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Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi

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Abstract Colonisation of plant roots by some arbuscular mycorrhizal (AM) fungi is reduced in the presence of sodium chloride (NaCl), probably due to a direct effect of NaCl on the fungi. However, there appear to be differences between the fungi in their ability to colonise plants in the presence of NaCl. This experiment tested the hypothesis that propagules of different isolates and species of AM fungi from saline and nonsaline soils would differ in their ability to germinate and grow in the presence of NaCl in the soil solution. Spores or pieces of root colonised by a range of AM fungi were incubated between filters buried in soil to which NaCl had been added at concentrations of 0, 150 or 300 mM in the soil solution. At regular intervals, filters were removed from the soil and both the percentage of propagules which had germinated and the length of proliferating hyphae were determined. Germination of spores of AM fungi studied was delayed in the presence of NaCl, but the fungi differed in the extent to which germination was inhibited. Two isolates of *Scutellospora calospora* reached maximum germination in 300 mM NaCl, but neither of two isolates of *Acaulospora laevis* germinated in the presence of NaCl. Germination of spores of the other fungi, including some isolated from saline soil, fell between these extremes. For some fungi, the specific rate of hyphal extension was reduced by NaCl. For others, the specific rate of growth was similar in the presence of NaCl to that in the control treatment, but overall production of hyphae was reduced in the NaCl treatments because germination was reduced.

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Introduction

Arbuscular mycorrhizas (AM) occur naturally in saline environments (Khan 1974; Allen and Cunningham 1983; Pond et al. 1984; Rozema et al. 1986; Ho 1987; Cooke and Lefor 1990; Sengupta and Chaudhuri 1990; van Duin et al. 1989; Hoefnagels et al. 1993; Johnson-Green et al. 1995) and have been shown to increase plant yield in saline soil (Hirrel and Gerdemann 1980; Ojala et al. 1983; Pond et al. 1984; Poss et al. 1985; Pfeiffer and Bloss 1988).

Some studies have shown that soil salinity reduces colonisation by AM fungi (Hirrel and Gerdemann 1980; Ojala et al. 1983; Poss et al. 1985; Duke et al. 1986; Rozema et al. 1986; Dixon et al. 1993; Johnson-Green et al. 1995; McMillen et al. 1998; Juniper 1996), and other studies have reported that colonisation was not reduced by salinity (Levy et al. 1983; Hartmond et al. 1987). Juniper (1996) found that two species (*Gigaspora decipiens* Hall and Abbott and *Scutellospora calospora* (Nicol. and Gerd.) Walker and Sanders) differed in the extent to which the presence of NaCl inhibited colonisation. Other studies have shown that some AM fungi adapt to different environmental conditions (Lambert et al. 1980; Hayman 1982; Gianinazzi-Pearson et al. 1985; Henkel et al. 1989; Stahl and Christensen 1991). Thus, differences between observations by different workers may be partly a reflection of differences between the fungi used and their ability to adapt to various environments. Most studies on mycorrhizas and soil salinity to date have not considered these differences. The presence of excess salts in the soil solution may limit the growth of an organism due to specific ion toxicity or osmotic stress. These factors will differ in relative importance depending on the species and concentration of ions and the tolerance to both stresses of the organism in question (Sterne et al. 1976; Brownell and Schneider 1985). Either stress may be more limiting at some stages of the life history of an organism compared with other stages.

Arbuscular mycorrhizal fungi are obligate symbionts. This imposes a severe limitation on our ability to distinguish their response to environmental variables from those to plant-mediated factors. However, spore germination and early growth of hyphae from spores can be studied without interactions with the plant (Daniels and Graham 1976; Hepper 1979; Daniels and Trappe 1980; Elias and Safir 1987; Gianinazzi-Pearson et al. 1989). Before primary colonisation of a plant root by an AM fungus can commence, fungal propagules in the soil must become hydrated and activated and produce a germ tube. One or more hyphae then extend through the soil and encounter a receptive root. The relative timing of these stages is highly dependent on the characteristics of the individual fungus. The extent to which colonisation by AM fungi is reduced in the presence of NaCl is dependent on the timing of the observation, such that inhibition is more noticeable in the early than in the later stages of the symbiosis (McMillen et al. 1998; Juniper 1996). It is, therefore, likely that the inhibition in these cases was due to an effect on primary rather than secondary (Wilson 1984) colonisation.

The fungi may also differ in the extent to which the early stages of growth are affected by the presence of NaCl. For example, Juniper (1996) found that the early colonisation of roots by *G. decipiens* was more inhibited by NaCl than was colonisation by *S. calospora*. It is not known whether this difference between the two species reflects their differing response to the effect of NaCl on germination of spores or on the proliferation of hyphae. Hirrel (1981) and Estaun (1989) reported that the presence of NaCl directly inhibited the germination of spores of AM fungi in solution and in agar, respectively. Hirrel's observations suggest that the presence of NaCl may have delayed (i.e. increased the duration of the stages of germination preceding germ tube emergence) rather than prevented germination. However, this point has not been resolved. Hirrel also noted that the presence of chloride ions increased the frequency of branching in *Gigaspora margarita*, but no data were given. Little is currently known of the separate effects of NaCl on hyphal extension or morphology.

Spores are not the only propagules of AM fungi. Some of these fungi can grow from fragments of hyphae in the soil or from pieces of dried colonised root (Tommerup and Abbott 1981). The relative tolerance of different types of propagules of the same fungal isolate to environmental stresses is not known. These tolerances may differ because of differences in wall structure between intra- and extra-radicle fungal structures and differing energy stores in propagules of disparate size.

The aim of this experiment was to determine the effect of NaCl in the growth medium on the germination of propagules and proliferation of hyphae of a variety of AM fungi isolated from various soils. It was hypothesized that in the presence of NaCl, the total number of propagules that germinated would be reduced and the rate of hyphal extension would be limited. Furthermore, it was expected that a range of tolerance would be observed (i.e. species, isolates and different types of propagule of the fungi would differ in their response to NaCl).

Materials and methods

General design

Spores or dry pieces of colonised root for a range of AM fungi established in pot culture were incubated between filters buried in soil moistened with a solution of either 0, 150 or 300 mM sodium chloride (NaCl). There were three replicates of each treatment for each of four harvest times. At regular intervals, replicate filters were stained and opened. The number of propagules with proliferating hyphae and the length of hyphae on each filter were determined.

Table 1 Details of isolates of arbuscular mycorrhizal fungi used in this study

Fungal species/authority	Isolate numbers		Original soil salinity
Acaulospora laevis Gerd. & Trappe	WUM 11-1	INVAM AU211	Nonsaline
Acaulospora laevis	WUM 11-4	INVAM AU221	Nonsaline
Archaeospora trappei (Ames & Lindermann) Morton & Redecher	WUM 19-1	INVAM AU219-1	Nonsaline
Gigaspora decipiens Hall & Abbott	WUM 6-1	INVAM AU102-3	Nonsaline
Glomus invermaium Hall	WUM 10-1	INVAM AU210	Nonsaline
Glomus sp.	WUM 2-1	INVAM AU202	Nonsaline
Glomus sp. Curragh 1	WUM 21-1		Saline
Glomus sp. Curragh 2	WUM 23-1		Saline
Glomus sp. Kakadu 1	WUM 58		Nonsaline
Glomus sp. Kakadu 2	WUM 59		Nonsaline
Scutellospora calospora (Nicol. & Gerd.) Walker and Sanders	WUM 12-2	INVAM AU212	Nonsaline
Scutellospora calospora	WUM 12-3	INVAM AU222	Nonsaline

Most isolates have two numbers: a number (WUM) from The University of Western Australia and an International Collection of Vesicular–Arbuscular Mycorrhizal Fungi (INVAM) number from the culture collection at INVAM, West Virginia University

Fungal isolates

The fungal isolates included in this study are shown in Table 1. All fungi had been pot-cultured at The University of Western Australia and maintained as described by Jasper et al. (1988); the pot cultures were at least 2 years old. For *Glomus invermaium* Hall and *Glomus* sp. Curragh 2, proliferation of hyphae from colonised pieces of root was assessed in addition to assessment of germination and growth of hyphae from spores. Air-dried roots from pot culture soil were ground into approximately 1-mm pieces and similar-sized pieces were selected and incubated for spores. For *G. invermaium*, the roots of the pot culture plants (*Sorghum* sp.) were 75% colonised. For *Glomus* sp. Curragh 2 the roots of the pot culture plants (*Trifolium subterraneum* L.) were 50% colonised.

Soil preparation and treatments

The surface, 10 cm (A horizon), was collected from a brown sand (Hill et al. 1979) under native vegetation at Lancelin, Western Australia ('Lancelin sand'). The air-dried soil was sieved to pass through a 4-mm mesh, steamed at 100°C for 1 h on each of two successive days and then dried at 60°C for 2 days prior to storage. Immediately before use, the soil was watered to field capacity (10% gravimetric water content) with a solution of NaCl (0, 150 or 300 mM) in water and was thoroughly mixed. These treatments correspond to 0, 0.88 and 1.75-g NaCl kg^{-1}, respectively.

Procedure

Spores were removed from soil from each air-dried pot-culture by wet sieving and decanting, followed by sequential centrifugation with kaolin and 50% sucrose (Tommerup and Kidby 1979). After thorough rinsing in deionised water, the spores were stored on filter paper at 4°C for a maximum of 24 h before use. No sterilants were used. For each replicate, 20 clean, uniform spores were transferred with ultra-fine forceps onto a sterile Millipore (0.45 μm pore size) filter marked into 3.6 mm squares. Another filter was placed over the spores to create a 'sandwich' (Tommerup and Kidby 1979). Each sandwich was placed in a 150-ml plastic tub containing 50 g of moist soil and covered with 50 g of the same soil and gently tamped to achieve a volume of approximately 70 ml. The tubs were closed with airtight lids and incubated in the dark at 20°C. *Glomus* spp., Kakadu 1 and Kakadu 2, did not germinate at this temperature and were later incubated at 30°C. Four harvests were conducted for each fungus, but the exact intervals between harvests differed slightly. At each harvest, the filter sandwiches were removed from the soil, brushed free of adhering particles, placed on dry filter paper in Petri dishes, and flooded with lactic-glycerol blue (modified from Phillips and Hayman 1970 by Gazey et al. 1992) for 48 h, after which they were blotted and allowed to dry in air. Each pair of filters was carefully separated and examined under a dissecting microscope at ×300 magnification.

Spores were considered to have germinated if the length of the germ tube exceeded the diameter of the spore. Hyphae in root pieces were considered to have regrown when proliferating hyphae visibly extended from the cut ends of the pieces of root. Total length of hyphae on each filter was estimated after counting the number of intercepts between hyphae and the grid lines on the filter (Tennant 1975). The length of hyphae on the two filters from a sandwich was pooled as one replicate and expressed as length per germinated spore or piece of root.

Due to uncertainty about the exact timing of germination of each of the fungi, precise rates of propagules specific hyphal extension could not be calculated. However, for each fungus, an approximation of the specific rate (R) of extension up to each harvest in each treatment was obtained by the formula:

$$R^{*}(L_2 - \mathrm{ML}_1)/(T_2 - T_1)$$

where R is specific rate of extension, T is days, L_2 is length of hyphae per germinated spore at T_2, and ML_1 is the mean length of hyphae per germinated spore for that treatment at T_1. The calculation was made for each harvest in which germination was observed. The mean values of R for each harvest were used to calculate a grand mean of R for each treatment, for each fungus. There were unequal numbers of data points contributing to each grand mean, and therefore, these data were not analysed statistically.

Statistical analyses

The fungi were treated separately in the analyses. For each harvest, treatments were compared by one-way ANOVA followed by pairwise comparisons of means after calculation of Fisher Least Significant Difference (LSD) and Dunnett t statistic. Percentage data were transformed by arcsin prior to analysis. No other transformations were necessary.

Results

Germination

Maximum germination and timing of germination differed greatly between the fungi. Maximum germination ranged from 15 to 90%, with one *Glomus* sp. reaching maximum germination by 7 days while *Archaeospora trappei* (Ames & Linderman) Morton & Redecker did not commence germination until after 14 days (Table 2). *Glomus* spp. Kakadu 1 and 2 had not germinated after 21 days at 20°C, but commenced germination within 7 days after transfer to 30°C. The experiment was repeated with these fungi at 30°C and, at this temperature, spores in the control

treatment reached maximum germination at 7 days after the start of the experiment.

For all fungi, maximum germination of spores was first reached in the 0-mM NaCl controls and delayed in varying degrees in the presence of NaCl (Table 2). Both isolates of *S. calospora* were the only fungi to reach maximum spore germination in the 300 mM treatment. For all other fungi, including spores of *Glomus* spp. isolated from the saline Curragh soil, the percentage of spores that germinated in the 300 mM treatment during the course of the experiment

Table 2 Percentage of maximum germination of spores of arbuscular mycorrhizal fungi incubated between millipore filters in the presence of 0-, 150- and 300-mM NaCl

Fungal isolate	Percentage of spores germinated (%)	Days	NaCl treatment (mM)		
			0	150	300
Acaulospora laevis WUM 11-1	73	14	0	0	0
		21	57	0	0
		28	52	0	0
		35	100	0	0
Acaulospora laevis 4 (WUM 11-4)	87	14	27	0	0
		21	90	0	0
		28	100	0	0
		35	98	0	0
Archaeospora trappei (WUM 19-1)	78	7	28	0	0
		11	57a	32b	0
		14	100a	43b	0
		21	81a	70a	8b
Gigaspora decipiens (WUM 6-1)	88	7	45a	21b	0
		9	87a	19b	0
		14	75a	53b	0
		21	100a	64b	6c
Glomus sp. (WUM 2-1)	48	9	21a	27a	0
		12	35a	38a	0
		14	62a	35a	0
		21	100a	97a	3b
Glomus sp. Kakadu 1	78	7	30	0	0
		14	55	0	0
		21	100a	2b	0
		28	94a	23b	0
Glomus sp. Kakadu 2	90	7	100	0	0
		14	96a	6b	0
		21	93a	22b	11b
		28	94a	50b	17c
Glomus sp. Curragh 1 (WUM 21-1)	32	7	42a	32a	0
		14	53a	16b	0
		21	84a	37b	0
		28	100a	37b	0
Glomus sp. Curragh 2 (WUM 23-1)	15	7	60a	40a	0
		14	80a	80a	0
		21	100a	80a	0
		28	100a	100a	0
Scutellospora calospora (WUM 12-2)	65	7	69a	62a	11b
		9	64a	59a	64a
		14	87a	74a	69a
		21	97a	100a	97a
Scutellospora calospora (WUM 12-3)	65	7	90a	74b	15c
		9	58a	62a	54a
		14	67a	72a	90a
		21	100a	85a	92a

Value for each treatment is a percentage of the maximum germination observed overall for that fungus. Within each row, values not followed by the same letter are significantly different ($p<0.05$). No statistical comparison was made within columns

was negligible. Spores of neither isolate of *Acaulospora laevis* Gerd. and Trappe germinated in either 150 or 300 mM NaCl within 35 days. A subsequent attempt was made to elicit germination of spores of *A. laevis* in soil watered with 50 mM NaCl, but no germination occurred within 40 days. Where two isolates from the same species were studied, the results were relatively consistent. Both isolates of *S. calospora* germinated in 300-mM NaCl, and neither isolate of *A. laevis* germinated in the presence of NaCl.

Proliferation of hyphae from colonised root pieces of *Glomus* sp. Curragh 2 (Table 3) was more robust in the presence of NaCl than was spore germination in the same isolate (Table 2) because intra-root propagules germinated in the presence of 300-mM NaCl but spores did not. NaCl-induced inhibition of growth of *G. invermaium* hyphae from colonised root pieces was not observed.

Hyphal extension

For all the fungi, the length of hyphae that had been produced per germinated spore at any given time was reduced in the presence of NaCl (Figs. 1 and 2; Table 4). For some fungi, the reduction in the amount of hyphae in the presence of increasing concentrations of NaCl was primarily associated with a delay in spore germination, while for other fungi, the specific *rate* of hyphal production was also substantially reduced in the presence of NaCl. Thus, for *G. decipiens* and both isolates of *S. calospora* (see Fig. 1a–c), increasing the concentration of NaCl clearly reduced the specific rate of hyphal production, whereas for *Glomus* sp., Kakadu 1, and *Glomus* sp. (isolate WUM 2) it did not (Table 4). For the latter, reduced lengths of hyphae in the NaCl treatments relative to the controls appeared to have been due to delayed germination rather than to an effect on the specific rate of growth.

Despite initial inhibition of hyphal extension in the salinity treatments, the rate of hyphal extension from spores of *Glomus* sp. (isolate WUM 2) in 150-mM NaCl increased relative to the control after 14 days, and the total length of hyphae was similar to that in the control at 21 days (Table 4). However, this was the only fungus for which this was observed. For all other fungi, the total length of hyphae per germinated spore in NaCl treatments was reduced relative to the control throughout the experiment.

For root pieces colonised by *G. invermaium*, there was little difference between lengths of hyphae formed per root piece in the three treatments, at any time. The hyphae barely grew after 7 days, so the total amount of hyphae remained almost static throughout the experiment (Fig. 2a). The rate of hyphal extension from pieces of root colonised by *Glomus* sp. Curragh 2 (Fig. 2b) was similar in 150- and 300-mM NaCl, but this was much slower than hyphal growth in the 0-mM NaCl control (Fig. 1d). The total length of hyphae produced from colonised root pieces was similar to that produced from spores of *Glomus* sp. Curragh 2 (compare Fig. 2b with Fig. 1d).

Hyphal diameters were not quantified in this experiment. However, hyphae of *G. decipiens* grown in the presence of NaCl appeared thicker and more highly branched than hyphae of the same fungus grown in NaCl-free soil. This was not observed for any other fungus in this experiment. For all other fungi, the hyphae appeared uniform in morphology in all treatments. Hyphal diameter and maximum length were highly related to the size of the spores of different species: the large spores of *G. decipiens* and *S. calospora* produced thick, rapidly growing hyphae, and small spores such as those of *Archaeospora trappei* produced fine, -slow-growing hyphae. Hyphae of distal branches were usually, but not always, finer than proximal hyphae. No distinct dimorphism was observed within any treatment, for any fungus, and there was a range in the diameters of hyphae formed on any filter.

Discussion

The addition of NaCl to soil inhibited germination of spores of 11 AM fungi. This finding is consistent with those of Hirrel (1981) and Estaun (1989), who observed germination of spores of *G. margarita* and *Glomus mosseae* to be substantially reduced in the presence of NaCl, in solution and agar, respectively. For most of the

Table 3 Percentage of maximum proliferation of arbuscular mycorrhizal fungi from colonised root pieces incubated between millipore filters in the presence of 0-, 150- and 300-mM NaCl

Fungal isolate	Percentage of root pieces with proliferating hyphae	Days	NaCl treatment (mM)		
			0	150	300
Glomus invermaium (WUM 10-1)	78	7	85a	87a	96a
		14	92a	91a	81a
		21	87a	87a	85a
		28	100a	64a	62a
Glomus sp. Curragh 2 (WUM 23-1)	22	7	100a	23b	46b
		14	69a	46a	8b
		21	100a	69a	69a
		28	93a	69a	46a

Value for each treatment is a percentage of the maximum proliferation observed overall for that fungus. Within each row, values not followed by the same letter are significantly different ($p<0.05$). No statistical comparison was made within columns

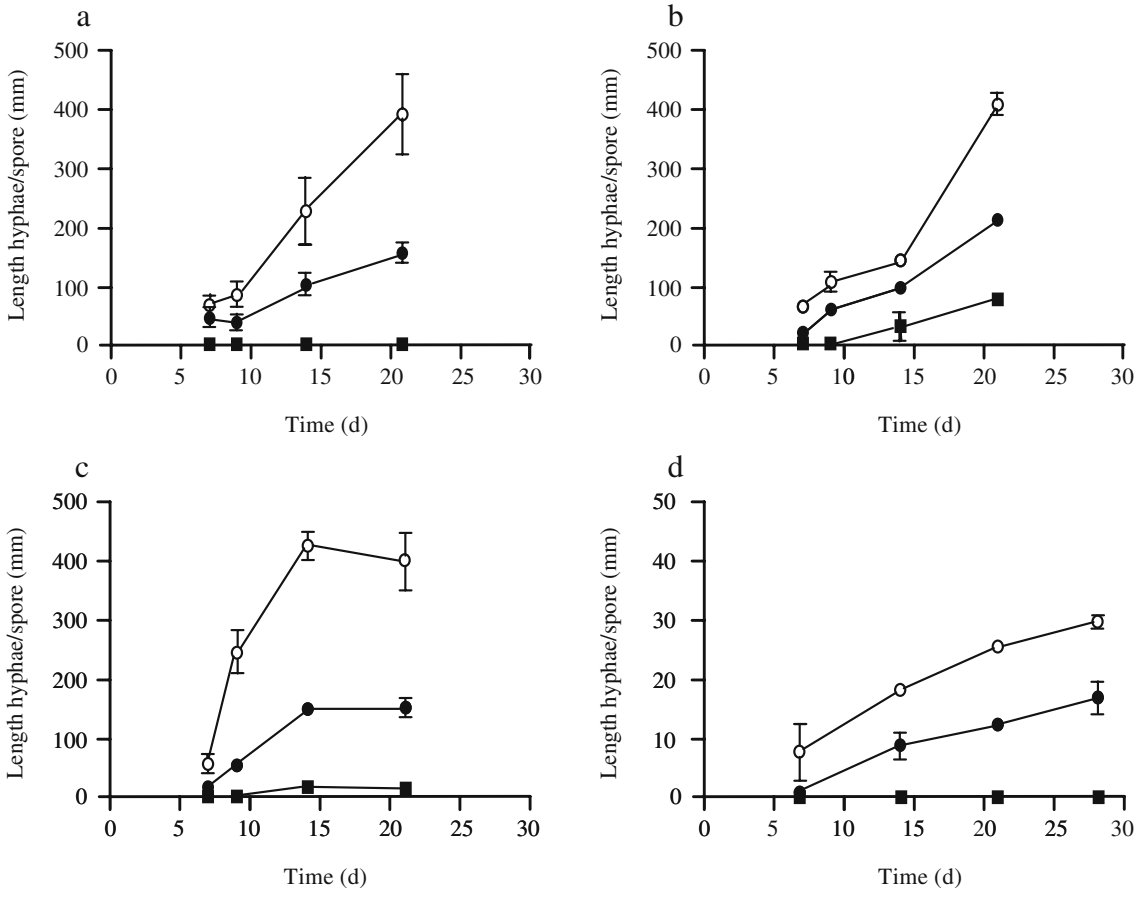

Fig. 1 Lengths of hyphae produced from spores of **a** *Gigaspora decipiens* (WUM 6), **b** *Scutellospora calospora* (WUM 12-2), **c** *Scutellospora calospora* (WUM 12-3) and **d** *Glomus* sp. Curragh 2 (WUM 23) after incubation in soil watered with solutions of 0 (○), 150 (●) and 300 (■) mM NaCl

fungi, germination was delayed rather than prevented in the presence of NaCl. That is, the length of incubation before the first appearance of a germ tube was longer with increasing concentrations of NaCl. The extent of the delay varied between the fungi.

The finding that germination was delayed in the presence of NaCl is consistent with Hirrel's (1981) note that previously ungerminated spores of *G. margarita* incubated in 170-mM NaCl began to germinate after 12 days, and the observations of Tommerup (1984) who found that germination time of *Glomus caledonium* and *A. laevis* was longer with decreasing (more negative) matric potential of the substrate. This finding also illustrates the importance of including multiple harvests in experiments

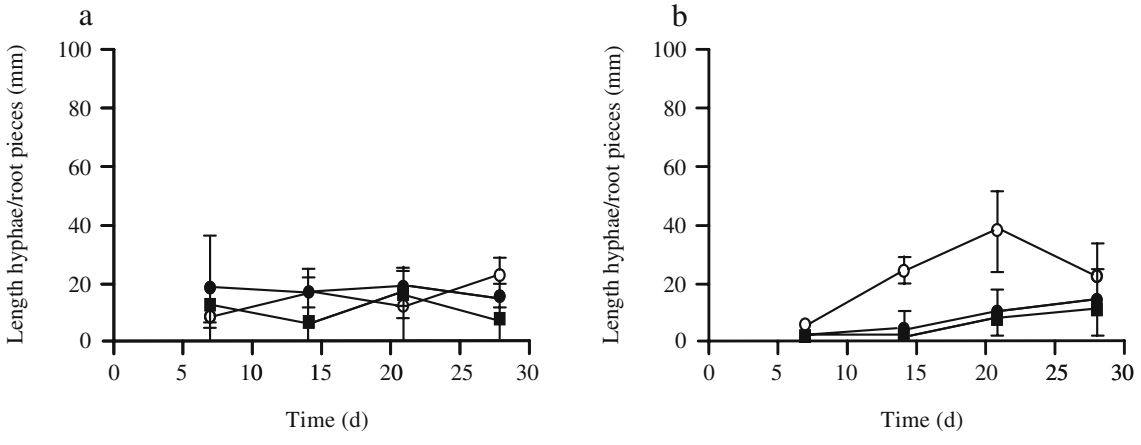

Fig. 2 Lengths of hyphae produced from pieces of dried root colonised by **a** *Glomus invermaium* (WUM 10) and **b** *Glomus* sp. Curragh 2 (WUM 23) after incubation in soil watered with solutions of 0 (○), 150 (●) and 300 (▪) mM NaCl

Table 4 Rates of hyphal extension from spores or pieces of colonised root of arbuscular mycorrhizal fungi, incubated between millipore filters in the presence of 0-, 150- and 300-mM NaCl

Fungal isolate	NaCl treatment (mM)		
	0	150	300
Spores			
Acaulospora laevis (WUM 11-1)	3.8		
Acaulospora laevis (WUM 11-4)	3.6		
Archaeospora trappei (WUM19-1)	0.4	0.2	0.2
Gigaspora decipiens (WUM6-1)	17.4	5.8	0.1
Glomus sp. (WUM 2)	3.0	4.4	6.7
Glomus sp. Kakadu 1	1.1	1.1	
Glomus sp. Kakadu 2	0.8	0.6	0.5
Glomus sp. Curragh 1 (WUM 21-1)	3.8	1.3	
Glomus sp. Curragh 2 (WUM23-1)	1.0	0.7	
Scutellospora calospora (WUM 12-3)	33.1	10.1	0.9
Scutellospora calospora (WUM 12-2)	19.2	11.5	3.0
Root pieces			
Glomus invermaium	0.8	0.6	0.7
Glomus sp. Curragh 2	0.8	0.5	0.2

Value for each treatment is the mean rate of extension (mm spore^{-1} day^{-1}) from all germinated spores or root pieces in that treatment, throughout the experiment

of this type. Had the spores been examined after only one harvest, interpretation would have been misleading.

The large difference between the fungi in their ability to germinate and grow in the presence of NaCl indicates a probable source of discrepancies between other studies where colonisation of plants by AM fungi was reduced (Poss et al. 1985; Hirrel and Gerdemann 1980; Ojala et al. 1983; Duke et al. 1986; Rozema et al. 1986; Dixon et al. 1993; McMillen et al. 1998; Juniper 1996) or not reduced (Levy et al. 1983; Hartmond et al. 1987) by salinity. Similarly, the confirmation that germination is delayed rather than prevented by NaCl, may have been a source of confusion where observation times varied. Colonisation may be reduced by NaCl early after inoculation, but not at later observation times (McMillen et al. 1998; Juniper 1996).

For most fungi used in this study, the specific rate of hyphal growth was reduced in the presence of NaCl. That is, hyphal growth of these fungi can be taken as being more sensitive to NaCl than spore germination, which was delayed but was not necessarily reduced in the presence of NaCl. For *Glomus* sp., Kakadu 1, the specific rate of growth was not reduced by NaCl. At each harvest, the amount of hyphae per germinated spore was reduced in the presence of NaCl, but this was due to the delay of germination in the salt relative to the control treatments. Once germination had commenced, similar amounts of hyphae were produced in the 150-mM NaCl treatment as were produced by control spores that had been germinated for the same period of time. Thus, hyphal growth of this fungus may have been less sensitive to NaCl than was spore germination.

The observation that intra-root propagules of *Glomus* sp. Curragh 2 regrew in the presence of 300-mM NaCl, but spores of the same isolate did not, may be an indication of different ecological roles for the two forms of propagule. It may also indicate a difference in energy status between them, or differences in the amount of water and energy required to initiate germination.

The period of time for which hyphae of AM fungi will continue to grow in the absence of contact with a plant and the maximum possible lengths of axenically produced hyphae, have not been determined (McMillen et al. 1998). Similarly, it has not been determined whether the total length of hyphae that a spore can produce would be reduced in the presence of NaCl. However, it is likely that osmoregulation costs energy that would otherwise be available for growth. It has not been determined whether, under conditions of osmotic stress, osmoregulation occurs at the significant expense of inoculum potential, or whether the net inoculum potential remains similar but a longer time is required to attain it. Reduction of the length of hyphae produced within a given time must diminish the probability that the hyphae will encounter and subsequently colonise a root within that time (McMillen et al. 1998). However, the persistence of a suppression of overall colonisation by a reduction of hyphal growth may ultimately depend on whether growth of the fungus was permanently "stunted" or temporarily delayed.

In this experiment, and in the studies by Hirrel (1981) and Estaun (1989), the spores were extracted from pot culture soil by wet-sieving and decanting. Therefore, the spores may have been fully hydrated prior to the imposition of NaCl treatments and the effects of NaCl on the initial stages of germination would not have been tested. Data presented by Tommerup (1984) indicated that initial hydration (the first stage of spore germination) and hyphal extension (the final stage) have higher requirements for water than the intermediate stages of activation and germ tube production. Spore germination where dry spores were

soaked with salt solution could, therefore, be less than the expected response of germination where spores were already hydrated before being exposed to NaCl, as in pot experiments using dry soil inoculum or in saline field soils during the first rain of the season. It is, therefore, possible that in this experiment, the use of previously hydrated spores resulted in more spores germinating than would have been observed had dry spores been subjected to the NaCl solutions.

It is not certain whether differences between species of AM fungi in this study were related to differences in size and other characteristics of their propagules. Spores of *G. decipiens* are very large in comparison to those of the *Glomus* species studied here, and this may have made them less susceptible to increasing concentrations of NaCl. Similarly, differences in the characteristics and abundance of the intra-root fungal structures among species may have contributed to differences in the rate of germination observed, especially if regrowth from these structures differed in response to NaCl. Furthermore, in the current study where fungi were grown in pot cultures using different plant species, the infectivity of the inoculum sources may have differed in their sensitivity to NaCl. These possibilities require further investigation.

Conclusion

In the presence of NaCl, the germination of spores of all AM fungi tested was delayed, and the specific rate of hyphal extension of most fungi was reduced. The extent to which spore germination was inhibited was not the same for all species investigated, but was similar for isolates of the same species. Variation in the rate of spore germination in NaCl ranged from no germination at all to maximum germination being attained in 300 mM NaCl. Propagules within colonised root pieces of one species germinated in 300 mM NaCl, but spores of the same fungi extracted from soil did not. This may indicate different ecological roles for the two types of propagule.

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