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Vegetation and Colonization Status of Mycorrhizal and Endophytic Fungi in Plant Species on Acidic Barren at Crater Basin of Volcano Esan in Hokkaido, Japan

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Abstract

We investigated the colonization status of mycorrhizal and endophytic fungi in roots of plants established on an acidic barren at volcano Esan, Hokkaido, Japan. In total, 87 individuals of 21 plant taxa were investigated in four different vegetation sites and the surrounding area. Ericaceous plants such as *Empetrum nigrum* var. *japonicum*, *Ledum palustre* var. *diversipilosum*, *Vaccinium vitis-idaea* var. *minus* and *Loiseleuria procumbens* were the main colonizers at the study sites and dominated under even the most severe conditions of low soil moisture and nutrient content. They were associated with ericoid mycorrhizal (ERM) fungi at all vegetation sites and erratically with dark septate endophytic (DSE) fungi. They commonly associated together with arbuscular mycorrhizal (AM) fungi only at sites where *Sasa senanensis* dominated. Among the ericaceous plants, only *Enkianthus campanulatus* associated with AM and DSE fungi and had no associations with ERM fungi. Other herbaceous and woody plants associated commonly with AM or erratically with DSE fungi, except for *Carex* sp. and *Polygonum* spp. which erratically associated with AM and DSE fungi. Mycorrhizal associations were common in representative vegetation on the crater basin of Mt. Esan. In particular, ERM associations might play a significant role in invasion and establishment of ericaceous plants, and also in the development of plant communities in the harsh environment at Mt. Esan.

Key words: Arbuscular mycorrhiza, Dark septate endophyte, Ericaceae, Ericoid mycorrhiza, *Enkianthus campanulatus*

Introduction

Mycorrhizal associations between plant roots and fungi are commonly observed in terrestrial forest ecosystems. Mycorrhizal associations have been classified into seven groups based on the type of fungi, the host plant, and fungal morphological characteristics (Smith and Read 1997). For example, most herbaceous plants associate with arbuscular mycorrhizal (AM) fungi and some woody plants belonging to families, such as Salicaceae, Betulaceae and Pinaceae, associate with ectomycorrhizal (ECM) fungi (Wang and Oiu 2006). Also, some families form peculiar types of mycorrhiza such as ericoid mycorrhiza (ERM) in Ericaceae (Wang and Oiu 2006). In general, mycorrhizal associations are beneficial to host plants because they enhance nutrient acquisition and drought tolerance, thus alleviating the stress of their host plants (Smith and Read 1997). These mutual associations are recognized as essential for healthy growth of plants. In particular, they play a significant role in plant establishment and development in some highly stressed environments, such as denuded areas under primary succession where environmental stresses, for example low soil nutrients, instability of the soil surface and drought, make plant establishment difficult (Nara and Hogetsu 2004, Nara 2006, Obase *et al*. 2007).

In the northern hemisphere, members of ericaceous plants often constitute important components of a hydrologically and altitudinally diverse range of habitats such as some forests (e.g., Bergero *et al*. 2000), heathland (e.g., Diaz *et al*. 2006), subalpine glacier forefront (Cázares *et al*. 2005), and exposed area of volcanic mountains (e.g., Tsuyuzaki *et al*. 2005), where severe soil conditions include poor nutrient status, low pH, poor drainage, or extreme temperatures. The presence of ERM associations was recognized as a key factor for the establishment of ericaceous plants in such extreme environments (Cairney and Meharg 2003; Diaz *et al*. 2006).

On the other hand, AM and ECM also play important roles in plant establishment on denuded areas. Several efforts have been made to describe the status of mycorrhizal associations of plants that undergo primary succession. These investigations have demonstrated that AM and ECM associations reestablish immediately after volcanic eruption (Allen *et al*. 1992; Obase *et al*. 2007; Obase *et al*. 2008) and are commonly observed under primary succession (Trowbridge and Jumpponen 2004; Tsuyuzaki *et al*. 2005). Also, they play an important role in the establishment of plants and in the developmental and successional processes of plant communities in volcanic areas (Fujiyoshi *et al*. 2005; Nara 2006). Therefore, mycorrhizal associations are an important factor for plant invasion and establishment

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under severe environmental conditions associated with primary succession. However, our knowledge of the mycorrhizal status of plants undergoing primary succession is incomplete.

The 1874 eruption of Mt. Esan in northern Japan produced pumice deposits that created an extensively exposed crater basin. In recent years, it was reported that various plants such as Ericaceae became established naturally and dominantly, and created unique vegetation in different areas near the crater basin (Munakata and Ikeda 1960). In this study, we determined the mycorrhizal status of plants from representative vegetation areas on volcano Esan, Hokkaido, Japan. Also, the mycorrhizal status of ericaceous plants that were the main colonizers in the study sites was examined.

Materials and Methods

Study area

Mt. Esan (41°48'N, 141°10'E, 618 m elevation) is an active volcano located in southwest Hokkaido, Japan. Mt. Esan started its volcanic activity about 40,000-50,000 years ago and has had repeated pyroclastic flows and landslides that have led to the formation of a lava dome. This has led to the existing complicated geographical features at surrounding craters. The most recent activity at Mt. Esan occurred in 1874. Phreatic eruptions and volcanic activity, such as ejection of volcanic gas and hydrothermal activity, have continued to date. The crater basin extends between Mt. Esan and Mt. Kaikou, which is covered by pyroclastic material such as pumice. Vegetation has developed differently depending on the distance from the crater and the topography (Munakata and Ikeda 1960). Natural vegetation surrounding the crater basin consists of various broadleaf woody plants such as *Quercus mongolica* var. *grosseserrata* (Blume) Rehd. et Wils and *Acer mono* Maxim. (Munakata and Ikeda 1960).

Climatic data for Kakkumi town (41°54'N, 140°58'E), which is located near Mt. Esan, indicates a mean annual precipitation of 1421 mm and a mean annual temperature of 8.3°C with temperatures ranging from -2.6°C in January to 20.4°C in August (Sapporo meteorological station).

Vegetation

Four study sites with representative vegetation structure were established on the crater basin from a preliminary survey (Figs. 1 and 2). One 5 m x 50 m rectangular plot consisting of ten 5 m x 5 m square quadrats was placed in each site, except for Site 1, where ten 5 x 5 m square quadrats were placed around the crater due to the complicated landform. In each quadrat, we recorded the percentage area covered by the plants and classified it into 6 cover classes (5: 75-100%, 4: 50-75%, 3: 25-50%, 2: 10-25%, 1: 1-10%, and +: less than 1%) in July 2004 according to the method of Braun-Blanquet (1964) with some

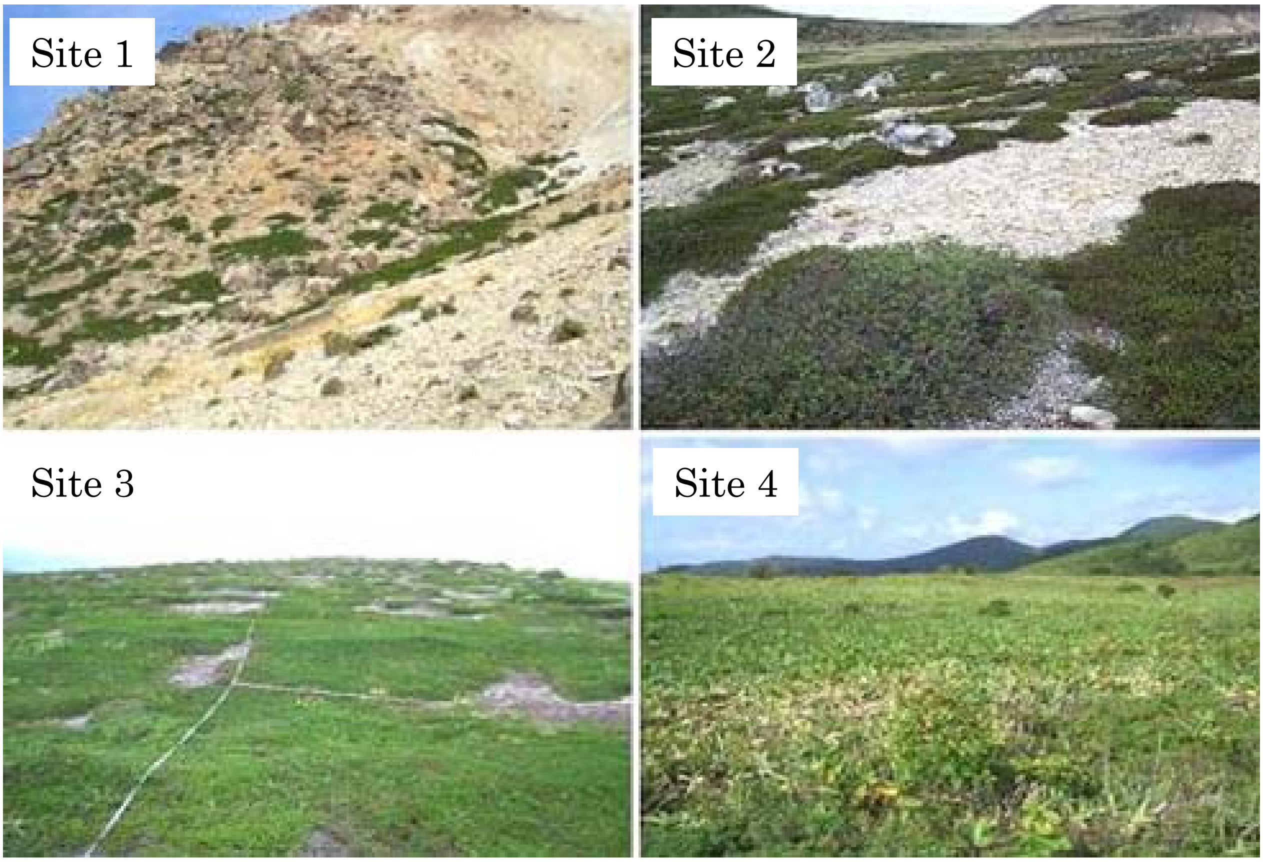

Fig. 1. Study sites in volcanic area of Mt. Esan in Hokkaido, Japan.

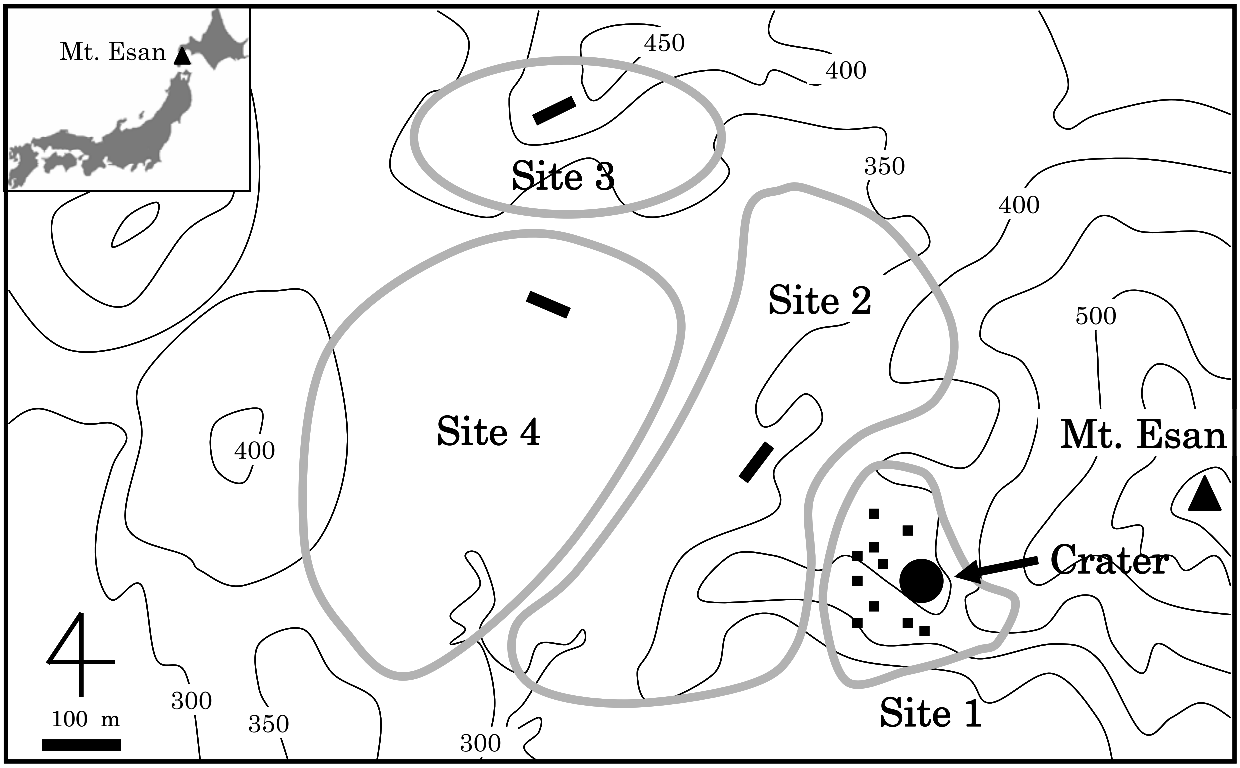

Fig. 2. Location of transects (side lines) or quadats (squares) in four study sites (enclosed by gray lines) in volcanic area of Mt. Esan in Hokkaido, Japan.

modifications. Next, we replaced each cover class with the median of percentage cover (5 → 87.5%, 4 → 62.5%, 3 → 37.5%, 2 → 17.5%, 1 → 5.5%, and + → 0.5%) and calculated the area covered by each plant species, which was defined as: (the sum of median in all quadrats in one plant species) / (total number of quadrats) / 87.5 x 100. The percentage of total plant cover in each quadrat was defined as: (the sum of areas covered by plant communities in quadrat) / (quadrat area = 25 m^2) x 100. The frequency of each plant species, which was defined as the number of quadrats in which the plant species was observed, was also calculated.

Five 100 ml soil cores (5 cm in depth) were sampled from the rhizosphere soil of each site. Soil water content, ratio of gravel, total C, total N and pH (H_2O) were measured in each sample and averaged for each site. Total C and N were measured by using the CN analyzer (SUMIGRAPH NC-1000, SHIMADZU, Kyoto).

Mycorrhizal and endophytic assessment

In September 2004, three seedlings of major plant species that were present at high frequencies (more than 4) or had a mean plant cover of more than 1.0 % at each site were selected and their lateral roots were sampled individually at each site. Also, three seedlings of major members of the Ericaceae that were not established at the study plots, but were representative colonizers on the crater basin at Mt. Esan, were randomly selected from outside of the study plots and subjected to lateral root sampling. Roots were separated from adhering soil by soaking and washing in tap water. Each root sample was stored in FAA solution (formaldehyde: acetic acid: ethyl alcohol: distilled water = 1:1:9:9) until microscopic investigation. Mycorrhizas were identified by root staining, as described by Phillips and Hayman (1970), with some modifications. Roots were rinsed with distilled water, cleared in 10% KOH for 10-20 min (3-5 min in case of fine roots) at 80°C, bleached in 2.5% H_2O_2 for 10-20 min (3-5 min in case of fine roots) at 60°C, acidified in 1% HCl at room temperature for 10 min, and stained using 0.1% Chlorazol Black E in lactoglycerine for 15 min at 80°C. Colonization by mycorrhizal fungi and root endophyte was identified by examining for the presence of typical structures, including vesicles or arbuscules for arbuscular mycorrhizal (AM) fungi, coiled intracellular hyphae for ericoid mycorrhizal (ERM) fungi, and thick walled darken hyphae or microsclerotia for dark septate endophytic (DSE) fungi. The frequencies of mycorrhizal or DSE associations were determined as the number of seedlings that were colonized by mycorrhizal or DSE fungi relative to the total number of seedlings observed.

Results

Vegetation

Frequencies, plant cover of each plant, and total plant cover at each site are presented in Table 1. In total, 7 herbaceous and 10 woody plant species were observed at the study sites. All woody plants except for *Sorbus commixta* Hedl. were shrubs. Only *Empetrum nigrum* var. *japonicum* K. Koch and *Ledum palustre* var. *diversipilosum* Nakai were commonly established at all sites. Site 1 is located adjacent to the craters, and plants were sparsely established at this site. Two ericaceous species, *E. nigrum* var. *japonicum* and *L. palustre* var. *diversipilosum*, *Polygonum weyrichii*Fr. Schm., and one unidentified Gramineae species were present at high frequencies, but displayed the lowest total plant cover (20.9%) at the study sites. In site 2, plant flora was similar to that at site 1, but the dominant plants *E. nigrum* var. *japonicum* and *L. palustre* var. *diversipilosum* showed high plant cover at 49.7% and 19.1%, respectively, representing most of the total plant cover (68.6%) at the site. In site 3, several ericaceous species such as *E. nigrum* var. *japonicum*, *L. palustre* var. *diversipilosum*, *Loiseleuria procumbens* (L.) Desv, and *Vaccinium vitis-idaea* var. *minus* Rodd. were dominantly observed. Each represented 54.9, 9.8, 26.9, and 9.0% of plant cover, respectively. In site 4, in addition to several ericaceous species that were also observed at other sites, *Sasa senanensis* Rhed. showed high plant cover at 54.3%. Total plant cover indicated 100%.

Table 1. Frequencies (F; n=10 in each site) and mean plant cover of each plant (C) and quadrat at study sites in volcanic area of Mt. Esan in Hokkaido, Japan

Family	Plant species	Site 1		Site 2		Site 3		Site 4	
		F	C	F	C	F	C	F	C
Anacardiaceae	*Rhus trichocarpa* Miq.	0	-	0	-	0	-	3	0.2
Aquifoliaceae	*Ilex sugerokii* Maxim. var. *brevipedunculata* (Maxim.) S. Y. Hu	0	-	0	-	0	-	3	1.3
Cyperaceae	*Carex* sp.	2	0.1	10	0.6	7	1	0	-
Empetraceae	*Empetrum nigrum* Linn. var. *japonicum* K. Koch	8	8	10	49.7	10	54.9	10	14.9
Ericaceae	*Enkianthus campanulatus* (Miq.) Nichols.	1	0.1	0	-	4	0.2	5	3.1
	Ledum palustre Linn. var. *diversipilosum* Nakai	9	4.2	10	19.1	10	9.8	10	82.9
	Loiseleuria procumbens (Linn.) Desv.	0	-	0	-	10	26.9	0	-
	Vaccinium vitis-idaea Linn. var. *minus* Lodd.	0	-	7	0.4	10	9	9	9.2
Gramineae	*Miscanthus sinensis* Anderss.	0	-	0	-	4	1.4	10	6.5
	Sasa senanensis (Franch. et Savat.) Rehder	0	-	0	-	0	-	10	54.3
	Gramineae sp.	8	6.6	0	-	0	-	0	-
Liliaceae	*Heloniopsis orientalis* (Thunb.) C. Tanaka	0	-	1	0.1	9	4.5	0	-
Polygonaceae	*Polygonum sachalinense* Fr. Schm.	2	0.1	5	0.3	0	-	10	4.8
	Polygonum weyrichii Fr. Schm.	7	6.6	7	1.5	0	-	0	-
Rosaceae	*Sorbus commixta* Hedl.	0	-	0	-	1	0.1	0	-
	Spiraea betulifolia Pall.	0	-	0	-	1	0.1	0	-
Saxifragaceae	*Hydrangea paniculata* Siebold	0	-	0	-	10	5.1	6	2.6
Mean plant cover (%) of quadrats		20.9±3.4		68.6±6.4		74.7±6.9		100.0±0.0	

Soil properties

The properties of the soil at each site are shown in Table 2. The soil pH values were low at all sites, ranging from 4.1 to 4.6. The soil water contents at site 1 (19.6%) and 2 (24.7%) were lower than at site 3 (34.7%) and 4 (45.1%). The gravel ratio was higher at site 1 (58.5%) and 2 (34.7%) than at site 3 (14.4%) and 4 (15.0%). Total C and N at site 1 (C = 0.3%, N = 0.02%) and 2 (C = 0.7%, N = 0.04%) were relatively lower than at site 3 (C = 3.4 %, N = 0.14 %) and 4 (C = 2.8 %, N = 0.08 %).

Colonization status of mycorrhizal and endophytic fungi

The frequencies of mycorrhizal or endophytic fungal associations in representative plants established in study plots are presented (Table 3 and Figs. 3, 4, 5). Most plants belonging to ericaceous species were heavily associated with ERM and erratically associated with DSE fungi at all study sites. They were also commonly associated with AM and DSE fungi only at site 4. Among ericaceous plants, only *Enkianthus campanulatus* Nichols. was associated with AM and DSE fungi, and not with ERM fungi. Other herbaceous

Table 2. Soil properties at each site in volcanic area of Mt. Esan in Hokkaido, Japan. Means and standard deviations are indicated

Site	Soil water content (%)	Ratio of >2.0 mm particle size	Total C (%)	Total N (%)	C/N	Soil pH
1	19.6±2.8	58.5±20.2	0.3±0.1	0.02±0.01	14.6±2.6	4.2±0.2
2	24.7±1.9	34.7±12.9	0.7±0.1	0.04±0.01	21.4±0.8	4.1±0.2
3	34.7±5.2	14.4±9.4	3.4±0.9	0.14±0.02	24.2±4.1	4.6±0.2
4	45.1±1.2	15.0±7.4	2.8±1.0	0.08±0.03	37.0±4.7	4.2±0.1

Table 3. Frequencies (n=3 for each plant at each site) of ericoid mycorrhizal (ER) and arbuscular mycorrhizal (A) association in plants that were established at study sites on Mt. Esan in Hokkaido, Japan. Frequencies of dark septate endophytic fungi (DSE) are also presented

Plant species	Site 1			Site 2			Site 3			Site 4		
	ER	A	DSE	ER	A	DSE	ER	A	DSE	ER	A	DSE
Gramineae												
Sasa senanensis										0	3	3
Miscanthus sinensis							0	3	1	0	3	1
Gramineae sp.	0	3	1									
Cyperaceae												
Carex sp.				0	0	3	0	3	1			
Liliaceae												
Heloniopsis orientalis							0	3	2			
Polygonaceae												
Polygonum weyrichii	0	0	0	0	0	2						
Polygonum sachalinense				0	0	1				0	3	1
Saxifragaceae												
Hydrangea paniculata							0	3	0	0	3	0
Empetraceae												
Empetrum nigrum var. *japonicum*	3	0	0	3	0	2	3	0	1	3	2	3
Aquifoliaceae												
Ilex sugerokii var. *brevipedunculata*										0	3	2
Ericaceae												
Ledum palustre var. *diversipilosum*	3	1	0	3	0	0	3	0	0	3	3	3
Loiseleuria procumbens							3	0	0			
Enkianthus campanulatus							0	3	1	0	3	2
Vaccinium vitis-idaea var. *minus*				3	0	1	3	0	0	3	3	1

and woody plants were associated commonly with AM and erratically with DSE fungi at all study sites, except for *Carex* sp. and *Polygonum* spp., which were erratically associated with AM and DSE fungi.

The frequencies of mycorrhizal associations in ericaceous species from outside of the study plots are presented in Table 4. Seven ericaceous species, *Tripetaleia bracteata*, *Rhododendron tschonoskii*, *Rhododendron kaempferi*, *Leucothoe grayana*, *Gaultheria miqueliana*, *Vaccinium smallii* and *Vaccinium oldhamii,* were newly observed. With the exception of *E. campanulatus*, most of the ericaceous species were associated commonly with ERM fungi.

Discussion

On the crater basin of Mt. Esan, dwarf shrubs of ericaceous plants such as *E. nigrum* var. *japonicum*, *L. palustre* var. *diversipilosum*, *V. vitis-idaea* var. *minus* and *L. procumbens* were the main colonizers and developed plant communities along the different vegetation areas (Table 1). Generally, alpine vegetation in Hokkaido is characterized by the domination of *Pinus pumila* (Itou 1987), but dwarf shrub communities that are dominated by ericaceous plants such as *L. procumbens*, *Arcterica nana* and *Vaccinium uliginosum* are also spread out like a carpet on wind-swept areas. It is known that the vegetation of volcanic areas often resembles that of alpine wind-swept areas, possibly because both areas have similar environments, and ericaceous plants spread out like a carpet or colonize patchily (Miyawaki 1997). The vegetation on the crater basin of Mt. Esan was characteristic of volcanic wind-swept dwarf shrub communities.

Even though a low pH and nutrient status exists on Mt. Esan (Table 2), ericaceous species become established and dominant because of their high tolerance to severe conditions. The presence of ERM associations is regarded as a key factor for the establishment of ericaceous species in such extreme environments (Cairney and Meharg 2003; Diaz *et al*. 2006). In the present study, all ericaceous species except for *E. campanulatus* associated with ERM fungi at all study sites, even at site 1 which had the most severe soil moisture and nutrient availability conditions among the study sites. Thus, ERM associations might play a significant role in invasion, establishment, and development of plant communities under the severe conditions on the crater basin of Mt. Esan.

On the other hand, AM associations were commonly observed in most herbaceous and woody plants at site 3 and 4. Only 1 out of 8 plant species that made associations with AM fungi established and formed AM associations under the most severe soil conditions at sites 1 and 2. *Carex* sp. and *Polygonum* spp. did not form AM associations at sites 1 or 2, but formed them at sites 3 and 4. Wang and Qiu (2006) reported that more than half of plant species in the genera *Carex* and *Polygonum* were facultatively AM or non-mycorrhizal. Thus, it seems that most AM plants could not invade the harsh environment on the crater basin. *Carex* and *Polygonum* might be more tolerant of soils with a poor nutrient and water content and not require AM associations to survive under these conditions. AM associations may play significant roles in plant establishment in relatively amended areas on the crater basin of Mt. Esan.

Table 4. Frequencies (n=3 or 2 in each plant) of ericoid mycorrhizal associations in plants of Empetraceae and Ericaceae at study sites on Mt. Esan in Hokkaido, Japan

Plant species	F
Empetraceae	
Empetrum nigrum var. *japonicum*	3/3
Ericaceae	
Enkianthus campanulatus	0/3
Gaultheria miqueliana Takeda	3/3
Ledum palustre var. *diversipilosum*	3/3
Leucothoe grayana Maxim. var. *oblongifolia* (Miq.) Ohwi	3/3
Loiseleuria procumbens	3/3
Rhododendron kaempferi Planch.	3/3
Rhododendron tschonoskii Maxim.	3/3
Tripetaleia bracteata Maxim.	3/3
Vaccinium oldhamii Thunb.	2/2
Vaccinium smallii A. Gray	3/3
Vaccinium vitis-idaea var. *minus*	3/3

Generally, AM fungi are not thought to occur in ericaceous plants (e.g., Harley and Harley 1987; Treu *et al*. 1996). However, some reports of this type of symbiosis (e.g., McGee 1986; Koske *et al*. 1990; Francis and Read 1995) have suggested that AM fungi have sufficient inoculum potential to penetrate nearby plants that are not normally associated with AM fungi. At site 4, in addition to ericaceous plants, *S. senanensis* showed high plant cover at 54.3%, which implies that it can coexist with ericaceous plants (Table 1). *Sasa senanensis* was also commonly associated with AM fungi (Table 3). It might be that the propagula of AM extended from *S. senanensis* to host plants, compulsorily colonized into the roots of adjacent ericaceous plants. On the other hand, Urcelay (2002) surveyed the status of mycorrhizal associations in *Gaultheria poeppiggi* DC in Central Argentina and revealed that ERM fungi were present in the terminal portions of the finer roots, whose sizes generally ranged from 60 to 120 µm, while AM fungi were present in wider roots whose sizes ranged from 80 to 250 µm. Therefore, the size of the section of root observed might influence the mycorrhizal status because most observations have focused on fine hair roots which constitute the main parts of roots used for ERM synthesis.

Although *E. campanulatus* associated with AM fungi, it was the only species not to associate with ERM fungi (Table 3 and 4). It has been reported that, among 15 ericaceous species, only *E. campanulatus* cannot form ERM associations following inoculation with the most common ERM fungi *Hymenoscyphus ericae* (Read) Korf & Kernan (Gorman and Starrett 2003). From field observations in the present study and an inoculation experiment described in a previous report, it seems that *E. campanulatus* intrinsically forms AM associations but not ERM associations. *Enkianthus* is known to be the most basal extant in the phylogenetic trees of ericaceous plant species (Anderberg 1992; Kron 1996; Kron *et al*. 1999). It was suggested that AM is an ancestral type of mycorrhizal association that occurred in all early diverging lineages of major clades of land plants (Brundrett 2002; Wang and Oiu 2006). Thus, *Enkianthus* might yet be in a transitional state between a plant species that forms only AM associations and a plant that has adapted to other types of mycorrhizal associations such as ERM.

Root colonization by DSE fungi was erratically observed in most plant species. Although very little is known about the host range, ecology and function of DSE fungi, several studies have shown various aspects of DSE fungi and their associations with colonized host plants. Jumpponen and Trappe (1998) reported that DSE colonization was observed in about 600 plant species, suggesting a very wide range of host plant species, or little or no host specificities. Also, DSE fungi are widely distributed in various ecosystems, ranging from tropical and temporal zones (Ahlich and Sieber 1996) to boreal forests (Summerbell 1989). In particular, the high abundance of DSE fungi in harsh environments such as alpine, arctic area (Olsson *et al*. 2004; Treu *et al*. 1996), and deglaciated areas (Trowbridge and Jumpponen 2004; Cázares et al. 2005) strongly suggests that they can colonize areas under severe conditions and also help in host plant establishment.

In conclusion, a variety of plants was established in areas in the vicinity of Mt. Esan and showed different mycorrhizal associations according to the location of the study plot. In particular, ericaceous plants and ERM associations played a significant role in the development of plant communities in the crater basin on Mt. Esan.

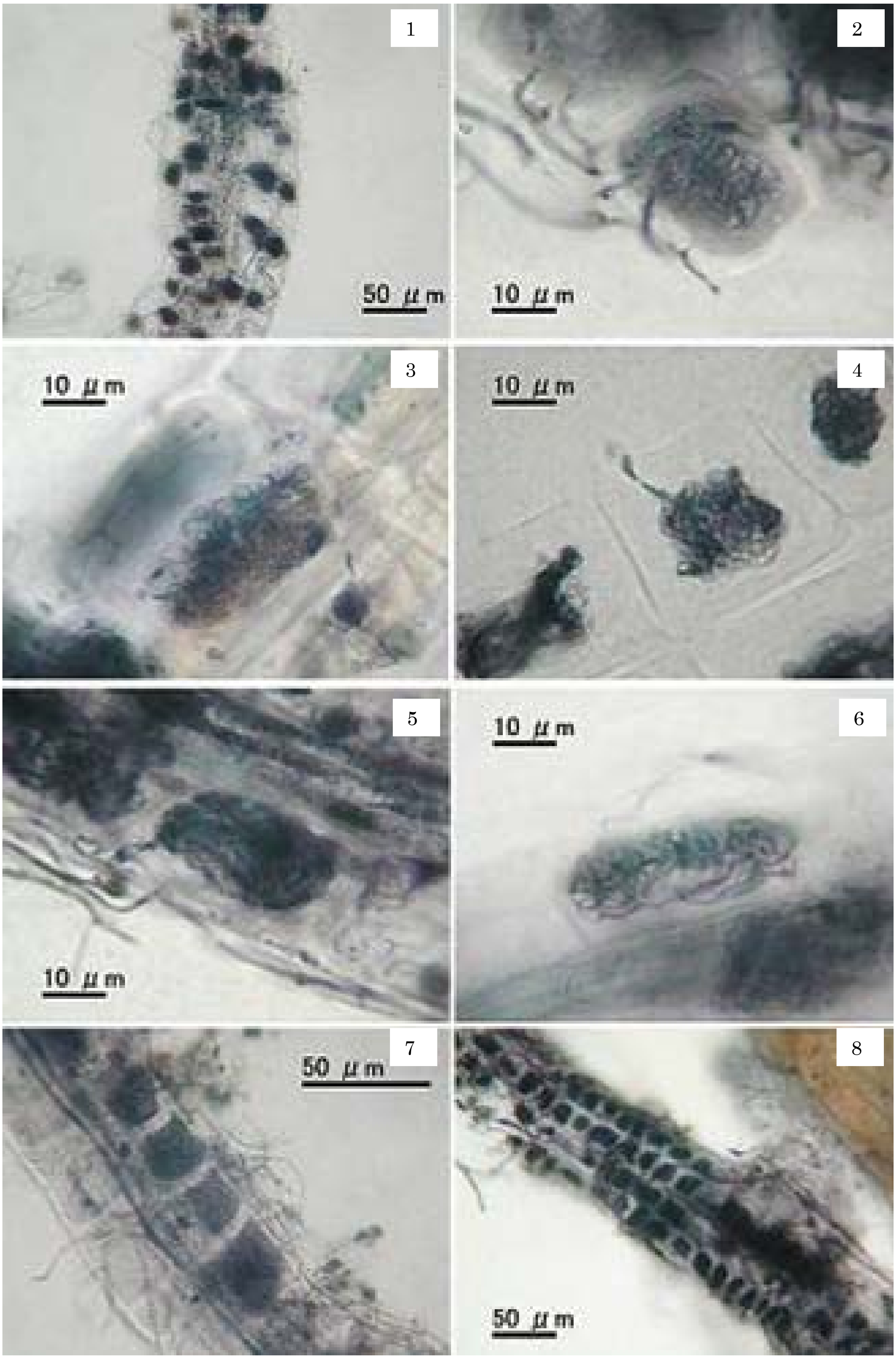

Fig. 3. Ericoid mycorrhizal colonization in roots of plants on crater basin of Mt. Esan, Hokkaido, Japan. 1; *Empetrum nigrum* var. *japonicum*, 2; *Gaultheria miqueliana*, 3; *Ledum palustre* var. *diversipilosum*, 4; *Loiseleuria procumbens*, 5; *Rhododendron kaempferi*, 6; *Rhododendron tschonoskii*, 7; *Tripetaleia bracteata*, 8; *Vaccinium vitis-idaea* var. *minus*.

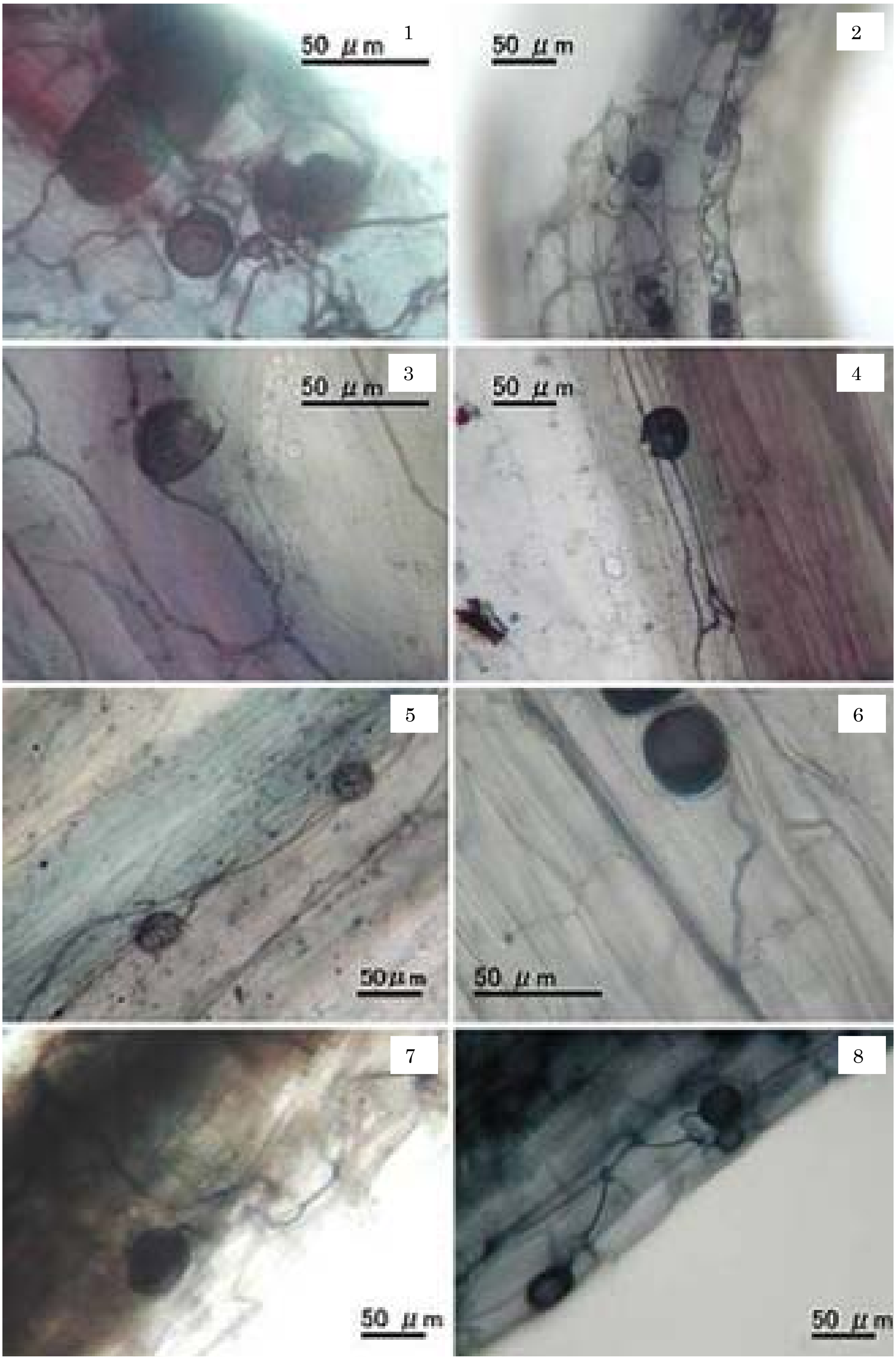

Fig. 4. Arbuscular mycorrhizal colonization in roots of plants on crater basin of Mt. Esan, Hokkaido, Japan. 1; *Sasa senanensis*, 2; *Miscanthus sinensis*, 3; *Carex* sp., 4; *Heloniopsis orientalis*, 5; *Polygonum sachalinense*, 6; *Hydrangea paniculata*, 7; *Empetrum nigrum* var. *japonicum*, 8; *Enkianthus campanulatus.*

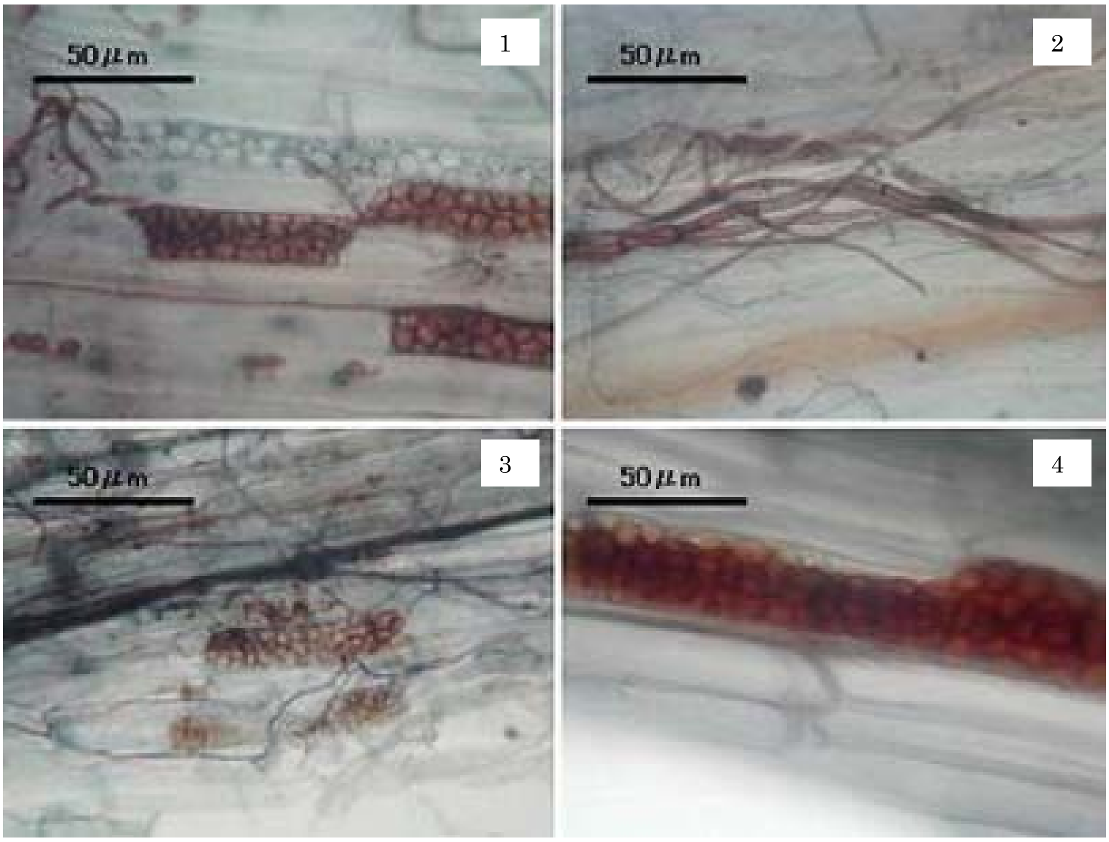

Fig. 5. Dark septate endophytic colonization in roots of plants on crater basin of Mt. Esan, Hokkaido, Japan. 1; *Sasa senanensis*, 2; *Carex* sp., 3; *Ledum palustre* var. *diversipilosum*., 4; *Hydrangea paniculata*.

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