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Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal)

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Abstract The factors which may influence temporal and spatial variation in plant arbuscular mycorrhizal (AM) colonization and propagule occurrence were evaluated in a Portuguese salt marsh poor in plant diversity. Two distinct sites were studied: a more-flooded (low marsh) and a less-flooded zone (high marsh). AM root colonization, AM fungal spore number and inoculum potential, soil edaphic parameters and tidal flooding time periods were analysed. Levels of AM colonization were considerable in *Aster tripolium* and *Inula crithmoides* but very low in *Puccinellia maritima* and non-existent in *Spartina maritima*, *Halimione portulacoides*, *Arthrocnemum fruticosum* and *Arthrocnemum perenne*. Fungal diversity was very low, with *Glomus geosporum* dominant at both marsh zones. Colonization showed no spatial variation within marsh zones but temporal variation was observed in the high marsh, dependent on plant phenological phases. In the low marsh, no significantly seasonal variation was observed. Apparently, plant phenological events were diluted by stressful conditions (e.g. flooding, salinity). Spore density was significantly different between marsh zones and showed temporal variation in both zones. This study showed that distribution of mycorrhizas in salt marsh is more dependent on host plant species than on environmental stresses.

Keywords Arbuscular mycorrhizas · Salt marshes · Temporal and spatial variation · Flooding · Salinity

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Introduction

Salt marshes are ecosystems where tidal flooding with seawater leads to a partial or total submergence of vegetation, high soil salinity and soil anoxia. Soil inundation creates anaerobic and chemically reduced conditions around plant roots, leading to oxygen deficiency and phytotoxin accumulation (Armstrong et al. 1991). As a result, plant growth and survival, species composition and zonation patterns are strongly influenced by flooding and the concentrations of salt and oxygen (Armstrong et al. 1985; Pennings and Callaway 1992). Generally, soil salinity, soil moisture and anaerobiosis decrease from the lower to the higher zone of a salt marsh, creating a zonated pattern in the vegetation. Salt marsh plants exhibit biochemical, morphological and physiological adaptations to waterlogging and salinity (Armstrong et al. 1991; Naidoo et al. 1992). Microorganisms in the root zones, particularly arbuscular mycorrhizal fungi (AMF), may enhance ecological adaptation of these plants, including pioneer plant colonizers, to salt marsh environments (Sengupta and Chaudhuri 1990; Khan and Belik 1995). Some evidence suggests that mycorrhizas improve plant tolerance to salinity (Jindal et al. 1993; Ruiz-Lozano et al. 1996), though other results show that mycorrhizal infection can be suppressed by high soil salinity (Pfeiffer and Bloss 1988; Juniper and Abbott 1993) and waterlogging (Harley and Smith 1983). Recently, Miller (1999) showed that flooding partially inhibits AM colonization of wetland grasses.

Despite the stressful environment, occurrence of arbuscular mycorrhizas has been reported in salt marsh plants in several countries, including the Netherlands (Rozema et al. 1986; Van Duin et al. 1990; Hildebrandt et al. 2001), Germany (Hildebrandt et al. 2001), Great Britain (Mason 1928; Read et al. 1976), France (Boullard 1958), United States (Cooke and Lefor 1990; Cooke et al. 1993; Hoefnagels et al. 1993; Brown and Bledsoe 1996), India (Sengupta and Chaudhuri 1990) and Pakistan (Khan 1974). No previous assessment has been made of Portuguese salt marshes.

In Mediterranean-type salt marshes, the characteristic climate (wet winters and hot and dry summers) causes salinity to fluctuate seasonally and along a gradient (Callaway et al. 1990). These zonal differences in abiotic characteristics cause plant zonation that, associated with plant phenology, may influence spatial and temporal patterns of arbuscular mycorrhizas.

Few studies have tried to identify the principal factors responsible for spatial and temporal variation in AM colonization of salt marsh plants. Plant phenology events and abiotic stresses, such as flooding and salinity, have been suggested (Rozema et al. 1986; Cooke and Lefor 1990; Van Duin et al. 1990; Brown and Bledsoe 1996). Brown and Bledsoe (1996) suggested that AM colonization is influenced by salinity in the higher marsh zone and by oxygen availability levels due to tidal inundation in the more flooded zones (low marsh). However, in a controlled experiment with the salt marsh plant *Aster tripolium*, Rozema et al. (1986) found a decrease in AM colonization with increased flooding but not with raised soil salinity.

This present study aimed to assess the AM status of plant species in a Portuguese salt marsh poor in plant diversity. The presence of AM infection in plant species posed, subsequently, two main questions: (1) did the incidence of AM colonization and spore density vary spatially according to different marsh zones with different tidal flooding regimes; (2) were AM colonization and spore density related to phenological events of plant species or to edaphic conditions? To answer these questions, plant species present in lower and higher areas of the salt marsh were evaluated in order to assess response of mycorrhizas to biotic and abiotic variables. Temporal and spatial variation in salinity, soil moisture, soil organic matter, redox potential and tidal flooding periods was assessed and compared to patterns of mycorrhizal colonization and spore density.

Materials and methods

Study site

This work was performed in a marsh of the Tagus estuary, Portugal. This is one of the largest estuaries of the European Atlantic coast, covering an area of 300 km^2 at low tide and 340 km^2 at spring high tide (Caçador et al. 1996). The climate is Mediterranean, characterized by warm and dry summers and cold and wet winters with 600–700 mm mean annual precipitation. The tides are semi-diurnal with tidal ranges from less than 1 m (neap) to more than 4 m (spring). The salinity varies between 5‰ upstream and 36‰ near the river outfall (Lisbon) (Caçador 1994). The estuary is exposed to high pollution due to the inflow of urban, industrial and agricultural effluents. The salt marsh vegetation acts as a sink concentrating high levels of heavy metals in the rhizosphere sediments (Caçador 1994). About 20 km^2 of the estuary are occupied by salt marshes, with Pancas being one of the largest, located on the left bank (38°49´N 08°57´W). This salt marsh links with agricultural lands in the higher marsh zone and to extensive intertidal mudflat areas in the lower marsh zone. The soils are clay (97% particle size <63 µm) and characteristics in the lower and higher marsh zone, respectively, are: pH 6.0 and 6.3; 2 and 3 g kg^{-1} total N; 900 and 792 mg kg^{-1} total P; 7,476 and 2,498 mg kg^{-1} total Ca; 1,813 and 1,902 mg kg^{-1} total K; 2,369 and 2,658 mg kg^{-1} total Mg. The halophytic vegetation of this marsh presents very low plant diversity compared with other northern European salt marshes. It also shows distinct and almost homogeneous plant species stand zones. The dominant species are *Spartina maritima* (Curtis) Fernald, *Arthrocnemum perenne* (Miller) Moss, *Arthrocnemum fruticosum* (L.) Moq., *Halimione portulacoides* (L.) Aellen and *Aster tripolium* L. The lower marsh zone (pioneer zone) of this salt marsh is regularly flooded with salt water and the higher marsh zone (more elevated site) is flooded by a channel system but only during high tides and high precipitation.

Two sampling areas of 300 m^2 were established, one in the lower marsh and another in the higher marsh zone. The plant species present were *Spartina maritima*, *Puccinellia maritima* (Huds.) Parl., *Halimione portulacoides*, *Arthrocnemum fruticosum* and *Aster tripolium* in the lower marsh sampling area and *Halimione portulacoides*, *Arthrocnemum fruticosum*, *Arthrocnemum perenne*, *Aster tripolium* and *Inula crithmoides* L. in the higher marsh sampling area.

Hours of tidal flooding

The tidal level above mean sea level required to flood each of the sampling areas was determined during some tides by means of stakes and on tide data published by the Hydrographical Institute. The mean time per day that vegetation was flooded at each sampling area was estimated using a model developed by Serôdio and Catarino (2000).

Plant root sampling

At each sampling area, plant roots were sampled at 2-month intervals between July 1996 and July 1997. For each survey, root systems and associated soil from three randomly selected plants of each species present in the sampling areas were carefully collected at low tide to a depth of approximately 20 cm. The samples were transported to the laboratory in plastic bags and stored at 4°C until processed.

Soil sampling

Soil samples from *Aster tripolium* stands in both sampling areas were also collected on the root sampling days. At each marsh zone, four sediment cores (7 cm in diameter, 18 cm long) were collected. After first removing the uppermost 1 cm sediment layer, the cores were transported to the laboratory in plastic bags and stored at 4°C until processed. In order to compare sediment redox potential between marsh zones, measurements were made in May and July 1997. After high tide, a platinum electrode was inserted into the rhizosphere sediment of *Aster tripolium* at a depth of 10 cm and the redox potential (Eh) was recorded after a stabilization period of 2 min. Four measurements were made in each zone on each sampling date.

AM root colonization

After careful rinsing with tap water, approximately 2 g (fresh weight) of roots was cleared and stained for analysis of colonization by AMF using a modified Phillips and Hayman (1970) procedure. The roots were cleared for 40–60 min (according to the species) in a 10% KOH solution at 90°C, placed in 10% HCl solution for 10 min and then stained with glycerol-trypan blue solution (0.05%) at 90°C for 20 min. The stained root samples were examined at ×45–100 magnification and quantification of root colonization by AMF was estimated by the gridline intersection method (Giovannetti and Mosse 1980). When it was difficult to discriminate between mycorrhizal structures and other fungi, the root pieces were examined microscopically at ×400 magnification.

Table 1 Time of tidal flooding of the vegetation, obtained from monthly values through the sampling periods, and edaphic characteristics and spore density in soils collected from *Aster tripolium* stands on seven sampling dates, except for redox potential which is from two sampling dates. The values are means with ranges in parentheses (*AT Aster tripolium*, *MPN* most probable number with confidence limits of 95%, *SM Spartina maritima*)

Variable	Low marsh	High marsh
Tidal flooding of the vegetation (min day^{-1})	94 (53–124)	9 (0–21)
Moisture (%)	77 (74–89)	45 (27–57)
Soil organic matter content (LOI) (%)	11.9 (10.5–13.1)	13.0 (9.2–18.1)
Salinity (‰)	18 (3–31)	12 (5–18)
Redox potential (mV)	194 (100–289)	402 (381–423)
Spore density (g^{-1} dry wt.)	6 (2–14)	13 (5–34)
MPN (propagules g^{-1} dry wt.) (AT)	0.75 (0.35–1.60)	1.74 (0.81–3.72)
MPN (propagules g^{-1} dry wt.) (SM)	0.17 (0.08–0.35)	

AMF spore population

AMF spores were isolated by wet sieving followed by sucrose gradient centrifugation (Daniels and Skipper 1982). From each core sample of the *Aster tripolium* rhizosphere, 50 g of sediment was sieved. The fraction collected in the last sieve (53 µm) was centrifuged in a 60% (w/v) sucrose solution for 2 min at 3,000 rpm. Spores were collected from the water-sucrose interface and poured through a sieve, rinsed with distilled water and transferred to a Petri dish. Spores were examined under a dissecting microscope and each morphotype quantified. Spore density was expressed as spore number per g dry weight of soil. Some spores of each morphotype were then prepared permanently for identification. Taxonomic identifications were made according to Banque Européenne des Glomales www.bio.ukc.ac.uk/beg.htm and Schenck and Pérez (1990).

Spore viability tests were performed to examine differences between marsh zones in samples isolated in July 1997. From each sample, 40 isolated spores were randomly collected, placed in iodonitrotetrazolium (INT) solution (1 mg ml^{-1}) and left at room temperature for 48 h (Walley and Germida 1995). Spores were checked for viability and the results expressed as viable percentage.

Soil analysis

Aliquots (10 g) of each soil core sample were heated at 80°C for 48 h then weighed. Soil moisture content was calculated as percent oven-dry weight of soil. Organic matter content was determined in 2 g of dried soil without roots of each sample by loss of ignition (LOI) at 600°C for 2 h (Otte 1991). Soil solution salinity was calculated in each soil sample. Aliquots (2 g) of dried soil were ground, sieved and diluted with deionized water (1:5). After overnight shaking, the soil solution was filtered and its salinity determined with a hand-held refractometer.

AM inoculum potential

The total number of AMF propagules in the salt marsh sediment was estimated using a soil-dilution method, the "most probable number" (MPN) method (Porter 1979). Sediments from the root zone of *Aster tripolium*, from higher and lower marsh zones, and from *Spartina maritima* from the lower marsh zone, collected in September 1996, were used. Each sediment sample was diluted with sterilized sand in a serial dilution of 4^0 to 4^{-6}. The control contained only sterilized sand. The mixture was placed in 120-ml containers and seedlings of sorghum were used as bait plants for AMF. Five replicates were used for each dilution level. The plants were grown in a greenhouse with a photoperiod of 14 h, 26/20°C (day/night air temperature) and a photosynthetic photon flux density of 200 µmol m^{-2} s^{-1} and watered as necessary. After 8 weeks, plant roots were collected and analysed for AM colonization as described above. The number of propagules was calculated according to Sieverding (1991).

Statistical analysis

Prior to statistical analysis, mycorrhizal colonization data were arcsin square root-transformed and data for spore number, LOI, soil salinity and number of tidal flooding hours of the vegetation over the last 14 days (number of days corresponding to a tidal cycle) were log-transformed (Zar 1984). Data on mycorrhizal colonization and spore densities were analysed by one-way analysis of variance (ANOVA) to test for differences between the two marsh zones and between sampling dates within each marsh zone. Significant results ($P<0.05$) were analysed by Duncan's test. MPN data were expressed with 95% confidence limits. Pearson product-moment correlation analysis was used to relate AM colonization and spore density with soil moisture, LOI, soil salinity and number of tidal flooding hours of the vegetation in each sampling date of *Aster tripolium* roots or soil rhizosphere in each marsh zone separately. Statistica software (StatSoft, Tulsa, USA) was used for this statistical analysis.

Results

Environmental information and soil analyses

In Pancas, lower marsh vegetation was much more flooded due to tides than higher marsh vegetation, as expected (Table 1). During June and December 1996 and May 1997, the higher marsh vegetation was not tidally flooded. The lower marsh exhibited higher mean soil moisture and salinity, and lower organic matter content (LOI) than the higher marsh zone (Table 1). Soil moisture is more influenced by tide than by seasonal precipitation. Most precipitation in the study period occurred in the winter months of December and January (data not shown). While the lower marsh zone showed a marked seasonal variation of salinity, the higher marsh zone did not. The lower marsh zone exhibited an average redox potential much lower than the higher marsh zone (Table 1).

AM root colonization

AMF were only present in root samples of three salt marsh plant species, *Aster tripolium*, *Inula crithmoides* and *Puccinellia maritima*, in both marsh zones (Table 2). The level of AM colonization in *Inula crithmoides* was similar to that in *Aster tripolium*, while *Puccinellia maritima* displayed little or no colonization (1% on aver-

Table 2 Range of root length colonization by AMF (%), with sampling every 2 months from July 1996 to July 1997 in all plant species present in the lower and higher marsh zones. *n*=21, except for *Inula crithmoides* where *n*=18 (*A* arbuscules, *V* vesicles)

Zone	Plant species	Plant family	Root length colonized (%)	Mycorrhizal structures
Lower	*Aster tripolium*	Asteraceae	1–56	VA
	Puccinellia maritima	Poaceae	0–6	V
	Spartina maritima	Poaceae	0	Absent
	Halimione portulacoides	Chenopodiaceae	0	Absent
	Arthrocnemum fruticosum	Chenopodiaceae	0	Absent
Higher	*Aster tripolium*	Asteraceae	3–63	VA
	Inula crithmoides	Asteraceae	6–62	VA
	Halimione portulacoides	Chenopodiaceae	0	Absent
	Arthrocnemum fruticosum	Chenopodiaceae	0	Absent
	Arthrocnemum perenne	Chenopodiaceae	0	Absent

Table 3 Summary of one-way analysis of variance of the effect of marsh zone (lower and higher) and sampling date on root AM colonization and soil spore density for *Aster tripolium* and of the effect of sampling date on root AM colonization for *Inula crithmoides*

		d.f.	Colonization *F* value	Spore density *F* value
Marsh zones	*Aster tripolium*	1	0.89	17.64***
Sampling date	*Aster tripolium* (low marsh)	6	1.51	4.79**
	Aster tripolium (high marsh)	6	3.70*	11.72***
	Inula crithmoides (high marsh)	5	6.54**	

*P<0.05; **P<0.01; ***P<0.001

Table 4 Pearson product-moment correlation coefficients at each sampling date between % of AM colonization and spore density in *Aster tripolium* samples of each marsh zone and soil moisture, soil organic matter content (LOI), soil salinity and number of tidal flooding hours of the vegetation over the last 14 days (number of days corresponding to a tidal cycle). Coefficients were calculated from means obtained for each sampling date (*n*=7)

		Moisture	LOI	Salinity	Flooding hours
Low marsh	% AM	–0.316	0.076	–0.007	0.776*
	Spore density	–0.119	0.590	–0.341	0.130
High marsh	% AM	–0.199	–0.259	0.820*	0.123
	Spore density	0.430	0.299	0.183	–0.219

*P<0.05

age). Mycorrhizal colonization was not observed in roots of *Spartina maritima*, *Halimione portulacoides*, *Arthrocnemum fruticosum* and *Arthrocnemum perenne* from any marsh zone on any sampling date (Table 2). In *Aster tripolium* and *Inula crithmoides*, both arbuscules and vesicles were always observed. Arbuscules were never observed in *Puccinellia maritima* (Table 2). This plant species was only colonized by AMF on two sampling dates, January and March, but with very low values of 5% and 2%, respectively (Fig. 1).

Aster tripolium was the only AMF-colonized plant species present in both marsh zones (Table 2). One-way analysis of variance was performed on colonization data to examine temporal and/or spatial patterns (Table 3). No significant variation in colonization as a function of salt marsh zone sampled was observed. On the contrary, significant variation in colonization was found in the higher marsh zone for both *Aster tripolium* and *Inula crithmoides* (Fig. 1 and Table 3). In this zone, colonization was higher in summer and autumn than in winter and spring sampled months (Fig. 1); this was particularly evident for *Aster tripolium*. In the lower marsh zone, colonization of *Aster tripolium* did not differ significantly between sampling dates (Fig. 1 and Table 3).

AMF spore population

The AMF spores found in the soil samples belonged exclusively to *Glomus* species and all of the species were present at both salt marsh zones. Spores of *G. geosporum* (Nicol. and Gerd.) Walker were the most common, accounting for more than 84% of the total AMF spore population. *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe was also present, along with two other unidentified *Glomus* species. Spore density varied both spatially and temporally (Tables 1 and 3). The patterns of temporal variation in spore density in the two salt marsh zones were similar (Fig. 2). Spores from both marsh zones were highly viable: 67% (±3 SE) in the higher marsh zone and 71% (±3 SE) in the lower marsh zone.

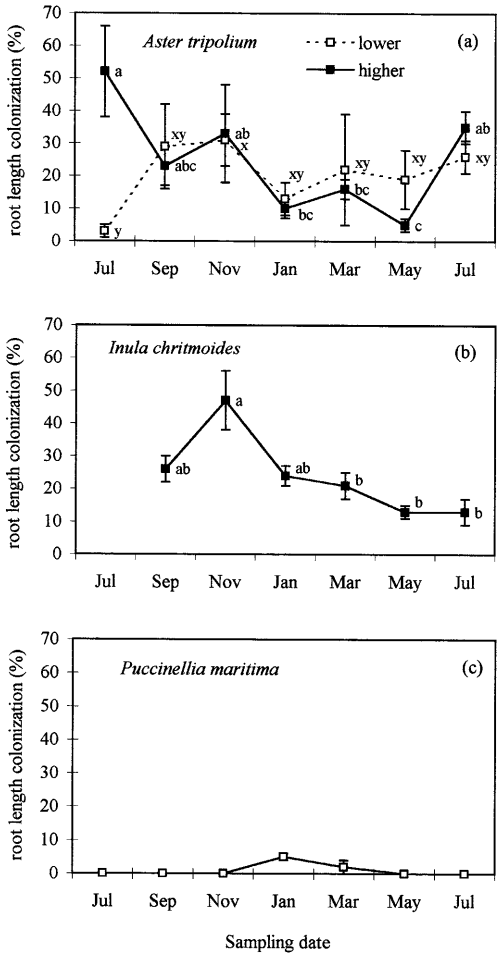

Fig. 1 Temporal and spatial variation of root length colonization by arbuscular mycorrhizal (AM) fungi on **a** *Aster tripolium* plants of the lower and higher marshes zones, **b** *Inula crithmoides* plants of the higher marsh zone and **c** *Puccinellia maritima* plants of the lower marsh zone. Values are means of three replicates per month and marsh zone ± SE. In each graph and within each marsh zone, values followed by the same letter are not significantly different at $P<0.05$ (Duncan's test)

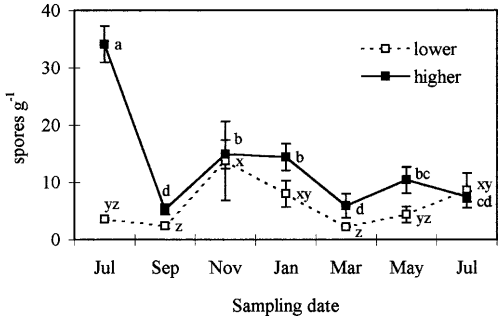

Fig. 2 Temporal and spatial variation on spore density (per g dry soil) in rhizosphere soil of *Aster tripolium* from the lower and higher marshes zones. Values are means of four replicates per month and marsh zone ±SE. Within each marsh zone, values followed by the same letter are not significantly different at $P<0.05$ (Duncan's test)

AM inoculum potential

The results of mycorrhizal infectivity determined by a soil-dilution method showed differences between soils tested. The soil collected from *Aster tripolium* stands was significantly more infective than the soil from *Spartina maritima* (Table 1) and that collected from the higher marsh zone had more infective propagules than that from the lower marsh zone (Table 1). The *Spartina maritima* soil had some infective propagules, although roots were never colonized (Table 2).

Effect of edaphic conditions

AM colonization and spore density were not related to soil moisture, LOI and salinity, except in the higher marsh zone, where AM colonization was positively correlated with soil salinity (Table 4). AM colonization in the low marsh was significantly positively correlated with the number of tidal flooding hours of the vegetation over the previous 14 days before sampling took place (Table 4).

Discussion

This study showed that arbuscular mycorrhizas were present in the Pancas salt marsh of the Tagus estuary and that colonization and propagules occurred in higher and lower marsh zones. However, not all plant species were colonized and only the species belonging to the Asteraceae family, *Aster tripolium* and *Inula crithmoides*, displayed high levels of colonization. The presence of a considerable level of colonization in *Aster tripolium* roots has also been reported in salt marshes by Rozema et al. (1986), Van Duin et al. (1989) and Hildebrandt et al. (2001). AM colonization of *Puccinellia maritima* occurred at very low levels and only in winter months, a period of slow plant growth. These data, together with the absence of arbuscules, suggest that this plant species is not functionally mycorrhizal.

All the plant species present in Pancas that were not colonized belong to the Chenopodiaceae family, with the exception of *Spartina maritima*, a member of Poaceae. Chenopodiaceae is usually referred to as non-mycorrhizal (Harley and Smith 1983). However, AM has also been found in some plants of this family, not only in other salt marshes but also in other types of ecosystems (e.g. Rozema et al. 1986; Van Duin et al. 1989; Sengupta and Chaudhuri 1990; Johnson-Green et al. 1995; Barrow et al. 1997; Hildebrandt et al. 2001). *S. maritima* may be referred to as a non-mycorrhizal species together with *S. alterniflora* and *S. anglica* (Rozema et al. 1986; Hildebrandt et al. 2001). In contrast, mycorrhizas have been observed in the *Spartina* species *S. patens* (Cooke and Lefor 1990; Hoefnagels et al. 1993) and *S. cynosuroides* (Hoefnagels et al. 1993).

AMF species diversity was very low, accompanying the low plant diversity of this salt marsh ecosystem

(Caçador 1994). Only *Glomus* species were found. *G. mosseae*, observed in Pancas salt marsh, has also been reported in other marshes (Sengupta and Chaudhuri 1990; Brown and Bledsoe 1996). Interestingly, the most abundant *Glomus* species was *G. geosporum*, as was previously found in Baltic salt marshes (Hildebrandt et al. 2001).

The evaluation of plant species present in two different sites in the marsh showed that the occurrence of AM colonization did not depend on position within the salt marsh nor, consequently, on the tidal flooding regimes which create different levels of anoxia around the roots, nor on salt gradients. This suggests that AM fungal distribution does not coincide with the zonation pattern of vegetation. The absence of any significant negative correlation between AM and salinity, moisture and tidal inundation of the vegetation seems to suggest that AMF can tolerate the flooding and salinity levels in the Pancas salt marsh.

The evaluation of biotic and abiotic factors responsible for AM seasonal patterns has shown that plant phenology is related to AM colonization in *Aster tripolium* and *Inula crithmoides* and spore density, more clearly in the higher marsh zone. The highest levels of colonization corresponded to the period of the highest plant growth and the flowering period in both species, summer and autumn, respectively, in agreement with the results of Van Duin et al. (1989). Similar seasonal patterns in spore density have been observed in aquatic plants (Khan 1974) but not for the halophyte *Jaumea carnosa* (Brown and Bledsoe 1996). These differences may be related either to the different behaviour of each AM fungal species, even in similar ecosystems (Klironomos et al. 1993), or to the influence of different environmental conditions.

AM colonization and spore distribution may also be influenced by tides, since *S. maritima*, a non-mycorrhizal plant which occupies a frontal isolated zone in the marsh, showed a reasonable number of propagules and a significantly positive correlation between AM colonization and tidal flooding hours.

In the lower marsh zone, the lack of seasonality and the small variation in the AM colonization in *Aster tripolium* suggest that either other factors influence fungal development more than plant phenology or other factors dilute its influence. Brown and Bledsoe (1996) also did not find significant seasonal variation in colonization in the most waterlogged zone of a salt marsh.

The presence of AM colonization in few plant species and in species that have a limited cover in all salt marsh vegetation suggests that mycorrhizas are of limited relevance for plant communities of the Tagus estuary salt marshes. However, since *Aster tripolium* is a species which occurs in less consolidated sediments and in more flooded areas, the presence of AM in this plant suggests that mycorrhizas have an important role during plant establishment and community development, at least, of this salt marsh species. Compared with observations in other salt marsh ecosystems (e.g. Rozema et al. 1986; Van Duin et al. 1990; Hildebrandt et al. 2001), it may be asked why so few plant species are mycorrhizal in this Portuguese salt marsh. Hypotheses that can be formulated for further study include the existence of high levels of phosphorous and the presence of few potential mycorrhizal plant families. In these harsh environments, the interaction between host plant species and abiotic factors is so complex that it is difficult to explain AM colonization patterns in terms of general factors common to all salt marshes.

The results of this study emphasize that host plant rather than environmental stress factors are responsible for AMF distribution. Like the plant species, AMF may have developed adaptive strategies to tolerate this stressful environment. The results of this study raise questions important to our understanding of the role of mycorrhizas in the ecology of salt marshes.

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References

Armstrong W, Wright EJ, Lythe S, Gaynard TJ (1985) Plant zonation and the effects of the spring-neap tidal cycle on soil aeration in a Humber salt marsh. J Ecol 73:323–339

Armstrong W, Justin SHFW, Beckett PM, Lythe S (1991) Root adaptation to soil waterlogging. Aquat Bot 39:57–73

Barrow JR, Havstad KM, McCaslin BD (1997) Fungal root endophytes in fourwing saltbush, *Atriplex canescens*, on arid rangelands of southwestern USA. Arid Soil Res Rehabil 11:177–185

Boullard B (1958) Les mycorhizes des espèces de contact marin et de contact salin. Revu Mycol 23:282–317

Brown AM, Bledsoe C (1996) Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. J Ecol 84:703–715

Caçador I (1994) Acumulação e retenção de metais pesados nos sapais do estuário do Tejo. PhD thesis, University of Lisbon, Portugal

Caçador I, Vale C, Catarino F (1996) Accumulation of Zn, Pb, Cu, Cr and Ni in sediments between roots of the Tagus Estuary salt marshes, Portugal. Estuar Coast Shelf Sci 42:393–403

Callaway RM, Jones S, Ferren WR, Parikh A (1990) Ecology of a Mediterranean-climate estuarine wetland at Carpinteria, California: plant distributions and soil salinity in the upper marsh. Can J Bot 68:1139–1146

Cooke JC, Lefor MW (1990) Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, Connecticut, USA. Environ Manage 14:131–137

Cooke JC, Butler RH, Madole G (1993) Some observations on the vertical distribution of vesicular-arbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. Mycologia 85:547–550

Daniels BA, Skipper HA (1982) Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minn, pp 29–35

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic, London

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 MH, Broome SW, Shafer SR (1993) Vesicular-arbuscular mycorrhizae in salt marshes in North Carolina. Estuaries 16:851–858

Jindal V, Atwal A, Sekhon BS, Singh R (1993) Effect of vesicular-arbuscular mycorrhizae on metabolism of moong plants under sodium chloride salinity. Plant Physiol Biochem 31:475–481

Johnson-Green PC, Kenkel NC, Booth T (1995) The distribution and phenology of arbuscular mycorrhizae along an inland salinity gradient. Can J Bot 73:1318–1327

Juniper S, Abbott LK (1993) Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45–57

Khan AG (1974) The occurrence of mycorrhizas in Halophytes, Hydrophytes and Xerophytes, and of Endogone spores in adjacent soils. J Gen Microbiol 81:7–14

Khan AG, Belik M (1995) Occurrence and ecological significance of mycorrhizal symbiosis in aquatic plants. In: Varma A, Hock B (eds) Mycorrhiza. Structure, function, molecular biology and biotechnology. Springer, Berlin Heidelberg New York, pp 627–666

Klironomos JN, Moutoglis P, Kendrick B, Widden P (1993) A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. Can J Bot 71:1472–1480

Mason E (1928) Note on the presence of mycorrhiza in the roots of salt marsh plants. New Phytol 27:193–195

Miller SP (1999) Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. New Phytol 145:145–155

Naidoo G, McKee KL, Mendelssohn IA (1992) Anatomical and metabolic responses to waterlogging and salinity in *Spartina alterniflora* and *S. patens* (Poaceae). Am J Bot 79:765–770

Otte ML (1991) Heavy metals and arsenic in vegetation of salt marshes and floodplains. PhD thesis, Vrije Universiteit, Amsterdam

Pennings SC, Callaway RM (1992) Salt marsh plant zonation: the relative importance of competition and physical factors. Ecology 73:681–690

Pfeiffer CM, Bloss HE (1988) Growth and nutrition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. New Phytol 108:315–321

Phillips JM, Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Porter WM (1979) The “most probable number” method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust J Soil Res 17:515–519

Read J, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. New Phytol 77:641–653

Rozema J, Arp W, Van Diggelen J, Van Esbroek M, Broekman R, Punte H (1986) Occurrence and ecological significance of vesicular-arbuscular mycorrhiza in the salt marsh environment. Acta Bot Neerl 35:457–467

Ruiz-Lozano JM, Azcón R, Gómez M (1996) Alleviation of salt stress by arbuscular mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol Plant 98:767–772

Schenck NC, Pérez Y (1990) Manual for the identification of VA mycorrhizal fungi, 3rd edn. Synergistic, Gainesville, Fla

Sengupta A, Chaudhuri S (1990) Vesicular-arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges River delta in West Bengal (India). Plant Soil 122:111–113

Serôdio J, Catarino F (2000) Modelling the primary productivity of intertidal microphytobenthos: time scales of variability and effects of migratory rhythms. Mar Ecol Prog Ser 192:13–30

Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystem. Technical Cooperation, Eschborn, Germany

Van Duin WE, Rozema J, Ernst WHO (1990) Seasonal and spatial variation in the occurrence of vesicular-arbuscular (VA) mycorrhiza in salt marsh plants. Agric Ecosyst Environ 29:107–110

Walley FL, Germida JJ (1995) Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tertrazolium salts as vital stains. Mycologia 87:273–279

Zar JH (1984) Biostatistical analysis. Prentice Hall, Englewood Cliffs, NJ