SHORT COMMUNICATION

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Arbuscular mycorrhizas on *Athyrium yokoscense* and *A. niponicum* grown at a lead-contaminated site

Abstract To investigate the occurrence of arbuscular mycorrhizas (AM) and their morphological types, four *Athyrium yokoscense* and five *A. niponicum* individuals were collected at a lead-contaminated site. Three of the *A. yokoscense* and four of the *A. niponicum* formed AM structures. However, the percentage of AM formation within both species differed significantly among individuals. Morphological types of most AM structures were the *Paris*-type, except for one *A. yokoscense* that formed both *Paris*-type and *Arum*-type-like structures in different parts of the same root section. These results demonstrate that the two *Athyrium* species form AM associations in lead-contaminated soil.

Key words Arbuscular mycorrhizas · *Arum*-type · Morphological types · *Paris*-type · Pteridophytes

Most land plants have mycorrhizal associations with soil fungi belonging to the Zygomycetes, Ascomycetes, and Basidiomycetes, and as many as 80% of flowering plants form arbuscular mycorrhizas (AM; Smith and Read 1997). Host specificity of AM fungi is generally lacking (Molina et al. 1992), and the fungi are known to form AM with pteridophytes, based on the presence or absence of mycorrhizal associations across taxa (e.g., Berch and Kendrick 1982; Harley and Harley 1987; Gemma et al. 1992; Zhao 2000; Zhang et al. 2004).

AM are divided into two morphological types: the *Paris*-type and the *Arum*-type, named after the host plants, *Paris quadrifolia* and *Arum maculatum*, in which each type was first found (Gallaud 1905 in Smith and Smith 1997). The *Paris*-type is characterized by the absence of intercellular hyphal growth in the root cortex, the presence of intracellular hyphal coils, and arbuscules as intercalary structures on the coils, whereas the *Arum*-type is characterized by the presence of intercellular hyphal growth and the formation of arbuscules in the intracellular hyphal branches. Morphological types of AM have been examined in many plant taxa (Cavagnaro et al. 2001; Kubota and Hyakumachi 2004; Yamato 2004), and those in pteridophytes have generally been recognized as *Paris*-type (Smith and Smith 1997).

In Japan, more than 630 species of pteridophytes belonging to 34 families have been described (Iwatsuki 1992), although few studies have examined mycorrhizal formation in pteridophytes (Maeda 1954; Fujiyoshi et al. 1999). In the present study, we focused on *Athyrium yokoscense.* This fern is known to be a metal-hyperaccumulating plant; it accumulates relatively higher levels of heavy metals, such as cadmium and zinc (Ishizawa et al. 1980) and lead (Sakai et al. 1991), within tissues at polluted compared to nonpolluted sites. Many species within the genus *Athyrium* have been shown to form AM (Maeda 1954; Harley and Harley 1987; Gemma et al. 1992; Zhao 2000; Zhang et al. 2004), but no study has examined whether *A. yokoscense* forms AM, especially at sites with contaminated heavy metals. Thus, researchers do not fully understand the colonization and functional significance of mycorrhizal fungi in *A. yokoscense* with respect to the accumulation of heavy metals from soil by plant tissues (Leyval et al. 1997). The aim of this study was to investigate the occurrence and morphological types of AM in *A. yokoscense* collected at a lead-contaminated site.

From July to November 2003, nine pteridophytes were sampled with surrounding soils at a lead-contaminated site. Four were identified to be *A. yokoscense* and the remaining five were *A. niponicum* (Table 1). *Reynoutria japonica*, *Hydrocotyle sibthorpioides*, *Gnaphalium affine*, *Oxalis corniculata,* and *Zanthoxylum ailanthoides* also grew sporadically at the site. (The exact location of our study site is not described because of an environmental controversy.) Lead contained in soils at the site was extracted with 1 N

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Table 1. Sampling habitat and occurrence of arbuscular mycorrhizas (AM) in *Athyrium yokoscense* and *A. niponicum*

Species	Individual no.	Habitat	AM percentage (%)[a]	Species χ^2 value
Athyrium yokoscense	h-1	Soil	40.5 (325/802)	
	h-2	Soil	16.4 (213/1300)	
	h-3	Soil	68.1 (417/612)	
	h-4	Soil	0 (0/500)	799.5*
A. niponicum	i-1	Soil	15.2 (142/936)	
	i-2	Soil	20.2 (252/1242)	
	i-a	Rock	23.8 (156/657)	
	i-b	Rock	4.0 (26/654)	
	i-c	Rock	0 (0/500)	223.2*

[a] Percentage of colonization = [(number of stained sections that crossed grid lines)/(number of root fragments that crossed grid lines)] × 100
* $P < 0.001$

HCl and elutes were adjusted at pH 4.5. Then, the concentration of lead was measured with an ion meter (F-23; Horiba, Tokyo, Japan) and averaged 6380 ± 1551 ppm (mean ± SE; $n = 14$), a significantly higher lead concentration than in undisturbed soils (<50 ppm in Japan; Fujikawa et al. 2000).

Root samples excised randomly from the distal part of the root systems were rinsed with tap water using an ultrasonic cleaner (VC-1; As One, Osaka, Japan) to remove soil particles. They were then cleaned in 10% (w/v) KOH in a microwave oven for up to 1 h with 700 W and bleached and stained with either 0.05% trypan blue or 0.03% chlorazol black E at 90°C for 10 min. Roots were squashed and checked for the presence of AM fungal structures under a Nomarski interference contrast light microscope at magnifications from 200 to 400×. When we confirmed arbuscules, arbuscule coils, or hyphal coils within the root cell, we identified the structures as AM. The samples stained with trypan blue were further cut into 5-mm- to 1-cm-long segments and spread onto a 1-cm-grid Petri dish under a dissecting microscope to estimate the percentage of root length colonized by AM fungi. The line intersection method was applied for the estimation (Tennant 1975). Root length of more than 78.6 cm was assessed counting at a minimum of 100 intersections each time. The measurement was repeated five times and pooled as one data point for each individual. The percentage of AM fungal colonization was calculated as [(number of stained sections that crossed grid lines)/(number of root fragments that crossed grid lines)] × 100.

Three of four *A. yokoscense* and four of five *A. niponicum* individuals formed AM structures (Table 1, Fig. 1a). However, the percent AM colonization in both species differed significantly among individuals within the species (χ^2 test, $P < 0.001$; Table 1). The percentage of root length colonized on *A. yokoscense* averaged 29.8% (range, 0%–68.1%) and that on *A. niponicum* averaged 18.1% (range, 0%–23.8%). For one *A. yokoscense* individual (h-4), we did not observe either the formation of AM structures or the growth of fungal hyphae within root tissues. Two individuals of *A. niponicum* (i-b and i-c) growing in the crevice of stone walls tended to form few or no AM structures within their root cells.

AM formation in the genus *Athyrium* is well recognized (Maeda 1954; Harley and Harley 1987; Gemma et al. 1992;

Zhao 2000; Zhang et al. 2004). However, few studies examined AM formation on *A. niponicum* in particular (Maeda 1954; Zhao 2000). Zhao (2000) showed no arbuscule formations on *A. niponicum* collected in southwest China, whereas Maeda (1954) determined that this species formed endomycorrhizas. As shown in the present study, contradictory results regarding AM formation within a species are common, with one individual forming AM structures and another not (Harley and Harley 1987; Smith and Smith 1997). To our knowledge, no study has been conducted on the mycorrhizal formation of *A. yokoscense,* and thus our findings are unique. Moreover, AM formation in *A. niponicum* and *A. yokoscense* at a lead-contaminated site is also a new record for the two species.

AM fungal structures of arbuscules, arbuscule coils, hyphal coils, or vesicles were observed in both *A. yokoscense* and *A. niponicum* (Fig. 1b–e). All individuals of both species formed the *Paris*-type AM, except for one *A. yokoscense* that formed the *Paris*-type in addition to a few *Arum*-type-like structures in different parts of the same root section (Fig. 1f). Although Maeda (1954) found that *A. niponicum* formed endomycorrhizas, he did not mention the morphological type of AM in this species. Zhang et al. (2004) recorded *A. wardii* to have *Paris*-type structures. Indeed, morphological types found on pteridophytes so far have been recorded as *Paris*-type AM. The different AM types may have different functions in the transfer of inorganic nutrients and organic carbons (Smith and Smith 1997). Moreover, Yamato (2004) indicated that AM morphological types may be influenced by environmental conditions. To examine whether metal accumulation in soils affects the development of the types in these two *Athyrium* species, future studies will examine individuals from nonpolluted sites for comparison with these results.

In this study, little hyphal growth was observed on the root surface of non-AM individuals, indicating the possibility of no AM fungal colonization. According to a previous study, the germination of AM fungal spores and the percentage of root colonization by the fungi decreased in soil highly contaminated with heavy metals (Del Val et al. 1999). We isolated the spores of AM fungi from the rhizosphere of the pteridophytes sampled, and we were able to identify several types of AM spores, including some from the genus *Glomus* (unpublished data). Several kinds of AM spores were also isolated from soil around a non-AM *A.*
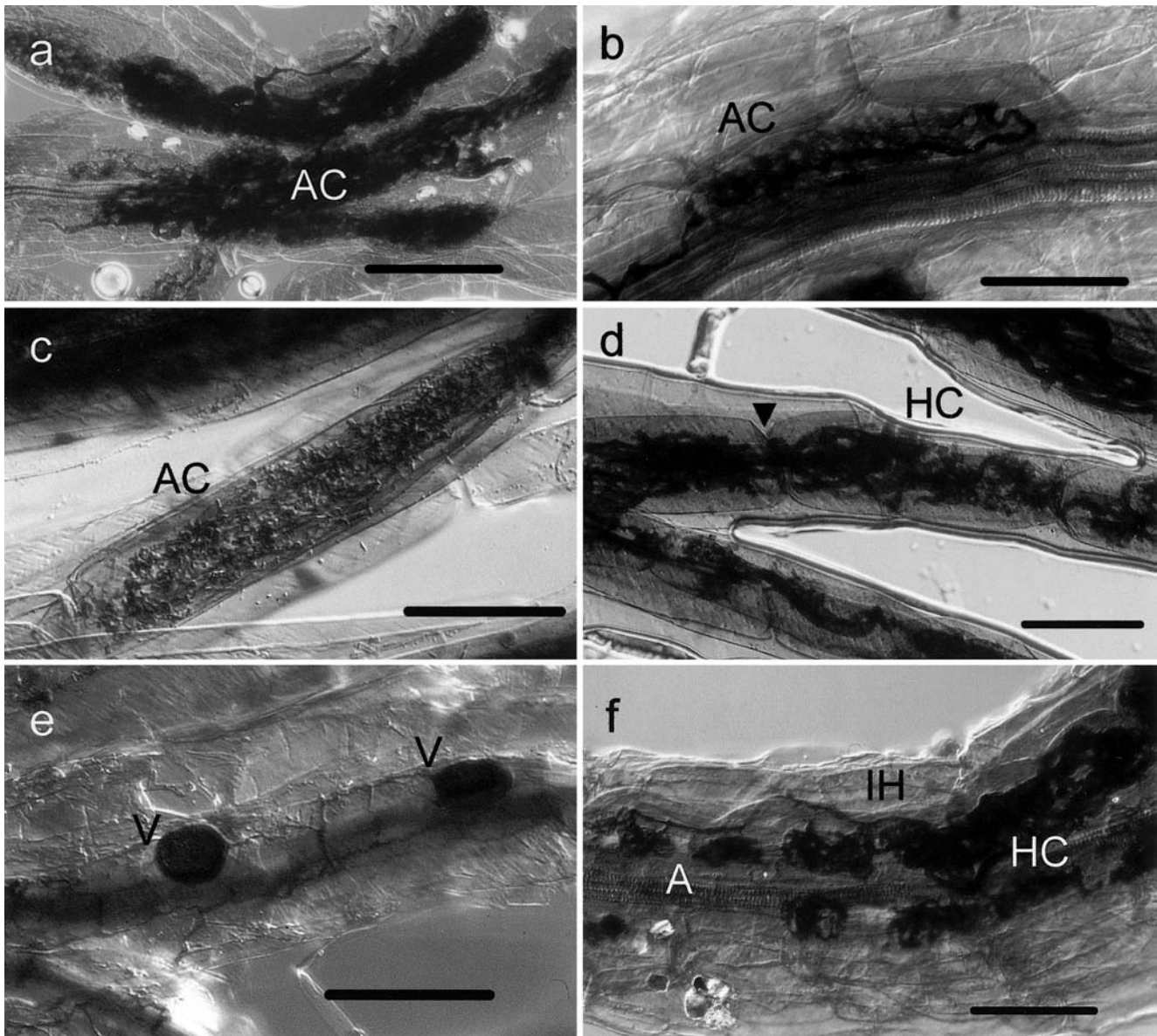
Fig. 1. Structures of arbuscular mycorrhizas formed on *Athyrium yokoscense* (**a, b, d–f**) and *A. niponicum* (**c**). *A*, arbuscule; *AC*, arbusculate coil; *HC*, hyphal coil; *IH*, intercellular hyphae; *V*, vesicle; *arrowhead*, cell-to-cell hyphal spread of colonization. *Bars* **a**, **f** 100 μm; **b–e** 50 μm

yokoscense, where the concentration of lead was relatively high. Therefore, high metal ion concentrations in soil may suppress the germination of AM fungal spores and/or the formation of AM. The absence of AM can also be explained by the growth substrate of pteridophytes. We found non-AM *A. niponicum* growing in the crevice of a stone wall. Berch and Kendrick (1982) reported no mycorrhizal formation on Canadian pteridophytes growing on bare rock or in the crevices of cliffs. Because AM fungi mainly inhabit the soil, the presence of little AM fungal inoculum in such substrates may be the underlying cause of few or no AM on the pteridophytes examined.

The ability of certain plants to absorb and accumulate high levels of metals can be utilized for phytoremediation (Macnair 2003). Some species of pteridophytes, including *A. yokoscense*, have been suggested as the candidate for phytoremediation (Ishizawa et al. 1980; Sakai et al. 1991; Ma et al. 2001). In this study, we showed that two *Athyrium* species grown at a site with relatively high lead concentrations formed AM. Malcová et al. (2003) indicated that the colonization of AM fungi had the potential to change the amount of metal transfer from soil to host plants. Further studies are needed to determine whether AM fungi associated with pteridophytes are functionally involved in metal accumulation.

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