Successional changes in mycorrhizal type in the pioneer plant communities of a subalpine volcanic desert on Mt. Fuji, Japan

Masaaki Fujiyoshi[1]*, Atsushi Kagawa[2], Takayuki Nakatsubo[3] and Takehiro Masuzawa[2]

[1]*Department of Human Development, School of Humanities and Culture, Tokai University, Kitakaname 1117, Hiratsuka 259-1292*
[2]*Department of Biology and Geosciences, Graduate School of Science and Technology, Shizuoka University, Ohya 836, Shizuoka 422-8017*
[3]*Department of Environmental Dynamics and Management, Graduate School of Biosphere Science, Hiroshima University, Kagamiyama 1–7–1, Higashi-Hiroshima 739-8521*
*Corresponding author. E-mail: masaakif@keyaki.cc.u-tokai.ac.jp

Abstract: Isolated island-like plant communities dominated by the perennial herb *Polygonum cuspidatum* are typical pioneer vegetation in the subalpine volcanic deserts of Japan. To study the relationship between mycorrhizal associations and plant community development, we conducted a survey of the mycorrhizal status of plants in subalpine island communities in a volcanic desert on Mt. Fuji. Roots of 45 native species, belonging to 23 families, were collected from island communities at 3 different successional stages: (I) pioneer communities dominated by *P. cuspidatum*, (II) mixed communities of herbs and grass, and (III) mixed communities of herbs and shrubs. Arbuscular mycorrhiza (AM) was the predominant mycorrhizal type in the early stages of community successions (I and II). *P. cuspidatum*, which had previously been reported as a non-mycorrhizal species, formed AM at the study site. In the later stage (III), 5 mycorrhizal types, AM, ecto-, ericoid, arbutoid and orchid mycorrhizal, were observed. Seven woody species had two types of mycorrhizas (AM and ectomycorrhiza). High spore densities of AM fungi were observed in the soil of the island communities, whereas few spores were observed in bare ground without vegetation cover. The average colonization levels of the AM fungi were within a wide range of 0.1 to 72%, independent of successional progress. These data show that marked increases in mycorrhizal types occur during the succession of island communities, although AM is the predominant mycorrhizal type throughout the successional process.

key words: arbuscular mycorrhiza, island community, Mt. Fuji, plant succession, volcanic desert

Introduction

Plant succession in alpine and subalpine volcanic zones takes place very slowly. There are a number of places where the vegetation has remained in the pioneer stage (volcanic desert) for several hundred years after a volcanic eruption (Yoshii, 1940; Miyawaki, 1971). Because of severe environmental conditions such as a short growing season (Maruta, 1976, 1983), soil dryness (Maruta, 1976), and low nutrient availability (Chiba and Hirose, 1993),

only a limited number of plant species can colonize in these habitats. The perennial herb *Polygonum cuspidatum* (＝*Reynoutria japonica*, Polygonaceae) and a related species (*P. sachalinense*) are among the most important first colonizers in subalpine volcanic deserts in Japan (Yoshii, 1940, 1942). These species form island-like isolated communities, which later successional species can colonize (Yoshii, 1942; Masuzawa and Suzuki, 1991; Masuzawa, 1995).

In the subalpine volcanic desert of Mt. Fuji, "island communities" at different successional stages can be observed, ranging from almost pure stands of the first colonizer *P. cuspidatum* to those that include later successional species. The pattern and mechanisms of succession have been studied by a number of ecologists (Maruta, 1976; Hirose and Tateno, 1984; Masuzawa and Suzuki, 1991; Masuzawa, 1995; Adachi *et al.*, 1996a, b; Nishitani and Masuzawa, 1996), and is known to begin with the colonization of *P. cuspidatum* on bare ground. As the *P. cuspidatum* stand spreads outward, the shoot density of *P. cuspidatum* decreases in its center (central die-back phenomenon) (Masuzawa, 1995; Adachi *et al.*, 1996a, b) thus providing a colonization site for later successional species. Adachi *et al.* (1996a, b) studied the mechanisms of the central die-back phenomenon in a volcanic desert on Mt. Fuji, and concluded that neither interspecific nor intraspecific competition are the likely primary cause; rather, central die-back is brought about intrinsically by the growth pattern of the *P. cuspidatum* rhizome systems.

However, soil nutrient conditions might also be important in determining successional processes in island communities. It has been reported that nitrogen increases during the central die-back phenomenon, accumulating especially in the central part of the island community (Hirose and Tateno, 1984; Tateno and Hirose, 1987). Under nitrogen limiting conditions, secondary colonizer plants such as *Aster ageratoides* var. *ovatus* and *Miscanthus oligostachyus* do not grow as well as *P. cuspidatum* (Chiba and Hirose, 1993). It is likely, therefore, that nutrient accumulation during the central die-back phenomenon has significant influence on the progress of primary succession. However, many uncertainties still exist as to the nutrient economy of pioneer plants and its relationship with successional processes, among which is the role of mycorrhizas.

It is well known that most terrestrial vascular plants form mycorrhizas (Maeda, 1954; Harley and Harley, 1987a, b; Smith and Read, 1997). Based on the anatomy and taxonomic positions of the partners, mycorrhizas can be divided into seven distinct types, namely ecto-, ectoendo-, arbuscular, ericoid, arbutoid, orchid, and monotropoid mycorrhizas (Harley, 1989; Smith and Read, 1997). There are some distinct qualitative differences in the nature of the substances that are moved in the different types of mycorrhizal associations. The ability of a host plant to access soil nutrients is largely dependent on its mycorrhizal type (Melin and Nilsson, 1953; Read and Stribley, 1973; Tinker, 1975; Smith and Read, 1997); therefore, it is possible to hypothesize that there might be a close relationship between the occurrence of different mycorrhizal types and the successional pattern in island communities. Although mycorrhiza occurrence in volcanic deserts has been studied by several authors (Allen and Allen, 1990; Allen *et al.*, 1992; Titus and Del Moral, 1998a, b; Titus and Tsuyazaki, 2002; Nara *et al.*, 2003a, b), little is known about the relationship between mycorrhizal type and successional patterns in island communities.

To study this relationship, we investigated the abundance of the different types of mycorrhiza associated with the herbaceous and woody plants growing in island communities in

a subalpine volcanic desert on Mt. Fuji. We also examined the AM colonization of plant roots and spore density of AM fungi in soil, because AM are the most abundant mycorrhizal type at this site.

Materials and methods

Study site

The study site was located on the southeast slope of Mt. Fuji between 1550 and 1650 m above sea level (35°20′N, 138°47′E) (Fig. 1). An accumulation of basaltic ejecta composed mainly of scoria (typically 2–30 mm width) and reaching 5 m at its deepest (Tsuya, 1971) from Mt. Hoei (2702 m), a parasitic volcano on the mid-slope of Mt. Fuji which formed in an eruption in 1707, extends widely over the southeast slope. From an altitude of 1400 m to the summit of Mt. Hoei this volcanic scoria desert is about 3–4 km wide.

The study site has a cool humid climate with an annual mean air temperature of 8.6°C
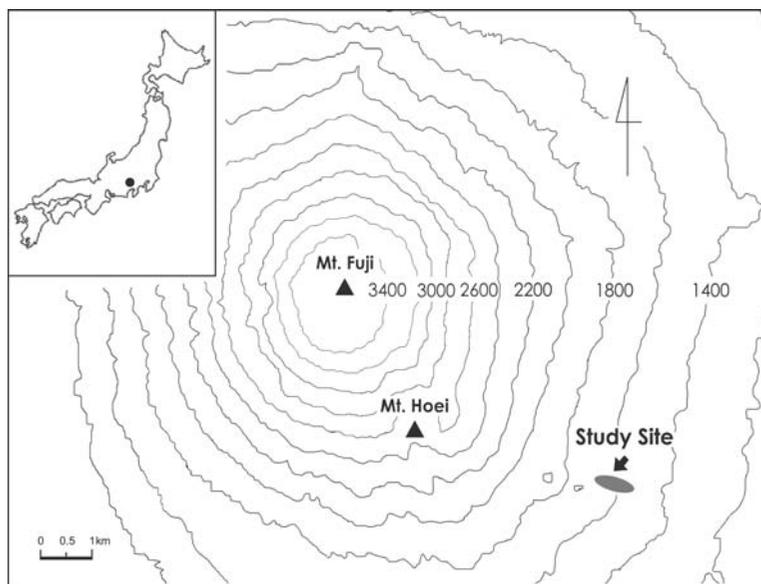
Fig. 1. Location of the study site on Mt. Fuji.

Table 1. Some physical and chemical properties of soil collected from the study site.

Stage	Soil density (g soil/cm³)	pH (H₂O)	Total C content (mg/cm³)	Total N content (mg/cm³)	Total P content (μg/cm³)	Solbule P content Olsen (μg/cm³)
B	0.4±0.04	6.0±0.06	0.6±0.06	0.1±0.02	24.3±7.2	0.7±0.51
Ⅰ	0.1±0.01	5.9±0.07	0.6±0.02	0.2±0.01	8.9±0.7	0.3±0.03
Ⅱ	0.2±0.01	5.8±0.04	1.3±0.05	0.5±0.02	18.4±1.1	0.5±0.12
Ⅲ	0.2±0.03	5.7±0.06	1.4±0.23	0.6±0.07	18.6±2.0	0.4±0.11

Data represent average ± SE (*n*=3).

and a monthly mean air temperature ranging from $-1.9°C$ in January to 19.1°C in August (Tateno and Hirose, 1987). The annual precipitation in 2003 in Gotenba, about 15 km southeast of the study site, was 3433 mm. Average soil pH (H_2O) levels are within a narrow range of 5.7 to 6.0 (Table 1), and soil carbon and nitrogen concentrations tend to increase with successional progress (Table 1). There were no significant differences in the soil bicarbonate-extractable P among successional stages including in the bare ground (B) (Table 1).

Vegetation

A number of island communities at different stages are scattered within the study site. During the study period, the diameter of the largest island community exceeded 20 m. The growing season usually begins in late April and ends in early October. Snowfall occurs from midwinter (December) to early spring (April) at which point avalanches sometimes destroy the vegetation.

In this study, plant materials were collected from island communities at three different successional stages (stages I, II, and III). The first stage (I) contained pioneer communities dominated by *P. cuspidatum* (Fig. 2I). Although a few other herbaceous plants were also found in these communities, their coverage ($<5\%$) was much smaller than that of *P. cuspidatum*. In stage II, *P. cuspidatum* occupied the peripheral area, while herbs and grass such as *A. ageratoides* var. *ovatus*, *Calamagrostis hakonensis* and *M. oligostachyus* were found in the central areas (Fig. 2II). The coverage of *P. cuspidatum* in stage II ranged from 40 to 60%. In a later stage (stage III), shrubs such as *Betula ermanii*, *Larix kaempferi* and *Salix reinii*
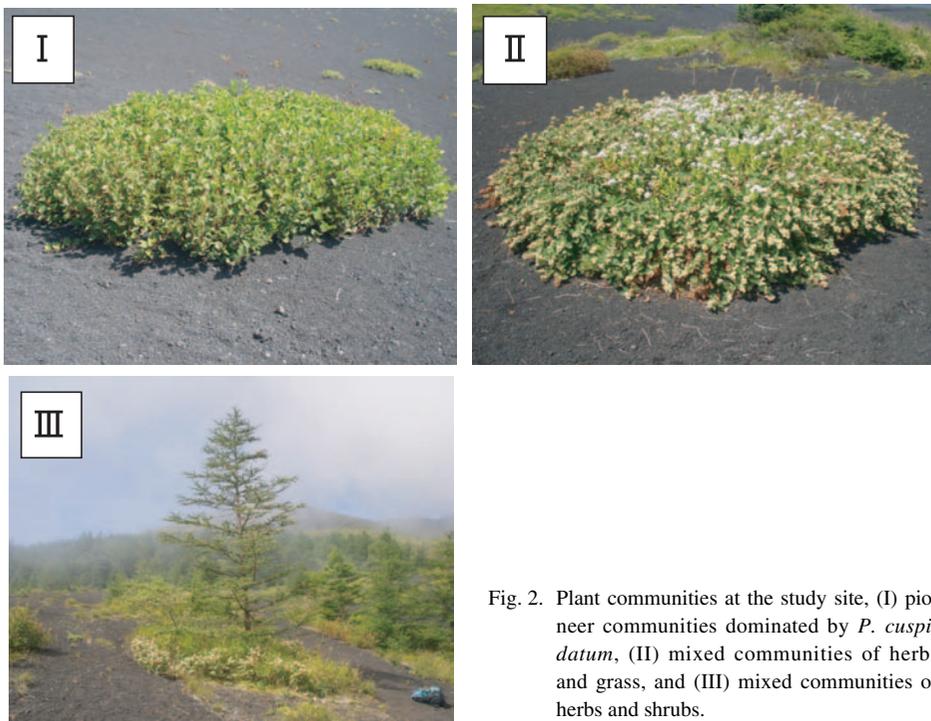
Fig. 2. Plant communities at the study site, (I) pioneer communities dominated by *P. cuspidatum*, (II) mixed communities of herbs and grass, and (III) mixed communities of herbs and shrubs.

colonized the central areas while various species of herbaceous plants including *P. cuspidatum* were observed in the community (Fig. 2III). The coverage of *P. cuspidatum* in stage III (＜10%) was much smaller than in the early stages (stages I and II).

Sampling of plant roots and soil

Three island communities belonging to each of the 3 successional stages (stages I–III) were selected for the present study. An average of 5 (1–18) specimens per plant species (total 45 plant species) were collected from the selected island communities from June to October 2003. For plant species that grew in different successional stages (stages I–III or II–III), the specimens were collected evenly from stages where it occurred. Root samples were carefully excavated with shoots and adhering soil. Any soil particles and organic debris adhering to the roots were carefully removed in a water bath using a paintbrush. Special care was taken to eliminate contaminants originating from the roots of other species.

To determine the density of AM fungal spores in the soil, three island communities representing each stage were selected. On August 23, 2003, three core soil samples (0–20 cm in depth) were randomly collected from each community. Soil samples collected from the bare ground were also examined for the presence of AM fungal spores. The soil samples were air-dried and passed through a 2-mm mesh sieve prior to analysis.

Observations of mycorrhizal type

Part of each root sample (0.01–10 g in dry weight) was used for mycorrhizal type identification. The roots were cut into 1–2 cm segments, washed with 2.5% (w/v) KOH solution, and stained with 0.05% (w/v) trypan blue in lactophenol according to the method of Koske and Gemma (1989). Root segments were viewed under light microscopy at either ×100 or ×400 and screened for mycorrhizal infection. If a fungal sheath-like structure was observed, a cross section of the root sample was viewed under light microscopy at ×100 or ×400 to determine whether it was ectomycorrhizal or not.

Mycorrhizal types were determined according to the criteria of Harley (1989). To avoid confusion with non-mycorrhizal fungi, which also have non-septae leading internal hyphae, AM fungi were recorded only when arbuscules or vesicles were present. Ectomycorrhizas were recorded when both a fungal sheath and hartig net were observed. Determinations of other mycorrhizal types were made by observations of the fungal sheath, hartig net, fungal coil, or a combination of these three structures. Each root system was examined for the presence or absence of each mycorrhizal type.

AM colonization

To estimate the extent of AM colonization, 10 randomly selected root fragments were mounted on slides. The presence or absence of AM colonization (internal hyphae, vesicles or arbuscles) was determined using light microscopy (×100) on intersecting vertical gridlines (McGonigle *et al*., 1990; Klironomos *et al*., 1996). AM colonization (*AC*) was quantified as follows:

$$AC = Ni/Nt \times 100 \ (\%),$$

where *Ni* is the number of vertical gridlines intersected by infected roots (AM) and *Nt* is the total number of vertical gridlines intersected by roots (both infected and noninfected).

Number of AM spores in soil

The number of spores in the soil samples was determined by a modification of the sucrose centrifugation method (Daniels and Skipper, 1982; Nishio, 1987). Three grams of air-dried soil was suspended in water and centrifuged at 1500 rpm for 5 min. The precipitate was then suspended in 50% sucrose and centrifuged at 1500 rpm for 1 min. The supernatant was poured into a 45-μm sieve and rinsed with distilled water. After washing the inner surface, the contents of the sieve were transferred to a petri dish containing distilled water. The number of spores in the petri dish was counted under a dissecting microscope ($\times$10–65).

Results

Forty-five species of 23 plant families representing most of the vegetation of the study site were examined for their mycorrhizal status. Most of these species had mycorrhizal associations of one or two types (Table 2).

The pioneer plant species that colonized the stage I communities were non-mycotrophic and/or AM plants (Table 2). Of these, *Arabis serrata* (Cruciferae) and *Polygonum weyrichii* var. *alpinum* (Polygonaceae) showed no mycorrhizal colonization in any cases. Plant species belonging to Cruciferae and Polygonaceae generally have non-mycorrhizal associations, although a few species with mycorrhizal associations have been reported (Harley and Harley, 1987a, b). The other Polygonaceous species at the study site, *P. cuspidatum*, showed an AM association in most cases though its colonization level was low (Table 2; Fig. 3).
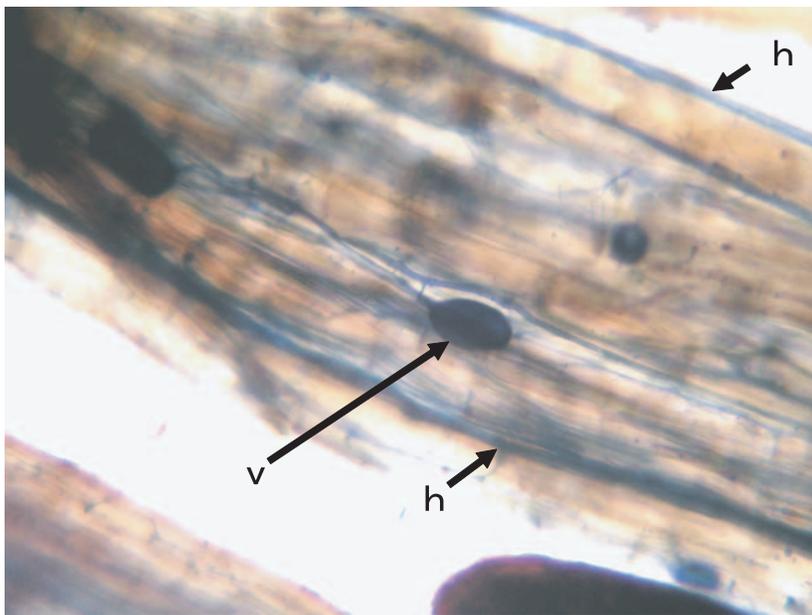
Fig. 3. *Polygonum cuspidatum* root infected by AM fungi in the stage II community. Shown are vesicle (v) and hyphae (h).

Table 2. Mycorrhizal type of plants growing in the volanic desert on Mt. Fuji.

Family	Species	Successional stages[a]			n[b]	Type of mycorrhizal association[c]						AM colonization level (%)[d]
		I	II	III		Non	Ecto	Arbutoid	Ericoid	Orchid	AM	
GRAMINEAE	*Calamagrostis hakonensis*	○	○	○	18						18	5±1
POLYGONACEAE	*Polygonum weyrichii* var. *alpinum*	○	○	○	5	5						0
POLYGONACEAE	*Polygonum cuspidatum*	○	○	○	9	1					8	2±1
CRUCIFERAE	*Arabis serrata*	○	○	○	5	5						0
CAMPANULACEAE	*Campanula punctata* var. *hondoensis*	○	○	○	5						5	3±1
COMPOSITAE	*Senecio nemorensis*	○	○	○	5						5	24±8
COMPOSITAE	*Cirsium purpuratum*	○	○	○	5						5	14±4
COMPOSITAE	*Picris hieracioides* var. *glabrescens*	○	○	○	5						5	7±2
GRAMINEAE	*Miscanthus oligostachyus*	○	○	○	5						5	3±1
RANUNCULACEAE	*Clematis stans*		○	○	5	1					4	55±15
ROSACEAE	*Spiraea japonica*		○	○	5	1					4	2±1
ROSACEAE	*Rosa luciae* var. *fujisanensis*		○	○	5						5	35±10
LEGUMINOSAE	*Hedysarum vicioides*		○	○	10						10	13±4
GUTTIFERAE	*Hypericum erectum* var. *caespitosum*		○	○	5						5	72±4
COMPOSITAE	*Aster ageratoides* var. *ovatus*		○	○	15						15	10±3
GENTIANACEAE	*Pterygocalyx volubilis*		○	○	5	2					3	0.2±0.1
PINACEAE	*Abies veitchii*			○	1		1				1	0.3
PINACEAE	*Larix kaempferi*			○	5		5				4	1±1
PINACEAE	*Pinus densiflora*			○	1		1				1	1
CYPERACEAE	*Carex* sp.			○	5	5						0
LILIACEAE	*Polygonatum falcatum*			○	1						1	29
LILIACEAE	*Aletris foliata*			○	5	3					2	0.3±0.2
ORCHIDACEAE	*Gymnadenia cucullata*			○	3	2				1		0
ORCHIDACEAE	*Spiranthes sinensis*			○	1					1		0
SALICACEAE	*Salix reinii*			○	5		5				4	0.1±0.1
SALICACEAE	*Salix integra*			○	2		2				1	0.2±0.2
SALICACEAE	*Salix* sp.			○	5		5				3	0.6±1

Family	Species	Colonization[a]	No. examined[b]	Non	Ecto	Arbutoid	Ericoid	Orchid	AM[c]	average ± SE[d]
BETULACEAE	*Betula ermanii*	○	5		5				1	0.1 ± 0.1
RANUNCULACEAE	*Clematis ochotensis*	○	1						1	34
ROSACEAE	*Fragaria nipponica*	○	5						5	65 ± 3
ROSACEAE	*Prunus incisa*	○	2						2	6 ± 6
ROSACEAE	*Sorbus commixta*	○	3		1				2	0.7 ± 0.4
LEGUMINOSAE	*Maackia amurensis* var. *buergeri*	○	1						1	2
LEGUMINOSAE	*Astragalus adsurgens*	○	5						5	5 ± 2
ACERACEAE	*Acer micranthum*	○	1						1	1
ELAEAGNACEAE	*Elaeagnus montana*	○	3						3	8 ± 5
CORNACEAE	*Cornus controversa*	○	1						1	20
PYROLACEAE	*Pyrola incarnata*	○	5			5			2	0.2 ± 0.1
ERICACEAE	*Leucothoe grayana* var. *venosa*	○	1				1			0
OLEACEAE	*Ligustrum tschonoskii*	○	3						3	1 ± 0.3
OLEACEAE	*Fraxinus lanuginosa*	○	1						1	0.1
SCROPHULARIACEAE	*Euphrasia iinumae* var. *idzuensis*	○	5	3					2	0.1 ± 0.1
COMPOSITAE	*Anaphalis margaritacea*	○	5						5	10 ± 5
COMPOSITAE	*Solidago virga-aurea* var. *asiatica*	○	5						5	4 ± 1
COMPOSITAE	*Artemisia princeps*	○	5						5	5 ± 2

a) colonization of plants
b) number of plants examined
c) number of plants assosiated with mycorrhiza, Non: non mycorrhiza, Ecto: ecto mycorrhiza, Arbutoid: arbutoid mycorrhiza, Ericoid: ericoid mycorrhiza, Orchid: orchid mycorrhiza, AM: arbuscular mycorrhiza.
d) average ± SE

The number of plant species in the stage II community was almost twice that in the stage I community. All species that had colonized the stage II communities, but not all those in stage I, showed AM colonization (Table 2). AM were found in species belonging to the following families: Campanulaceae, Compositae, Gentianaceae, Gramineae, Guttiferae, Leguminosae, Ranunculaceae and Rosaceae (Table 2). The average colonization levels of AM fungi ranged from 0.2 (*Pterygocalyx volubilis*) to 72% (*Hypericum erectum* var. *caespitosum*). Some dominant plants such as *C. hakonensis, M. oligostachyus* and *P. cuspidatum*, showed very low levels of AM colonization ($<$5%).

In the later stage (stage III), ecto-, ericoid, arbutoid and orchid mycorrhizal species were observed in the island communities. However, AM species were still dominant and a few non-mycorrhizal plants were also found. As in stages I and II, AM colonization levels varied widely among species (Table 2). Seven woody species (*Abies veitchii*, *B. ermanii*, *L. kaempferi*, *Pinus densiflora*, *Salix integra*, *S. reinii*, *Salix* sp.) and one herbaceous species (*Pyrola incarnate*) had two types of mycorrhizas (AM and ectomycorrhiza, or AM and arbutoid mycorrhiza) (Table 2).
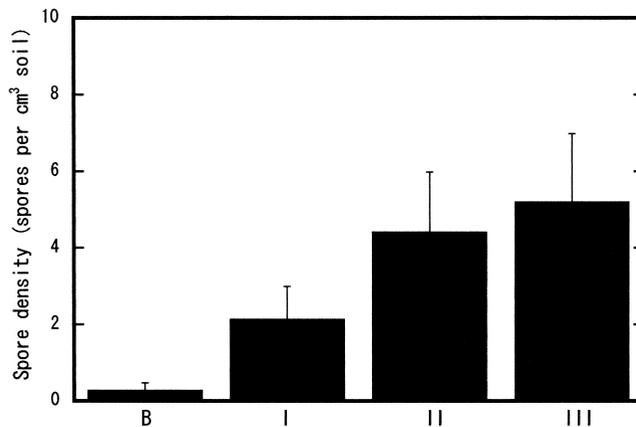
Fig. 4. AM fungal spore density per cm^3 soil. Vertical bars represent SE (n=9).

The spore density of AM fungi in soil samples collected from different successional stages (bare ground and stages I–III) is shown in Fig. 4. The effect of successional stages on the spore density was significant (ANOVA $P<$0.05). Soil samples from the bare ground contained few spores, whereas larger numbers of spores were observed in samples collected from the island communities. The average spore number per cm^3 soil of stage I communities was 2.1, which was comparable to those in the later stages (stages II and III). The average spore numbers did not differ significantly among island communities of different stages (Fig. 4).

Discussion

Mycorrhiza establishment and colonization in volcanic deserts has been studied by several authors (Allen *et al*., 1984, 1987, 1992; Allen, 1988, 1991; Allen and Allen, 1990; Titus

and Del Moral, 1998a, b; Titus *et al*., 1998; Titus and Tsuyazaki, 2002). In the volcanic desert of Mount St. Helens, non-mycotrophic and/or facultatively mycotrophic (AM) plants were the first colonizers, followed by obligatory mycotrophic plants such as ectomycorrhizal species after the fungal populations had established in the soil (Allen and Allen, 1990; Allen, 1991). On the other hand, Haruki and Tsuyuzaki (2001) reported that *Betula*, *Populus* and *Salix* species were the first colonizers in the volcanic desert of Mt. Usu, Hokkaido. These plants are known as ectomycorrhizal species (Harley and Harley, 1987a, b). Successional changes in the island communities of this study site are similar to those on Mount St. Helens in that obligatory mycotrophic plants (ecto-, ericoid, arbutoid and orchid mycorrhizal species) colonized only in the later stage. However, most of the species that had colonized the stage II and III communities also showed AM colonization. In addition, in the stage III community, seven woody species (Betulaceae, Pinaceae and Salicaceae) and one herbaceous species (Pyrolaceae) had two types of mycorrhizas (AM and ectomycorrhiza, or AM and arbutoid mycorrhiza). A similar dual colonization of ectomycorrhiza and AM within the same root system has been reported for several tree species belonging to Pinaceae and Salicaceae (Lapeyrie and Chilvers, 1985; Chilvers *et al*., 1987; Cazares and Trappe, 1993; Cazares and Smith, 1996; Horton *et al*., 1998; Fujiyoshi *et al*., 1999).

The results of this study show that AM is the predominant mycorrhizal type throughout the successional process of the island communities. However, this does not necessarily mean that they are important for the growth and survival of plants in the island communities. Although it is well accepted that AM fungi have significant effects on the growth and survival of the host plant (for example, Smith and Read, 1997), the sensitivity of plants to AM colonization varies widely depending on species as well as on soil nutrient conditions. In this study, AM colonization levels varied widely among species irrespective of the successional stage. Some species, including dominant plants such as *C. hakonensis*, *M. oligostachyus* and *P. cuspidatum*, showed very low levels of colonization (<5%). These low colonization levels might indicate low sensitivity of these plants to AM colonization, since a positive relationship between AM colonization level and the growth response of the host plant to AM colonization has been reported (Hirata *et al*., 1988; Kelly *et al*., 2001).

Nitrogen availability is also important in determining the effect of AM associations on plant growth. A number of studies have reported that the direct effects of AM colonization on plant growth are not detected in nitrogen-limited conditions (Titus and Del Moral, 1998a; Sasaki *et al*., 2001; Funatsu *et al*., 2004). This is possibly due to the fact that the major effect of AM association is the enhancement of phosphorus, not of nitrogen (Smith and Read, 1997). Since there is evidence that the growth of some plant species in island communities is limited by nitrogen availability (Hirose and Tateno, 1984; Tateno and Hirose, 1987), it is doubtful that AM associations are actually beneficial to host plants in such areas.

Although the effects of AM fungi on the growth and survival of host plants require further clarification, the presence of the host plant is clearly important for the reproduction of AM fungi because AM fungi obligately depend on the host plant for their carbon source (for example, Smith and Read, 1997). At this study site, high spore densities were observed in soils of the island communities dominated by *P. cuspidatum*, whereas few spores were observed in bare ground without vegetation cover. The average spore numbers in stage I communities were comparable to those in later stages (stages II and III). These findings suggest that island communities dominated by *P. cuspidatum* are important in facilitating the

reproduction of AM fungi, and AM fungal populations establish rapidly in the subalpine volcanic desert.

Acknowledgments

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