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Vesicular-arbuscular mycorrhizal fungi in coastal dune plant communities
I. Spore formation of *Glomus* spp. predominates under a patch of *Elymus mollis**

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Vesicular-arbuscular (VA) mycorrhizal fungi in pure patches of coastal dune plants *Elymus mollis, Wedelia prostrata* and *Zoysia macrostachya* were examined for frequency of occurrence and number of spores of VA mycorrhizal fungi over one year. Six species in three genera of VA mycorrhizal fungi were recovered. Under a patch of *E. mollis*, spores of *Acaulospora* sp. 1, *Glomus tortuosum, Glomus* sp. 1, *Glomus* sp. 2 and *Scutellospora gregaria* were recovered. Spores of *Glomus* spp. were most common. In patches of *W. prostrata* and *Z. macrostachya* spores of *Acaulospora* sp. 1, *G. tortuosum, Glomus* sp. 1, *Glomus* sp. 2, *S. gregaria* and *Scutellospora* sp. 1 were found.

Key Words——*Acaulospora*; coastal sand dunes; *Glomus*; *Scutellospora*; VA mycorrhizal fungi.

Introduction

Many plant species form vesicular-arbuscular (VA) mycorrhizae. Plants growing in most habitats are found with this fungal association (Harley and Smith, 1983). The fungi have been extensively examined in coastal dune plant systems (Nicolson, 1960; Nicolson and Johnston, 1979; Koske, 1975, 1981, 1987; Koske and Halvorson, 1981; Giovannetti and Nicolson, 1983; Giovannetti, 1985; Sylvia, 1986; Gemma and Koske, 1988; Gemma et al., 1989; Friese and Koske, 1991). Sand dune systems are particularly interesting because the fungal symbiont advances towards the water line just behind the edge of the vegetation. Factors like permanent seawater spray, instability of sands, intermittent drought, high temperatures and infertile soil lead to specialized plant communities. The same factors may influence the VA mycorrhizal fungi found at these sites.

Early studies of mycorrhizae in dune plant systems examined fungal infection of plants (Nicolson, 1960). In Japan, Asai (1934) noted that *Wedelia prostrata* (Hook. et Arn.) Hemsl. (Compositae) formed VA mycorrhizae. Subsequently, many VA mycorrhizal fungi in dune systems were identified (Koske, 1981, 1987; Koske and Halvorson, 1981; Sylvia, 1986; Friese and Koske, 1991). However, we could find no other information on the VA mycorrhizal fungi in Japanese dune systems. The rhizomatous perennial beach grass *Elymus mollis* Trin. (Gramineae) is found at the edge of the vegetation in sand dune communities. *Wedelia prostrata* and *Zoysia macrostachya* Franch. et Savat. (Gramineae) are both perennial coastal dune plants commonly found growing on the foredune and dune behind *E. mollis*.

The most stressful point of the habitat for VA mycorrhizal fungi, the edge of the plant community, has not been examined in detail. We report here studies on the VA mycorrhizal fungi found under *E. mollis, W. prostrata* and *Z. macrostachya* to determine whether specific relations exist between coastal dune plant species and VA mycorrhizal fungal spore formation.

Materials and Methods

The study site was a barrier beach in Hasaki, Ibaraki Prefecture (140° 43′ E; 35° 51′ N). The barrier beach faces the Pacific Ocean. One pure patch of each of

Table 1. Area, altitude, distance from shoreline and soil pH of the three coastal dune plant stands in Hasaki beach.

Host	Area (m^2)	Altitude (m)[a]	Distance from the sea (m)[b]	pH
Elymus mollis	1730	1.7	36	8.27
Wedelia prostrata	3.5	8.0	80	7.69
Zoysia macrostachya	90	4.8	100	7.98

[a] The altitude of the highest point in each patch.
[b] The shortest distance between each patch and the shoreline.

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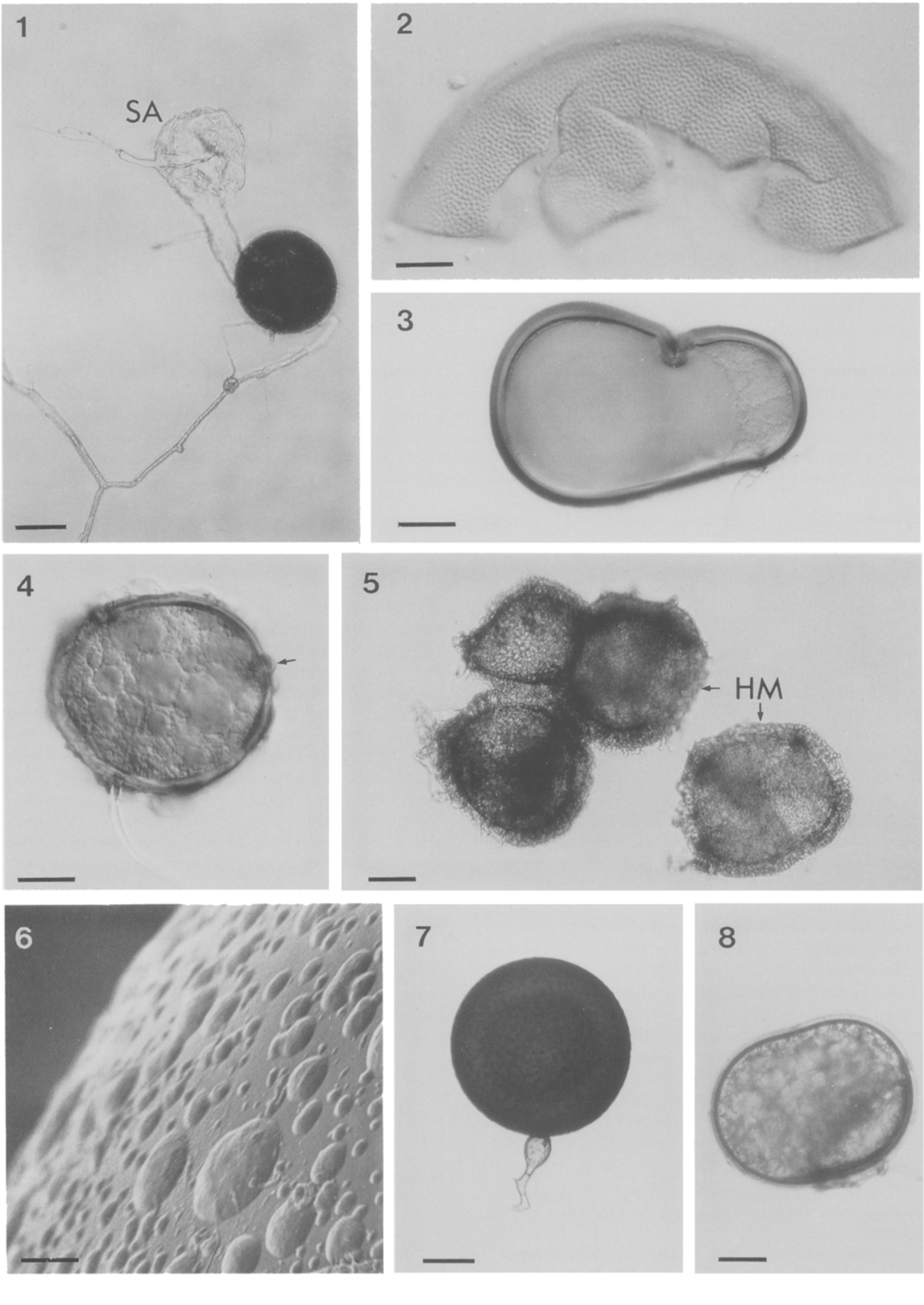
1
SA
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HM
HM
6
7
8

three species of coastal dune plants, *E. mollis, W. prostrata* and *Z. macrostachya* was chosen for examination of VA mycorrhizal fungal spores produced in sand. *E. mollis* had the largest patch and was closest to the shoreline. The other two patches were on the ridge of a sand dune which lay parallel to the shoreline (Table 1).

Sand samples were collected monthly from June 1990 to May 1991. At each collection one litre of sand from each patch was removed. In each patch the sample was collected from the root zone. Samples were extracted at 30-40 cm deep from the *E. mollis* patch and 10-20 cm deep from the *W. prostrata* and *Z. macrostachya* patches. Samples containing sand and roots were placed in polyethylene bags and were stored at 5°C for one week until processed. Sample pH was determined in a slurry of 1 : 2.5 (w/v) of soil to 1 N KCl with a pH glass electrode (Beckmann Φ43 pH meter) following gentle shaking for one hour. Spores of VA mycorrhizal fungi were collected by the wet-sieving and decanting method (Gerdemann and Nicolson, 1963) using 1000 g of sand and sieves of 53, 106 and 1680 μm mesh. Spores were counted and identified using dissecting and compound microscopes. Only spores with contents were used. The frequency of occurrence of each species was determined by calculating the proportion of the 12 samples in which each fungus was recovered.

Results and Discussion

In the patch of *E. mollis* spore formation of *Acaulospora* sp. 1, *G. tortuosum* Schenck et Smith, *Glomus* sp. 1, *Glomus* sp. 2 and *S. greagaria* was recognized; in *W. prostrata* : *Acaulospora* sp. 1, *Glomus* sp. 1, *Glomus* sp. 2, *S. gregaria* (Schenck et Nicolson) Walker et Sanders and *Scutellospora* sp. 1; and in *Z. macrostachya*: *Acaulospora* sp. 1, *G. tortuosum, Glomus* sp. 1, *Glomus* sp. 2, *S. gregaria, Scutellospora* sp. 1. These species are described in detail below.

VA mycorrhizal fungi

Acaulospora sp. 1 Figs. 1, 2

Spores formed singly in soil or sometimes observed in dead matter, globose to subglobose and broadly ellipsoid, 90-130×95-160 μm, pale olive to light brown. Sporogenous saccule hyaline; circular rimmed saccule attachment 12-14 μm in diameter. Spore wall consisting of five walls, 8.5-10.0 μm thick. Outermost wall 1 laminated, pale olive, 4-6 μm thick. Surface of wall 1 pitted (each pits 1-2 μm wide). Walls 2 and 3 membranous, hyaline, >0.5 μm and 0.5 μm thick, respectively. Wall 4 flexible, hyaline, 2-3 μm thick. Innermost wall 5 membranous, hyaline, >0.5 μm thick and turning red in Melzer's reagent.

Spores of *Acaulospora* sp. 1 had wall 4 which was 2-3 μm thick and wall 5. This characteristic differed from those of *A. scrobiculata* Trappe. However, the other morphological characteristics of *Acaulospora* sp. 1 were consistent with *A. scrobiculata. Acaulospora scrobiculata* was also reported from sand dune plants, *Ammophila breviligulata* Fern., *Solidago sempervirens* L., *Lathyrus japonicus* var. *glaber* (Ser.) Fern. and *Myrica pennsylvanica* Loisel in the Atlantic coast of the United States of America (Koske, 1981, 1987). It seems possible that *Acaulospora* sp. 1 is closely related to *A. scrobiculata*.

Glomus tortuosum Schenck et Smith Fig. 5

Spores formed singly in soil but occasionally adhering in clods, pale brown to dull yellow-brown, subglobose to ellipsoidal, 120-170×170-240 μm wide. A composite spore wall consisting of one wall and a hyphal mantle appressed to the spore surface. Hyphae of the hyphal mantle sinuous, 2-4 μm wide; the walls 0.5 μm thick. At the points of attachment, the subtending hyphae 10-14 μm wide and the pore remaining open.

Glomus sp. 1 Fig. 3

Spores formed singly in soil, globose to ellipsoid and irregular, 60-130×100-170 μm, orange-yellow. A composite spore wall consisting of three walls, 5-7 μm thick. Outermost wall 1 unit, hyaline, >0.5 μm thick. Wall layer 1 might be evanescent. Wall 2 laminated, yellow-brown, 4-6 μm thick. Innermost wall 3 membranous, hyaline, >1 μm thick. Subtending hyphae at point of attachment 4-11 μm wide; the walls 0.5 μm thick; subtending hyphae formed by wall 2, usually straight and simple. The pore at point of attachment remains open.

Glomus sp. 1 had three walls and a subtending hypha which easily collapsed. This species was different from other known *Glomus* species. It is probably a new species.

Glomus sp. 2 Fig. 4

Spores formed singly in soil, globose to subglobose and irregular, light brown to brown, 80-130 μm. A composite spore wall consisting of 2 or 4 walls, 6-8 μm thick. Outermost wall 1 unit, brown, 2 μm thick. Often ornament-like blisters found on the surface of the spore wall. The blisters 1.5-3.0 μm high, 2.5-6.0 μm in diameter at the base; some blisters broadly ellipsoid, 2.5-6.5×7.0-7.5 μm at the base. Wall 2 laminated, light brown, 3-6 μm thick. Walls 3 and 4 adherent, both membranous, each 0.5 μm thick. Subtending hyphae at point of attachment 6-12 μm wide, usually straight and simple, continuous to wall 2; the wall 0.5 μm thick, pore at point of attachment remaining open.

Figs. 1, 2. *Acaulospora* sp. 1.
1. Spore with sporogenous saccule (SA), 2. Ornamentation (pits) on a crushed spore surface. Scale bar=50 μm in Fig. 1, scale bar=30 μm in Fig. 2.

Figs. 3-5. *Glomus* sp. 1, *Glomus* sp. 2 and *Glomus tortuosum*.
3. *Glomus* sp. 1. 4. *Glomus* sp. 2; Blister-like ornament (arrow) on the spore surface. 5. *G. tortuosum*; Single and in cluster with a hyphal mantle (HM) as a spore surface ornament. Scale bar=30 μm in Figs. 3, 4, scale bar=50 μm in Fig. 5.

Figs. 6-8. *Scutellospora gregaria* and *Scutellospora* sp. 1.
6, 7. *S. gregaria*. 6. Wart like projections on the spore surface observed under SEM. 8. *Scutellospora* sp. 1. Scale bar =5 μm in Fig. 6, scale bar=100 μm in Fig. 7, scale bar=50 μm in Fig. 8.

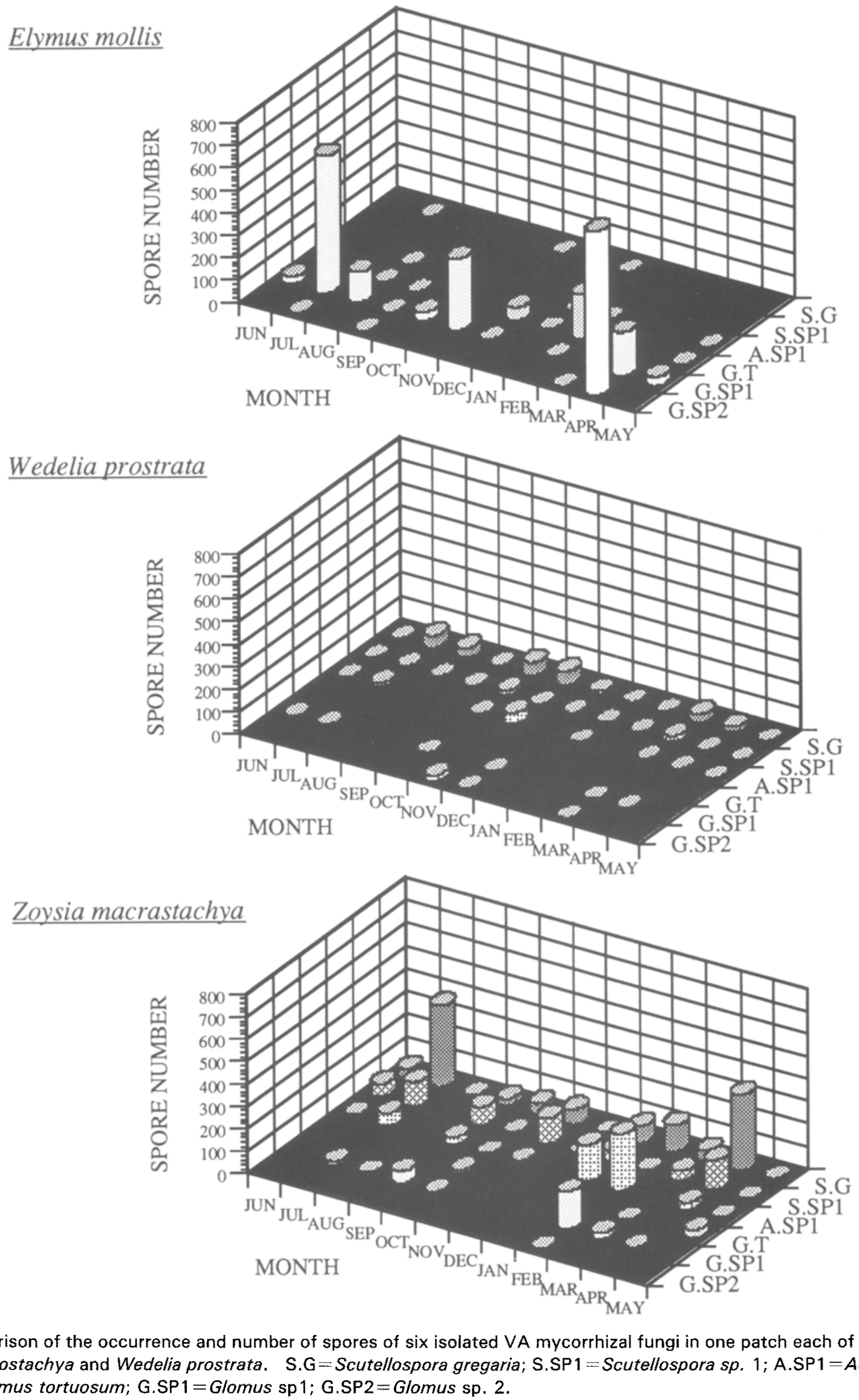

Fig. 9. Comparison of the occurrence and number of spores of six isolated VA mycorrhizal fungi in one patch each of *Elymus mollis, Zoysia macrostachya* and *Wedelia prostrata*. S.G=*Scutellospora gregaria*; S.SP1=*Scutellospora sp.* 1; A.SP1=*Acaulospora* sp. 1; G.T=*Glomus tortuosum*; G.SP1=*Glomus* sp1; G.SP2=*Glomus* sp. 2.

Spores of *Glomus* sp. 2 were similar to the spores of *G. pustulatum* Koske, Friese, Walker et Dalpé. However, *G. pustulatum* lacked the innermost wall 4.

Thus *Glomus* sp. 2 was not consistent with previously described fungal species. However, we believe that this species is closely related to *G. pustulatum*, which has been recorded from sand dunes by Koske et al. (1986).

Scutellospora gregaria (Schenck et Nicolson) Walker et Sanders Figs. 6, 7

Spores formed singly in soil, globose to subglobose, red-brown, 200-370×210-370 μm. A composite spore wall consisting of three walls 10-12 μm thick. Outermost wall 1 unit, hyaline, 0.5 μm thick. Wart-like projections found on the surface of the spore wall, 1-6 μm in diameter at the base, >1 μm high. Wall 2 laminate, red-brown, 2-4 μm thick. Wall 3 laminated, pale brown, 6-7 μm thick. Innermost wall 4 membranous, hyaline, 1-2 μm thick. Bulbous cell of subtending hypha 48-56 μm wide; the wall 2-3 μm thick, pale red-brown to pale brown.

Scutellospora sp. 1 Fig. 8

Spores formed singly in soil, globose to subglobose and ellipsoid, hyaline, 130-250×170-260 μm. A composite spore wall consisting of five walls, 11-12 μm thick. Outermost wall 1 hyaline, laminated 5-9 μm thick with smooth surface. Walls 2 and 3 hyaline, membranous, each up to 1 μm thick; occasionally 3 thicker than 2. Wall 4 hyaline, flexible, 3.5-5.0 μm thick. The innermost wall 5, hyaline, membranous, >0.5 μm thick. The outermost wall 1 and innermost wall 5 turning purple-red and red in Melzer's reagent, respectively. Bulbous cell of the subtending hypha 36-48 μm wide; the wall 1-2 μm thick, pale brown-yellow.

The spores of *Scutellospora* sp. 1 resembled those of *S. weresubiae* Koske et Walker, but the spores of *Scutellospora* sp. 1 were hyaline not pink. The outermost wall layer of *S. weresubiae* was a very thin (>0.5 μm), hyaline, unit wall (Koske and Walker, 1986), but in fresh materials from the sampling site this unit wall was absent.

Relationships between host plants and VA mycorrhizal fungi Six species in three genera of VA mycorrhizal fungi were recovered from the three beach plant patches. In the patch of *E. mollis*, *Glomus* spp. were most frequently found and had the highest total spore number (2379 spores). Among *Glomus* spp. spore production of *Glomus* sp. 1 predominated. Although spores of *Acaulospora* and *Scutellospora* spp. were also found, either frequency of occurrence or spore number was considerably lower than *Glomus* spp. *Acaulospora* and *Scutellospora* spp. were more frequently recorded under both *W. prostrata* and *Z. macrostachya* patches. *Glomus* spp. were less comonly recovered from these two patches. The highest total spore numbers of *Acaulospora* (557 spores) and *Scutellospora* spp. (1750 spores) were obtained from the *Z. macrostachya* patch (Table 2, Fig. 9). No seasonality was observed in spore numbers or occurrence of VA mycorrhizal fungi from June 1990 to May 1991 (Fig. 9).

Table 2. Total spore numbers and frequency of occurrence of six VA mycorrhizal fungi in the three coastal dune plant patches in Hasaki beach over one year.

Fungal species	*Elymus*	*Wedelia*	*Zoysia*
G. tortuosum	274 (50)[a]	0 (0)	58 (25)
Glomus sp.1	1374 (75)	20 (50)	274 (58)
Glomus sp.2	731 (33)	38 (25)	1 (8)
S. gregaria	4 (25)	306 (100)	1143 (100)
Scutellospora sp.1	0 (0)	78 (100)	607 (92)
Acaulospora sp.1	4 (33)	97 (67)	557 (75)

[a] frequency in percent (100% means spore of that species was recovered every month).

The predominance of *Glomus* spp. under *E. mollis* in Hasaki beach but not under *W. prostrata* or *Z. macrostachya* may be due to either:

1) a host-fungus specificity between *E. mollis* and *Glomus* spp.

2) environmental factors such as pH or salinity influencing the occurrence of spores of the fungi.

Host specificity has not been observed in associations between VA mycorrhizal fungi and their host plants (Harley and Smith, 1983). However, environmental factors have been found to influence the fungi. Some species are only found in acidic or alkaline soils and many fungi will not grow when transferred across gradients of pH (Robson and Abbott, 1989).

Robson and Abbott (1989) found that infection by *Acaulospora leavis* appears to be greater in acid soils, while infection by *G. mossae* and some other *Glomus* spp. appears to be greater in alkaline soils. The ecological importance of these differences is unclear and the relationship between *E. mollis* and *Glomus* requires further investigation.

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