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<tr>
<td>Citation</td>
<td>Environmental Pollution, 139(3): 421-432</td>
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Growth and photosynthetic responses of two pine species (*Pinus koraiensis* and *P. rigida*) in a polluted industrial region in Korea

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Abstract

We investigated the effects of pollutants on two pine species (*Pinus koraiensis* and *P. rigida*) in an industrial region in Korea, using a physiological approach. The concentrations of fluorine (F) and chlorine (Cl) in the atmosphere, in 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 pine needles were significantly higher, and essential elements and chlorophyll in needles were significantly lower at the damaged site than at the control site. The photosynthetic capacities, shoot length and survival statistics of needles of the two pines were all significantly reduced at the damaged site compared to the control site, especially *P. rigida*. Based on our comparison of photosynthetic responses and the concentrations of F, Cl and Mn in needles of the two pine species, *P. koraiensis* is more resistant to excess Mn in its needles than *P. rigida*.

Keywords: Photosynthetic capacity; Fluoride; Chlorine; *Pinus koraiensis*; *Pinus rigida*; Needle life span
Introduction

Pine trees are planted in cities, at roadsides for casting shade, and are used for rehabilitating degraded regions in Korea. The Korean pine (*Pinus koraiensis* Sieb. et Zucc.) and the Pitch pine (*Pinus rigida* Miller) are the most important afforestation tree species in Korean forests, since they can grow in low-nutrient or barren soil, including shallow soil and sandy or gravelly soil. To rehabilitate degraded forested areas, *P. rigida* was introduced in 1906 from North America (Kim, 1999). This species has good resistance against O$_3$ and soil acidification (Burns and Honkala, 1990). In recent years, however, the resulting pine forests have been in decline near to industrial areas and large cities (Choi et al., 2003, 2005; Kim et al., 2003; Lee et al., 1996).

In most Korean forests, the soil is gravelly and not particularly fertile, and is also easily acidified since it derives from granite (Lee at al., 1998). Large amounts of chemicals, such as lime and nitrogen fertilizer, have been administered to reduce or reverse soil degradation, but the decline is continuing (Choi, 2003; Yoo et al., 1998).

What causes this decline in pine forests? Environmental pollutants, including SO$_2$, O$_3$, NO$_x$, usually reduce plant growth (Fornasiero, 2003; Kayama et al., 2003; Matyssek et al., 1995a; Shindo, 2002a, b; Sucoff, 1975) through their negative effects on photosynthetic function (Darral, 1989; Furukawa, 1991; Heber et al., 1995; Hinrichsen, 1986; Lambers et al., 1998; Larcher, 2003; Mansfield, 1998; Matyssek el al., 1995b; Weber et al., 1994), on leaf stomatal conductance (Winner, 1981), on chlorophyll content and leaf longevity (Matyssek et al., 1993a, b; Reich, 1983; Reich et al., 1995) and through wet and dry deposition (Choi et al. 2005; Izuta, 1998; Izuta et al., 2001).

Our preliminary surveys 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). Near the damaged site, there are many industrial plants, including glassworks, steelworks,
brickworks, ceramics plants, dye works and fuel coal combustion. These plants usually emit NO\textsubscript{x} and SO\textsubscript{2} as well as F and Cl (Supharungsun and Wainwright, 1982). Of these, fluoride (F) seems to be the most toxic (Aluminiumindustriens Miljösekretariat, 1993; Statens forurensningstilsyn, 1992). For about half a century, forest ecosystems in Korea have faced heavy industrial pollution, mainly SO\textsubscript{2}, NO\textsubscript{x}, and acid rain. In recent years the level of toxic pollutants emitted into the atmosphere has fallen (Ministry of Environment, 2002), but pine species have been still been in decline. What kinds of pollutants are most harmful to pine forests?

According to our preliminary surveys, the photosynthetic rates at light saturation and ambient CO\textsubscript{2} of pine saplings (Pinus koraiensis and P. rigida) in polluted industrial areas are significantly lower than in clear university forests (Choi et al., 2003; Kayama et al., 2004). The soil pH is lower in polluted areas and usually contains soluble manganese (Mn) (Kitao et al., 1997a). Recently, Choi et al. (2005) found that P. koraiensis is more resistant against soil acidification, based on the (Ca+Mg+K)/Al ratio, than P. densiflora. We therefore hypothesize that reduced growth and photosynthetic capacity of pines at the damaged site is related to excess Mn accumulation in pine needles and to F as well as Cl. Which pine species are most resistant against soil acidification and air pollutants?

To tackle these questions, we measured the growth performance and photosynthetic activity in situ of pine saplings planted in polluted and clear areas, taking climatic factors into account.
MATERIALS AND METHODS

Study sites
To compare the effect of pollutants on growth and physiological changes, we chose two plantation sites of Korean pine (*Pinus koraiensis* Sieb. et Zucc.) and Pitch pine (*Pinus rigida* Miller). The control site is southeast of Seoul city in Korea, and the damaged site is to the southwest of the city. The control site of *P. koraiensis* and *P. rigida* trees was in the Toi-chon Experimental Forest of Kyunghee University (37.2N, 127.1E), where air pollution is low and is close to clean-air conditions (regarded as “control”) (Kwangju, Kyunggido, Korea). The damaged site was located in the Ansan industrial region (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.

We chose three study plots for the control site, and six for the damaged site. Three of the plots at the damaged site were on two slopes (a west-facing slope and a south-facing slope) in the region. 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 radiation between the control and damaged sites during the study period (Korea Meteorological Administration, 2002a). The altitude of the control site was 90m and that of the damaged site was 50m. The annual mean precipitation at the control and damaged sites was respectively 1,468mm and 1,204mm, and the mean temperature during the growing season (April to October) was 18.9 and 19.2°C (Korea Meteorological Administration, 2002b). The rainy season in Korea runs from July to September: more than 60% of the annual precipitation falls in these three months (Korea meteorological Administration, 2002b, 2003).
Plant materials

Needles of *Pinus koraiensis* and *P. rigida* for chemical analysis were collected from plantations at the control and damaged sites. Sample needles were taken randomly from sun-exposed branches of five individual trees per species in early September 2002. The sample needles were divided into current-year 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 (since they were planted at three years old). The tree height for each species varied between 1.6m and 2.0m.

Analysis of soil chemistry

We measured the soil pH, and the concentrations of nitrogen (N), exchangeable phosphorus (P), base cations (Ca, Mg, K) and heavy metal (Mn) in soil. The soil pH was determined from a soil suspension using a pH meter (CH-8603, Mettler-Toledo, Greifensee, Switzerland). The suspension was made by mixing 20g fresh soil and 50ml distilled water in a 100ml 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 sodium bicarbonate (Olsen and Sommers, 1982), and Ca, Mg and K were extracted from 2.5g dry soil with 50ml of 1 N ammonium acetate solution after shaking for 1 h. Mn was extracted from 5.0g of dry soil with 0.1N HCl after shaking for 1 h at 30°C. P, Ca, Mg, K and Mn in soil were all measured from these prepared solutions using a ICP (IRIS, Jarrel Ash, Franklin, MA).

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

To evaluate shoot growth, we measured the length of leader shoots for 20 samples (4 branches
from each of 5 trees) of *P. koraiensis* and *P. rigida* at the control and damaged sites in mid-September 2002, once the leader shoot was observed to be fully grown. We also measured needle longevity on four branches of five individual trees of each pine species at each study site. The branches were selected from a sun-exposed crown of trees on the forest edge at a height of about 1.2 - 1.5m. The main branch was divided for our purposes 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 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 formula: SN (%) = RN/TN × 100, where RN and TN respectively denote the number of retained and total needles (estimated by multiplying the number of needles borne in a bundle by the number of scars) (Kayama et al., 2002).

We also dug out 0.07 - 0.1m of the tip portion of the roots of the five individual trees of both pines at about 0.1m depth at both sites, to search for infection by ectomycorrhiza. We observed the root tips 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.

**Measurement of photosynthesis**

The photosynthetic capacities of five trees of *P. koraiensis* and of *P. rigida* were measured in mid-September 2002 at the control and damaged sites using two methods. In the first method, we examined the photosynthetic light response curve of current year needles in sunny crown trees at the edge of the forest at a height of around 1.3m. We used a portable gas analyzer for these measurements (H4A, ADC BioScientific, Hoddesdon, U.K.); the ambient temperature was 23 - 25°C and the ambient CO₂ concentration was 35.5 - 36.0Pa. Between 15 and 20 needles of two pines were covered with a conifer chamber (137 cm³, ADC BioScientific, Hoddesdon, U.K.), and
the photosynthetic rate was measured. Supplementary light was provided by a halogen lamp (WALZ, Effeltrich, Germany). We changed the photosynthetic photon flux density (PPFD) from high to low to dark (2000, 1000, 500, 100 and 0 μmol·m⁻²·s⁻¹) using cloth shades (Krary, Osaka, Japan). After determining the photosynthetic light curve, we measured the width and length of needles using vernier calipers, and then calculated the net photosynthetic rate per unit area. Photosynthetic light response curves were drawn from the resulting data, using the formula (Thornley, 1976):

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

where \( P \) is the net photosynthetic rate, \( \phi \) is the initial slope of the curve, \( I \) is the incident PPFD, \( P_{\text{max}} \) 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 \( A_{\text{max}} \) of the unshaded value.

The second method of measuring photosynthetic capacity looked at the \( A/C_i \) (\( A \)=photosynthetic rate, \( C_i \)=intercellular CO₂ 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 CO₂ assimilation rate was measured at light saturation, under a photosynthetic photon flux density (PPFD) of 1,000 - 1,200 μmol·m⁻²·s⁻¹ provided by a cool halogen lamp (WALZ, Effeltrich, Germany). The leaf temperature was 25ºC and the relative humidity was 50 - 70%. Needles were allowed to acclimatize to their surroundings for 10 minutes before measurement began, after which we began the determination with CO₂ concentrations of 15 - 150Pa. Distinct processes regulate the rate of CO₂ assimilation at low and high \( C_i \) concentrations (Farquhar and Sharkey, 1982; Matyssek et al., 1993b; Sharkey, 1985). The initial slope of the \( A/C_i \) curve is proportional to the carboxylation activity of Rubisco (i.e., the carboxylation efficiency). The RuBP regeneration rate was estimated from the \( A/C_i \) curve. The rationale is that the CO₂
assimilation rate at high CO$_2$ concentrations is limited by the regeneration rate of ribulose-1, 5-bisphosphate (RuBP) (Lambers et al., 1998).

4

**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 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 using 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 (Alvarez, 1995; Greenhalgh and Riley, 1961; Shindo, 2002a).

**Absorption of fluoride and chlorine in the atmosphere**

We 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 5×15cm 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 per plot were established at the control and damaged sites, placed under shelter but with good ventilation, and were exposed to the wind for one month.
Analysis of fluoride and chlorine in precipitation and soil water

We collected precipitation and soil water samples from each site, making three replications. Precipitation was collected from April to November 2003. Soil water was collected from depths of 0.1, 0.3 and 0.6m (using a 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).

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 and compared between species and sites by the $t$-test (Li, 1964) using the Stat View 5.0 software (SAS Institute, Cary, NC, USA). Relations between $P_{sat}$ and Mn, Cl or F were estimated using (S)MATR (Falster et al., 2003).

Results

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

Table 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 1); this difference is statistically significant ($p<0.05$). The concentration of Cl in the atmosphere exhibited a similar pattern ($p<0.01$).

The concentration of Cl and F in precipitation was significantly higher at the damaged site than at the control site, at least during early and late summer ($p<0.05$) (Figure 1). The amount of trapped Cl and F scarcely differed between the two slopes in the damaged area. 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 shows the soil condition at the control and damaged sites. Soil was more acidified at the damaged site than at the control site, but there was no statistical difference in Al concentration between the control and damaged sites. 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. We therefore averaged the two slopes as representing the damaged site. Exchangeable base cations (Mg, K) and P were significantly lower at the damaged site than at the control site ($P<0.05$). However, concentrations of N and Ca were higher at the damaged site than at the control site.

Concentrations of Cl and F in soil water showed similar trends to concentrations in precipitation, and were significantly higher at the damaged site than at the control site ($p<0.05$) (Fig. 2). 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 0.3m from the surface was significantly higher at the damaged site than at the control site, but at 0.6m depth there was no significant difference between the sites. For Cl, the concentration in soil water at 0.1, 0.3 and 0.6m depth at the damaged site was significantly higher than at the control site ($P<0.001$).

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

Shoots were shorter at the damaged site than at the control site, having length 0.26 - 0.34m (control site) and 0.14 - 0.22m (damaged site) for *P. koraiensis* and 0.23 - 0.28m (control site) and 0.14 - 0.19m (damaged site) for *P. rigida* (Fig. 3). These figures correspond to reductions of 24 - 47% for *P. koraiensis* and 26 - 48% for *P. rigida*, and attained statistical significance ($p<0.01$).
Figure 4 shows the survival of needles of *P. koraiensis* and *P. rigida*. Both shed their needles earlier at the damaged site than at the control site. *P. koraiensis* retained 60% more 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* was significantly worse at the damaged site 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).

**Concentrations of elements in needles**

Table 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 fell with needle age in both species at both sites. Calcium (Ca) accumulated in needles with aging, especially at the damaged site.

The concentration of manganese (Mn) in needles was higher at the damaged site than at the control site in both species, but especially *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 were significantly higher at the damaged site than at the control site (*p*<0.05). The Cl and F concentrations of needles increased with needle age in both species, especially at the damaged site.
Chlorophyll concentration in needles

Chlorophyll (a+b) and chlorophyll b concentrations in 1 to 3-year-old needles of *P. koraiensis* at the control site maintain levels of ca. 1.5μmol·g⁻¹ (a+b) and ca. 0.25μmol·g⁻¹ (b), respectively, but concentrations at the damaged site fell rapidly from 1.60 to 0.11μmol·g⁻¹ (a+b) and from 0.23 to 0.04μmol·g⁻¹ (b) with needle age (see Fig. 5). Chlorophyll (a+b) and b concentrations were significantly lower for *P. koraiensis* at the damaged site 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 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).

Photosynthetic responses

The net photosynthetic rate of 1-year-old needles saturated at approximately 1300μmol·m⁻²·s⁻¹ PPFD for both *P. koraiensis* and *P. rigida* at the control site; at the damaged site saturation was reached at approximately 800μmol·m⁻²·s⁻¹ PPFD for *P. koraiensis* and 600μmol·m⁻²·s⁻¹ PPFD for *P. rigida* (Fig. 6). The photosynthetic rate at light saturation (Pₘₐₓ) was significantly lower for both species at the damaged site than at the control site (*P. koraiensis*, 3.97 to 2.55μmol·m⁻²·s⁻¹; *P. rigida* 3.29 to 1.38μmol·m⁻²·s⁻¹; *p*<0.01). Moreover, the apparent quantum yield (Φ) was significantly lower at the damaged site than at the control site (i.e., 0.02 to 0.012μmolCO₂·m⁻²·Pa⁻¹·CO₂·s⁻¹ for *P. koraiensis*, and 0.011 to 0.007μmolCO₂·m⁻²·Pa⁻¹·CO₂·s⁻¹ for *P. rigida*; see Table 4).

Figure 7 shows the photosynthetic dependence of intercellular CO₂ (Ci) at light saturation, (A/Ci), for *P. koraiensis* and *P. rigida* 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 μmol·m⁻²·s⁻¹ to 3 μmol·m⁻²·s⁻¹. Also, the carboxylation efficiency (CE) of the A/Cᵢ curve for *P. rigida* at the damaged site is significantly less than at the control site (0.06 μmol·m⁻²·s⁻¹/Pa and 0.14 μmol·m⁻²·s⁻¹/Pa; *p* < 0.001; see Table 4). However, we did not find any clear reduction in the CE of the A/Cᵢ curve of *P. koraiensis* at the damaged site relative to the control site. The CE was more suppressed in *P. rigida* than in *P. koraiensis*. The RuBP regeneration rates of *P. koraiensis* and *P. rigida* at the damaged site were less than at the control site (Table 4). From the A/Cᵢ curve, the reduction in each parameter was smaller in *P. koraiensis* than in *P. rigida*.

**Relation between photosynthesis and Mn, Cl and F concentrations**

We found that *P*ₘₐₜ decreased with increasing Mn concentration in needles in both species (Fig. 8). The gradient of the plot of *P*ₘₐₜ versus F and *P*ₘₐₜ versus Cl also showed a similar tendency. In all cases the regression lines fell more steeply for *P. rigida*. The gradient of the plot of *P*ₘₐₜ versus Mn differed significantly between the species (*P* < 0.01).

**Discussion**

There were no statistical differences in soil pH and Al concentration in soils between the control and damaged sites. We therefore focused on other factors affecting photosynthetic function (Table 2). We found that *P*ₘₐₜ decreased with accumulation of Mn, Cl or F in needles of both species. The gradient of the regression lines for *P. rigida* was steeper than for *P. koraiensis* (Fig. 8), although *P. koraiensis* accumulated more of these elements in needles than *P. rigida*. Fluoride is absorbed via the stomata, transported by transpirational flow in the apoplast, and can accumulate at toxic levels in the tips and margins of the leaves since there is no removal.
mechanism. Moreover, F that is 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 reduces the rate
of mycorrhizal colonization (Duke et al., 1986), as we 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 concentrations in
precipitation did not differ between the sites from June to September (Fig. 1). These pollutants are
washed out by the high precipitation between June and September, which 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 the sea. At the damaged site, the Cl concentration 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).
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 PS II (Nable et al., 1988; Kitao et al., 1997a,b). Enhanced accumulation of Mn, Cl and/or F in needles therefore reduces the carboxylation efficiency in Rubisco, the RuBP regeneration rate, and also $P_{sat}$, 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 3, Fig. 5).

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, development of mycorrhizae is likely 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 sunlight energy in photosynthesis. P deficiency in chloroplasts usually 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; Harley and Sharkey, 1991; Lauer et al., 1989). Nutrient deficiency in plants is accompanied by a reduction in the rate of CO$_2$ assimilation, in turn reducing shoot growth and accelerating needle loss (Fig. 3, 4) (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 stemming from environmental stressors such as F and excess Mn and Cl. Needle loss and premature senescence due to air pollutants such as SO$_2$, O$_3$, NO$_x$, or acid deposition, have been reported in pine, birch and poplar (Reich, 1983; Reich et al., 1995; Matyssek et al., 1993a, b).
The toxicity of these pollutants is mainly due to their interference with respiration and photosynthetic function (Heber et al., 1995; Lambers et al., 1998; Matyssek et al., 1995a, b; Pukacki, 2000). The energy status of damaged trees may then be reduced (Ernst, 1976; Ernst and Joosse-van Damme, 1983) by smaller uptake of mineral nutrients (Adams, 1981; Larcher, 2003).

We conclude that reduction in growth of both pine species at the damaged site is the result of various physiological stresses induced by air pollutants. In particular, Cl and F discharged from nearby industrial regions suppresses growth of *P. koraiensis* and *P. rigida*. *P. rigida* is less resistant against pollutants and excess Mn in needles than *P. koraiensis*, and needle loss at the damaged site was more severe in *P. rigida* than in *P. koraiensis*.

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Table 1. The concentration of fluorine in the air from the control site and damaged site (Unit=μgF·dm²·LTP/month, mean±S.D.), and the concentration of chlorine (Unit=ppm, mean±S.D., *P<0.05, **P<0.01, ***P<0.001) (- : No sample was collected).

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>**Control</td>
<td>29.52±2.41</td>
<td>36.79±6.99</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Damaged</td>
<td>97.02±28.43</td>
<td><strong>103.76±41.52</strong></td>
<td>*124.71±22.06</td>
</tr>
<tr>
<td>Cl</td>
<td>**Control</td>
<td>0.28±0.02</td>
<td>0.09±0.03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Damaged</td>
<td>0.45±0.05</td>
<td><strong>0.87±0.30</strong></td>
<td><strong>1.13±0.68</strong></td>
</tr>
</tbody>
</table>

Table 2. The soil pH and concentration of soil chemical elements of A and B stratum at the control and damaged site. (*P<0.05, **P<0.01, ***P<0.001) (Unit of C and N = mg·100mg⁻¹; Ca, Mg, K and Mn = mg·100g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Damaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>A 5.03±2.20 B 5.25±2.36</td>
<td>A 4.69±0.17 B 4.64±0.07</td>
</tr>
<tr>
<td>N</td>
<td>A 0.14±0.04 B 0.06±0.02 *</td>
<td>A 0.24±0.12 B 0.12±0.03</td>
</tr>
<tr>
<td>Ca</td>
<td>A 5.39±0.64 B 2.78±0.48</td>
<td>A 6.10±1.84 B 2.89±1.18</td>
</tr>
<tr>
<td>Mg</td>
<td>A 6.20±0.51 *** B 3.84±0.49 *</td>
<td>A 3.73±0.15 B 2.71±0.38</td>
</tr>
<tr>
<td>K</td>
<td>A 40.20±8.25 ** B 17.47±3.47</td>
<td>A 17.40±4.73 B 24.55±7.27</td>
</tr>
<tr>
<td>P</td>
<td>A 2.42±0.25 * B 2.35±0.21 ***</td>
<td>A 1.29±0.88 B 0.20±0.11</td>
</tr>
<tr>
<td>Al</td>
<td>A 20.45±0.76 B 19.74±2.81</td>
<td>A 18.69±1.25 B 20.65±1.26</td>
</tr>
<tr>
<td>Mn</td>
<td>A 3.16±0.66 ** B 1.59±0.44 ***</td>
<td>A 10.56±3.86 B 13.29±1.55</td>
</tr>
</tbody>
</table>
Table 3. Concentration of elements in every age of needles of *P. koraiensis* and *P. rigida* (Unit = mg·g⁻¹ and μg·g⁻¹ *P<0.05, **P<0.01, ***P<0.001) (C: control site, D: Damaged site) (-: No needle was collected).

<table>
<thead>
<tr>
<th>Needle age</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td><em>P. koraiensis</em></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (mg·g⁻¹)</td>
<td>C</td>
<td>2.93±0.83</td>
<td>3.55±0.89</td>
<td>3.28±0.27**</td>
<td>2.14±0.37</td>
<td>3.00±0.71</td>
<td>4.09±1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3.21±0.27</td>
<td>5.20±0.33</td>
<td>7.05±0.98</td>
<td>1.73±0.10</td>
<td>3.03±0.26</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mg (mg·g⁻¹)</td>
<td>C</td>
<td>1.03±0.13</td>
<td>1.07±0.16</td>
<td>1.05±0.10*</td>
<td>1.16±0.13***</td>
<td>1.02±0.01**</td>
<td>1.12±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.88±0.05</td>
<td>0.82±0.10</td>
<td>0.81±0.09</td>
<td>0.61±0.04</td>
<td>0.63±0.10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>K (mg·g⁻¹)</td>
<td>C</td>
<td>5.49±0.52</td>
<td>4.92±0.39*</td>
<td>5.25±1.41*</td>
<td>4.36±0.26*</td>
<td>3.75±0.51**</td>
<td>3.45±0.95</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>4.32±1.01</td>
<td>3.83±0.47</td>
<td>3.08±0.49</td>
<td>3.44±0.42</td>
<td>1.67±0.22</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P (mg·g⁻¹)</td>
<td>C</td>
<td>3.22±0.46</td>
<td>2.61±0.38</td>
<td>2.61±0.19**</td>
<td>2.44±0.28**</td>
<td>2.36±0.33**</td>
<td>2.08±0.33</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>2.63±0.19</td>
<td>2.28±0.39</td>
<td>1.74±0.10</td>
<td>1.83±0.09</td>
<td>1.49±0.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mn (mg·g⁻¹)</td>
<td>C</td>
<td>0.38±0.11*</td>
<td>0.38±0.17*</td>
<td>0.44±0.14**</td>
<td>0.34±0.06</td>
<td>0.62±0.16</td>
<td>0.73±0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1.98±0.69</td>
<td>2.55±0.93</td>
<td>2.24±0.71</td>
<td>0.44±0.04</td>
<td>0.91±0.20</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cl (μg·g⁻¹)</td>
<td>C</td>
<td>0.57±0.35**</td>
<td>0.56±0.20***</td>
<td>0.70±0.25***</td>
<td>0.85±0.36*</td>
<td>0.71±0.18**</td>
<td>0.83±0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1.43±0.30</td>
<td>2.39±0.36</td>
<td>3.84±0.92</td>
<td>1.77±0.42</td>
<td>3.26±0.86</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F (μg·g⁻¹)</td>
<td>C</td>
<td>13.60±5.91**</td>
<td>20.17±15.86**</td>
<td>20.58±1.46**</td>
<td>11.26±1.86***</td>
<td>24.13±1.75**</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>31.55±2.01</td>
<td>72.97±11.23</td>
<td>94.33±21.80</td>
<td>37.81±4.05</td>
<td>102.65±24.40</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Parameter estimates as functions of the A/C_i curves and light curves. P_{sat} is light-saturated net photosynthesis (μmol·m^{-2}·s^{-1}), Φ is apparent quantum yield (μmolCO_2·m^{-2}·Pa^{-1}·CO_2·s^{-1}), CE is carboxylation efficiency (μmol·m^{-2}·s^{-1}/Pa), and RuBP regeneration is the rate of RuBP regeneration (μmol·m^{-2}·s^{-1}). (*P<0.05, **P<0.01, ***P<0.001)

<table>
<thead>
<tr>
<th></th>
<th>P_{sat}</th>
<th>Φ</th>
<th>CE</th>
<th>RuBP regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. koraiensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.97±0.16 ***</td>
<td>0.019±0.001 **</td>
<td>0.20±0.0008</td>
<td>35.91±5.00</td>
</tr>
<tr>
<td>Damaged</td>
<td>2.55±0.05</td>
<td>0.012±0.0008</td>
<td>0.19±0.01</td>
<td>23.71±5.30</td>
</tr>
<tr>
<td><em>P. rigida</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.29±0.36 **</td>
<td>0.011±0.0005 **</td>
<td>0.14±0.004 ***</td>
<td>37.16±2.90</td>
</tr>
<tr>
<td>Damaged</td>
<td>1.38±0.51</td>
<td>0.007±0.0005</td>
<td>0.06±0.001</td>
<td>32.34±0.01</td>
</tr>
</tbody>
</table>
Figure 1. The concentration of F and Cl in the precipitation from April to November 2003 at the control and damaged site.
Figure 2. Example of concentration of F and Cl in the soil water at the control and damaged site.
Figure 3. 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*<0.05, **P*<0.01, ***P*<0.001.
Figure 4. 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.
Figure 5. 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.
Figure 6. Photosynthetic light response curves of 1-year-old needles at the control and damaged site for *P. koraiensis* and *P. rigida*. 
Figure 7. The CO$_2$ assimilation (A) response curve to intercellular CO$_2$ concentration ($C_i$) of current year needles at the control and damaged sites for _P. koraiensis_ and _P. rigida_.

- _P. koraiensis_
- _P. rigida_

- **Control**
- **Damaged**
Figure 8. The relationship between concentration of Mn, F or Cl in needle and photosynthetic rate at light saturation (P<sub>sat</sub>) of <i>P. koraiensis</i> and <i>P. rigida</i> at the control and damaged sites. Circle mark is control site and triangle mark is damaged site, and open symbols show <i>P. koraiensis</i> (<i>P.k</i>) and closed ones mean <i>P. rigida</i> (<i>P.r</i>).

Regression lines:

- **P. koraiensis (P.k):**
  - Mn: y = -0.7405x + 3.430, R<sup>2</sup> = 0.568
  - Cl: y = -0.7482x + 3.608, R<sup>2</sup> = 0.783
  - F: y = -0.0272x + 3.561, R<sup>2</sup> = 0.555

- **P. rigida (P.r):**
  - Mn: y = -2.5124x + 3.534, R<sup>2</sup> = 0.353
  - Cl: y = -0.9702x + 3.676, R<sup>2</sup> = 0.808
  - F: y = -0.0417x + 3.342, R<sup>2</sup> = 0.688

<Regression lines>