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ORIGINAL PAPER

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Morphological types of arbuscular mycorrhizal fungi in roots of weeds on vacant land

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Abstract Morphological types of arbuscular mycorrhizal (AM) fungi in weeds of vacant land were examined in spring and autumn. In total, 33 plant species belonging to 28 genera in 13 families were examined. The number of plant species with *Arum*-type AM was higher than those with *Paris*- or intermediate types in both seasons. Thus, *Arum*-type colonization may be beneficial for fast-growing plant species on vacant land. There was a strong relationship between plant identity and AM morphological type, as the colonization types were mostly distinguished at the plant family level.

Keywords Arbuscular mycorrhiza · Morphological type · *Paris*-type · *Arum*-type · Intermediate type

Introduction

Arbuscular mycorrhizal (AM) fungi (Order Glomales) form symbiotic associations with approximately 80% of terrestrial flowering plants (Harley and Smith 1983). The morphology of AM can be divided mainly into two types, *Arum* and *Paris*, first described by Gallaud (1905). In the *Arum*-type colonization, AM fungi form extensive intercellular hyphae in well-developed air spaces between cortical cells and invaginate the cells as short side branches to form arbuscules. In contrast, in the *Paris*-type, colonization spreads directly from cell to cell in the root. This is further characterized by the absence of intercellular hyphae and the development of intracellular hyphal coils that frequently have intercalary arbuscules.

Although functional differences between the two types have not been elucidated, Brundrett and Kendrick (1990a) found that the spreading rate of colonization was slower in *Paris*- than in *Arum*-type. They suggested that slower colonization by AM fungi is beneficial for the host plant to keep the energy supply to the fungi at a manageable level for plants growing slowly in an environment such as woodland (Brundrett and Kendrick 1990b). Cavagnaro et al. (2001a) also demonstrated a slow rate of colonization spread in *Paris*-type between *Asphodelus fistulosus* and *Glomus coronatum*. Furthermore, Yamato and Iwasaki (2002) found dominance of *Paris*-type AM among herbaceous plants growing on forest floors. There may be some relationship between AM morphological type and vegetation environment or plant habitat and further investigation of this relationship could be helpful in elucidating functional differences between the two types.

Contrary to the dominance of *Paris*-type AM in forest floor plants (Yamato and Iwasaki 2002), it appears that *Arum*-type AM are dominant among weeds growing on vacant land. In order to test this hypothesis, many weed plants growing on vacant land were examined for AM morphological type. The relationship between AM morphological type and the ecological habitat of host plants is discussed. The effect of the plant species on AM morphological type is also discussed, using a current scheme for plant phylogeny.

Materials and methods

A sampling plot of 25 m^2 (5 m × 5 m) was prepared on vacant land at the Biological Environment Institute, Kansai Environmental Engineering Center Co. Ltd, which is located in Uji city, Kyoto prefecture at 35 m above sea level. The statistical data for temperature and precipitation in Kyoto prefecture in 1971–2000 were obtained from the Meteorological Agency, Japan (Fig. 1). The vegetation is typical of fields in the Kansai region and similar vegetation was observed in surrounding areas. Plants were sampled twice, on 10 May and 10 September 2001, in order to examine them in both spring and autumn vegetative states, respectively. Weeding was performed between sampling times at the beginning of July using a mowing machine, which was done regularly every 3 months. Several specimens of all plant species found in the plot were collected and examined for AM formation. The roots were fixed and preserved in formalin-acetic acid-alcohol, stained according to the method of Brundrett et al. (1984) with slight modification, cleared by autoclaving at 121°C for 10 min in 10% KOH solution

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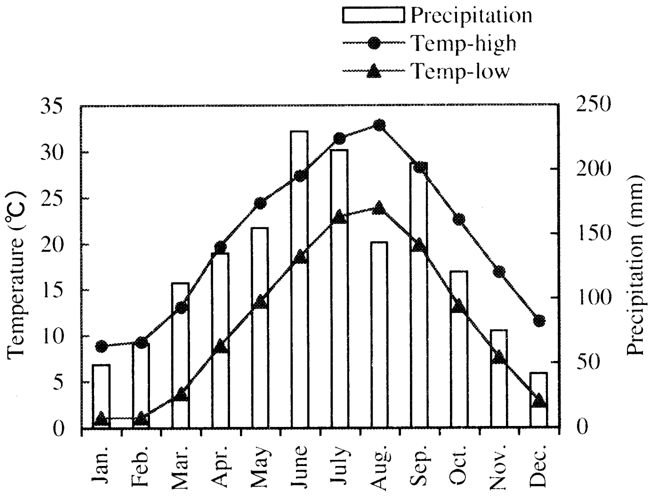


Fig. 1 Mean monthly temperature and precipitation in Kyoto from 1971 to 2000 (Meteorological Agency, Japan)

and stained with 0.1% Chlorazol black E at 95°C for 90 min. They were then squashed and observed under a Nomarski interference-contrast microscope (Leica Leitz DMR, Wetzlar) to detect AM and distinguish AM morphological types. At least 20 cm of root from three individuals was examined for each plant species. The numbers of plant species with each AM morphological type were compared at each sampling time.

The classification system of flowering plants used here corresponds to that of the Angiosperm Phylogeny Group (APG 1998). The relationship between the classification of the plants and AM morphological type was also derived from a current plant phylogeny scheme inferred from 18S rDNA, *rbcL* and *atpB* sequences (Soltis et al. 2000).

Results

The examined vegetation was typical of vacant land in the Kansai region, consisting of species in the Iridaceae, Commelinaceae, Poaceae, Cyperaceae, Amaranthaceae, Caryophyllaceae, Polygonaceae, Vitaceae, Rosaceae, Fabaceae, Oxalidaceae, Geraniaceae, Primulaceae, Scrophulariaceae, Acanthaceae, Lamiaceae, Rubiaceae and Asteraceae. In total, 40 species belonging to 34 genera in 18 families were examined. Among them, 33 species belonging to 28 genera in 13 families were found to have AM colonization (Fig. 2). AM colonization was not found in 7 species of the Caryophyllaceae, Polygonaceae, Amaranthaceae, Commelinaceae and Cyperaceae (Fig. 2) that are known as non-mycorrhizal or weakly mycorrhizal plant families (Tester et al. 1987). Some of the examined AM plant species with both intercellular hyphae and the intracellular spread of hyphal coils were categorized as intermediate type. There was no difference in the AM morphological type of the same plant species at different sampling times.

Arum-type colonization was found to be dominant at each sampling time. In the spring sampling, 28 species were collected of which 24 species were colonized by AM fungi. Among them, *Arum*-, *Paris*- and intermediate type were found in 17, 5 and 2 species, respectively (Fig. 2). In the autumn sampling, 26 species were collected, of which 20 species were colonized by AM fungi. Among them, *Arum*-, *Paris*- and intermediate types were found in 13, 1 and 6 species, respectively.

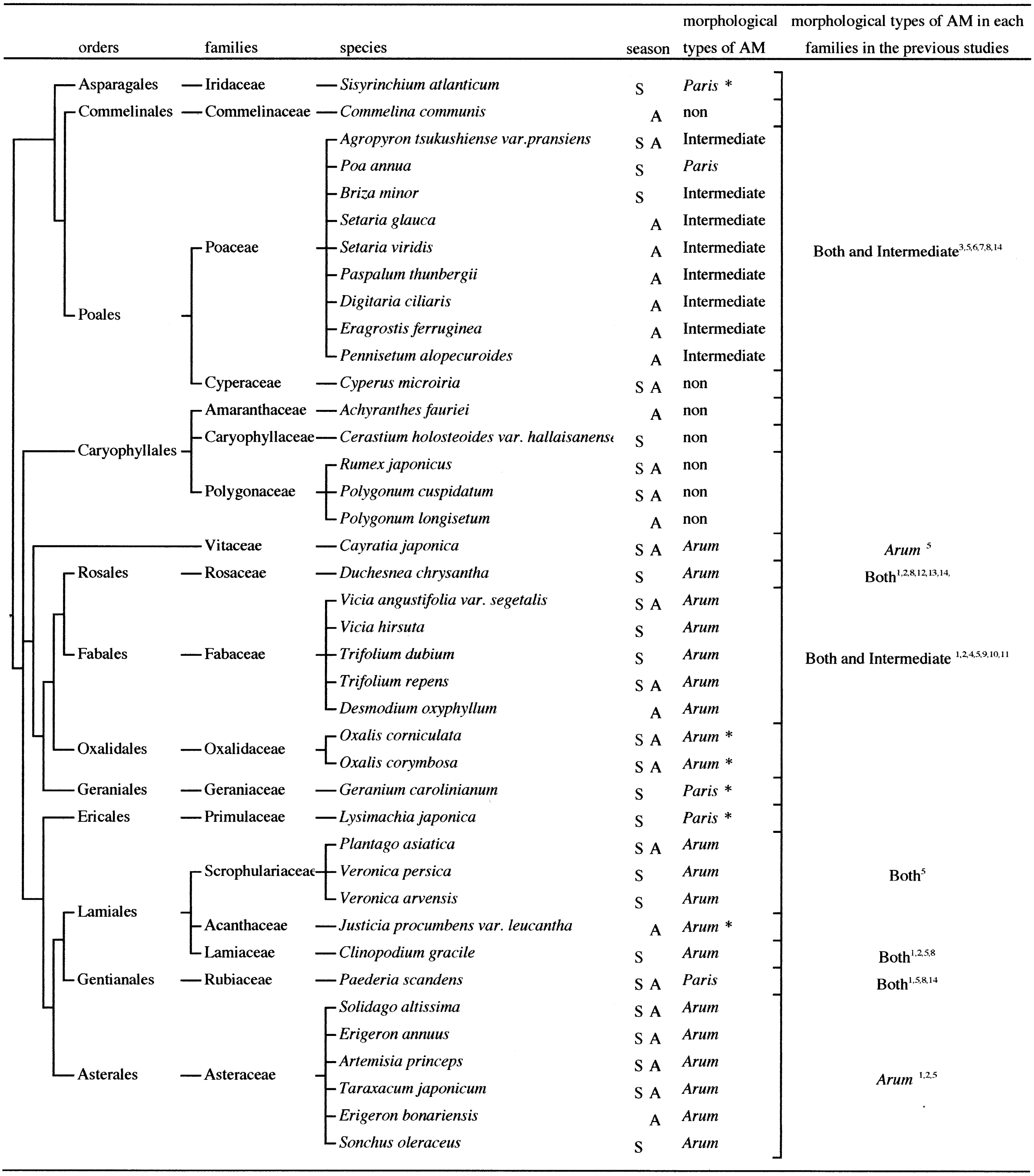
Morphological types of AM were distinguished at the plant order level and were mostly determined at a family level, except for Poaceae (Fig. 2). In this family, most of the examined species were categorized as intermediate, in which both typical *Paris*- and intermediate type colonization was found even in individual plants, depending on the position in the root (Figs. 3, 4).

To our knowledge, *Arum*-type in Oxalidaceae, Acanthaceae (Figs. 5, 6) and *Paris*-type in Iridaceae, Geraniaceae and Primulaceae (Figs. 7, 8, 9) are new records.

Discussion

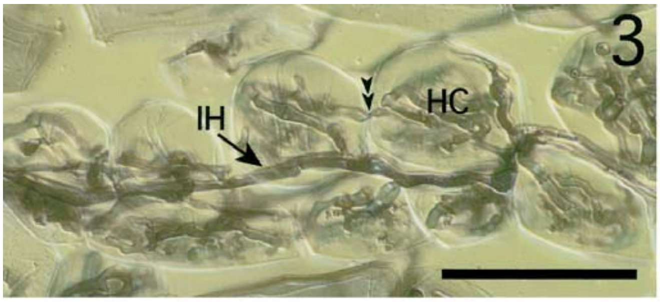
Arum-type colonization was shown to be dominant among the weed species examined in this study. Since the vegetation at the sampling site was typical of vacant land, *Arum*-type dominance may be a general characteristic of most vegetation on vacant land in this region. O'Connor et al. (2001) also found that *Paris*-type colonization was rare among AM plants examined in the southern Simpson Desert in Australia. It is also well known that most cultivated herbs grown without any shading form *Arum*-type AM (Smith and Smith 1997). This contrasts with a previous study in which *Paris*-type AM was seen to be dominant in the herbaceous understorey plants in deciduous broad-leaved forest of the Kansai region (Yamato and Iwasaki 2002). One of the most conspicuous environmental differences between these sites is the light intensity, which strongly affects photosynthetic activity and plant growth. This suggests that *Arum*-type colonization is appropriate for fast-growing plant species. Root growth rate of such fast-growing plants is considered to be relatively high. The high spreading rate of colonization by *Arum*-type (Brundrett and Kendrick 1990a) may be one reason for the aptitude of *Arum*-type AM for fast-growing plants, as indicated by Brundrett and Kendrick (1990b). The number of *Paris*-type plant species was greater in spring than in autumn. Plant growth rate in spring is considered in general to be slower than in autumn, because temperature and light intensity are low during several months before the spring sampling. This may be a reason for the more frequent appearance of *Paris*-type plants in the spring sampling.

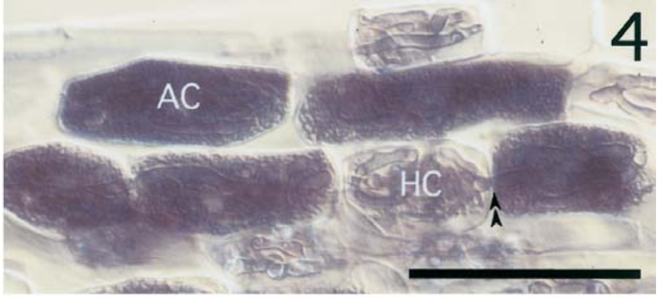
It has been shown that AM morphological type is controlled by the host plant (Gerdemann 1965; Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983). A strong link between plant identity and AM morphology was also shown at the present field site, where AM morphological type was determined mostly at the plant family level. However, some inconsistency of morphological type occurred at the family level in comparison with previous studies. All of the examined species from Asteraceae and Fabaceae formed typical *Arum*-type in this study, but

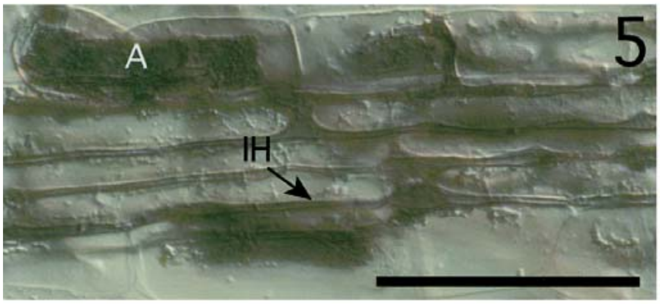


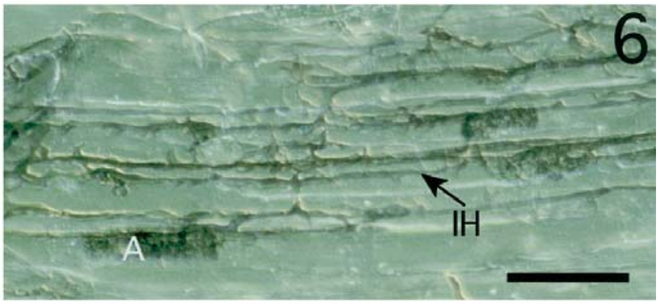
orders	families	species	season	morphological types of AM	morphological types of AM in each families in the previous studies
Asparagales	Iridaceae	*Sisyrinchium atlanticum*	S	*Paris* *	
Commelinales	Commelinaceae	*Commelina communis*	A	non	
Poales	Poaceae	*Agropyron tsukushiense var.pransiens*	S A	Intermediate	Both and Intermediate[3,5,6,7,8,14]
		Poa annua	S	*Paris*	
		Briza minor	S	Intermediate	
		Setaria glauca	A	Intermediate	
		Setaria viridis	A	Intermediate	
		Paspalum thunbergii	A	Intermediate	
		Digitaria ciliaris	A	Intermediate	
		Eragrostis ferruginea	A	Intermediate	
		Pennisetum alopecuroides	A	Intermediate	
	Cyperaceae	*Cyperus microiria*	S A	non	
Caryophyllales	Amaranthaceae	*Achyranthes fauriei*	A	non	
	Caryophyllaceae	*Cerastium holosteoides var. hallaisanense*	S	non	
	Polygonaceae	*Rumex japonicus*	S A	non	
		Polygonum cuspidatum	S A	non	
		Polygonum longisetum	A	non	
	Vitaceae	*Cayratia japonica*	S A	*Arum*	*Arum* [5]
Rosales	Rosaceae	*Duchesnea chrysantha*	S	*Arum*	Both[1,2,8,12,13,14,]
Fabales	Fabaceae	*Vicia angustifolia var. segetalis*	S A	*Arum*	Both and Intermediate [1,2,4,5,9,10,11]
		Vicia hirsuta	S	*Arum*	
		Trifolium dubium	S	*Arum*	
		Trifolium repens	S A	*Arum*	
		Desmodium oxyphyllum	A	*Arum*	
Oxalidales	Oxalidaceae	*Oxalis corniculata*	S A	*Arum* *	
		Oxalis corymbosa	S A	*Arum* *	
Geraniales	Geraniaceae	*Geranium carolinianum*	S	*Paris* *	
Ericales	Primulaceae	*Lysimachia japonica*	S	*Paris* *	
Lamiales	Scrophulariaceae	*Plantago asiatica*	S A	*Arum*	Both[5]
		Veronica persica	S	*Arum*	
		Veronica arvensis	S	*Arum*	
	Acanthaceae	*Justicia procumbens var. leucantha*	A	*Arum* *	
	Lamiaceae	*Clinopodium gracile*	S	*Arum*	Both[1,2,5,8]
Gentianales	Rubiaceae	*Paederia scandens*	S A	*Paris*	Both[1,5,8,14]
Asterales	Asteraceae	*Solidago altissima*	S A	*Arum*	*Arum* [1,2,5]
		Erigeron annuus	S A	*Arum*	
		Artemisia princeps	S A	*Arum*	
		Taraxacum japonicum	S A	*Arum*	
		Erigeron bonariensis	A	*Arum*	
		Sonchus oleraceus	S	*Arum*	

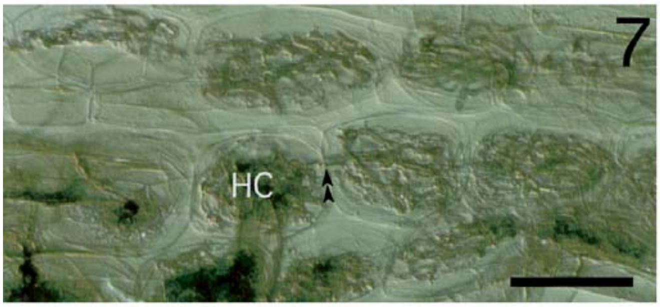
Fig. 2 Morphological types of arbuscular mycorrhizal (AM) fungi examined in this study with a current phylogeny scheme. The ordinal names correspond to those used in APG (1998). The applied phylogeny is that of Soltis et al. (2000), which is inferred from 18S rDNA, *rbcL*, and *atpB* sequences. The AM morphological type of each plant family in previous studies are also shown. References: [1]Janse (1897) [2]Gallaud (1905), [3]Endrigkeit (1937), [4]Asai (1944), [5]Johnston (1949), [6]Nicolson (1959), [7]Gerdemann (1965), [8]Steltz (1968), [9]Abbott and Robson (1978), [10]Holley and Peterson (1979), [11]Carling and Brown (1982), [12]Brundrett et al. (1990), [13]Widden (1996), [14]Yamato and Iwasaki (2002). References to some early papers in the list are as cited by Smith and Smith (1997) (*S* spring, *A* autumn, * new record of the AM morphological type in each plant family)

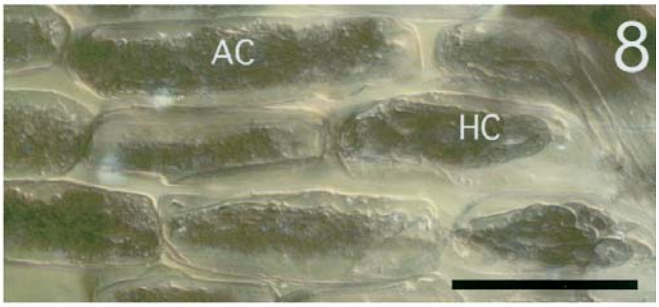












Figs. 3–9 Representative micrographs showing morphology of mycorrhizal association (*IH* Intercellular hyphae, *HC* hyphal coil, *AC* arbuscular hyphal coil, *double arrowheads* cell to cell spread of colonization); *bar* 100 μm

Fig. 3 Mycorrhizal associations in *Pennisetum alopecuroides* (Poaceae)

Fig. 4 Mycorrhizal associations in *Pennisetum alopecuroides* (Poaceae)

Fig. 5 Mycorrhizal associations in *Oxalis corniculata* (Oxalidaceae)

Fig. 6 Mycorrhizal associations in *Justicia procumbens* var. leucantha (Acanthaceae)

Fig. 7 Mycorrhizal associations in *Sisyrinchium atlanticum* (Iridaceae)

Fig. 8 Mycorrhizal associations in *Lysimachina japonica* (Primulaceae)

Fig. 9 Mycorrhizal associations in *Geranium carolinianum* (Geraniaceae)

Louis (1990) found hyphal coils in some species from these families in a survey of AM colonization on coastal reclaimed land in Singapore. A review by Smith and Smith (1997) also reported that *Paris*- and *Arum*-types coexist in some plant families. Effects of the fungal species may be a reason for this inconsistency. Abbott (1982) compared the morphology of 10 AM species colonizing *Trifolium subterraneum* and found that *Acaulospora* formed predominantly intracellular hyphae throughout the cortex, though some others formed intercellular hyphae in the inner cortex. Recently, the effect of fungal species on AM morphological type was shown clearly by Cavagnaro et al. (2001b), who demonstrated that both AM morphological types were formed in *Lycopersicon esculentum* (wild-type tomato) depending on the fungal species. Therefore, it is probable that AM morphological type is determined by both plant and fungal identity, but that most plants incline to one morphological type. Hence, it is also probable that plants from the location studied are dominated by AM fungi which tend to form *Arum*-type, and that different morphological types may be found in some plant species at other sites. Additionally, environmental factors such as temperature, light intensity and soil moisture content may influence AM morphology, as indicated by Cavagnaro et al. (2001b), as these factors affect the growth and morphology of roots.

In order to evaluate the generality of the *Arum*-type dominance seen here in weeds on vacant land, it is necessary to examine a wider range of plant species colonized by AM fungi in this kind of vegetation. Such a study would help clarify the ecological significance of the *Arum*-type AM in the environment and may lead to the elucidation of functional or physiological differences between the morphological types.

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