The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal

R.S. Oliveira · J.C. Dodd · P.M.L. Castro

Abstract Roots of *Phragmites australis* from three polluted soils and sediments (a periodically flooded stream bank containing organic pollutants, a high-pH drying sedimentation pond and an acidic, periodically flooded sand polluted by industrial effluents) were sampled over a 1-year cycle of plant growth to assess the degree of colonisation by arbuscular mycorrhizal fungi (AMF). At the dry sedimentation pond, root samples of *Juncus effusus* and *Salix atrocinerea* were also taken to assess the presence of AMF throughout the year. Root colonisation was low (<5% root length colonised) but arbuscule presence peaked in *P. australis* during the spring and autumn prior to flowering. These changes in arbuscule abundance were also seen in a parallel greenhouse trial using seed taken from one of the sites. Roots of *J. effusus* contained mainly vesicular colonisation but arbuscule activity peaked during the winter months (December–March). *S. atrocinerea* roots were found to be ectomycorrhizal throughout the year but the fine feeder roots were colonised by AMF. The results confirm that semi-aquatics, like *P. australis*, can become arbuscular mycorrhizal but that this status changes during the year depending on soil moisture content and plant phenology. The influence of AMF in these polluted soils is uncertain but the potential exists to establish a more diverse plant ecosystem during the landscaping of these areas (phytostabilisation) by management of adapted plant and AMF ecotypes.

Keywords *Phragmites australis* · Arbuscular mycorrhizas · Pollution · Sedimentation pond · *Salix atrocinerea*

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Introduction

Various wetland plants have been reported to be either non-mycorrhizal (Harley 1970) or mycorrhizal (Khan 1993), e.g. *Carex stricta* and *Typha latifolia* (Wetzel and van der Valk 1996). Read et al. (1976) found that colonisation levels in aquatic plants were low and/or colonisation occurred during dry periods. Whilst functional arbuscules, hyphal coils and storage vesicles have been found, the importance of the association to plant growth, survival and fitness is unclear. Wetland systems are open to input of nutrients and heavy metals from adjacent agricultural land and industrial and urban areas. They may be sensitive to these inputs or they may be able to attenuate the pollutants. Previous studies have shown that the intraradical and extraradical mycelium of adapted AMF isolates are capable of sequestering heavy metals (Griffoen 1994).

The use of plant microbe-based systems (phytoremediation) for the treatment of contaminated soil and industrial effluents has been increasing in recent years (Cunningham et al. 1997). *Phragmites australis* Trin (common reed), for instance, has been used in constructed wetlands designed to treat effluents containing nitrophenols (Dias 1998) and waste landfill leachates (Trautmann et al. 1989; Maehlum 1995). *P. australis* is an important species in the zone between land and water in many global wetlands (Ostendorp 1989) but has been little studied with respect to its mycorrhizal status in polluted marginal lands. Anthropogenic sites have been shown to encourage invasion by *P. australis* (Roman et al. 1984) as it thrives in disturbed areas (e.g. drying sedimentation ponds or river banks) as well as pristine sites.

Table 1 Chemical properties of the soil/sediment from the three assessed sites in the uppermost 15-cm layer. For the metals, the values presented refer to the total amount of each element after sample pre-treatment (digestion under strongly acidic conditions) and subsequent determination by atomic, absorption spectrometry

Chemical properties	Site A	Site B	Site C
Conductivity (µS/cm)	278.0 [b]	5,500.0 [b]	258.0 [b]
pH (H_2O)	7.1–8.1 [b]	12.6 [b]	4.1 [b]
Total organic carbon (g/kg)	46.5 [a]	5.1 [c]	40.0 [d]
N (g/kg)	2.9 [b]	0.3 [c]	0.6 [b]
P (mg/kg)	4.6 [b]	36.0 [c]	12.5 [b]
K (mg/kg)	151.0 [b]	12.0 [b]	3.8 [d]
Na (mg/kg)	2,500 [b]	51,000 [c]	3.1 [d]
Mg (mg/kg)	0.4 [b]	0.7 [c]	29.6 [d]
Ca (mg/kg)	0.1 [b]	48,100 [c]	28.2 [d]
Total metals (mg/kg)			
Zn	898.9 (125–3,620) [a]	8.7 [c]	39.2 [d]
Pb	835.4 (16–3,740) [a]	71.3 [c]	18.2 [d]
Hg	66.6 (0.30–275) [a]	0.1 [c]	3.5 (0.73–8.90) [a]
As	1495.9 (45–5,620) [a]	<0.9 [b]	237.4 (35–955) [a]
Cr	26.0 [b]	17.3 [c]	7.0 [b]
Ni	37.3 [b]	4.3 [c]	3.5 [d]
Cu	0.0 [b]	23.0 [c]	10.0 [d]
Fe (g/kg)	16.8 [b]	1.3 [c]	9.2 [d]

[a] From Atkins (1999) (average and range)
[b] Determined during this study
[c] From Balsa et al. (1996)
[d] From Leitão et al. (1994)

It may help establish a rhizosphere of adapted microorganisms, including AMF, which then allows invasion by mycotrophic plants when soils or sediments dry out. The aim of this present work was to survey three sites in Northern Portugal during a 1-year growth period to assess the mycorrhizal status of *P. australis* and two other wetland species growing in polluted substrata or exposed to pollutants.

Materials and methods

Field survey

Three sites (Table 1) located in Estarreja, Northern Portugal (40045′16″N, 8034′17″W) were sampled between April 1999 and March 2000.

Site A – located on the bank of a stream, contaminated with zinc (Zn), arsenate (As), lead (Pb) and mercury (Hg) from the discharge of liquid and solid residues in the area. Atkins (1999) showed that As, Pb, Zn and Hg levels on the bank to a depth of 50 cm (Table 1) were above the limits established by EC Directive 86/278/EC for soil and by the Dutch system (Gieseler 1987) usually used as a reference for Portugal. The soil on the top of the bank had a pH of 8.1 to a depth of 10 cm, whilst the lower section (a saturated sediment) had a pH of 7.1 to a depth of 10 cm. The composition of the sediment was sand (52%) and silt (32%) with a clay content of 8%. The lower part of the bank was periodically covered by the stream, whilst the top of the bank was not and hence water contents varied. The bank had abundant vegetation mainly comprising *P. australis* with small reeds and grasses; *P. australis* was the only species found on the lower sections of the bank. Samples of root were taken from the top and lower section of the bank.

Site B – located on a 10-ha dry sedimentation pond deposit resulting from the production of polyvinylchloride (PVC) over a 25-year period. A thorough study of the chemical composition of the site was carried out by Balsa et al. (1996) (Table 1). The composition of the sediment (pH 12.6) in the upper 50 cm was mainly $Ca(OH)_2$ (80% of total soil) with low levels of organic carbon, N and P. The site had scant vegetation, comprising mainly small trees (100 cm high) of *Pinus* spp. and *Salix atrocinerea* Brot., together with less frequent *Pteridium aquilinum* (L.) Kuhn, *Conyza bonariensis* (L.) Cronq, *P. australis* and *Juncus effusus* L (Balsa et al. 1996).

Site C – adjacent to site B with a small stream and a periodically flooded soil having a pH of 4.1 to a depth of 10 cm. Chemical analysis of the site (Leitão et al. 1994) (Table 1) showing high levels of As and Hg was confirmed later by Atkins (1999). The soil was permeable, mostly sand (83%) with clay 8% and a silt content of 9%. Vegetation was abundant, mainly comprising *Pinus* spp, *Acacia melanoxylon* Aiton, *P. australis* and *J. effusus*.

Additional soil analyses (Table 1) were carried out by a certified analytical company (DPM, Lisbon, Portugal). K, Na, Mg, Ca, Cu, Fe, Ni, Cr, Zn, Pb and Hg were determined using standard atomic absorption spectrometry techniques after digestion in HNO_3 + $HClO_4$ + HF (Pratt 1965). As was measured colorimetrically (Hesse 1971). N was determined using the Kjeldahl method (Bremner and Mulvaney 1982). P was measured by molecular absorption spectrometry after digestion with HNO_3 + $HClO_4$ (Johnson 1990). The pH was determined in water and the electrical conductivity in ultra-pure water. Water contents were determined after Hesse (1971).

Root sampling and preparation for staining

Roots of *P. australis*, *S. atrocinerea* and *J. effusus* were sampled on eight occasions between April 1999 and March 2000. Three plants were chosen at random at each site, 3 m apart at site A and within a 10 m^2 area at sites B and C, and excavated to a depth of 15 cm. Root systems were harvested carefully to avoid major damage to the feeder roots and washed in the laboratory to remove all substratum. Fine lateral roots were cut into 1-cm pieces, heated for 20 min at 120°C in 10% KOH in a pressure cooker to remove the cytoplasm and then stained using the standard protocol of Phillips and Hayman (1972) but with a somewhat longer incubation in 2% HCl. Root pieces were examined under a dissecting microscope and adequately stained roots were mounted on glass slides for examination with a compound microscope (×100–400). The presence of structures characteristic of arbuscular mycorrhizas, such as arbuscules and vesicles, was taken as a sign of colonisation. Percent root length colonised was always less than 5% based on a grid line intersect evaluation (data not presented), as found in previous studies with aquatic plants (Rickerl et al. 1994; Wetzel and van der Valk 1998). Changes in mycorrhizal colonisation intensity with time were expressed using a sliding scale (Table 2). Colonisation was estimated for each sample by examining 25 1-cm pieces of root.

Greenhouse trial

A greenhouse trial was established in July 1999 in a temperature- and light-controlled greenhouse (minimum 19°C/maximum 37°C, 600–800 µmol/m^2/s, relative humidity 49–80%) at the Internation-

Table 2 Arbuscular mycorrhizal fungal colonisation over a 1-year life-cycle of two semi-aquatic herbaceous plants (*Phragmites australis*, *Juncus effusus*) and one tree (*Salix atrocinerea*) growing at three sites with differing pollutant levels in the Estarreja region of Northern Portugal. Colonisation intensity: *0* no colonisation, *1* less than 25% of the area of colonised root cortex with typical arbuscules/hyphae/vesicles, *2* approximately 25–75%, *3* approximately 75–100% (*A* arbuscules, *H* hyphae, *lower* lower section of the bank, *ND* no data, *top* top of the bank, *V* vesicles)

Harvest time	Plant species	Site	AMF colonisation structures	AMF colonisation intensity
April 1999	*Phragmites australis*	A lower	–	0
	P. australis	A top	A	1
	P. australis	B	H, V, A	3
	P. australis	C	–	0
	Salix atrocinerea	B	–	0
	Juncus effusus	B	ND	ND
May 1999	*P. australis*	A lower	–	0
	P. australis	A top	H, V, A	1
	P. australis	B	H, V, A	1
	P. australis	C	–	0
	S. atrocinerea	B	–	0
	J. effusus	B	H, V, A	2
November 1999	*P. australis*	A lower	–	0
	P. australis	A top	H, V, A	2
	P. australis	B	H, V, A	3
	P. australis	C	H, V, A	3
	S. atrocinerea	B	H, V	1
	J. effusus	B	–	0
December 1999	*P. australis*	A lower	–	0
	P. australis	A top	H, V, A	3
	P. australis	B	–	0
	P. australis	C	–	0
	S. atrocinerea	B	–	0
	J. effusus	B	H, V	3
January 2000	*P. australis*	A lower	–	0
	P. australis	A top	H, A	1
	P. australis	B	H, V, A	2
	P. australis	C	–	0
	S. atrocinerea	B	H, V, A	2
	J. effusus	B	H, V	3
March 2000	*P. australis*	A lower	–	0
	P. australis	A top	H, V, A	2
	P. australis	B	H, V, A	3
	P. australis	C	–	0
	S. atrocinerea	B	–	0
	J. effusus	B	H, V, A	2

al Institute of Biotechnology/Research School of Biosciences, University of Kent). Seeds of *P. australis* collected from flowering plants at the top of the bank at site A during April 1999 were cold shocked at 4°C for 3 days before germination on moist, sterile sand at 25°C for 10 days. Ten seedlings were transplanted into treatment pots (12 cm in diameter, 11 cm tall) containing an attapulgite clay growth medium (Agsorb 8/16, Oil-Dri, Wisbech, UK). Four pots received 200 ml of an AMF inoculum (colonised root fragments, hyphae and spores) of *Glomus geosporum* BEG11 (Nicol. & Gerd.) Walker or *Glomus etunicatum* BEG137 Becker & Gerdemann (*BEG* European Bank of Glomales, wwwbio.ukc.ac.uk/beg) as a layer half-way down the pot and four pots were left uninoculated. The AMF were produced in open pot culture in attapulgite clay on onion (*Allium cepa* L.) and were chosen because of their ability in previous greenhouse trials to colonise rapidly many host roots (Dodd, personal observation). The surface of the soil in the pots was covered with a 0.5-cm layer of polypropylene homopolymer black beads (Cookson Ltd., Congleton, UK) to decrease evaporation and prevent algal growth. The plants were watered regularly with deionised water and fed weekly with 1.4 g/l Vitafeed 102 (Vitax Ltd, Leicester, UK) containing 18% N, 0% P and 36% K with trace elements. Irrigation was used to bring the water holding capacity of the pot substratum to 80% of maximum. Plants were grown under supplementary lighting for 7 months, at which time the root systems were sampled and stained for arbuscular mycorrhizas as above.

Results

Field survey

The water content of the soil on the upper bank at site A varied during the year between 36% and 50% (w/v), depending on rainfall (Fig. 1) and on the lower bank from 76% to 100% (w/v). At site A, *P. australis* plants growing in sediment closest to the water were never mycorrhizal (Table 2). Those at the top of the bank were always colonised and contained dense arbuscular colonisation in November 1999 and March 2000. At site B, the water content varied from 11% to 47% (w/v) during the year, again depending on rainfall. The colonised *P. australis* roots extracted from site B had frequent patches of dense arbuscular colonisation in April (Figs. 2, 3), in November, January and March 2000 (Table 2). The water content at site C varied from 25% to 100% (w/v) during the year following the rainfall minimum and maximum (Fig. 1). Roots of *P. australis* at site C were always free of colonisation, except for one sampling in Novem-

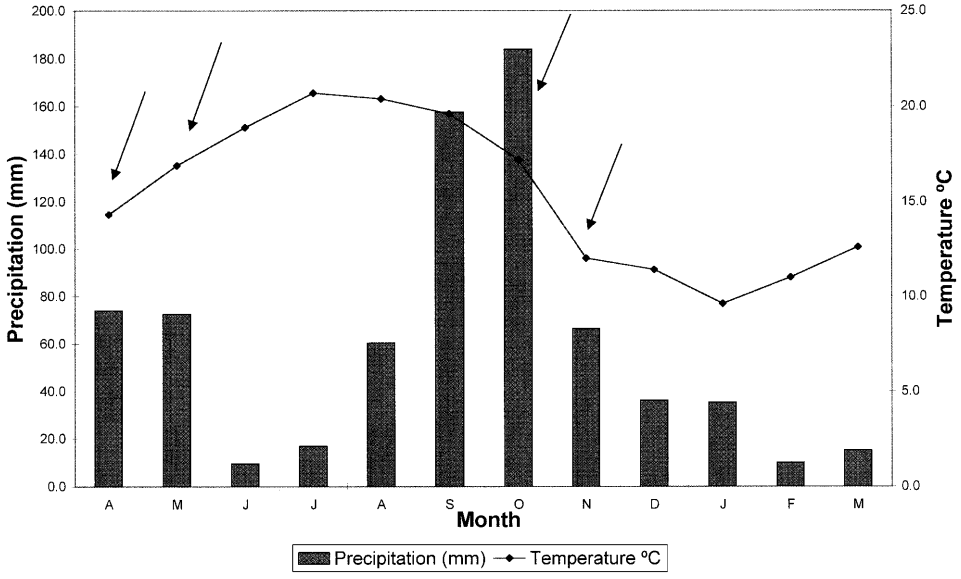


Fig. 1 Monthly temperature and precipitation profiles in the region of Estarreja, Northern Portugal during 1999–2000. The presented data are mean values. The flowering months for *Phragmites australis* at sites A and C are marked with *arrows*

ber 1999 (Table 2). Plants of *J. effusus* were sampled principally at site B and were found to be arbuscular mycorrhizal (Fig. 4) in May and December 1999 and in January and March 2000 (Table 2). Colonisation was dominated by vesicle production in *J. effusus* but arbuscules were occasionally found. Plants of *J. effusus* at site C were never mycorrhizal (data not presented). The rainfall during the November–March period (1999–2000) was significantly less than the 30-year average and may have caused soil water levels to fall far lower than in an average year (Fig. 1). Roots of *S. atrocinerea* at site B were ectomycorrhizal at each sampling (data not presented) and colonised by AMF in June 1999 (Fig. 5), October 1999, November 1999 and January 2000 (Table 2).

Greenhouse trial

Few areas of colonisation were seen in inoculated roots before December 1999 (5 months after sowing) and there was no difference in the growth of plants in different treatments. At final harvest, however, consistent low levels (1–5%) of root colonisation were observed in plants inoculated with either *G. geosporum* BEG11 (Fig. 6) or *G. etunicatum* BEG137. Colonisation comprised dense areas of arbuscules and vesicles (both inter- and intracellular) and occurred in all root orders except the largest primary roots and rhizomes. Colonisation was most frequent and dense in the smallest roots containing aerenchyma tissue (Fig. 6). These results paralleled those from the field, where the finer feeder roots emerging from the adventitious roots of the plant or the same roots emerging from subterranean rhizomes were the main sites of colonisation under favourable environmental conditions (i.e. reduced soil moisture, elevated temperature and plant reproductive phase).

Discussion

The results of the field survey show that the semi-aquatic *P. australis* can be colonised by AMF and that colonisation intensity depends on the stage in the plant life-cycle coupled with the apparent onset of water stress in the soil. Sampling sequentially throughout the yearly life-cycle of the plant showed that arbuscules were most frequent around the time of the two periods of flower production in the drier soils at sites A and B. The same trend was noted in the greenhouse trial. Most other studies based on single harvests have led to differences of opinion about the mycorrhizal status of different aquatic plants (Sondergaard and Laegaard 1977; Rickerl et al. 1994; Cooke and Lefor 1998). Enhanced mycorrhization was more obvious in the present study when soils had reduced water contents and higher mean temperatures, e.g. on top of the bank at site A and in the old sedimentation

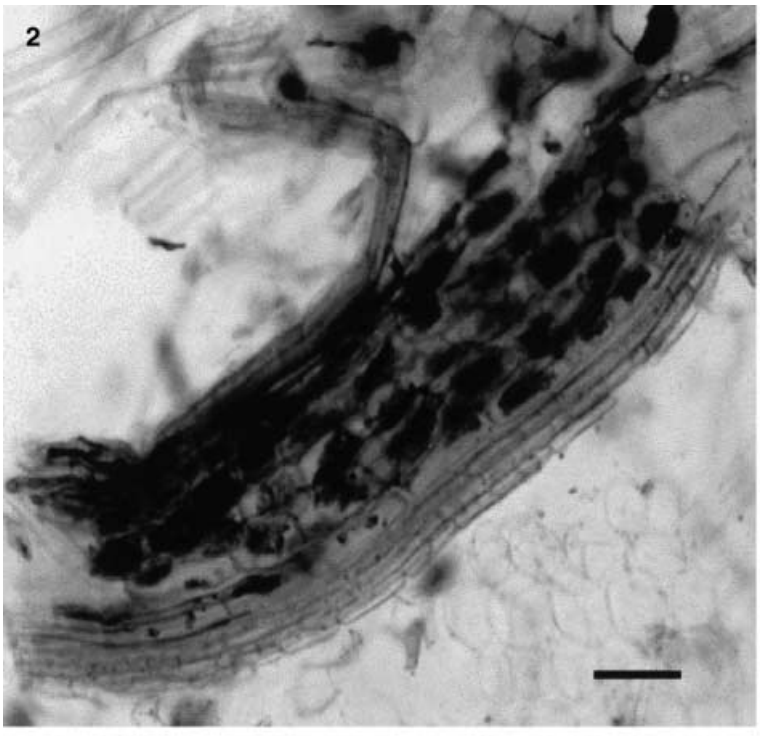
Fig. 2 Fine feeder root of *P. australis* collected at site B in April 1999 and stained with trypan blue. Patches of dense arbuscular colonisation are visible (category 3 on colonisation intensity scale); *bar* 100 µm ▶

Fig. 3 Close-up of arbuscules and vesicles present in *P. australis* roots collected at site B in April 1999 and stained with trypan blue; *bar* 50 µm

Fig. 4 Root of *Juncus effusus* collected at site B in May 1999. Both arbuscules (*A*) and vesicles (*V*) are present in the limited colonisation of the root cortex (category 2 on colonisation intensity scale); *bar* 100 µm

Fig. 5 AMF colonisation (*V* vesicles) in the fine lateral roots of *Salix atrocinerea* collected at site B in June 1999 (category 2 on colonisation intensity scale); *bar* 200 µm

Fig. 6 Six-month-old root of *P. australis* colonised by *Glomus geosporum* BEG11 in the greenhouse trial. Extraradical mycelium (*ERM*) is evident from the intraradical colonisation of roots with aerenchyma (*ACM*) tissue (category 2 on colonisation intensity scale); *bar* 200 µm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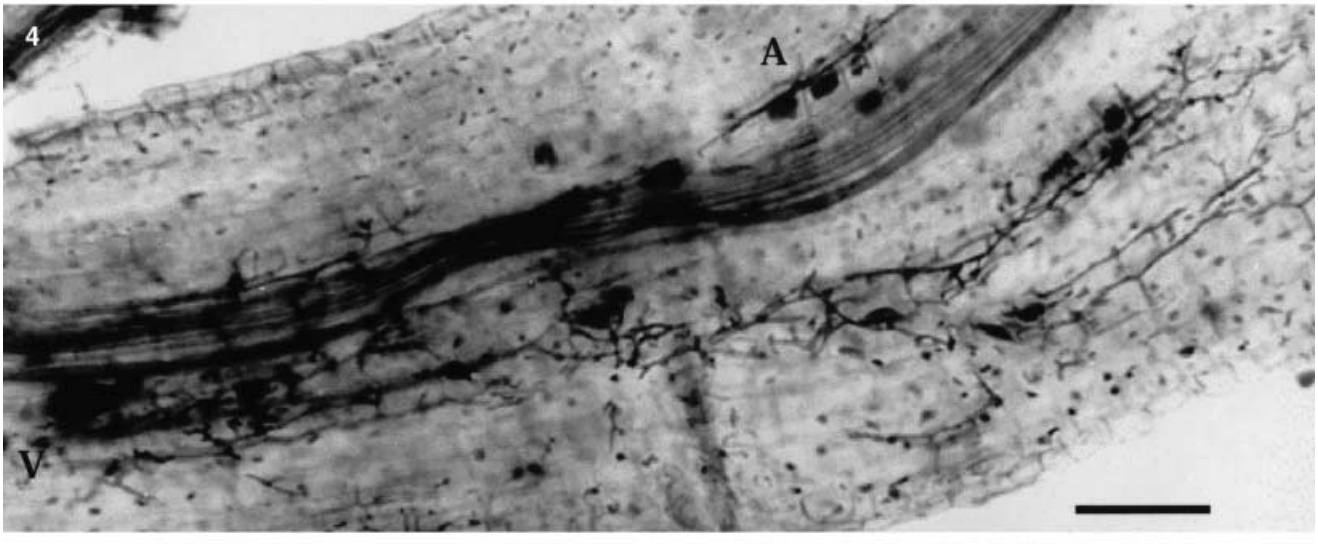
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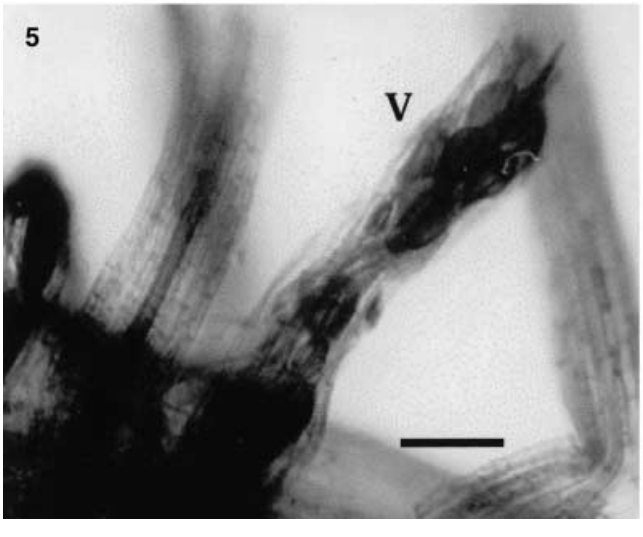
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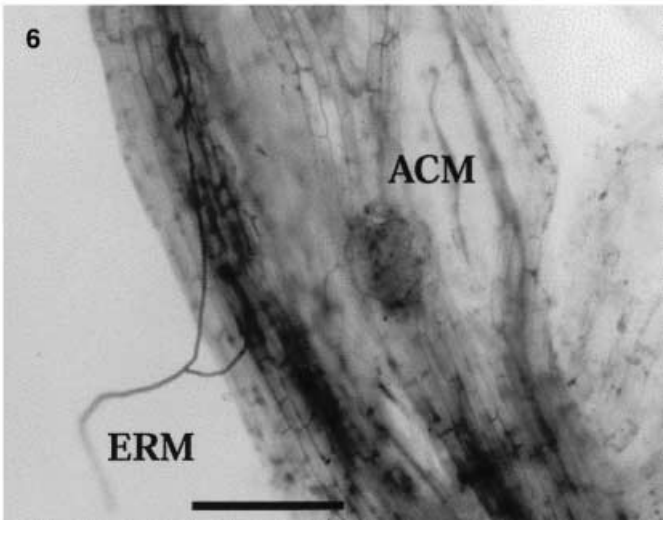
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ACM
ERM

pond at site B. This confirms previous observations that water level reductions in wetlands increase mycorrhization and that this frequently coincides with invasion by other mycotrophic plant species (Stevens and Peterson 1996). Plants that were frequently flooded, at site C and at the bottom of the bank at site A, had limited AMF colonisation throughout the year. In field studies of *Lythrum salicaria* L. (purple loosestrife) along an existing water gradient, Stevens and Peterson (1996) also noted that hyphal and arbuscular colonisation levels were significantly higher ($P<0.05$) in the dry and intermediate regions of the gradient than in the wet regions.

Isolates of *Glomus intraradices* Schenck & Smith and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe have been identified from trap cultures of *P. australis* taken from the polluted sediment at the bottom of the bank at site A (Dodd, unpublished data). Thus, even in sites where *P. australis* root systems are frequently flooded, AMF can survive (site B) until favourable conditions occur (soil drying) for colonisation of roots. When soils dried out in the early summer months and during the onset of flowering of *P. australis* (April–May 1999 and October–November 1999) due to higher mean temperatures and reduced rainfall (Fig. 1), the colonisation intensity of AMF increased (increase in arbuscules) at sites A (top of bank) and B. Plants at site B, however, did not produce flowers due to poor vegetative growth, but underground rhizomes stretched for up to 10 m from the mother plant. Thus, generalisations that arbuscule occurrence is infrequent or rare (Cantelmos and Ehrenfeld 1999) should be treated with caution, as should the assertion that this reflects a switch between mutualism and parasitism. Other dark-septate fungi have been noted to colonise the roots of *P. australis* under flooded soil conditions and their role deserves further attention (Haselwandter and Read 1982; Miller et al. 1999).

The colonisation of *P. australis* observed in the field was apparently not influenced by soil pH (site A pH 7.1, site B pH 12.6). *P. australis* is especially common in alkaline and brackish (slightly saline) environments (Haslam 1972) and can also thrive in highly acidic wetlands. It would appear, therefore, that colonisation occurred across the neutral to alkaline pH range but that the length of time that plant roots were submerged was a defining factor for restricting mycorrhization. Low pH and high soil moisture at site C were found to be least conducive to AMF colonisation of *P. australis*, as previously reported for *Carex* spp. (Miller et al. 1999), another semi-aquatic plant.

Fine feeder roots of *P. australis* were the primary sites of colonisation by AMF, whether they emerged from the roots of new seedlings or subterranean rhizomes. The amount of aerenchymous tissue also apparently declined in these roots in drier soils, as noted previously for other wetland species (Brix 1989). It seems, therefore, that finer roots become the primary niche for AMF when produced during flowering in drying soils and sediments. The results of the greenhouse trials support this conclusion: inoculated seedlings of *P. australis* (seeds collected from site A) only became mycorrhizal after 6 months immediately prior to flower production, despite the relatively low water content of the growth substratum.

Two other species, *J. effusus* and *S. atrocinerea* were found growing as pioneers on a barren former sedimentation pond (site B) and both became mycorrhizal during the study year. The *S. atrocinerea* plants had both ectomycorrhizas (not quantified) and arbuscular mycorrhizas, although levels of colonisation were low.

All three plant species survived on an extremely high pH sediment (12.6). These plant ecotypes may have great potential for the revegetation of spoil sites when planted with their adapted mycorrhizal fungal partners. A semi-natural habitat could, therefore, be established to landscape or phytostabilise former sedimentation ponds. In other work on dry sedimentation ponds in Poland, mycotrophic plant species comprised over 88% of the vegetation at the site of a 32-year-old soda factory (Pawlowska 1991). The use of AMF via inoculation or management has been proposed for other industrial sites and supports the important role of AMF in increasing plant diversity under adverse environmental conditions (Vangronsveld et al. 1996).

Recent work on the genus *Carex* (sedges) found the degree of mycotrophy to vary greatly (Miller et al. 1999). Some *Carex* species were obligate mycotrophs, a group were non-mycorrhizal, whilst edaphic factors influenced colonisation in others. It is becoming apparent in wetlands undergoing frequent wet and dry cycles that the establishment of mycorrhizas is more common in species thought to be generally non-mycorrhizal (Rickerl et al. 1994). Whether this is the result of changes in the susceptibility of roots in response to drying of the soils or sediments is uncertain. In conclusion, there is great potential for the management of semi-aquatic plants like *P. australis* and other species on polluted wetlands (streams and sedimentation ponds) in the initial remediation of these sites and for the encouragement of colonisation (re-invasion) by mycorrhizal fungi through gradual soil drying in the short-term. This approach could provide a management strategy for landscaping (phytostabilisation) of polluted sites by increasing the rate of establishment of mycotrophic species (Vangronsveld et al. 1996).

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