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Mycorrhizal Development and Growth Stimulation of *Pinus thunbergii* Seedlings Inoculated with *Pisolithus tinctorius* at Two Soil Mixtures Treated with Six Nitrogen Levels[1]*

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培壤土 및 窒素 施肥 水準이 모래밭 버섯菌(*Pisolithus tinctorius*)을 接種한 海松(*Pinus thunbergii*)苗의 生長과 菌根 形成에 미치는 影響[1]

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ABSTRACT

This study was carried out for observation of growth, mycorrhizal formation and nutrient absorption of *Pinus thunbergii* seedlings treated with two soil mixtures and various nitrogen levels after inoculation with mycorrhizal fungus, *Pisolithus tinctorius*.

1. Seedlings grown on vermiculite applied with 50-150 μg/ml nitrogen levels were well developed with pinnate type and cluster-like mycorrhizae. But seedlings on sandy loam had monopodial type in addition to the above-mentioned two types.
2. Optimum fertilization level for mycorrhizal formation is 50 or 150 μg/ml N that showed best mycorrhizal formation of 86.4(±3.14)% or 73.0(±7.21)%, respectively, but increased nitrogen levels decreased formation of mycorrhizal short roots.
3. Seedlings applied with 450 μg/ml nitrogen level decreased in net assimilation rate (NAR) and crop growth rate(CGR) during early growth of the seedlings, and they were increased since Aug. when nutrient application was stopped.
4. Inorganic nutrient absorption was increased more in seedlings grown on vermiculite and inoculated growth medium than those grown on sandy loam and noninoculated one, and it was gradually increased with increasing nitrogen increasing nitrogen level until 350μg/ml. But 450μg/ml nitrogen level rather reduced absorption of nutrient.

Key words : Pinus thunbergii ; Pisolithus tinctorius ; *ectomycorrhizal fungi* ; *vermiculite*.

要 約

本 硏究는 海松苗木에 모래밭버섯(*Pisolithus tinctorius*) 菌을 人工 接種한 後 두개의 混合土와 6개의

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窒素水準으로 處理된 實生苗의 生長과 菌根 形成 그리고 養料吸收關係를 推定키 爲해 實施되었다.

1) 50-150μg/ml N.로 施肥된 vermiculite에서 자란 實生苗는 pinnate type과 cluster-like type이 잘 發達하였으나, 砂質壤土는 monopodial, cluster-like type 그리고 pinnate type이 發達하였다.

2) 菌根形成의 適正施肥水準은 50 또는 150μg/ml로 菌根形成이 各各 86.4(±3.14)%와 73.0(±7.2)%였다. 그러나 窒素水準이 增加하면 菌根形成이 減少하였다.

3) 450μg/ml N.로 施肥된 實生苗는 純 同化率(NAR)과 個體生長率(CGR)이 生長初期에는 減少하였으나 窒素施肥가 멈춘 8月 以後에는 다시 增加하였다.

4) 實生苗의 無機養料 吸收는 砂質壤土 및 無接種區에 비해 vermiculite와 菌根處理區에서 增加하였다. 그리고 吸收率은 350μg/ml 窒素水準 까지는 窒素施肥 增加에 따라 漸進的으로 增加하였으나 450μg/ml 窒素水準에서는 도리어 吸收가 制限되었다.

INTRODUCTION

Pinus thunbergii is mainly distributed on the south coast of korea and a part of Japan, and this species is important forest resource that contribute to rural people with wood fiber, fuel and shelter-belt in the coast. But it requires ectomycorrhizal fungal association on its feeder roots for normal growth and development in natural forest enviroment.

Formation of mycorrhizal short roots in various important forest species must be accomplished because it promotes beneficial mechanism such as stimulation of nutrient absorption[4], detention of pathogenic infection by production of antibiotics, decrease of injury by nematodes, buffer of soil toxin by formation of fungal mantle[9,10], of host resistance to drought and extreme soil pH, and promotion of seedling growth by production of growth regulator[16,22].

These chlorophyllous host plants supply the symbiotic fungi with exudates such as carbohydrate, amino acid, vitamines and other organic substances.

The *Pisolithus tinctorius* of these species has shown a broad host range and wide distribution including korea and 38 states of U.S.A.[7,11] Seedling inoculated with specific ectomycorrhizal fungi can increase survival and growth of seedlings planted on cutover lander, former treeless areas, and disturbed or adverse sites such as raining spoils.

McArdle (1932)[13] showed that mycorrhizae on conifers had been well formed in cultures where nitrogen was include in the nutrient solution. Fortintin[2] et al. (1980) and Warrington[23] (1982) found that typical ectomycorrhizae with mantle and Hartignet were obtained within 5 days after inoculation with *P. tinctorius*. Marx (1977)[12] reported that development of ectomycorrhizae depend on sucrose concentration of root cell if high level of phosphorus was fertilized in soil, and that mycorrhizal formation was significantly inhibited when sucrose deficiency was generated by high level of nutrient on rhizosphere.

The purpose of this experiment was to estimate the growth stimulation and mycorrhizal formation of *P. thunbergii* seedlings inoculated with *P. tinctorious* at different soil growth medium and to compare nutrient absorption between two treatments inoculated with or without *P. tinctorius* ectomycorrhizal fungi.

MATERIALS AND METHODS

This study was accomplished in a glasshouse at the Chonnam National University located at Kwang Ju, Chonnam province. Conditions within the greenhouse followed the guidelines developed by Tinus and McDonald[22].

P. tinctorius-ectomycorrhizae super strain #250 was obtained from Institute for Mycorrhizal Research and Development in Athens, Georgia, U.S.A. This inoculum was cultivated in sterile petridish with modified Melin-Norkrans (MMN)

medium at 25℃ for about two wks, and was transferred to preautoclaved one-liter glass bottle filled with 750ml mixture of ground peat moss passed a 20-mesh sieve and coarse vermiculite passed a 2 mm sieve(1 : 24 v/v).

Each bottle already received 400ml MMN solution was stoppered with cotton and autocleaved for 30 minutes at 15psi(121℃) The inoculum grown in peat-vermiculite substrates using techniques described by Marx[10] (1969) was removed from the bottles and bundled in cheesecloth and rinsed under deionizer water for two minutes to remove excess nutrient out of the inoculum. Excessive water was removed by squeezing the inoculum in cheesecloth, and then the inoculum was stored in refrigerator(4℃) until accomplished inoculation into pots. 250μg/ml -polyethylene pots used container of *P. thunbergii* seedlings were inoculated by throughly up about 3 cc of the inoculum into soil growth medium that prepared with two kinds of mixture soils such as vermiculite and peat moss(1 : 1 v/v) and sandy loam and peat moss(1 : 1 v/v).

The sandy loam has been steam-sterilized for 5 hours at 105℃ before the inoculation and vermiculite has been fermigated with cylon for seven days and then volatilized as many as seven days.

The seedling pots have been taken to laboratory from greenhouse and carefully washed by tap water. Mycorrhizal roots were cutted with scissors in water and then transfered into FAA fixation solution. Removed by the vaccum treatment the air in root tissue, mycorrhizae root tips were received tertiary butyl alcohol schedule in order to dehydration of root tips before they vere embedded in paraffin wax. And then mycorrhizae were serially sectioned 10-12um by the rotary microtome. Präparat was made up double-stained -cutting tips to processed 0.5% safranin and 0.5% astrablue[8].

Plant Analysis[25]

Plant was collected from each of the container where experimental seedlings were grown under the same treatment and then bulked together for analysis on Sept.30.

As soon as sampling, all plant samples were oven dried at 80℃ for 24 hrs in order to prohibit plant metabolism and then all samples classified with two parts, leaf and root, were oven dried at 80℃ for 24 hrs. They were stored at desiccator for 48 hrs. Sample grounded by wind mill passed 20-mesh sieve.

Analysis of inorganic matters, K, Na and Ca were accomplished by atomic absorption spectrophometer(Model type : Pyeunicam 9000 AAS), total N. by Kjeldahl method, P. by Vanadate method (foliar). Data were collected for 16 wks. from June to Sept. They are divided into daily and major observation. Growth analysis were completed by Hoshino (1976)[6] methods.

A. Crop growth rate(CGR) $= dw/dt$

$= Wp_2 - Wp_1 / t_2 - t_1 \ (g/m^2/day)$.

B. Net assimilation rate(NAR) $= (Wp_2 - Wp_1)\ (L_n L_2 - L_n L_1 / (t_2 - t_1)(L_2 - L_1)\ (g/m^2/day)$.

C. Specific leaf area(SPA) $= L/WL\ (m^2/g)$.

Elucidation of used abbreviated form

t=time. Wp=individual total dry weight.

L=leaf area. WL=leaf dry weight.

Analysis of variance[24] were made on data investigated by the treatment passed through F -test and then figured it as a way of growth curve of Sekijima[20] (1968).

RESULTS AND DISCUSSION

Mycorrhizal Observation

Mycorrhizal formation of 5-month-old *P. thunbergii* seedlings after sowing on the greenhouse was significantly affected by nitrogen concentration and even soil growth medium (Table 1).

Fertilizer concentrations and soil growth medium types affected a significant effect on the number and the percent of mycorrhizal short roots. Seedlings grown on vermiculite fertilized with 50 ug/ml N. level showed high percent of mycorrh-

Table 1. Summarized characterization of *Pinus thunbergii* plus *Pisolithus tinctorius* mycorrhizae.

Treatment		Mycorrhizal Morphology			Ectomy-corrhizae	Rhizo-morph condition
		Mono-podial type	Fern-like type	Cluster-like type	%	
Vermiculite	0	+	+++	++	72.45	++
+p.t.	50	−	+++	+++	89.54	+++
	150	−	+	+++	83.26	+++
	250	−	+++	++	56.19	++
	350	+	++	++	46.33	+
	450	+	+	−	47.29	+
Sandy loam	0	++	+++	+	65.27	+
+p.t.	50	++	++	+++	80.26	+++
	150	+++	++	++	65.78	++
	250	−	++	++	59.04	+
	350	+	++	+	31.21	+
	450	−	+	−	25.33	−

− absent + present +++ present in large numbers
++ present in small numbers ++++ mass incidence

izal formation and they were higher by about 10% than that of sandy loam in equal N. level. Mycorrhizal morphology was mainly accomplished with pinnate and cluster-like type in vermiculite and with monopodial and pinnate on sandy loam, so that there were significant differences in seedling growth result from mycorrhizal short roots.

Sandy loam soil showed lower mycorrhizal short roots than those of vermiculite. But medium application(150μg/ml) of nitrogen level on *P. thunbergii* seedling pots did not show significant difference according to the soil growth mediums. High nitrogen levels on vermiculite has shown that mycorrhizal short root on *P. thunbergii* seedlings was restricted by accumulation of nitrogen concentration.

The optimum level of nitrogen in mycorrhizal formation in this experiment was observed that it was 50μg/ml N. level on vermiculite and sandy loam soil, respectively. But total dry weight of seedlings was increased from 50ug/ml to 350ug/ml N. levels. Development of mycorrhizal short root (Table 2.) on primary lateral root showed significant increase on seedlings treated with mycorrhizal fungi. Slankes (1973)[19] suggested that auxins produced by mycorrhizal fungi increased the number of feeder root. Mycorrhizal formation rates in each treatment block were calculated by percent of mycorrhizal short root[10] caused by infection of feeder root to total short roots[3,9,17,18].

Short root and ectomycorrhizal development according to seasonal nitrogen fertilization levels has shown to be significantly increasing during June to July, but decreasing in August because of accumulation of nitrogen sources, NH_4NO_3 level in soil and stress of environment by high temperature in greenhouse. But later in September when the application of nitrogen stopped, they were significantly increased again(Fig. 1).

Such phenomenon is similar to the results reported by Oh (1986)[15].

Growth of *P. thunbergii* Seedlings

In this study, seedlings inoculated with *P. tinctorius* showed that the stem length was significantly promoted than that of non-inoculated seedlings. But seedling height appeared to be influenced more or less by application of N. Accordingly, 150μg/ml N. level enhanced seedling growth and mycorrhizae formation[1,18].

P. thunbergii seedlings applied with high nitrogen level of 450μg/ml were even inhibited not only formation of mycorrhizae in short root but

Table 2. Length and number of lateral roots and number of short roots and mycorrhizae per 1cm lateral roots of *Pinus thunbergii* seedlings grown on sandy loam and vermiculite soil inoculated by *Pisolithus tinctorius* treated with various nitorgen levels.

Inoculation, Soil medium, N. Concentration.		No. of primary lateral roots/plant [1]	No. of short roots/cm	No. of mycorrhizae /cm	Total primary lateral root length/plant
Vermiculite	0	26.33^{o}	18.30^{b}	11.08^{cd}	118.17^{j}
+ Pt	50	38.00^{a}	19.35^{a}	17.00^{a}	159.20^{d}
	150	38.00^{a}	17.65^{bc}	14.30^{b}	182.57^{g}
	250	33.33^{def}	22.11^{fg}	7.20^{fg}	193.20^{e}
	350	34.66^{bcd}	22.43^{fg}	6.09^{ghijk}	172.20^{b}
	450	35.00^{abc}	10.09^{jkl}	4.80^{klm}	150.50^{c}
Sandy loam	0	37.00^{ab}	14.09^{cd}	10.45^{cde}	120.0^{hj}
+ Pt	50	35.00^{abcd}	13.72^{cde}	11.36^{c}	177.43^{eb}
	150	37.00^{ab}	12.00^{fghi}	7.53^{f}	190.20^{a}
	250	35.55^{abcd}	12.05^{fgh}	6.20^{fghijk}	140.20^{ef}
	350	32.67^{efg}	10.23^{jkl}	3.40^{pqr}	132.07^{g}
	450	27.67^{mn}	10.09^{jkl}	2.25^{rs}	123.83^{hi}
Vermiculite	0	32.33^{efgh}	10.23^{jkl}	4.7^{klm}	135.47^{f}
+Non. Pt	50	28.00^{m}	14.34^{c}	6.11^{fgh}	143.07^{c}
	150	31.00^{ij}	12.53^{f}	6.71^{fgh}	176.13^{ab}
	250	31.67^{fghi}	14.07^{cde}	4.61^{jklm}	165.40^{bc}
	350	28.67^{l}	12.07^{fgh}	4.61^{jklm}	141.33^{ef}
	450	30.00^{jk}	10.36^{j}	1.31^{s}	122.63^{hi}
Sandy loam	0	25.00^{p}	8.61^{mno}	6.26^{fghij}	130.30^{g}
+Non. Pt	50	27.00^{n}	9.27^{jklmn}	3.74^{nopq}	136.50^{ef}
	150	25.00^{p}	9.43^{jklmn}	4.03^{mnop}	136.27^{ef}
	250	25.00^{p}	8.38^{o}	1.13^{s}	144.00^{e}
	350	25.67^{o}	9.88^{jklm}	1.22^{s}	140.10^{ef}
	450	25.33^{o}	6.14^{o}	0.75^{s}	132.07^{h}

[1] All values within a given column and within a given inoculation followed by the same letter do not differ significantly at the 5% level by Duncan's New Multiple Range Test.

growth of seedling, because 450ug/ml N. level might be operated soil toxin in conifer species, even though its level could be stimulated growth of hardwood seedlings[15].

Growth of height in *P. thunbergii* seedlings grown on vermiculite showed more significant enhancement than that of seedlings grown on sandy loam at low concentration because vermiculite soil possessed high absorption capacity of nutrient and water in a expansion lattice from (cation exchange capacity 100-150ml/100g in vermiculite). Weight ratio of vermiculite is significantly low as compared to that of sandy loam. It could be usefully used to pots of transplantation seedlings.

Stem length of noninfected seedling grown on vermiculite and sandy loam was increased according to nitrogen levels. Even negative application of nitrogen proceeded seedling growth until 45 days after germination, its growth was ceased early and stunted by deficiency of nutrient. But mycorrhizal seedlings enhanced growth of height because nutrient absorption might be stimulated by mycorrhizal infection[9,10,14,15]. Formation of mycorrhizae and growth of seedlings, if optimum nutrient application were accomplished as much deficient quantity of seedling growth, were enhanced in early growth period[23].

As shown earlier, growth of seedling and formation of mycorrhizae were almost accom-

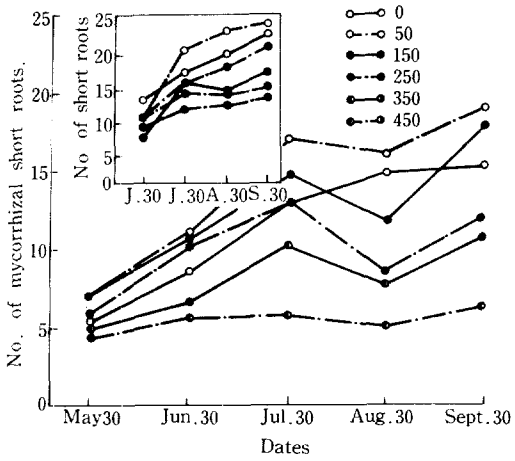

Fig. 1. Seasonal variation in number of short roots and mycorrhizae of *P. thunbergii* seedlings grown on vermiculite inoculated by *P. tinctorius* and at various nitrogen levels.

plished or finished in early time and then development of fungal mantle of ectomycorrhizae was stimulated by carbohydrate that was accumulated into host organization with photosynthesis[12]. Even though there was not significant difference according to nitrogen levels except for 0μg/ml and 450 ug/ml N. level, stem length of seedlings was almost finished in August. Stem length of mycorrhizal seedlings applied with nitrogen sources grown on vermiculite show to be a little bit higher than that of sandy loam. But even though seedling growth was enhanced according to increasing of N. level, 450μg/ml N. level inhibits seedling growth about 10% compared with 350μg/ml.

These differences in seedling growth were probably seemed to be cased by detention of mycorrhizal activity with accumulation of N[1].

Leaf Area

Leaf area was significantly different among the nitrogen concentration, soil medium and infection of mycorrhizae.

During the initial growth, it was appeared to be more influenced by the application of nitrogen than soil growth medium, but 450μg/ml, high nitrogen level, inhibited increasing of leaf area. Growth of seedlings planted in vermiculite was generally increased length and number of leaves more differently than that of sandy loam. Seedlings applied with 150μg/ml N. level were greatly superior compared with those of noninoculated seedlings (LAI increased through elapsed time after germination).

The imporvement of leaf area might be resulted from the absorption availabilty of cation by mycorrhizal short roots. Seedlings grown on inoculated soil showed high percentage of mycorrhizal fungi in contrast with noninoculated soil. It was observed that leaf area was effected with nitrogen level, soil growth medium and mycorrhizal formation.

Leaf area must be accomplished with nitrogen fertilization and mycorrhizal inoculation in which collected with super strain. Such phenomenon is similar to the results reported by Ekwebelam et al (1983)[1].

Total Dry Weight

As shown in table 3, total dry weight of seedlings applied by nitrogen fertilizer and inoculated with mycorrhizal fungi was significantly enhanced regardless of difference of soil growth medium. Seedlings applied with 350ug/ml nitrogen levels were the best among the ones applied with other nitrogen levels. The total dry weight of seedlings applied with 450μg/ml N. levels was inhibited because its level reacted with soil inhibitor with stimulation in soil growth medium. This phenomenon disappeared when nitrogen application was ended. Seedlings applied with 0μg/ml N. level were shown to be lower than those of other nitrogen levels because *P. thunbergii* seedlings were not grown until optimum nitrogen level was applied in soil growth medium.

Seedlings applied with 350μg/ml nitrogen level were increased stem dry weight and leaf dry weight, but over-charged nitrogen, 450μg/ml, level inhibits number and percentage of mycorrhizal short roots from increasing (Fig. 2).

Because its high nitrogen level may be occurred

Table 3. Growth of *Pinus thunbergii* seedlings inoculated with and without *Pisolithus tinctorius* and by fertilization levels and soil medium types on Sept. 1987.

Inoculation, Soil medium and N. Concentration.		No. of leaves	Leaf area cm^2	Shoot length cm	Leaf dry wt. mg	Stem dry wt. mg	Root dry wt. mg	Total dry wt. mg	Root collar diameter mm	T/R ratio
Vermiculite	0	132.33^{fg}	50.39^{ij}	9.30^{j}	182.76^{g}	59.19^{i}	93.00^{hi}	335.64^{f}	1.52^{fgh}	2.60^{g}
+ Pt	50	195.33^{de}	92.37^{f}	14.23^{de}	326.43^{de}	146.24^{cd}	228.09^{a}	700.74^{fg}	2.00^{bcd}	2.01^{h}
	150	201.00^{cde}	121.73^{bc}	14.70^{d}	414.49^{bc}	147.91^{c}	133.51^{cd}	695.88^{c}	2.00^{bcd}	4.21^{def}
	250	226.00^{a}	160.19^{a}	20.50^{a}	438.06^{b}	162.26^{c}	126.26^{e}	717.91^{bc}	2.04^{ab}	4.75^{a}
	350	236.00^{a}	170.00^{a}	18.46^{a}	407.10^{bc}	141.95^{cd}	108.90^{fi}	657.70^{e}	2.98^{a}	5.04^{c}
	450	117.00^{g}	148.00^{a}	17.06^{b}	410.03^{bc}	150.63^{bc}	98.69^{h}	749.17^{b}	2.07^{a}	5.68^{c}
Sandy loam	0	130.67^{g}	79.89^{h}	9.53^{j}	210.87^{g}	58.62^{i}	85.42^{i}	354.87^{f}	1.51^{fgh}	3. 6^{fg}
+ Pt	50	184.00^{ef}	128.45^{bc}	12.77^{gh}	265.21^{fg}	106.59^{fg}	147.72^{c}	519.52^{e}	2.07^{a}	2.52^{g}
	150	208.67^{c}	147.09^{a}	15.70^{c}	304.32^{ef}	99.39^{g}	113.34^{ef}	517.05^{e}	1.82^{efg}	3.55^{ef}
	250	225.67^{ab}	156.92^{a}	14.57^{d}	392.16^{bcd}	130.79^{de}	78.14^{i}	601.09^{f}	1.89^{def}	6.69^{b}
	350	224.00^{ab}	158.60^{a}	14.57^{d}	445.40^{b}	132.02^{d}	102.47^{gh}	680.00^{cd}	1.93^{def}	5.64^{c}
	450	184.33^{def}	134.04^{a}	13.67^{fgh}	329.43^{de}	136.00^{d}	104.07^{gh}	569.50^{g}	1.87^{def}	4.47^{d}
Vermiculite	0	105.66^{g}	77.50^{f}	10.43^{ij}	188.84^{g}	73.43^{j}	125.92^{a}	387.80^{f}	1.44^{fghi}	2.08^{h}
+Non. Pt	50	174.33^{f}	102.96^{e}	13.13^{gh}	426.29^{bc}	173.04^{ab}	166.82^{b}	766.19^{b}	2.06^{ab}	3.59^{ef}
	150	189.67^{def}	146.09^{a}	14.93^{d}	431.01^{b}	107.02^{f}	125.90^{e}	659.90^{e}	1.88^{def}	6.66^{b}
	250	202.67^{cd}	163.28^{a}	13.97^{f}	476.17^{ab}	115.49^{e}	136.54^{c}	728.15^{bc}	1.96^{def}	6.53^{b}
	350	192.00^{de}	132.55^{ab}	13.93^{fgh}	503.34^{a}	107.09^{f}	146.13^{c}	756.56^{b}	1.22^{ghi}	6.23^{bc}
	450	215.00^{b}	92.15^{f}	12.90^{gh}	529.12^{a}	126.00^{e}	105.89^{fg}	804.00^{a}	2.01^{abc}	9.02^{a}
Sandy loam	0	106.33^{g}	57.89^{ij}	9.9 ^{j}	243.86^{g}	76.76^{h}	67.45^{i}	388.10^{f}	1.53^{efgh}	4.75^{d}
-Non. Pt	50	149.67^{fg}	84.58^{g}	11.40^{hi}	340.82^{cde}	105.23^{fg}	104.90^{fg}	550.92^{g}	1.85^{def}	4.25^{def}
	150	178.66^{ef}	118.15^{d}	12.47^{gh}	380.05^{cd}	107.19^{f}	129.25^{cde}	616.48^{f}	1.82^{ef}	3.77^{ef}
	250	199.67^{cde}	170.98^{a}	14.00^{a}	389.06^{bcd}	185.08^{a}	103.28^{gh}	677.64^{d}	1.97^{cde}	7.50^{ab}
	350	213.00^{b}	147.19^{a}	14.80^{d}	424.14^{bc}	197.10^{a}	98.35^{h}	719.59^{bc}	1.96^{cde}	8.35^{a}
	450	172.00^{fg}	89.92^{g}	11.90^{hi}	316.02^{e}	190.83^{a}	104.32^{fgh}	711.0 ^{e}	1.92^{def}	6.73^{b}

[1] All values within a given column and within a given inoculation followed by the same letter do not differ significantly at the 5% level by Duncan's New Multiple Range Test.

and released by watering and decomposition, mycorrhizal formation was reformed by passage of time and then completely formed later on. However mycorrhizal short root and root dry weight were decreased by increasing the nitrogen levels in contrast with shoot growth. Growth of seedling grown on vermiculite and sandy loam was different from each other, regardless of fertilization due to difference of particle size of soil growth medium. Seedlings grown on vermiculite were greatly increased total dry weight, as shown earlier.

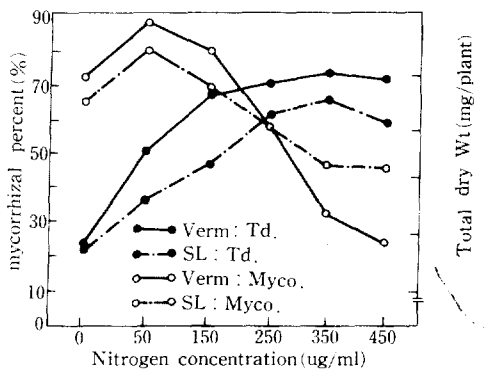

Fig. 2. The relation between no. of mycorrhizal short roots and total dry wt. of *P. thunbergii* seedlings applied by various nitrogen levels on vermiculite soil.

Growth Analysis of *P. thunbergii* Seedlings

The effect of photosynthesis in growth of *P. thunbergii* seedlings in greenhouse has given an

explanation with methods of growth analysis producted by Sekijema as shown at Fig. 3.

Seedlings applied with 50-450 μg/ml N. levels regardless of infection and growth medium showed increase of LAI according to elapsed time in contrast with specfic leaf area. Leaf development of *P. thunbergii* seedlings was accomplished in the end of Aug. But control of N. has early been restricted by N. deficiency of growth medium. At 450μg/ml N. levels, CGR and NAR showed significant decrease at earlier growth stage, and then total dry weight has been increased at last growth stage, Sept, 30, after the termination of nitrogen application.

In other nitrogen levels, NAR and CGR decreased because of growth of old leaves and stress of environment such as high temperature. SLA was likely to decrease according to elapsed time. Pattern of growth like this was similar to that of woody plant.

According to Hatchel and Marx (1987), long leaf pine(*Pinus palustris* Mill) had better survival and growth as it was inoculated with *P. tinctorious* through seven growing seasons but the effect of fertilizer was not significantly advantageous for both activity and condition of mycorrhizae.

In this experiment, application of N. fertilizer and inoculation of mycorrhizal fungi gave an effect of the seedling growth even though growth

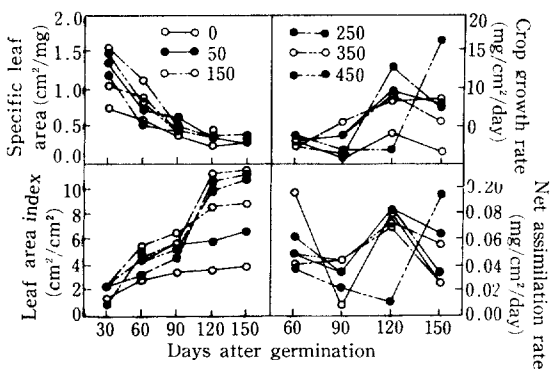

Fig. 3. Growth parameters of *Pinus thunbergii* seedlings is affected with inoculation of *P. tinctorius*, and nitrogen levels on Verm. soil types.

of *P. thunbergii* seedling was significantly stimulated by 50-350 μg/ml nitrogen application. Its case could be affected by elements such as insoluble P. absorbed by mycorrhizal fungi. Mean values of root collar diameter in 5-month-old seedling grown on infected soil were significantly different. Seedlings grown on inoculated soil regardless of medium showed that increasing nitrogen level could increase both shoot and root collar diameter. The increase of root collar diameter would be resulted from shoot growth that was stimulated by nitrogen application and *P. tinctorius* inoculation.

This phenomenon was similar to the results reported by Thedorou and Bowen[21] (1969) in that high nitorgen levels inhibit mycorrhizal fungi growth but stimulate development of shoot growth. The difference in growth among media after nitrogen fertilization may be due to difference of mycorrhizal formation or cation exchange capacity in soil growth media.

Nutrient Absorption

Even though total nitrogen level analyzed in *P. thunbergii* seedlings did not show a significant difference (Table 4), nitrogen concentration of leaf fertilized with low nitrogen level, Oug/ml, was rather reduced. At the vermiculite inoculated with *P. tinctorius*, the results analyzed of micro-nutrient were recorded to be higher by 143, 159, 181, 182 and 161% than those of sandy loam soil in responce to 0, 50, 150, 250, 350 and 450μg/ml nitrogen application level, respectively.

Phosphorus concentration in leaves of seedlings mainly depended upon mycorrhizal formation. Phosphorus concentration on leaves of seedlings that mycorrhizal formation was accomplished in short roots was kept higher than that of non-inoculated seedlings in each soil type. *P. thunbergii* seedlings applied high nitrogen levels should be received a little inhibition because of inhibit of root development. Fertilized and infected seedlings were kept with a high concentration of total nitrogen in leaves rather than the non

Table 4. Foliar concentration of elements in *P. thunbergii* one-year-old seedling inoculated with or without *P. tinctorius*.

Inoculation soil medium N, concentration		N	P	K	Ca	Mg	Na
				%			
vermiculite	0	1.43[k]	0.36[h]	0.93[m]	1.43[j]	0.96[ij]	1.07[gh]
+ Pt	50	1.59[g]	0.57[d]	1.45[j]	1.24[jk]	1.41[e]	1.06[gh]
	150	1.81[bc]	0.63[b]	1.55[j]	1.24[k]	1.35[e]	1.32[de]
	250	1.82[b]	0.60[c]	2.73[a]	2.49[a]	1.54[cd]	1.34[d]
	350	1.88[a]	0.69[a]	2.42[d]	2.19[c]	1.96[a]	1.37[cd]
	450	1.61[f]	0.21[l]	1.48[j]	2.28[b]	1.46[d]	1.31[de]
sandy loam	0	1.32[l]	0.34[hij]	1.87[f]	1.76[g]	1.22[g]	1.07[gh]
+ Pt	50	1.53[h]	0.42[fg]	0.99[m]	1.57[h]	1.26[f]	1.10[g]
	150	1.59[g]	0.60[c]	1.68[h]	1.43[i]	1.06[i]	1.41[c]
	250	1.46[j]	0.35[hi]	1.42[j]	1.46[hi]	1.43[de]	1.67[a]
	350	1.43[k]	0.36[h]	2.63[b]	1.50[h]	1.48[d]	1.63[a]
	450	1.53[h]	0.24[k]	2.47[c]	1.90[c]	1.62[b]	1.54[b]
vermiculite	0	1.69[e]	0.25[k]	2.01[e]	1.32[j]	0.99[h]	1.08[g]
+Non. Pt	50	1.48[i]	0.35[hi]	1.01[lm]	1.88[f]	1.55[c]	1.12[g]
	150	1.59[g]	0.38[g]	1.76[g]	1.71[g]	1.40[e]	1.42[c]
	250	1.60[fg]	0.43[f]	1.84[f]	2.01[d]	1.40[e]	1.22[fg]
	350	1.53[h]	0.53[e]	2.03[e]	2.04[d]	1.53[cd]	1.45[c]
	450	1.43[k]	0.52[e]	2.72[a]	2.03[d]	1.94[a]	1.09[g]
sandy loam	0	1.69[e]	0.24[k]	1.35[k]	1.49[j]	1.14[h]	0.99[j]
+Non. Pt	50	1.62[f]	0.32[ij]	1.26[l]	1.76[g]	1.18[gh]	1.26[ef]
	150	1.80[bc]	0.39[g]	1.79[g]	1.84[f]	1.27[f]	1.26[ef]
	250	1.77[d]	0.33[ij]	1.49[j]	1.74[g]	1.15[gh]	1.29[e]
	350	1.80[bc]	0.43[f]	1.16[l]	1.91[e]	1.06[i]	1.27[ef]
	450	1.24[m]	0.43[f]	1.25[l]	1.26[jk]	1.08[i]	1.23[fg]

[1] All values within a given column and within a given inoculation followed by the same letter do not differ significantly at the 5% level by Duncan's New Multiple Range Test.

-fertilized and non-infected seedlings. The result is due to the applied nitrogen sources or mycorrhizal formation. These results were similar to the results reported by Ruehle et[18] al.(1983).

Potassium concentration of seedlings inhibited mycorrhizal formation has been recorded higher by 1.98 than that of seedlings developed with ectomycorrhizae.

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