Effects of Nitrogen and Phosphorus Fertilization on Ectomycorrhiza Development, N-Fixation and Growth of Red Alder Seedlings[*]

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窒素와 燐酸 施肥가 루브라 오리나무(*Alnus rubra* Bong.) 苗木의 外生菌根發達과 窒素固定 및 生長에 미치는 影響[*]

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ABSTRACT

Red alder(*Alnus rubra* Bong.) seedlings inoculated with *Frankia* only or both *Frankia* and spores of *Alpova diplophloeus*(Zeller & Dodge) Trappe & Smith were grown in a greenhouse for ten weeks. The ten-week-old seedlings were fertilized with six nitrogen(N) and phosphorus(P) fertility regimes (no fertilization, 10mM NH_4NO_3, 50mM NH_4NO_3, 5mM KH_2PO_4, 10mM NH_4NO_3+5mM KH_2PO_4, and 50mM NH_4NO_3+5mM KH_2PO_4) three times a week for ten weeks.

The higher N-fertilization significantly increased mycorrhiza formation by greenhouse contaminant mycorrhizal fungi, but decreased N-fixation and P concentration in nodule tissues. P-fertilization significantly increased nodule and shoot dry weight, and P concentration in plant tissues. When N was highly fertilized, however, the P-fertilization effect disappeared in nodule P concentration but doubled in leaf P concentration. *A. diplophloeus* inoculation significantly increased diameter growth and CO_2 exchange rate, but decreased leaf dry weight. Our results suggest that the higher N- or P-fertilization affect nitrogenase activity and mycorrhizal development but the effects are changed by their interactions.

Key words : Alnus rubra, Frankia, Alpova diplophloeus, *N-fixation, Ectomycorrhiza, N-P-fertilization*

要　約

루브라 오리나무(*Alnus rubra* Bong.) 播種苗에 *Frankia* 窒素固定菌과 *Alpova diplophloeus* 外生菌根菌을 接種한 후 온실에서 10주 동안 길렀다. 이 苗木에 窒素와 燐酸을 여섯 가지로 조합(對照, 10mM NH_4NO_3, 50mM NH_4NO_3, 5mM KH_2PO_4, 10mM NH_4NO_3+5mM KH_2PO_4, 50mM NH_4NO_3 +5mM KH_2PO_4)하여 매주 3회씩 10주 동안 施肥하였다.

高濃度의 窒素 施肥는 온실에서 오염된 균에 의한 菌根 形成을 증가시켰으나, 窒素固定과 뿌리혹內 燐酸 濃度는 감소시켰다. 燐酸 施肥는 뿌리혹과 묘목의 지상부 생장, 그리고 植物體 組織內 燐酸 濃度를 증가시켰다. 한편 高濃度의 窒素 施肥는, 葉內 燐酸 濃度에 있어서는 燐酸 施肥 효과를 倍加시

컸으나, 뿌리혹內의 燐酸 濃度에 있어서는 燐酸 施肥 효과를 없어지게 하였다. *A. diplophloeus* 外生菌根菌 接種으로는 묘목의 直徑生長과 光合成이 증가되었으나 잎 생장은 감소하였다. 이 결과는 高濃度의 窒素나 燐酸 施肥가 窒素固定과 菌根 발달에 영향을 주지만, 그 효과는 窒素와 燐酸의 相互作用으로 일정하지 않게 됨을 나타낸다.

INTRODUCTION

Given the coevolved nature of the *Alnus*-mycorrhizal fungus-*Frankia* tripartite symbioses, it is possible that the three organisms interact synergistically to enhance the performance of the union. Even though the mycorrhizal one of the tripartite symbioses has been proved highly beneficial(Rose and Youngberg, 1981 ; Barea and Azcon-Aguilar, 1983 ; Gardner *et al.*, 1984), however, Koo *et al.*(1995) found that *Frankia* nodule is more important for *A. diplophloeus* mycorrhiza formation and their host growth than the mycorrhiza is for the nodule formation and the plant growth. Molina et al.(1994) stated that mycorrhizal development likewise improves growth and phosphorus status, but during the first year of seedling growth, increases in growth due to ectomycorrhizal development were small compared to the actinorrhizal benefit alone. N-fixing *Comptonia* and *Myrica* sp. well sustained phosphorus absorption in a local soil even without forming mycorrhizae(Berliner and Torrey, 1989). Visser *et al.* (1991) also demonstrated highly significant role of N-fixing nodules for the growth of actinorhizal and mycorrhizal Elaeagnaceae species outplanted on oil sands tailings.

In general, both actinorhizal and mycorrhizal symbioses are sensitive to fertilization, especially with high N and P. For example, N addition reduced nodule formation and nitrogenase activity (Burgess and Peterson, 1987), and even N-fixation in *Frankia* pure cultures(Tjepkema *et al.*, 1981), whereas P addition enhanced nodule formation (Seiler and McCromick, 1982). Mycorrhizae are well known for enhancing P uptake in infertile soils, but P fertilization often reduces mycorrhiza formation and symbiotic effectiveness(Thomas *et al.*, 1982). Nitrogen fertilization also induced reduction in ectomycorrhizal development(Brunner and Scheidegger, 1994 ; Dighton and Jansen, 1991).

Little is known about the effect of N and P on the development and function of alder tripartite symbioses. Therefore, the objective of this study was to examine the effects of N and P fertilization on mycorrhiza formation, nodulation, nitrogen fixation, photosynthesis and growth of nodulated red alder seedlings with/without *A. diplophloeus* (Zeller and Dodge) Trappe & Smith spore inoculation.

MATERIALS AND METHODS

Biological materials

Red alder seeds(Brown Seed Company, Vancouver, Washington) were selected for uniform size by dry sieving. *Frankia* was isolated by the filtration method(Benson, 1982) from nodules of one-year-old red alder seedlings collected at the US Forest Service Cascade Head Experimental Forest near the Oregon coast. The isolates were cultured for one month in N-free BAP liquid medium(Murry *et al.*, 1984). Sporocarps of *A. diplophloeus*, a hypogeous ectomycorrhizal fungus specific to alder(Molina, 1981), were collected under young red alder trees at the Cascade Head Experimental Forest and stored at -18℃ until used.

Seedling growth conditions

Rooting substrate was a 2:1:1 mixture of ca. 60-year-old alder forest soil, coarse vermiculite and peatmoss. Nutrients in the mixture were 0.252% N, 80ppm tatal P, 11ppm available P, 352ppm K, 1570ppm Ca and 400ppm Mg after autoclaving for 2hrs. Red alder seeds surface-sterilized in 30% H_2O_2 for 15 minutes were directly planted in leach tubes(3.2cm diameter x 20cm long, 165ml) and grown in a greenhouse with day/night temperatures of 24/18℃ with supplemental light from sodium vapor lamps for 16 hours a day. Light intensity was photosynthetic photon flux density(PPFD) of $510 \pm 26 \mu$

mol/㎡/s at noon on a clear day. Seeds started to germinate in five days and seedlings were thinned to one per tube after two weeks. After thinning, all seedlings were inoculated with one-month-old *Frankia* cultures by injecting 1㎕ packed cell volume diluted in 1ml of sterile distilled water per seedling. Half of the seedlings received five million *A. diplophloeus* spores diluted in 5ml of distilled sterile water per tube. Seedlings were watered to saturation every morning during the experiment.

Experimental design

The experiment was a $2 \times 3 \times 2$ factorial design with eight replications each combination of factors. The first factor was *A. dipophloeus* spore treatment with two levels : live or dead spore inoculation. The second factor was N-fertilization with three levels : no fertilization, 5ml of 10mM NH_4NO_3 and 5ml of 50mM NH_4NO_3 for each seedling. The third factor was P-fertilization with two levels : no fertilization and 5ml of 5mM KH_2PO_4. The fertilizations were applied three times a week from when the seedlings were 10-week-old. Seedlings were repositioned every other week to even out location effects in the greenhouse.

Data collection

When the seedlings were 20 weeks old, ten weeks after the fertilization, nitrogenase activity, seedling growth, and symbiosis developemnt were measured for eight seedlings per inoculation treatment. Apparent photosynthetic activity of a seedling was measured as CO_2 exchange rates with a LI6000 portable photosynthesis system (LI-COR, Inc, Lincoln, Nebraska) for 16cm² leaf area on the 4th leaf from the top of the seedling. At the same time stomatal conductance, initial transpiration rate, and initial internal CO_2 concentration were obtained from the equation stored in the system. During the measurements a respiration mask connected to a vacuum was used to remove CO_2 respired by the experimenter. To measure *in situ* acetylene reduction rates, we followed the procedures of Koo et al(1995). The physiological activities were measured under the

temperatures of 22 to 24℃ and PPFD of 480 to 520㎛ol/㎡/s between noon to 2pm.

Height, diameter and the dry weights of shoot and root were determined for each seedling. Dry weight was obtained after drying the tissues at 65℃ to a constant weight. A mean value of mycorrhiza formation for each plant was calculated from three root subsamples collected at 2.5 to 5cm, 7.5 to 10cm, and 12.5 to 15cm along the length of the root plug. From each subsample 50 to 100 short roots were counted and mycorrhizal formation was calculated as percentage.

Leaf areas were measured with a LI3100 Area Meter(LI-COR, Inc, Lincoln, Nebraska). Specific leaf dry weight was calculated by dividing the leaf dry weight with the leaf area of each plant. To determine the concentrations of total N and P in leaves, feeder roots(<ca. 2mm diameter) and nodules, samples from two seedlings within a treatment were combined and determined by autoanalyzer after Kjeldahl digestion. Feeder root samples were collected by carefully rubbing the dried root system.

Data analysis

Data were analyzed by GLM(General Linear Models) procedures in SAS(SAS Institute Inc. Cary, North Carolina) to test the treatment effects for each parameter. To test a significance between the means of the treatments Duncan's multiple range-test which is programmed in the MEANS statement in SAS was applied.

RESULTS

When they were 10 weeks old, prior to N and P ferilization, *A. diplophloeus* inoculated seedlings were significantly taller in height and greater in total nitrogenase activity than non-inoculated seedlings(Table 1). This was true even though all seedlings became mycorrhizal with greenhouse contaminating fungi(mostly *Thelephora* sp.), by ca. 50% on noninoculated seedlings. At the end of the experiment, all seedlings were mycorrhizal to a high percentage with either contaminant fungi or combination of *A. diplophloeus*+contaminant fungi. And the seed-

Table 1. Growth of *Alnus rubra* seedlings for ten weeks in a greenhouse before fertilization treatments. Seedlings were inoculated with *Frankia* only(F) or *Frankia* and *Alpova diplophloeus* spores(FA) when they were two weeks old.

Parameter	Inoculation	
	F	FA
Alpova diplophloeus mycorrhizae(%)	0.0	19.0±5.0
Total mycorrhizae(%)	54.0±7.2	39.6±6.8
Height(cm)	23.7±0.8	28.8±0.9**
Diameter(mm)	4.0±0.2	4.1±1.0
Shoot dry weight(g)	1.09±0.09	1.25±0.06
Root dry weight(g)	0.43±0.05	0.47±0.05
Shoot/Root ratio	2.82±0.28	2.80±0.19
Leaf dry weight(g)	0.50±0.03	0.56±0.04
Leaf area(cm^2)	119.0±8.0	128.0±10.0
Nodule dry weight(mg)	38.3±3.8	41.5±2.1
Total nitrogenase activity (μmol C_2H_2 reduced/g dry nodule/hr)	7.7±0.3	10.1±0.9*
Specific nitrogenase activity (μmol C_2H_2 reduced/g dry nodule/hr)	233.0±41.0	245.0±24.0
CO_2 exchange rate(mg CO_2/m^2/s)	0.22±0.03	0.23±0.02

Values are means of 9 or 10 samples ± standard error.
* and ** are significantly different at $p<0.05$ and $0<0.01$ by Duncan's test, respectively.

lings started to produce paler and smaller leaves probably due to depleted nutrients in the tubes. Nevertheless, the ANOVA showed *A. diplophloeus* inoculation continued to affect significantly total mycorrhiza formation, stem diameter, specific nitrogenase activity, leaf area and leaf dry weight, root dry weight, shoot/root ratio, and CO_2 exchange rate(Table 2). N-fertilization was also an influential treatment with significant effects on mycorrhiza formation, nodulation, N-fixation, leaf parameters, shoot dry weight, and N and P concentration in tissues. P-fertilization affected nodulation, specific nitrogenase activity, shoot dry weight, CO_2 exchange rate and P concentration in tissues. The interaction effect of N and P fertilization was significant in mycorrhizal development, nodulation, total nitrogenase activity, leaf area and P contents in tissues (Table 2).

Total mycorrhiza formation by both *A. diplophloeus* and contaminant fungi, 58 to 96%, was significantly increased by N-fertilization but not by P-fertilization(Fig. 1A, Table 2). *A. diplophloeus* mycorrhiza formation ranged 15 to 62% was significantly decreased by the higher

N-fertilization. However, when P was added, the N effect on mycorrhiza disappeared.

Nodule development measured by its dry weight was significantly decreased by the higher N-fertilization by 57%(Fig. 1D). On the other hand, the nodule development was increased by P-fertilization by 50%, but this P effect became non-significant by N-fertilization. Total nitrogenase activity was significantly and drastically reduced by the higher N-fertilization from 9.8 to 1.7μmol C_2H_2 reduced/plant/hr(Fig. 1E). Specific nitrogenase activity was significantly decreased by *A. diplophloeus* inoculation, and N- and P-fertilization(Fig. 1F). Thus, N-fertilization affected N-fixation differently from the other two treatments, by reducing both nodule developemnt and specific nitrogenase activity, while P-fertilization and the fungus inoculation increased nodule development and decreased specific nitrogenase activity.

CO_2 exchange rate(CER)(Fig. 3A), stomatal conductance(Fig. 3B) and transpiration rate(Fig. 3C) were significantly increased by *A. diplophloeus* inoculation(Table 2). CER was also significantly increased by P-fertilization. However,

Table 2. Significance for ANOVA for mycorrhiza formation, growth parameters, N-fixation rate and photosynthetic activities of mycorrhizal and actinorhizal *Alnus rubra* seedlings 10 weeks after N- and P-fertilization.

Parameter	A	N	P	AxN	NxP	AxP	AxNxP
Total mycorrhizae	**	**	— —	**	— —	— —	— —
A. diplophloeus mycorrhizae	— —	— —	— —	— —	**	— —	— —
Height	— —	— —	— —	— —	— —	— —	— —
Diameter	**	— —	— —	— —	— —	— —	— —
Nodule dry weight	— —	**	**	— —	**	— —	— —
Total nitrogenase activity	— —	**	— —	— —	*	— —	— —
Specific nitrogenase activity	*	**	*	— —	— —	— —	— —
Leaf area	**	*	— —	— —	*	— —	— —
Leaf dry weight	**	**	— —	— —	— —	— —	— —
Specific Leaf dry weight	— —	**	— —	— —	— —	— —	— —
Shoot dry weight	— —	**	**	— —	— —	— —	— —
Root dry weight	*	— —	— —	— —	— —	— —	— —
Shoot/Root ratio	*	— —	— —	— —	— —	— —	— —
CO_2 exchange rate	**	— —	*	— —	— —	— —	— —
Stomatal conductance	**	— —	— —	— —	— —	— —	— —
Transpiration rate	**	— —	— —	— —	— —	— —	— —
Internal [CO_2]	— —	— —	— —	— —	— —	— —	— —
Leaf N	— —	**	— —	— —	— —	*	— —
Root N	*	**	— —	— —	*	— —	— —
Nodule N	— —	— —	— —	— —	— —	— —	— —
Leaf P	*	**	**	— —	**	— —	— —
Root P	— —	— —	**	— —	— —	— —	— —
Nodule P	*	**	**	— —	**	— —	— —

A : *Alpova diplophloeus* spore inoculation, N : nitrogen fertilization, P : phosphorus fertilization.
'— —' is not significant. '*' and '**' are significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

internal CO_2 concentration was not significantly affected by any treatment(Fig. 3D).

Height growth was not significantly affected by any treatment(Fig. 1B, Table 2), whereas diameter growth was significantly increased by *A. diplophloeus* inoculation from 6.2 to 6.5mm (Fig. 1C, Table 2). Leaf development measured as leaf dry weight and leaf area was significantly decreased by *A. diplophloeus* inoculation and the higher N-fertilization(Figs. 2A and 2B). Specific leaf dry weight was significantly decreased only by the higher N-fertilization(Fig. 2C). Shoot dry weight was significantly decreased by the higher N-fertilization but increased by P-fertilization (Fig. 2D). Root dry weight was significantly increased by *A. diplophloeus* inoculation(Fig. 2E), resulting in significantly reduced shoot/root ratio(Fig. 2F).

N concentration in the leaf and root tissues were significantly increased by the higher N-fertilization but the N in the nodules was not affected by the treatment(Tables 2 and 3). The higher N-fertilization effect in the root N was more prominant than in the leaf N. However, nodule N was not affected by any treatment.

The P concentration in the leaf tissues was significantly increased but decreased in the nodule by the higher N-fertilization(Tables 2 and 3). The P concentration significantly increased in the leaf, root and nodule tissues by P-fertiliztion. The P in the root was increased by ca. 100 to 150% by the P-fertilization, whereas the P in the leaf and nodules were increased by only 8 to 80%. The P-fertilization effect in the leaf was doubled by the higher N-fertilization. On the other hand, nodule P concentration was gradually decreased as the N-fertilization level increased and the degree of the P decrease was
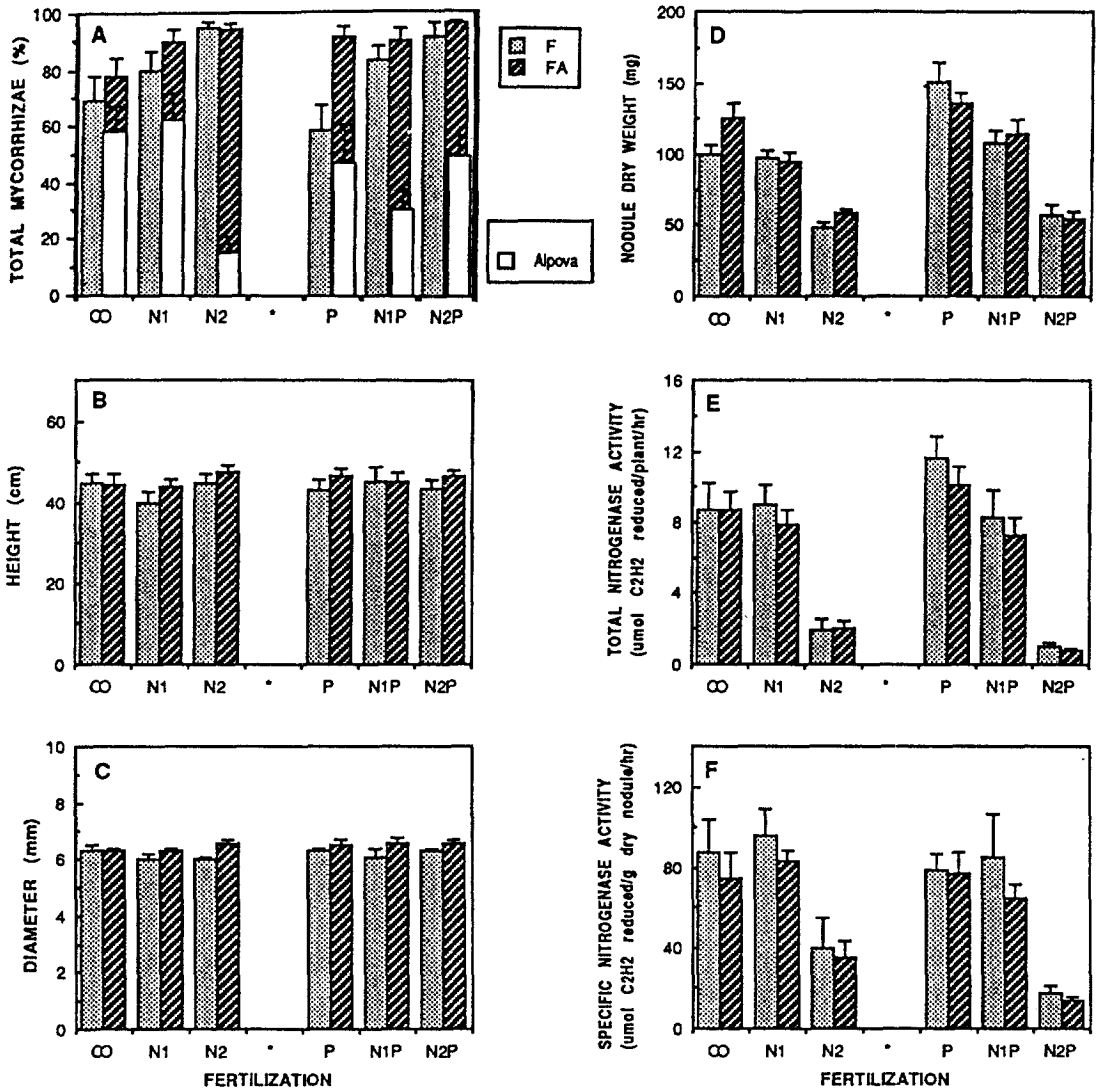
Fig. 1. A-F. N- and P-fertilization effects on the mycorrhiza formation (A) ; open bars show *Alpova diplophloeus* mycorrhiza percentage, height (B), diameter (C), nodule dry weight (D), total nitrogenase activity (E) and specific nitrogenase activity (F) of 20-week-old *Alnus rubra* seedlings grown in a greenhouse. In the legend, F=*Frankia* pure culture and dead *Alpova* spore inoculation ; FA=*Frankia* and live *Alpova* spore inoculation. On the horizontal, CO=no fertilization ; N1=5ml of 10mM NH_4NO_3 ; N2=5ml of 50mM NH_4NO_3 P=5ml of 5mM KH_2PO_4, N1P=combination of N1 and P treatments, N2P=combination of N2 and P treatments. Seedlings received the fertilizer three times a week for 10 weeks.

greater with P-fertilization than without P.

DISCUSSION

These results support other studies showing

that N-fertilization decreases N-fixation but P-fertilization increases it and that mycorrhizae improve plant growth. But a positive ecto-mycorrhizal effect provided by *A. diplophloeus* in 10 weeks old seedlings became unclear because
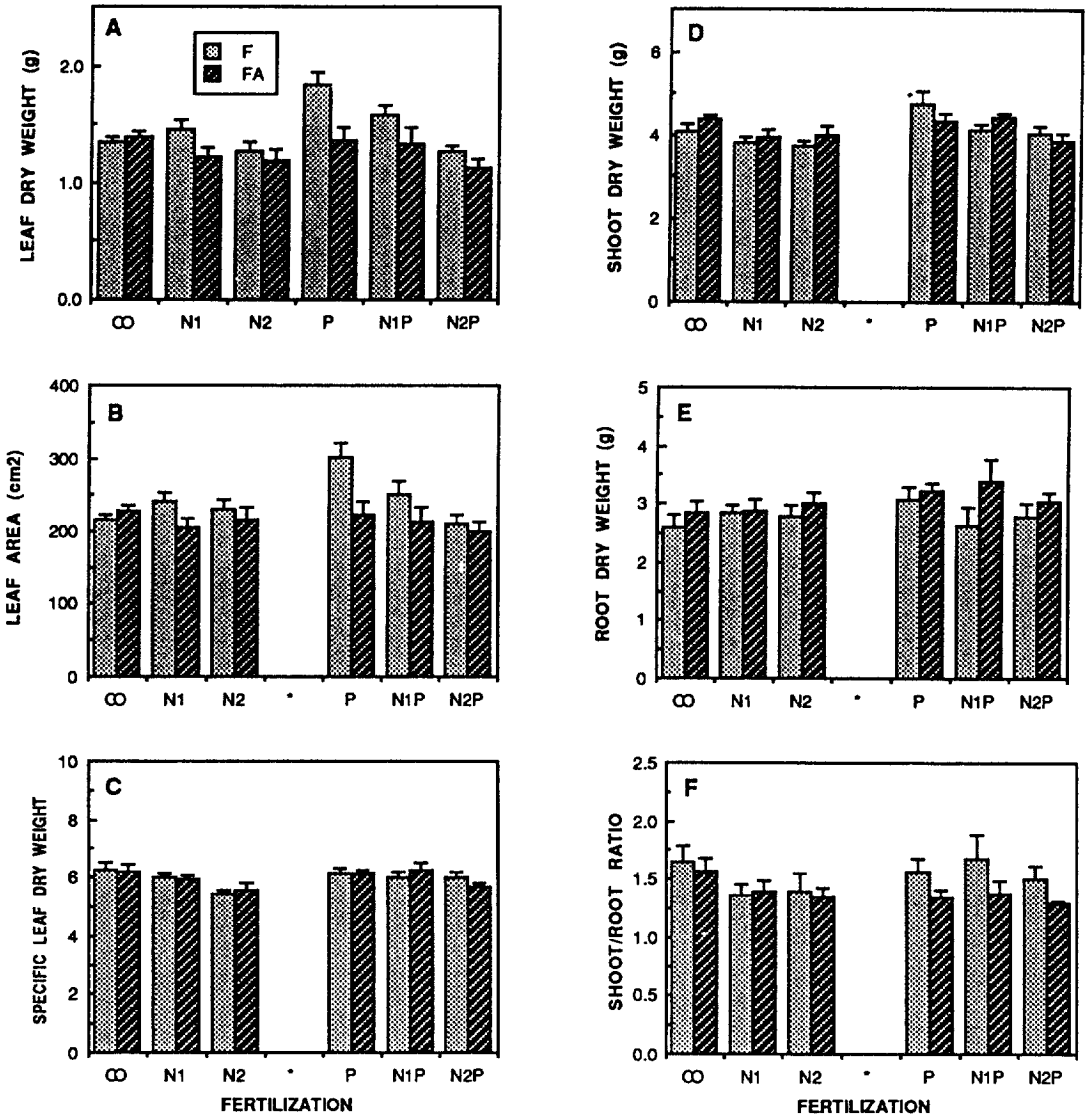
Fig. 2. A-F. N- and P-fertilization effects on the leaf dry weight (A), leaf area (B), specific leaf dry weight (C), shoot dry weight (D), root dry weight (E) and shoot/root ratio (F) of 20-week-old *Alnus rubra* seedlings grown in a greenhouse.

of greenhouse contaminating fungi.

N-fertilization effects on N-fixation could be shown only when the concentration and the period of the fertilization were sufficiently high or long. The ten-week-long and strong N-fertilization treatment greatly reduced nodule formation, total nitrogenase activity and specific nitrogenase activity. This negative effects of N-fertilization on N-fixation were explained by damaged *Frankia* vesicles in the nodules(Huss-Danell *et al.*, 1982)

and by reduced numbers of endophyte vesicles per unit area of the infected cells(Burgess and Peterson, 1987).

However, it is possible that N-fertilization reduces N-fixation by hindering nitrogenase activity. The reduced P concentration in nodules by N-fertilization indicates that carbohydrate metabolism and energy transfer in nodules was reduced. At the same time unaffected N concentrations and reduced specific nitrogenase activity
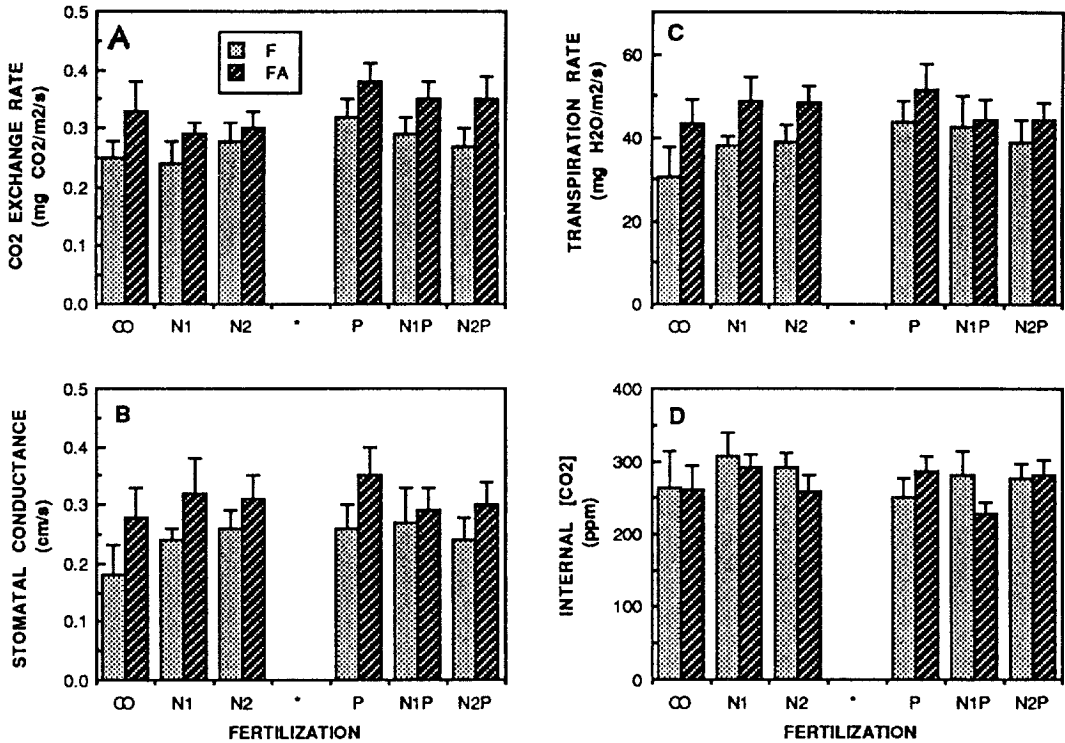
Fig. 3. A-D. N- and P-fertilization effects on the CO_2 exchange rate (A), stomatal conductance (B), transpiration rate (C) and internal CO_2 concentration (D) of 20-week-old *Alnus rubra* seedlings grown in a greenhouse.

Table 3. N and P concentration in the leaf, root and nodule tissues of 20-week-old *Alnus rubra* seedlings fertilized for 10 weeks. Seedlings were inoculated with *Frankia* only(F) or *Frankia* and *Alpova diplophloeus* spores(FA) when they were two weeks old.

Fertilization	Leaf		Root		Nodule	
	F	FA	F	FA	F	FA
			N(%)			
CO	1.82±0.05	1.73±0.05	1.79±0.10	1.59±0.03	2.58±0.21	2.73±0.14
N1	1.80±0.03	1.74±0.09	1.81±0.14	1.83±0.03	2.41±0.13	3.06±0.20
N2	1.93±0.02	2.03±0.08	2.88±0.04	2.33±0.14	2.80±0.11	2.77±0.17
P	1.69±0.03	1.87±0.04	1.60±0.02	1.63±0.09	2.52±0.04	2.79±0.20
N1P	1.80±0.05	1.78±0.09	1.94±0.02	1.82±0.06	3.07±0.21	2.55±0.23
N2P	1.94±0.03	2.13±0.04	2.24±0.04	2.38±0.08	2.91±0.17	3.06±0.18
			P(%)			
CO	0.10±0.01	0.11±0.01	0.15±0.01	0.14±0.01	0.16±0.01	0.15±0.01
N1	0.09±0.01	0.12±0.01	0.12±0.01	0.15±0.01	0.13±0.01	0.14±0.01
N2	0.11±0.01	0.12±0.01	0.14±0.01	0.13±0.01	0.12±0.01	0.11±0.01
P	0.14±0.01	0.14±0.01	0.32±0.03	0.28±0.01	0.19±0.01	0.18±0.01
N1P	0.15±0.01	0.15±0.01	0.34±0.03	0.32±0.01	0.20±0.01	0.18±0.01
N2P	0.18±0.01	0.21±0.01	0.29±0.01	0.33±0.02	0.13±0.01	0.13±0.01

CO=no fertilization ; N1=5ml of 1mM NH_4NO_3 fertilization ; N2=5ml of 50mM NH_4NO_3 ;
P=5mM KH_2PO_4 fertilization ; N1P=N1+P ; N2P=N2+P.
Values are the means of three to four samples±standard error.

in the nodule by the N-fertilization may indicate that proteins(nitrogenase) exist in the tissue but they are not active. This may suggest that plants allocate minerals and carbohydrate to a sink demand within a plant system. This supports Huss-Danell and Hahlin(1988) who addressed that ammonium treatment reduced translocation of carbon to *Frankia* vesicles in nodules to cause a reduced metabolic rate in the nodule.

Some experimental application of nitrogen caused reductions in ectomycorrhizal infection (Wallander and Nylund, 1992, Brunner and Sheidegger, 1994) and in fruiting body production of ectomycorrhizal fungi(Brandrud, 1995). However, N-fertilization increased total ectomycorrhiza formation by greenhouse contaminating fungus such as *Thelephora terrestris*, but only high level N-fertilization decreased *A. diplophloeus* mycorrhiza formation in our experiment. This supports other studies that nitrogen has caused increased mycorrhiza infection in heather(Carpom *et al.*, 1995) and in Douglas-fir(Gorissen, *et al.*, 1991). The result that *Frankia* nodule formation increased *A. diplophloeus* mycorrhiza development in alder seedlings(Koo, *et al.*, 1995) also supports the positive effects of nitrogen on mycorrhizae.

Mycorrhiza response to P-fertilization differs depending on host plants, fungal species and soil characteristics. In our experiment, even though P-fertilization increased the P concentration in feeder roots by over 100%, it did not significantly decrease mycorrhiza formation. Generally, P fertilization or high soil P levels could substitute for beneficial mycorrhizal effects on plant growth in an ectomycorrhizal plant(Thomas *et al.*, 1982) and in vesicular-arbuscular mycorrhizal plants(Pacovsky *et al.*, 1986 ; Asimi *et al.*, 1980). On the one hand, however, *Thelephora terrestris* and *Laccaria laccata* mycorrhizae infection on *Pinus silvestris* was not significantly changed but *Hebeloma crustuliniforme* mycorrhizae formation was increased by high P-fertilization in a greenhouse(Tyminska *et al.*, 1986). On the other hand, *Thelephora terrestris* mycorrhizae on *Picea sitchensis* even increased slightly in high P-soil, although the fungus enhanced host growth only in low P-soil(Thomas *et al.*, 1982). Thus,

P-fertilization effects on ectomycorrhizae are less clear cut.

P-fertilization effects on mycorrhiza formation may depend on the degree of the demand by the symbionts or on the N/P ratio in the soil. P deficiency occurs as an N-fixing plant grows: as total plant N increases, P is depleted in the soil. Thus, high soil P effects on mycorrhiza formation can diminish as the plant grows to deplete the soil P. For example, in nodulated soybean, vesicular arbuscular mycorrhizal infection in the high P-treatment was delayed by 14 days compared to the low P-treatment, and both high and low P treated seedlings had similar root P concentration and mycorrhiza formation(Fredeen and Terry, 1988).

Frankia was more critical for seedling growth than mycorrhizal fungi(Koo, *et al.*, 1995). In preliminary *Frankia* inoculation trials, the authors found that non-nodulated and non-N-fertilized red alder seedlings remained stunted and formed only trace of *A. diplophloeus* ectomycorrhizae later with a thinn mantle(unpublished data). Furthermore, when *Frankia* inoculation was delayed, mycorrhiza formation remained low until nodulation began, and the length of feeder root colonized by mycorrhizal fungi was shorter than when the symbiont was inoculated earlier(Koo, unpublished data). Sempalvalan *et al.*(1995) also stated that *Glomus* mycorrhizal colonization was lower when *Frankia* inoculation was delayed in *Casuarina* species. Although Visser *et al.*(1991) did not differentiate between the relative contributions of *Frankia* and mycorrhizae to the growth of actinorhizal *Elaeagnus* seedlings, the growth of the seedlings outplanted on oil sands tailings was strongly correlated($r^2 = 0.88$) with nodule dry weight. In this regard, further investigation is needed on how *Frankia* nodulation mediates mycorrhiza development on actinorhizal plants.

Fredeen and Terry(1988) demonstrated that CO_2 exchange rates per unit leaf area did not differ between mycorrhizal and nonmycorrhizal soybean plants. They suggested that VA mycorrhizal colonization increased production of photosynthate due to an increase in the rate of leaf surface expansion. In our experiment, however, *A.*

diplophloeus inoculated seedlings were smaller in leaf growth but larger in shoot and root growth as well as photosynthetic activity. This can be explained by the roles of mycorrhizae in nutrient exploitation, effects of limited rooting substrate and mechanisms of photosynthate sink demand. Before starting the fertilization treatment, *A. diplophloeus* inoculated seedlings were generally larger in all the growth parameters. But the growth difference gradually decreased with age (data were not shown), probably due to depletion of soil nutrients. As a result, the inoculated plants produced even smaller leaves than the noninoculated ones at the end of the experiment. It would seem that maintaining the larger biomass of non-photosynthetic tissues induce compensating higher photosynthetic rates in smaller leaves.

In conclusion, N-fertilization inhibited nitrogenase activity in red alder but promote total mycorrhiza formation. The N-fertilized host plant allocated the unused P in nodules, which had been used for energy transfer for N-fixation process otherwise, to the leaf where photosynthesis and metabolism were active. In contrast, P fertilization promotes N-fixation, but its effect was strongly controlled by the higher N-fertilization. *A. diplophloeus* mycorrhizae could increase seedling growth but slightly only in some parameters while rooting substrate was not depleted. This may mean the importance of mycorrhizae in alder will increase in the nature as the N status of the plant improves by N-fixation.

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REFERENCES

1. Asimi, S., P.V. Gianinazzi and S. Gianinazzi. 1980. Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhiza and *Rhizobium* in soybeans. Can. J. Bot. 58 : 2200-2205.

2. Barea, J.M. and C. Azcon-Aguilar. 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. Adv. Agron. 36 : 1-54.

3. Benson, D.R. 1982. Isolation of *Frankia* strains from alder actinorhizal root nodules. Appl. Environ. Microbiol. 44 : 461-465.

4. Berliner, R. and J.G. Torrey. 1989. On tripartite *Frankia*-mycorrhizal associations in the Myricaceae. Can. J. Bot. 67 : 1708-1712.

5. Brandrud, J.E. 1995. The effects of experimental nitrogen addition on the ectomycorrhizal fungus flora in an oligotrophic spruce forest at Gradsjon, Sweden. Forest Ecology and Management 71 : 111-122.

6. Brunner, I. and C. Scheidegger. 1994. Effects of high nitrogen concentrations on ectomycorrhizal structure and growth of seedlings of *Picea abies*(L.) Karst. New Phytol. 129 : 83-95.

7. Burgess, D. and R.L. Peterson. 1987. Effect of nutrient conditions on root nodule development in *Alnus japonica*. Can. J. Bot. 65 : 1658-1670.

8. Carpon, S.J.M., W. Song, D.J. Read and J.A. Lee. 1995. The effect of repeated nitrogen fertilization on mycorrhizal infection in heather(*Calluna vulgaris*(L.) Hull. New Phytol. 129 : 605-609.

9. Dighton, J. and A.E. Jansen. 1991. Atmospheric pollutants and ectomycorrhizae : more questions than answers? Environmental Pollution 73 : 179-204.

10. Fredeen, A.L. and N. Terry. 1988. Influence of vesicular-arbuscular mycorrhizal infection and soil phosphorus level on growth and carbon metabolism of soybean. Can. J. Bot. 66 : 2311-2316.

11. Gardner, I.C., J.M. Clelland and A. Scott. 1984. Mycorrhizal improvement in nonleguminous nitrogen fixation associations with particular reference to *Hippophae rhamnoides* L. Plant and Soil 78 : 189-199.

12. Gorissen, A., N.N. Joosten and A.E. Jansen. 1991. Effects of ozone and ammonium sulphate on carbon partitioning to mycorrhizal roots of juvenile Douglas-fir. New

Phytol. 119 : 243-250.

13. Huss-Danell, K., A. Sellstedt, A. Jlower-Ellis. and M. Sjostrom. 1982. Ammonium effects on function and structure of nitrogen fixing root nodules of *Alnus incana*(L.) Moench. Planta 156 : 332-340.

14. Huss-Danell, K. and A.-S. Hahlin. 1988. Nitrogenase activity decay and energy supply in *Frankia* after addition of ammonium to the host plant *Alnus incana*. Physiologia Planta. 74 : 745-751.

15. Koo, C.D., R.J. Molina and S.L. Miller. 1995. Effects of light and inoculation of *Frankia* and *Alpova diplophloeus* on the tripartite symbioses development in *Alnus rubra* Bong. seedlings. Jour. Korean For. Soc. 84 : 306-318

16. Molina, R. 1981. Ectomycorrhizal specificity in the genus *Alnus*. Can. J. Bot. 58 : 325-334.

17. Molina, R., D. Myrold and C.Y. Li. 1994. Root symbioses of red alder : Technological opportunities for enhanced regeneration and soil improvement. *In* The Biology and Management of Red Alder. edited by D.E. Hibbs and D.S. DeBell and R.F. Tarrant. Oregon State University Press, Corvallis, Oregon. pp23-46.

18. Murry, M.A., M.S. Fontaine and J.G. Torrey. 1984. Growth kinetics and nitrogenase induction in *Frankia* sp. HFPArI3 grown in batch culture. Plant and Soil 78 : 61-78.

19. Pacovsky, R.S., J.J. Bethlenfalvay and E.A. Paul. 1986. Comparison between P-fertilized and mycorrhizal plants. Crop Sci. 26 : 151-156.

20. Rose, S.L. and C.T. Youngberg. 1981. Tripartite association in snowbrush(*Ceanothus vulutinus*) : Effect of vesicular-arbuscular my-corrhizae on growth, nodulation, and nitrogen fixation. Can. J. Bot. 59 : 34-39.

21. Seiler, J.R. and L.H. McCormick. 1982. Effects of soil acidity and phosphorus on the growth and nodule development of black alder. Can. J. For. Res. 12 : 576-581.

22. Sempavalan, J., C.T. Wheeler and J.E. Hooker. 1995. Lack of competition between *Frankia* and *Glomus* for infection and colonization of roots of *Casuarina equisetifolia*(L.). New Phytol. 130 : 429-436.

23. Thomas, G.W., C.A. Clarke, B. Mosse and R.M. Jackson. 1982. Source of phosphate taken up from two soils by mycorrhizal (*Thelephora terrestris*) and non-mycorrhizal *Picea sitchensis* seedlings. Soil Biol. Biochem. 14 : 73-75.

24. Tjepkema, J.D., W. Ormerod and J.G. Torrey. 1981. Factors affecting vesicle formation and acetylene reduction(nitrogenase activity) in *Frankia* sp. CpI1. Can. J. Microbiol. 27 : 815-823.

25. Tyminska, A., J. Le Tacon and J. Chadoeuf. 1986. Effect of three ectomycorrhizal fungi on growth and phosphorus uptake of *Pinus silvestris* seedlings at increasing phosphorus levels. Can. J. Bot. 64 : 2753-2757.

26. Visser, S., R.M. Danielson and D. Parkinson. 1991. Field performance of *Elaeagnus commutata* and *Shepherdia canadensis*(Elaeagnaceae) inoculated with soil containing *Frankia* and vesicular-arbuscular mycorrhizal fungi. Can. J. Bot. 69 : 1321-1328.

27. Wallander, H. and J.E. Nylund. 1991. Effects of excess nitrogen on carbohydrate concentration and mycorrhizal development of *Pinus sylvestris* L. seedlings. New Phytol. 119 : 405-411.