

DOI: 10.1002/iroh.200510827

WANG KAI<sup>1</sup> and ZHAO ZHIWEI<sup>\*,2</sup>

<sup>1</sup>Key Laboratory for Conservation and Utilization of Bio-resources, Yunnan University Kunming, 650091 P.R. China, e-mail: wangkai0535@yahoo.com.cn

<sup>2</sup>Key Laboratory for Conservation and Utilization of Bio-resources, Yunnan University Kunming, 650091 P.R. China, e-mail: zhaozhw@ynu.edu.cn

## Occurrence of Arbuscular Mycorrhizas and Dark Septate Endophytes in Hydrophytes from Lakes and Streams in Southwest China

*key words:* arbuscular mycorrhiza, dark septate endophytes, hydrophytes

### Abstract

In this study, the colonization of arbuscular mycorrhizas (AM) and dark septate endophytes (DSE) in 140 specimens of 32 hydrophytes collected from four lakes and four streams in southwest China were investigated. The arbuscular mycorrhizal fungi (AMF) and DSE colonization in these hydrophytes were rare. Typical AM structures were observed in one of the 25 hydrophytic species collected in lakes and six of the 17 species collected in streams.

Spores of 10 identified AMF species and an unidentified *Acaulospora* sp. were isolated from the sediments. The identified AMF came from the four genera, *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. *Glomus* and *G. mosseae* were the dominant genus and species respectively in these aquatic environments.

The presence of DSE in hydrophytes was recorded for the first time. DSE occurred in one of the 25 hydrophyte species collected in lakes and three of the 17 species collected in streams.

### 1. Introduction

Arbuscular mycorrhiza (AM) association is a relatively ubiquitous, long lasting, highly compatible mutualism from which both partners derive benefit in terrestrial ecosystems. It occurs in about 83% of the dicotyledonous and 79% of the monocotyledonous species tested in terrestrial plants (TRAPPE, 1987). In aquatic plants, AM was first documented by SØNDERGAARD and LAEGAARD (1977). Subsequently, the occurrences of AM in hydrophytes in different areas were reported (BAGYARAJ *et al.*, 1979; CHAUBAL *et al.*, 1982; CLAYTON and BAGYARAJ, 1984; FARMER, 1985; FARMER, 1988; RAGUPATHY *et al.*, 1990; KHAN, 1993; BECK-NIELSEN and MADSEN, 2001).

Aquatic plants are essential in aquatic ecosystems and provide oxygen, food, shelter, and protect shorelines and stream banks from eroding. The association between AMF and aquatic plants may mediate coexistence of aquatic plant species and keep balance of the hydrophytes community, as in terrestrial ecosystems (HART *et al.*, 2003). Thus AM may be an ecologically and functionally significant factor, which may also be important in the maintaining and restoring processes of aquatic ecosystems.

Dark septate endophytes (DSE) refer to a miscellaneous group of ascomycetous and anamorphic fungi. Once DSE colonized the roots of the plants, they form characterized inter-

\* Corresponding author

and intracellular structures including a superficial net of hyphae, penetration into the cortical layer, microsclerotia and, occasionally, a partial mantle (JUMPPONEN and TRAPPE, 1998). DSE can be easily distinguished from AM fungal hyphae by their dark red-brown to dark brown colour, thicker lateral wall and frequent septa. So far, DSE have been found in many terrestrial ecosystems such as arctic (JUMPPONEN and TRAPPE, 1998), alpine environments (READ and HASELWANDTER, 1981), acidic organic soils (WURZBURGER and BLEDSOE, 2001), neotropical cloud forests (RAINS *et al.*, 2003), peat bogs and fen meadows (FUCHS and HASELEANDTER, 2004). Whereas, little has been documented about the occurrence of DSE in hydrophytes.

Collectively, many investigations on AM of hydrophytes have been done in different areas but in China. This study presents the first step towards describing the AMF and DSE colonization status of aquatic plants in southwest China. The specific objectives of this study were to determine whether AMF and DSE colonize hydrophytes in lakes and streams in southwest China, and the composition of AMF spores in the sediments collected from the arbuscular mycorrhized hydrophyte rhizospheres.

## 2. Materials and Methods

### 2.1. Study Site

Study sites were located at four lakes situated in Kunming (25°04' N, 102°73' E), Chengjiang (24°68' N, 102°91' E), Shiping (23°73' N, 102°48' E) and Tonghai (24°09' N, 102°75' E), and four streams situated in Jianshui (23°64' N, 102°79' E) and Songming (25°35' N, 103°03' E) in Yunnan province, southwest China. The trophic status of both lakes and streams ranged from oligotrophic to eutrophic.

### 2.2. Sampling

Hydrophytes examined were sampled during the summer of 2004. Samples were collected in water between the surface (free floating plants) and a depth of 60 cm.

For each species in both lakes and streams, at least 3 replications were sampled except for *Polygonum hydropiper* and *Oenanthe decumbens* with 2 replications. After plants and sediments (if accessible) were taken from the water, they were separated *in situ*. Entire roots were cut off from the plant and sediment samples were put into plastic bags, respectively. Root samples (with root tips) were fixed in 1/2 formalin-aceto-alcohol (FAA, BERCH and KENDRICK, 1982) and stored at 4 °C until examination.

### 2.3. Root Sample Treatment

Roots were taken out from 1/2 FAA, washed several times in running tap water, cut into 3 cm pieces, and bleached in 10% (w/v) KOH by heating to approximately 90 °C in a water bath for 20–30 min, the time depending on the size/structure of the roots and their pigmentation. Then, the cooled root samples were washed and stained with 0.5% acid fuchsin (BERCH and KENDRICK, 1982). After destaining, at least fifty root segments were examined for each specimen, and the segments colonized by AMF and DSE were counted and the frequency of AMF colonization was expressed as the ratio of the colonized root segments to the total number examined (READ *et al.*, 1976). Mycorrhizal coverage of colonized segments was determined using the method modified by MCGONIGLE *et al.* (1990) under a compound-light microscope (OLYMPUS-BX51) at magnification  $\times 200$ . One hundred and fifty intersections were observed for each specimen.

### 2.4. Sediment Treatment

Sediment samples were wet-sieved and decanted (GERDEMANN and NICOLSON, 1963) to recover AMF spores. Spore identification was based on morphological characters with reference to the descriptions provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (<http://invam.caf.wvu.edu>) and the original species descriptions.

## 3. Results

### 3.1. AMF Colonization of Hydrophytes

In total, 140 specimens of 32 hydrophyte species were investigated (Table 1). Life forms of aquatic plants included free-floating, rooted-floating, emergent and submerged species. Typical AM structures (arbuscule and vesicle) were observed in one of the 25 hydrophyte species collected in lakes and six out of the 17 species collected from streams (Fig. 1). Most of colonized samples showed low frequency of AMF colonization and mycorrhizal coverage (below 25%) (Tables 1 and 2). The highest colonized frequency and mycorrhizal coverage were observed respectively in emergent plants *Impatiens aquatilis* (45%) and *Rotala rotundifolia* (35%), both of which were collected in streams (Table 2).

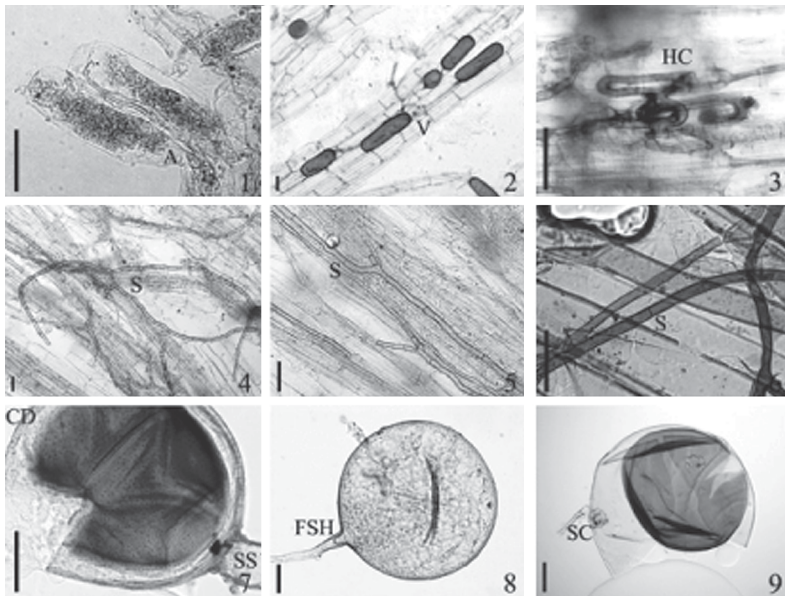


Figure 1. AMF and DSE structures in the roots of hydrophytes, and some identified AMF spores: (1) Typical arbuscules (A); (2) Vesicles (V) in the roots; (3) Hyphal coils (HC) of AMF in the roots; (4–6) Dark septate endophytes (DSE) in the roots; Septates (S) of the hyphae; (7) *Acaulospora scrobiculata*: Concave depressions (CD) on the spore wall surface and sporiferous saccule (SS) attached to the spore; (8) *Glomus mosseae*: Funnel-like subtending hypha (FSH); (9) *Scutellospora calospora*: Suspensor-like cell. bar = 40  $\mu$ m.

Table 1. Frequency of AMF colonization, mycorrhizal coverage, root hair presence and DSE frequency in aquatic plants collected from the lakes<sup>1</sup>.

Species	Samples	Frequency	Mycorrhizal coverage	Root hair	DSE frequency
Free-floating					
<i>Eichhornia crassipes</i>	5	0	0	abundant	0
<i>Lemna minor</i>	8	0	0	absent	0
Rooted-floating					
<i>Hydrocharis dubia</i>	3	0	0	absent	0
<i>Nymphaea tetragona</i>	3	0	0	abundant	0
<i>Nymphoides peltatum</i>	3	0	0	sparse	0
<i>Trapa quadrispinosa</i>	3	0	0	abundant	0
Emergent					
<i>Alisma plantago-aquatica</i> subsp. <i>orientale</i>	3	0	0	sparse	0
<i>Gladiolus gandavensis</i>	3	0	0	abundant	0
<i>Nelumbo nucifera</i>	3	0	0	abundant	0
<i>Oenanthe decumbens</i>	2	0	0	abundant	0.03 ± 0.02
<i>Phragmites communis</i>	3	0	0	abundant	0
<i>Polygonum hydropiper</i>	2	0	0	abundant	0
<i>P. lapathifolium</i> var. <i>salicifolium</i>	3	0.08 ± 0.03	0.06 ± 0.05	abundant	0
<i>Sagittaria trifolia</i>	3	0	0	abundant	0
<i>Typha orientalis</i>	3	0	0	abundant	0
<i>Zizania caduciflora</i>	3	0	0	sparse	0
Submerged					
<i>Ceratophyllum demersum</i>	5	0	0	absent	0
<i>Hydrilla verticillata</i>	3	0	0	absent	0
<i>Myriophyllum spicatum</i>	4	0	0	abundant	0
<i>Potamogeton lucens</i>	3	0	0	absent	0
<i>P. malaianus</i>	3	0	0	absent	0
<i>P. oxyphyllum</i>	3	0	0	absent	0
<i>P. pectinatus</i>	6	0	0	absent	0
<i>P. perfoliatus</i>	3	0	0	abundant	0
<i>Vallisneria spiralis</i>	4	0	0	absent	0

<sup>1</sup> The frequency is expressed as the relative number of root segments colonized by AMF/DSE and the mycorrhizal coverage as the relative area (in percentage) of the projected root surface covered by fungal structures. Mean ± S.D., n = 2–3 (each sample consisted of 50 root segments at least).

### 3.2. AMF Colonization in Hydrophytes with Different Root Hair Presence

Based on the degree of root hair presence, all aquatic plants were divided into three groups: absent, sparse, and abundant. Of the 7 arbuscular mycorrhizal hydrophytes, four species (*Impatiens aquatilis*, *Polygonum lapathifolium* var. *salicifolium*, *Rotala rotundifolia*, *Sagittaria trifolia*) had abundant root hairs whereas three species (*Alisma plantago-aquatica* subsp. *orientale*, *Cardamine multijuga* var. *multijuga*, *Sagittaria sagittifolia*) had sparse root hairs (Tables 1 and 2).

Table 2. Frequency of AMF colonization, mycorrhizal coverage, root hair presence and DSE frequency in aquatic plants collected from the streams<sup>1</sup>.

Species	Samples	Frequency	Mycorrhizal coverage	Root hair	DSE frequency
Free-floating					
<i>Marsilea quadrifolia</i>	5	0	0	abundant	0
Rooted-floating					
<i>Marsilea crenata</i>	3	0	0	abundant	0
<i>Potamogeton tepperi</i>	3	0	0	abundant	0.03 ± 0.02
Emergent					
<i>Alisma plantago-aquatica</i> subsp. <i>orientale</i>	3	0.11 ± 0.03	0.09 ± 0.03	sparse	0
<i>Elatine ambigua</i>	3	0	0	absent	0
<i>Impatiens aquatilis</i>	3	0.45 ± 0.22	0.24 ± 0.10	abundant	0
<i>Nelumbo nucifera</i>	3	0	0	abundant	0
<i>Rotala rotundifolia</i>	3	0.34 ± 0.24	0.35 ± 0.06	abundant	0.23 ± 0.13
<i>Sagittaria sagittifolia</i>	3	0.17 ± 0.09	0.22 ± 0.05	sparse	0
<i>S. trifolia</i>	3	0.03 ± 0.02	0.03 ± 0.02	abundant	0
<i>Typha orientalis</i>	3	0	0	abundant	0
<i>Zizania caduciflora</i>	3	0	0	sparse	0
Submerged					
<i>Cardamine multijuga</i> var. <i>multijuga</i>	3	0.10 ± 0.00	0.02 ± 0.01	sparse	0
<i>Ceratophyllum demersum</i>	3	0	0	absent	0
<i>Hydrilla verticillata</i>	3	0	0	absent	0.03 ± 0.02
<i>Potamogeton malaianus</i>	3	0	0	absent	0
<i>Vallisneria natans</i>	3	0	0	absent	0

<sup>1</sup> The frequency is expressed as the relative number of root segments colonized by AMF/DSE and the mycorrhizal coverage as the relative area (in percentage) of the projected root surface covered by fungal structures. Mean ± S.D., n = 3 (each sample consisted of 50 root segments at least).

### 3.3. AMF Spores Isolated from the Sediments

Four hundred and nine AMF spores (or sporocarps) were wet-sieved from the 36 sediment samples, from which *Acaulospora scrobiculata*, *A. turberculata*, *Gigaspora gigantea*, *Glomus aggregatum*, *G. claroideum*, *G. clarum*, *G. geosprum*, *G. mosseae*, *G. rubiforme*, *Scutellospora calospora*, and an unidentified *Acaulospora* sp., were identified (Fig. 1). Among the 4 identified AMF genera, *Glomus* spores/sporocarps (397) accounted for 97%, *Acaulospora* (7), *Gigaspora* (4) and *Scutellospora* (1) respectively for 1.8%, 1%, and 0.2%.

### 3.4. DSE Colonization of Hydrophytes

DSE were present in one of the 25 hydrophyte species collected in lakes and three of the 17 species collected in streams (Tables 1 and 2; (Fig. 1). Among all DSE colonized hydrophytes, *Oenanthe decumbens* collected in lake grew emergent, and *Hydrilla verticillata*, *Potamogeton tepperi*, and *Rotala rotundifolia* collected in streams grew submerged, rooted-floating and emergent, respectively (Tables 1 and 2). *Rotala rotundifolia* collected in streams harbored the highest DSE colonization of 23% (Table 2).

#### 4. Discussion

In this study, only 22% of the aquatic plants were arbuscular mycorrhized. This was similar to the results reported by CHAUBAL *et al.* (1982) who found that 29% of the aquatic plants investigated was mycorrhizal and concluded that AM occurrence was a rare phenomenon in aquatic tropical plants. It was interesting that *P. lapathifolium* var. *salicifolium* and *C. multijuga* var. *multijuga* came from Polygonaceae and Cruciferae, respectively, which were thought to rarely form AM in terrestrial ecosystems (BOLAN, 1991; ST. JOHN, 1996) and were typically arbuscular mycorrhized in this study. Of all sampled species, none of isoetids was observed to be colonized by AMF. Whereas, emergent species accounted for 86% of all colonized species and had the highest AMF colonization and frequency (Tables 1 and 2). This was different from the results of BECK-NIELSON and MADSEN (2001), who documented that isoetids had the highest colonization among all infected species whereas emergent plants had the lowest AMF colonization. Isoetids should theoretically be easier to form association with AMF, due to their highly compatible characters. Isoetids can transport most of the photosynthetically produced oxygen through lacunae to the roots where a major part is released to the sediment (SAND-JENSEN *et al.*, 1982; CHRISTENSEN *et al.*, 1994; PEDERSEN *et al.*, 1995). This creates a basic living condition for the aerobic metabolism of arbuscular mycorrhizal fungi (AMF) (HARLEY and SMITH, 1983; TANNER and CLAYTON, 1985). The formation of mycorrhizal-isoetid associations can facilitate the phosphorus uptake to the isoetids either through the hyphae beyond the plaque (WIGAND and STEVENSON, 1997) or through the organic acids released by the mycorrhizal fungi which can make the solid-phase phosphorus mobilized again and become available to the host (BOLAN, 1991). An explanation for the increased colonization of emergent plants might be that in the present study these all were collected in shallower water where there was a relatively high redox potential in the sediment (compared with the deeper part of the water) and a potentially greater photosynthetic efficiency of the hosts. Higher photosynthetic efficiency of the hosts and redox potential were considered respectively to facilitate the colonization of AMF indirectly and directly (KHAN, 1993; WIGAND *et al.*, 1998; BECK-NIELSON and MADSEN, 2001).

Nutrient status of the sediment and water body may have a notable impact on the establishment of AM in a definite hydrophyte. *A. plantago-aquatica* subsp. *orientale*, *C. multijuga* var. *multijuga*, *S. trifolia*, *T. orientalis*, and *V. natans* were sampled both in lakes and streams in this study. AMF colonization were found in three of these species, *A. plantago-aquatica* subsp. *orientale*, *C. multijuga* var. *multijuga* and *S. trifolia*, collected in the streams but not in lakes. Nutrient content in the water body and sediment may great influence on the establishment of AM, as nutrients were more easily accumulated in lentic water ecosystem than in flowing water ecosystem. Besides, it has been found that mycorrhizal colonization tends to correlate both with low tissue phosphorus content of the aquatic plants as well as with low sediment nutrient concentration (NIELSEN *et al.*, 2004).

Our findings contradicted those of earlier studies that macrophytes without and with sparse root hairs were heavily colonized by AMF, and those with abundant root hairs had no AMF colonization (SØNDERGAARD and LAEGARRD, 1977; CLAYTON and BAGYARAJ, 1984). In present study, the majority of species without root hairs or with sparse root hairs were not colonized by AMF. The results of this study were in agreement with those reports that there was not an obvious relationship between the root hair presence and AMF colonization in aquatic plants (CLAYTON and BAGYARAJ, 1984; FARMER, 1985; FARMER, 1988).

Species of *Glomus* were predominant among four AMF genera identified from the aquatic sediments. This result was consistent with previous reports coming from terrestrial ecosystems (BEVER *et al.*, 1996; ZHAO *et al.*, 2003; LI and ZHAO, 2005). Moreover, it should be noted that *G. mosseae* accounted for 71% of *Glomus* and 69% of the total spores, respectively. *Glomus* has usually been found to be the dominant AMF genus in many land environments. *Glomus mosseae* is also the most common species in terrestrial ecosystems and



is often used as a representative of AMF in research and application. It seems that the composition of AMF in aquatic ecosystems is similar to that in terrestrial ecosystems.

The DSE colonization was similar to that by AMF, in that the majority of DSE colonized hydrophytes were collected in streams, and the level of colonization was low (Tables 1 and 2). This confirmed that aquatic environment is also habitat for DSE. It was noted that DSE and AMF co-occurred in *Rotala rotundifolia* for the first time in aquatic environment in this study, while the co-colonization of AMF and DSE in many other plants have often been observed in terrestrial ecosystems (LI *et al.*, 2005; RUOTSALAINEN *et al.*, 2002; RAINS *et al.*, 2003). In addition, DSE have also been reported in roots of grasses and shrubs from cold environments (TREU *et al.*, 1996) and xeric habitats (BARROW and AALTONEN, 2001; BARROW, 2003). The extensive colonization of these plants by DSE has been suggested to be indicative of a significant ecological function for the fungi, for example, a “mycorrhizal function” of nutrient and water acquisition (BARROW and AALTONEN, 2001; BARROW, 2003). The occurrence of DSE in the hydrophytes, however, suggests water acquisition is not a primary role for DSE. Arguments concerning to the function of DSE colonization are changeful, as DSE association showed highly variable effects on host performance or host tissue nutrient concentrations (JUMPPONEN, 2001). ADDY *et al.*, (2005) considered that the designation of DSE was misleading (GRÜNIG and SIEBER, 2005) when it was used to imply taxonomic relatedness or similarity in physiological or ecological function. More detail studies of interactions between identified taxa of DSE and host plants are necessary to elucidate the functional basis of these symbioses in terrestrial ecosystems as well as in aquatic ecosystems.

## 5. Acknowledgements

The authors thank Prof. ZHU WEIMING (Biology Department of Yunnan University, China) for his help to identify the hydrophytes. We would like to express our appreciation to LI TAO, LI JIANPING, LI LINGFEI, YANG ANNA, ZHAO DANDAN and LIANG CHANGCONG (Key Laboratory for Conservation and Utilization of Bio-resources, Yunnan University) for their help to collect samples. This research was financially supported by the National Nature Science Foundation of China (NSFC: 30360003) and Natural Science Foundation of Yunnan Province (2001C0001Z).

## 6. References

- ADDY, H. D., M. M. PIERCEY and R. S. CURRAH, 2005: Microfungal endophytes in roots. – *Can. J. Bot.* **83**: 1–13.
- BAGYARAJ, D. J., A. MANJUNATH and R. B. PATIL, 1979: Occurrence of vesicular-arbuscular mycorrhizas in some tropical aquatic plants. – *Trans. Br. Mycol. Soc.* **72**: 164–167.
- BARROW, J. R. and R. E. AALTONEN, 2001: Evaluation of the internal colonization of *Atriplex canescens* (PURSH) NUTT. roots by dark septate fungi and the influence of host physiological activity. – *Mycorrhiza* **11**: 199–205.
- BARROW, J. R., 2003: A typical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. – *Mycorrhiza* **13**: 239–247.
- BECK-NIELSEN, K. B., R. KJØLLER, P. A. OLSSON, P. F. SCHWEIGER, F. Ø. ANDERSEN and S. ROSENDAHL, 2004: Colonization and molecular diversity of arbuscular mycorrhizal fungi in the aquatic plants *Littorella uniflora* and *Lobelia dortmanna* in southern Sweden. – *Mycol. Res.* **108**: 616–625.
- BERCH, S. M. and W. B. KENDRICK, 1982: Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. – *Mycologia* **74**: 769–776.
- BEVER, J. D., J. B. MORTON, J. ANTONOVICS and P. R. SCHULTZ, 1996: Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. – *J. Ecol.* **84**: 71–82.
- BOLAN, N. S., 1991: A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants. – *Plant Soil* **134**: 189–207.

- CHAUBAL, R., G. D. SHARMA and R. R. MISHRA, 1982: Vesicular-arbuscular mycorrhiza in subtropical aquatic and marshy plant communities. – *Proc. Indian. Acad. Sci. (Plant Sci.)* **91**: 69–77.
- CHRISTENSEN, P. B., N. P. REVSBECH and K. SAND-JENSEN, 1994: Microsensor analysis of oxygen in the rhizosphere of the aquatic macrophyte *Littorella uniflora* (L.) ASCHERSON. – *Plant Physiol.* **105**: 847–852.
- CLAYTON, J. S. and D. J. BAGYARAJ, 1984: Vesicular-arbuscular mycorrhizas in submerged aquatic plants of New Zealand. – *Aquat. Bot.* **19**: 251–262.
- FARMER, A. M., 1985: The occurrence of vesicular-arbuscular mycorrhiza on isoetid-type submerged aquatic macrophytes under naturally varying condition. – *Aquat. Bot.* **21**: 245–249.
- FARMER, A. M., 1988: Vesicular-arbuscular mycorrhiza in submerged *Isoetes*. – *Mycologist* **2**: 74.
- FUCHS, B. and K. HASELWANDTER, 2004: Red list plants: colonization by arbuscular mycorrhizal fungi and dark septate endophytes. – *Mycorrhiza* **14**: 277–281.
- GERDEMANN, J. W. and T. H. NICOLSON, 1963: Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. – *Trans. Br. Mycol. Soc.* **46**: 235–244.
- GRÜNING, C. R. and T. N. SIEBER, 2005: Molecular and phenotypic description of the widespread root symbiont *Acephala aplanata* gen. et sp. nov., formerly known as dark-septate endophyte Type 1. – *Mycologia* **97**: 628–640.
- HARLEY, J. L. and S. E. SMITH, 1983: *Mycorrhizal symbiosis*. – Academic Press, London UK
- HART, M. M., R. J. READER and J. K. KLIRONOMOS, 2003: Plant coexistence mediated by arbuscular mycorrhizal fungi. – *Trans. Ecol. Evol.* **18**: 418–423.
- JUMPPONEN, A. and J. M. TRAPPE, 1998: Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. – *New Phytol.* **140**: 295–310.
- JUMPPONEN, A., 2001: Dark septate endophytes-are they mycorrhizal? – *Mycorrhiza* **11**: 207–211.
- KHAN, A. G., 1993: Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. – *Mycorrhiza* **3**: 31–38.
- LI, L. F., A. N. YANG and Z. W. ZHAO, 2005: Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. – *FEMS Microbiol. Ecol.* **54**: 367–373.
- LI, T. and Z. ZHIWEI, 2005: Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China. – *Appl. Soil. Ecol.* **29**: 135–141.
- MCGONIGLE, T. P., M. H. MILLER, D. G. EVANS, G. L. FAIRCHILD and J. A. SWAN, 1990: A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. – *New Phytol.* **115**: 495–501.
- NIELSEN, D. B. and T. V. MADSEN, 2001: Occurrence of vesicular-arbuscular mycorrhiza in aquatic macrophytes from lakes and streams. – *Aquat. Bot.* **71**: 141–148.
- PEDERSEN, O., K. SAND-JENSEN and N. P. REVSBECH, 1995: Diel pulses of O<sub>2</sub> and CO<sub>2</sub> in sandy lake sediments inhabited by *Lobelia dortmanna*. – *Ecology* **76**: 1536–1545.
- RAGUPATHY, S., V. MOHANKUMAR and A. MAHADEVAN, 1990: Occurrence of vesicular- arbuscular mycorrhizas in tropical hydrophytes. – *Aquat. Bot.* **36**: 287–291.
- RAINS, K. C., N. M. NADKARNI and C. S. BLEDSOE, 2003: Epiphytic and terrestrial mycorrhizas in a lower montane Costa Rican cloud forest. – *Mycorrhiza* **13**: 257–264.
- READ, D. J., H. K. KOUCHEKI and J. HODGSON, 1976: Vesicular–arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. – *New Phytol.* **77**: 641–653.
- READ, D. J. and K. HASELWANDTER, 1981: Observations on the mycorrhizal status of some alpine plant communities. – *New Phytol.* **88**: 341–352.
- RUOTSALAINEN, A. L., H. VÄRE and M. VESTBERG, 2002: Seasonality of root fungal colonization in low-alpine herbs. – *Mycorrhiza* **12**: 29–36.
- SAND-JENSEN, K., C. PRAHL and H. STOKLHOLM, 1982: Oxygen release from roots of submerged aquatic macrophytes. – *Oikos* **38**: 349–354.
- SØNDERGAARD, M. and S. LAEGAARD, 1977: Vesicular-arbuscular mycorrhiza in some aquatic vascular plants. – *Nature* **268**: 232–233.
- ST. JOHN, T. V., 1996: Mycorrhizal inoculation: advice for growers and restorationists. – *Hortus West* **7**: 10.
- TANNER, C. C. and J. S. CLAYTON, 1985: Vesicular-arbuscular mycorrhiza studies with a submerged aquatic plant. – *Trans. Br. Mycol. Soc.* **85**: 683–688.
- TRAPPE, J. M., 1987: Phylogenetic and ecological aspects of mycotrophy in angiosperms from an evolutionary standpoint. – *In*: SAFIR, G. R. (Ed.), *Ecophysiology of VA Mycorrhiza*. – CRC Press, Boca Raton, pp. 5–25.



- TREU, R., G. A. LAURSEN, S. L. STEPHENSON, J. C. LANDOLT and R. DENSMORE, 1996: Mycorrhizae from Denali National Park and Preserve, Alaska. – *Mycorrhiza* **6**: 21–29.
- WIGAND, C. and J. C. STEVENSON, 1997: Facilitation of phosphate assimilation by aquatic mycorrhizae of *Vallisneria americana* Michx. – *Hydrobiologia* **342/343**: 35–41.
- WIGAND, C., F. Ø. ANDERSEN, K. K. CHRISTENSEN, M. HOLMER and H. S. JENSEN, 1998: Endomycorrhizae of isoetids along a biogeochemical gradient. – *Limnol. Oceanogr.* **43**: 508–515.
- WURZBURGER, N. and C. S. BLEDSOE, 2001: Comparison of ericoid and ectomycorrhizal colonization and ectomycorrhizal morphotypes in mixed conifer and pygmy forests on the northern California coast. – *Can. J. Bot.* **79**: 1202–1210.
- ZHAO, Z. W., G. H. WANG and L. YANG, 2003: Biodiversity of arbuscular mycorrhizal fungi in a tropical rainforest of Xishuangbanna, southwest China. – *Fungal Diversity* **13**: 233–242.

Manuscript received July 1st, 2005; revised December 1st, 2005; accepted December 13th, 2005