



Title	Comparison of growth characteristics and tolerance to serpentine soil of three ectomycorrhizal spruce seedlings in northern Japan
Author(s)	Kayama, Masazumi; Choi, Dongsu; Tobita, Hiroyuki; Utsugi, Hajime; Kitao, Mitsutoshi; Maruyama, Yutaka; Nomura, Mutsumi; Koike, Takayoshi
Citation	Trees - Structure and Function, 20(4), 430-440 https://doi.org/10.1007/s00468-006-0057-3
Issue Date	2006-06
Doc URL	http://hdl.handle.net/2115/14464
Type	article (author version)
Note	The original publication is available at www.springerlink.com .
File Information	Kayama_Trees3.pdf



[Instructions for use](#)

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

3

4 Authors: Masazumi Kayama, Dongsu Choi, Hiroyuki Tobita, Hajime Utsugi,
5 Mitsutoshi Kitao, Yutaka Maruyama, Mutsumi Nomura and Takayoshi Koike

6

7 M. Kayama · D. Choi · M. Nomura · T. Koike

8 Hokkaido University Forests, FSC, Sapporo 060-0809, Japan.

9 Present address of M. Kayama: Kyushu Research Center, Forestry and Forest Products

10 Research Institute, 4-11-16 Kurokami, Kumamoto 860-0862, Japan.

11

12 H. Tobita · H. Utsugi · M. Kitao

13 Hokkaido Research Center, Forestry and Forest Products Research Institute,

14 Hitsujigaoka 7, Toyohira, Sapporo 062-8516, Japan

15

16 Y. Maruyama

17 Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki

18 305-8687, Japan.

19

20

1 Corresponding author: Takayoshi Koike

2 Address of corresponding author:

3 Hokkaido University Forests, FSC

4 Kita 9 Nishi 9, Kita-ku, Sapporo 060-0809, Japan

5 Tel: +81-11-706-3854

6 Fax: +81-11-706-3450

7 E-mail: tkoike@exfor.agr.hokudai.ac.jp

8

9

10

11

12

13

14

15

16

17

18

19

20

1 **Abstract**

2

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

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

17

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

20

1 **Introduction**

2

3 In many parts of the world, serpentinite, an ultramafic rock, is outcropped, and
4 particular flora is distributed in such regions (Brooks 1987; Roberts and Proctor 1991).

5 Serpentine soil deriving from weathering of serpentinite is characterised by an excess
6 of elements such as Ni, Cr, and Mg, a low Ca/Mg ratio, and low levels of several

7 essential plant nutrients (Proctor 1971; Brooks 1987). Plant species grown on
8 serpentine soil require high tolerance against the toxic metals in this soil (Brooks 1987;

9 Roberts and Proctor 1991). The tolerance mechanisms of plants that adapt to high
10 concentrations of toxic metals in soils generally involve either restricting metal uptake

11 and translocation (exclusion) or accumulating the metal in a non-toxic form
12 (accumulation) (Baker 1981; 1987). Some plants can survive in serpentine soil by

13 symbiosis with microbes (Panaccione et al. 2001; Wardle 2002; Moser et al. 2005).

14 Ectomycorrhizal symbiosis might be important in excluding toxic metals. Differing
15 ectomycorrhizal species are in symbiosis with woody species on serpentine and

16 non-serpentine soil (Panaccione et al. 2001), and endemic fungus species also exist on
17 serpentine soil (Maas and Stuntz 1969; Moser et al. 2005). Ectomycorrhizal species in

18 serpentine soil have the capacity to protect against toxic metals (Panaccione et al.
19 2001). Ectomycorrhizal fungi can exclude toxic metals by (1) binding to the hyphal

20 sheath, (2) reducing apoplastic mobility as a result of hydrophobicity of fungal sheath,

1 (3) chelating by organic acids, and (4) binding to the external mycelium (Gadd 1993;
2 Jentschke and Goldbold 2000). When ectomycorrhizae are inoculated into the roots of
3 woody species, the concentration of Ni in the leaves decreases, and root growth is
4 accelerated despite the high concentration of Ni (Dixon 1988; Dixon and Buschena
5 1988; Jones and Hutchinson 1986; 1988a; b; Jones et al. 1988; Wilkins 1991).

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

1 are inhibited (Shimada 1972).

2 On serpentine soil in northern Japan, the Sakhalin spruce (*Picea glehnii* Masters) is

3 dominant (Tatewaki 1958; Nakata and Kojima 1987; Matsuda 1989). *P. glehnii* can

4 grow in various infertile environments (Tatewaki 1958; Matsuda 1989). Moreover,

5 Yezo spruce (*P. jezoensis* Carr.) is a dominant coniferous species in the mesic region of

6 northern Japan (Miyawaki 1988). In addition, The Norway spruce (*P. abies* (L.) Karst.)

7 was introduced from southwestern Germany in the early 1900s for timber production,

8 and is well adapted to mesic sites in northern Japan (Kubota and Fukuchi 1981). *P.*

9 *jezoensis* and *P. abies* are usually grown on fertile soils (Miyawaki 1988; Nikolov and

10 Helmisaari 1992), and are rarely found on serpentine soils (Brooks 1987; Nakata and

11 Kojima 1987).

12 In *P. glehnii* grown in serpentine soil, the concentration of Ni in needles is lower

13 than in other species (Blandon et al. 1994; Kayama et al. 2002). It follows that *P.*

14 *glehnii* acts as a Ni excluder (Kayama et al. 2005). The total dry mass of *P. glehnii* is

15 almost the same on serpentine and non-serpentine soil. Moreover, the concentration of

16 Ni in needles and roots of *P. glehnii* was lower than in the other two species, and

17 ectomycorrhizal symbiosis may help to exclude Ni. In contrast, growth of *P. jezoensis*

18 and *P. abies* on serpentine soil was significantly less than in non-serpentine soil. Ni

19 accumulated in needles and roots of *P. jezoensis* and *P. abies* on serpentine soil, so that

20 these spruces may suffer from Ni toxicity. However, serpentine soil is characterized

1 not only by excess accumulation of Mg and Ni but also by deficiency of nutrients, so
2 that spruces grown on serpentine soil showed deficiencies of various nutrients.
3 Moreover, the concentration of nitrogen in needles, which is positively correlated with
4 the photosynthetic capacity (Field and Mooney 1986, Lambers et al. 1998), was lower
5 for the three spruce species on serpentine soil than on non-serpentine soil. We
6 therefore were unable to determine whether the suppression of growth of *P. jezoensis*
7 and *P. abies* was due to toxicity of Ni and Mg or inadequacy of nutrients.

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

15

16

17 **Materials and Methods**

18 Study site

19

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

1 Experimental Forest (TEF) maintained by Hokkaido University (N44°55', E142°00',
2 16m a.s.l.). The mean annual precipitation from 1996 to 2000 was 1062 mm yr⁻¹
3 (Takagi et al. 2001). The annual mean, maximum and minimum temperatures were
4 respectively 5.3 °C, 26.1 °C and -21.8 °C from 1996 to 2000, as measured by a thermo
5 recorder at the Toikanbetsu meteorological station (Takagi et al. 2001). The
6 experimental site was approximately 1.5 km from the meteorological station.

7

8 Experimental nurseries

9

10 We prepared eight experimental nurseries, four on serpentine conditions and four on
11 non-serpentine (i.e., control) conditions. Based on the FAO-UNESCO system, the
12 control soil was classified as Cambisol (Teshio, unpublished data). To establish the
13 nursery containing serpentine soil, we collected 3000 kg of serpentine soil using a
14 power shovel from a serpentine region in TEF (N45°05', E142°06', 100m a.s.l.) and
15 transported it to the nursery using a dump truck. The size of each nursery was 2 × 10 m.

16 We selected four nurseries at random and added serpentine soil to them to comprise
17 330g of serpentine soil per 1 kg of Cambisol. After mixing, the soils in each
18 experimental nursery were cultivated using a tractor to reduce soil heterogeneity, and
19 eight nurseries were prepared. Each nursery was separated by approximately 5 m to
20 prevent soil cross-contamination.

1

2 Plant materials

3

4 We studied the *Picea glehnii* Masters, *P. jezoensis* Carr., and *P. abies* (L.) Karst.

5 Preliminary research indicates that needle longevity is about two years for seedlings of

6 the three spruces grown in fertile nurseries, and suppression of growth of seedlings on

7 serpentine soil became apparent from the second year (Kayama et al. 2005). The

8 experiment had therefore to last at least two years.

9 Seeds of *P. glehnii* Masters and *P. jezoensis* Carr. were selected from a similar

10 habitat in the central part of Hokkaido, governed by the National Forestry Research

11 Institute, where the soil was non-serpentine. Second generation seeds of *P. abies* have

12 now been produced in central Hokkaido. We used four-year-old seedlings of *P. glehnii*

13 and *P. jezoensis*, and two-year-old *P. abies* seedlings, because we could not obtain

14 seedlings of the species of equal age. In May 1999, 128 seedlings of each spruce

15 species were removed from the nursery and transported to the Nukanan experimental

16 nurseries of TEF.

17 During transportation, the roots of each seedling were protected by moist paper

18 towels (Kimitowel, Crecia Co., Tokyo, Japan). 16 seedlings of each spruce species were

19 planted on each of the four nurseries of serpentine and control. After planting, each

20 plot was weeded periodically. The water status was monitored by time domain

1 reflectometry (TRIME-FM, IMKO Micromodultechnik GmbH, Ettlingen, Germany).
2 The water content at field capacity of serpentine and brown forest soils was 48 %, and
3 water was supplied if the water content fell below 35 %.

4

5 Analysis of soil chemistry

6

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

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

1 (Sumigraph NC-800, Sumika Chemical Analysis Service, Tokyo, Japan; Japanese
2 Society of Soil Science and Plant Nutrition 1997). Exchangeable phosphorus was
3 separated using dilute acid fluoride (Kuo 1996), shaking for 1 minute. Exchangeable
4 base cations (Ca, Mg, K, Na) were quantified by mixing 2.5 g of dry soil with 50 ml of
5 1 N ammonium acetate solution, shaking for 1 h (Thomas 1982). Nickel was
6 determined by the DTPA method (Baker and Amacher 1982), and chromium by the
7 nitric acid method (Reisenauer 1982). Phosphorus, base cations, and heavy metals in
8 the extracted solutions were analysed by an inductively coupled plasma (ICP) analyser
9 (IRIS, Jarrel ash, Franklin, MA, USA; Thompson and Walsh 1989). We also analysed a
10 standard solution of elements between every 40 samples, and corrected the data
11 accordingly.

12

13 Measurement of seedling growth

14

15 To determine the growth characteristics of seedlings of the three spruce species, we
16 measured the dry mass of needles, stems and branches, and roots. Four seedlings of
17 each spruce species were harvested from four plots of the two types of nursery in May
18 1999, May 2000, and Apr. 2001 (initial, 13th, and 24th month harvest). The roots of
19 the harvested seedlings were carefully washed three times with water to remove soil,
20 and then with distilled water by an ultrasonic washer (US-2A, As One Co., Osaka,

1 Japan) for 15 minutes. The washed seedlings were divided into shoot (organs above
2 ground) and root components, and shoots were divided into components by age. Each
3 component was put into its own envelope and was oven-dried at 80 °C for four days.
4 The dry masses of the various components were then determined.

5 To estimate needle longevity, the survival of needles (SN) was calculated at the
6 24th month harvest. The total needle dry mass and mass per needle were determined
7 by weighing for needles of each age at the three harvest periods, and the SN was
8 calculated as follows (Kayama et al. 2005):

9
$$SN = (FTNM / ITNM) \times (INM / FNM) \times 100,$$

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

13
14 Measurement of photosynthetic rate

15
16 We measured the photosynthetic rate of two-year-old needles taken from the sunny
17 crown of seedlings, since two years are needed for new needles to reach their
18 maximum photosynthetic rate (Hom and Oechel 1983). Three individuals of three
19 species of spruce planted on four plots of the two nursery types were used to measure
20 the photosynthetic rate in August 2000. These measurements were conducted using a

1 portable gas analyzer (H4A, ADC-Analytical Development Company, U.K.) under
2 steady-state conditions, an ambient temperature of 23-26 °C, and ambient CO₂
3 concentration of 35.5-36.0 Pa. Supplementary light was provided by a halogen lamp
4 (WALZ, Effeltrich, Germany). We changed the photosynthetic photon flux density
5 (PPFD) from high to low using shade cloths (Krary, Osaka, Japan). After measuring
6 the photosynthetic rate, we measured the needle-projected area using the image
7 scanner (FB636U, Canon, Japan), and calculated the net photosynthetic rate per unit
8 area. From the photosynthetic data, light-dependent photosynthesis curves were
9 calculated as follows (Thornley 1976):

10
$$Pn = Pmax [1 - e^{-(\alpha I / Pmax)}] - R,$$

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

15

16 Measurement of the rate of symbiosis with ectomycorrhizae

17

18 To measure the extent of ectomycorrhizal symbiosis, we selected ten lateral roots for
19 each seedling at random; over 500 short roots (viz. < 5 cm in length) diverging from
20 these were observed. The percentage of ectomycorrhizal symbiosis was determined

1 before drying for sixteen seedlings of the three spruce species grown in the two types
2 of nursery. For assessment of ectomycorrhizae, roots of seedlings were harvested in the
3 initial and 24th months, and were carefully washed free of soils under gently flowing
4 water. The root systems were observed under a stereomicroscope, and the numbers of
5 symbiotic and non-symbiotic short roots were counted (Quoreshi and Timmers 1998).
6 The percentage of short roots made a symbiosis with ectomycorrhizae was then
7 calculated.

8

9 Analysis of nutrition in plants

10

11 We determined the concentrations of N, P, K, Ca, Mg, and Ni in two-year-old
12 needles and roots. We also analysed these elements in two-year-old needles for which
13 the photosynthetic rate had been measured. Two-year-old needles were used, by which
14 time their maximum physiological activity had been reached (Hom and Oechel 1983).

15 The dried samples were ground to a fine powder using a sample mill (WB-1, Osaka
16 Chemical Co., Osaka, Japan). The concentration of N was determined using a NC
17 analyser. To determine the concentrations of the other elements (P, K, Ca, Mg, and Ni),
18 dried samples were digested by the HNO₃-HCl-H₂O₂ method (Goto 1990) and
19 analysed using ICP (IRIS, Jarrel ash, Franklin, MA, USA). We also analysed standard
20 solutions of each element between every 40 samples to verify the reliability of the

1 analysis. Mg and Ni were also analysed in part of the roots at the 24th month harvest.
2 Roots of seedlings of the three spruce species planted on serpentine soil were
3 separated using a sieve of mesh diameter 1.0 mm, and categorized into thin roots (<
4 1.0 mm diameter) and thick roots (1.0 > mm diameter) at the 24th month harvest. Thin
5 and thick roots were digested separately, and were analysed for N, P, K, Ca, Mg, and
6 Ni.

7

8 Statistical analysis

9

10 Stat View 5.0 (SAS Institute Inc.) was used for statistical analysis of all parameters.
11 The mean dry mass of each organ, survival of needles, percentage made a symbiosis
12 with ectomycorrhizae, and concentrations of elements in needles and roots, were all
13 examined using t-tests. The mean values for the three spruce species were compared
14 between the serpentine and brown forest soils. In the analysis of soil chemical
15 properties, depth and soil type were tested by repeated measures of ANOVA. The
16 symbols *, **, and *** indicate a statistical significance of $P<0.05$, $P<0.01$ and
17 $P<0.001$, respectively.

18 The mean values of the concentrations of Mg and Ni in needles and roots grown on
19 serpentine nursery were examined using a Tukey test. From Fig. 5, the mean values for
20 the two types of roots of the three spruces were compared. Different letters of the

1 alphabet, such as a, b, and c, indicate a statistical significance of $P<0.05$.
2 We also ran simple regression analyses using Stat View 5.0 to estimate the relations
3 between physiological parameters. In Fig. 6, we examine the relation between the
4 concentration of Ni and the photosynthetic rate at light saturation for each species. We
5 also examined ANCOVA among the regression lines of three spruce species. The
6 symbol *** indicates a statistical relationship of a regression line at $P<0.001$.

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

9

10 **Results**

11 Soil chemical properties

12

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

20 In the original serpentine soil, the concentration of Mg was about 70 mg 100g⁻¹

1 higher, and Ni were 5.4 mg 100g⁻¹ higher than those of soils from serpentine nurseries.
2 By contrast, the concentrations of many nutrients were low compare with soils from
3 two types of nurseries.

4

5 Growth characteristics

6

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

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

1

2 Photosynthetic capacity

3

4 The photosynthetic rate (Pn) of 2-year-old needles of *P. glehnii* and *P. jezoensis*

5 saturated at approximately $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD in each type of nursery (Fig. 5). For

6 *P. abies*, Pn in serpentine nurseries saturated at around $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD, but in

7 control nursery saturation occurred at only around $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD.

8 Compared with the Pn value at light saturation, Pn in serpentine nurseries was 1.6

9 $\mu\text{mol m}^{-2}\text{s}^{-1}$ lower for *P. jezoensis*, and $0.7 \mu\text{mol m}^{-2}\text{s}^{-1}$ lower for *P. abies*, than in

10 control nurseries ($P<0.01$). In contrast, the Pn of *P. glehnii* at high PPFD was $2.6 \mu\text{mol}$

11 $\text{m}^{-2}\text{s}^{-1}$ in each type of nursery, with no significant difference. Moreover, the initial

12 gradient of the light photosynthetic curve for *P. abies* and *P. glehnii* in serpentine

13 nurseries was lower than that at the healthy site.

14

15 The percentage of ectomycorrhizal symbiosis

16

17 At the initial harvest, the percentages of ectomycorrhizal symbiosis with roots of *P.*

18 *glehnii*, *P. jezoensis*, and *P. abies* were respectively 43.5%, 44.4%, and 40.3%.

19 Ectomycorrhizal development for the three spruces did not change by the 13th month

20 harvest (data not shown). By the 24th month, the percentages for the three spruces had

1 increased to values varying from 75 to 81% (Fig. 4). In a comparison between
2 serpentine and control nurseries, the percentage of ectomycorrhizal symbiosis
3 percentage of *P. glehnii* and *P. jezoensis* did not differ significantly. In contrast, the
4 percentage of ectomycorrhizal symbiosis for *P. abies* in serpentine nurseries was
5 significantly lower than in control nurseries ($P<0.01$).

6

7 Element concentrations

8

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

1 rate were no significantly difference those in the 24th month (data not shown).
2 Concentrations of N and P in roots of *P. glehnii* were higher in serpentine nurseries
3 than in controls at the 13th month (Table 3, $P<0.05$). In contrast, concentrations of P, K,
4 and Ca in roots of *P. abies* were significantly lower in serpentine nurseries than in
5 control nurseries at the 13th month ($P<0.05$). Concentration of K in roots of *P.*
6 *jezoensis* was significantly lower in serpentine nurseries than in control nurseries from
7 the 13th month ($P<0.05$).

8 Concentrations of Mg and Ni in roots of the three spruces were significantly higher
9 in serpentine nurseries than in control nurseries at the 24th month (Table 3, $P<0.05$),
10 and these elements were significantly higher in thin roots than in thick roots (Fig. 5,
11 $P<0.01$). In particular, the Ni concentration in thin roots of *P. glehnii* grown in
12 serpentine nurseries was $2.0 \mu\text{mol g}^{-1}$ DW, the lowest value among the three spruces
13 ($P<0.05$).

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

20

1 **Discussion**

2

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

16 Also, the concentration of Ni and Mg in thin roots of *P. glehnii* in serpentine soil
17 was the lowest among the three spruces (Fig. 5). Other mechanisms of excluding toxic
18 metal for ectomycorrhizae are (1) reducing apoplastic mobility as a result of
19 hydrophobicity of fungal sheath, (2) chelating by organic acids, and (3) binding to the
20 external mycelium (Jentschke and Goldbold 2000). It seems that ectomycorrhizae

1 making a symbiosis with roots of *P. glehnii* may have high excluding capacity of Ni; as
2 a result, Ni in thin roots was the lowest among the three spruces. However, the
3 percentage of ectomycorrhizal symbiosis at the 13th month harvest was about 44 % for
4 roots of *P. glehnii* in serpentine nursery. It appears that roots with no ectomycorrhizal
5 association may have absorbed Mg and Ni, so that concentrations of Mg and Ni in
6 needles and roots of *P. glehnii* were increased at the 13th month harvest. Similar trend
7 was verified that the total content of Mg and Ni in *P. glehnii* increased drastically at
8 the harvest when ectomycorrhizal colonisation was poor (Kayama et al. 2005).

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

1 than that of other spruces.

2 Also, the photosynthetic rate at light saturation was reduced for *P. jezoensis* and *P.*
3 *abies* grown in serpentine nurseries (Fig. 3). The toxicity of Ni reduces photosynthetic
4 capacity (Carlson 1975; Rauser and Dumbroff 1981; Morgutti et al. 1984; Jones and
5 Hutchinson 1988a). Our results also suggest a negative correlation between
6 concentrations of Ni and Psat for *P. jezoensis* and *P. abies* (Fig. 6). Especially, the
7 gradient of the regression lines between Ni and Psat was larger for *P. jezoensis* than for
8 other spruces (Fig. 6). Consequently, photosynthetic capacity of *P. jezoensis* may be
9 depressed by a modicum of Ni accumulation. In a comparison of woody species, the
10 photosynthetic rate of paper birch was suppressed by a Ni concentration of $125 \mu\text{g g}^{-1}$
11 ($2.13 \mu\text{mol g}^{-1}$) in needles (Jones and Hutchinson 1988a; b). We find here that
12 suppression of the photosynthetic rate begins at a concentration of $0.3 \mu\text{mol g}^{-1}$ Ni in
13 needles in *P. jezoensis*, and at $0.6 \mu\text{mol g}^{-1}$ Ni in *P. abies* (Fig. 6). We believe that
14 depression of photosynthetic capacity probably runs in proportion to the nickel
15 concentration in leaves at low concentrations.

16 Furthermore, uptake of K by *P. jezoensis* in serpentine nurseries was suppressed
17 even when K was present in soil in high concentrations (Tables 2, 3). In general,
18 inhibition of K uptake is a major toxic effect of Ni (Pandolfini et al. 1992; Baccouch et
19 al. 1998), and Ni-induced K deficiency is a key consequence of serpentine soil (Millar
20 and Cumming 2000). Therefore, *P. jezoensis*, which is sensitive to Ni toxicity, may

1 suffer from K deficiency. K deficiency leads to reduce photosynthetic capacity
2 (Marschner 1995), so that *P. jezoensis* in serpentine nurseries may accelerate reduction
3 of photosynthetic capacity. *P. abies* in serpentine nurseries had reduced concentrations
4 of P, K, and Ca in roots in the 13th month (Table 3). Toxicity of Ni reduced the uptake
5 of several nutrients as well as K: also P and Ca (Millar & Cumming 2000). *P. abies* in
6 serpentine nurseries absorbed large amounts of Ni from the 13th month, presumably
7 inhibiting the uptake of P, K, and Ca from that time.

8 The ectomycorrhizal colonization percentage in *P. abies* was significantly lower in
9 serpentine nurseries than in control nurseries (Fig. 4). Of the three spruces, the
10 concentration of Ni in needles was greatest in *P. abies* (Table 2). It appears that Ni
11 uptake and transfer to needles of *P. abies* is not suppressed due to low ectomycorrhizal
12 colonisation. In contrast, the ectomycorrhizal colonization of *P. jezoensis* was high
13 percentage same as *P. glehnii*, and no significantly difference between the two types of
14 nursery (Fig. 4). However, *P. jezoensis* in serpentine nurseries accumulated large
15 amount of Ni in roots compared with *P. glehnii* (Table 2). One of possibility is that the
16 excluding capacity of Ni may be lower for ectomycorrhizae of *P. jezoensis* than that of
17 *P. glehnii*. In fact, only two types of ectomycorrhizae were in symbiosis for roots of
18 young seedlings of *P. jezoensis* grown on various habitats (Takahashi 1991). By
19 contrast, young seedling of *P. glehnii* grown on various habitats was detected over 30
20 types of ectomycorrhizae (Kasuya 1995). A capacity excluding toxic metals was high

1 when several ectomycorrhizal species are inoculated (Choi 2005). It seems that
2 symbiosis with various type of ectomycorrhizae may have strong effects to exclude Ni
3 for *P. glehnii*.

4 On effects of other elements, high concentration of Mg contained in soils of
5 serpentine nurseries was detected in plant organ of *P. jezoensis* and *P. abies* (Table 2,
6 3). Concentration of Mg in roots inhibited activities of various enzymes was over 1 %
7 plant^{-1} ($412 \mu\text{mol g}^{-1}$; Shimada 1972). Concentration of Mg in thin roots of *P. jezoensis*
8 and *P. abies* in serpentine nursery was about $230 \mu\text{mol g}^{-1}$; therefore, effects of excess
9 Mg for activity of enzymes in root of *P. jezoensis* and *P. abies* in serpentine are
10 probably little. Moreover, photosynthetic capacity was decreased by accumulation over
11 1.2 % plant^{-1} ($494 \mu\text{mol g}^{-1}$) of Mg (Rao et al. 1987). Also, concentration of Ca in leaf
12 was decreased by excess Mg (Shimada 1972; Ushijima et al. 2004; Kobayashi et al.
13 2005). However, maximum concentration of Mg in needles was $188 \mu\text{mol g}^{-1}$ for *P.*
14 *jezoensis* (data not shown), and concentration of Ca in needles of *P. jezoensis* and *P.*
15 *abies* in serpentine nursery was not decreased (Table 2). Therefore, effects of toxicity
16 of excess Mg for aboveorgan of *P. jezoensis* and *P. abies* are probably less than Ni.

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

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

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

13

14

15 **Acknowledgements**

16

17 We thank Prof. K. Sasa, Prof. F. Satoh, Dr. S. Uemura and Dr. Y. Akibayashi for
18 valuable comments on this study. We are grateful to the technical staff of Teshio
19 Experimental Forest, Hokkaido University for their excellent technical assistance.
20 Thanks are due to Dr. S. Kitaoka and Ms. Y. Yanagihara for preparation of the

1 nurseries and sampling of the seedlings. We also thank Ms. M. Ohbuchi for technical
2 assistance with the analysis. Financial support of M.K. and T.K. by JSPS and the Japan
3 Science Society is gratefully acknowledged.

4

5 **References**

6

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

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

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

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

15 Baker DE, Amacher MC (1982) Nickel, Copper, Zinc, and Cadmium. In: Page AL,
16 Miller RH, Keeney DR (eds) Methods of soil analysis, Part 2. Chemical and
17 microbiological properties. 2 ed. Soil Science Society of America Inc., Madison, pp
18 323-336

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

- 1 Bull Hokkaido Univ For 51:1-13
- 2 Brooks RR (1987) Serpentine and its vegetation. Dioscorides Press, Portland
- 3 Carlson RW, Bazzaz FA, Rolfe GL (1975) The effect of heavy metals on plants II. Net
- 4 photosynthesis and transpiration of whole corn and sunflower plants treated with Pb,
- 5 Cd, Ni, and Ti. Environ Pollut 7:241-246
- 6 Chiarucci A, Maccherini S, Bonini I, De Dominicis V (1998) Effects of nutrient
- 7 addition on species diversity and ground cover of “serpentine” vegetation. Plant
- 8 Biosys 132:143-150
- 9 Choi DS (2005) Ecophysiological study on growth of the ectomycorrhizal conifer
- 10 species in Korea treated with soil acidification and elevated CO₂. Ph.D. dissertation,
- 11 Hokkaido University, Sapporo, Japan.
- 12 Cocucci SM, Morgutti S (1986) Stimulation of proton extrusion by K⁺ and divalent
- 13 cations (Ni²⁺, Co²⁺, Zn²⁺) in maize root segments. Physiol Plant 68:497-501
- 14 Dixon RK (1988) Response of ectomycorrhizal *Quercus rubra* to soil cadmium, nickel
- 15 and lead. Soil Biol Biochem 20:555-559
- 16 Dixon RK, Buschena CA (1988) Response of ectomycorrhizal *Pinus banksiana* and
- 17 *Picea glauca* to heavy metals in soil. Plant Soil 105:265-271
- 18 Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. In:
- 19 Givnish TJ (ed) On the economy of plant form and function. Cambridge University
- 20 Press, Cambridge, pp 25-54

- 1 Gabbrielli R, Pandolfini T, Vergnano O, Palandri MR (1990) Comparison of two
2 serpentine species with different nickel tolerance strategies. Plant Soil 122:271-277
- 3 Gadd GM (1993) Tansley Review No. 47. Interactions of fungi with toxic metals. New
4 Phytol 124:25-60
- 5 Goto S (1990) Digestion method. In: Editorial Committee of Methods for Experiments
6 in Plant Nutrition (eds) Manual of plant nutrition. Hakuyusha, Tokyo, pp 125-128 (in
7 Japanese)
- 8 Hom JL, Oechel WC (1983) The photosynthetic capacity, nutrient content, and nutrient
9 use efficiency of different needle age-classes of black spruce (*Picea mariana*) found in
10 interior Alaska. Can J For Res 13:834-839
- 11 Japanese Society of Soil Science and Plant Nutrition (1997) Manual of soil
12 environments. Hakuyusha, Tokyo (in Japanese)
- 13 Jentschke G, Godbold DL (2000) Metal toxicity and ectomycorrhizas. Physiol Plant
14 109:107-116
- 15 Jones MD, Hutchinson TC (1986) The effect of mycorrhizal inflection on the response
16 of *Betula papyrifera* to nickel and copper. New Phytol 102:429-442
- 17 Jones MD, Hutchinson TC (1988a) Nickel toxicity in mycorrhizal birch seedlings
18 infected with *Lactarius rufus* or *Scleroderma flavidum*. I Effects on growth,
19 photosynthesis, respiration and transpiration. New Phytol 108:451-459
- 20 Jones MD, Hutchinson TC (1988b) Nickel toxicity in mycorrhizal birch seedlings

- 1 infected with *Lactarius rufus* or *Scleroderma flavidum*. II Uptake of nickel, calcium,
2 magnesium, phosphorus and iron. New Phytol 108:461-470
- 3 Jones MD, Dainty J, Hutchinson TC (1988) The effect of infection by *Lactarius rufus*
4 and *Scleroderma flavidum* on the uptake of ^{63}Ni by paper birth. Can J Bot 66:934-960
- 5 Kasuya MCM (1995) Ecological and physiological studies on ectomycorrhizae of *Picea*
6 *glehnii* (Fr. Schm.) Masters. Ph.D. dissertation, Hokkaido University, Sapporo, Japan.
- 7 Kayama M, Sasa K, Koike T (2002) Needle life span, photosynthetic rate, and nutrient
8 concentration of *Picea glehnii*, *P. jezoensis*, and *P. abies* planted on serpentine soil in
9 Northern Japan. Tree Physiology 22:707-716
- 10 Kayama M, Quoreshi AM, Uemura S, Koike T (2005) Differences in growth
11 characteristics and dynamics of elements absorbed in seedlings of three spruce species
12 raised on serpentine soil in northern Japan. Ann Bot 95:661-672
- 13 Kobayashi H, Masaoka Y, Sato S (2005) Effects of excess magnesium on the growth
14 and mineral content of rice and *Echinochloa*. Plant Prod Sci 8:38-43
- 15 Kubota Y, Fukuchi M (1981) Genus of *Picea*. In: Asakawa S, Katsuta M, Yokoyama Y
16 (eds) Seeds of woody plants in Japan. Japan Forest Tree Breeding Association, Tokyo,
17 pp 42-51 (in Japanese)
- 18 Kuo S (1996) Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loepert RH,
19 Soltanpour PN, Tabatabai MA, Johnson CT, Sumner ME (eds) Methods of soil
20 analysis, Part 3. Soil Science Society of America Inc., Madison, pp 869-919

- 1 Lambers H, Chapin III FS, Pons TL (1998) Plant physiological ecology.
- 2 Springer-Verlag, New York
- 3 Lee J, Reeves RD, Brooks RR, Jaffre T (1978) The relationship between nickel and
- 4 citric acid in some nickel-accumulating plants. Phytochem 17:1033-1035
- 5 L'Huillier L, d'Auzac J, Durand M, Michaud-Ferrière N (1996) Nickel effects on two
- 6 maize (*Zea mays*) cultivars: growth, structure, Ni concentration, and localization. Can
- 7 J Bot 74:1547-1554
- 8 Maas JL, Stuntz DE (1969) Mycoecology on serpentine soil. Mycologia 61:1106-1116
- 9 Marschner H (1995) Mineral nutrition of higher plants, 2 ed. Academic Press, London
- 10 Matsuda K (1989) Regeneration and growth in the *Picea glehnii* forest. Res Bull
- 11 Hokkaido Univ For 46:595-717 (in Japanese and English summary)
- 12 Miller SP, Cumming JR (2000) Effects of serpentine soil factors on Virginia pine (*Pinus*
- 13 *virginiana*). Tree Physiol 20:1129-1135
- 14 Miyawaki A (1988) Vegetation of Japan, Hokkaido. Sibundo, Tokyo (in Japanese and
- 15 English summary)
- 16 Mizuno N (1979) Studies on chemical characteristics of serpentine soils and mineral
- 17 deficiencies and toxicities of crops. Rep Hokkaido Pref Agr Exp Sta 29:1-79 (in
- 18 Japanese and English summary)
- 19 Molas J (2002) Changes of chloroplast ultrastructure and total chlorophyll
- 20 concentration in cabbage leaves caused by excess of organic Ni (II) complexes.

- 1 Environ Exp Bot 47:115-126
- 2 Morgutti S, Sacchi GA, Cocucci SM (1984) Effects of Ni²⁺ on proton extrusion, dark
- 3 CO₂ fixation and malate synthesis in maize roots. Physiol Plant 60:70-74
- 4 Moser AM, Petersen CA, D'Allura JA, Southworth D (2005) Comparison of
- 5 ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and non-serpentine
- 6 soils in southwestern Oregon. Am J Bot 92:224-230
- 7 Nakata M, Kojima S (1987) Effects of serpentine substrate on vegetation and soil
- 8 development with special reference to *Picea glehnii* in Teshio district, Hokkaido,
- 9 Japan. For Ecol Manage 20:265-290
- 10 Nikolov N, Helminssari H (1992) Silvics of the circumpolar boreal forest tree species.
- 11 In: Shugart HH, Leemans R, Bonan GB (eds) A systems analysis of the global boreal
- 12 forest. Cambridge University Press, Cambridge, pp 13-84
- 13 Quoreshi AM, Timmer VR (1998) Exponential fertilization increases nutrient uptake
- 14 and ectomycorrhizal development of black spruce seedlings. Can J For Res 28:674-682
- 15 Pandolfini T, Gabrielli R, Comparini C (1992) Nickel toxicity and peroxidase activity in
- 16 seedlings of *Triticum aestivum* L. Plant Cell Environ 15:719-725
- 17 Panaccione DG, Sheets NL, Miller SP, Cumming JR (2001) Diversity of *Cenococcum*
- 18 *geophilum* isolated from serpentine and non-serpentine soils. Mycologia 93:645-652
- 19 Proctor J (1971) The plant ecology of serpentine III. The influence of a high calcium /
- 20 magnesium ratio and high nickel and chromium levels in some British and Swedish

- 1 serpentine soil. J Ecol 59:827-842
- 2 Rao M, Sharp RE, Boyer J (1987) Leaf Magnesium alters photosynthetic response to
- 3 low water potentials in sunflower. Plant Physiol 84:1214-1219
- 4 Rauser WE, Dumbroff EB (1981) Effects of excess cobalt, nickel and zinc on the water
- 5 relations of *Phaseolus vulgaris*. Environ Exp Bot 21:249-255
- 6 Reisenauer HM (1982) Chromium. In: Page AL, Miller RH, Keeney DR (eds) Methods
- 7 of soil analysis, Part 2. Chemical and microbiological properties. 2 ed. Soil Science
- 8 Society of America Inc., Madison, pp 337-346
- 9 Roberts BA, Proctor J (1992) The ecology of areas with serpentized rocks. Kluwer,
- 10 Dordrecht
- 11 Shimada N (1972) Studies on the excess injury of magnesium in the crops. Trans Fac
- 12 Hort Chiba Univ 6:1-105 (in Japanese and English summary)
- 13 Takagi K, Sasa K, Satoh F, Nomura M, Komiya K, Takahashi H, Hohjo H, Kaneko K,
- 14 Ichikawa K, Nakajima J, Ashiya D, Ishida N, Okuda A, Naniwa A, Okamoto T (2001)
- 15 Meteorological characteristics of northern Hokkaido University Forests during
- 16 1995-1999. Res Bull Hokkaido Univ For 58:29-36 (in Japanese and English summary)
- 17 Takahashi I (1991) Studies of the mycofloral succession on Yezo spruce (*Picea*
- 18 *jezoensis* Carr.) in various stages of growth, with special reference to the role of fungi
- 19 in the natural regeneration of the host. Bull Tokyo Univ For 86:201-273
- 20 Tatewaki M (1958) Forest ecology of the islands of the North Pacific Ocean. J Fac Agr

- 1 Hokkaido Univ 50:371-486
- 2 Thomas GW (1982) Exchangeable cations. In: Page AL, Miller RH, Keeney DR (eds)
- 3 Methods of soil analysis, Part 2. Chemical and microbiological properties. 2 ed. Soil
- 4 Science Society of America Inc., Madison, pp 159-165
- 5 Thompson M, Walsh JN (1989) Handbook of inductively coupled plasma spectrometry.
- 6 2 ed. Blackie Academic & Professional, Glasgow.
- 7 Thornley JHM (1976) Mathematical models in plant physiology. Academic Press,
- 8 London
- 9 Tilstone GH, Macnair MR (1997) Nickel tolerance and copper-nickel co-tolerance in
- 10 *Mimulus guttatus* from copper mine and serpentine habitats. Plant Soil 191:173-180
- 11 Ushijima T, Kaneko A, Yamamoto T (2004) Magnesium excess – mediated chlorosis in
- 12 leaves of cucumber grown on soils of granodiorite origin under a plastic house. J Japan
- 13 Soc Hour Sci 73:476-483 (in Japanese and English summary)
- 14 Van Reeuwijk LP (1993) Procedures for soil analysis. International soil reference and
- 15 information centre, Wageningen
- 16 Wardle DA (2002) Communities and ecosystems: Linkage the aboveground and
- 17 belowground components. Princeton University Press, Princeton, Oxford
- 18 Wilkins DA (1991) The influence of sheathing (ecto-) mycorrhizas of trees on the
- 19 uptake and toxicity of metals. Agr Ecosys Environ 35:245-260
- 20 Yang X, Baligar VC, Martens DC, Clark RB (1996) Plant tolerance to nickel toxicity. II

1 Nickel effects on influx and transport of mineral in four plant species. J Plant Nutr
2 19:265-279

3

4 **Figure legends**

5

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

10

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

13

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

17

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

1

2

3 Fig. 5. Concentrations of Mg and Ni in two classes of roots of seedlings of three spruce
4 species planted in serpentine nursery at 24th month harvest (Mean + SD, n=10).

5 Different letters indicate significant differences between values ($P<0.05$, Tukey test)

6

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

11

12

13

14

15

16

17

18

19

20

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 \pm 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 \pm 0.24	1.97 \pm 0.17	0.206 \pm 0.019	12.5 \pm 1.4	240 \pm 16
3 cm	6.38 \pm 0.32	1.96 \pm 0.12	0.211 \pm 0.017	13.0 \pm 0.7	274 \pm 27
15 cm					
Control nursery					
3 cm	5.70 \pm 0.05	2.07 \pm 0.09	0.222 \pm 0.010	12.9 \pm 0.7	285 \pm 8
15 cm	5.88 \pm 0.09	1.98 \pm 0.11	0.206 \pm 0.008	13.0 \pm 1.7	278 \pm 24
Serpentine soil					
	7.44 \pm 0.11	0.09 \pm 0.02	0.009 \pm 0.001	0.9 \pm 0.3	4 \pm 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 \times N	n.s.	n.s.	n.s.	n.s.	n.s.
Mg (mg 100g ⁻¹)	Mg (mg 100g ⁻¹)	K (mg 100g ⁻¹)	Na (mg 100g ⁻¹)	Ni (mg 100g ⁻¹)	Cr (mg 100g ⁻¹)
Serpentine					
nursery	71 \pm 6	36.7 \pm 4.6	5.85 \pm 0.54	2.61 \pm 0.61	0.89 \pm 0.02
3 cm	73 \pm 7	30.4 \pm 3.2	6.54 \pm 0.60	2.23 \pm 0.53	0.95 \pm 0.13
15 cm					
Control nursery					
3 cm	45 \pm 2	37.3 \pm 2.1	6.80 \pm 1.15	0.51 \pm 0.03	0.86 \pm 0.13
15 cm	46 \pm 3	33.5 \pm 2.3	7.31 \pm 0.20	0.56 \pm 0.06	1.00 \pm 0.10
Serpentine soil					
	140 \pm 9	2.1 \pm 1.2	1.14 \pm 0.08	7.81 \pm 0.35	1.38 \pm 0.16
Statistical test					
Depth (D)	n.s.	**	n.s.	n.s.	n.s.
Nursery type (N)	***	n.s.	*	***	n.s.
D \times 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 \pm SD, n=16). Asterisks indicates significant effects by t-test *= $P<0.05$, **= $P<0.01$, ***= $P<0.001$.

Species	N		P		K		
	Months	($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)	
<i>P. glehnii</i>		S	C	S	C	S	C
	0	926 \pm 69		55 \pm 10		81 \pm 10	
	13	776 \pm 45	685 \pm 45	34 \pm 5	31 \pm 8	78 \pm 4	78 \pm 9
	24	1037 \pm 168	1097 \pm 80	52 \pm 7	48 \pm 8	76 \pm 14*	53 \pm 7
<i>P. jezoensis</i>		S	C	S	C	S	C
	0	1130 \pm 80		62 \pm 7		88 \pm 19	
	13	873 \pm 20	932 \pm 110	41 \pm 3	45 \pm 4	59 \pm 11*	86 \pm 13
	24	1045 \pm 185	1208 \pm 105	55 \pm 6	63 \pm 6	71 \pm 10*	86 \pm 5
<i>P. abies</i>		S	C	S	C	S	C
	0	583 \pm 94		60 \pm 8		111 \pm 38	
	13	1119 \pm 92	1174 \pm 31	47 \pm 4	51 \pm 4	55 \pm 8	57 \pm 12
	24	1026 \pm 108	1129 \pm 92	46 \pm 9	55 \pm 9	90 \pm 14	91 \pm 14
		Ca		Mg		Ni	
		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)	
<i>P. glehnii</i>		S	C	S	C	S	C
	0	296 \pm 58		28 \pm 4		0.08 \pm 0.03	
	13	292 \pm 55	256 \pm 51	79 \pm 20*	35 \pm 8	0.26 \pm 0.10*	0.08 \pm 0.02
	24	198 \pm 19	206 \pm 20	88 \pm 58	29 \pm 7	0.39 \pm 0.20	0.18 \pm 0.07
<i>P. jezoensis</i>		S	C	S	C	S	C
	0	309 \pm 24		34 \pm 10		0.07 \pm 0.04	
	13	227 \pm 14	257 \pm 39	83 \pm 35**	26 \pm 5	0.25 \pm 0.10**	0.07 \pm 0.01
	24	233 \pm 15	257 \pm 34	87 \pm 61*	30 \pm 1	0.51 \pm 0.28*	0.19 \pm 0.04
<i>P. abies</i>		S	C	S	C	S	C
	0	163 \pm 29		47 \pm 10		0.14 \pm 0.03	
	13	232 \pm 17	271 \pm 35	107 \pm 21**	48 \pm 3	0.54 \pm 0.17**	0.09 \pm 0.06
	24	392 \pm 57	436 \pm 72	135 \pm 23***	48 \pm 4	0.86 \pm 0.31**	0.28 \pm 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 \pm SD, n=16). Asterisks indicate significant effects by t-test: * $=P<0.05$, ** $=P<0.01$, *** $=P<0.001$.

Species	N		P		K		
	Months	($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)	
<i>P. glehnii</i>		S	C	S	C	S	C
	0		535 \pm 156		49 \pm 13		72 \pm 8
	13	679 \pm 88*	537 \pm 73	45 \pm 11*	30 \pm 5	58 \pm 6	49 \pm 14
	24	703 \pm 108	707 \pm 137	56 \pm 3	55 \pm 9	105 \pm 16	94 \pm 11
<i>P. jezoensis</i>		S	C	S	C	S	C
	0		575 \pm 71		38 \pm 6		56 \pm 7
	13	612 \pm 66	617 \pm 65	40 \pm 6	37 \pm 3	43 \pm 6*	52 \pm 2
	24	718 \pm 103		50 \pm 4	57 \pm 7	86 \pm 10*	111 \pm 12
			852 \pm 127				
<i>P. abies</i>		S	C	S	C	S	C
	0		404 \pm 21		27 \pm 6		44 \pm 6
	13	908 \pm 125	904 \pm 84	27 \pm 2*** 50 \pm 10		24 \pm 2***	37 \pm 4
	24	786 \pm 38	836 \pm 31	64 \pm 2*** 67 \pm 6		116 \pm 9	115 \pm 15
		Ca		Mg		Ni	
		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)		($\mu\text{mol g}^{-1}$ DM)	
<i>P. glehnii</i>		S	C	S	C	S	C
	0		103 \pm 16		44 \pm 12		0.15 \pm 0.07
	13	100 \pm 6	87 \pm 10	51 \pm 11*	73 \pm 19	0.41 \pm 0.10** 0.22 \pm 0.04	
	24	128 \pm 16	99 \pm 5	82 \pm 32*	45 \pm 3	0.64 \pm 0.35	0.34 \pm 0.08
<i>P. jezoensis</i>		S	C	S	C	S	C
	0		114 \pm 17		36 \pm 5		0.23 \pm 0.09
	13	103 \pm 6	105 \pm 9	77 \pm 37	54 \pm 12	0.69 \pm 0.27	0.38 \pm 0.06
	24	118 \pm 7	112 \pm 6	102 \pm 34** 43 \pm 3		1.12 \pm 0.34** 0.43 \pm 0.15	
<i>P. abies</i>		S	C	S	C	S	C
	0		88 \pm 13		29 \pm 5		0.33 \pm 0.16
	13	72 \pm 7**	92 \pm 8	101 \pm 24**	39 \pm 3	0.79 \pm 0.20** 0.35 \pm 0.08	
	24	120 \pm 20	122 \pm 7	104 \pm 25*** 45 \pm 6		1.07 \pm 0.15** 0.57 \pm 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.

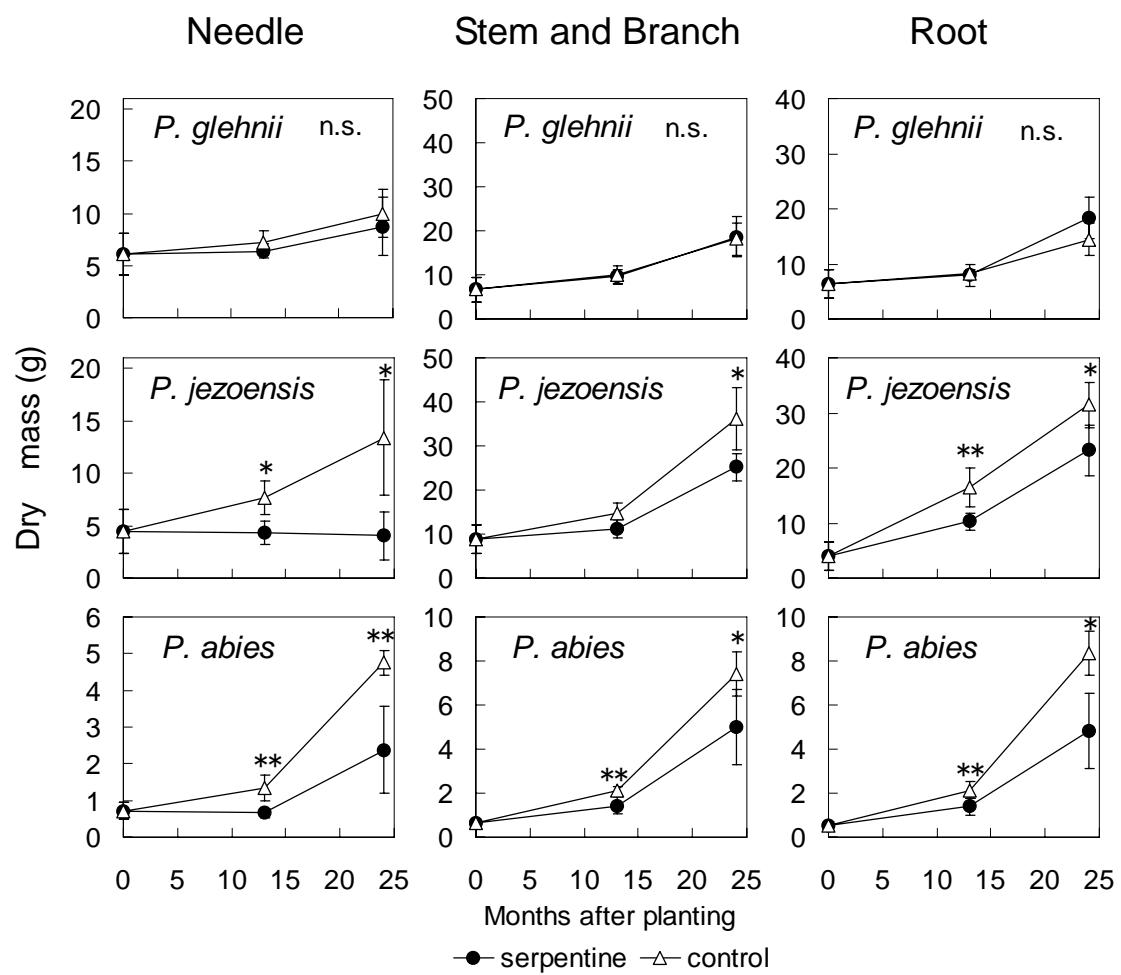


Fig. 1.

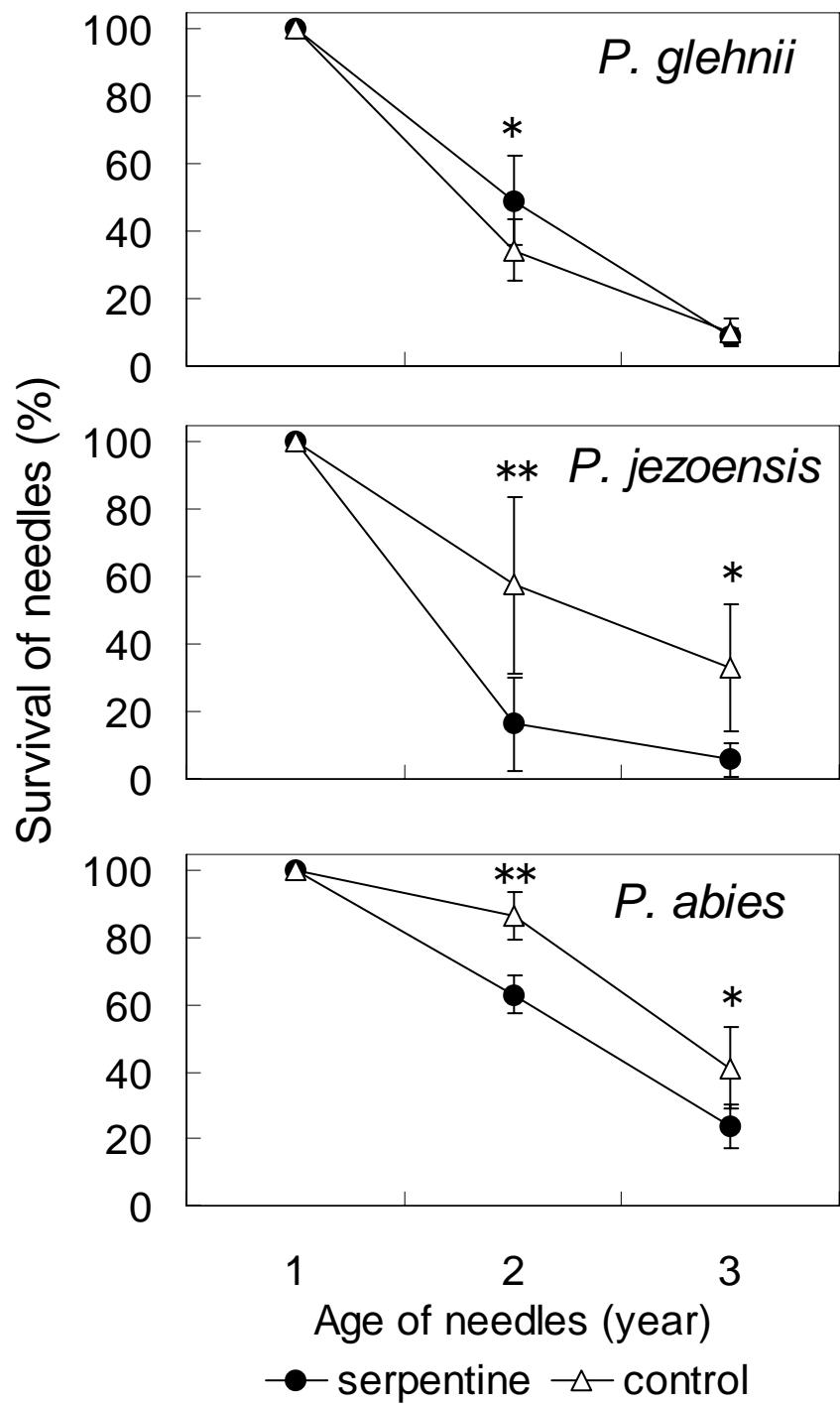


Fig. 2.

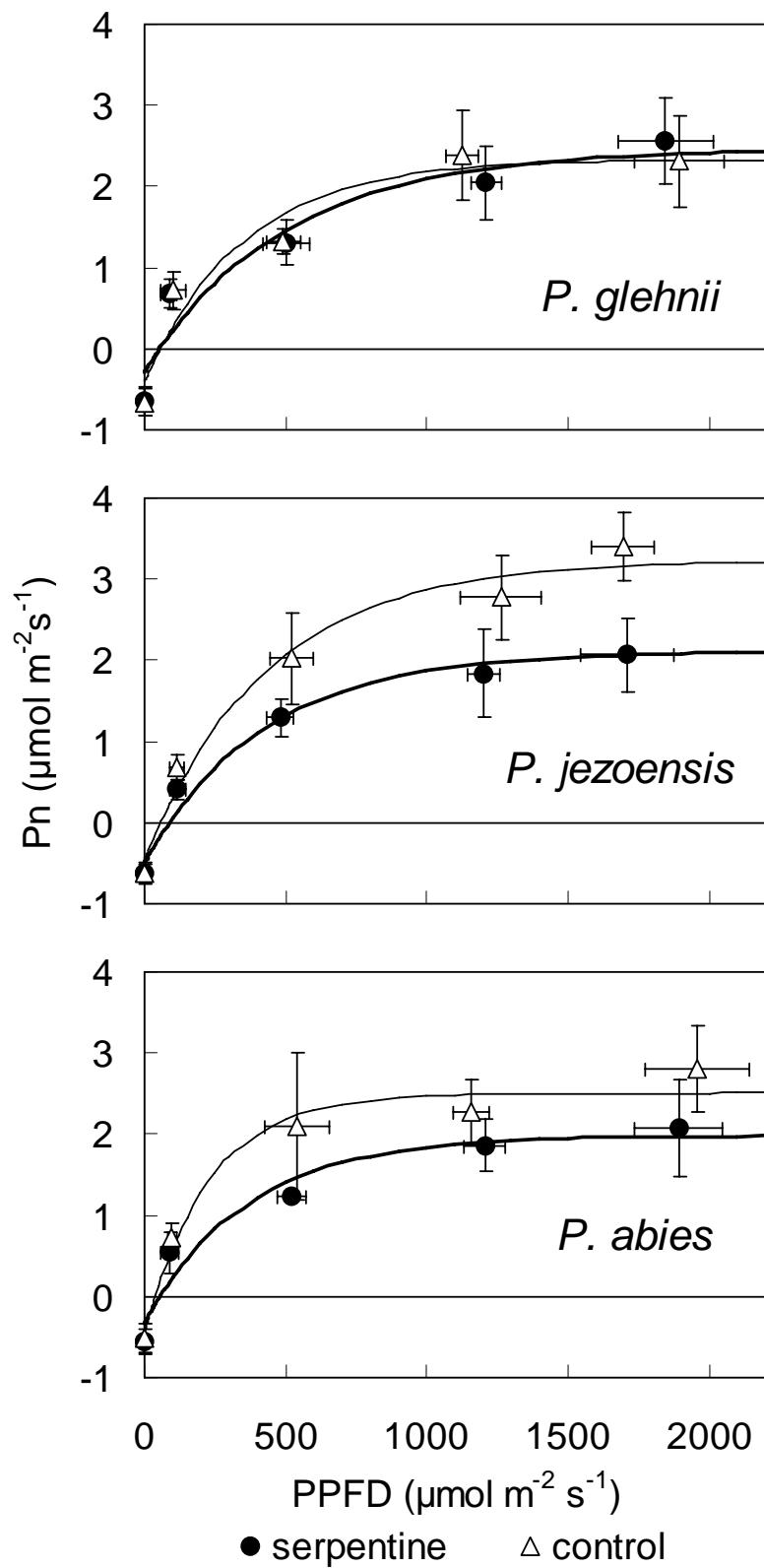


Fig. 3.

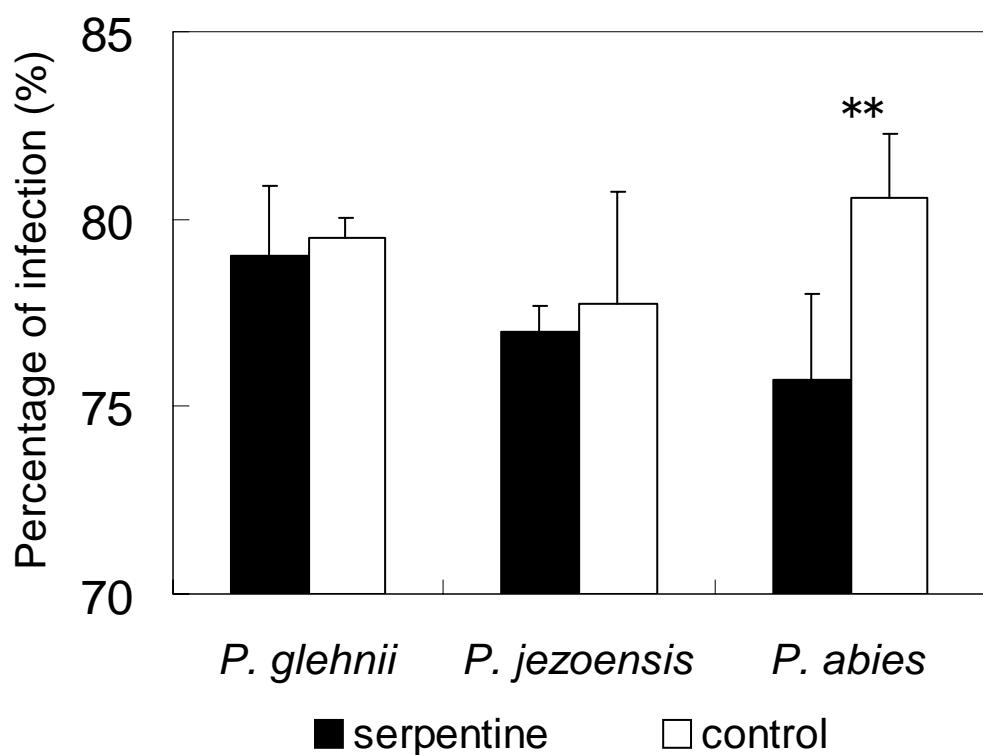


Fig. 4.

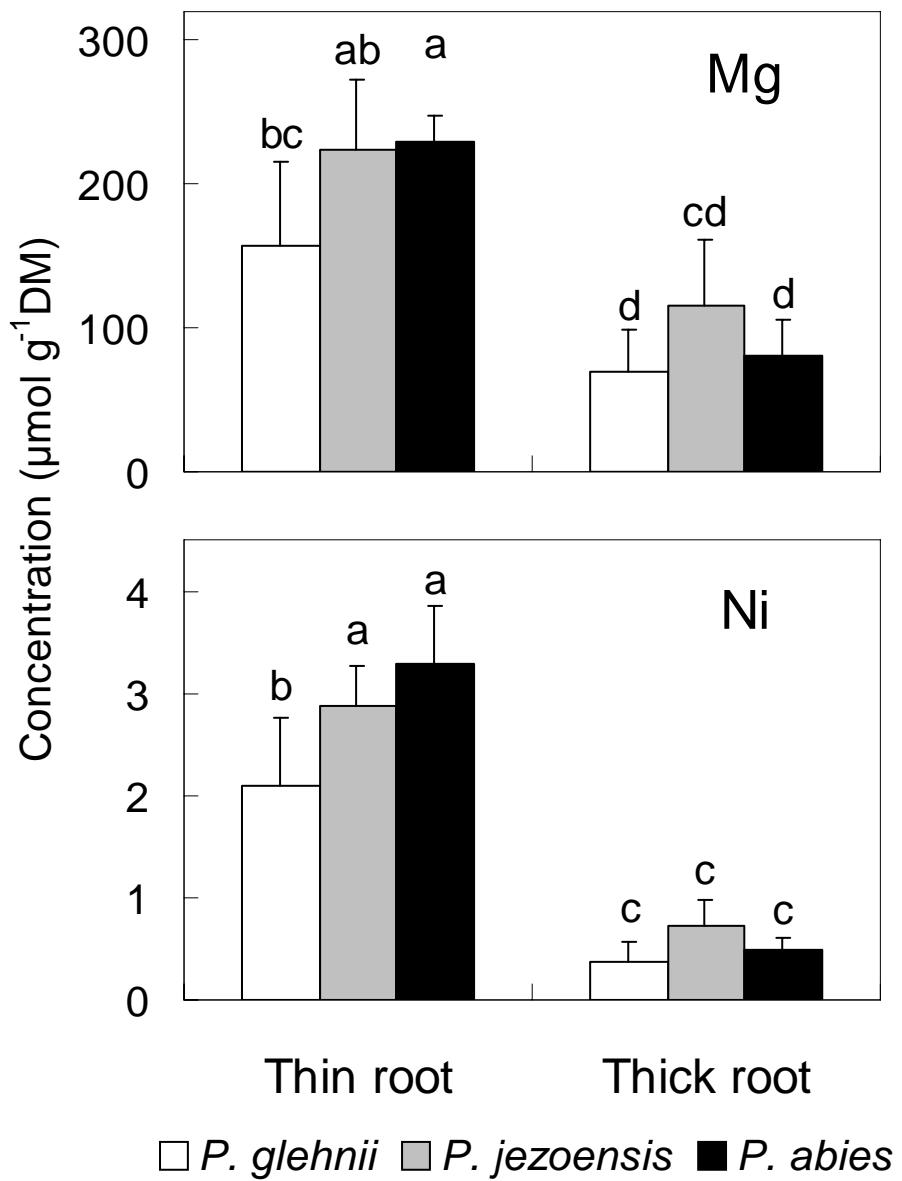


Fig. 5.

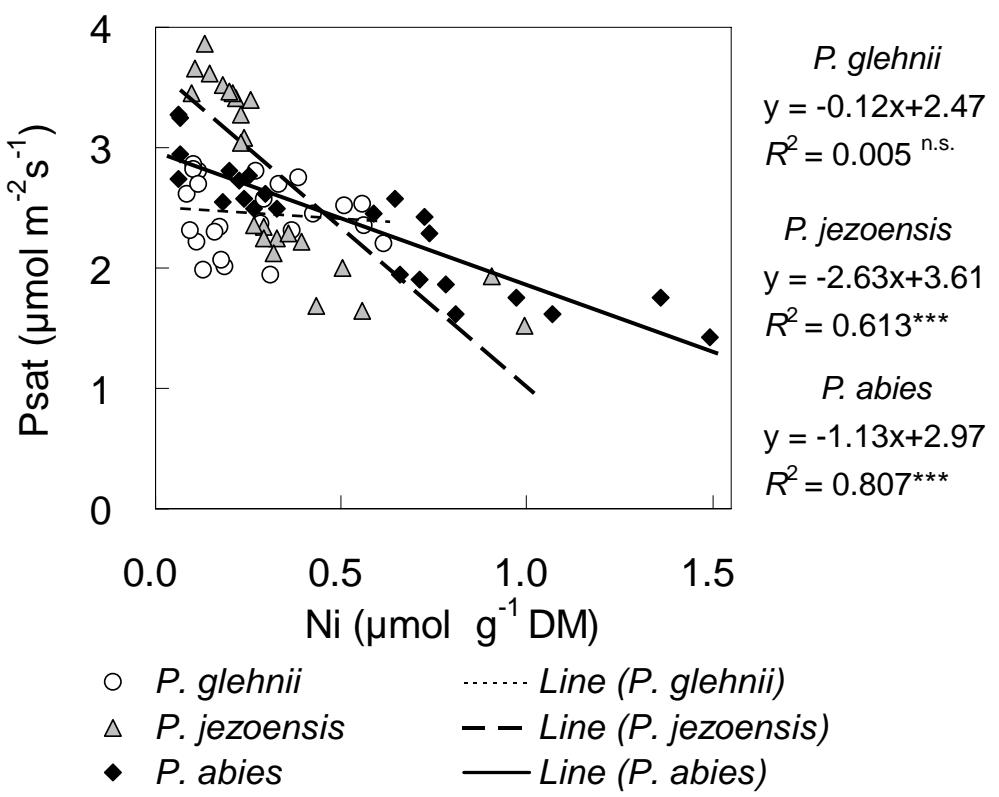


Fig. 6.