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Short Communication

Vesicular-arbuscular mycorrhizal fungi in coastal dune plant communities
II. Spore formation of *Glomus* spp. predominates under geographically separated patches of *Elymus mollis**

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Five study sites in Japan with pure patches of *Elymus mollis* near to the shoreline were chosen. Abundance of spore formation of *Glomus* spp. under *E. mollis* patches were studied at four sites to understand the factors determining the development of VA mycorrhizal fungal communities at the edge of coastal sand dune vegetation. At each study site, eight soil samples from two soil depths were collected four times (three times for "Niigata") during a year. Spores of *Glomus* spp. predominated at every site, but species composition of *Glomus* spp. differed from site to site. In a further experiment to measure the soil depth of the highest spore densities under *E. mollis,* the highest density was found at depths where rhizomes of *E. mollis* were present.

Key Words——coastal sand dune; *Elymus mollis*; *Glomus* spp.; VA mycorrhizal fungi.

Vesicular-arbuscular (VA) mycorrhizae are found in many ecosystems of the world (Harley and Smith, 1983). For ecological studies of VA mycorrhizal fungi, coastal sand dune ecosystems seem attractive (Nicolson, 1960; Koske, 1975, 1981, 1987; Nicolson and Johnston, 1979; Koske and Halvorson, 1981; Giovannetti and Nicolson, 1983; Giovannetti, 1985; Silvia, 1986). Because the sand dune ecosystem is a stressful habitat for both plants and fungi, VA mycorrhizal fungi may play an important role for pioneer plants in this ecosystem. It is easier to isolate VA mycorrhizal fungal spores and hyphae from sand than from soils with clay or organic matter.

An ecological study in a barrier sand dune found no evidence that any of the VA mycorrhizal fungi were better competitors than others, and it was considered that environmental factors and host plant characteristics appeared more important for determining VA mycorrhizal fungal distribution (Koske, 1981). However, when one VA mycorrhizal fungal species formed many spores in a soil sample of sand dunes, there was low sporulation of other VA mycorrhizal fungal species, suggesting antagonistic interactions between the species (Gemma et al., 1989).

An early study of the vertical distribution of soil fungi showed that most species occurred most abundantly in the surface layers of the soil, but few species were commoner in subsurface layers (Warcup, 1951).

The perennial grass *Elymus mollis* Trin. [=*Leymus mollis* (Trin.) Pilger] is a typical beach plant which may prefer to grow on saline or alkaline soil, and is widely distributed along the Pacific coasts of the Northern Hemisphere with southern limits of Japan and the U.S.A. (Osada, 1989). *E. mollis* is often planted to stabilize sand dunes for land reclamation in Japan.

Recently, we reported the predominance of spore formation of *Glomus* spp. in a patch of *E. mollis* at "Hasaki," Ibaraki Prefecture (Abe et al., 1994). In the current study we examined a further three *E. mollis* patches, growing at the edge of the coastal dune vegetation in geographically separated sand dune communities, to determine factors which allow the production of high spore numbers of *Glomus* spp. as compared with other genera.

Five study sites were chosen where pure patches of *E. mollis* on primary foredunes were present: 1. "Hasaki" (35°51′N, 140°43′E), Ibaraki Prefecture; 2. "Souma" (37°47′N, 140°59′E), Fukushima Prefecture; 3. "Futtu" (35°19′N, 139°49′E), Chiba Prefecture; 4. "Shirako" (35°27′N, 140°24′E), Chiba Prefecture and 5. "Niigata" (37°49′N, 138°51′E), Niigata Prefecture (Table 1). Sites 1, 2 and 4 were along the Pacific coast, site 5 on the Sea of Japan, and site 3 faced Tokyo Bay.

Elymus mollis does not predominate on primary foredunes in our study sites. *Carex kobomugi* Ohwi or *Calystegia soldanella* (Linn.) Roem. & Schult. is dominant on foredunes near the shoreline at Azigaura, Ibaraki

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Table 1. Study site description (All data from sampling in January, 1991).

Study site	Area (m²)[a]	Distance (m)[b]	Depth of rhizomes of *Elymus mollis* (cm)
"Hasaki"	400	30	30-50
"Souma"	40	9	20-25
"Futtu"	390	5	25-40
"Niigata"[c]	40	30	20-40
"Shirako"	720	70	20-50

[a] Area of each patch of *E. mollis*.
[b] The shortest distance between shoreline and each patch of *E. mollis*.
[c] Data from sampling in May, 1991.

Prefecture (Kachi and Hirose, 1979), and also at "Hasaki," "Shirako" and "Souma." At "Niigata," *Messerschmidia sibirica* (Linn.) Linn. is codominate with *C. kobomugi* and *C. soldanella* (Miura and Maruyama, 1983). The *E. mollis* patch at "Futtu," is the southernmost study site, is characterized by green leaves throughout the year.

The vertical distribution of spores of VA mycorrhizal fungi in two *E. mollis* patches was studied to determine the soil depth of the highest densities of spores. A pit of approx. 1.5 m^2 in area was dug in an *E. mollis* patch at "Hasaki" on 13 October 1989 and "Shirako" on 1 November 1989. Each pit was dug to the depth of the groundwater level: 100 cm at "Hasaki" and 140 cm at "Shirako." Soil samples (each 500 g) were collected at intervals of 10 cm, beginning with the topsoil (0 cm). Spores of VA mycorrhizal fungi were collected by the wet-sieving and decanting method using sieves of 50, 105 and 1680 μm. The remaining suspension with spores was filtered using a Buchner funnel, and spores on the filter paper (Advantec No. 2, diam of 90 mm) were counted with the aid of a dissecting microscope at 20–40x (Koske and Walker, 1984). Spore numbers were transformed with log (x+1).

VA mycorrhizal fungi under geographically separated *E. mollis* patches were studied at "Hasaki," "Souma," "Futtu" and "Niigata." Samples were taken in each of the four seasons of 1991, except for "Niigata," where no winter sampling was made. At each sampling site, a pure *E. mollis* patch was chosen from among those growing nearest to the seashore. Four pits, each 1-2 m, apart were dug in each patch. Two soil samples, each of about 1000 ml, were removed from each of the four pits and placed in polyethylene bags. The first soil sample was collected at the depth of 40 cm below ground, where rhizomes of *E. mollis* are present (Table 1), and the second at 80 cm. An exception was at "Souma," where rhizomes were not deeper than 25 cm. Here soil samples were removed from 20 cm and 40 cm below ground.

For identification of species of VA mycorrhizal fungi, subsamples of 1000 g fresh weight from each winter soil sample (1000 ml) were removed and stored at 5°C until processed. Spores of VA mycorrhizal fungi were collected from 24 soil samples from three study sites (4 samples at each depth) in two steps. First step was the wet-sieving and decanting method (Gerdemann and Nicolson, 1963) using sieves of 38, 106 and 500 μm mesh, and the second step was centrifugation in 40% sucrose. After wet-sieving and decanting the spore suspension was centrifuged, first with water (about 1200×*g* for 5 min), then with 40% sucrose suspension (about 650×*g* for 1 min). Putative healthy spores with lipidic contents were identified using dissecting (20–63x) and compound (100–1000x) microscopes.

For the other three seasons, 72 soil samples from four study sites (3 samples at each depth) were collected. Subsamples of 200 g fresh weight were removed from the 1000 ml soil samples each of which had been mixed thoroughly. Spores were collected with the wet-sieving and decanting method, but without centrifugation, as outlined above and the VA mycorrhizal fungal genera were identified by "healthy" spores.

There was a predominance of *Glomus* spp. spores under *E. mollis* patches, not only at "Hasaki" as reported (Abe et al., 1994), but also at "Souma" and "Futtu" at all

Table 2. Occurence of VA mycorrhizal fungal species under four geographically separated *Elymus mollis* patches in winter 1991.

Fungal species	Study site, soil depth and number of samples containing spores[a]					
	"Hasaki"		"Souma"		"Futtu"	
	40 cm	80 cm	20 cm	40 cm	40 cm	80 cm
Scutellospora gregaria	0	0	0	0	0	0
Scutellospora sp. 1	0	0	0	0	0	0
Acaulospora sp. 1	0	0	0	0	0	0
Glomus aggregatum	0	0	1	0	0	0
G. tortuosum	0	0	0	0	0	1
Glomus sp. 1 (yellow)	0	0	1	1	0	0
Glomus sp. 2 (pustule)	2	2	2	3	0	0
Glomus sp. 3 (evanescent)	1	1	0	0	0	0
Glomus sp. 4 (pale brown)	0	0	3	1	4	4

[a] Totally 4 soil samples were observed.

Table 3. Occurrence of VA mycorrhizal fungal genera under four geographically separated *Elymus mollis* patches.

Genus	Study site and date of soil sampling														
	"Hasaki"				"Souma"				"Futtu"				"Niigata"		
	27 Jan[a]	26 Apr	23 Aug	26 Oct	31 Jan	5 May	22 Aug	6 Nov	16 Jan	26 Apr	23 Aug	2 Nov	1 May	12 Aug	30 Oct
Scutellospora spp.	—[b]	+	—	—	—	—	—	—	—	—	—	—	—	—	+
Acaulospora spp.	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Glomus spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] All collected in 1991.
[b] +: spores present in one of three samples; —: no spore.

four times, and at "Niigata" at all three times (Tables 2, 3).

Scutellospora spp. or *Acaulospora* sp. 1 were found in two soil samples from "Hasaki" collected on 26 April and 26 November, and in one soil sample from "Niigata" collected on 30 October. In each sample, the number of spores (1-2) of these species was low.

Species composition and predominance of *Glomus* spp. differed between "Hasaki," "Souma" and "Futtu" (Table 3). At "Futtu," spores of *Glomus* sp. 4 predominated (Table 2).

A study of vertical distribution showed that most spores occurred at a depth of 30 cm at "Hasaki" (1437 spores/500 g soil) and 60 cm at "Shirako" (3279 spores/500 g soil). The median depth of rhizomes of *E. mollis* at "Hasaki" and at "Shirako" was 45 cm (Fig. 1), close to the depth with the highest densities of spores. No spores were collected in the topsoil at 0 cm. This observation suggests the need to examine the depth of rhizomes and roots of host plants in dune ex-

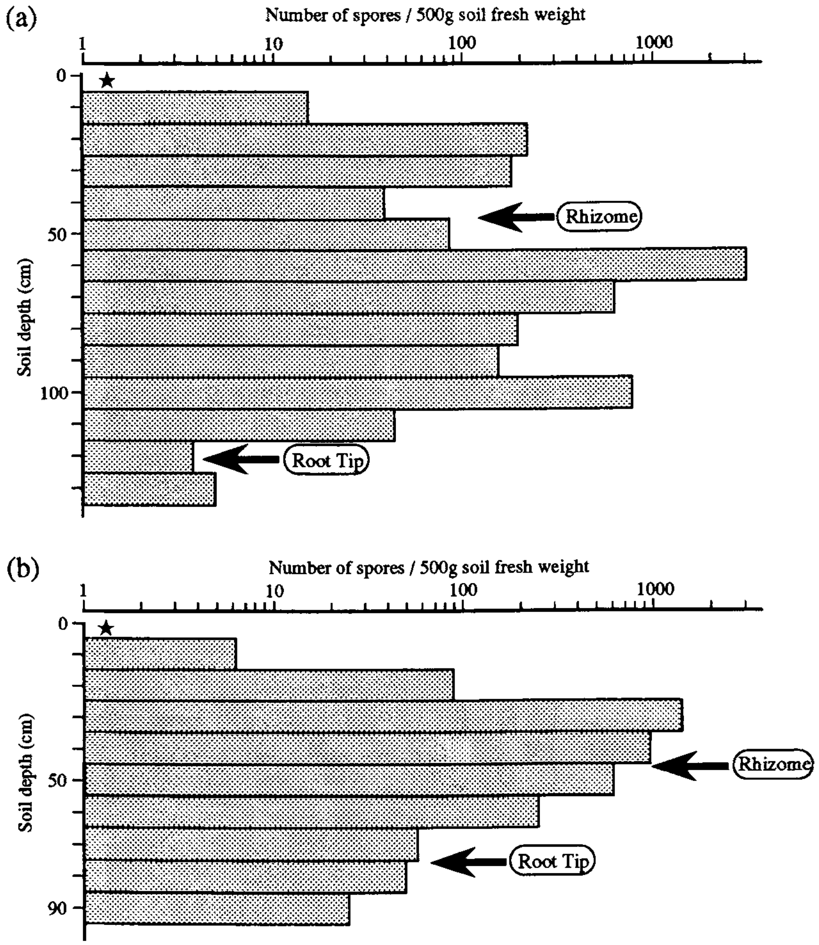

Fig. 1. Vertical spore distribution of VA mycorrhizal fungi under *Elymus mollis* plants at "Shirako" (a) and "Hasaki" (b). The median soil depth between the shallowest and the deepest rhizome (Rhizome) and the deepest root tip (Root Tip) are marked. ★: No spores collected.

periments. The deepest spores were found at 130 cm soil depth at "Shirako," which is below the deepest root tip, but unfortunately these spores were not identified. A study of prairie plants of the Tallgrass Prairie in the U.S.A. revealed that *Baptisia* sp. was colonized by VA mycorrhizal fungi under 200 cm soil depth, and spores of *G. fasciculatum* (Nicol. & Gerd.) Gerd. & Trappe were found 220 cm below ground (Zajicek et al., 1986). However, a study of the spatial dispersion of spores of VA mycorrhizal fungi under *Ammophila breviligulata* Fern. found that spore density was not correlated with the presence or abundance of roots or rhizomes of this plant. Roots were not able to be collected below 35 cm (Friese and Koske, 1991). Both *E. mollis* and *A. breviligulata* are beachgrasses, but their root systems and root structures are very different: the former has deep roots and the latter shallow. This structural difference in the roots may correlate with the distance between plants and shoreline: *E. mollis* is closer and *A. breviligulata* farther from the shoreline.

A study of a 355 km transect along the barrier dunes of the northern Atlantic coast of U.S.A. showed that the three dominant VA mycorrhizal fungal species did not change along the transect (Koske, 1987), but the composition of fungal species differed and the number of species increased in the southern zone of the transect. It was suggested that the determining factor for VA mycorrhizal fungal communities is temperature, which either affects the fungi directly or is indirectly mediated through the host plants.

Recently we reported a comparison of VA mycorrhizal fungal communities under patches of three different beach plants, *Z. macrostachya, W. prostrata* and *E. mollis*, at "Hasaki." We observed that spore formation of *Glomus* spp. predominated under *E. mollis* and discussed the determining factors of this phenomenon which are biotic ones such as host-fungus specificity between *E. mollis* and *Glomus* spp., or abiotic ones like soil pH or salinity influencing the occurrence of spores (Abe et al., 1994). In the current study we show that spore formation of different species of the genus *Glomus* is very common under plants of *E. mollis* near the shoreline and is independent of the geographical location. Sometimes a few spores of *Scutellospora* spp. or *Acaulospora* sp. 1 occur in patches of *E. mollis*, and patches which are approx. 100 m from the shoreline have often many spores of *Scutellospora* spp., *Acaulospora* sp. 1 and *Glomus* spp. (Abe, unpublished). *E. mollis* is able to associate with spores of species of *Scutellospora* and *Acaulospora*, depending on where it is growing (Abe, unpublished). We suggest that determining factors for the fungal community at the edge of coastal dune vegetation, such as under patches of *E. mollis*, are abiotic (e.g., soil pH or salinity) rather than biotic (e.g., competition between VA mycorrhizal fungi, or specificity of VA mycorrhizal fungi and host plants). Further investigations are needed to determine these factors and to understand the mechanisms of these factors in such an ecosystem.

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