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## The Study on Mycorrhizal Status of Current-Year *Acer mono* Seedlings

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### Abstract

We investigated mycorrhizal status of current-year *Acer mono* seedlings at different regenerated sites. The arbuscular mycorrhizae (AM) formation degree, spore density, available phosphorus (AP) and total nitrogen (TN) contents of samples were tested. Our results indicated that current-year *A. mono* seedlings may not be obligatory mycorrhizal species since AM colonization was not found in some of the seedlings in the bare sites. The spore density in the soils can play an important role in AM formation since it has significantly positive correlations with the frequency of vesicles and hyphae. AP and TN contents in the soil also have close relationships with the frequency of hyphae in roots.

*Key words:* *Acer mono*, arbuscular mycorrhizae, current-year seedlings, spore density

### Introduction

*Acer mono* is an important species which popularly distributed across Japan. It can regenerate under the forest canopy because of its tolerance to shade and also invade bare sites which created by various disturbances. Species of the family *Aceraceae* could form arbuscular mycorrhizae (AM) (Brundrett *et al.*, 1990). Although Yamato and Iwasaki (2002) reported on the AM of five species from this family in Japan, no other information is available on the *A. mono* and its mycorrhizal fungi.

Plants can generally be divided into three categories, i.e., obligatory mycorrhizal, facultative mycorrhizal and non-mycorrhizal, to reflect the varying degrees of benefits received from mycorrhizal associations (Brundrett, 2002). Smith and Read (1997) reported that the density of the inoculum, temperature, light, and the availability of nutrients, particularly phosphorus, are important environmental factors that influence the percent colonization. To understand the characteristic features of mycorrhizal formation in *A. mono* seedlings, it is necessary to investigate the mycorrhizal status of seedlings established in various locations.

We investigated the degree of AM formation, AM spore density and soil nutrients in the rhizosphere to assess the relationships between mycorrhizal fungi and its current-year seedlings of *A. mono* from various forest types and disturbed sites.

### Materials and methods

#### Study sites and sampling procedure

We selected eight sites in Hokkaido, Japan (Df, Db1, Db2, Af1, Cf, Af2, Rf, and Bf), where seedlings of *A. mono* had naturally regenerated.

Df ; Slightly disturbed forest area in the Nishiyama crater group on Mt. Usu, which erupted in 2000, in Abuta, Hokkaido (42° 32' N, 140° 50' E). Broad-leaved forest dominated by *Quercus crispula* Blume and *A. mono*.

Db1 ; Disturbed bare site near the Nishiyama crater

group on Mt. Usu.

Db2 ; Disturbed bare gully formed on the sediment surface from the eruption in the Nishiyama crater group on Mt. Usu.

Af1 ; *A. mono* forest in the Hiyama Research Forest of Hokkaido University, Kaminokuni, Hokkaido (41° 45' N, 140° 09' E).

Cf ; Artificial Japanese cedar forest in the Hiyama Research Forest.

Af2 ; *A. mono* forest along the Ishizaki coast in Kaminokuni, Hokkaido (41° 42' N, 140° 01' E).

Rf ; Riparian forest dominated by willows (*Salix* spp.) along the Izari River in Eniwa, Hokkaido (42° 46' N, 141° 15' E).

Bf ; Broad-leaved forest in the Misumai Research Forest of Hokkaido University, Sapporo, Hokkaido (42° 57' N, 141° 57' E).

In September and October 2003, 4 to 11 current-year seedlings of *A. mono* and their rhizospheric soils were carefully sampled at each site. The roots were carefully washed under running water and then fixed into FAA solution (formalin:acetic acid:ethyl alcohol:water = 1:1:9:9). Rhizospheric soils sampled here were around their seedlings within a radius of about 10 cm. All samples were stored at 4°C in plastic bags sorted by sites until they were used for enumeration of AM fungal spores and determinations of soil characteristics.

#### AM assessment

The roots were stained according to Brundrett *et al.* (1996) with procedural modifications as follows. Roots were cleared in 10% KOH for 20 min at 80°C, bleached in 2.5% H<sub>2</sub>O<sub>2</sub> for 15 min at 80°C, then treated with 1.0% HCl for 10 min at room temperature, and stained in 0.05% Trypan blue solution for 15 min at 80°C. They were then mounted on slide glasses and examined under a light microscope. AM characteristics (arbuscules, vesicles, and hyphae) were observed, and the amount of intra- and intercellular hyphae were

ranked in one of five levels based on visual examination (-: no AM structures were found, ±: very few AM structures were found, + ... +++: increasing abundance of AM structures).

### Separating AM fungal spores from soil

AM fungal spores were separated from rhizospheric soil samples of each site according to Brundrett *et al.* (1996). About 20 g of soils were wet-sieved with 500-, 250-, 106-, and 53- $\mu$ m mesh sieves, respectively. Soils that remained on the 250-, 106-, and 53- $\mu$ m mesh sieves were centrifuged with water for 5 min at 438 xG. After removing the supernatant, the samples were centrifuged with 50ml of 50% sucrose solution for 1 min at 438xG. The supernatants were filtered through membrane filter. Separated spores on the filter were counted under the stereomicroscope. Spore density is expressed as the number of spores per 100 g of air-dried soils.

### Determination of soil nutrients

Available phosphorus (AP) and total nitrogen (TN) were determined in rhizospheric soil samples from each site. Samples were air-dried and then grounded. 2 g of soil were used to estimate available phosphorus according to Truog (1930). Total nitrogen content in soils were analyzed by using automatic gas chromatography (Elemental Analyzer NCS 2500; ThermoQuest, Austin, TX, USA).

### Statistical analysis

To identify differences among sites, data from the AM assessment were subjected to a Kruskal–Wallis test (SPSS software version 12.0.1; SPSS Inc., Chicago, IL, USA). When significant differences occurred among sites, data were analyzed using a *post hoc* Steel–Dwass test. To assess the correlation between vesicles, hyphae, spore density, and soil nutrients, data were also examined with a Kendall correlation analysis (SPSS software version 12.0.1).

### Results and discussion

There had not arbuscules and only very few vesicles were found in the roots of current-year *A. mono* seedlings at 8 sites (Table 1). However, the presence of AM was confirmed because we observed coiled hyphae, which are the features of *Paris*-type hyphae of AM, in the root cortical cells. This result corresponded to findings of Brundrett *et al.* (1990) and Yamato and Iwasaki (2002). They also reported similar results for other species of *Aceraceae*. Some seedlings had neither vesicles nor AM hyphae. We found significantly larger amounts of hyphae in the roots of seedlings collected in the forests (Af1, Af2, and Rf), but few in bare sites (Db1 and Db2; Table 1). No AM colonization was found in some of the examined seedlings in the bare sites. These results suggest that current-year *A. mono* seedlings may not be obligatory mycorrhizal.

The AP contents of rhizospheric soil in bare sites (Db1 and Db2) were very high, however, the levels of AM colonization were low (Table 1). No correlations were observed between the amount of vesicles and AP,

whereas a negative correlation was found between AP and the amount of hyphae or spore density (Table 2). Amijee *et al.* (1989) demonstrated that mycorrhizal formation is inhibited as the content of phosphorus in the soil increases in a laboratory experiment. The data from our field study were consistent with this laboratory experiment. No nitrogen was detected in Db1 and Db2 sites, where seedlings showed low levels of AM colonization. A positive correlation was observed between TN in the soil and the amount of hyphae in the roots or spore density (Table 2). These results suggested that nitrogen may be a critical factor to affect the formation of hyphae in the roots. AM colonization may not occur in the absence of nitrogen, but if nitrogen is available to a certain degree, then available phosphorus could be a limiting factor for AM colonization.

We observed positive correlations between the frequency of vesicles and spore density, and also between the frequency of hyphae and spore density (Table 2). It indicated that spore density in the soils can play an important role in AM formation. Spores produced by AM fungi in the rhizosphere may have caused high spore density. However, in this case, spores were not thought to have been produced by AM fungi in seedling roots because seedlings collected in this study were current-year individuals. These seedlings had likely germinated between the end of spring and early summer, and were sampled between the end of summer and early autumn of the same year. Therefore, the spores that existed in the rhizosphere of seedlings are thought to be derived from other plants. Moreover, high spore densities and high frequencies of mycorrhizal formation were observed in the forest sites (Df, Af1, Cf, Af2, Rf, and Bf). Consequently, current-year *A. mono* seedlings are thought to be able to form mycorrhizae because of the abundant source of inoculum from spores that were produced by other plants. The significant differences of plant coverage between the forest sites and bared sites resulted in the different spore density.

Schroeder and Janos (2004) demonstrated that phosphorus availability, intraspecific density, and their interaction significantly modify plant responses to AM. In forests, AM formation may take place because the inoculum is abundant and competition for nutrient acquisition occurs. Guadarrama *et al.* (2004) also reported that competition was diminished in the presence of AM fungi for *Stemmadenia donnel-smithii*, which is a late successional species like *A. mono*. In addition, Smith and Read (1997) described that the density of inoculum, temperature, light, and the availability of nutrients, particularly phosphorus, are important environmental factors on percent colonization. In this study, we obtained results that supported the reports mentioned above. Our study suggests that AP, AM spore density, and environmental factors, such site characteristics, are important factors that influence the mycorrhizal status of *A. mono*. And *A. mono* is a non-obligatory (facultative) mycorrhizal tree species.

Table 1. Relative frequency of AM colonization, spore density and chemical properties of soils in the study sites

Site*	Vesicles		Hyphae		Spore density (spores/100 g)	Available P (mgP <sub>2</sub> O <sub>5</sub> /kg)	Total N (mg/g)
Df	1/4	(-)	4/4	(++)	709	302.43	0.12
Db1	0/6	(-)	4/6	(± <sup>a</sup> )	0	251.03	0.00
Db2	0/11	(- <sup>a</sup> )	7/11	(+ <sup>a</sup> )	6	350.43	0.00
Af1	1/8	(-)	8/8	(++ <sup>b</sup> )	1201	41.50	7.81
Cf	0/4	(-)	4/4	(+++)	1394	38.07	6.36
Af2	7/11	(± <sup>bc</sup> )	11/11	(+++ <sup>b</sup> )	914	117.27	8.98
Rf	6/8	(+ <sup>b</sup> )	8/8	(++ <sup>b</sup> )	1285	69.67	0.89
Bf	0/10	(- <sup>ac</sup> )	10/10	(++)	494	55.93	5.39

The denominator indicates the total number of seedlings observed and the numerator indicates the number of seedlings that showed AM colonization. Symbols indicate the relative frequency of AM structure. -: no AM structure was found; ±: very few AM structures were found; + ... +++: increasing abundance of AM structures. Symbols in a column followed by different letters are significantly different at  $P < 0.01$

\*Abbreviations: Df: disturbed forest area, Db: disturbed bare area, Af: *Acer mono* forest, Cf: cedar forest, Rf: riparian forest, Bf: broad-leaved forest

Table 2. Kendall correlations ( $\tau$ ) between vesicles, hyphae, or spore density and soil properties in the study sites (AP: available phosphoric acid, TN: total nitrogen)

	Vesicles	Hyphae	Spore density	AP	TN
Vesicles	-	0.361**	0.347**	-0.008	0.243*
Hyphae	-	-	0.600**	-0.371**	0.597**
Spore density	-	-	-	-0.535**	0.441**

\* $P < 0.05$ , \*\* $P < 0.01$

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