own, this is often highly beneficial (Newman 1988, Perry *et al.* 1992). It has been suggested that rapid and early integration of seedlings into adult ectomycorrhizal

root systems has benefits in carbon transfer from donor to receptor trees (Simard *et al.* 1997). On the other hand, charcoal inhibits the development of ectomycorrhizae. There remain many open questions about forest rehabilitation practices.

## Chapter 6

### Synopsis

1. Climate change, specifically global warming because of increasing atmospheric CO<sub>2</sub> and atmospheric acid deposition, is a serious environmental concern not only in Europe, North America and Japan but also in Asia, Africa and Latin America. Since a rapid industrial development and urbanization is taking place in these areas. Air pollution from wet and dry deposition can directly harm plant physiological activity and growth. Moreover, wet and dry deposition of acid substances causes acidification of forest soil by increased leaching of base cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>) from the rhizosphere soil, and dissolution of phytotoxic metals such as Al and Mn. Atmospheric deposition can therefore have harmful effects on forest ecosystems, including soil acidification, nutrient imbalances, carbohydrate content and growth. Moreover, the atmospheric carbon dioxide concentration (CO<sub>2</sub>) continues to rise, affecting the ecosystem through increased availability of CO<sub>2</sub> for photosynthesis. However, photosynthesis down-regulation occurs by constraints on nutrients, especially P or N and reduced sink strength. Atmospheric deposition, soil acidification and rising atmospheric CO<sub>2</sub> concentration can therefore reduce tree physiological activity and growth. Ectomycorrhizae have a symbiotic relationship with trees. They improve the uptake by plants of water and nutrients, particularly nitrogen and phosphorus, from the soil solution by enlarging the absorptive surface of the root, with widely ramified hyphae. Moreover, ectomycorrhizal associations can reduce the toxicity of metals such as Al, Mn, Zn, Cu and Ni, the effects being dependent on the mycorrhizal fungi and the host species of plant. In view of these environmental changes and ectomycorrhizal characteristics, this study focused on the effects of changing climate on the physiological activity and growth of pine species, and investigated the role of ectomycorrhizae.

2. The concentrations of fluorine (F) and chlorine (Cl) in the atmosphere, precipitation and soil water at the damaged site were all significantly higher than at a control site. Moreover, the concentrations of F, Cl and Mn in needles of the two pine species were significantly higher at the damaged site than at the control site, and concentrations of essential elements (Mg, K, P) and chlorophyll in needles were significantly lower at the damaged site than at the control site. Ectomycorrhizal infection of the two pine species at the control site was 30% higher than at the damaged site. Almost no ectomycorrhiza infection was found in either species at the damaged site. The shoot length and survival statistics of needles of the two pines were significantly lower at the damaged site than at the

control site. In particular, growth of shoots and needle longevity of *P. koraiensis* are larger than for *P. rigida* at the damaged site. The photosynthetic responses (light-saturated net photosynthesis ( $P_{\text{sat}}$ ), apparent quantum yield ( $\Phi$ ), carboxylation efficiency (CE) and RuBP regeneration rate) of both pines were significantly lower at the damaged site than at the control site. The present comparison of photosynthetic responses and the concentrations of F, Cl and Mn in needles of the two pine species clearly shows that *P. koraiensis* is more tolerant to excess Mn in its needles than *P. rigida*.

3. With increasing amounts of  $\text{H}^+$  added to the soil, the water-soluble concentrations of Ca, Mg, K, Al and Mn increased, especially below a soil pH of 3.8. Development of ectomycorrhizae, i.e. *Pisolithus tinctorius* (*Pt*), Ectodrench (EC) or *Cenococcum geophilum* (*Cg*), with *Pinus densiflora*, *P. koraiensis*, *P. rigida* and *Larix kaempferi* seedlings was significantly greater in the 10 and 30  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments than control treatments. The mycorrhizal development in 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments was significantly lower than the control, 10 and 30  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments. The concentrations of Al in roots and Mn in needles of each species increased with increasing amount of  $\text{H}^+$  added to the soil. Also, the concentration of N in needles of each seedling was higher in all treatments than controls. The maximum net photosynthetic rate at saturated  $\text{CO}_2$  concentration ( $P_{\text{max}}$ ) of each seedling was greater with 10  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatment than controls, but it fell above 30  $\text{mmolH}^+\cdot\text{kg}^{-1}$ , especially in the 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments. However, the concentration of Al in roots and Mn in needles of ectomycorrhizal seedlings of each species was significantly less than corresponding non-ectomycorrhizal (NE) seedlings. The  $P_{\text{max}}$  of ectomycorrhizal seedlings of each species was significantly greater than corresponding non-ectomycorrhizal seedlings. The dry mass of the whole plant of NE, *Pt*, EC or *Cg* of each species increased in the 10 and 30  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments, but was significantly less in the 60 and 90  $\text{mmolH}^+\cdot\text{kg}^{-1}$  treatments than other treatments. Moreover, the relative total dry mass (TDM) of *P. koraiensis* and *L. kaempferi* seedlings was approximately 40% at a BC/Al ratio of 1.0, but that of *P. rigida* and *P. densiflora* was approximately 50% at the same BC/Al ratio.

4. The effects of elevated  $\text{CO}_2$  concentration on *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings were estimated, specifically their physiological responses and growth with ectomycorrhizae, i.e. *Pt*, EC or *Cg*. Seedlings were grown under ambient ( $360 \mu\text{mol}\cdot\text{mol}^{-1}$ ) or elevated ( $720 \mu\text{mol}\cdot\text{mol}^{-1}$ )  $\text{CO}_2$  concentrations in a phytotron with or without ectomycorrhizae for 18 weeks. The ectomycorrhizal development of each species was significantly higher under elevated  $\text{CO}_2$  concentration than at ambient levels. The P concentration in needles and roots following inoculation with *Pt*, EC or *Cg* was significantly higher in each species than without

inoculation at both  $\text{CO}_2$  concentrations. Moreover,  $P_{\text{max}}$ , CE and the RuBP regeneration rate for each species inoculated with *Pt*, EC or *Cg* were significantly higher than for non-inoculated seedlings at both  $\text{CO}_2$  concentrations. Stomatal limitation in each species at each  $\text{CO}_2$  concentration decreased relative to non-inoculated seedlings, especially in *P. densiflora*. Non-ectomycorrhizal seedlings of each species showed photosynthetic down-regulation, but ectomycorrhizal seedlings did not. Charcoal mixed with forest soil significantly increased the germination rate of *P. rigida*, *P. densiflora* and *L. kaempferi* seeds, but significantly reduced the ectomycorrhizal infection rate of these seedlings.

5. Air pollutants lead to soil acidification and imbalance of soil nutrients. Air pollutants and soil acidification reduce physiological activity and tree growth. Moreover, higher atmospheric  $\text{CO}_2$  concentrations lead to photosynthetic down-regulation. In contrast, ectomycorrhizal inoculation increased the uptake of nutrients and water, and protected the plant against phytotoxic metals. Seedlings of each species inoculated with ectomycorrhizae consequently showed increased physiological activities and dry mass growth under soil acidification, and did not show photosynthetic down-regulation under elevated  $\text{CO}_2$  concentrations. Mixed ectomycorrhiza, EC, were particularly effective. Also, charcoal mixed with the soil increased the germination rate by helping to maintain the soil moisture and temperature and keeping the soil loose increase aperture of soil, but it obstructs development of ectomycorrhizae.

#### Acknowledgements

This paper is partly modified from the doctoral dissertation of Graduate School of Agriculture, Hokkaido University. I would like to express my profound appreciation to my supervisor, Prof. Takayoshi Koike, for introducing me to tree ecophysiology for constructive discussions and encouraging me to find my own ways and for teaching me how to write scientific papers. I also appreciate Prof. Mitsuru Osaki, Prof. Takashi Hirano and Prof. Kaichiro Sasa for their invaluable comments on the study and paper publication. I also thank Dr. Yutaka Tamai for his stimulating comments on my thesis and each manuscript of mine, supporting the microscope and many proper guidance. I am very much appreciated Dr. Hideaki Shibata for his stimulating comments on data and chemicals analysis, supporting the equipments and careful revision on my whole manuscript. I really thank Dr. Ali M. Qureshi and Dr. Laiye Qu for introducing me to ectomycorrhizal fungi, for teaching techniques, writing papers and thinking way. I appreciate Dr. Masazumi Kayama for helping my work and teaching techniques of chemical analysis. I thank Dr. Martin Dobbertin, Dr. Yuzo Sano and Dr. Masato Shibuya for valuable advice and comments on this study.

I would like to give hearty thanks to my Korean supervisor, Prof. Hyun-O Jin, for his directions, suggestions, discussion on my scientific issues and



continuous encouragement from my master program to the Ph.D. research. Special thanks are also due to Prof. Youngchai Kim, Dr. Choonghwa Lee, Prof. Ryo Funada, Prof. Takeshi Izuta and Dr. Hiroto Toda for their suggestion, many previous advice and information.

Deep thanks are also due to all staff of Hokkaido University Forests, Field Science Center for northern Biosphere for their supporting on my study. Thanks are also due to all the friends and colleagues in the our groups, Dr. Tomoaki Ichie, Dr. Takatoshi Nakamura, Dr. Satoshi Kitaoka, Dr. Sawako Matsuki, Dr. Wenjie Wang, Mr. Donghun Ji, Mr. Norikazu Eguchi, Ms. Ikuko Endo, Ms. Yuko Sakuma and Mr. Takanori Shibata, for a nice time and good work environment. Without their faithfully generous help in the field and good comments in the seminar, I couldn't finish the thesis so smoothly.

I thank Dr. Yoko Watanabe for her guidance on the anatomical studies and encouragement. Thanks are also due to Mr. Eunwha Lee and Jingyu Han for assistance with the fieldwork. Thanks owe to Dr. Yoshinori Kitahashi, who has kindly given me help on daily life in Japan and also discussion on scientific matters. I appreciate all the staff in FFPRI (Forest and Forest Products Research Institute, Sapporo), Dr. Yutaka Maruyama, Dr. Mitsutoshi Kitao, Dr. Hiroyuki Tobita, etc. for their kind support on my works and suggestions.

Thanks are also due to my parents, Mr. Kwangjun Choi and Mrs. Haebun Kim, for supporting and encouraging my overseas study in Hokkaido University, the oldest agriculture school in Japan.

Finally, I deeply appreciate the permission of Elsevier Ltd. for their kind permission of adaptation of Figures and Tables in papers of Choi *et al.* (2006).

## References

- Adams, F. (1981) Nutritional imbalances and constraints to plant growth on acid soils. *Journal of Plant Nutrition*, 4: 81-87.
- Adam, N. R., Wall, G. W., Kimball, B. A., Idso, S. B. and Webber, A. N. (2004) Photosynthetic down-regulation over long-term CO<sub>2</sub> enrichment in leaves of sour orange (*Citrus aurantium*) trees. *New Phytol.*, 163: 341-347.
- Ahonen-Jonnarh, U., Göransson, A. and Finlay, R. D. (2003) Growth and nutrient uptake of ectomycorrhizal *Pinus sylvestris* seedlings in a natural substrate treated with elevated Al concentrations. *Tree Physiology*, 23: 157-167.
- Allen, M. F. (ed.) (1992) *Mycorrhizal Functioning*. Chapman & Hall, New York.
- Allen, M. F. (1996) *The Ecology of Mycorrhizae*. University of Cambridge, New York.
- Aluminiumindustriens Miljøsekretariat (1993) *Utslippsdata m.m. for primæraluminiumverk I Norge til og med Oslo: AMS Nr.*, 42: 239-94.
- Alvarez, R. (1995) Standard solutions and certified reference materials. *In: Cunniff, P. (Ed.) Official Methods of Analysis of AOAC*, Vol. 1, 16<sup>th</sup> ed. AOAC International, Gaithersburg, MD, A1-A11.
- Amarantus, M. P. (1994) Mycorrhizas, forest disturbance and regeneration in the pacific northwestern United States. *In: Read, D. J., Lewis, D. H., Fitter, A. H. and Alexander, I. J. (eds.) Mycorrhizas in ecosystems*, CAB International, Singapore, 202-207.
- Barnes, J. D., Eamus, D. and Brown, K. A. (1990) The influence of ozone, acid mist and soil nutrient status on Norway spruce (*Picea abies* (L.) Karst. ). *New phytologist*, 115: 149-156.
- Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S. and Davison, A. W. (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany*, 32: 85-100.
- Barnes, B. V., Zak, D. R., Denton, S. R. and Spurr, S. H. (1998) *Forest Ecology* 4<sup>th</sup> ed. John Wiley & Sons, New York, pp. 774.
- Bazzaz, F. A. (1990) The responses of natural ecosystems to the rising global CO<sub>2</sub> levels. *Annual Review of Ecology and Systematics*, 21: 167-196.
- Berntson, G. M., Bazzaz, F. A. (1996) Below-ground positive and negative feedbacks on CO<sub>2</sub> growth enhancement. *Plant and Soil*, 187: 119-131.
- Bolan, N. S. (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*, 134: 189-207.
- Brewer, R. F., Sutherland, F. H. and Guillemet, F. F. (1960) Sorption of fluorine by citrus foliage from equivalent solutions of HF, NaF, NH<sub>4</sub>F and H<sub>2</sub>SiF<sub>6</sub>. *American Society for Horticultural Science*, 76: 215-219.
- Brooks, A. (1986) Effects of phosphorus nutrition on ribulose-1,5-bisphosphate carboxylase activation, photosynthetic quantum yield and amounts of some Calvin-cycle metabolites in spinach leaves. *Australian Journal of Plant Physiology*, 13: 221-237.
- Brown, M. T. and Wilkins, D. A. (1985) Zinc tolerance in *Betula*. *New Phytologist*, 99: 91-100.
- Brundrett, M. C. (1991) Mycorrhizas in natural ecosystems. *Advances in Ecological Research*, 21: 171-313.
- Burns, R. M. and Honkala, B. H. (1990) *Silvics of North America: 1. Conifers*. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. Vol. 1: 456-462.
- Ceulemans, R. and Mousseau, M. (1994) Effects of elevated atmospheric CO<sub>2</sub> on woody plants. *New Phytologist*, 127: 425-446.
- Choi, D. S., Kayama M., Jin H.O, Lee C.H. and Koike T. (2003) Growth and physiological responses of two pine species grown under polluted Ansan industrial region in Korea. *Transaction of Hokkaido Branch of the Japanese Forestry Society*, 51: 58-60. (in Japanese).
- Choi, D. S., Jin, H. O, Lee, C. W., Kim, Y. C. and Kayama, M. (2005a) Effect of soil acidification on the growth of Korean pine (*Pinus koraiensis*) seedlings in a granite-derived forest soil. *Environmental Science*, 12: 33-47.
- Choi, D. S., Quoreshi, A. M., Maruyama, Y. Jin, H. O and Koike, T. (2005b) Effect of ectomycorrhizal

- infection on growth and photosynthetic characteristics of *Pinus densiflora* seedlings grown under elevated CO<sub>2</sub> concentrations. *Photosynthetica*, 43: 223-229.
- Choi, D. S., Quoreshi, A. M., Jin, H. O., Maruyama, Y. and Koike, T. (2005c) Mycorrhizal activities in *Pinus densiflora*, *P. koraiensis* and *L. kaempferi* raised under high CO<sub>2</sub> in relation to water use efficiency. *Phyton*, 45: 139-144.
- Choi, D. S., Kayama, M., Jin, H. O., Lee, C. H., Izuta, T. and Koike, T. (2006) Growth and photosynthetic responses of two pine species (*Pinus koraiensis* and *P. rigida*) planted in a polluted industrial region in Korea. *Environmental Pollution*, 139: 421-432.
- Choi, Y. A. (2003) Sanrimji Vol 7. National Forestry Cooperatives Federation, Seoul, pp 127. (in Korean).
- Coleman, J. S., McConnaughay, K. D. M. and Bazzaz, F. A. (1993) Elevated CO<sub>2</sub> and plant nitrogen-use: Is reduced tissue nitrogen concentration size-dependent? *Oecologia*, 93: 195-200.
- Conroy, J. P., Smillie, R. M., Küppers, M., Bevege, D. J. and Barlow, E. W. (1986) Chlorophyll *a* fluorescence and photosynthetic and growth response of *Pinus radiata* to phosphorus deficiency, drought stress and high CO<sub>2</sub>. *Plant Physiol.*, 91: 423-429.
- Cronan, C. S. and Grigal, D. F. (1995) Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality*, 24: 209-226.
- Cumming, J. R. and Wenstein, L. H. 1990. Aluminium-mycorrhizal interactions in the physiology of pitch pine seedlings. *Plant and Soil*, 125: 7-18.
- Darral, N. M., 1989. The effects of air pollutants on physiological processes in plants. *Plant, Cell and Environment*, 12: 1-30.
- DeBano, L. F., Neary, D. G. and Ffolliott, P. F. (1998) Fire's effects on ecosystems. John Wiley & Sons, New York. pp. 333.
- Deuck, Th. A., Zuin, a. and Elderson, J. (1998) Influence of ammonia and ozone on growth and drought sensitivity of *Pinus sylvestris*. *Atmospheric Environment*, 32: 545-550.
- Dighton, J. and Skeffington, R.A. (1987) Effects of artificial acid precipitation on the mycorrhizas of Scots pine seedlings. *New Phytologist*, 107: 191-202.
- Duke, E. R., Johnson, C. R. and Koch, K. E. (1986) Accumulation of phosphorus, dry matter and betaine during NaCl stress of sprit-root citrus seedlings colonized with vesicular-arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytologist*, 104: 583-590.
- Duñabeitia, M. K., Hormilla, S. Garcia-Plazaola, J. I. Txarterina, K. Arteche, U. and Becerril, J. M. (2004) Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D. Don. *Mycorrhiza*, 14: 11-18.
- Eamus, D. (1991) The interaction of rising CO<sub>2</sub> and temperatures with water use efficiency. *Plant, Cell and Environment*, 14: 843-852.
- Eguchi, N., Fukatsu E., Funada R., Tobita H., Kitao M., Maruyama Y. and Koike T. (2004) Changes in morphology, anatomy, and photosynthetic capacity on needles of Japanese larch (*Larix Kaempferi*) seedlings grown in high CO<sub>2</sub> concentrations. *Photosynthetica*, 42: 173-178.
- Ericsson, T., Göransson, A., Van Oene, H. and Gobran, G. (1995) Interactions between aluminium, calcium and magnesium - Impacts on nutrition and grown of forest trees. *Ecological Bulletins*, 44: 191-196.
- Ernst, W. H. O. (1976) Physiological and biochemical aspects of metal tolerance. In: Mansfield I. A. (ed.) *Effects of air pollutants on plant*, Cambridge University Press, Cambridge, 115-133.
- Ernst, W. H. O. and Joosse-van Damme E. N. G. (1983) *Umweltbelastung durch Mineralstoffe biologische Effekte*. Stuttgart, New York, Gustav Fischer Verlag.
- Farquhar, G. D., Caemmerer, S. von and Berry, J. A. (1980) A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta*, 149: 78-90.
- Farquhar, G. D. and Sharkey, T. D. (1982) Stomatal conductance and photosynthesis. *Annual Review Plant Physiology*, 33: 317-345.
- Falster, D. S., Warton, D. I. And Wright, I. J. (2003) (S)MATR: Standardised major axis tests and routines. Versin 1.0. <http://www.bio.mq.edu.au/ecology/SMART>.
- Farrar, J. F. and Williams, M. L. (1991) The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. *Plant Cell Environment*, 14: 819-830.
- Field, C. and Mooney, A. (1983) Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. *Oecologia*, 56: 348-355.
- Field, C., Merino, J. and Mooney, H. A. (1983) Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia*, 60: 384-389.
- Field, C. B., Jackson, R. B. and Mooney, H. A. (1995) Stomatal responses to increased CO<sub>2</sub>: implication from the plant to the global scale. *Plant, Cell and Environment*, 18: 1214-1225.
- Finlay, R. D. (1992) Uptake and mycelial translocation of nutrients by ectmycorrhizal fungi. In: Read, D. J. Lewis, D. H. Fitter, A. H. and Alexander, I. J. C.A.B. (eds.) *Mycorrhizas in Ecosystems*. International Wallingford, 91-97.
- Finlay, R. D. (1995) Interactions between soil acidification, plant growth and nutrient uptake in ectomycorrhizal associations of forest trees. *Ecol. Bull.*, 44: 197-214.
- Fisher, R. F. and Binkley, D. (2000) *Ecology and management of Forest Soils*. 3<sup>rd</sup> ed, John Wiley & Sons, New York.
- Fornasiero, R. B. (2003) Fluorides effects on

- Hypericum perforatum* plants: first field observations. *Plant Science*, 165: 507-513.
- Foy, C.D., Chaney, R.L. and White, W.C. (1978) The physiology of metal toxicity in plants. *Annual Review of plant physiology*, 29: 511-566.
- Fredeen, A. L., Raab, T. K., Rao, I. M. and Terry, N. (1990) Effects of phosphorus nutrition on photosynthesis in *Glycine max* (L.) Merr. *Planta*, 191: 399-405.
- Furukawa, A. (1991) Inhibition of photosynthesis of *Populus euramericana* and *Helianthus annuus* by SO<sub>2</sub>, NO<sub>2</sub> and O<sub>3</sub>. *Ecological Research*, 6: 79-86
- Gavito, M. E., Curtis, P. S., Mikkelsen, T. N. and Jakobsen, I. (2000) Atmospheric CO<sub>2</sub> and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. *J. Exp. Bot.*, 51: 1931-1938.
- Godbold, D. L., Jentschke, G., winter, S. and Marschner, P. (1998) Ectomycorrhizas and amelioration of metal stress in forest tree. *Chemosphere*, 36: 757-762.
- Gratani, L., Crescente, M. F. and Petrucci, M. (2000) Relationship between leaf life span and photosynthetic activity of *Quercus ilex* in polluted urban area (Rome). *Environmental pollution*, 110: 19-28.
- Greenhalgh, R. and Riley, J. P. (1961) The Determination of Fluoriden in Natural water, with Particular Reference to Sea Water. *Analytica chimica acta*, 25: 179-188.
- Griffin, K. L., Tissue, D. T., Turnbull, M. H. and Whitehead, D. (2000) The onset of photosynthetic acclimation to elevated CO<sub>2</sub> partial pressure in field-grown *Pinus radiata* D. Don. After 4 years. *Plant, Cell and Environment*, 23: 1089-1098.
- Grodziński, W., Greszta, J., Laskowski, R., Maryński, M. and Rozen, A. (1990) Effect of the chemical composition of industrial dusts on forest floor organic matter accumulation. *Water, Air and Soil Pollution*, 53: 169-178.
- Grulke, N. E., Andersen, C. P., Fenn, M. e. and Miller, P. R. 1998. Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the san Bernardino Mountains, California. *Environmental Pollution*, 103: 63-73.
- Guehl, J. M., Mousain, D., Falconnet, G. and Gruez, J. (1990) Growth, carbon dioxide assimilation capacity and water-use efficiency of *Pinus pinea* L. seedlings inoculated with different ectomycorrhizal fungi. *Ann. Sci. Forestières*, 47: 91-100.
- Hallbacken, L. and Tamm, C. O. (1986) Changes in soil acidity from 1927 to 1982-84 in a forest area of south-west Sweden. *Scand. J. For. Res.*, 1: 219-232.
- Harley, P. C. and Sharkey, T. D. (1991) An improved model of C<sub>3</sub> photosynthesis at high CO<sub>2</sub>: reversed O<sub>2</sub> sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research*, 27: 169-178.
- Havranek, Von W. M., Pfeifhofer, H. and Grill, D. (1990) Pigmentgehalte und gaswechsel von tief- und hochlagenfichten nach chronischer ozonbelastung. *Forstwiss. Centralbl.*, 109: 200-209.
- Heber, U., Kaiser, W., Luwe, M., Kindermann, G., Veljovic-Javonovic, S., Yin, Z., Pfanz, H. and Slovick, S. (1995) Air pollution, photosynthesis and forest decline: interactions and consequences. *In: Schulze, E. -D., Caldwell, M. M. (eds.)*, *Ecophysiology of Photosynthesis*, Springer-Verlag, Berlin, 279-298.
- Hecht-Buchholz, C., Jorns, C.A. and Keil, P. (1987) Effect of excess aluminium and manganese on Norway spruce seedlings as related to magnesium nutrition. *Journal of Plant Nutrition*, 10: 1103-1110.
- Hensen, C. W., Lynch, J. and Ottosen, C. O. (1998) Response to phosphorus availability during vegetative and reproductive growth of *Chrysanthemum*: I. Whole-plant carbon dioxide exchange. *J. am. Soc. horticult. Sci.*, 123: 215-222.
- Hinrichsen, D. (1986) Multiple pollutants and forest decline. *Ambio*, 15: 258-265.
- Houghton, J. T., Jenkins, G. J. and Ephraums, J. J. (1990) *Climate change; The IPCC scientific assessment*. Cambridge; Intergovernmental Panel on Climate Change, Cambridge University Press.
- Houghton, R. A. (1995) Land-use change and the carbon cycle. *Global Change Biology*, 1: 275-287.
- Hunt, R. (1978) *Plant Growth Analysis*. Edward Arnold Publishing, London, 8-25.
- Hutchinson, T. C. (1980) Effects of acid leaching on cation loss from soil. *In: Hutchinson, T. C. and Havas, M. (eds.)* *Effects of acid precipitation on terrestrial ecosystems*. Plenum Press, 481-497.
- IPCC. (1996) *Climate Change 1995: The Science of Climate Change, Summary for Policymakers*. Cambridge University Press, New York.
- Idso, S. B. and Kimball, B. A. (1991) Downward regulation of photosynthesis and growth at high CO<sub>2</sub> levels. *Plant Physiol.*, 96: 990-992.
- Ineichen, K., Wiemken, V. and Wiemken, A. (1995) Shoots, roots and ectomycorrhizal formation of pine seedlings at elevated atmospheric carbon dioxide. *Plant, Cell and Environment*, 18: 703-707.
- Izuta, T., Noguchi, K., Aoki, M. and Totsuka, T. (1995) Effects of excess manganese on growth, water content and nutrient status of Japanese cedar seedlings. *Environmental Sciences*, 3: 209-220.
- Izuta, T. and Totsuka, T. (1996) Effects of Soil Acidification on Growth of *Cryptomeria japonica* Seedlings. *Proceedings of the International Symposium on Acidic Deposition and Its Impacts*. 157-164.
- Izuta, T., Seki, T. and Totsuka, T. (1996a) Growth and nutrient status of *Betula platyphylla* seedlings grown in andosol or brown forest soil acidified by adding H<sub>2</sub>SO<sub>4</sub> solution. *Environmental Sciences*, 4: 233-247.
- Izuta, T., Yamada, A., Miwa, M., Aoki, M. and Totsuka, T. (1996b) Effects of Low pH and Excess Al on Growth, Water content and Nutrient Status of

- Japanese cedar Seedlings. *Environmental Sciences*, 4: 113-125.
- Izuta, T. (1998) Ecophysiological responses of Japanese forest tree species to ozone, simulated acid rain and soil acidification. *J. Plant Res.*, 111: 471-480.
- Izuta, T., Yamaoka, T., Nakaji, T., Yonekura, T., Yokoyama, M., Matsumura, H., Ishida, S., Yazaki, K., Funada, R. and Koike, T. (2001) Growth, net photosynthetic rate, nutrient status and secondary xylem anatomical characteristics of *Fagus crenata* seedlings grown in brown forest soil acidified with H<sub>2</sub>SO<sub>4</sub> solution. *Water Air and Soil Pollution*, 130: 1007-1012.
- Jach, M. E. and Ceulemans, R. (2000) Effects of season, needle age and elevated atmospheric CO<sub>2</sub> on photosynthesis in Scots pine (*Pinus sylvestris*). *Tree Physiol.*, 20: 145-157.
- Jacob, J. and Lawlor, D. W. (1991) Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize, and wheat plants. *Jornal of Experimental Botany*, 42: 1003-1011.
- Jacobson, J. S., Weinstein, L. H., McCune, D. C. and Hitchcock, A. E. (1966) The accumulation of fluorine by plants. *Journal of the Air Pollution Control Association*, 16: 412-417.
- Janos, D. P. (1980) Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, 61: 151-162.
- Janos, D. P. (1996) Mycorrhizas, succession and rehabilitation of deforested lands in the humid tropics. *In: Frankland, J. C., Magan, N. and Gadd, G. M. (eds.) Fungi and environmental change.* Cambridge University Press, Cambridge, UK, 129-161.
- Jentschke, G. and Godbold, D. L. (2000) Metal toxicity and ectomycorrhizas. *Physiol. Plant.*, 109: 107-116.
- Jin, H.O., Jung, D.Y. and Lee, C.W. (1999) Water chemistry profiles under Korean white pine (*Pinus koraiensis* S. et Z.) stand. *Korean Journal of Agricultural and Forest Meteorology*, 1: 110-118.
- Kayama, M., Sasa, K. and Koike, T. (2002) Needle life span, photosynthetic rate, and nutrient concentration of *Picea glehnii*, *P. jezoensis*, and *P. abies* planted on serpentine soil in Northern Japan. *Tree Physiology*, 22: 707-716.
- Kayama, M., Quoreshi, A. M., Kitaoka, O., Kitahashi, Y., Sakamoto, Y., Maruyama, Y., Kitao, M., and Koike, T. (2003) Effects of deicing salt on the vitality and health of two spruce species, *Picea abies* Karts., and *Picea glehnii* Masters planted along roadsides in northern Japan. *Environmental Pollution*, 124: 127-137.
- Kayama, M., Choi, D., Jin, H., Lee, C., and Koike, T. (2004) The decline and growth characteristics of two pine species planted in Ansan industrial complex, Korea. *Northern Forestry (Hoppo ringyo)*, 56: 269-272. (in Japanese).
- Kayama, M., Quoreshi, A.M., Uemura, S., and Koike, T. (2005) Differences in growth characteristics and dynamics of elements absorbed in seedlings of three spruce species raised on serpentine soil in northern Japan. *Annals of Botany*, 95: 661-671.
- Kelly, J. M., Schaedle, M., Thornton, F. C. and Joslin, J. D. (1990) Sensitivity of deciduous and coniferous tree species to Al: II. Red oak, Sugar maple, and European beech. *Journal of Environal Quality*, 19: 172-179.
- Kim, G. T. (1986) Effects of simulated acid rain on growth and physiological characteristics of *Ginkgo biloba* L. seedlings and on chemical properties of the tested soil. *In: Ph. D. dissertation, Seoul National University, Seoul*, pp. 46. (in Korean).
- Kim, T. W. (1999) *The Woody Plants of Korea in color.* Kyo-Hak Publishing, Seoul, pp. 30-33. (in Korean).
- Kim, Y. C., Jin H. O., Choi D. S., Lee C. H. and Koike T. (2003) Effects of artificially acidified soils on the growth and nutrient status of *Pinus Koraiensis* seedlings. *Journal of Korean forest society*, 92: 642-649. (in Korean).
- Kirschbaum, M. U. F. and Tompkins, D. (1990) Photosynthetic responses to phosphorus nutrition in *Eucalyptus grandis* seedlings. *Australian journal of plant physiology*, 17: 527-535.
- Kitao, M., Lei, T. T. and Koike, T. (1997a) Comparison of photosynthetic responses to manganese toxicity of deciduous broad-leaved trees in northern Japan. *Environmental Pollution*, 97: 113-118.
- Kitao, M., Lei, T. T. and Koike, T. (1997b) Effects of manganese toxicity on photosynthesis of white birch (*Betula platyphylla* var. japonica) seedlings. *Physiologia Plantarum*, 101: 249-256.
- Kochian, L. (1995) Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 46: 237-260.
- Kohno, Y., Matsumura, H. and Kobayashi, T. (1995) Effect of simulated acid rain on the growth of Japanese Conifers grown with or without fertilizer. *Water Air and Soil Pollution*, 85: 1305-1310.
- Koike, T. (1995) Effects of CO<sub>2</sub> in interaction with temperature and soil fertility on the foliar phenology of alder, birch, and maple seedlings. *Can. J. Bot.*, 73: 149-157.
- Koike, T., Yazaki, K., Funada, R., Kitao, M., Maruyama, Y., Takahashi, K., Maximov, T.C. and Ivanov, B.I. (2000) Photosynthetic characteristics of Dahurian larch, Scotch pine and white birch seedlings native to eastern Siberia raised under elevated CO<sub>2</sub>. *Eurasian J. Forest Res.*, 1: 31-37.
- Korea Meteorological Administration (2002a) *Annual Climatological Report.* Dongjin, Seoul, 59-160. (in Korean).
- Korea Meteorological Administration (2002b) *Annual Report of Automatic Weather System Data.* Dongjin, Seoul, pp. 202-205. (consultation of web site : [http://www.kma.go.kr/weather/climate/sum/sum\\_year\\_frame.html](http://www.kma.go.kr/weather/climate/sum/sum_year_frame.html)). (in Korean).
- Korea Meteorological Administration (2003) *Annual Report of Automatic Weather System Data.* Dongjin, Seoul, 210-213. (in Korean).
- Kruger, E. and Sucoff, E. (1989) Growth and nutrient status of *Quercus rubra* L. in response to Al and

- Ca. Journal of Experimental Botany, 40: 653-658.
- Lambers, H., Chapin III, F. S. and Pons, T. L. (1998) Plant Physiological Ecology. Springer-Verlag, New York, pp. 540.
- Lamersdorf, N. P. and Mayer, M. (1993) Nutrient cycling and acidification of a northwest German forest site with high atmospheric nitrogen deposition. For. Ecol. Manag., 62: 323-354.
- Larcher, W. (2003) Physiological Plant Ecology. 4<sup>th</sup> ed, Springer-Verlag, New York.
- Lauer, M. J., Pallardy, S. G., Blevins, D. G. and Randall, D. D. (1989) Whole leaf carbon exchange characteristics of phosphate deficient soybeans (*Glycine max* L.). Plant Physiology, 91: 848-854.
- Lee, C. H., Izuta, T., Aoki, M., Totsuka, T. and Kato, H. (1998) Growth and photosynthetic responses of red pine seedlings grown in brown forest soil acidified by adding H<sub>2</sub>SO<sub>4</sub> solution. Jpn. J. Soil Sci. Plant Nutr., 69: 59-61. (in Japanese).
- Lee, C. H., Yoo, J. H., Kim, Y. K., Byun, J. K., Kim, C. S., Lee, S. W. and Lee, B. S. (1999) Growth of *Pinus densiflora* seedlings in artificially acidified soils. FRI Journal of Forest Science, 61: 90-96 (in Korean).
- Lee, K. J., Cho, W. and Han, B. H. (1996) Restoration and Status of Urban Ecosystem in Seoul - Plant Community Structure in Forest Area. Korean Journal of Environmental and Ecology, 10: 113-127. (in Korean).
- Lewis, J. D., Griffen, K. L., Thomas, R. B. and Strain, B. R. (1994) Phosphorus supply affects the photosynthetic capacity of loblolly pine grown in elevated carbon dioxide. Tree Physiology, 14: 229-244.
- Li, J. C. R. (1964) Analysis of variance versus Chi-square test. In: Edwards Brothers, Ann Arbor, Statistical inference, 471-484.
- Macfie, S.M. and Taylor, G.J. (1992) The effects of excess manganese on photosynthetic rate and concentration of chlorophyll in *Triticum aestivum* growth in solution culture. Physiology Plant, 85: 467-475.
- Mansfield, T. A. (1998) Stomata and plant water relations: does air pollution create problems. Environmental Pollution, 101: 1-11.
- Marschner, H. and Dell, B. (1994) Nutrient uptake in mycorrhizal symbiosis. Plant and Soil, 159: 89-102.
- Marschner, H. (1995) Mineral nutrition of higher plants, 2<sup>nd</sup>ed, Academic Press, New York.
- Marx, D. H., Cordell, C. E. Kenney, D. S., Mexal, J. G., Artman, J. D. Riffle, J. W. and Molina, R. J. (1984) Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root seedlings. Forest Sci. Monogr., 25: 1-101.
- Matyssek, R., Günthardt-Goerg, M. S., Landolt, W. and Keller, T. (1993a) Whole-plant growth and leaf formation in ozonated hybrid poplar (*Populus X euramericana*). Environmental Pollution, 81: 207-212.
- Matyssek, R. Keller, T. and Koike, T. (1993b) Branch growth and leaf gas exchange of *Populus tremula* exposed to low ozone concentrations throughout two growing seasons. Environmental Pollution, 79: 1-7.
- Matyssek, R., Günthardt-Goerg, M., Maurer, S. and Keller, T. (1995a) Nighttime exposure to ozone reduces whole-plant production in *Betula pendula*. Tree physiology, 15: 159-165.
- Matyssek, R., Reich, P., Oren, R. and Winner, W. E. (1995b) Response mechanisms of conifers to air pollutants. In: Ecophysiology of coniferous forests. In: Smith, W. K. and Hinckley, T. M. (eds), Academic Press, San Diego, CA, 255-308.
- Miller, B. L., Allemendinger, D. F., Johnson, F. and Poley, D. (1953) Lime papers and indicator plants in fluorine air pollution investigations. Journal of Agricultural and food chemistry, 1: 526-529.
- Ministry of Environment (2002) Environmental statistics yearbook vol. 15. Adcore, Seoul, 142-203. (in Korean).
- Miwa, M., Izuta, T. and Totsuka, T. (1994) Effects of soil acidification on the growth of Japanese cedar seedlings grown in three soils from different parent materials. Journal of Japan Society of Air Pollution, 29: 254-263.
- Miyake, H., Kamei, N., Izuta, T. and Totsuka, T. (1991) Effects of aluminium on the growth of hydroponically grown seedlings of *Cryptomeria japonica* D. Don. Man and Environ., 17: 10-16. (in Japanese with English summary).
- Moor, P. D. (1995) Too much of a good thing. Nature, 374: 117-118.
- Moroto, M., Mashimo, Y. and Harata Y. (1987) Soil properties and growth of Japanese red pine (*Pinus densiflora*) in the hilly and low-mountainous region of central Japan. J. of jap. Forestry Soc., 69: 371-378. (in Japanese with English summary).
- Mousseau, M., Dufrene, E., El Kohen, A., Epron, D., Godard, D., Liozon, R., Pontailier, J.Y. and Saugier, B. (1996) Growth strategy and tree response to elevated CO<sub>2</sub>: A comparison of beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa* Mill.). In: Koch, G. W. and Mooney, G. A. (eds.) Carbon Dioxide and Terrestrial Ecosystems. Academic Press, New York, 71-86.
- Nable, R. O., Houtz, R. L. and Cheniae, G. M. (1988) Early inhibition of photosynthesis during development of Mn toxicity in tobacco. Plant Physiology, 86: 1136-1142.
- Nakaji, T. (2002) Ecophysiological study on the effects of nitrogen load and elevated ozone on *Pinus densiflora* seedlings. In: Ph.D. Tokyo University of Agriculture and Technology, Tokyo, Japan. (in Japanese).
- Newman, E. J. (1988) Mycorrhizal links between plants: their functioning and ecological significance. Adv. Ecol. Res., 18: 243-266.
- Nilson, J. (1987) Critical loads for sulphur and nitrogen. In Proc. Int. Symp. Air Pollution and Ecosystems, Grenoble, France, 85-91.
- Norby, R. J., O'Neill, E. G., Hood, W. G., Luxmoore, R. J. (1987) Carbon allocation, root exudation and

- mycorrhizal colonization of *Pinus echinata* seedlings grown under CO<sub>2</sub> enrichment. *Tree Physiol.*, 3: 203-210.
- Norby, R. J., Wullschleger, S. D. and Gunderson, C. A. (1996) Tree responses to elevated CO<sub>2</sub> and implications for forest. *In*: Koch, G. A. (ed.) *Carbon Dioxide and Terrestrial Ecosystems*, Academic Press, New York, 1-21.
- Okita, T. (1996) *Shinban Sanseiu (Acid Rain)*. Hakuyuu-sha, Tokyo, pp. 309.
- Olsen, S. R. and Sommers, L. E. (1982) Phosphorus. *In*: Page, A. L., Miller, R. H. and Keeney, D. R. (eds.) *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*. 2<sup>nd</sup>, Soil Science Society of America, Madison, WI, 403-430.
- Paynter, V. A., Reardon, J. C. and Shelburne, V. B. (1991) Carbohydrate changes in short-leaf pine (*Pinus echinata*) needles exposed to acid rain and ozone. *Can. J. For. Res.*, 21: 666-671.
- Perry, D. A., Bell, T. and Amaranthus, M. P. (1992) Mycorrhizal fungi in mixed-species forests and other tales of positive feedback, redundancy and stability. *In*: Cannell, M. R., Malcolm, D. C., Robertson, P. A. (eds.) *Ecology of mixed species stands of trees*. Blackwell, Oxford, 151-174.
- Poorter, H. (1993) Interspecific variation in the growth response of plants to an elevated ambient CO<sub>2</sub> concentration. *Vegetatio*, 104/105: 77-97.
- Pukacki, P. M. (2000) Effects of sulphur, fluoride and heavy metal pollution on the chlorophyll fluorescence of Scots pine (*Pinus sylvestris* L.) needles. *Dendrobiology*, 45: 83-88.
- Qu, L., Qureshi, A. M., Iwase, K., Tamai, Y., Funada, R. and Koike, T. (2003) *In vitro* ectomycorrhiza formation on two larch species of seedlings with six different fungal species. *Eurasian J. For. Res.*, 6: 65-73.
- Qureshi A. M. (2003) Nutritional Preconditioning and Ectomycorrhizal Formation of *Picea mariana* (Mill.) B.S.P. seedling. *Eurasian Journal of Forest Research*, 6: 1-63.
- Qureshi, A. M., Maruyama, Y. and Koike, T. (2003) The role of mycorrhiza in forest ecosystems under CO<sub>2</sub>-enriched atmosphere. *Eurasian Journal of Forest Research*, 6: 171-176.
- Reich, P. B. (1983) Effects of low concentrations of O<sub>3</sub> on net photosynthesis, dark respiration and chlorophyll contents in spring hybrid poplar leaves. *Plant Physiology*, 73: 291-296.
- Reich, P. B., Koike, T., Gower, S. T. and Schoettle, A. W. (1995) Causes and consequences of variation in conifer leaf life-span. *In*: Smith, W. K. and Hinckley, T. M. (eds.), *Academic Press, San Diego, CA*, 225-254.
- Reid, C. P. P., Kidd, F. A. and Ekwebelam, S. A. (1983) Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. *Plant Soil*, 71: 415-432.
- Rengel, Z. (1992) Role of calcium in aluminium toxicity. *New Phytologist*, 121: 499-513.
- Rey, A. and Jarvis, P. G. (1997) Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO<sub>2</sub> exposure. *Ann. Bot.*, 80: 809-816.
- Rogers, H. H., Runion, G. B. and Krupa, S. V. (1994) Plant responses to atmospheric CO<sub>2</sub> enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, 83: 155-189.
- Rogers, H. H., Prior, S. A., Runion, G. B. and Mitchell, R. J. (1996) Root to shoot ratio of crops as influenced by CO<sub>2</sub>. *Plant and Soil*, 187: 229-248.
- Rogers, A., Fischer, B. U., Bryant, J., Frehner, M., Blum, H., Raines, C. A. and Long, S.P. (1998) Acclimation of photosynthesis to elevated CO<sub>2</sub> under low-nitrogen nutrition is affected by the capacity for assimilate utilization. *Perennial ryegrass under free-air CO<sub>2</sub> enrichment*. *Plant Physiol.*, 118: 683-689.
- Rollwagen, B.A. and Zasoski, R.J. (1988) Nitrogen source effects on rhizosphere pH and nutrient accumulation by Pacific Northwest conifers. *Plant and Soil*, 105: 79-86.
- Rouhier, H. and Read, D. J. (1998) Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedlings of *Pinus sylvestris*. *Environ. exp. Bot.*, 40: 237-246.
- Rouhier, H. and Read, D. J. (1999) Plant and fungal responses to elevated atmospheric CO<sub>2</sub> in mycorrhizal seedlings of *Betula pendula*. *Environ. exp. Bot.*, 42: 231-241.
- Rousseau, J. V. D. and Reid, C. P. P. (1990) Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. *Forest Sci.*, 36: 101-112.
- Runion, G. B., Mitchell, R. J., Rogers, H. H., Prior, S. A. and Counts, T. K. (1997) Effects of nitrogen and water limitation and elevated atmospheric CO<sub>2</sub> on ectomycorrhiza of longleaf pine. *New Phytol.*, 137: 681-689.
- Sandermann, H., Wellburn, A. R. and Heath, R. L. (eds.) (1997) *Forest decline and ozone - A comparison of controlled chamber and field experiments (Ecological studies Vol. 127)*. Springer-Verlag, Berlin, Heidelberg.
- SAS Institute Inc. (1998) *SAS/STAT user's guide' 6.03 ed.* SAS Institute INC. Cary, N.C.
- Schaedle, M., Thornton, F. C., Raynal, D. J. and tepper, H. B. (1989) Response of tree seedlings to aluminum. *Tree physiology*, 5: 337-356.
- Seegmüller, S. and Rennenberg, H. (1994) Interactive effects of mycorrhization and elevated carbon dioxide on growth of young pedunculate oak (*Quercus robur* L.) trees. *Plant and Soil*, 167: 325-329.
- Sharkey, T. D. (1985a) Photosynthesis in intact leaves of C3 plants: Physics, physiology and rate limitations. *The Botanical Review*, 51: 53-105.
- Sharkey, T. D. (1985b) Photosynthesis of cotton plants exposed to elevated levels of carbon dioxide in the field. *Photosynth. Res.*, 12: 191-203.
- Sharkey, T. D., Socias, X. and Loreto, F. (1994) CO<sub>2</sub> effects on photosynthetic and product synthesis and feedback. *In*: Alscher, R.G. and Wellbern, A.R.

- (eds.): Plant Responses to the Gaseous Environment, Chapman & Hall, London - New York, 55-78.
- Shinano, T., Lei, T. T., Kawamukai, T., Inoue, M. T., Koike, T. and Tadano, T. (1996) Dimethylsulfoxide method for the extraction of chlorophyll a and b from the leaves of wheat, field bean, dwarf bamboo, and oak. *Photosynthetica*, 32: 409-415.
- Shindo, K. (2002a) Effects of hydrogen fluoride on growth of plants (I), *Journal of Japan Society for atmospheric Environment*, 37: 245-255. (in Japanese).
- Shindo, K. (2002b) Effects of hydrogen fluoride on Growth of plants (II), *Journal of Japan Society for atmospheric Environment*, 37: 256-264. (in Japanese).
- Simard, S. W., Perry, D. A., Jones, M. D., Myrold, D. D., Durall, D. M. and Molina, R. (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388: 579-582.
- Smith, S. E. and Read, D. J. (1997) *Mycorrhizal Symbiosis*. - Academic Press, San Diego.
- Staddon, P. L., Fitter, A. H. and Robinson, D. (1999) Effects of mycorrhizal colonization and elevated atmospheric carbon dioxide on carbon fixation and below-ground carbon partitioning in *Plantago lanceolata*. *J. Exp. Bot.*, 50: 853-860.
- Statens forurensningstilsyn (1992) Virkninger av luft-forurensning på helse og miljø. Anbefalte luftkvalitets-kriterier. Rapport nr. 92, 16.
- Stitt, M. (1991) Rising CO<sub>2</sub> levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment*, 14: 741-762.
- Stulen, I. and den Hertog, J. (1993) Root growth and functioning under atmospheric CO<sub>2</sub> enrichment. *Vegetatio*, 104/105: 99-115.
- Sucoff, E. (1975) Effect of deicing salts on woody vegetation along Minnesota roads. *Technical Bulletin*, 303: 1-49.
- Supharungsun, S. and Wainwright, M. (1982) Determination and absorption of fluoride in atmospheric-polluted soils. *Bulletin of Environmental Contamination of Toxicology*, 28: 632-636.
- Suresh, R., Foy, C.D. and Weidner, J.R. (1987) Effects of excess soil manganese on stomatal function in two soybean cultivars. *Journal of Plant Nutrition*, 10: 749-760.
- Sverdrup, H., de Vries, W. and Henriksen, A. (1990) Mapping Critical Loads' Miljörappport 1990:14, Nordic Council of Ministers, Copenhagen, pp. 124.
- Sverdrup, H., Warfvinge, P. and Nihlgård, B. (1994) Assessment of soil acidification effects on forest growth in Sweden. *Water Air and Soil Pollution*, 78: 1-36.
- Terashima, I. (1992) Anatomy of non-uniform leaf photosynthesis. *Photosynth. Res.*, 31: 195-212.
- Thompson, G. W. and Medve, R. J. (1984) Effects of aluminum and manganese on the growth of ectomycorrhizal fungi. *Applied and Environmental Microbiology*, 48: 556-560.
- Thornley, J. H. M. (1976) *Mathematical models in plant physiology*. Academic Press, London, pp. 318.
- Thornton, F. C., Pier, P. A. and McDuffie, C. Jr. (1990) response of growth, photosynthesis, and mineral nutrition of red spruce seedlings to ozone and acidic cloud deposition. *Environ. Exp. Bot.*, 30: 313-323.
- Tissue, D. T., Thomas, R. B. and Strain, B. R. (1996) Growth and photosynthesis of loblolly pine (*Pinus taeda*) after exposure to elevated CO<sub>2</sub> for 19 months in the field. *Tree Physiol.*, 16: 49-59.
- Trappe, J. M. (1988) Lessons from alpine fungi. *Mycologia*, 80: 1-10.
- Treshow, M. and Anderson, F. K. (1989) *Plant stress from air pollution*. Chichester, John Wiley & Sons Ltd.
- Ulrich, B., Mayer, R. and Khanna, P. K. (1980) Chemical changes due to acid precipitation in a loss-derived soil in Central Europe. *Soil Sci.*, 130: 193-199.
- Ulrich, B. (1983) Soil acidity and its relation to acid deposition. *In: Ulrich, B. and Pankrath, J. (eds.) Effects of accumulation of air pollution in forest ecosystems*. Reidel, Dordrecht, Boston, London, 127-146.
- Ulrich, B. (1989) Effects of acidic precipitation on forest ecosystems in Europe. *In: Adriano, D. C. and Johnson, H. J. (eds.) Acidic Precipitation, Volume 2: Biological and Ecological effects*, Springer-Verlag, New York, 189-272.
- Ulrich, B. (1990) *Waldsterben* : Forest decline in West Germany. *Environmental Science and Technology*, 24: 436-441.
- Van Breemen, N., Burrough, P. A., Velthorst, E. J., Van Dobben, H. F., Toke de Wit, Ridder, T. B. and Reijnders, H. F. R. (1982) Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, 299: 548-550.
- Vogen, C. S. and Curtis, P. S. (1995) Leaf gas exchange and nitrogen dynamics of N<sub>2</sub>-fixing, field-grown *Alnus glutinosa* under elevated atmospheric CO<sub>2</sub>. *Global Change Biol.*, 1: 55-61.
- Wardlaw, I. F. (1990) The control of carbon partitioning in plants. *New Phytol.*, 116: 341-381.
- Weber, J. A., Tingey, D. T. and Andersen, C. P. (1994) Plant response to air pollution. *In: Wilkinson, R. E. (ed.) Plant-Environment Interactions*. Marcel Dekker Inc, New York, 357-389.
- Wilkins, D. A. (1991) The influence of sheathing (ecto)mycorrhizas of trees on the uptake and toxicity of metals. *Agric. Ecosyst. Environ.*, 35: 245-260.
- Winner, W. E. (1981) The effects of SO<sub>2</sub> on photosynthesis and stomatal behavior of Mediterranean-climate shrubs and herbs. *In: Margaris, N. S. and Mooney, N. A. (eds.) Components of Productivity of Mediterranean Climate Regions. Basic and Applied Aspects*. Dr. W. Junk Publishers, The Hague, 91-103.
- Wright, D. P., Scholes, J. D., Read, D. J. and Rolfe, S. A. (2000) Changes in carbon allocation and

- expression of carbon transporter genes in *Betula pendula* Roth. colonized by the ectomycorrhizal fungus *Paxillus involutus* (Batsch) Fr. *Plant, Cell and Environ.*, 23: 39-49.
- Wyman, R. L. (1991) *Global climate change and life on earth*. New York, Chapman & Hall.
- Yang, L., Izuta, T., Aoki, M. and Totsuka, T. (1996) Effects of SO<sub>2</sub> and soil acidification, alone and in combination, on growth of mason pine seedlings. *Journal of Japan Society for Atmospheric Environment*, 31: 1-10. (in Japanese with English summary).
- Yoo, J. H., Byun, J. K., Kim, C. S., Lee, C. H., Kim, Y. K. and Lee, W. K. (1998) Effects of lime, magnesium sulfate, and compound fertilizers on soil chemical properties of acidified forest soils. *Journal of Korean Forest for Society*, 87: 341-346. (in Korean with English summary).
- Yoshida, M. and Kawahata, Y. (1988) Neutralization mechanism of acid rain by soil. *Jpn. J. soil Sci. Plant Nur.*, 59: 413-415. (in Japanese).
- Zhang, J., Ferdinand, J. A., Vanderheyden, D. J., Skelly, J. M. and Innes, J. L. (2001) Variation of gas exchange within native plant species of Switzerland and relationships with ozone injury: An open-top experiment. *Environmental pollution*, 113: 177-185.