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SHORT NOTE

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Arbuscular mycorrhizal colonization of the dominant plant species in primary successional volcanic deserts on the Southeast slope of Mount Fuji

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Abstract Arbuscular mycorrhizal (AM) colonization was observed on four plant species in primary successional volcanic deserts on the Southeast slope of Mount Fuji. The AM colonization of the dominant species, *Polygonum cuspidatum*, contradicts the conclusion that Polygonaceae are often regarded as being non-mycorrhizal species. The secondary dominant species, *Polygonum weyrichii* var. *alpinum*, formed no mycorrhizas. The roots of *Cirsium purpuratum*, *Clematis stans* and *Campanula punctata* ssp. *hondoensis*, showed a higher percentage of AM colonization than *P. cuspidatum*. AM colonization and spore density in the rhizosphere soil of *P. cuspidatum* significantly decreased as elevation increased. AM colonization in roots of *Cirsium purpuratum* and *Clematis stans* also tended to decrease with increased altitudes. *Cirsium purpuratum* and *Campanula punctata* ssp. *hondoensis* formed single structural types of *Arum*- and *Paris*-type, respectively, whereas *P. cuspidatum* and *Clematis stans* formed both *Arum*- and *Paris*-type morphologies.

Keywords Arbuscular mycorrhizal colonization · Primary succession · Volcanic desert · *Arum* · *Paris*

Introduction

It is well known that symbiotic associations of plants with mycorrhizal fungi can greatly enhance nutrient availability as well as water uptake by the plant hosts (Smith and Read 1997). Therefore, mycorrhizal symbiosis may play a crucial role in barren lands such as primary succession sites, where the accumulated amount of available nutrients is limited. The effects of arbuscular mycorrhizal (AM) fungi on vegetative succession have been studied by many researchers, e.g., Miller (1979), Allen and Allen (1980), and Allen et al. (1992). A basic model of primary succession suggests that non- or facultatively mycotrophic plants are the first invaders, followed by obligatory mycotrophic plants, which invade after AM populations have been established in the soil (Janos 1980). Allen and coworkers (Allen et al. 1984; Allen 1987) reported, however, that post-eruption re-establishment of AM occurred quickly on Mount St. Helens, and that the plant invaders of successional vegetation were all mycotrophic plants. Allen (1991) suggested that mycorrhizal status and the progress of vegetative succession would vary with different environmental conditions in terms of water and nutrients. Although AM fungal colonization is thought to be an important factor in the process of vegetative succession, relatively little about the AM fungus community and its patterns in primary succession has been documented.

The Southeast slope of Mount Fuji is still in primary succession, and vegetation is distributed patchily on the volcanic deserts. Although Nara et al. have identified both sporocarp (Nara et al. 2003a) and underground (Nara et al. 2003b) ectomycorrhizal (ECM) fungal communities associated with *Salix reinii* Franch. et Savat—the dominant woody plant and first ECM host species to colonize this site—there is no scientific information on AM fungal succession on this site.

For this investigation of succession and AM formation in a volcanic desert on Mt. Fuji, root samples of five species of herbaceous plants at several elevations were collected and examined microscopically.

Materials and methods

Research site

Mount Fuji, the highest mountain in Japan, last erupted in 1707, when its Southeast slope was completely covered by scoria with a thickness of about 10 m. The vegetation at

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Table 1. Root hair length, percentage arbuscular mycorrhizal (AM) colonization, and spore density of the dominant plant species in primary successional volcanic deserts on the Southeast slope of Mount Fuji. Values are means ±SE, and different letters within a column differ statistically according to Tukey's HSD tests after one-way ANOVA at $P<0.05$

		Polygonum cuspidatum		*Cirsium purpuratum*	*Clematisstans*^{e}	*Campanula punctata* ssp. *hondoensis*^{f}	*Polygonum weyrichii* var. *alpinum*
Root hair length (μm)		302.7±27.0 b		50.3±11.7 d	98.7±19.9 cd	172.7±14.3 c	876.3±26.0 a
		AM colonization (%)	Spore density (spore/g dry soil)	AM colonization (%)	AM colonization (%)	AM colonization (%)	AM colonization (%)
Altitude (m)	1,500	25.0±4.7 a	2.25±0.68 a	84.0±7.0 a	64.8±12.3 a	74.6±2.3 a	0
	1,600	17.6±3.3 a	2.08±0.62 a	86.8±5.6 a	69.0±12.3 a	72.8±7.6 a	0
	1,700	3.3±1.1 b	0.28±0.15 b	97.5±1.9 a	40.4±9.0 a		0
	1,930	2.0±0.9 b	0.03±0.02 b	23.0±8.6 b			0

^{e}No *Clematis stans* was found at 1,930 m

^{f}No *Campanula punctata* ssp. *hondoensis* was found at 1,700 and 1,930 m

the time of eruption was completely destroyed, and is now recovering. Our research sites were at altitudes from 1,500 to 1,930 m above sea level on this slope, the geographical location of which is 35°20′18″N, 138°47′23″E. The dominant plant species *Polygonum cuspidatum* Sieb. et Zucc., was distributed patchily on the scoria. Various patches of this plant formed by vegetative and sexual reproduction provided subsequent plant invaders with a relatively stable habitat (Adachi et al. 1996; Zhou et al. 2003). Another dominant plant species *Polygonum weyrichii* Fr. Schm. var. *alpinum* Maxim. also was patchily distributed on this slope, but its patches were smaller than those of *P. cuspidatum*. Many plant species often seen in other disturbed areas invaded this open site from higher elevations (Ohsawa 1984). Many other pioneer species of herbaceous plants, such as *Cirsium purpuratum* (Maxim.) Matsum., *Clematis stans* Sieb. et Zucc., *Campanula punctata* Lam. subsp. *hondoensis* Kitam., grow within the patches of *P. cuspidatum* or colonize bare ground.

Sampling of soil and roots

Sampling of soil and roots was conducted from August to September 2003. Samples of soil and roots of *P. cuspidatum* were taken from four altitudes of 1,500, 1,600, 1,700 and 1,930 m. No vegetation was observed on the slope above 1,930 m. At each of the four altitudes, 30 samples of soil and roots of *P. cuspidatum* from ten patches (i.e., three samples from each patch) were randomly taken. The sample size of soil and roots was 10×10×10 cm. Root samples of four other species of herbaceous plants, *P. weyrichii* var. *alpinum*, *Cirsium purpuratum*, *Clematis stans*, and *Campanula punctata* ssp. *hondoensis*, also were taken from ten plants at each altitude, respectively. *Clematis stans* and *Campanula punctata* ssp. *hondoensis* were not found above 1,700 m and 1,600 m, respectively; root samples of *Clematis stans* were taken from altitudes of 1,500, 1,600, 1,700 m, and those of *Campanula punctata* ssp. *hondoensis* were taken only from altitudes of 1,500 and 1,600 m.

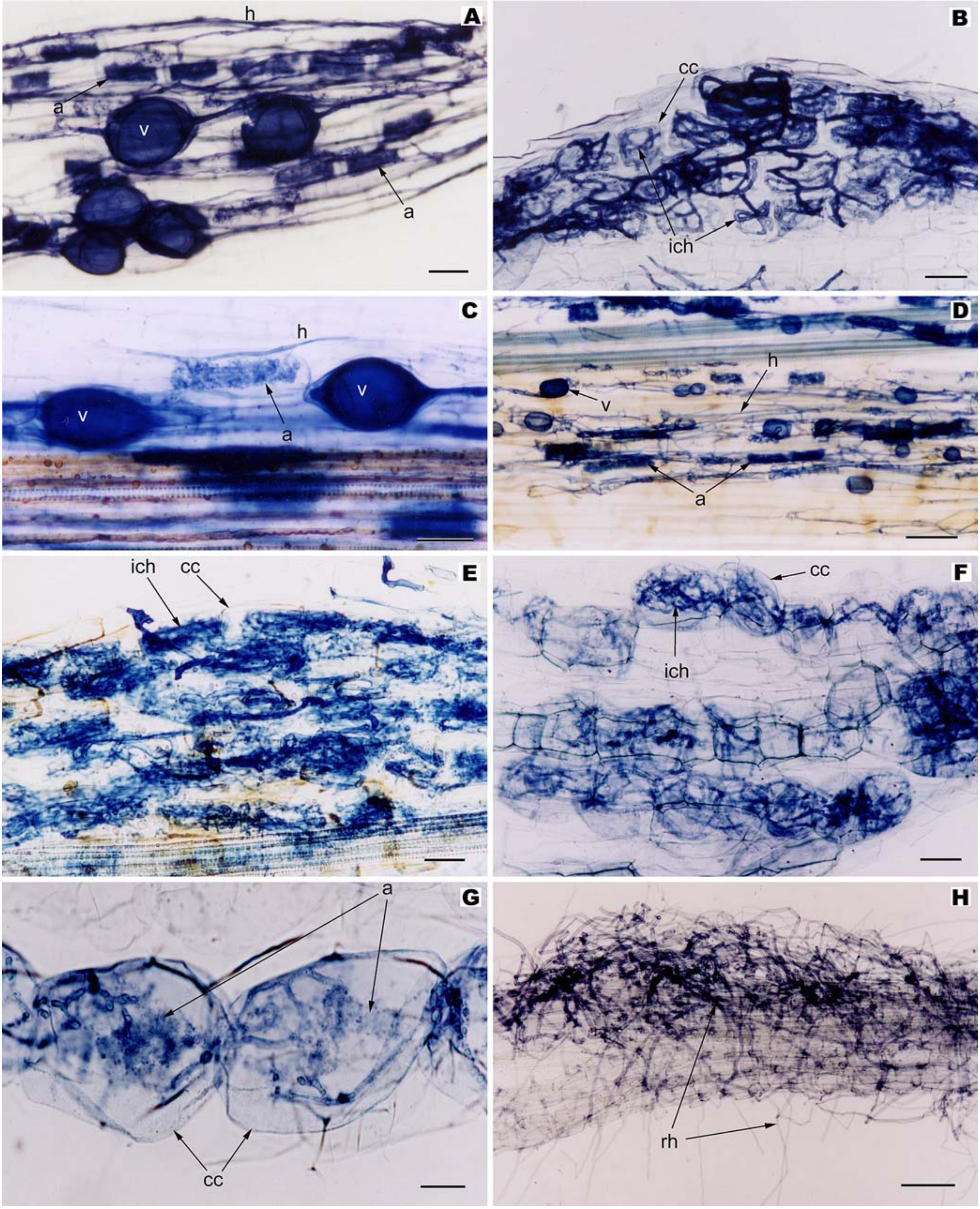
Determination of spore density and AM colonization

Glomalean fungus spores were separated from each sample of *P. cuspidatum* soil by wet sieving and sucrose centrifugation methods according to Brundrett et al. (1996). A portion of each root sample was washed free of soil and stained with Trypan blue following by clearing in 10% KOH, and assessed for AM colonization using the gridline intersect method (Giovannetti and Mosse 1980) as well as for root hair length of non-mycorrhizal roots under a light microscope. Spore density was calculated as spore number per gram of air dry soil.

Statistical analyses

The data of percentage AM colonization and also of spore density were logarithmically transformed, and then one-way ANOVAs followed by Tukey's HSD (honestly significant difference) tests were performed to test for effects of elevation on percentage AM colonization and spore density of each plant species, and to test for significant difference of measured root hair length among

Fig. 1 Microscope photographs of arbuscular mycorrhiza (AM) in ▶ the roots of *Polygonum cuspidatum* (**A**, **B**), *Cirsium purpuratum* (**C**), *Clematis stans* (**D**, **E**) and *Campanula punctata* ssp. *hondoensis* (**F**, **G**). **A** *Arum*-type mycorrhizal colonization of *P. cuspidatum* with intercellular hyphae (*h*), arbuscules (*a*) and vesicles (*v*). **B** *Paris*-type mycorrhizal colonization of *P. cuspidatum* showing extensive development of intracellular coiled hyphae (*ich*) spreading directly from cell (*cc*) to cell within the cortex. **C** *Arum*-type mycorrhizal colonization of *Cirsium purpuratum* with intercellular hyphae (*h*), arbuscules (*a*) and vesicles (*v*). **D** *Arum*-type mycorrhizal colonization of *Clematis stans* with intercellular hyphae (*h*), arbuscules (*a*) and vesicles (*v*). **E** *Paris*-type mycorrhizal colonization of *Clematis stans* showing intracellular coiled hyphae (*ich*) in the cortical cells (*cc*). **F** *Paris*-type mycorrhizal colonization of *Campanula punctata* ssp. *hondoensis* showing intracellular coiled hyphae (*ich*) in the cortical cells (*cc*). **G** Arbuscules (*a*) in the cortical cells (*cc*) of *Campanula punctata* ssp. *hondoensis*. **H** Root of *Polygonum weyrichii* var. *alpinum* with extremely well developed root hairs (*rh*). *Bars* **A**–**C**, **E**, **F** 20 μm; **D**, **H** 50 μm; **G** 10 μm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plant species, at $P<0.05$ using the statistical software StatFlex 4.0 for Windows (A-tech, Chiba, Japan).

Results

Table 1 shows root hair length, percentage AM colonization of each plant species, and spore density in the rhizosphere of *P. cuspidatum*. No mycorrhizas were observed on *P. weyrichii* var. *alpinum*. The percentage AM colonization of *P. cuspidatum* was lower than that of the other three species at each of the four altitudes, with *Cirsium purpuratum* showing the highest percentage AM colonization among the four species. Root hair length of non-mycorrhizal roots was significantly different among five plant species. The two *Polygonum* species, especially *P. weyrichii* var. *alpinum*, showed more well-developed root hairs than other three plant species (Fig. 1). Moreover, AM colonization of *P. cuspidatum* was significantly lower at the high altitudes (1,700 and 1,930 m) than at the low altitudes (1,500 and 1,600 m). *Cirsium purpuratum* also showed a significant decrease in AM colonization at 1,930 m.

Figure 1 shows the morphological structures of AM of the four plant species. In some sections of root material of *P. cuspidatum*, *Arum*-type AM with intercellular hyphal colonization, arbuscules, and vesicles were observed (Fig. 1A), but in other root sections of this plant, *Paris*-type AM with developed intracellular coiled hyphae spreading directly from cell to cell within the cortex were also identified (Fig. 1B). In *Cirsium purpuratum*, only *Arum*-type with intercellular hyphal colonization, arbuscules and vesicles was found (Fig. 1C). Both *Arum*- (Fig. 1D) and *Paris*-type (Fig. 1E) AM were found in the roots of *Clematis stans*. Only *Paris*-type AM was found in the roots of *Campanula punctata* ssp. *hondoensis* (Fig. 1F,G). In the roots of this plant, intracellular coiled hyphae were extended to the neighboring cells in the cortex (Fig. 1F). Arbuscules grew from these coiled hyphae in the cortical cells (Fig. 1G). The roots of *P. weyrichii* var. *alpinum* showed extremely well developed root hairs with lengths of more than 800 μm, but no AM were observed in this species (Fig. 1H).

Discussion

Symbiotic associations with AM fungi were found in *P. cuspidatum*, *Cirsium purpuratum*, *Clematis stans* and *Campanula punctata* ssp. *hondoensis* in primary successional volcanic deserts on Mount Fuji. The observation of AM on *P. cuspidatum* contradicts the conclusion that Polygonaceae are often regarded as being non-mycorrhizal despite several reports of ectomycorrhizal species (Massicotte et al. 1998; Titus and Tsuyuzaki 2002; Yamato 2004).

P. cuspidatum showed a low percentage AM colonization, and even non-mycorrhizal plants grew well and formed well developed patches, indicating that *P. cuspidatum* is a facultatively mycotrophic plant. No mycorrhiza formation by *P. weyrichii* var. *alpinum* was observed, signifying that this species is a non-mycotrophic plant. Baylis (1975) suggested that plant species of Magnoliales, which are usually lacking in root hairs, are likely dependent on AM fungi for nutrient uptake, whereas graminoid roots, which are relatively fine with well developed root hairs, may be independent of AM formation. This theory was reconfirmed with some tropical tree species by St John (1980). It was also found in our study that the non-mycotrophic plant, *P. weyrichii* var. *alpinum*, had roots with extremely well developed root hairs. Our results also showed that AM colonization and spore density of *P. cuspidatum* decreased significantly as altitude increased. A similar tendency was also observed in the AM colonization of *Cirsium purpuratum* and *Clematis stans*. This suggests that AM establishment might be more difficult at higher altitudes than at lower altitudes.

The survey of mycorrhizal structures of four plants indicated that both *Arum*- and *Paris*-type AM were found to be formed during primary succession on the Southeast slope of Mount Fuji. Mycorrhizas of *Cirsium purpuratum* and *Campanula punctata* ssp. *hondoensis* were formed a single structural type of *Arum*- and *Paris*-type, respectively, whereas *P. cuspidatum* and *Clematis stans* formed both *Arum*- and *Paris*-type morphologies. In a review of the literature, Smith and Smith (1997) revealed that *Paris*-types occur more frequently in natural plant communities. In a recent study, Dickson (2004) reported a continuum of mycorrhizal structures ranging from *Arum* to *Paris* in a series of combinations of 12 plants/6 fungi, depending upon both the host plant and the fungus. ‘Intermediate’ morphology or both *Arum*- and *Paris*-type were recorded even in the same plant species. Our survey of mycorrhizal structures in the present study has supported these observations. Further investigation by inoculation experiments of each plant/fungus combination and by molecular biological techniques will be helpful in clarifying the morphological types of AM in this primary successional area.

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