Full title: Comparison of growth characteristics and tolerance to serpentine soil of three ectomycorrhizal spruce seedlings in northern Japan

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Abstract

Picea glehnii is distributed widely on serpentine soils in northern Japan. Serpentine soil is characterised by the presence of heavy metals (Ni, Cr) and excessive Mg; these elements often suppress plant growth. We have examined the tolerance to serpentine soil and its effects on growth of *P. glehnii*, *P. jezoensis* (distributed in the same region) and *P. abies* (planted for timber production).

The dry mass of each organ was not reduced in *P. glehnii* planted in serpentine soil contained nursery (serpentine nursery). In contrast, growth of *P. jezoensis* and *P. abies* was suppressed. Concentrations of Ni and Mg in needles and roots of *P. glehnii* planted in serpentine nursery were the lowest of the three species. Moreover, the photosynthetic rate of *P. glehnii* planted in the serpentine nursery was not reduced. *P. glehnii* has high capability to maintain low concentration of Ni, and ectomycorrhizal symbiosis may have a positive effect to excluding Ni. As a result, *P. glehnii* has a high tolerance against Ni toxicity, and its photosynthetic capacity is not suppressed by accumulation of Ni.

Key words: spruce, serpentine soil, photosynthetic capacity, ectomycorrhiza, nutrient physiology.

Introduction

In many parts of the world, serpentinite, an ultramafic rock, is outcropped, and particular flora is distributed in such regions (Brooks 1987; Roberts and Proctor 1991). Serpentine soil deriving from weathering of serpentinite is characterised by an excess of elements such as Ni, Cr, and Mg, a low Ca/Mg ratio, and low levels of several essential plant nutrients (Proctor 1971; Brooks 1987). Plant species grown on serpentine soil require high tolerance against the toxic metals in this soil (Brooks 1987; Roberts and Proctor 1991). The tolerance mechanisms of plants that adapt to high concentrations of toxic metals in soils generally involve either restricting metal uptake and translocation (exclusion) or accumulating the metal in a non-toxic form (accumulation) (Baker 1981; 1987). Some plants can survive in serpentine soil by symbiosis with microbes (Panaccione et al. 2001; Wardle 2002; Moser et al. 2005).

Ectomycorrhizal symbiosis might be important in excluding toxic metals. Differing ectomycorrhizal species are in symbiosis with woody species on serpentine and non-serpentine soil (Panaccione et al. 2001), and endemic fungus species also exist on serpentine soil (Maas and Stuntz 1969; Moser et al. 2005). Ectomycorrhizal species in serpentine soil have the capacity to protect against toxic metals (Panaccione et al. 2001). Ectomycorrhizal fungi can exclude toxic metals by (1) binding to the hyphal sheath, (2) reducing apoplastic mobility as a result of hydrophobicity of fungal sheath,

(3) chelating by organic acids, and (4) binding to the external mycelium (Gadd 1993; Jentschke and Goldbold 2000). When ectomycorrhizae are inoculated into the roots of woody species, the concentration of Ni in the leaves decreases, and root growth is accelerated despite the high concentration of Ni (Dixon 1988; Dixon and Buschena 1988; Jones and Hutchinson 1986; 1988a; b; Jones et al. 1988; Wilkins 1991).

Many plant species that have no tolerance against serpentine soil suffer from toxicity. Excess Ni has a negative effect on plasma membrane polarisation, ion uptake and translocation, cell mitotic activity, and carbon partitioning in roots (Lee et al. 1978, Cocucci and Morgutti 1986; Gabbrielli et al. 1990; Yang et al. 1996). Consequently, root growth is inhibited by the increased uptake of Ni (Dixon and Buschena 1988; Jones and Hutchinson 1986; 1988a; Jones et al. 1988; Yang et al. 1996; Tilstone and Macnair 1997; Miller and Cumming 2000). Moreover, when Ni is transported to leaves, Ni is accumulated into the chloroplasts (L'Huillier 1996; Molas 2002), and photosynthetic capacity and concentration of chlorophyll are decreased (Carlson 1975; Rauser and Dumbroff 1981; Morgutti et al. 1984; Jones and Hutchinson 1988a; Miller and Cumming 2000; Molas 2002). A high Mg concentration may lead to substitution of extra-cellular Ca by Mg via mass action, altering cell wall stability and plasma membrane permeability (Marschner 1995). Root growth and uptake of Ca are inhibited by excess Mg (Shimada 1972; Ushijima et al. 2004; Kobayashi et al. 2005). Moreover, excess Mg is accumulated in mitochondria of roots, and activities of various enzymes are inhibited (Shimada 1972).

On serpentine soil in northern Japan, the Sakhalin spruce (*Picea glehnii* Masters) is dominant (Tatewaki 1958; Nakata and Kojima 1987; Matsuda 1989). *P. glehnii* can grow in various infertile environments (Tatewaki 1958; Matsuda 1989). Moreover, Yezo spruce (*P. jezoensis* Carr.) is a dominant coniferous species in the mesic region of northern Japan (Miyawaki 1988). In addition, The Norway spruce (*P. abies* (L.) Karst.) was introduced from southwestern Germany in the early 1900s for timber production, and is well adapted to mesic sites in northern Japan (Kubota and Fukuchi 1981). *P. jezoensis* and *P. abies* are usually grown on fertile soils (Miyawaki 1988; Nikolov and Helmisaari 1992), and are rarely found on serpentine soils (Brooks 1987; Nakata and Kojima 1987).

In *P. glehnii* grown in serpentine soil, the concentration of Ni in needles is lower than in other species (Blandon et al. 1994; Kayama et al. 2002). It follows that *P. glehnii* acts as a Ni excluder (Kayama et al. 2005). The total dry mass of *P. glehnii* is almost the same on serpentine and non-serpentine soil. Moreover, the concentration of Ni in needles and roots of *P. glehnii* was lower than in the other two species, and ectomycorrhizal symbiosis may help to exclude Ni. In contrast, growth of *P. jezoensis* and *P. abies* on serpentine soil was significantly less than in non-serpentine soil. Ni accumulated in needles and roots of *P. jezoensis* and *P. abies* on serpentine soil, so that these spruces may suffer from Ni toxicity. However, serpentine soil is characterized not only by excess accumulation of Mg and Ni but also by deficiency of nutrients, so that spruces grown on serpentine soil showed deficiencies of various nutrients. Moreover, the concentration of nitrogen in needles, which is positively correlated with the photosynthetic capacity (Field and Mooney 1986, Lambers et al. 1998), was lower for the three spruce species on serpentine soil than on non-serpentine soil. We therefore were unable to determine whether the suppression of growth of *P. jezoensis* and *P. abies* was due to toxicity of Ni and Mg or inadequacy of nutrients.

We hypothesize that *P. glehnii* grown on serpentine soil has a high capability to inhibit uptake of Ni and Mg, so that it is able to maintain a high photosynthetic capacity without adverse toxic effects of Mg and Ni. Here, we analysed if mineral uptake or metal resistance of *P. glehnii* differed from those of *P. jezoensis* and *P. abies*, and could these factors explain its better performance on serpentine soils. Our results indicate that both factors are important and together make *P. glehnii* a superior spruce species for serpentine soil plantations.

Materials and Methods

Study site

The experimental site was located in the Nukanan experimental nursery of Teshio

Experimental Forest (TEF) maintained by Hokkaido University (N44°55', E142°00', 16m a.s.l.). The mean annual precipitation from 1996 to 2000 was 1062 mm yr^{-1} (Takagi et al. 2001). The annual mean, maximum and minimum temperatures were respectively 5.3 °C, 26.1 °C and –21.8 °C from 1996 to 2000, as measured by a thermo recorder at the Toikanbetsu meteorological station (Takagi et al. 2001). The experimental site was approximately 1.5 km from the meteorological station.

Experimental nurseries

We prepared eight experimental nurseries, four on serpentine conditions and four on non-serpentine (i.e., control) conditions. Based on the FAO-UNESCO system, the control soil was classified as Cambisol (Teshio, unpublished data). To establish the nursery containing serpentine soil, we collected 3000 kg of serpentine soil using a power shovel from a serpentine region in TEF (N45°05', E142°06', 100m a.s.l.) and transported it to the nursery using a dump truck. The size of each nursery was 2 × 10 m. We selected four nurseries at random and added serpentine soil to them to comprise 330g of serpentine soil per 1 kg of Cambisol. After mixing, the soils in each experimental nursery were cultivated using a tractor to reduce soil heterogeneity, and eight nurseries were prepared. Each nursery was separated by approximately 5 m to prevent soil cross-contamination.

Plant materials

We studied the *Picea glehnii* Masters, *P. jezoensis* Carr., and *P. abies* (L.) Karst. Preliminary research indicates that needle longevity is about two years for seedlings of the three spruces grown in fertile nurseries, and suppression of growth of seedlings on serpentine soil became apparent from the second year (Kayama et al. 2005). The experiment had therefore to last at least two years.

Seeds of *P. glehnii* Masters and *P. jezoensis* Carr. were selected from a similar habitat in the central part of Hokkaido, governed by the National Forestry Research Institute, where the soil was non-serpentine. Second generation seeds of *P. abies* have now been produced in central Hokkaido. We used four-year-old seedlings of *P. glehnii* and *P. jezoensis*, and two-year-old *P. abies* seedlings, because we could not obtain seedlings of the species of equal age. In May 1999, 128 seedlings of each spruce species were removed from the nursery and transported to the Nukanan experimental nurseries of TEF.

During transportation, the roots of each seedling were protected by moist paper towels (Kimtowel, Crecia Co., Tokyo, Japan). 16 seedlings of each spruce species were planted on each of the four nurseries of serpentine and control. After planting, each plot was weeded periodically. The water status was monitored by time domain reflectometry (TRIME-FM, IMKO Micromodultechnik GmbH, Ettlingen, Germany). The water content at field capacity of serpentine and brown forest soils was 48 %, and water was supplied if the water content fell below 35 %.

Analysis of soil chemistry

We measured soil properties including pH and concentrations of carbon, nitrogen, exchangeable phosphorus, base cations, and heavy metals. The soils in each experimental plot were sampled at 3 cm and 15 cm depth every month in each nursery to measure the soil pH and concentrations of elements. There was almost no variation with time during the experimental period. We analysed the soil chemistry in detail in Oct. 2000, and four soil samples at depths of 3 and 15 cm were collected from four plots of serpentine and four from control nurseries. We also analysed the serpentine soil that was added to the nurseries; four samples were collected at 3 cm depth from the excavation site in Oct. 2000.

To determine soil pH, 25 ml of distilled water was added to 10 g fresh soil to make a homogenized mixture (Van Reeuwijk 1993). This mixture was then shaken for 1 h and the soil pH was measured using a pH meter (HM30G, DKK-TOA Co., Tokyo, Japan). Prior to chemical analysis, soil samples were oven dried at 105 °C for 24 h. The carbon and nitrogen content of the dried soils were determined using a NC analyser

(Sumigraph NC-800, Sumika Chemical Analysis Service, Tokyo, Japan; Japanese Society of Soil Science and Plant Nutrition 1997). Exchangeable phosphorus was separated using dilute acid fluoride (Kuo 1996), shaking for 1 minute. Exchangeable base cations (Ca, Mg, K, Na) were quantified by mixing 2.5 g of dry soil with 50 ml of 1 N ammonium acetate solution, shaking for 1 h (Thomas 1982). Nickel was determined by the DTPA method (Baker and Amacher 1982), and chromium by the nitric acid method (Reisenauer 1982). Phosphorus, base cations, and heavy metals in the extracted solutions were analysed by an inductivity coupled plasma (ICP) analyser (IRIS, Jarrel ash, Franklin, MA, USA; Thompson and Walsh 1989). We also analysed a standard solution of elements between every 40 samples, and corrected the data accordingly.

Measurement of seedling growth

To determine the growth characteristics of seedlings of the three spruce species, we measured the dry mass of needles, stems and branches, and roots. Four seedlings of each spruce species were harvested from four plots of the two types of nursery in May 1999, May 2000, and Apr. 2001 (initial, 13th, and 24th month harvest). The roots of the harvested seedlings were carefully washed three times with water to remove soil, and then with distilled water by an ultrasonic washer (US-2A, As One Co., Osaka, Japan) for 15 minutes. The washed seedlings were divided into shoot (organs above ground) and root components, and shoots were divided into components by age. Each component was put into its own envelope and was oven-dried at 80 °C for four days. The dry masses of the various components were then determined.

To estimate needle longevity, the survival of needles (SN) was calculated at the 24th month harvest. The total needle dry mass and mass per needle were determined by weighing for needles of each age at the three harvest periods, and the SN was calculated as follows (Kayama et al. 2005):

$$SN = (FTNM / ITNM) \times (INM / FNM) \times 100,$$

where FTNM is the total needle dry mass in the final period, ITNM is the total needle dry mass in the initial period, INM is the dry mass per needle in the initial period, and FNM is the dry mass per needle in the final period.

Measurement of photosynthetic rate

We measured the photosynthetic rate of two-year-old needles taken from the sunny crown of seedlings, since two years are needed for new needles to reach their maximum photosynthetic rate (Hom and Oechel 1983). Three individuals of three species of spruce planted on four plots of the two nursery types were used to measure the photosynthetic rate in August 2000. These measurements were conducted using a portable gas analyzer (H4A, ADC-Analytical Development Company, U.K.) under steady-state conditions, an ambient temperature of 23-26 °C, and ambient CO_2 concentration of 35.5-36.0 Pa. Supplementary light was provided by a halogen lamp (WALZ, Effeltrich, Germany). We changed the photosynthetic photon flux density (PPFD) from high to low using shade cloths (Krary, Osaka, Japan). After measuring the photosynthetic rate, we measured the needle-projected area using the image scanner (FB636U, Canon, Japan), and calculated the net photosynthetic rate per unit area. From the photosynthetic data, light-dependent photosynthesis curves were calculated as follows (Thornley 1976):

$$Pn = Pmax\ [1\text{-}e^{-(\alpha I / Pmax)}] - R,$$

where Pn is the net photosynthetic rate, Pmax is the maximum photosynthetic rate at light saturation, α is the initial gradient of the curve, I is the PPFD, and R is the respiration rate at 0 μmol $m^{-2}s^{-1}$ PPFD. After measurement of the needle-projected area, the needles were put into their own envelopes and oven-dried at 80 °C for four days.

Measurement of the rate of symbiosis with ectomycorrhizae

To measure the extent of ectomycorrhizal symbiosis, we selected ten lateral roots for each seedling at random; over 500 short roots (viz. < 5 cm in length) diverging from these were observed. The percentage of ectomycorrhizal symbiosis was determined before drying for sixteen seedlings of the three spruce species grown in the two types of nursery. For assessment of ectomycorrhizae, roots of seedlings were harvested in the initial and 24th months, and were carefully washed free of soils under gently flowing water. The root systems were observed under a stereomicroscope, and the numbers of symbiotic and non-symbiotic short roots were counted (Quoreshi and Timmers 1998). The percentage of short roots made a symbiosis with ectomycorrhizae was then calculated.

Analysis of nutrition in plants

We determined the concentrations of N, P, K, Ca, Mg, and Ni in two-year-old needles and roots. We also analysed these elements in two-year-old needles for which the photosynthetic rate had been measured. Two-year-old needles were used, by which time their maximum physiological activity had been reached (Hom and Oechel 1983). The dried samples were ground to a fine powder using a sample mill (WB-1, Osaka Chemical Co., Osaka, Japan). The concentration of N was determined using a NC analyser. To determine the concentrations of the other elements (P, K, Ca, Mg, and Ni), dried samples were digested by the HNO_3-HCl-H_2O_2 method (Goto 1990) and analysed using ICP (IRIS, Jarrel ash, Franklin, MA, USA). We also analysed standard solutions of each element between every 40 samples to verify the reliability of the analysis. Mg and Ni were also analysed in part of the roots at the 24th month harvest.

Roots of seedlings of the three spruce species planted on serpentine soil were separated using a sieve of mesh diameter 1.0 mm, and categorized into thin roots (< 1.0 mm diameter) and thick roots (1.0 > mm diameter) at the 24th month harvest. Thin and thick roots were digested separately, and were analysed for N, P, K, Ca, Mg, and Ni.

Statistical analysis

Stat View 5.0 (SAS Institute Inc.) was used for statistical analysis of all parameters. The mean dry mass of each organ, survival of needles, percentage made a symbiosis with ectomycorrhizae, and concentrations of elements in needles and roots, were all examined using t-tests. The mean values for the three spruce species were compared between the serpentine and brown forest soils. In the analysis of soil chemical properties, depth and soil type were tested by repeated measures of ANOVA. The symbols *, **, and *** indicate a statistical significance of $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

The mean values of the concentrations of Mg and Ni in needles and roots grown on serpentine nursery were examined using a Tukey test. From Fig. 5, the mean values for the two types of roots of the three spruces were compared. Different letters of the alphabet, such as a, b, and c, indicate a statistical significance of $P<0.05$.

We also ran simple regression analyses using Stat View 5.0 to estimate the relations between physiological parameters. In Fig. 6, we examine the relation between the concentration of Ni and the photosynthetic rate at light saturation for each species. We also examined ANCOVA among the regression lines of three spruce species. The symbol *** indicates a statistical relationship of a regression line at $P<0.001$.

All mean values of the same type across the four nurseries were examined by a single ANOVA; no significant differences were found between the four nurseries.

Results

Soil chemical properties

Table 1 lists the chemical properties of the soils of two nurseries. Soils from serpentine nurseries had a higher pH (6.4) than controls (5.8, $P<0.001$). Concentrations of Mg and Ni were higher in soils from serpentine nurseries than those in control ones ($P<0.001$). In contrast, the concentrations of Na were higher in soils from control nurseries than those in serpentine ones ($P<0.05$). Other nutrients, such as N, P, K and Ca, did not differ significantly between soils from serpentine and control nurseries. The concentration of K was significantly higher at 15 cm depth than at 3 cm ($P<0.01$).

In the original serpentine soil, the concentration of Mg was about 70 mg $100g^{-1}$

higher, and Ni were 5.4 mg $100g^{-1}$ higher than those of soils from serpentine nurseries. By contrast, the concentrations of many nutrients were low compare with soils from two types of nurseries.

Growth characteristics

The dry masses of each organ of *P. glehnii* did not differ significantly between the two nurseries in any month (Fig. 1). In contrast, the dry masses of each organ of *P. abies* in serpentine nurseries were smaller than in control nurseries until the 13th month ($P<0.05$). For *P. jezoensis*, the needle and root dry mass in serpentine nurseries were smaller than in control nurseries after the 13th month ($P<0.05$). At the 24th month, the dry masses of each organ of *P. jezoensis* in serpentine nurseries were smaller than in control nurseries ($P<0.05$). In particular, the needle dry mass of *P. jezoensis* in serpentine nurseries did not increase, even by the 24th month.

Survival of needles (SN) of the three spruce species declined with needle aging (Fig. 2). The SN for *P. glehnii* in serpentine nurseries was higher for two-year-old needles than on brown forest soil ($P<0.05$). In contrast, the SN for *P. jezoensis* and *P. abies* in serpentine nurseries was lower than in control nurseries for two-year-old needles ($P<0.05$). The SN for *P. jezoensis* in serpentine nurseries decreased drastically; only 16% of two-year-old needles remained.

Photosynthetic capacity

The photosynthetic rate (Pn) of 2-year-old needles of *P. glehnii* and *P. jezoensis* saturated at approximately 1500 μmol $m^{-2}s^{-1}$ PPFD in each type of nursery (Fig. 5). For *P. abies*, Pn in serpentine nurseries saturated at around 1500 μmol $m^{-2}s^{-1}$ PPFD, but in control nursery saturation occurred at only around 1000 μmol $m^{-2}s^{-1}$ PPFD.

Compared with the Pn value at light saturation, Pn in serpentine nurseries was 1.6 μmol $m^{-2}s^{-1}$ lower for *P. jezoensis*, and 0.7 μmol $m^{-2}s^{-1}$ lower for *P. abies*, than in control nurseries (P<0.01). In contrast, the Pn of *P. glehnii* at high PPFD was 2.6 μmol $m^{-2}s^{-1}$ in each type of nursery, with no significant difference. Moreover, the initial gradient of the light photosynthetic curve for *P. abies* and *P. glehnii* in serpentine nurseries was lower than that at the healthy site.

The percentage of ectomycorrhizal symbiosis

At the initial harvest, the percentages of ectomycorrhizal symbiosis with roots of *P. glehnii*, *P. jezoensis*, and *P. abies* were respectively 43.5%, 44.4%, and 40.3%. Ectomycorrhizal development for the three spruces did not change by the 13th month harvest (data not shown). By the 24th month, the percentages for the three spruces had increased to values varying from 75 to 81% (Fig. 4). In a comparison between serpentine and control nurseries, the percentage of ectomycorrhizal symbiosis percentage of *P. glehnii* and *P. jezoensis* did not differ significantly. In contrast, the percentage of ectomycorrhizal symbiosis for *P. abies* in serpentine nurseries was significantly lower than in control nurseries ($P<0.01$).

Element concentrations

The concentrations of N, P, and Ca in needles of the three spruces did not differ significantly between the two types of nursery (Table 2). The concentration of K was significantly reduced for *P. jezoensis* in serpentine nurseries from 13th months, whereas *P. glehnii* maintained its significantly high concentration ($P<0.05$). In contrast, the concentrations of Mg and Ni in needles of the three spruces were significantly higher in serpentine nurseries than in control nurseries from 13th months ($P<0.05$). However, concentrations of these elements for *P. glehnii* needles in the 24th month were no significantly difference between two types of nursery. Compared with three spruces within serpentine nursery, concentration of Ni in 24th month was significantly higher for *P. abies* than that for *P. glehnii* and *P. jezoensis* ($P<0.05$), whereas concentration of Mg was no significantly difference between the three spruces. The concentrations of elements in needles sampled after measurement of the photosynthetic rate were no significantly difference those in the 24th month (data not shown).

Concentrations of N and P in roots of *P. glehnii* were higher in serpentine nurseries than in controls at the 13th month (Table 3, $P<0.05$). In contrast, concentrations of P, K, and Ca in roots of *P. abies* were significantly lower in serpentine nurseries than in control nurseries at the 13th month ($P<0.05$). Concentration of K in roots of *P. jezoensis* was significantly lower in serpentine nurseries than in control nurseries from the 13th month ($P<0.05$).

Concentrations of Mg and Ni in roots of the three spruces were significantly higher in serpentine nurseries than in control nurseries at the 24th month (Table 3, $P<0.05$), and these elements were significantly higher in thin roots than in thick roots (Fig. 5, $P<0.01$). In particular, the Ni concentration in thin roots of *P. glehnii* grown in serpentine nurseries was 2.0 μmol g^{-1} DW, the lowest value among the three spruces ($P<0.05$).

To verify the effect of Ni on photosynthetic capacity, we examined the relation between photosynthetic rate and concentration of Ni in needles (Fig. 6). There was a negative correlation between concentrations of Ni and Psat for *P. jezoensis* and *P. abies* ($P<0.001$), but no significant relation for *P. glehnii*. Regression lines for the three spruces showed significant differences between concentrations of Ni and Psat ($P<0.01$).

Discussion

We found that the growth of seedlings of *P. glehnii* in serpentine nurseries was not suppressed by the serpentine soil (Fig. 1). The concentration of Ni in needles of *P. glehnii* in serpentine nurseries was less than in *P. abies* (Table 2). Moreover, the extent of ectomycorrhizal colonization of *P. glehnii* did not differ significantly between the two types of nursery (Fig. 4). In general, ectomycorrhizal fungi can bind toxic metals to the hyphal sheath (Gadd 1993; Jentschke and Goldbold 2000). Concentration of Ni and Mg for *P. glehnii* in serpentine nursery was higher in thin roots than that in thick ones (Fig. 5). Thin roots contained hyphal sheath of ectomycorrhizal fungi. Therefore, *P. glehnii* in serpentine nurseries probably bind Ni and Mg to hyphal sheath of ectomycorrhizae, and uptake of these metals is suppressed. Furthermore, the photosynthetic capacity of *P. glehnii* in serpentine nurseries was almost the same rate between two nurseries (Fig. 2). *P. glehnii* in serpentine nurseries therefore can obtain high photosynthetic capacity by suppression of uptake of Ni.

Also, the concentration of Ni and Mg in thin roots of *P. glehnii* in serpentine soil was the lowest among the three spruces (Fig. 5). Other mechanisms of excluding toxic metal for ectomycorrhizae are (1) reducing apoplastic mobility as a result of hydrophobicity of fungal sheath, (2) chelating by organic acids, and (3) binding to the external mycelium (Jentschke and Goldbold 2000). It seems that ectomycorrhizae making a symbiosis with roots of *P. glehnii* may have high excluding capacity of Ni; as a result, Ni in thin roots was the lowest among the three spruces. However, the percentage of ectomycorrhizal symbiosis at the 13th month harvest was about 44 % for roots of *P. glehnii* in serpentine nursery. It appears that roots with no ectomycorrhizal association may have absorbed Mg and Ni, so that concentrations of Mg and Ni in needles and roots of *P. glehnii* were increased at the 13th month harvest. Similar trend was verified that the total content of Mg and Ni in *P. glehnii* increased drastically at the harvest when ectomycorrhizal colonisation was poor (Kayama et al. 2005).

By contrast, growth of *P. jezoensis* and *P. abies* in serpentine nurseries was suppressed (Fig. 1) by toxicity of Ni even though the concentration of nutrients in soils from serpentine nurseries was almost the same as in control nurseries (Table 1). Moreover, the decrease in needle dry mass with aging was sharper for *P. jezoensis* and *P. abies* in serpentine nurseries than in control nurseries (Fig. 2). In general, the shoot dry mass decreased by accumulation of Ni in needles (Dixon and Buschera 1988; Millar and Cumming 2000; Ahonen-Jonnarth and Finlay 2001; Kayama et al. 2005); especially, *Pinus banksiana* were suppressed its growth by accumulation only 7 ppm plant^{-1} (0.12 μg g^{-1}) Ni in needles (Dixon and Buschera 1988). Growth of *P. jezoensis* and *P. abies* was therefore suppressed and they lost their needles due to the toxicity of Ni. In particular, two-year-old needles of *P. jezoensis* in serpentine nursery were shed dramatically (Fig. 2). Needles of *P. jezoensis* may be more sensitive to toxicity of Ni than that of other spruces.

Also, the photosynthetic rate at light saturation was reduced for *P. jezoensis* and *P. abies* grown in serpentine nurseries (Fig. 3). The toxicity of Ni reduces photosynthetic capacity (Carlson 1975; Rauser and Dumbroff 1981; Morgutti et al. 1984; Jones and Hutchinson 1988a). Our results also suggest a negative correlation between concentrations of Ni and Psat for *P. jezoensis* and *P. abies* (Fig. 6). Especially, the gradient of the regression lines between Ni and Psat was larger for *P. jezoensis* than for other spruces (Fig. 6). Consequently, photosynthetic capacity of *P. jezoensis* may be depressed by a modicum of Ni accumulation. In a comparison of woody species, the photosynthetic rate of paper birch was suppressed by a Ni concentration of 125 $\mu g\ g^{-1}$ (2.13 $\mu mol\ g^{-1}$) in needles (Jones and Hutchinson 1988a; b). We find here that suppression of the photosynthetic rate begins at a concentration of 0.3 $\mu mol\ g^{-1}$ Ni in needles in *P. jezoensis*, and at 0.6 $\mu mol\ g^{-1}$ Ni in *P. abies* (Fig. 6). We believe that depression of photosynthetic capacity probably runs in proportion to the nickel concentration in leaves at low concentrations.

Furthermore, uptake of K by *P. jezoensis* in serpentine nurseries was suppressed even when K was present in soil in high concentrations (Tables 2, 3). In general, inhibition of K uptake is a major toxic effect of Ni (Pandolfini et al. 1992; Baccouch et al. 1998), and Ni-induced K deficiency is a key consequence of serpentine soil (Millar and Cumming 2000). Therefore, *P. jezoensis*, which is sensitive to Ni toxicity, may suffer from K deficiency. K deficiency leads to reduce photosynthetic capacity (Marschner 1995), so that *P. jezoensis* in serpentine nurseries may accelerate reduction of photosynthetic capacity. *P. abies* in serpentine nurseries had reduced concentrations of P, K, and Ca in roots in the 13th month (Table 3). Toxicity of Ni reduced the uptake of several nutrients as well as K: also P and Ca (Millar & Cumming 2000). *P. abies* in serpentine nurseries absorbed large amounts of Ni from the 13th month, presumably inhibiting the uptake of P, K, and Ca from that time.

The ectomycorrhizal colonization percentage in *P. abies* was significantly lower in serpentine nurseries than in control nurseries (Fig. 4). Of the three spruces, the concentration of Ni in needles was greatest in *P. abies* (Table 2). It appears that Ni uptake and transfer to needles of *P. abies* is not suppressed due to low ectomycorrhizal colonisation. In contrast, the ectomycorrhizal colonization of *P. jezoensis* was high percentage same as *P. glehnii*, and no significantly difference between the two types of nursery (Fig. 4). However, *P. jezoensis* in serpentine nurseries accumulated large amount of Ni in roots compared with *P. glehnii* (Table 2). One of possibility is that the excluding capacity of Ni may be lower for ectomycorrhizae of *P. jezoensis* than that of *P. glehnii*. In fact, only two types of ectomycorrhizae were in symbiosis for roots of young seedlings of *P. jezoensis* grown on various habitats (Takahashi 1991). By contrast, young seedling of *P. glehnii* grown on various habitats was detected over 30 types of ectomycorrhizae (Kasuya 1995). A capacity excluding toxic metals was high when several ectomycorrhizal species are inoculated (Choi 2005). It seems that symbiosis with various type of ectomycorrhizae may have strong effects to exclude Ni for *P. glehnii*.

On effects of other elements, high concentration of Mg contained in soils of serpentine nurseries was detected in plant organ of *P. jezoensis* and *P. abies* (Table 2, 3). Concentration of Mg in roots inhibited activities of various enzymes was over 1 % $plant^{-1}$ (412 μmol g^{-1}; Shimada 1972). Concentration of Mg in thin roots of *P. jezoensis* and *P. abies* in serpentine nursery was about 230 μmol g^{-1}; therefore, effects of excess Mg for activity of enzymes in root of *P. jezoensis* and *P. abies* in serpentine are probably little. Moreover, photosynthetic capacity was decreased by accumulation over 1.2 % $plant^{-1}$ (494 μmol g^{-1}) of Mg (Rao et al. 1987). Also, concentration of Ca in leaf was decreased by excess Mg (Shimada 1972; Ushijima et al. 2004; Kobayashi et al. 2005). However, maximum concentration of Mg in needles was 188 μmol g^{-1} for *P. jezoensis* (data not shown), and concentration of Ca in needles of *P. jezoensis* and *P. abies* in serpentine nursery was not decreased (Table 2). Therefore, effects of toxicity of excess Mg for aboveorgan of *P. jezoensis* and *P. abies* are probably less than Ni.

Previously, we have compared the growth characteristics of *P. glehnii*, *P. jezoensis* and *P. abies* planted on the original serpentine soil used here (Kayama et al. 2005). The concentrations of Mg and Ni in roots were lower than in previous research. However, the concentrations of Mg and Ni in needles were almost the same there as here.

Nutrients in the soil in serpentine nurseries were clearly more abundant than in the 'pure' serpentine soil. In previous studies, when calcium was added to serpentine soil, uptake of Ni and Mg were decreased (Chiarucci et al. 1998), and concentrations of Ni and Mg in leaves also decreased (Mizuno 1979; Brooks 1987). However, the present results suggest that large amounts of Ca in soil are not correlated with reduction in the uptake of Ni and Mg. Consequently, the presence of soil nutrients in abundance may not compensate for the toxicity of serpentine soil.

Finally, we conclude that *P. glehnii* has a high capability to exclude Ni due to symbiosis with ectomycorrhizae. This symbiosis might inhibit transportation of Ni from roots to needles. Moreover, *P. glehnii* has a high tolerance against Ni toxicity, and its photosynthetic capacity is not suppressed by accumulation of Ni. Consequently, *P. glehnii* can survive in serpentine regions without suffering from toxicity of the soil.

Acknowledgements

We thank Prof. K. Sasa, Prof. F. Satoh, Dr. S. Uemura and Dr. Y. Akibayashi for valuable comments on this study. We are grateful to the technical staff of Teshio Experimental Forest, Hokkaido University for their excellent technical assistance. Thanks are due to Dr. S. Kitaoka and Ms. Y. Yanagihara for preparation of the nurseries and sampling of the seedlings. We also thank Ms. M. Ohbuchi for technical assistance with the analysis. Financial support of M.K. and T.K. by JSPS and the Japan Science Society is gratefully acknowledged.

References

Ahonen-Jonnarth U, Finlay RD (2001) Effects of elevated nickel and cadmium concentrations on growth and nutrient uptake of mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Plant Soil 236:129-138

Baccouch S, Chaoui A, El Ferjari E (1998) Nickel toxicity: effects on growth and metabolism of maize. J Plant Nutr 21:577-588

Baker AJM (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643-654

Baker AJM (1987) Metal tolerance. New Phytol 106: 93-111

Baker DE, Amacher MC (1982) Nickel, Copper, Zinc, and Cadmium. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Chemical and microbiological properties. 2 ed. Soil Science Society of America Inc., Madison, pp 323-336

Blandon DMZ, Satoh F, Matsuda K, Sasa K, Igarashi T (1994) The mineral condition of soils and tree species in serpentine and non-serpentine areas of northern Hokkaido. Res

Bull Hokkaido Univ For 51:1-13

Brooks RR (1987) Serpentine and its vegetation. Dioscorides Press, Portland

Carlson RW, Bazzaz FA, Rolfe GL (1975) The effect of heavy metals on plants II. Net photosynthesis and transpiration of whole corn and sunflower plants treated with Pb, Cd, Ni, and Ti. Environ Pollut **7**:241-246

Chiarucci A, Maccherini S, Bonini I, De Dominicis V (1998) Effects of nutrient addition on species diversity and ground cover of “serpentine” vegetation. Plant Biosys 132:143-150

Choi DS (2005) Ecophysiological study on growth of the ectomycorrhizal conifer species in Korea treated with soil acidification and elevated CO_2. Ph.D. dissertation, Hokkaido University, Sapporo, Japan.

Cocucci SM, Morgutti S (1986) Stimulation of proton extrusion by K^+ and divalent cations (Ni^{2+}, Co^{2+}, Zn^{2+}) in maize root segments. Physiol Plant 68:497-501

Dixon RK (1988) Response of ectomycorrhizal Quercus rubra to soil cadmium, nickel and lead. Soil Biol Biochem 20:555-559

Dixon RK, Buschena CA (1988) Response of ectomycorrhizal *Pinus banksiana* and *Picea glauca* to heavy metals in soil. Plant Soil 105:265-271

Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 25-54

Gabbrielli R, Pandolfini T, Vergnano O, Palandri MR (1990) Comparison of two serpentine species with different nickel tolerance strategies. Plant Soil 122:271-277

Gadd GM (1993) Tansley Review No. 47. Interactions of fungi with toxic metals. New Phytol 124:25-60

Goto S (1990) Digestion method. In: Editorial Committee of Methods for Experiments in Plant Nutrition (eds) Manual of plant nutrition. Hakuyusha, Tokyo, pp 125-128 (in Japanese)

Hom JL, Oechel WC (1983) The photosynthetic capacity, nutrient content, and nutrient use efficiency of different needle age-classes of black spruce (*Picea mariana*) found in interior Alaska. Can J For Res 13:834-839

Japanese Society of Soil Science and Plant Nutrition (1997) Manual of soil environments. Hakuyusha, Tokyo (in Japanese)

Jentschke G., Godbold DL (2000) Metal toxicity and ectomycorrhizas. Physiol Plant 109:107-116

Jones MD, Hutchinson TC (1986) The effect of mycorrhizal inflection on the response of *Betula papyrifera* to nickel and copper. New Phytol 102:429-442

Jones MD, Hutchinson TC (1988a) Nickel toxicity in mycorrhizal birch seedlings infected with *Lactarius rufus* or *Scleroderma flavidum*. I Effects on growth, photosynthesis, respiration and transpiration. New Phytol 108:451-459

Jones MD, Hutchinson TC (1988b) Nickel toxicity in mycorrhizal birch seedlings infected with *Lactarius rufus* or *Scleroderma flavidum*. II Uptake of nickel, calcium, magnesium, phosphorus and iron. New Phytol 108:461-470

Jones MD, Dainty J, Hutchinson TC (1988) The effect of infection by *Lactarius rufus* and *Scleroderma flavidum* on the uptake of ^{63}Ni by paper birth. Can J Bot 66:934-960

Kasuya MCM (1995) Ecological and physiological studies on ectomycorrhizae of *Picea glehnii* (Fr. Schm.) Masters. Ph.D. dissertation, Hokkaido University, Sapporo, Japan.

Kayama M, Sasa K, 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 AM, Uemura S, 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. Ann Bot 95:661-672

Kobayashi H, Masaoka Y, Sato S (2005) Effects of excess magnesium on the growth and mineral content of rice and *Echinochloa*. Plant Prod Sci 8:38-43

Kubota Y, Fukuchi M (1981) Genus of *Picea*. In: Asakawa S, Katsuta M, Yokoyama Y (eds) Seeds of woody plants in Japan. Japan Forest Tree Breeding Association, Tokyo, pp 42-51 (in Japanese)

Kuo S (1996) Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnson CT, Sumner ME (eds) Methods of soil analysis, Part 3. Soil Science Society of America Inc., Madison, pp 869-919

Lambers H, Chapin III FS, Pons TL (1998) Plant physiological ecology. Springer-Verlag, New York

Lee J, Reeves RD, Brooks RR, Jaffre T (1978) The relationship between nickel and citric acid in some nickel-accumulating plants. Phytochem 17:1033-1035

L'Huillier L, d'Auzac J, Durand M, Michaud-Ferrière N (1996) Nickel effects on two maize (*Zea mays*) cultivars: growth, structure, Ni concentration, and localization. Can J Bot 74:1547-1554

Maas JL, Stuntz DE (1969) Mycoecology on serpentine soil. Mycologia 61:1106-1116

Marschner H (1995) Mineral nutrition of higher plants, 2 ed. Academic Press, London

Matsuda K (1989) Regeneration and growth in the *Picea glehnii* forest. Res Bull Hokkaido Univ For 46:595-717 (in Japanese and English summary)

Miller SP, Cumming JR (2000) Effects of serpentine soil factors on Virginia pine (*Pinus virginiana*). Tree Physiol 20:1129-1135

Miyawaki A (1988) Vegetation of Japan, Hokkaido. Sibundo, Tokyo (in Japanese and English summary)

Mizuno N (1979) Studies on chemical characteristics of serpentine soils and mineral deficiencies and toxicities of crops. Rep Hokkaido Pref Agr Exp Sta 29:1-79 (in Japanese and English summary)

Molas J (2002) Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni (II) complexes.

Environ Exp Bot 47:115-126
Morgutti S, Sacchi GA, Cocucci SM (1984) Effects of Ni^{2+} on proton extrusion, dark CO_2 fixation and malate synthesis in maize roots. Physiol Plant 60:70-74
Moser AM, Petersen CA, D'Allura JA, Southworth D (2005) Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and non-serpentine soils in southwestern Oregon. Am J Bot 92:224-230
Nakata M, Kojima S (1987) Effects of serpentine substrate on vegetation and soil development with special reference to *Picea glehnii* in Teshio district, Hokkaido, Japan. For Ecol Manage 20:265-290
Nikolov N, Helmmissari H (1992) Silvics of the circumpolar boreal forest tree species. In: Shugart HH, Leemans R, Bonan GB (eds) A systems analysis of the global boreal forest. Cambridge University Press, Cambridge, pp 13-84
Quoreshi AM, Timmer VR (1998) Exponential fertilization increases nutrient uptake and ectomycorrhizal development of black spruce seedlings. Can J For Res 28:674-682
Pandolfini T, Gabrielli R, Comparini C (1992) Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. Plant Cell Environ 15:719-725
Panaccione DG, Sheets NL, Miller SP, Cumming JR (2001) Diversity of *Cenococcum geophilum* isolated from serpentine and non-serpentine soils. Mycologia 93:645-652
Proctor J (1971) The plant ecology of serpentine III. The influence of a high calcium / magnesium ratio and high nickel and chromium levels in some British and Swedish

serpentine soil. J Ecol 59:827-842

Rao M, Sharp RE, Boyer J (1987) Leaf Magnesium alters photosynthetic response to low water potentials in sunflower. Plant Physiol 84:1214-1219

Rauser WE, Dumbroff EB (1981) Effects of excess cobalt, nickel and zinc on the water relations of *Phaseolus vulgaris*. Environ Exp Bot 21:249-255

Reisenauer HM (1982) Chromium. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Chemical and microbiological properties. 2 ed. Soil Science Society of America Inc., Madison, pp 337-346

Roberts BA, Proctor J (1992) The ecology of areas with serpentinized rocks. Kluwer, Dordrecht

Shimada N (1972) Studies on the excess injury of magnesium in the crops. Trans Fac Hort Chiba Univ 6:1-105 (in Japanese and English summary)

Takagi K, Sasa K, Satoh F, Nomura M, Komiya K, Takahashi H, Hohjo H, Kaneko K, Ichikawa K, Nakajima J, Ashiya D, Ishida N, Okuda A, Naniwa A, Okamoto T (2001) Meteorological characteristics of northern Hokkaido University Forests during 1995-1999. Res Bull Hokkaido Univ For 58:29-36 (in Japanese and English summary)

Takahashi I (1991) Studies of the mycofloral succession on Yezo spruce (*Picea jezoensis* Carr.) in various stages of growth, with special reference to the role of fungi in the natural regeneration of the host. Bull Tokyo Univ For 86:201-273

Tatewaki M (1958) Forest ecology of the islands of the North Pacific Ocean. J Fac Agr

Hokkaido Univ 50:371-486

Thomas GW (1982) Exchangeable cations. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Chemical and microbiological properties. 2 ed. Soil Science Society of America Inc., Madison, pp 159-165

Thompson M, Walsh JN (1989) Handbook of inductively coupled plasma spectrometry. 2 ed. Blackie Academic & Professional, Glasgow.

Thornley JHM (1976) Mathematical models in plant physiology. Academic Press, London

Tilistone GH, Macnair MR (1997) Nickel tolerance and copper-nickel co-tolerance in *Mimulus guttatus* from copper mine and serpentine habitats. Plant Soil 191:173-180

Ushijima T, Kaneko A, Yamamoto T (2004) Magnesium excess – mediated chlorosis in leaves of cucumber grown on soils of granodiorite origin under a plastic house. J Japan Soc Hour Sci 73:476-483 (in Japanese and English summary)

Van Reeuwijk LP (1993) Procedures for soil analysis. International soil reference and information centre, Wagningen

Wardle DA (2002) Communities and ecosystems: Linkage the aboveground and belowground components. Princeton University Press, Princeton, Oxford

Wilkins DA (1991) The influence of sheathing (ecto-) mycorrhizas of trees on the uptake and toxicity of metals. Agr Ecosys Environ 35:245-260

Yang X, Baligar VC, Martens DC, Clark RB (1996) Plant tolerance to nickel toxicity. II

Nickel effects on influx and transport of mineral in four plant species. J Plant Nutr 19:265-279

Figure legends

Fig. 1. Dry mass of needle, stem and branch, and root for seedlings of three spruce species planted on two types of soil (Mean ± SD, n=16). Vertical scales on graphs of each organ are smaller for *P. abies* than for *P. glehnii* and *P. jezoensis*. *=*P*<0.05, **=*P*<0.01 and ***=*P*<0.001

Fig. 2. Survival of needles (SN) of three spruce species planted on two types of soil (Mean ± SD, n=16). *=*P*<0.05, **=*P*<0.01 and ***=*P*<0.001

Fig. 3. Ectomycorrhiza infection percentage for short root (< 5 mm in length) seedlings of three spruce species planted on two types of soil (17th and 28th months; Mean + SD, n=16). *=*P*<0.05, and **=*P*<0.01

Fig. 4. Ectomycorrhizal infection percentage for short root (< 5 mm in length) seedlings of three spruce species planted in two types of nursery (24th months; Mean + SD, n=10). **=*P*<0.01

Fig. 5. Concentrations of Mg and Ni in two classes of roots of seedlings of three spruce species planted in serpentine nursery at 24th month harvest (Mean + SD, n=10). Different letters indicate significant differences between values (*P*<0.05, Tukey test)

Fig. 6. Relation between concentration of nickel and photosynthetic rate at light saturation (Psat) for two-year-old needles of three spruce seedlings planted in serpentine and control nurseries (n=12). Asterisks show a significant relation according to the regression line (****P*<0.001).

Table 1. Chemical properties of soils from the two types of nurseries and serpentine soil. Soils were sampled at depths of 3 and 15 cm (Mean ± SD, n=16). Mean values of chemical properties were analyzed as two-factor ANOVA. The soils were dried prior to analysis. Asterisks indicate significant effects: *=*P*<0.05, **=*P*<0.01, ***=*P*<0.001.

	pH	C ($g\ 100g^{-1}$)	N ($g\ 100g^{-1}$)	P ($mg\ 100g^{-1}$)	Ca ($mg\ 100g^{-1}$)
Serpentine					
nursery	6.32±0.24	1.97±0.17	0.206±0.019	12.5±1.4	240±16
3 cm	6.38±0.32	1.96±0.12	0.211±0.017	13.0±0.7	274±27
15 cm					
Control nursery					
3 cm	5.70±0.05	2.07±0.09	0.222±0.010	12.9±0.7	285± 8
15 cm	5.88±0.09	1.98±0.11	0.206±0.008	13.0±1.7	278±24
Serpentine soil					
	7.44±0.11	0.09±0.02	0.009±0.001	0.9±0.3	4± 1
Statistical test					
Depth (D)	n.s.	n.s.	n.s.	n.s.	n.s.
Nursery type (N)	***	n.s.	n.s.	n.s.	n.s.
D × N	n.s.	n.s.	n.s.	n.s.	n.s.
	Mg ($mg\ 100g^{-1}$)	K ($mg\ 100g^{-1}$)	Na ($mg\ 100g^{-1}$)	Ni ($mg\ 100g^{-1}$)	Cr ($mg\ 100g^{-1}$)
Serpentine					
nursery	71±6	36.7±4.6	5.85±0.54	2.61±0.61	0.89±0.02
3 cm	73±7	30.4±3.2	6.54±0.60	2.23±0.53	0.95±0.13
15 cm					
Control nursery					
3 cm	45± 2	37.3±2.1	6.80±1.15	0.51±0.03	0.86±0.13
15 cm	46± 3	33.5±2.3	7.31±0.20	0.56±0.06	1.00±0.10
Serpentine soil					
	140±9	2.1±1.2	1.14±0.08	7.81±0.35	1.38±0.16
Statistical test					
Depth (D)	n.s.	**	n.s.	n.s.	n.s.
Nursery type (N)	***	n.s.	*	***	n.s.
D × N	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2. Concentrations of elements (N, P, K, Ca, Mg and Ni) in two-year-old needles for seedlings of three spruce species planted on a serpentine (S) and control (C) nurseries (Mean ±SD, n=16). Asterisks indicates significant effects by t-test *=P<0.05, **=P<0.01, ***=P<0.001.

Species Months	N (μmol g^{-1} DM)		P (μmol g^{-1} DM)		K (μmol g^{-1} DM)	
P. glehnii	S	C	S	C	S	C
0	926± 69		55±10		81±10	
13	776± 45	685± 45	34± 5	31± 8	78± 4	78± 9
24	1037±168	1097± 80	52± 7	48± 8	76±14*	53±7
P. jezoensis	S	C	S	C	S	C
0	1130± 80		62± 7		88±19	
13	873± 20	932±110	41± 3	45± 4	59±11*	86±13
24	1045±185	1208±105	55± 6	63± 6	71±10*	86± 5
P. abies	S	C	S	C	S	C
0	583± 94		60± 8		111±38	
13	1119±92	1174±31	47± 4	51± 4	55± 8	57±12
24	1026±108	1129±92	46± 9	55± 9	90±14	91±14
	Ca (μmol g^{-1} DM)		Mg (μmol g^{-1} DM)		Ni (μmol g^{-1} DM)	
P. glehnii	S	C	S	C	S	C
0	296±58		28± 4		0.08±0.03	
13	292±55	256±51	79±20*	35± 8	0.26±0.10*	0.08±0.02
24	198±19	206±20	88±58	29± 7	0.39±0.20	0.18±0.07
P. jezoensis	S	C	S	C	S	C
0	309±24		34±10		0.07±0.04	
13	227±14	257±39	83±35**	26± 5	0.25±0.10**	0.07±0.01
24	233±15	257±34	87±61*	30± 1	0.51±0.28*	0.19±0.04
P. abies	S	C	S	C	S	C
0	163±29		47±10		0.14±0.03	
13	232±17	271±35	107±21**	48± 3	0.54±0.17**	0.09±0.06
24	392±57	436±72	135±23***	48± 4	0.86±0.31**	0.28±0.05

Note. "S" means the serpentine nursery, and "C" means the control nursery.

Table 3. Concentrations of elements (N, P, K, Ca, Mg and Ni) in roots for seedlings of three spruce species planted on a serpentine (S) and control (C) nurseries (Mean ± SD, n=16). Asterisks indicate significant effects by t-test: *=P<0.05, **=P<0.01, ***=P<0.001.

Species Months	N (μmol g^{-1} DM)		P (μmol g^{-1} DM)		K (μmol g^{-1} DM)	
P. glehnii	S	C	S	C	S	C
0	535±156		49±13		72± 8	
13	679± 88*	537± 73	45±11*	30± 5	58± 6	49±14
24	703±108	707±137	56± 3	55± 9	105±16	94±11
P. jezoensis	S	C	S	C	S	C
0	575± 71		38± 6		56± 7	
13	612± 66	617± 65	40± 6	37± 3	43± 6*	52± 2
24	718±103	852±127	50± 4	57± 7	86±10*	111±12
P. abies	S	C	S	C	S	C
0	404± 21		27± 6		44± 6	
13	908±125	904± 84	27± 2***	50±10	24± 2***	37±4
24	786± 38	836± 31	64± 2***	67± 6	116±9	115±15

	Ca (μmol g^{-1} DM)		Mg (μmol g^{-1} DM)		Ni (μmol g^{-1} DM)	
P. glehnii	S	C	S	C	S	C
0	103±16		44±12		0.15±0.07	
13	100± 6	87±10	51±11*	73±19	0.41±0.10**	0.22±0.04
24	128±16	99± 5	82±32*	45± 3	0.64±0.35	0.34±0.08
P. jezoensis	S	C	S	C	S	C
0	114±17		36± 5		0.23±0.09	
13	103± 6	105± 9	77±37	54±12	0.69±0.27	0.38±0.06
24	118± 7	112± 6	102±34**	43± 3	1.12±0.34**	0.43±0.15
P. abies	S	C	S	C	S	C
0	88±13		29± 5		0.33±0.16	
13	72± 7**	92± 8	101±24**	39± 3	0.79±0.20**	0.35±0.08
24	120±20	122± 7	104±25***	45± 6	1.07±0.15**	0.57±0.13

Note. "S" means the serpentine nursery, and "C" means the control nursery. Concentrations of elements showed the average between values of thin and thick roots.

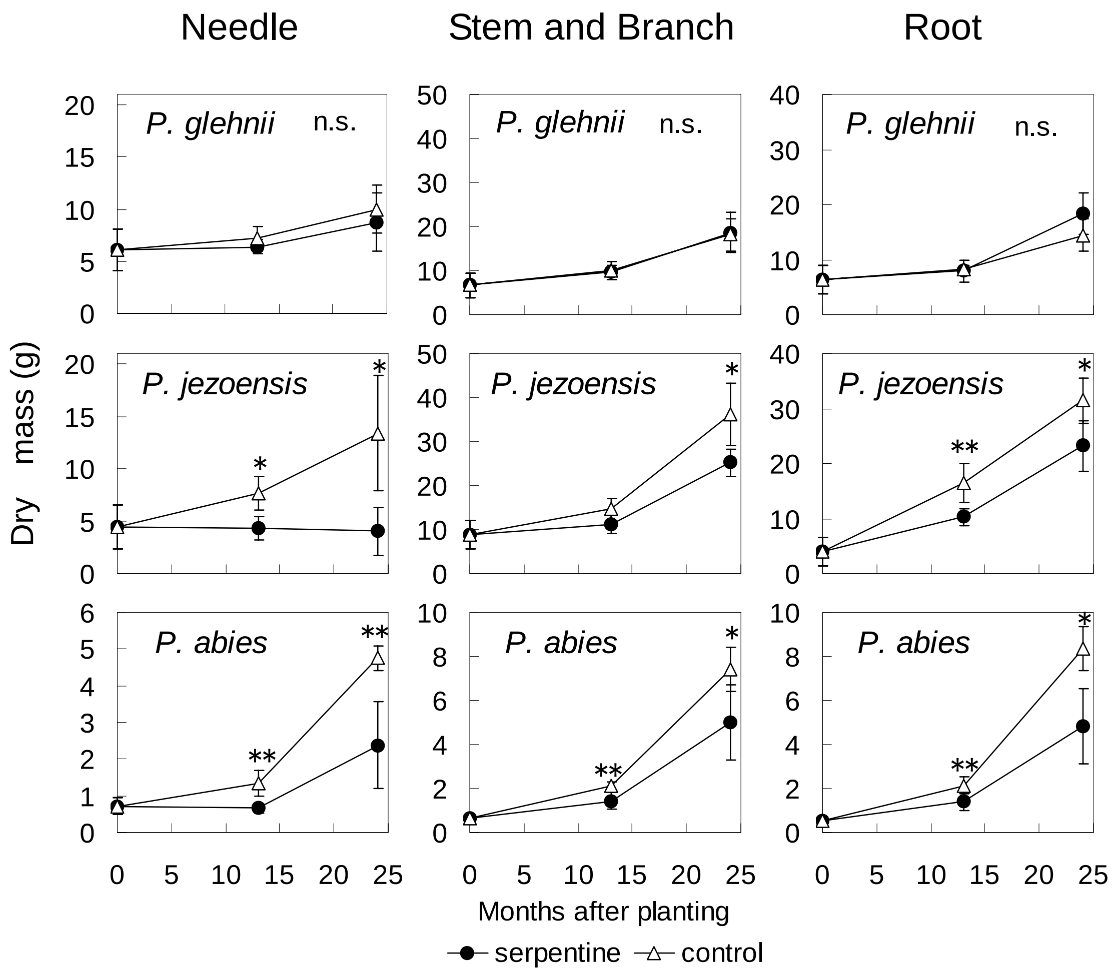

Needle
Stem and Branch
Root
P. glehnii
n.s.
P. jezoensis
P. abies
Dry mass (g)
Months after planting
serpentine
control

Fig. 1.

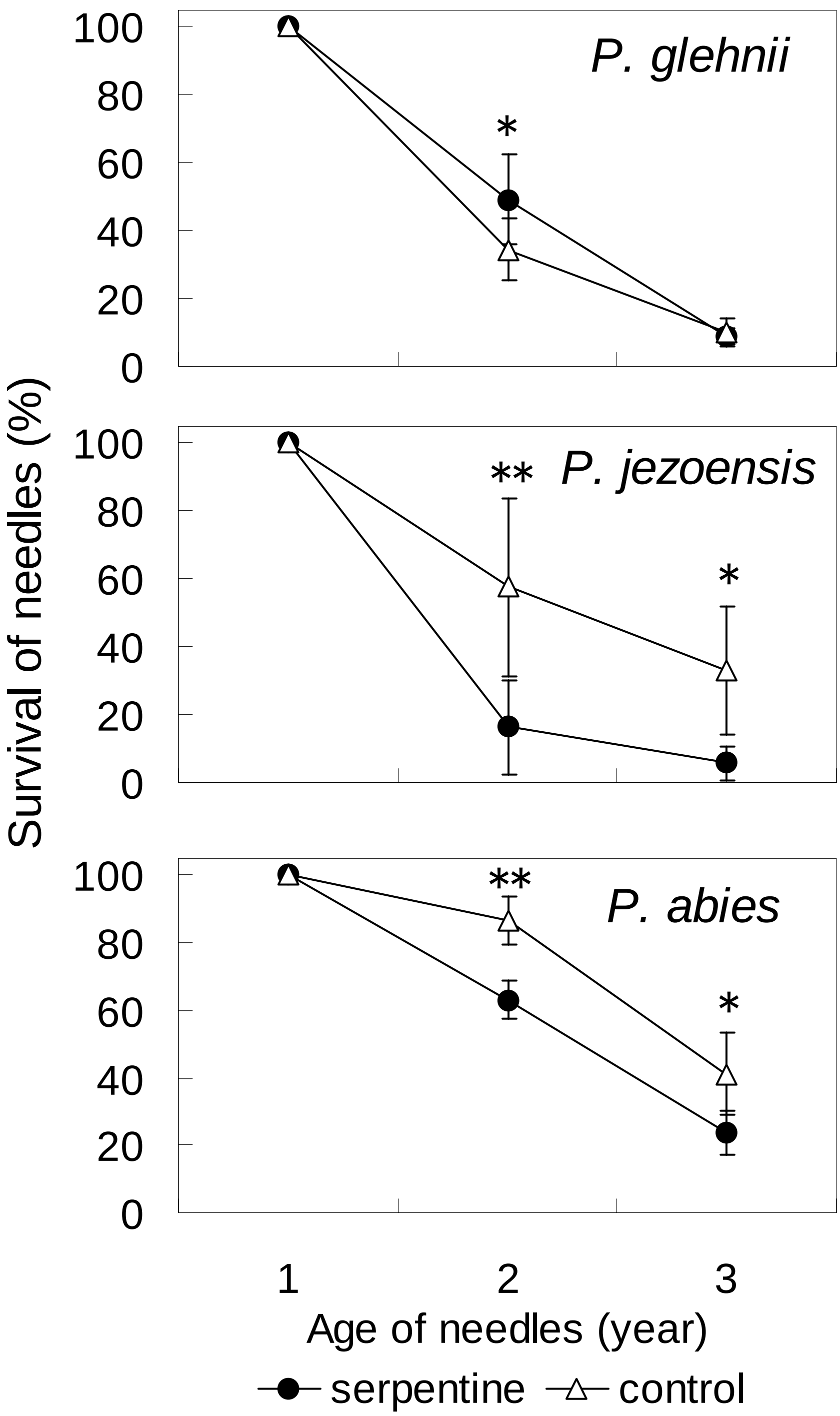
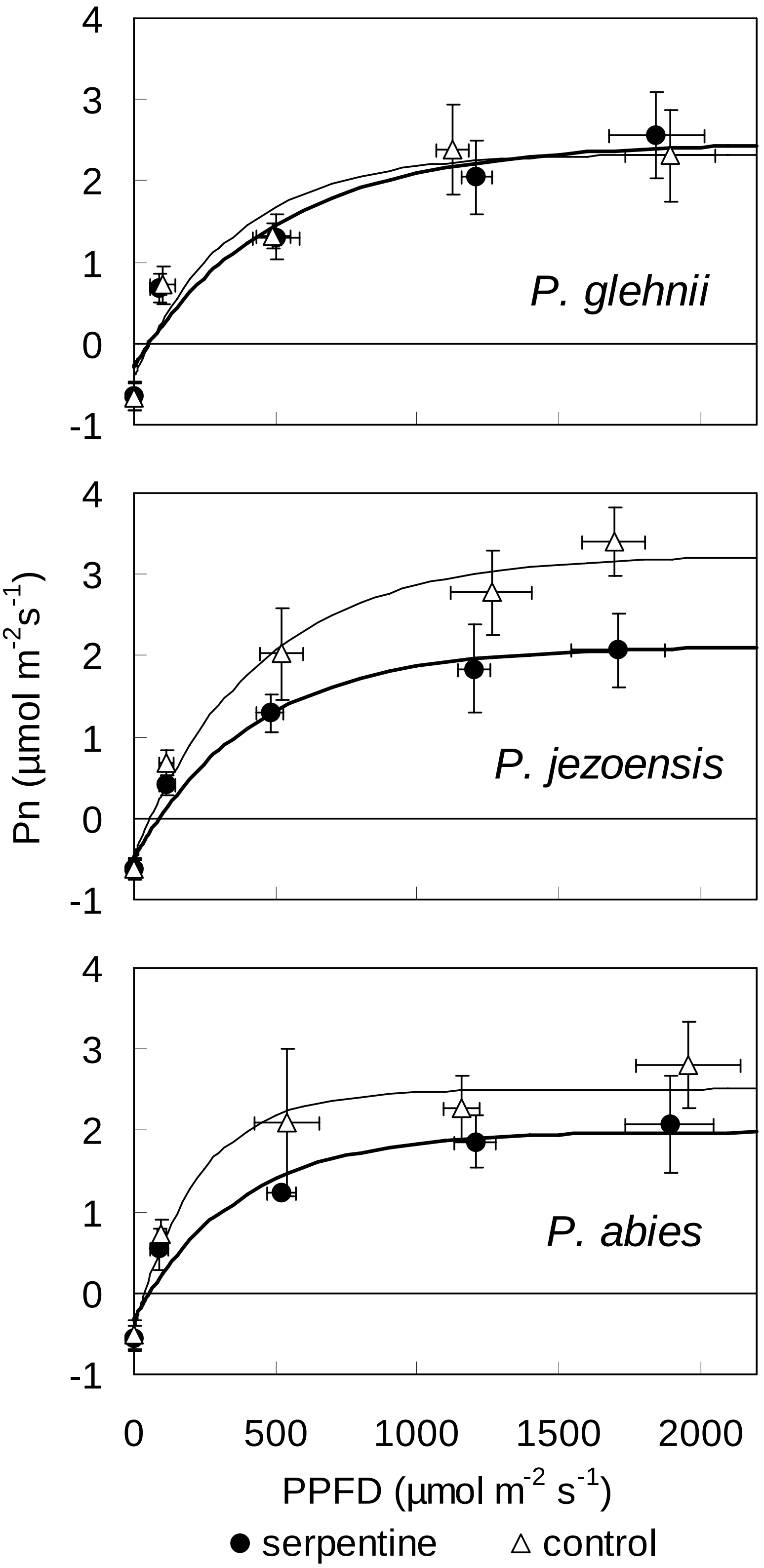

P. glehnii
P. jezoensis
P. abies
Survival of needles (%)
Age of needles (year)
100
80
60
40
20
0
1
2
3
*
**
serpentine
control

Fig. 2.



4
3
2
1
0
-1
P. glehnii
P. jezoensis
P. abies
Pn (µmol m-2s-1)
0
500
1000
1500
2000
PPFD (µmol m-2 s-1)
● serpentine
△ control

Fig. 3.

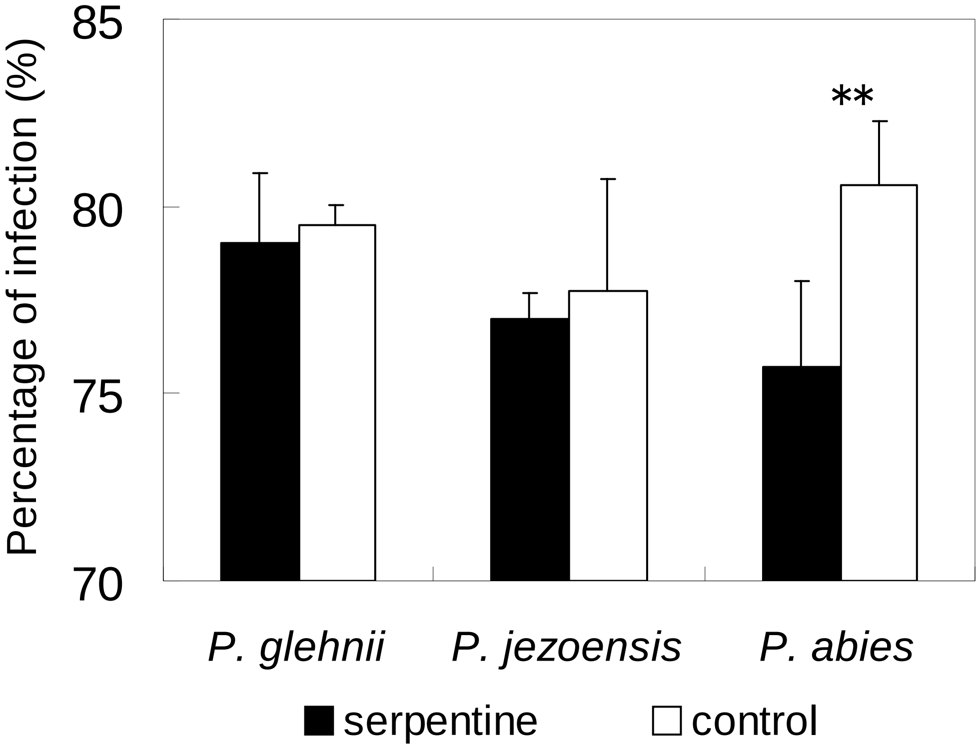

Percentage of infection (%)
85
80
75
70
**
P. glehnii
P. jezoensis
P. abies
serpentine
control

Fig. 4.

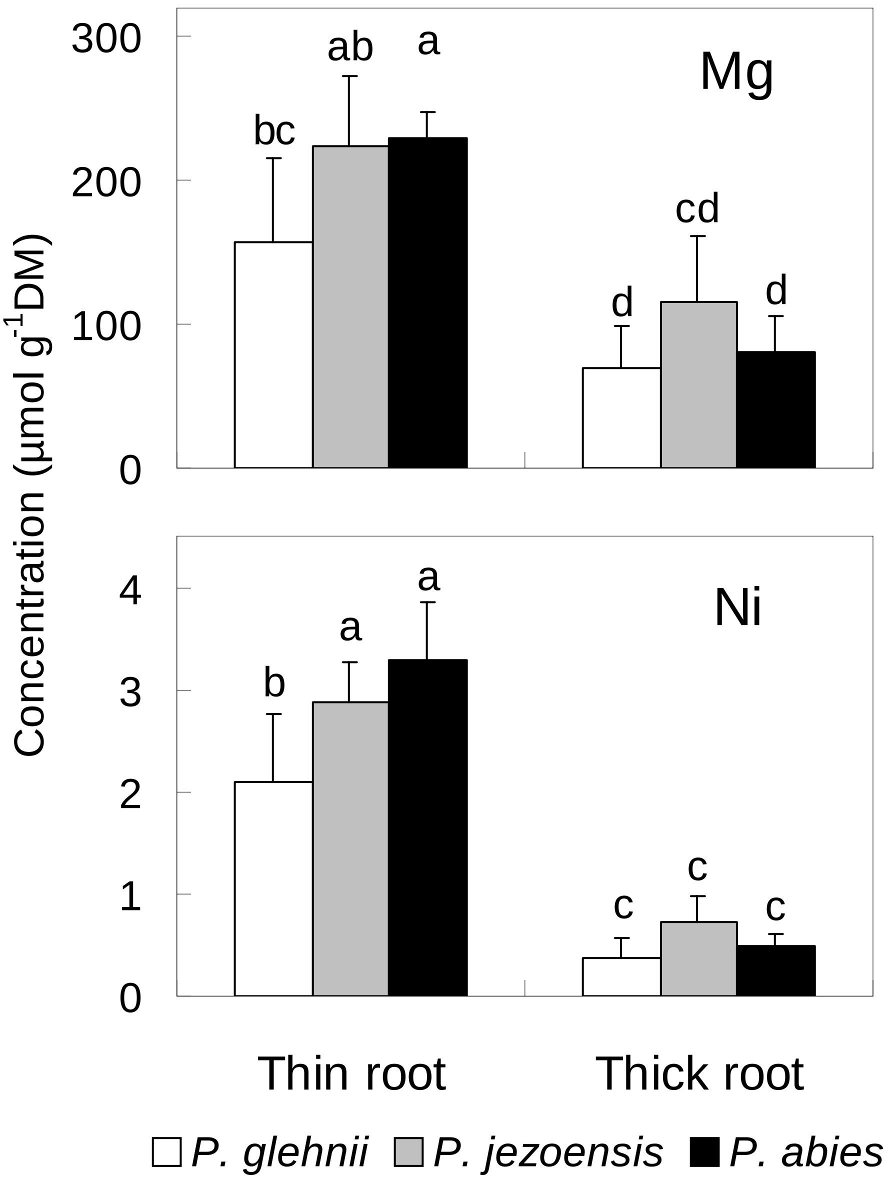

300
200
100
0
4
3
2
1
0
Concentration (µmol g-1DM)
Mg
Ni
bc
ab
a
cd
d
d
b
a
a
c
c
c
Thin root
Thick root
P. glehnii
P. jezoensis
P. abies

Fig. 5.

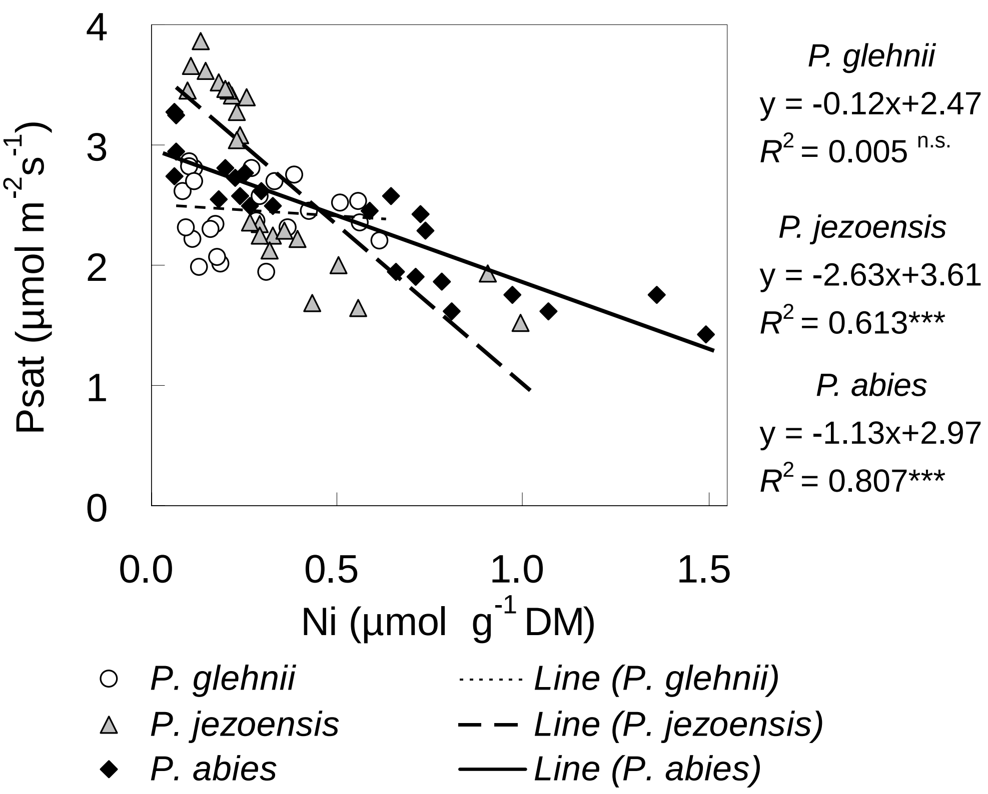

4
3
2
1
0
Psat (µmol m-2 s-1)
0.0
0.5
1.0
1.5
Ni (µmol g-1 DM)
P. glehnii
y = -0.12x+2.47
R2 = 0.005 n.s.
P. jezoensis
y = -2.63x+3.61
R2 = 0.613***
P. abies
y = -1.13x+2.97
R2 = 0.807***
P. glehnii
P. jezoensis
P. abies
Line (P. glehnii)
Line (P. jezoensis)
Line (P. abies)

Fig. 6.