

Diversity of arbuscular mycorrhizal symbiosis in plants from coastal habitats

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Abstract

The aim of the present study was to examine the occurrence of mycorrhizal symbiosis in roots of coastal plants of temperate region and to search for structures related to possible functional status of the symbiosis. A total of 29 plant species belonging to 18 families and 27 genera were examined. The presented data clearly show that in spite of sometimes low intensity of mycorrhization, roots of all coastal plants studied possess functional structures of arbuscular mycorrhizal symbiosis. It can be suggested that fluctuating and low intensity of mycorrhizal symbiosis is caused by unfavourable environmental conditions within the vegetation season. For salt marsh plants, increased soil salinity is one of the environmental factors leading to decreased intensity of arbuscular mycorrhizal symbiosis.

Key words: arbuscular mycorrhiza; coastal habitats; morphology.

Abbreviations: AM, arbuscular mycorrhiza.

Introduction

The majority of wild plant species in natural ecosystems possess mycorrhizal symbiosis, a mutual beneficial interaction between a fungus and a plant. It is widely accepted that the fungus provides the plant with nutrients and water in exchange of reduced carbon compounds from photosynthesis (Brundrett 2009). However, some plant families are considered nonmycorrhizal. Many families of nonmycorrhizal plants rely on alternative strategies of mineral acquisition, while the other are thought to occur in habitats unsuitable for mycorrhizal fungi (Brundrett 2009). According to recent data, only 53 from 336 Angiosperm families are nonmycorrhizal, with another 40 having variable nonmycorrhizal-mycorrhizal status (Brundrett 2009). In total, 86% of all flowering plants are considered mycorrhizal.

The so called facultatively mycorrhizal plant species are thought to be characterized by inconsistent mycorrhization or low levels of colonization (below 25%; Brundrett 1991). However, this is not a truly physiological term, as some studies indicate that even a low level of mycorrhizal symbiosis may have beneficial effect on the host plant (Wilson, Hartnett 1998; Gange, Ayres 1999).

Arbuscular mycorrhiza (AM) is a main type of mycorrhizal symbiosis for herbaceous plants. High mycorrhizal status of plants both on sand dunes (Koske, Halvorson 1981; Logan et al. 1989; Little, Maun 1997; Beena et al. 2001; Çakan, Karataş 2006) and, to a lesser extent, coastal

salt marsh (Hoefnagels et al. 1993; Carvalho et al. 2001; Hildebrandt et al. 2001) has been confirmed. One of the historically oldest studies on mycorrhizal status of salt marsh plants mentions the following mycorrhizal species: *Plantago coronopus*, *Plantago maritima*, *Aster tripolium*, *Glaux maritima*, *Armeria maritima*, *Cochleria officinalis*, *Agrostis alba* and *Glyceria maritima* (Mason 1928). On the other hand, *Salicornia europaea*, *Spergularia marginata*, *Triglochin maritimum*, *Juncus maritimus* and *Juncus gerardi* were described as non-mycorrhizal.

While measurement of mycorrhizal intensity and frequency by counting indicative structures in root fragments allows to consider quantitative aspects of mycorrhizal symbiosis, analysis of different morphological structures also gains insight into the functional differences of the symbiosis. Assessment of the functional status can be important in considering the status of mycorrhizal symbiosis for a particular host plant. The two major morphological types of AM are classified according to characteristic differences in fungal development within a root. An extensive formation of intracellular structures in the form of hyphal coils and arbuscular coils is found only in *Paris*-type, while in *Arum*-type, hyphae spread between the root cortical cells intercellularly, penetrating cells only to form arbuscules (Smith, Smith 1997). It has been suggested that the *Paris*-type AM is characteristic of plants in low-nutrient and high-stress conditions, while the *Arum*-type is associated with fast growing plants (Brundrett, Kendrick 1990). However, co-occurrence of the both types has also

been noted (Kubota et al. 2005).

An active exchange process between the symbionts morphologically is associated with the presence of highly branched hyphal structures and arbuscules while the abundance of vesicles indicates storage of resources (Brundrett 1991). The presence of vesicles as well as internal and external hyphae in plant roots can be used to indicate active mycorrhizal symbiosis (Brundrett 1991). Vesicles are hyphal swellings that act as storage organs of AM, accumulating lipids. Additional presence of arbuscules and intracellular hyphal coils indicates high functionality of symbiosis (Brundrett 1991). However, not all genera of AM fungi form intraradical vesicles. Development of sporocarps and spores on extraradical hyphae or within internal vesicles indicates high abundance of organic carbon, allowing successful reproduction (Brundrett 1991). However, colonization of senescing roots by saprobic or parasitic fungi can be mistaken for mycorrhizal symbiosis (Glenn et al. 1985; Brundrett 1991).

The aim of the present study was to examine the presence of mycorrhizal symbiosis in roots of coastal plants of the temperate region and to search for structures related to the possible functional status of the symbiosis. A total of 29 plant species belonging to 18 families and 27 genera were examined.

Materials and methods

Study species and their putative mycorrhizal status

A number of plant species were chosen for mycorrhizal analysis from coastal habitats of the Baltic Sea in the territory of Latvia. The plants represented 18 families and 27 genera of vascular plants. Both relatively dry (coastal dunes, dune forest, dry coastal meadow) and relatively wet (sandy beach, wet coastal meadow, salt marsh) coastal habitats were considered. A list of species sampled with the respective families, characteristic habitat and sampling sites is presented in Table 1. Information is provided below on the putative mycorrhizal status of families and species used in the respective study.

Cyperaceae is thought as lacking mycorrhizal symbiosis (Harley, Harley 1987) or with 74% of species nonmycorrhizal (Newman, Reddell 1987), *Bolboschoenus maritimus* as non-mycorrhizal.

Juncaceae is regarded as a non-mycorrhizal family, with 56% of species nonmycorrhizal (Newman, Reddell 1987). *Juncus garardi* as occasionally mycorrhizal; no information is available on *Juncus balticus* (Harley, Harley 1987).

Juncaginaceae in general is characterized as variable mycorrhizal (Brundrett 2009). *Triglochin maritimum* is characterized as only occasionally mycorrhizal (Harley, Harley 1987).

Liliaceae is described as mostly mycorrhizal, genus *Tofieldia* as mycorrhizal (Harley, Harley 1987).

Poaceae is considered as mycorrhizal, *Phleum arena-*

rium as non-mycorrhizal, species of genus *Puccinellia* as sometimes mycorrhizal. *Puccinellia maritima*, a plant species also characteristic for littoral saline habitats, has been reported to be non-mycorrhizal, occasionally only with a slight infection (Gray, Scott 1977).

Apiaceae is considered as mycorrhizal (Harley, Harley 1987), *Eryngium maritimum* and *Hydrocotyle vulgaris* as mostly mycorrhizal (Harley, Harley 1987).

Asteraceae is considered as a mycorrhizal family, with only 6% nonmycorrhizal species (Newman, Reddell 1987). *Aster tripolium* is described as always mycorrhizal (Harley, Harley 1987) and the genus *Tragopogon* as mycorrhizal (Harley, Harley 1987).

Only 8% of species are considered mycorrhizal within the family Brassicaceae (Newman, Reddell 1987). Several species of *Alyssum* are described as nonmycorrhizal, including *Alyssum montanum*, a close relative of *Alyssum gmelinii* (Pawlowska et al. 1996; Çakan, Karataş 2006).

Caryophyllaceae is usually regarded as a non-mycorrhizal family (Harley, Harley 1987). About 50% of the species from Caryophyllaceae are described as nonmycorrhizal (Newman, Reddell 1987). Most species of genus *Silene* are described as non-mycorrhizal (Harley, Harley 1987). *Spergularia marina* and *Spergularia media* are described as sometimes mycorrhizal (Harley, Harley 1987).

In total, 61% of species from the family Cehnopodiaceae are considered nonmycorrhizal (Newman, Reddell 1987). The genus *Atriplex* has been regarded as non-mycorrhizal, but mycorrhizal symbiosis is reported for several species (Harley, Harley 1987; Brundrett 2009).

Euphorbiaceae and the genus *Euphorbia* are known as mycorrhizal (Harley, Harley 1987).

Fabaceae is considered as a mycorrhizal family, and the genera *Anthyllis*, *Lathyrus*, *Trifolium* as mycorrhizal (Harley, Harley 1987). Only 4% of species are considered nonmycorrhizal (Newman, Reddell 1987). However, *Trifolium fragiferum* as non-mycorrhizal (Harley, Harley 1987).

Gentianaceae are mostly mycorrhizal, with some species of *Centaurium* mycorrhizal (Harley, Harley 1987). Members of the Geraniaceae are described as mostly mycorrhizal, *Geranium molle* as mycorrhizal (Harley, Harley 1987).

Plantaginaceae is considered as mycorrhizal and *Plantago maritima* as nearly always mycorrhizal (Harley, Harley 1987).

The Primulaceae family and *Glaux maritima* are considered as mycorrhizal (Harley, Harley 1987).

Ranunculaceae is described as mostly mycorrhizal family, with 11% of species considered nonmycorrhizal (Newman, Reddell 1987), and *Ranunculus bulbosus* as having fungal hyphae (Harley, Harley 1987).

Scrophulariaceae is considered as mostly mycorrhizal, with 11% of nonmycorrhizal species (Newman, Reddell 1987). The genus *Linaria* is characterized as sometimes mycorrhizal (Harley, Harley 1987).

Table 1. Species sampled in the present study with characteristic habitat and sites

Family	Species	Habitat	Site	Coordinates
Cyperaceae	<i>Bolboschoenus maritimus</i>	sandy beach	Lielupe estuary	57°00; 23°56
	<i>Blysmus rufus</i>	salt marsh	Mērsrags	57°20; 23°08
	<i>Carex ligerica</i>	dry coastal meadow	Ainaži	57°50; 24°20
	<i>Carex reichenbachii</i>	dune forest	Mērsrags	57°20; 23°08
			Miķeltornis	57°36; 21°59
Juncaceae	<i>Juncus balticus</i>	sandy beach	Lielupe estuary	57°00; 23°56
			lake Būšnieka	57°26; 21°39
			Liepene	57°30; 21°39
	<i>Juncus gerardi</i>	salt marsh	Mērsrags	57°20; 23°08
Juncaginaceae	<i>Triglochin maritimum</i>	salt marsh	Mērsrags	57°20; 23°08
			lake Liepājas	56°30; 21°02
Liliaceae	<i>Tofieldia calyculata</i>	dune forest	Oviši	57°34; 21°43
Poaceae	<i>Phleum arenarium</i>	dry coastal meadow	Oviši	57°34; 21°44
	<i>Puccinellia capillaris</i>	salt marsh	Lielupe estuary	57°00; 23°56
Apiaceae	<i>Eryngium maritimum</i>	dunes	Užava	57°15; 21°25
			Ziemupe	56°48; 21°04
	<i>Hydrocotyle vulgaris</i>	salt marsh	Mērsrags	57°20; 23°08
			lake Engures	57°16; 23°08
			lake Būšnieka	57°26; 21°39
Asteraceae	<i>Aster tripolium</i>	salt marsh	lake Liepājas	56°30; 21°02
	<i>Tragopogon heterospermus</i>	dunes	Jūrmalciems	56°19; 20°59
Brassicaceae	<i>Alyssum gmelinii</i>	dunes	Jūrmalciems	56°19; 20°59
			Nida	56°07; 21°03
			Jaunupe	57°30; 21°39
			Užava	57°15; 21°25
Caryophyllaceae	<i>Gypsophila paniculata</i>	dry coastal meadow	Jūrmalciems	56°19; 20°59
	<i>Silene borysthena</i>	dunes /	Jūrmalciems	56°19; 20°59
		dry coastal meadow		
	<i>Spergularia salina</i>	salt marsh	Ainaži	57°52; 24°21
			lake Liepājas	56°30; 21°02
Chenopodiaceae	<i>Atriplex calotheca</i>	sandy beach	Lielupe estuary	57°00; 23°56
			Ainaži	57°53; 24°21
Euphorbiaceae	<i>Euphorbia palustris</i>	wet coastal meadow	lake Slokas	56°58; 23°33
Fabaceae	<i>Anthyllis maritima</i>	dunes	Pape	56°08; 21°02
	<i>Lathyrus maritimus</i>	dunes	Jūrmalciems	56°19; 20°59
	<i>Trifolium fragiferum</i>	wet coastal meadow / salt marsh	lake Liepājas	56°30; 21°02
Gentianaceae	<i>Centaurium litorale</i>	dunes /	Lielupe estuary	57°00; 23°56
		dry coastal meadow	Ainaži	57°52; 24°21
Geraniaceae	<i>Geranium molle</i>	dry coastal meadow	Oviši	57°34; 21°44
Plantaginaceae	<i>Plantago maritima</i>	salt marsh	lake Liepājas	56°30; 21°02
Primulaceae	<i>Glaux maritima</i>	salt marsh	Mērsrags	57°20; 23°08
			lake Liepājas	56°30; 21°02
Ranunculaceae	<i>Ranunculus bulbosus</i>	dry coastal meadow	Oviši	57°34; 21°44
			Jaunupe	57°30; 21°39
Scrophulariaceae	<i>Linaria loeselii</i>	dunes	Nida	56°06; 21°03
			Oviši	57°34; 21°43

Collection of root samples and analysis of mycorrhizal symbiosis

Root samples were collected during the vegetation season of 2005 and 2006. One characteristic sample was collected for every plant species from a particular site in a respective year. Minimum damage strategy of sampling were used whenever possible, digging a hole from the side and not destroying the individual. Plant roots together with soil were placed in sealed polyethylene bags and transported to the laboratory. Samples were stored in a refrigerator at 4 °C until analysis.

Root samples were prepared for analysis by boiling in 10% KOH and staining with trypan blue as described previously (Druva-Lusite et al. 2008). Samples were stored in lactoglycerol. Root fragments were mounted on glass slides and examined under a Nikon Eclipse E200 light microscope.

Photographs of mycorrhizal structures were taken by a digital camera. Mycorrhizal colonization (abundance of hyphae, vesicles and arbuscules) was estimated after Trouvelot et al. (1986) as described previously (Druva-Lusite et al. 2008).

Results

Relatively dry coastal habitats

In total, 15 plant species characteristic for relatively dry coastal habitats (dunes, dune forest, dry coastal meadow) were analyzed for the presence of mycorrhizal symbiosis in root samples.

Hyphae of AM fungus seen in root fragments of dune forest plant *Carex reichenbachii* were mostly linear as characteristic for the *Arum*-type AM (Fig. 1A). Vesicles were mainly irregularly-shaped. The presence of other fungal edophytes was evident in root cells of *Carex reichenbachii* (Fig. 1B). However, no arbuscules were present.

In root fragments of the dry coastal meadow species *Carex ligerica*, hyphae of AM fungus were linear, corresponding to the *Arum*-type (Fig. 2A, B). Vesicles were regularly-shaped (Fig. 2A, B). No arbuscules or other fungal structures were found in roots of *Carex ligerica*.

Relatively small coils were formed by hyphae in root fragments of the coastal dune forest species *Tofieldia calyculata* as characteristic for the *Paris*-type (Fig. 3A). Vesicles were mainly regularly-shaped. While there were

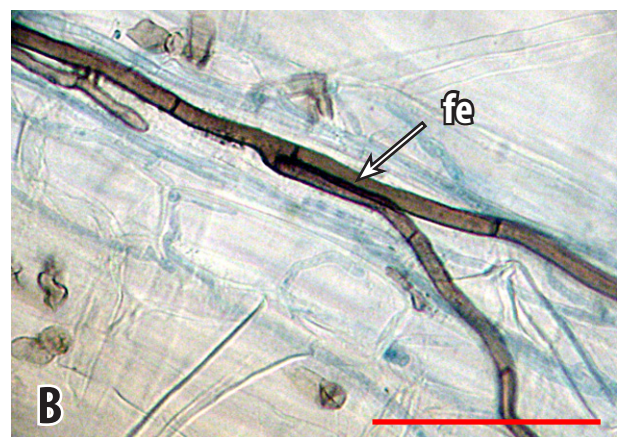
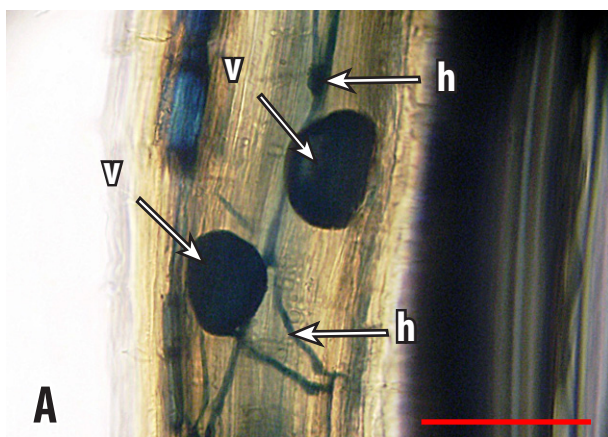


Fig. 1. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a dune forest plant *Carex reichenbachii*. h, hyphae; v, vesicles; fe, other fungal endophytes. Bar indicates 100 µm.

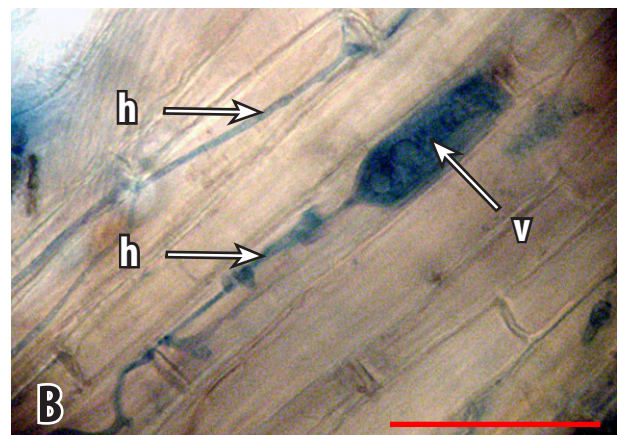
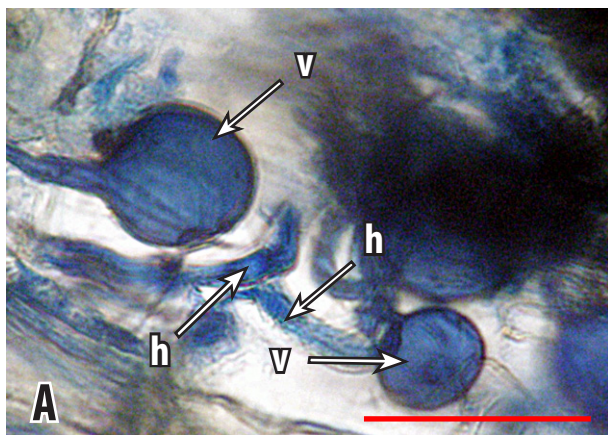


Fig. 2. Structures of arbuscular mycorrhiza in root fragments of a dry coastal meadow plant *Carex ligerica*. h, hyphae; v, vesicles. Bar indicates 100 µm.

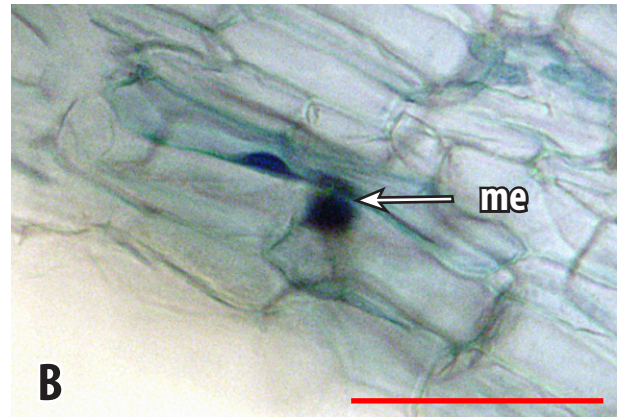
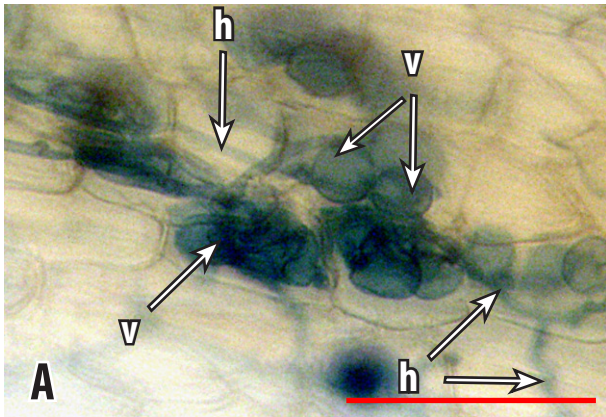


Fig. 3. Structures of arbuscular mycorrhiza and other mycorrhizal endophytes in root fragments of a dune forest plant *Tofieldia calyculata*. h, hyphae; v, vesicles; me, mycorrhizal endophytes. Bar indicates 100 μ m.

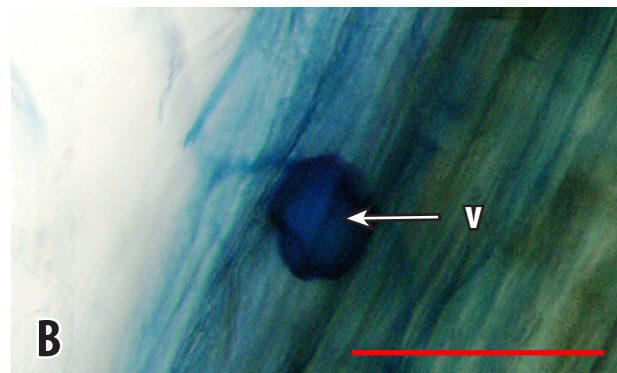
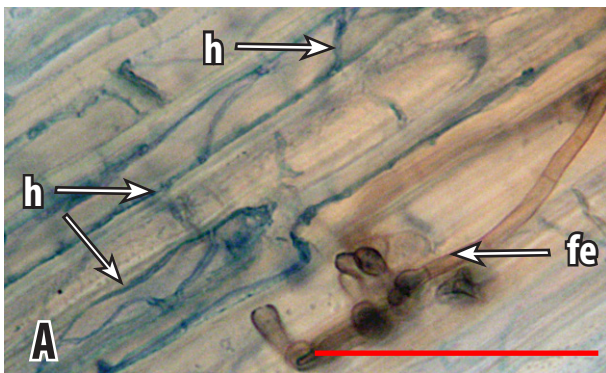


Fig. 4. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a dry coastal meadow plant *Phleum arenarium*. h, hyphae; v, vesicles; fe, other fungal endophytes. Bar indicates 100 μ m.

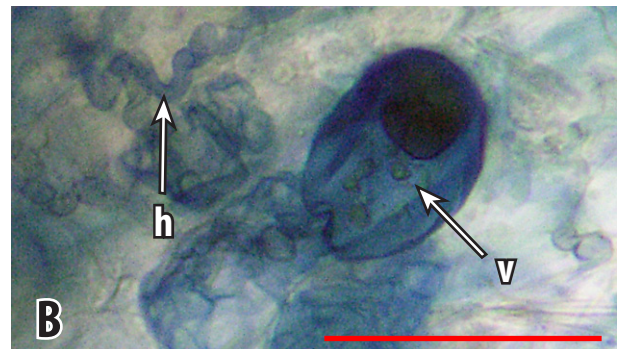
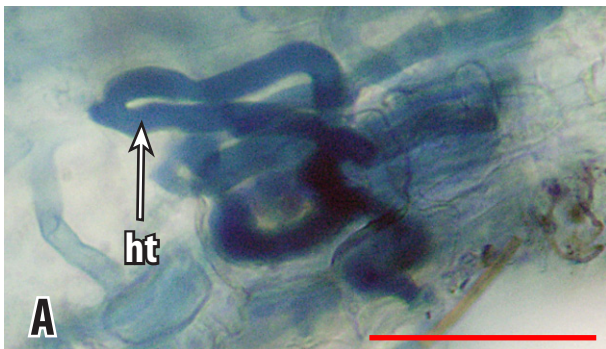


Fig. 5. Structures of arbuscular mycorrhiza in root fragments of a dune plant *Eryngium maritimum*. h, hyphae; ht, hyphal coils; v, vesicles. Bar indicates 100 μ m.

no arbusculae in root samples, structures of other fungal endophytes (similar to *Glomus tenue*) were clearly visible (Fig. 3B).

The dry coastal meadow plant *Phleum arenarium* had linear hyphae of AM fungi in root fragments, corresponding to *Arum*-type mycorrhizae (Fig. 4A). Vesicles were regularly-shaped (Fig. 4B). Structures of other fungal endophytes were visible, while no arbuscules were present (Fig. 4A).

In root fragments of coastal dune plant *Eryngium*

maritimum, after penetrating root cortical cells, AM fungi formed pronounced hyphal coils of the *Paris*-type (Fig. 5A). Both regularly- and irregularly-shaped vesicles were present with clearly pronounced lipid storage bodies (Fig. 5B). There were no arbuscules present in root fragments of *Eryngium maritimum*.

Swelling of external hyphae of AM fungi, followed by apresorium formation and subsequent penetration inside roots were seen on root fragments of the dune plant *Tragopogon heterospermus* (Fig. 6A). Linearity of hyphae

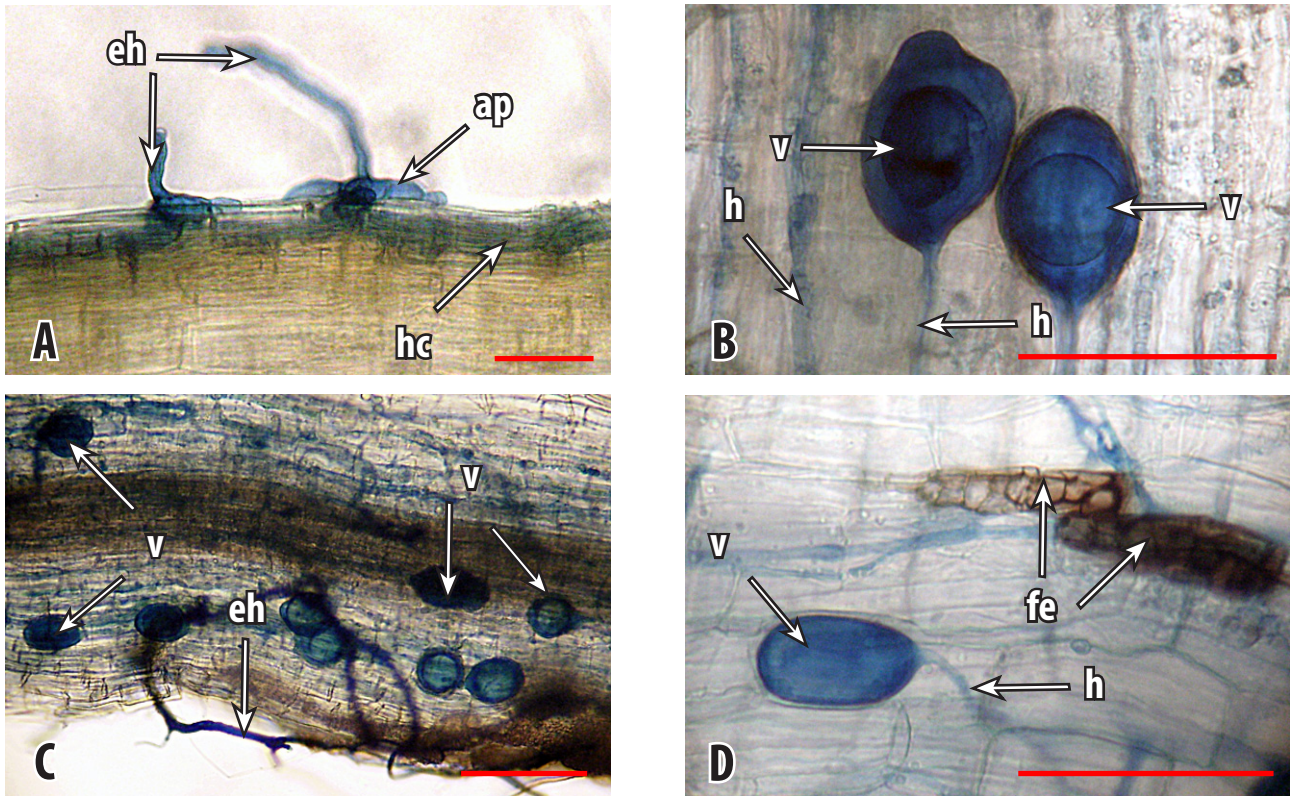


Fig. 6. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a dune plant *Tragopogon heterospermus*. h, hyphae; eh, external hyphae; hc, hyphal coils; v, vesicles; ap, aporesorium; fe, other fungal endophytes. Bar indicates 100 µm.

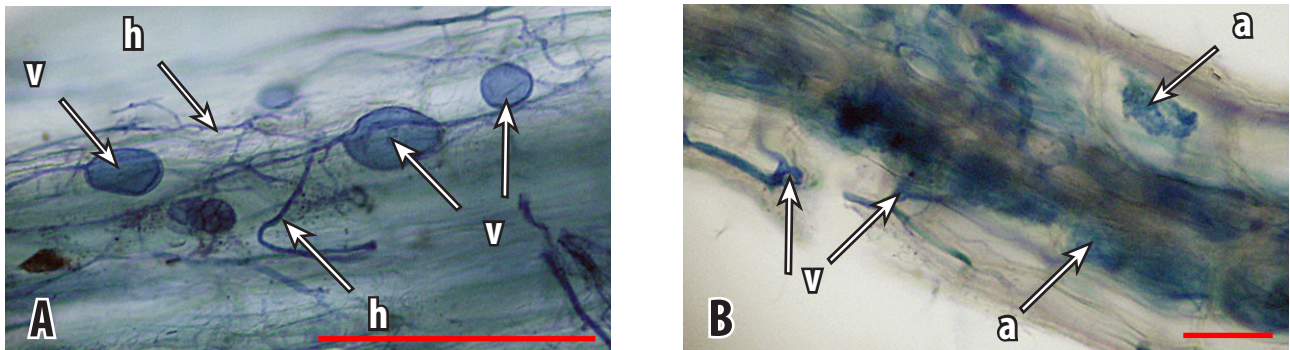


Fig. 7. Structures of arbuscular mycorrhiza in root fragments of a dune plant *Alyssum gmelinii*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 µm.

suggested they belong to the *Arum*-type AM. Vesicles were both regularly- and irregularly-shaped (Fig. 6B, C, D). Part of the vesicles contained storage lipid bodies (Fig. 6B). The presence of fungal endophytes was seen (Fig. 6D). However, no arbuscule formation was evident in roots of *Tragopogon heterospermus*.

Both *Arum*-type (linear hyphae) and *Paris*-type (intracellular structures, spreading from cell to cell) AM were observed in root fragments of the coastal dune plant *Alyssum gmelinii* (Fig. 7). Vesicles were both regular- as well as irregular-shaped. Arbuscules in roots had finely branched structure (Fig. 7B). No other fungal endophytes were present.

In root fragments of the dune and dry coastal meadow species *Gypsophila paniculata*, there were linear hyphae of AM fungi corresponding to the *Arum*-type (Fig. 8A). Both regularly- as well as irregularly-shaped vesicles were present. While no arbuscules were found in roots, the presence of other fungal endophytes was evident (Fig. 8B).

AM fungal hyphae in roots of the dune and dry coastal meadow plant *Silene borysthenica* formed relatively small coils, corresponding to the *Paris*-type AM (Fig. 9). Vesicles were both regularly- and irregularly-shaped. No arbuscules or other fungal endophyte structures were found in roots.

Hyphal coils characteristic for the *Paris*-type AM were evident in root fragments of the dune plant *Anthyllis*

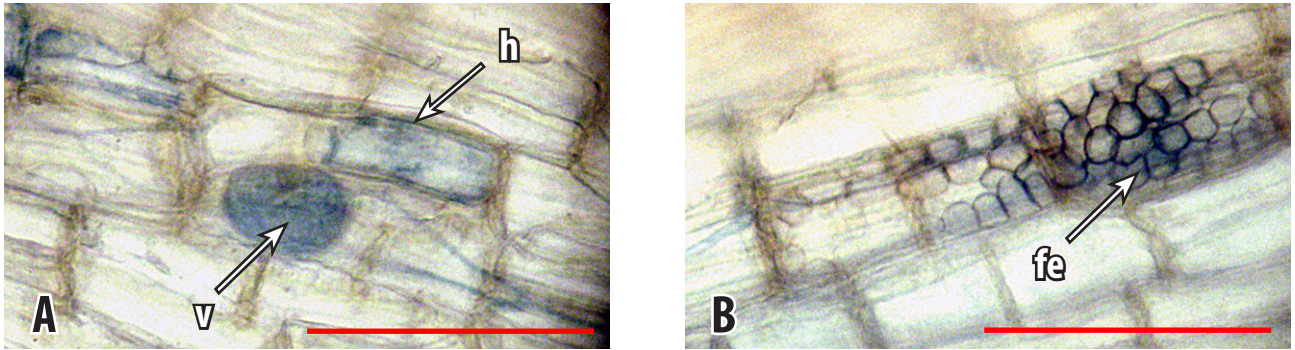


Fig. 8. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a dune and dry coastal meadow plant *Gypsophila paniculata*. h, hyphae; v, vesicles; fe, other fungal endophytes. Bar indicates 100 μ m.

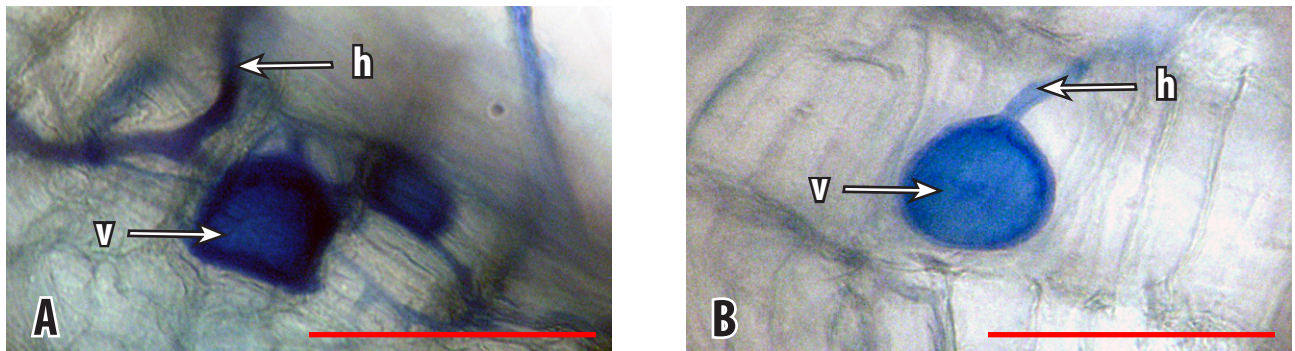


Fig. 9. Structures of arbuscular mycorrhiza in root fragments of a dune and dry coastal meadow plant *Silene borysthenica*. h, hyphae; v, vesicles. Bar indicates 100 μ m.

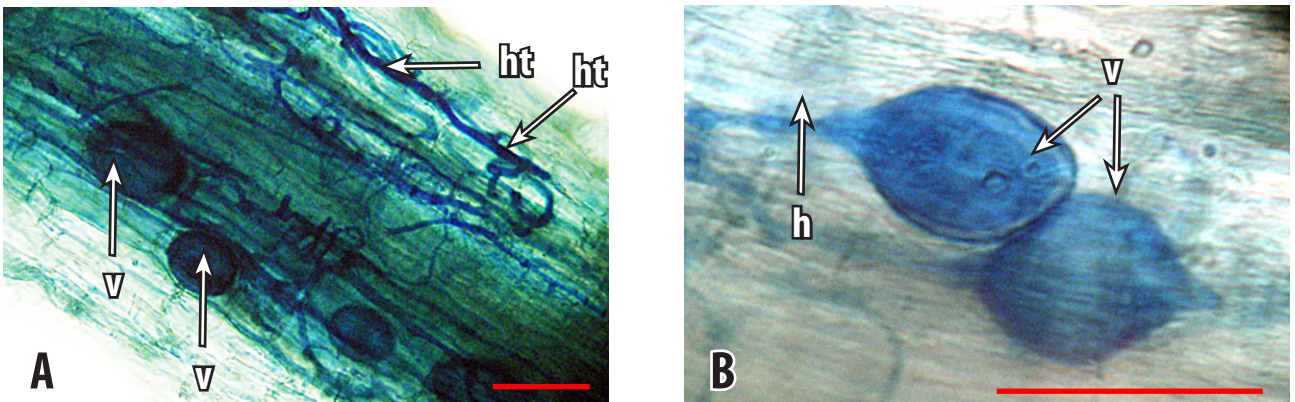


Fig. 10. Structures of arbuscular mycorrhiza in root fragments of a dune plant *Anthyllis maritima*. h, hyphae; ht, hyphal coils; v, vesicles. Bar indicates 100 μ m.

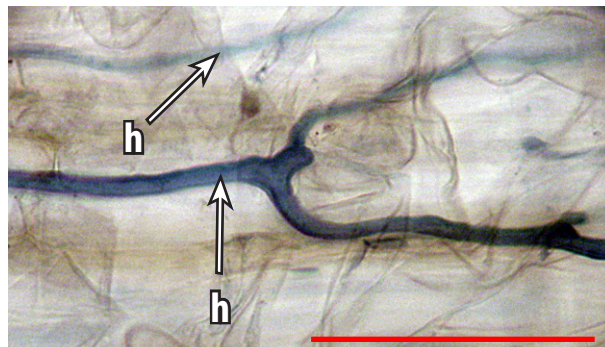


Fig. 11. Structures of arbuscular mycorrhiza in root fragments of a dune plant *Lathyrus maritimus*. h, hyphae. Bar indicates 100 μ m.

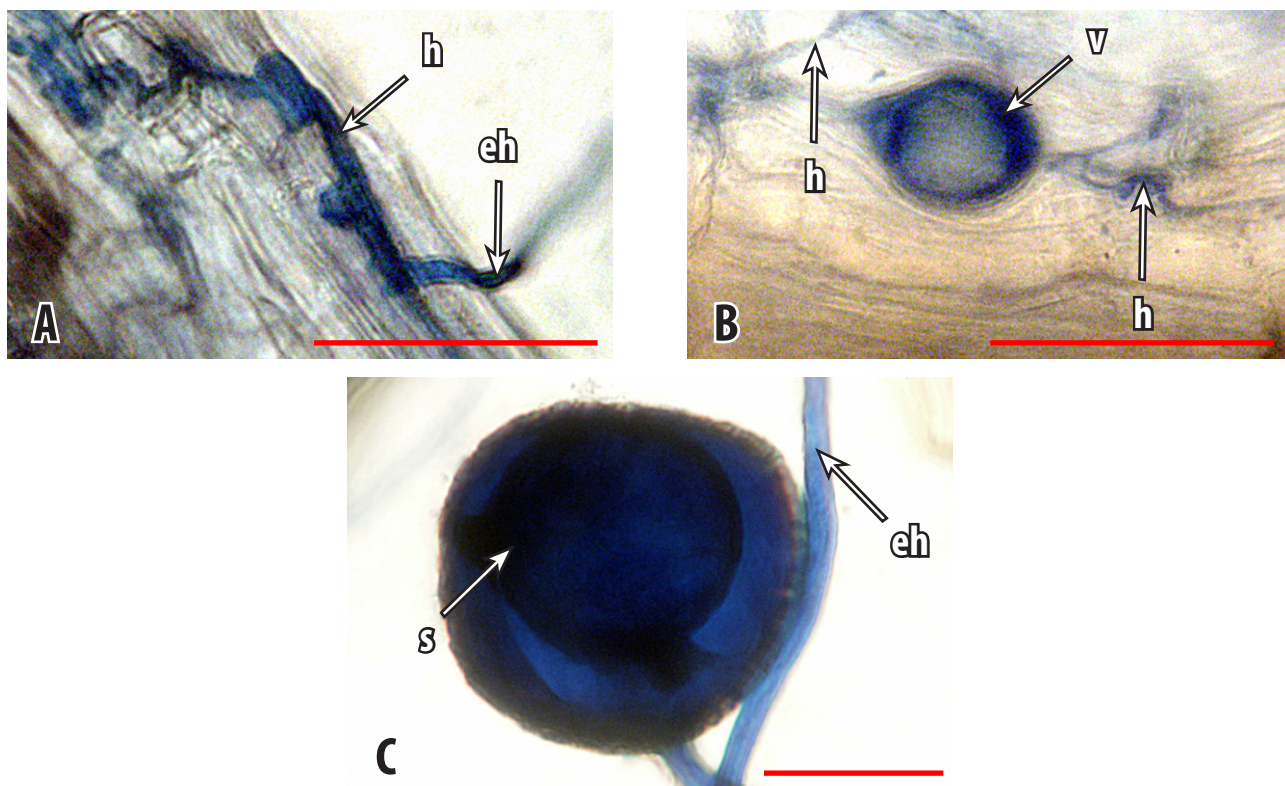


Fig. 12. Structures of arbuscular mycorrhiza in root fragments of a dune and dry coastal meadow plant *Centaurium littorale*. h, hyphae; eh, external hyphae; v, vesicles; s, spore. Bar indicates 100 μm.

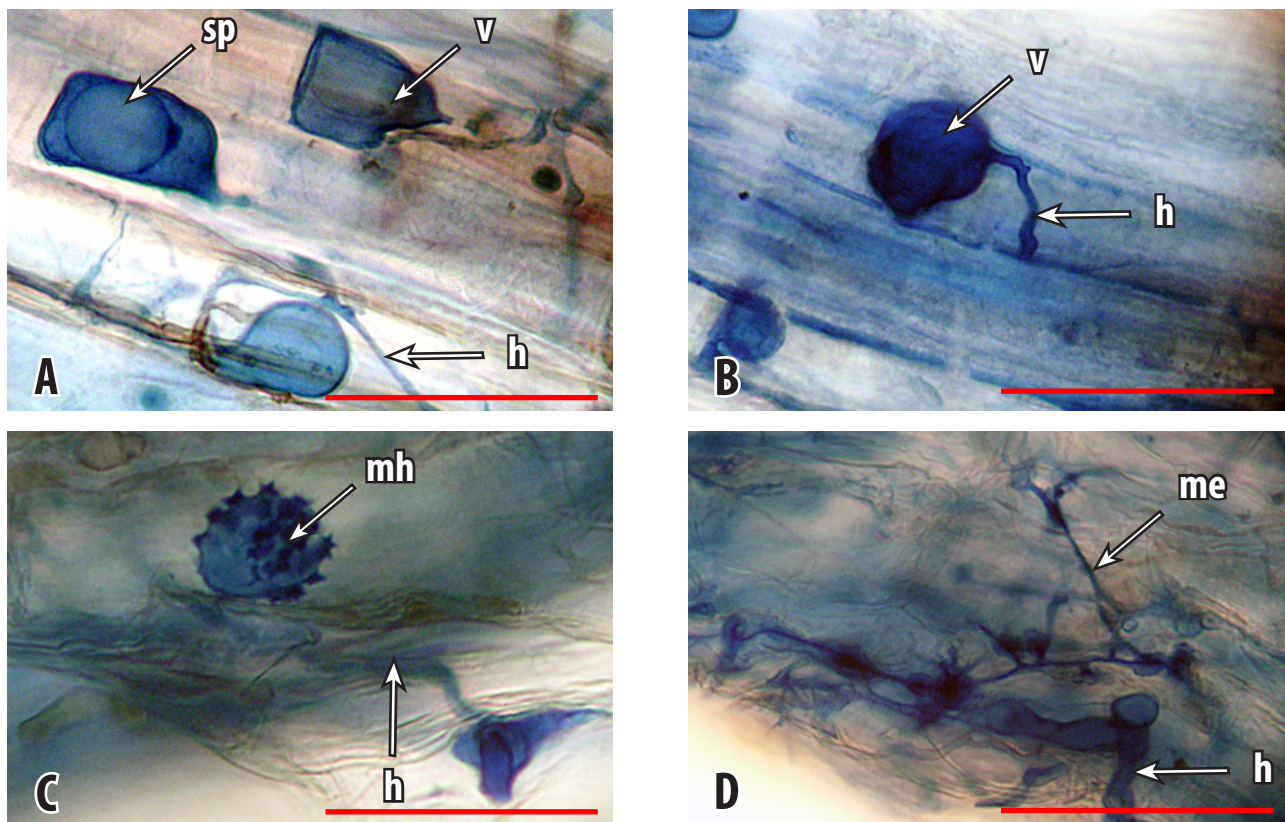


Fig. 13. Structures of arbuscular mycorrhiza and endophytes in root fragments of a dry coastal meadow plant *Geranium molle*. h, hyphae; v, vesicles; me, mycorrhizal endophytes; mh, mycorrhiza helper; sp, sporocarp. Bar indicates 100 μm.

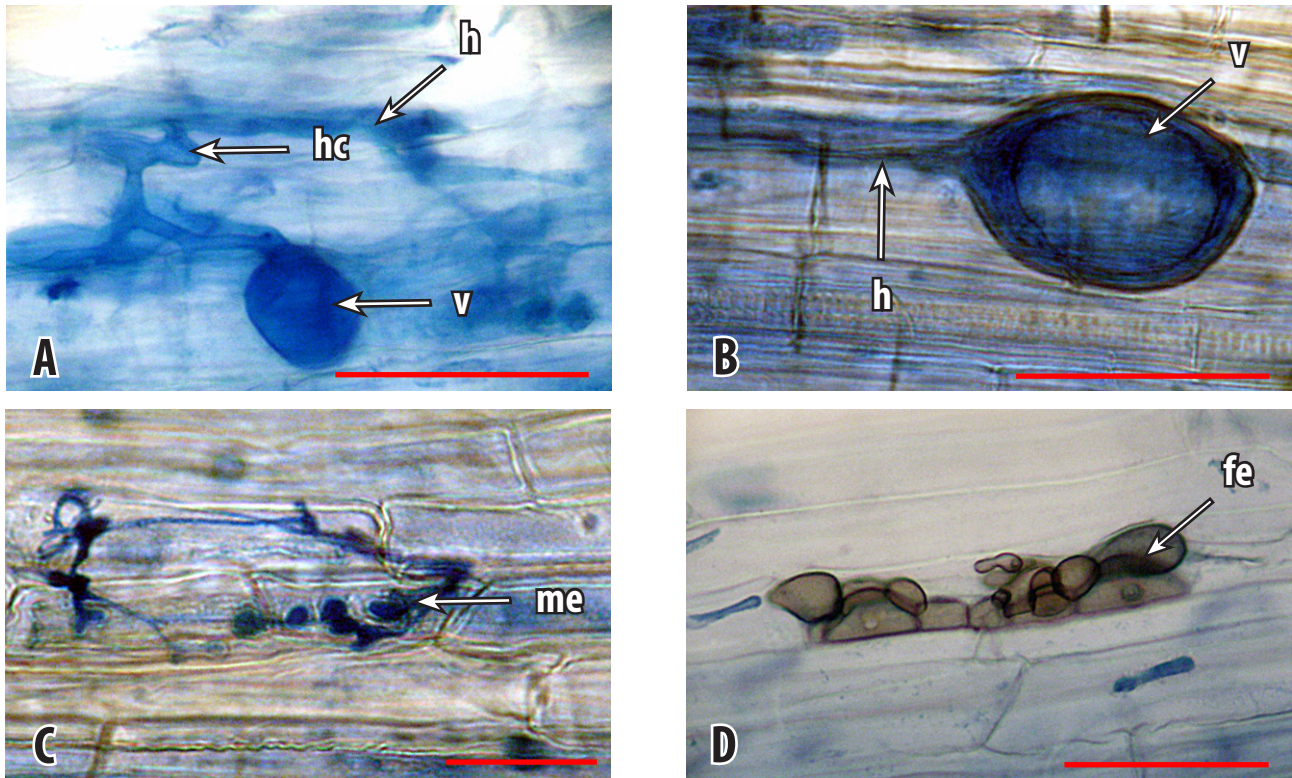


Fig. 14. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a dry coastal meadow plant *Ranunculus bulbosus*. h, hyphae; hc, hyphal coils; v, vesicles; me, mycorrhizal endophytes; fe, other fungal endophytes. Bar indicates 100 μ m.

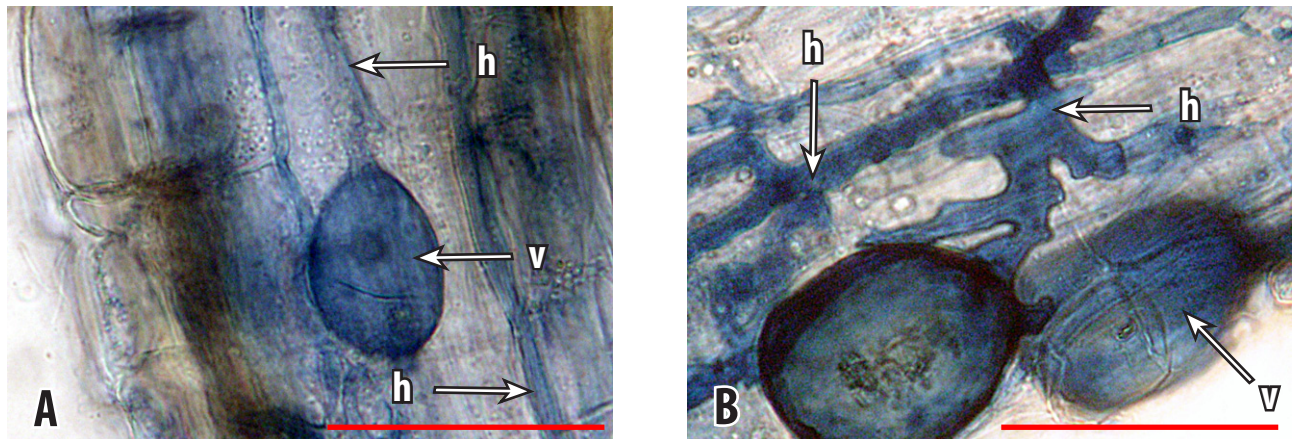


Fig. 15. Structures of arbuscular mycorrhiza in root fragments of a dune plant *Linaria loeselii*. h, hyphae; v, vesicles. Bar indicates 100 μ m.

maritima (Fig. 10A). Only regularly-shaped vesicles were present (Fig. 10B). No arbuscules or other fungal endophyte structures were found.

The dune plant *Lathyrus maritimus* possessed linear mycorrhizal hyphae in root fragments, corresponding to the *Arum*-type (Fig. 11). No other structures (vesicles, arbuscules, other fungal endophytes) were visible.

In root fragments of the dune and dry coastal meadow species *Centaureum litorale* fungal hyphae of AM after penetration in cortical tissues branched dichotomously and spread linearly, according to the *Arum*-type AM (Fig.

12A). Vesicles were regularly-shaped, with numerous lipid storage bodies (Fig. 12B). Formation of spores was evident (Fig. 12C). No arbuscules or other fungal endophyte structures were found in roots of *Centaureum litorale*.

In root fragments of *Geranium molle*, a species from a dry coastal meadow, hyphae of AM fungi formed coils characteristic for the *Paris*-type (Fig. 13). Vesicles were irregularly-shaped (Fig. 13A, B). Formation of spores within vesicles was evident in several root samples (Fig. 13A). Vesicles with storage lipid bodies were also found. Root fragments of *Geranium molle* also possessed specific

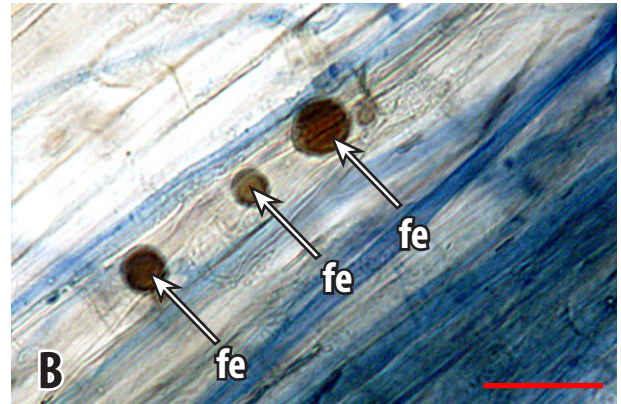
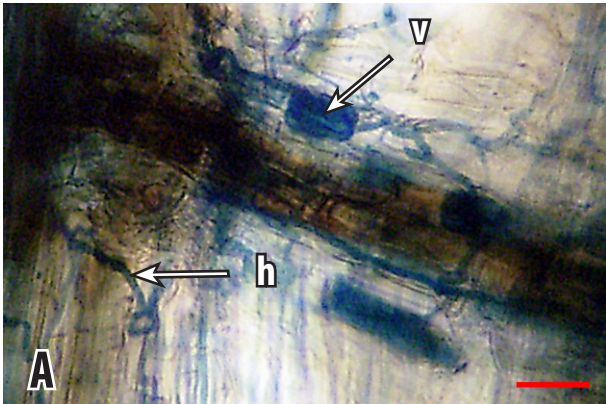


Fig. 16. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a beach plant *Bolboschoenus maritimus*. h, hyphae; v, vesicles; fe, other fungal endophytes. Bar indicates 100 μ m.

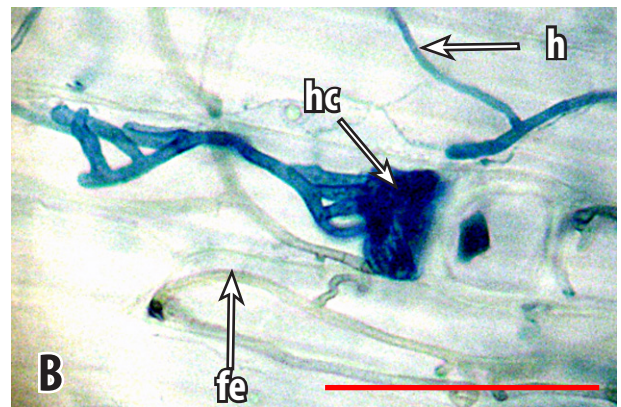
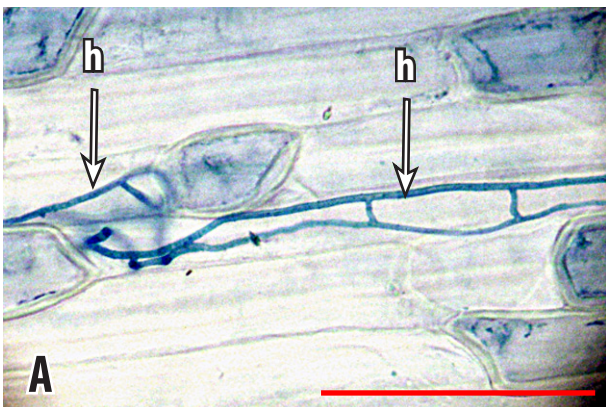


Fig. 17. Structures of arbuscular mycorrhiza and other fungal endophytes in root fragments of a salt marsh plant *Blysmus rufus*. h, hyphae; hc, hyphal coils; fe, other fungal endophytes. Bar indicates 100 μ m.

structures similar to the AM endophyte *Glomus tenue* (Fig. 13D). A characteristic feature of AM structures in roots of *Geranium molle* was formation of new hyphae from swelled or unswelled hyphae, with irregular hyphal expansions at the ends (Fig. 13D). In addition, formation of a vesicle-like body covered with special M-shaped thorns was evident (Fig. 13C). This structure possibly presented a AM helper body. No arbuscules were found in roots of *Geranium molle*.

AM fungal hyphae formed small hyphal coils characteristic for the *Paris*-type AM in root fragments of the dry coastal meadow plant *Ranunculus bulbosus* (Fig. 14A). Vesicles were regularly-shaped, with formation of lipid storage bodies in some of them (Fig. 14B). Structures like linear hyphae with uneven hyphal expansions, similar to those in the AM endophyte *Glomus tenue* were found in root samples of *Ranunculus bulbosus* (Fig. 14C). Structures of other fungal endophytes were also found (Fig. 14D), while no arbuscules were found.

AM fungal hyphae in root fragments of dune plant *Linaria loeselii* was linear, according to the *Arum*-type (Fig. 15A). Vesicles were regularly-shaped, with a relatively small number of storage lipid bodies (Fig. 15B). No arbuscules or other fungal endophyte structures were found in roots of *Linaria loeselii*.

Relatively wet coastal habitats

In total, 14 plant species, native to relatively wet coastal habitats (beach, wet coastal meadow, salt marsh), were analyzed for a presence of mycorrhizal symbiosis in root samples.

The beach species *Bolboschoenus maritimus* had AM fungal hyphae crossing cells linearly, corresponding to the *Arum*-type AM (Fig. 16). However, formation of small hyphal coils, as in the *Paris*-type, was also evident. Vesicles were irregularly-shaped (Fig. 16A). There were no arbuscules in root fragments of *Bolboschoenus maritimus*, while structures of other fungal endophytes were present (Fig. 16B).

Both linear hyphae and hyphal coils were seen in root fragments of the salt marsh plant *Blysmus rufus*, corresponding to both *Arum* and *Paris* types of AM (Fig. 17A, B). No arbuscules or vesicles were found. However, structures of other fungal endophytes were evident (Fig. 17B).

Arum-type linear hyphae of AM were found in root fragments of the beach species *Juncus balticus* (Fig. 18A). Both irregularly- and regularly-shaped vesicles were present, many of them with storage lipid bodies. Characteristic vesicle-like structures were seen in root tissues, covered

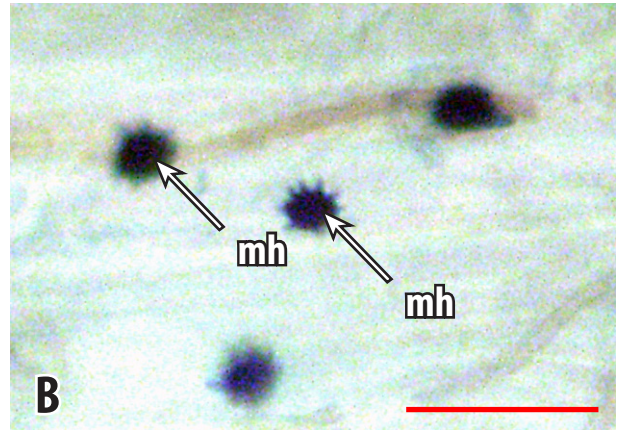
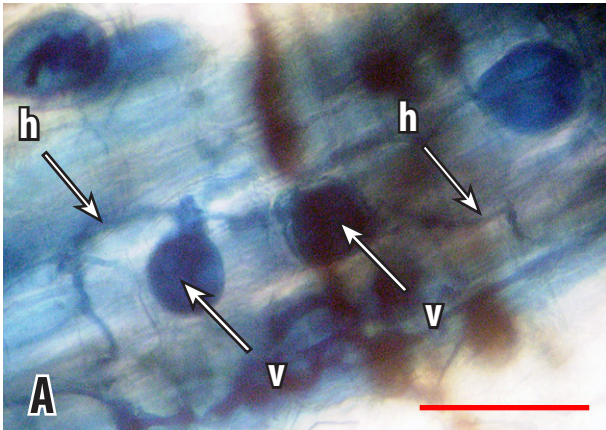


Fig. 18. Structures of arbuscular mycorrhiza in root fragments of a beach plant *Juncus balticus*. h, hyphae; v, vesicles; mh, mycorrhizal helper structures. Bar indicates 100 μ m.

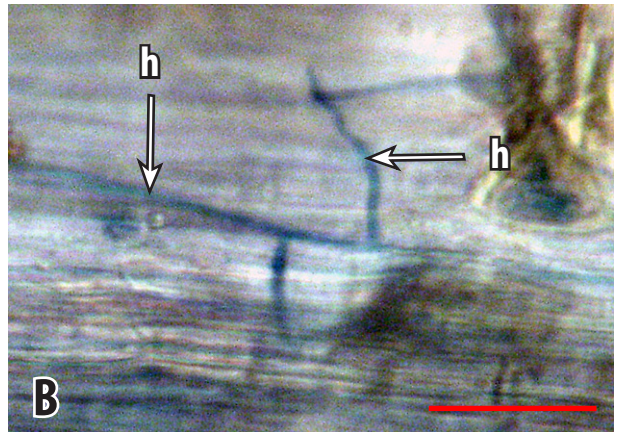
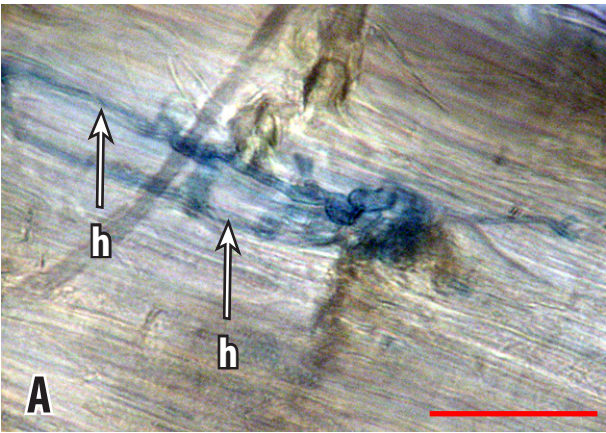


Fig. 19. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Juncus gerardii*. h, hyphae. Bar indicates 100 μ m.

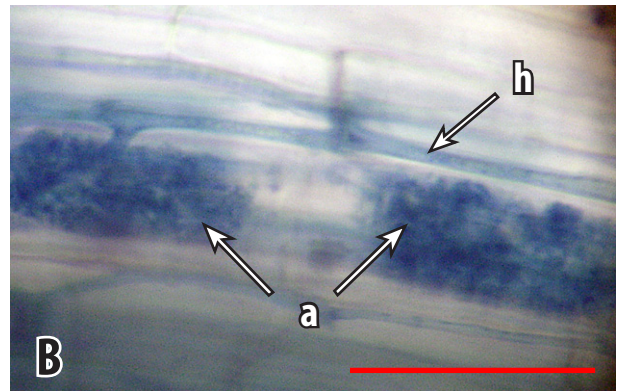
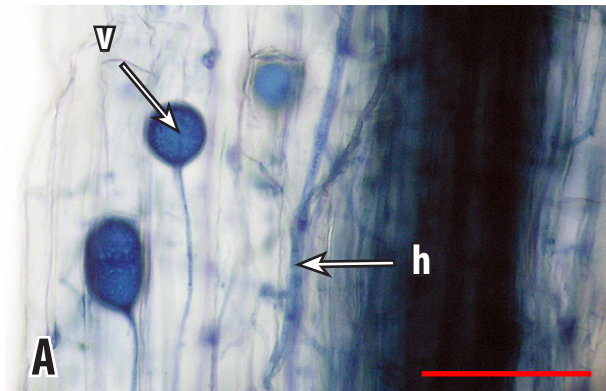


Fig. 20. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Triglochin maritimum*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 μ m.

with small thorn-shaped protrusions (Fig. 18B). These suggested putative mycorrhizal helper structures, although no connecting hyphae were evident. No arbuscules were found in root fragments of *Juncus balticus*.

Another species of the same genus, *Juncus gerardi*, a characteristic plant of salt marshes, had linearly distributed AM fungal hyphae, which corresponds to the *Arum*-type AM (Fig. 19). There were no vesicles or arbuscules present.

Linear, *Arum*-type fungal AM hyphae were found also in root fragments of another salt marsh species *Triglochin maritimum* (Fig. 20A). Vesicles were regularly-shaped, with included storage lipid bodies (Fig. 20A). Fine dichotomously-branched arbuscules were present in root cells (Fig. 20B).

The beach plant *Puccinellia capillaris* had linear AM fungal hyphae of the *Arum*-type in root fragments (Fig.

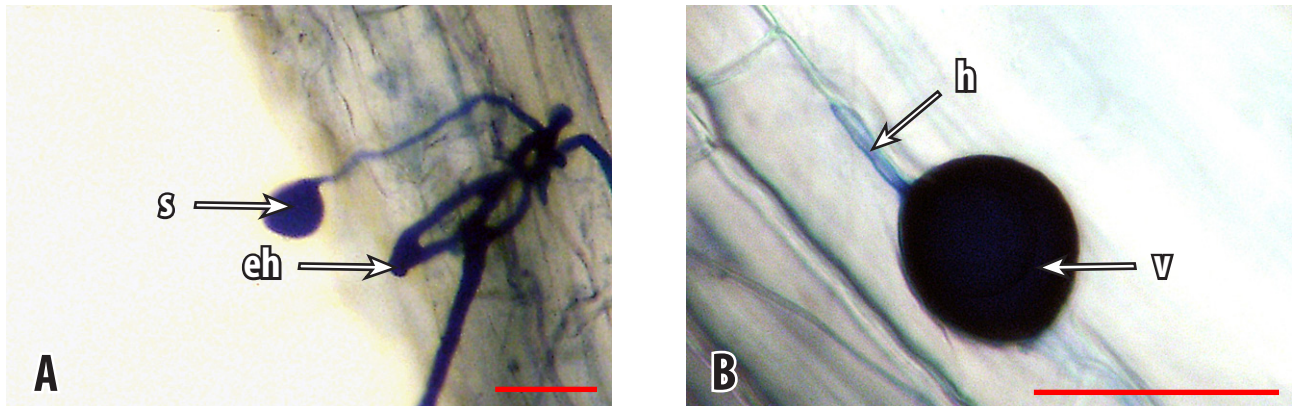


Fig. 21. Structures of arbuscular mycorrhiza in root fragments of a beach plant *Puccinellia capillaris*. h, hyphae; eh, external hyphae; v, vesicles; s, spore. Bar indicates 100 µm.

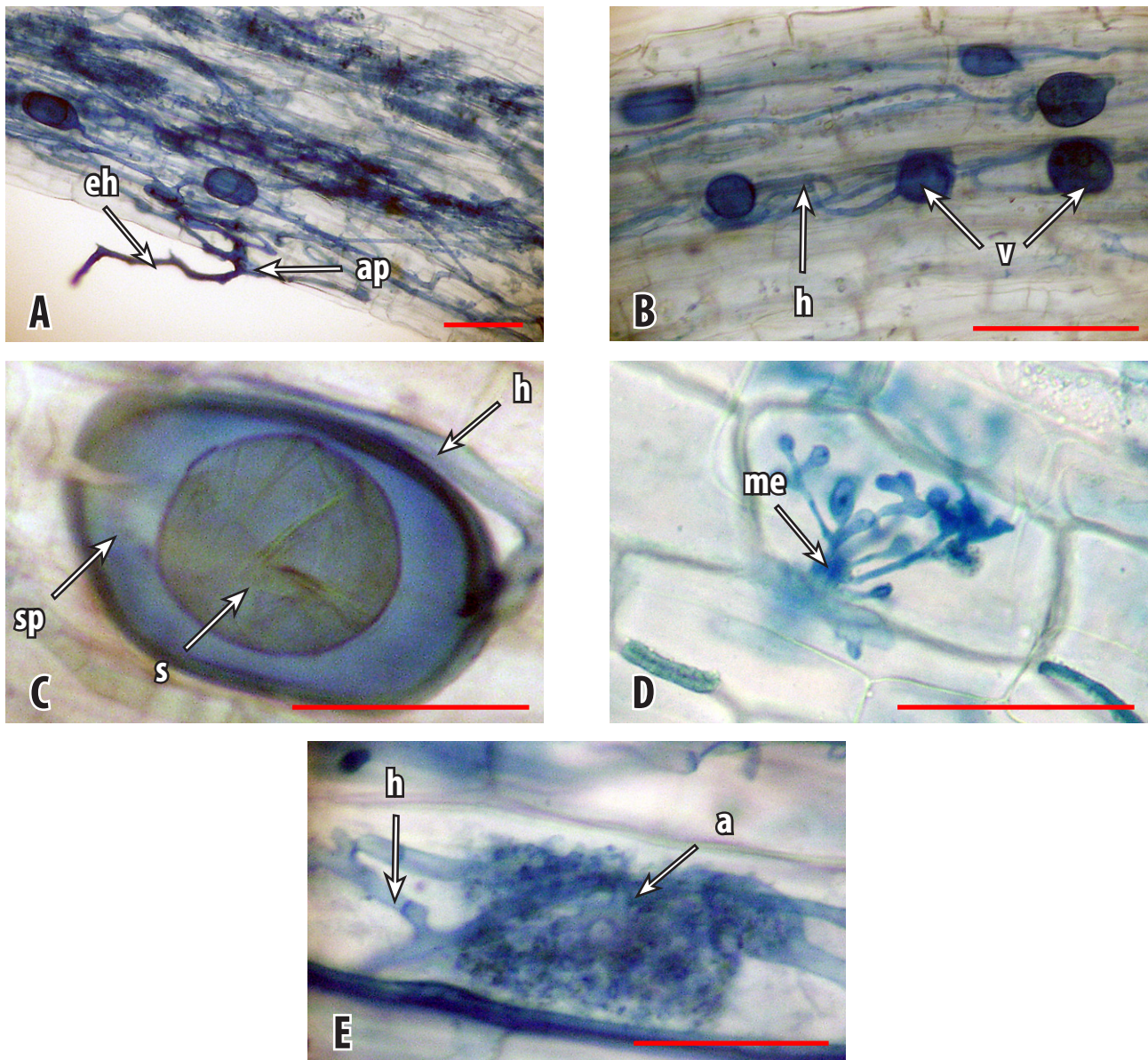


Fig. 22. Structures of arbuscular mycorrhiza in root fragments of a beach plant *Hydrocotyle vulgaris*. h, hyphae; eh, external hyphae; v, vesicles; ap, apresorium; a, arbuscules; s, spore; sp, sporocarp; me, mycorrhizal endophyte. Bar indicates 100 µm.

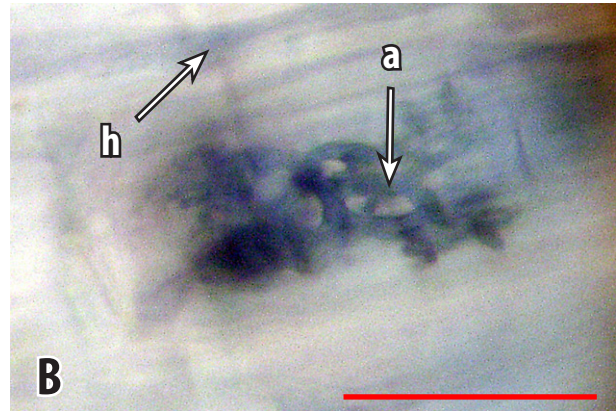
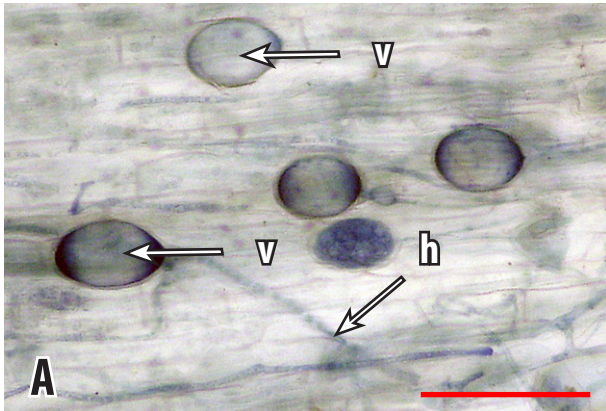


Fig. 23. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Aster tripolium*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 μ m.

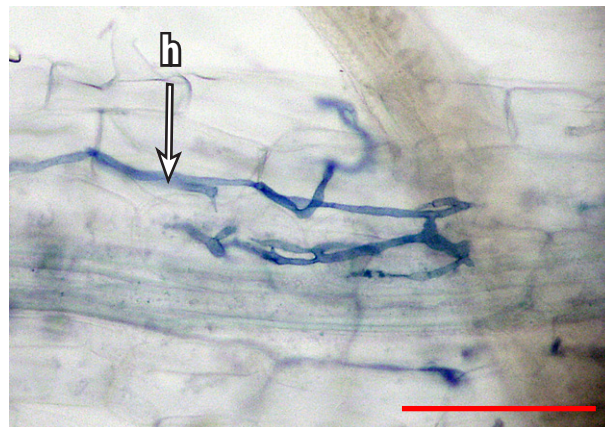


Fig. 24. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Spergularia salina*. h, hyphae. Bar indicates 100 μ m.

21A). Vesicles were both irregularly- and regularly-shaped (Fig. 21B). Formation of spores was evident in some vesicles (Fig. 21A).

In root fragments of a clonal plant from salt marshes, *Hydrocotyle vulgaris*, both linear hyphae as well as hyphal coils were found, corresponding to both *Arum* and *Paris* types of AM (Fig. 22 A, B). The internal hyphae formed aporesium and, after penetration into root cortex, spread within tissues by dichotomous branching (Fig. 22A). Vesicles were both irregularly- and regularly-shaped. Some vesicles had formed intercellular sporocarps, where spore formation occurred (Fig. 22 C). Arbuscules in root fragments of *Hydrocotyle vulgaris* were formed by relatively long and finely branched hyphae (Fig. 22E). In several root fragments deformed structures were present, formed from swelled hyphae (Fig. 22D). These structures had irregular hyphal expansions, similar to small vesicles. Each deformed structure was located within a single cell. More likely, the structures represented the AM endophyte *Glomus tenue* or were salinity-induced modifications of AM fungal hyphae.

Linear hyphae crossing tissues through extracellular space, as characteristic of the *Arum*-type AM, were found in root fragments of the salt marsh species *Aster tripolium*

(Fig. 23A). Vesicles were both irregularly- and regularly-shaped. Location of structural lipid bodies within vesicles were seen. Arbuscules in roots of *Aster tripolium* plants were relatively little branched (Fig. 23B).

Both linear hyphae and relatively small hyphal coils, characteristic for both *Arum* and *Paris* types of AM, were present in root fragments of the salt marsh plant *Spergularia salina* (Fig. 24). No other AM or fungal structures were evident.

In root fragments of the beach plant *Atriplex calotheca*, linear hyphae of AM fungi were present, corresponding to the *Arum*-type (Fig. 25). Both irregularly- and regularly-shaped vesicles were found, some of them contained high amounts of storage lipids, indicating possible spore formation (Fig. 25B). No arbuscules or other fungal structures were seen in roots of *Atriplex calotheca*.

Hyphae in root fragments of the wet coastal meadow plant *Euphorbia palustris* were both of *Arum* (linear hyphae) and *Paris* (hyphal coils) type (Fig. 26). Vesicles were irregularly-shaped. There were finely branched arbuscules in roots (Fig. 26B).

Linear fungal hyphae, characteristic for the *Arum*-type AM, were present in root fragments of the clonal species

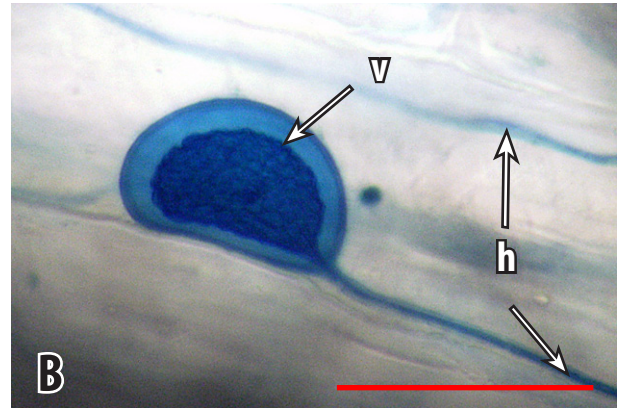
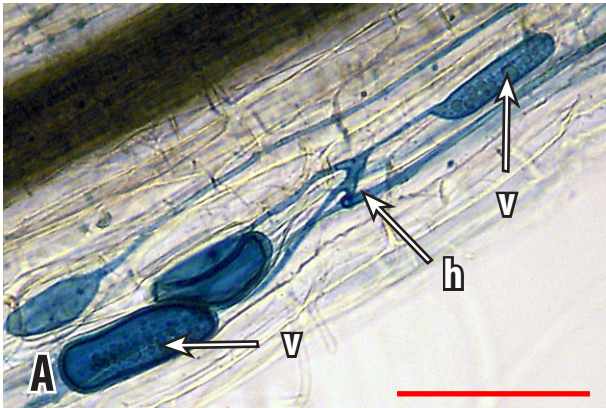


Fig. 25. Structures of arbuscular mycorrhiza in root fragments of a beach plant *Atriplex calotheca*. h, hyphae; v, vesicles. Bar indicates 100 μ m.

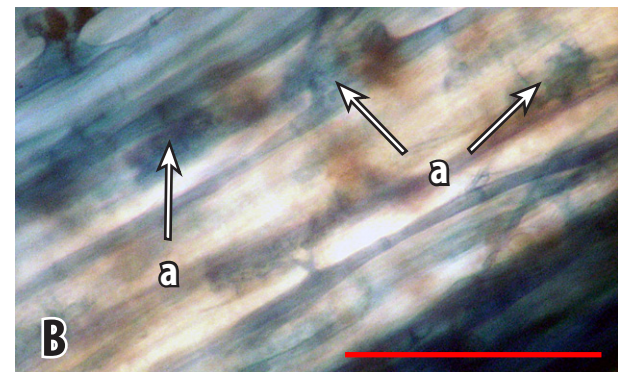
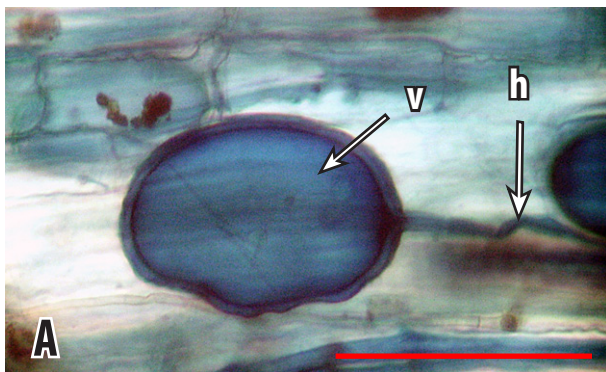


Fig. 26. Structures of arbuscular mycorrhiza in root fragments of a wet coastal meadow plant *Euphorbia palustris*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 μ m.

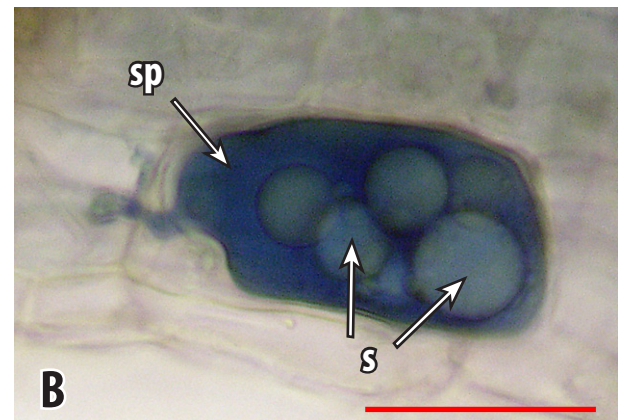
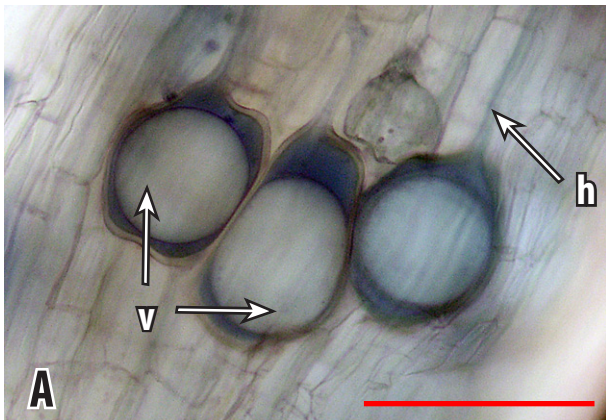


Fig. 27. Structures of arbuscular mycorrhiza in root fragments of a salt marsh and wet coastal meadow plant *Trifolium fragiferum*. h, hyphae; v, vesicles; s, spore; sp, sporocarp. Bar indicates 100 μ m.

Trifolium fragiferum from wet coastal meadows and salt marshes (Fig. 27A). Vesicles were irregularly-shaped, many with storage lipid bodies. Formation of intercellular sporocarps from vesicles with a number of new spores were seen (Fig. 27B). No arbuscules or other fungal structures were present in roots of *Trifolium fragiferum*.

Plantago maritima, a typical salt marsh plant, had linear hyphae of the *Arum*-type in root fragments (Fig.

28A). Vesicles were irregularly-shaped. In different root fragments morphologically various arbuscular structures were present. Some arbuscules were finely and abundantly branched (Fig. 28A), while others had relatively shorter branches (Fig. 28B).

AM fungal hyphae in root fragments of the salt marsh plant *Glaux maritima* were both linear (*Arum*-type AM, Fig. 29A) as well as forming coils (*Paris*-type AM; Fig.

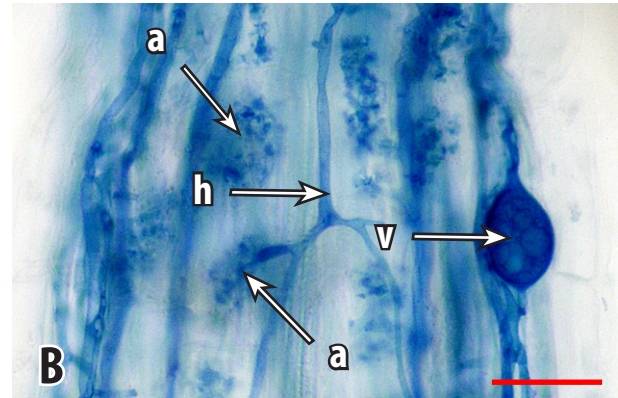
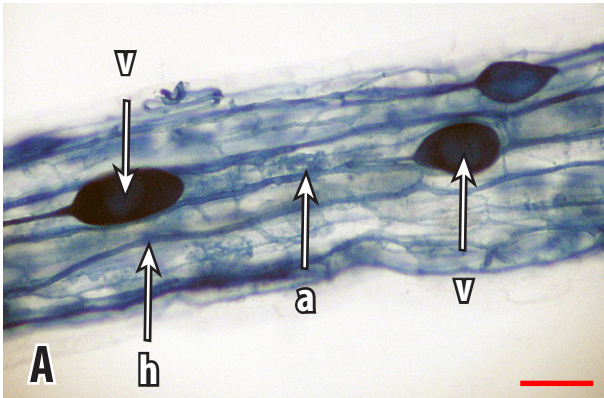


Fig. 28. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Plantago maritima*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 μ m.

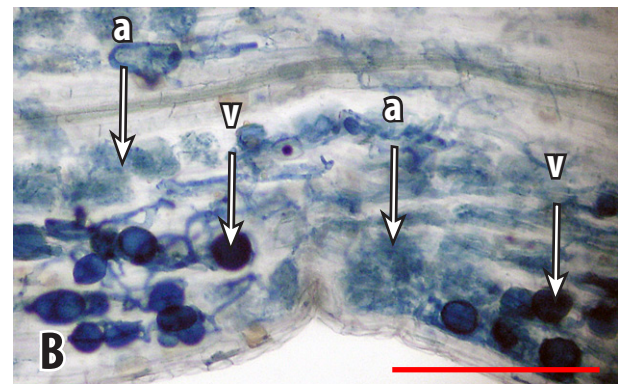
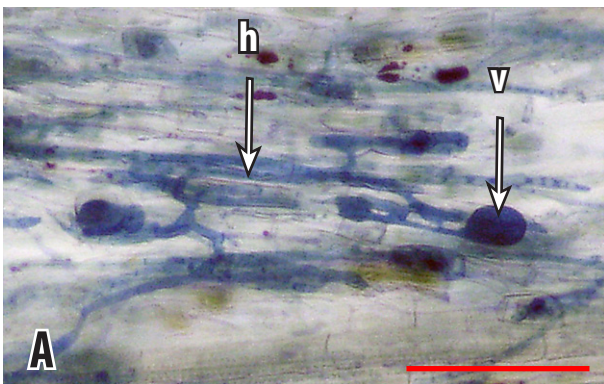


Fig. 29. Structures of arbuscular mycorrhiza in root fragments of a salt marsh plant *Glaux maritima*. h, hyphae; v, vesicles; a, arbuscules. Bar indicates 100 μ m.

29B). Vesicles were regularly-shaped, some with significant accumulation of storage lipids. Arbuscules in root tissues of *Glaux maritima* were formed by finely dichotomously branched hyphae (Fig. 29B).

Intensity of mycorrhizal symbiosis

In order to obtain an information on relative abundance of mycorrhizal symbiosis in roots of coastal plants native to different habitats, as well as to estimate the possible impact of seasonal environmental conditions, intensity of AM symbiosis in roots of 29 plant species from several locations were analyzed in two successive vegetation seasons (Fig. 30). Plants from relatively dry habitats (dunes, dune forest, dry coastal meadow) were characterized by a low average intensity of symbiosis. Ten species out of 15 had an intensity of mycorrhizal symbiosis of 10% or less. The highest intensity was evident in roots of the dune plant *Eryngium maritimum* and dry coastal meadow plants *Geranium molle*, *Phelum arenarium* and *Ranunculus bulbosus*. The majority of species from relatively dry coastal habitats had little variation in the intensity of mycorrhizal symbiosis between vegetation seasons and different sites, usually not exceeding natural biological variability (Fig. 30A). However, for *Ranunculus bulbosus* and *Tragopogon*

heterospermus the differences in intensity between samples were more than 50%.

In contrast, several plant species (*Aster tripolium*, *Atriplex calotheca*, *Glaux maritima*, *Hydrocotyle vulgaris*, *Juncus balticus*, *Trifolium fragiferum*) from relatively wet coastal habitats (sandy beach, wet coastal meadow, salt marsh) showed significant variation in intensity of mycorrhizal symbiosis between vegetation seasons and at different locations (Fig. 30B). The differences probably reflected higher environmental heterogeneity in relatively wet coastal habitats, possibly related to water regime and fluctuations in soil salinity.

Discussion

Coastal dunes are relatively nutrient-poor habitats, and many plants growing there have nutrient and water conservation strategies as well as adaptations favouring nutrient uptake, mycorrhizal symbiosis being one of them (Logan et al. 1989). In contrast, salt marsh habitats along a sea coast are considered more nutrient-rich. However, abiotic environmental conditions (periodic soil flooding and high salinity) are possible constraints for successful development of mycorrhizal symbiosis (Carvalho et al.

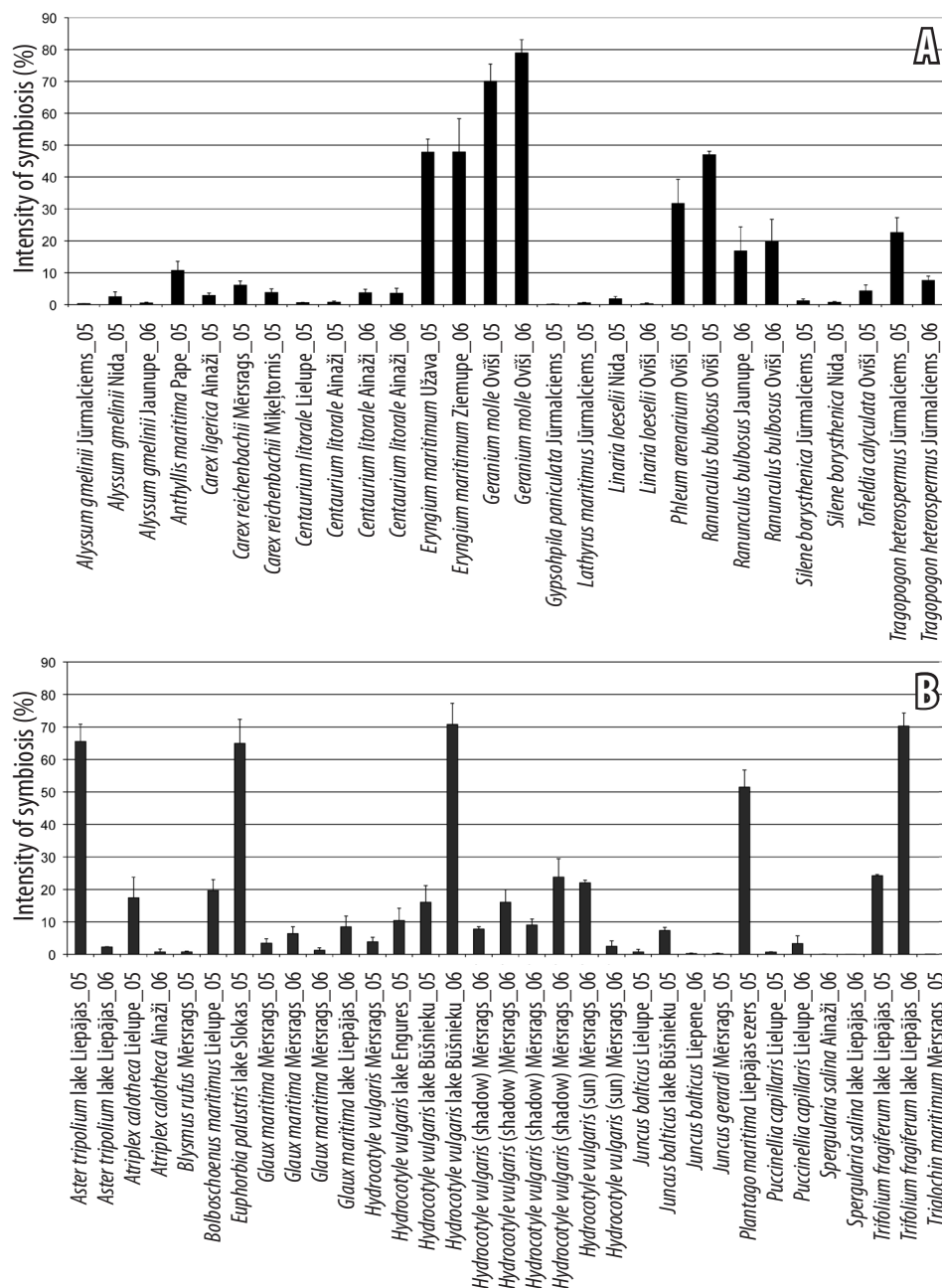


Fig. 30. Intensity of mycorrhizal symbiosis in root fragments of plants from relatively dry (A) and relatively wet (B) coastal habitats within two successive vegetation seasons (2005 and 2006). Data are means from three independent measurements for every species at a particular site $\pm$ SE.

2001; Hildebrandt et al. 2001).

All plant species in relatively wet coastal habitats had *Arum*-type AM symbiosis. Several of them (*Bolboschoenus maritimus*, *Blysmus rufus*, *Euphorbia palustris*, *Glaux maritima*, *Hydrocotyle vulgaris*, and *Spergularia salina*) in addition possessed also *Paris*-type AM, with formation of pronounced hyphal coils. In contrast, in plant roots from relatively dry coastal habitats, both AM types were found only in the dune plant *Alyssum gmelinii*. While it is generally accepted that host plant identity affects respective

morphology of AM symbiosis (Ahlu et al. 2005), it seems that in conditions of highly heterogeneous habitats (e.a., salt marsh), environmental constraints can affect the morphology of symbiosis. Also, the effect of the fungal genome on morphology of AM due to changes in fungal component can not be ruled out.

All analyzed plant species from both relatively dry and wet coastal habitats showed AM fungal structures in their roots. The majority of studied coastal plant species showed characteristic AM structures (vesicles, arbuscules, hyphal

coils) in root samples, suggesting a functionally active state of the symbiosis. Only two plants, the salt marsh species *Juncus gerardi* and dune species *Lathyrus maritimus*, had only linear hyphae without formation of hyphal coils or any other AM structures. Two salt marsh species (*Blysmus rufus* and *Spergularia salina*) possessed both linear hyphae and hyphal coils but no other AM structures. Consequently, AM symbiosis is abundant among coastal plant species, indicating to the universal role of the symbiosis in heterogeneous coastal habitats.

In the present study, several plant species from nonmycorrhizal families or even considered nonmycorrhizal by themselves, were found to have AM structures in their roots. Root fragments of *Bolboschoenus maritimus* and *Silene borysthena* had hyphal coils and vesicles, *Phleum arenarium* and *Trifolium fragiferum* had vesicles, and *Alyssum gmelinii* had hyphal coils, vesicles and arbuscules, suggesting functionally active symbiotic interactions.

Several salt marsh species, considered only occasionally mycorrhizal, e.g., *Spergularia salina* and *Triglochin maritimum*, showed functional AM structures in the present study. No mycorrhizal structures were found in roots of *Spergularia salina* from an inland salt marsh near Kraków, Poland, with a soil EC ranging from 10.6 to 31.2 dS m⁻¹ (Grzybowska 2004). However, individual plants of *Spergularia salina* showed a distinct colonization ranging from 2 to 19% in a number of Central European salt marshes with soil EC around 11.5 dS m⁻¹ (Hildebrandt et al. 2001). In the present study, both linear hyphae and hyphal coils were found in roots of *Spergularia salina* with very low intensity (less than 1%). However, when soil salinity in the salt marsh decreased from 20 dS m⁻¹ to below 7 dS m⁻¹, intensity of mycorrhizal colonization in roots of *Spergularia salina* sharply increased up to 65% (Druva-Lusite et al., unpublished data). Consequently, the fungal component of symbiosis is less tolerant to soil salinity in comparison to the host plant, leading to inhibition of mycorrhizal development at a range of soil salinity at which *Spergularia salina* frequently grows.

Another typical halophytic species, considered non-mycorrhizal, is *Triglochin maritimum*. Together with another typical non-mycorrhizal plant, *Juncus gerardi*, *Triglochin maritimum* from several Central European salt marshes did not show any indication of AM colonization (Hildebrandt et al. 2001). In the present study, linear hyphae, vesicles with storage lipid inclusions, as well as arbuscules were found, indicating presence of functionally active mycorrhizal symbiosis. Intensity of the symbiosis was, however, at a low level. Similarly, *Juncus gerardi* showed presence only of linear intercellular hyphae with a low intensity of symbiosis (less than 1%). However, similar to *Spergularia salina*, mycorrhizal intensity in roots of *Triglochin maritimum* increased up to 30% in conditions of decreased soil salinity (Druva-Lusite et al., unpublished data). Moreover, intensity of arbuscule distribution in root

fragments increased up to 24% at the end of the vegetation season.

The presented data clearly show that in spite of sometimes low intensity of mycorrhization, roots of all coastal plants studied possess functional structures of AM symbiosis. It can be suggested that fluctuating and low intensity of mycorrhizal symbiosis is caused by unfavourable environmental conditions within the vegetation season. The effect can vary according to the AM fungus-host plant combination. As was shown in studies with *Aster tripolium*, AM colonization is more affected by salinity than by flooding (Carvalho et al. 2003). Consequently, for salt marsh plants, increased soil salinity can be suggested as one of the main environmental factors leading to decreased intensity of AM symbiosis.

References

- Beena K.R., Arun A.B., Raviraja N.S., Sridhar K.R. 2001. Association of arbuscular mycorrhizal fungi with plants of coastal sand dunes of west coast of India. *Trop. Ecol.* 42: 213–222.
- Brundrett M. 1991. Mycorrhizas in natural ecosystems. In: Begon M., Fitter A.H., Macfayden A. (eds) *Advances in Ecological Research*, Vol. 21. P. 171–313.
- Brundrett M.C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320: 37–77.
- Brundrett M.C., Kendrick W.B. 1990. The roots and mycorrhizae of herbaceous woodland plants. II. Structural aspects of morphology. *New Phytol.* 114: 457–479.
- Çakan H., Karataş Ç. 2006. Interactions between mycorrhizal colonization and plant life forms along the successional gradient of coastal sand dunes in the eastern Mediterranean, Turkey. *Ecol. Res.* 21: 301–310.
- Carvalho LM, Caçador I, Martins-Loução MA 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza* 11: 303–309.
- Carvalho L.M., Correia P.M., Caçador I., Martins-Loução M.A. 2003. Effects of salinity and flooding on the infectivity of salt marsh arbuscular mycorrhizal fungi in *Aster tripolium* L. *Biol. Fertil. Soils* 38: 137–143.
- Druva-Lūsīte I., Karlsons A., Osvalde A., Nečajeva J., Ievinsh G. 2008. Photosynthetic performance and mycorrhizal symbiosis of a coastal marsh plant, *Glaux maritima*, in conditions of fluctuating soil salinity. *Acta Univ. Latv.* 745: 155–164.
- Gange A.C., Ayres R.L. 1999. On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. *Oikos* 87: 615–621.
- Glenn M.G., Shew F.S., Williams P.H. 1985. Hyphal penetration of Brassica (Cruciferae) roots by a vesicular-arbuscular mycorrhizal fungus. *New Phytol.* 99: 463–472.
- Gray A.J., Scott R. 1977. Biological flora of the British Isles. *Puccinellia maritima* (Huds) Parl. *J. Ecol.* 65: 699–716.
- Grzybowska B. 2004. Arbuscular mycorrhiza of herbs colonizing a salt affected area near Kraków (Poland). *Acta Soc. Bot. Polon.* 73: 247–253.

- Harley J.L., Harley E.L. 1987. A check-list of mycorrhiza in the British flora. *New Phytol.* 106: 1–102.
- Hildebrandt U., Janetta K., Ouziad F., Renne B., Nawrath K., Bothe H. 2001. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. *Mycorrhiza* 10: 175–183.
- Hoefnagels M.H., Broome S.W., Shafer S.R. 1993. Vesicular-arbuscular mycorrhizae in salt marshes in North Carolina. *Estuaries* 16: 851–858.
- Koske R.E., Halvorson W.L. 1981. Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Can. J. Bot.* 59: 1413–1422.
- Kubota M., McGonigle T.P., Hyakumachi M. 2005. Co-occurrence of *Arum*- and *Paris*-type morphologies of arbuscular mycorrhizae in cucumber and tomato. *Mycorrhiza* 15: 73–77.
- Little L.R., Maun A. 1997. Relationship among plant-parasitic nematodes, mycorrhizal fungi and the dominant vegetation of a sand dune system. *Ecoscience* 4: 67–74.
- Logan V.S., Clarke P.J., Allaway W.G. 1989. Mycorrhizas and root attributes of plants of coastal sand-dunes of New South Wales. *Aust. J. Plant Physiol.* 16: 141–146.
- Mason E. 1928. Note on the presence of mycorrhiza in the roots of salt marsh plants. *New Phytol.* 27: 193–195.
- Newman E.I., Reddell P. 1987. The distribution of mycorrhizas among families of vascular plants. *New Phytol.* 106: 745–751.
- Pawlowska T.E., Blaszkowski J., Rühling A. 1996. The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza* 6: 499–505.
- Smith F.A., Smith S.E. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytol.* 137: 373–388.
- Trouvelot A., Kough J.L., Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V., Gianinazzi S. (eds) *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press, Paris, pp. 217–221.
- Wilson G.W.T., Hartnett D.C. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *Am. J. Bot.* 85: 1732–1738.