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NOTE

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Mycorrhizal synthesis of four ectomycorrhizal fungi in potted *Populus maximowiczii* seedlings

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Abstract Ectomycorrhizal (ECM) syntheses between four ECM fungi, *Laccaria amethystina*, *Hebeloma mesophaeum*, *Thelephora terrestris*, and *Tomentella* sp., and *Populus maximowiczii* seedlings that are known to form ECM at a denuded area of Mt. Usu were performed in volcanic debris in a controlled growth chamber. The percentage of ECM colonization and seedling growth were determined 3 months after inoculation. Seedlings were successfully colonized by the inoculated ECM fungi with low contamination ratios. Seedling height and biomass were larger in the inoculated seedlings than in the control, although the effects of inoculation on seedling growth varied with the ECM fungus.

Key words Ectomycorrhiza · Inoculation · Mt. Usu · Poplar · Volcanic debris

On March 31, 2000, Mt. Usu, Hokkaido, Japan, experienced a devastating eruption that deposited a deep layer of volcanic ash around craters and almost completely destroyed approximately 71 ha of natural vegetation. We investigated the reestablishment process of primary vegetation in denuded areas of Mt. Usu and demonstrated that Salicaceae such as *Populus maximowiczii* A. Henry dominantly establishes there. Further investigation revealed that those Salicaceae seedlings are commonly associated with certain ectomycorrhizal (ECM) fungal taxa such as *Laccaria*, *Hebeloma*, and Thelephoraceae (Obase et al. 2007a,b). In this study, we performed mycorrhization experiments between these ECM fungi as inoculum and *P. maximowiczii* seedlings as the woody plant hosts in volcanic ash soil substrate. The purpose of this study was to examine the abilities of different ECM fungal species to colonize *P. maximowiczii* and to determine the effect of ECM colonization on host plant growth.

Volcanic debris as a soil substrate was collected from denuded areas of Mt. Usu., heat-sterilized at 110°C for 48 h in a dry oven, and then sieved through a 5.0-mm mesh. Soil pH (H_2O) was 4.9, and total N, P, and K contents were <0.1, 1.4, and 6.6 g/kg, respectively.

Seeds of *P. maximowiczii* were collected in July 2005, from a single tree in Abuta-cho, Hokkaido, Japan. Two to three seeds were sown in each plastic pot (25 mm diameter, 120 mm depth) filled with heat-sterilized river sand and thinned out after germination. Five hundred seedlings prepared in this way were grown at 25°C under 11 000 lux with a 16-h photoperiod in a growth chamber. No nutrient was applied to the soil substrate during the experiment.

Four ECM fungi that are known to form ECM with *P. maximowiczii* seedlings at a denuded area of Mt. Usu (Obase et al. 2007a,b) were used in this experiment. *Laccaria amethystina* Cooke and *Hebeloma mesophaeum* (Pers.) Quél were isolated from basidiocarps collected under mature trees of *P. maximowiczii* and *Salix integra* Thunb. near a denuded area of the Nishiyama-A (N-A) crater on Mt. Usu. *Thelephora terrestris* Fr. and *Tomentella* sp. (named Thelephoraceae 1; Obase et al. 2007a) were isolated from ECM on a *Salix sachalinensis* Fr. Schm. seedling established in a denuded area of the N-A crater. Pure cultures of these fungi were grown on plates of modified Melin-Norkrans (MMN) agar medium (Marx 1969) containing glucose instead of sucrose. After 1 month growth on MMN agar medium, mycelial plugs approximately 1 × 1 cm were placed in MMN liquid medium in 200-ml Erlenmeyer flasks and incubated for 1 month at 25°C in the dark.

ECM synthesis were conducted in a growth chamber. Of 500 45-day-old seedlings, 100 were used for ECM synthesis. Another 5 seedlings were used to confirm that no ECM were formed before inoculation. For ECM synthesis, seedlings were carefully picked out from plastic pots without damaging the roots. The roots were soaked in modified MMN liquid medium containing cultured ECM fungal mycelia. Control seedlings were soaked in modified MMN liquid medium with no ECM mycelia. Each inoculated

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Table 1. Ectomycorrhizal (ECM) colonization status and plant growth of *Populus maximowiczii* seedlings in relationship to different fungal treatment[1]

ECM inoculation treatment	ECM colonization by the fungi				Plant height (cm) at the following incubation period (month)				Dry weight (mg)			Shoot weight/ height
	Inoculated		Contaminated		0	1	2	3	Shoot	Root	Total	
	Colonization[2]	n^3	Col.	n								
Tomentella sp.	63.1 ± 15.5^{c}	20	0	0	2.5 ± 0.6^{a}	6.0 ± 1.6^{ab}	10.4 ± 2.4^{b}	14.7 ± 2.5^{b}	210.1 ± 70.1^{b}	84.7 ± 32.0^{b}	294.7 ± 100.0^{b}	13.8 ± 4.3^{b}
Thelephora terrestris	50.3 ± 14.3^{b}	20	0.1 ± 0.2	1	2.5 ± 0.5^{a}	6.6 ± 1.6^{ab}	12.4 ± 2.6^{c}	17.7 ± 3.7^{c}	318.4 ± 124.8^{c}	94.6 ± 58.5^{b}	413.0 ± 180.9^{bc}	17.1 ± 5.2^{bc}
Hebeloma mesophaeum	38.8 ± 12.3^{b}	20	2.8 ± 8.5	4	2.5 ± 0.6^{a}	7.0 ± 1.6^{b}	13.0 ± 3.8^{c}	18.0 ± 3.4^{c}	379.4 ± 167.0^{c}	120.8 ± 57.6^{b}	500.2 ± 218.7^{c}	20.3 ± 6.1^{cd}
Laccaria amethystina	61.1 ± 12.6^{c}	20	0	0	2.5 ± 0.6^{a}	6.3 ± 1.5^{ab}	12.5 ± 1.9^{c}	16.3 ± 2.1^{bc}	359.3 ± 100.3^{c}	165.0 ± 64.0^{c}	524.3 ± 156.4^{c}	22.2 ± 6.2^{d}
Control	0^{a}	0	0	0	2.4 ± 0.6^{a}	5.6 ± 1.2^{a}	8.6 ± 2.7^{a}	11.1 ± 2.5^{a}	91.5 ± 34.3^{a}	28.8 ± 14.4^{a}	120.2 ± 47.1^{a}	8.6 ± 2.6^{a}

[1]Mean values ± standard deviation
[2]Colonization (Col.) ratio
[3]Number of seedlings with ECM
Different letters indicate significant differences at $P < 0.05$ (Tukey–HSD test)

seedling was planted in a plastic pot (60 × 60 mm width and depth, 100 mm height) filled with heat-sterilized volcanic debris and placed in a growth chamber at 25°C under 11 000 lux with a 16-h photoperiod.

Seedling height was measured monthly for three times, and ECM colonization and seedling biomass were examined once at 3 months after inoculation. Each seedling was separated into roots and shoots. Shoots were oven-dried at 60°C for 48 h and weighed. The ECM were categorized into each morphotype based on morphological characterization (Ingleby et al. 1990). When the synthesized ECM were morphologically identical with those in our previous study (Obase et al. 2007b) and other descriptions (Brand 1988; Agerer and Weiss 1990; Ingleby et al. 1990), we concluded that the inoculated fungi had successfully formed ECM. The number of seedlings colonized by each morphotype was counted for each inoculation treatment. The percentage of ECM colonization was estimated as the proportion of ECM tips of each morphotype relative to the total number of root tips on the seedling.

All data were subjected to one-way analysis of variance (ANOVA). Differences among inoculations were determined using the Tukey–HSD (honestly significant difference) test ($P < 0.05$). All analyses were performed with the SPSS version 10.0.05 J for Windows (SPSS Japan, Tokyo, Japan).

All seedlings formed ECM with inoculated fungal species. It is known that some macroscopic features of ECM, such as color, are variable depending on environmental conditions such as soil substrate type and age of the ECM (e.g., Egli et al. 1993). However, the morphological characteristics of each ECM morphotype examined in this study were almost identical to previous descriptive reports. The number of ECM seedlings and the percentages of ECM colonization in roots of *P. maximowiczii* are shown in Table 1. All inoculated seedlings formed the intended ECM with high percentages of colonization ranging from 38.8% to 63.1% and with very little contamination. No ECM was formed in the control.

One month after inoculation, seedlings associated with *H. mesophaeum* were significantly higher than the control (see Table 1). At 3 months, all the inoculated seedlings were 1.3 to 1.6 times higher than the control dry weights of shoots and roots, and the total dry weights of the inoculated seedlings were all significantly greater than those of the controls. The ratio of plant height to shoot weight was also significantly higher in all the inoculated seedlings as compared with the control. Thus, all inoculated ECM fungi promoted seedling growth in both height and weight, contributing to the establishment on volcanic debris. The growth promotion of host plant by ECM colonization is possibly related to increased efficiency of nutrient acquisition, such as N and P, from volcanic debris. Other reports have also shown growth promotion of *Populus* spp. by the inoculated ECM fungi *Laccaria* (Baum et al. 2002) and *Paxillus* (Langenfeld-Heyser et al. 2007).

On a denuded area of Mt. Usu, all 1-year-old seedlings of willow and poplar formed ECM (Obase et al. 2007a,b). The present study shows that *L. amethystina*, *H. mesopha-*

eum, *T. terrestris*, and *Tomentella* sp. that associated with pioneer woody plants at the site had ECM associations with *P. maximowiczii*. Although the inoculation effects on seedling growth varied among the ECM fungi, all these fungi enhanced the growth of seedlings in an N-deficient volcanic debris substrate in a controlled environment. Further investigations, such as pot experiments using nonsterilized volcanic debris and ECM-inoculated seedlings, are needed to determine the practical availability and potential advantage of this method.

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References

Agerer R, Weiss M (1990) *Thelephora terrestris*. In: Agerer R (ed) Colour atlas of ectomycorrhizae, plate 48. Einhorn-Verlag, Schwäbisch Gmünd

Baum C, Stetter U, Makeschin F (2002) Growth response of *Populus trichocarpa* to inoculation by the ectomycorrhizal fungus *Laccaria laccata* in a pot and a field experiment. For Ecol Manag 163:1–8

Brand F (1988) *Laccaria amethystina*. In: Agerer R (ed) Colour atlas of ectomycorrhizae, plate 18. Einhorn-Verlag, Schwäbisch Gmünd

Egli S, Amiet M, Zollinger M, Schneider B (1993) Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. Trees 7:123–129

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE research publication no. 5. HMSO, London

Langenfeld-Heyser R, Gao J, Ducic T, Tachd P, Lu CF, Fritz E, Gafur A, Polle A (2007) *Paxillus involutus* mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar *Populus* × *canescens*. Mycorrhiza 17:121–131

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Obase K, Tamai Y, Yajima T, Miyamoto T (2007a) Mycorrhizal associations in woody plant species at the Mt. Usu volcano, Japan. Mycorrhiza 17:209–215

Obase K, Tamai Y, Yajima T, Miyamoto T (2007b) Morphological characteristics of ectomycorrhizas found in willow and poplar seedlings established in the area devastated by the volcanic eruption of Mt. Usu, Hokkaido, Japan in 2000. Eurasian J For Res 10:173–178