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# Salt stress affects in vitro growth and in situ symbioses of ectomycorrhizal fungi

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**Abstract.** Ten isolates of six species of ectomycorrhizal fungi were grown in vitro at nine concentrations of three sodium salts (NaCl, $Na_2SO_4$, $Na_3C_6H_5O_7$) for 4 weeks. Colony diamater, biomass and protein content of fungi were evaluated. Isolates of *Pisolithus tinctorius* and *Suillus luteus* were more tolerant of NaCl and $Na_2SO_4$ than of $Na_3C_6H_5O_7$. Fungi in the genera *Cenococcum, Laccaria,* and *Thelephora* were highly intolerant of $Na_3C_6H_5O_7$ and $Na_2SO_4$ in vitro. Biomass and protein content of fungi generally declined with increasing substrate salinity in solution culture. In situ ectomycorrhizal colonization by *Laccara laccata* and *P. tinctorius* and the dry weight of *Pinus taeda* seedlings were significantly reduced by 80 mM NaCl after 14 weeks. Only select ectomycorrhizal fungi appear capable of growth and symbiosis in saline soils.

**Key words:** *Pisolithus tinctorius* - *Laccaria laccata* - *Cenococcum geophilum* - *Thelephora terrestris* - Salt stress

## Introduction

Saline soils occupy approximately 7% of the earth's land surface and agronomic and forest crop production of these sites is relatively low (Yeo 1983; Barrett-Lennard et al. 1986; Jain et al. 1989). Properties of saline soils which inhibit or reduce plant survival and development include unfavorable pH, imbalance of essential cations and anions, and altered soil structure and texture which reduce aeration and water holding capacity (Abrol and Sandhu 1985; Bettenay 1986). The introduction of mycorrhizal fungi on some of these sites with unfavorable saline soil conditions may improve early plant survival and growth (Reddell et al. 1986; Jain et al. 1989; van der Moezel et al. 1989).

The salt tolerance of selected plant genera has been evaluated in controlled experiments (Sands and Clarke 1977; Greenway and Munns 1980; Reddell et al. 1986; Hennessey et al. 1989). However, in many of these studies, plants were supplied with adequate mineral nutrients and grown hydroponically or in artificial soil media (Yeo 1983). The effects of salinity on some symbiotic relationships (e.g., actinorrhizal or legume *Rhizobium* symbioses) have been assessed in a preliminary manner (Reddell et al. 1986; Marcar et al. 1991).

Plants relying on symbiotic relationships for adequate mineral nutrition and water uptake may differ in salt tolerance, depending on host and symbiont tolerance to soil salinity (Reddell et al. 1986; Perry et al. 1987; Marcar et al. 1991). The relative sensitivity of symbiotic fungi and host feeder roots to sodium salts, individually or as their composite ectomycorrhizal association, have not been evaluated. In addition to negative impacts on the host plant, extreme soil salinity could adversely affect mycorrhizal propagules in the rhizosphere, fungal colonization of feeder roots, and/or ectomycorrhizal structure and function (Reddell 1986; Hennessey et al. 1989). Preliminary investigations suggest rhizosphere organisms may be influenced by salt accumulation in soils (Barrett-Lennard et al. 1986). For example, the Basidiomycotina, a class with many ectomycorrhizal fungi, are highly intolerant of salt stress in vitro (Tresner and Hayes 1971). Salt tolerance may be important to the long-term survival, reproduction and spread of mycorrhizal fungi in saline soils (Perry et al. 1987; Jain et al. 1989).

The purpose of this study was to evaluate: (1) the influence of sodium salts on the survival, growth and metabolism of ten isolates of ectomycorrhizal fungi in vitro, and (2) the in situ salt tolerance of the loblolly pine (*Pinus taeda* L.) inoculated with select ectomycorrhizae.

## Materials and methods

The effect of salinity on ectomycorrhizal fungi was evaluated using three methods: (1) an in vitro study of fungal colony growth; (2) an in vitro analysis of fungi biomass production and protein

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**Table 1.** Ectomycorrhizal fungi used in experiments in vitro and in situ

| Isolate | Isolate no. | Source and isolation date |
|---|---|---|
| *Genococcum geophilum* (Cg) Fr. | 146 | *Quercus alba,* Md., USA, isolated by E. Hacskaylo from ectomycorrhiza |
| | 155 | *Quercus alba,* Md., USA, isolated by E. Hacskaylo from ectomycorrhiza |
| *Laccaria laccata* (Ll) (Scop.: Fr.) | 256 | *Pseudotsuga menziesii,* Oreg., USA , isolated by R. Molina |
| *Pisolithus tinctorius* (Pt) (Pers.) Coker & Couch | 250 | *Pinus taeda,* Ga., USA, isolated by W. Daniel from sporocarp tissue |
| | 301 | *P. taeda,* Ga., USA, isolated by D. Marx from sporocarp tissue |
| *Suillus luteus* (Sl) (L.: Fr.) S. F. Gray | 1 | *Pinus banksiana,* Minn., USA, isolated from ectomycorrhiza by R. Dixon |
| | 244 | *Pinus nigra,* Me., USA, isolated by W. Otrosina from sporocarp tissue |
| | 1088 | *Pinus resinosa,* Minn., USA, isolated by E. Stewart from sporocarp tisue |
| *S. tomentosus* (St) (Kauff.) Sing., Snell & Dick) | 1 | *Pinus resinosa,* Minn., USA, isolated by E. Stewart from sporocarp tissue |
| *Thelephora terrestris* (Tt) (Ehrh.) Fr. | 223 | *Pinus ocarpus,* Brazil, isolated by T. Krugner from ectomycorrhiza |

content; and (3) an in situ assessment of ectomycorrhizal colonization of loblolly pine (*P. taeda* L.). The ectomycorrhizal fungi used in the experiment and their cultural history are listed in Table 1. Loblolly pine was chosen as the host plant because of its known compatibility with the fungi tested and its widespread distribution on a variety of soil series worldwide. The fungi were selected for their potential utility in revegetation or reforestation programs, especially on substandard soils (Jain et al. 1989; Marx et al. 1991).

## *In vitro colony analyses*

The test fungi were cultured on modified Melin-Norkrans medium (MMN) (Marx 1969). MMN contains 0.05 g $CaCl_2$, 0.025 g NaCl, 0.5 g $KH_2PO_4$, 0.25 g $(NH_4)_2HPO_4$, 0.15 g $MgSO_4 \cdot 7H_2O$, 1.2 ml of 1% $FeCl_3$, 100 µg thiamine HCl, 3 g malt extract, and 10 g glucose per liter of distilled water. Agar was added to the MMN formulation at a concentration of 15 g/l. After autoclaving, the pH of the agar substrate was 5.5 to 5.7. Fungi were passed through loblolly pine seedlings and maintained on MMN medium prior to initiation of the experiments (Marx and Bryan 1975).

A factorial experiment was employed: ten isolates of ectomycorrhizal fungi were treated with three sodium salts at nine salt concentrations. Treatment combinations were replicated four times. Sodium chloride (NaCl) sodium sulphate ($Na_2SO_4$), and sodium citrate ($Na_3C_6H_5O_7$) were the test salts. Selection of test salts was based on prior experiments with fungi in the Basidiomycotina (Tresner and Hayes 1971) and occurrence of these salts in saline soils (Barrett-Lennard et al. 1986). In addition to the control, salts were added to MMN agar medium at eight concentrations, (10, 20, 30, 40, 60, 80, 100 and 120 mM). The range of salt concentrations tested was based on prior experimentation with rhizosphere organisms (Reddell et al. 1986; Marcar et al. 1991) and field assessments of soil salinity (Barrett-Lennard et al. 1986).

In each plate, two 6-mm diameter discs of mycelium agar inoculum were placed on agar MMN and incubated in darkness at 28 °C (Tresner and Hayes 1971). Colony diameter growth of fungi was measured weekly. The experiment was terminated after 4 weeks.

## *In vitro analysis in solution culture*

Biomass and protein content of the ectomycorrhizal fungi were evaluated in vitro in MMN liquid culture (Marx 1969). Protein content was monitored as a relative index of fungal metabolism (Tresner and Hayes 1971). The experimental design was factorial: three isolates of fungi [*Pisolithus tinctorius* (Pt) 250, *Suillus luteus* (Sl) 1088 and *Thelephora terrestris* (Tt) 223], three sodium salts (NaCl, $Na_2SO_4$, and $Na_3C_6H_5O_7$) and three salt concentrations (0, 30 and 120 mM). Each treatment combination was replicated four times.

Two 6-mm diameter discs of the test fungi were placed in sterile incubation vessels. Each vessel contained 100 ml of MMN liquid medium treated with the sodium salt solutions. Cultures were incubated at 28 °C 4 weeks. At the end of the experiment, mycelium was harvested to determine dry weight and water soluble protein content (Lowry et al. 1951).

## *In situ analyses*

Vegetative mycelial inocula of *T. terrestris* 223, *P. tinctorius* 301 and *L. laccata* 256 were produced using methods described by Marx and Bryan (1975). The three fungi were passed through loblolly pine and maintained on MMN agar prior to inoculum production. The inoculum was produced in the peat moss-vermiculite matrix containing MMN solution in 2-l jars for 14 weeks. The inocula was thoroughly leached before adding to the growth medium of seedling containers. The vegetative mycelial inoculum was mixed into the growing medium at a ratio of 1:25 (v/v) (Marx et al. 1991).

Seedlings of loblolly pine (*P. taeda* L.) were grown in 2-l pots containing a sandy loam soil using methods described by Dixon and Hiol Hiol (1992). The experimental design was a factorial arrangement of these fungi treatments and five salt treatments: 0, 20, 40, 60 and 80 mM of NaCl assigned to randomized blocks. Salts were applied as a solution to growth medium in weekly applications using methods described by Reddell et al. (1986). Each treatment combination was replicated seven times. Growth medium pH was 4.8–5.0, with a cation exchange capacity of 3.45 meg/100 g, an organic matter content of 1.5%, and P, $NO_3$, $NH_4$, Fe, Mn, Zn, B, Mo and Cu contents of 6, 2, 6, 3, 1, 0.3, 0.04, 0.01, and 0.1 ppm, respectively.

The study was implemented in a glasshouse using methods described by Dixon and Hiol Hiol (1992). Daily photoperiod and photosynthetically active radiation (PAR) were 14 h and 800 $\mu E \cdot m^2 \cdot s$, respectively. Ambient temperature and relative humidity ranged from 22 to 32 °C and from 45 to 90%, respectively.

Seedlings received deionized water as needed and were fertilized with half-strength Hoagland nutrient solution weekly (Hoagland and Aron 1950).

The study was terminated after 14 weeks and plants were harvested. Seedling root and shoot weight were measured after oven drying (75 °C, 72 h). Ectomycorrhizal colonization of seedling primary lateral roots was quantified using methods described by Marx et al. (1991). The percentage of ectomycorrhizal inoculation was computed as a ratio of the colonized lateral roots to total lateral roots (Dixon and Hiol Hiol 1992). The presence of a Hartig net was confirmed following microscopic examination of ectomycorrhizal short roots excised from seedling lateral roots.

Data from the three studies were subjected to analysis of variance and Duncan's multiple range test.

## Results

### *In vitro colony analysis*

The growth of ectomycorrhizal fungi varied widely with increasing NaCl concentrations (Table 2). Tolerance to NaCl varied significantly within a species and between isolates of the same species. For example, Sl 1088 had a significantly larger colony diameter compared to Sl 234 and Sl 244. At 30 to 120 mM NaCl, tolerance of fungi was as follows: Pt 301 > Pt 250 > Sl 1088 > Sl 234 > St > Ll > Tt > Cg. In some treatments low concentrations of NaCl stimulated the growth of the test fungi.

The effect of $Na_2SO_4$ on different ectomycorrhizal fungi was similar to that of NaCl with few exceptions (Table 3). $Na_3C_6H_5O_7$ significantly reduced colony growth of the test ectomycorrhizal fungi compared to NaCl and $Na_2SO_4$ (Table 4). The addition of $Na_3C_6H_5O_7$ to the growth media virtually inhibited fungal growth at concentrations greater than 30 mM.

### *In vitro analysis in solution culture*

The dry weight of *Pisolithus*, *Suillus* and *Thelephora* isolates grown in liquid culture varied significantly with the type of sodium salt and concentration (Table 5). Mycelial dry weight of Pt 250 was greater than Sl 1088 at 120 mM NaCl. In the $Na_2SO_4$ treatment, mycelia dry weight of Pt 250 and Sl 1088 did not vary significantly. The dry weight of Tt 223 was significantly reduced by NaCl and $Na_2SO_4$ relative to the other fungi. In the $Na_2C_6H_5O_7$ treatment, the dry weight of all test fungi significantly decreased with increasing salt concentrations.

**Table 2.** In vitro colony diameter growth (mm) of ten ectomycorrhizal fungi after 4 weeks on MMN media with nine concentrations of NaCl. Means followed by a common letter within a row are not significantly different by Duncan's multiple range test ($P=0.05$)

| | NaCl (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolates | 0 | 10 | 20 | 30 | 40 | 60 | 80 | 100 | 120 |
| Cg 146 | 18.5a | 14.8ab | 15.5ab | 14.0b | 15.5ab | 17.0ab | 14.5ab | 15.3b | 13.8b |
| Cg 155 | 14.5ab | 14.8a | 15.5a | 15.0a | 15.3a | 16.3b | 15.5a | 11.3b | — |
| Ll 256 | 14.8cd | 19.3a | 18.8ab | 17.5abc | 29.3a | 17.8abc | 14.3d | 18.3abc | 13.0d |
| Pt 250 | 61.8ab | 60.0c | 64.8abc | 65.0ab | 54.8d | 55.8d | 57.8cd | 64.3abc | 67.0a |
| Pt 301 | 64.8ab | 65.0a | 62.0abc | 59.8cd | 61.0bcd | 64.8ab | 58.8cd | 62.0abc | 53.3d |
| Sl 1 | 17.5b | 19.3b | 16.5b | 24.5a | 16.5b | 16.3b | 18.8b | 18.8b | 19.0b |
| Sl 244 | 15.3b | 13.3b | 15.8b | 16.8a | 17.3a | 15.8ab | 19.7a | 17.0a | 15.8ab |
| Sl 1088 | 68.8cd | 72.8a | 73.5a | 71.5a | 71.8a | 70.3a | 58.5d | 62.0a | 44.8d |
| St 1 | 21.0b | 20.3b | 20.0b | 21.5ab | 21.8ab | 23.8a | 24.0a | 25.0a | 18.8b |
| Tt 223 | 37.0a | 34.5ab | 29.8a | 28.0c | 22.0d | 19.2d | 13.8c | 28.5c | 14.0d |

**Table 3.** In vitro colony diameter growth (mm) of ten ectomycorrhizal fungi after 4 weeks on MMN media with nine concentrations of $Na_2SO_4$. Means followed by a common letter within a row are not significantly different by Duncan's multiple range test ($P=0.05$)

| | $Na_2SO_4$ (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolates | 0 | 10 | 20 | 30 | 40 | 60 | 80 | 100 | 120 |
| Cg 155 | 17.3a | 16.3a | 15.0a | 13.0a | 12.0a | 9.3b | 9.8b | 9.8b | 7.0b |
| Ll 256 | 17.0a | 15.8a | 15.3a | 15.8a | 12.3a | 15.5a | 11.7ab | 12.0a | 11.3b |
| Pt 250 | 75.3a | 56.8cd | 58.5bc | 63.3b | 51.3d | 46.8d | 39.3d | 47.0d | 32.8d |
| Pt 301 | 67.0a | 60.0b | 54.8bc | 51.8c | 51.8c | 53.5c | 50.5c | 52.8d | 51.0c |
| Sl 244 | 25.3b | 19.8c | 21.5c | 59.8cd | 24.0bc | 23.5c | 25.3b | 32.3ac | 29.5ab |
| Sl 1 | 23.8b | 22.8b | 18.8c | 20.3bc | 26.8a | 31.8b | 26.0a | 25.0ab | 19.0c |
| Sl 1088 | 64.8bc | 44.8d | 50.5d | 56.5d | 60.5cd | 70.3a | 69.5a | 68.5a | 66.0ab |
| St 1 | 18.5a | 18.3a | 18.3a | 18.3a | 19.8a | 23.5a | 22.3a | 22.8a | 22.8a |
| Tt 223 | 34.0a | 29.8ab | 30.0a | 25.3bc | 22.8c | 22.5c | 22.0cd | 18.5d | 18.0d |

**Table 4.** In vitro colony diameter growth (mm) of ten ectomycorrhizal fungi after 4 weeks on MMN media with nine concentrations of $Na_3C_6H_5O_7$. Means followed by a common letter within a row are not significantly different by Duncan's multiple range test ($P=0.05$)

| | $Na_3C_6H_5O_7$ (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolates | 0 | 10 | 20 | 30 | 40 | 60 | 80 | 100 | 120 |
| Cg 146 | 19.8a | 8.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b |
| Cg 155 | 18.3a | 8.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b | 7.0b |
| Ll 256 | 18.3d | 32.8b | 40.5a | 41.5a | 37.3ab | 30.5c | 34.3b | 32.5bc | 24.3d |
| Pt 250 | 75.0a | 72.5a | 55.8b | 36.0c | 25.3d | 16.3d | 11.3d | 9.3d | 9.0d |
| Pt 301 | 59.0a | 56.5a | 55.0a | 42.3b | 26.3c | 16.5d | 12.0d | 10.3d | 8.8d |
| Sl 1 | 16.0c | 24.8a | 25.5a | 21.8ab | 18.5bc | 13.0c | 11.3cd | 9.5d | 8.3d |
| Sl 244 | 20.8d | 36.8a | 34.3ab | 35.0a | 29.5bc | 27.3c | 20.5a | 18.5d | 15.8d |
| Sl 1088 | 61.5b | 76.3a | 70.0ab | 63.3b | 45.0ac | 12.0d | 10.5d | 8.0d | 7.0d |
| St 1 | 20.8bc | 31.3a | 21.7b | 23.8b | 21.5b | 15.5cd | 13.8d | 11.3d | 18.8d |
| Tt 233 | 39.5c | 58.5a | 45.5b | 33.3d | 16.5d | 8.8d | 8.0d | 7.3d | 7.0d |

**Table 5.** Biomass (mg) of ectomycorrhizal fungi grown in vitro with three concentrations of different sodium salts. Means followed by a common letter within a column are not significantly different by Duncan's multiple range test ($P=0.05$). Tr, Trace amount

| | | Salt | | |
|---|---|---|---|---|
| Species | Concentration (mM) | NaCl | $Na_2SO_4$ | $Na_3C_6H_5O_7$ |
| Sl 1088 | 0 | 35cd | 60a | 47b |
| | 30 | 47bc | 49ab | 47b |
| | 120 | 49b | 51a | 3d |
| Pt 250 | 0 | 63b | 50a | 42b |
| | 30 | 70a | 65a | 85a |
| | 120 | 82a | 35bc | 39bc |
| Tt 223 | 0 | 16d | 16d | 17cd |
| | 30 | 19d | 14c | Tr |
| | 120 | 18d | 20c | Tr |

The soluble protein content of Sl 1088 and Tt 223 mycelia increased with increasing levels of NaCl and $Na_2SO_4$, whereas the protein content of Pt 250 decreased at 120 mM NaCl or $Na_2SO_4$ (Table 6). In the $Na_3C_6H_5O_7$ treatment, the protein content of the fungal isolates varied widely.

### *In situ analysis of ectomycorrhizal relationships*

*P. taeda* seedlings inoculated with *P. tinctorius*, *L. laccata*, and *T. terrestris* developed abundant ectomycorrhizae in the absence of sodium salts (Table 7). Ectomycorrhizal colonization by *T. terrestris* was significantly reduced when the salt concentration exceeded 40 mM. In contrast, ectomycorrhizal development of *P. taeda* by *P. tinctorius* and *L. laccata* was not reduced until growth medium solution salt concentrations increased to 80 mM.

The dry weight of ectomycorrhizal *P. taeda* seedlings peaked at 20–40 mM salt (Table 7). Growth medium salt concentrations greater than 60 mM reduced the dry weight of seedlings colonized by all three fungi. Seedling phosphorus concentration was greater in plants inoculated with *P. tinctorius* and *L. laccata*. The phosphorus concentration of seedlings significantly declined when soil solution salt concentrations were 40–60 mM.

## Discussion

The in vitro salt tolerance of the test fungi varied significantly. The genera *Pisolithus*, *Laccaria* and *Suillus* appeared more tolerant of sodium salts than *Thelephora* or *Cenococcum*. In prior studies, genera within the Ascomycotina and Basidiomycotina were uniformly intolerant of sodium salts including NaCl (Tresner and Hayes 1971). In contrast to earlier assessments, differences in salt tolerance of isolates within a species were apparent in this study. The presence of NaCl actually stimulated colony growth of some test fungi.

The morphological and physiological mechanism(s) of salt tolerance in soil-borne fungi have not been elucidated (Griffin 1977; Hennessey et al. 1989). In this study, fungal species with relatively thick, well-develop-

**Table 6.** Protein content (%) of ectomycorrhizal fungi grown in vitro with three concentrations of three different sodium salts. Means followed by a common letter within a column are not significantly different by Duncan's multiple range test ($P=0.05$)

| | | Salt | | |
|---|---|---|---|---|
| Species | Concentration (mM) | NaCl | $Na_2SO_4$ | $Na_3C_6H_5O_7$ |
| Sl 1088 | 0 | 0.13b | 0.09c | 0.06a |
| | 30 | 0.26b | 0.13c | 0.4b |
| | 120 | 0.63b | 0.54b | 0.5b |
| Pt 250 | 0 | 1.13ab | 0.85b | 0.25b |
| | 30 | 1.59a | 1.65a | 1.88b |
| | 120 | 0.15b | 0.68b | 0.51b |
| Tt 223 | 0 | 0.21b | 0.37c | 0.28b |
| | 30 | 0.27b | 0.37bc | 0.01a |
| | 120 | 0.41b | 0.41b | 0.01a |

**Table 7.** Dry weight and ectomycorrhizal colonization of loblolly pine seedlings inoculated with three fungi and grown at five NaCl concentrations. Means followed by a common letter are not significantly different by Duncan's multiple range test ($P=0.05$)

| Ectomycorrhizal fungi | NaCl | Seedling | | Ectomycorrhizal colonization (%) |
|---|---|---|---|---|
| | | Dry wt (mg) | P % | |
| *Thelephora terrestris* | 0 | 281ef | 0.22bc | 42a |
| | 20 | 319cd | 0.24b | 39ab |
| | 40 | 283ef | 0.20c | 16ef |
| | 60 | 261f | 0.22bc | 11f |
| | 80 | 282ef | 0.23bc | 9f |
| *Pisolithus tinctorius* | 0 | 300de | 0.24bc | 37abc |
| | 20 | 351ab | 0.29a | 21e |
| | 40 | 398a | 0.26a | 30cd |
| | 60 | 329bcd | 0.27a | 22e |
| | 80 | 256f | 0.28a | 24de |
| *Laccaria laccata* | 0 | 296de | 0.25ab | 36abc |
| | 20 | 341bc | 0.27a | 30cd |
| | 40 | 395a | 0.28a | 29cd |
| | 60 | 307de | 0.29a | 22e |
| | 80 | 216g | 0.27a | 32bcd |

ed cell walls such as *C. geophilum* were highly sensitive to salts. Isolates with relatively thin cell walls (e.g., *Laccaria*) were generally tolerant of salt stress. Thus, morphological characteristics alone do not appear to be a primary factor in salt stress tolerance (Brownlee et al. 1983).

Studies of salt tolerance in plants reveal several mechanisms for NaCl exclusion from roots and, to a lesser extent, compartmentalization of salts in cell walls or vacuoles of root and shoot tissue (Greenway and Munns 1980; Yeo 1983; Reddell et al. 1986). Foster and Sands (1977) observed salt deposits in hypodermal cells of needles in salt-stressed radiata pine (*Pinus radiata*). Similarly, Reddell et al. (1986) and Dixon (1988) observed that *Frankia* and *Suillus* species compartementalized salt and toxic metals in vacuoles and cell walls, thus partially excluding these agents from metabolic pathways. The reduction in protein content of salt intolerant fungi in this study suggests that salinity influenced cytoplasmic metabolic activity (Greenway and Munns 1980; Yeo 1983).

Mexal and Reid (1973) observed that salt content influences osmotic properties of the fungal substrate. Increasing salt content enhances the water potential of the substrate and reduces growth of fungal colonies (Mexal and Reid 1973; Griffin 1979; Wilson and Griffin 1979). The salt concentrations tested in this study may have influenced the water potential gradient of the substrate to fungi. Ectomycorrhizal fungi and other rhizosphere organisms have demonstrated metabolic activity at water potentials which wilt the host plant (Mexal and Reid 1973; Hennessey et al. 1989). Shemakhanova (1967) and Mexal and Reid (1973) suggested that fungus tolerance of salt stress and low water potential may involve cytoplasmic osmoregulation.

Compartmentalized accumulation of salts in vacuoles and cell walls in ectomycorrhizal fungi and *Frankia* suggests these organisms have the capability to compensate for shifts in osmotic potential (Yeo 1983; Reddell et al. 1986). A number of organic compounds (e.g., organic acids, proteins) may provide the necessary extra osmotic potential which prevents plasmolysis (Mexal and Reid 1973; Foster and Sands 1977; Yeo 1983). In this study, protein content generally decreased with increasing salt concentration of liquid culture.

With relatively tolerant ectomycorrhizal symbionts (e.g. *P. tinctorius*), increasing soil solution salinity had little effect on plant growth and development. Seedlings inoculated with *Pisolithus* and *Laccaria* had root/shoot ratios of plants with balanced nutrition. In contrast, stunted seedlings grown in soils with high salt content had root/shoot ratios often found in nutritionally deficient plants (Harley and Smith 1983). Moreover, P concentration of seedlings declined at high salt concentrations, especially in those plants inoculated with *T. terrestris*. Jennings (1964) reported that exposure of *Fagus* ectomycorrhizae to NaCl reduced plabnt $PO_4$ uptake.

Ectomycorrhizal fungi improve in situ plant tolerance to soil drought, nutrient deficiency, heavy metal toxicity ands extremes of pH (Harley and Smith 1983). The results of this study suggest that selected ectomycorrhizal fungi can tolerate soil salt-stress conditions encountered in the field (Jain et al. 1989; Marcar et al. 1991). Moreover, some ectomycorrhizal symbionts may help improve host tolerance of saline soil. Previous qualitative assessments suggest that some rhizophere organisms are more tolerant of soil salt others (Barrett-Lennard et al. 1986; Reddell et al. 1986).

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## References

Abrol IP, Sandhu SS (1985) Growth responses of *Eucalyptus tereticornis* and *Acacia nilotica* to selected methods of site preparation in a highly sodic soil. Int Tree Crops J 3:171–183

Barrett-Lennard EG, Malcolm CV, Stern WR, Wilkins SM (eds) (1986) Forage and fuel production from salt-affected wasteland. Elsevier, New York

Bettenay E (1986) Salt affected soils in Australia. Reclam Reveg Res 5:167–179

Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71:433–443

Dixon RK (1988) Response of ectomycorrhizal *Quercus rubra* to soil cadmium, nickel and lead. Soil Biol Biochem 20:555–559

Dixon RK, Hiol Hiol F (1992) Mineral nutrition and growth of *Eucalyptus camaldulensis* and *Pinus caribaea* inoculated with *Pisolithus tinctorius* and *Thelephora terrestris*. Commun Soil Sci Plant Anal 23:1387–1396

Foster RC, Sands R (1977) Response of radiata pine to salt stress. II. Localization of chloride. Aust J Plant Physiol 4:863–875

Greenway H, Munns R (1980) Mechanisms of salt tolerance in non-halophytes. Annu Rev Plant Physiol 31:149–190

Griffin DM (1977) Water potential and wood-decay fungi. Annu Rev Phytopathol 15:319–329

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, New York

Hennessey TC, Vishniac HS, Lorenzi EM, Williams JC (1989) Dinitrogen fixation in a water-stressed *Alnus* clone is limited by host xerotolerance. Plant Soil 118:89–96

Hoagland DR, Arnon DI (1950) The water culture method of growing plants without soil. California Agricultural Experiment Station Circular 347

Jain RK, Paliwal K, Dixon RK, Gjerstad DH (1989) Improving productivity of multipurpose trees on substandard soils in India. J For 87:38–42

Jennings DH (1964) The effect of cations on the absorption of phosphate by breech mycorrhizal roots. New Phytol 63:348–357

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin-Phenol reagent. J Biol Chem 193:265–275

Marcar NE, Dart P, Sweeney C (1991) Effect of root-zone salinity on growth and chemical composition of *Acacia ampliceps* BR Maslin, *A. auriculiformis* A. Cunn. ex Benth, and *A. mangium* Willd. at two nitrogen levels. New Phytol 119:567–573

Marx DH (1969) The influence of ectotropic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. For Sci 21:245–254

Marx DH, Ruehle JL, Cordell CE (1991) Methods for studying nursery and field response of trees to specific ectomycorrhizae. In: Norris JR, Read DT, Varma AK (eds) Methods in microbiology, vol 23. Academic Press, New York, pp 383–411

Mexal J, Reid CPP (1973) The growth of selected mycorrhizal fungi in response to induced water stress. Can J Bot 51:1579–1588

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizosphere and reforestation: current knowledge and research needs. Can J For Res 17:929–940

Reddell P, Foster RC, Bowen GD (1986) The effects of sodium chloride on growth and nitrogen fixation in *Casuarina obesa* Miq. New Phytol 102:397–408

Sands R, Clarke ARP (1977) Response of radiata pine to salt stress. I. Water relations, osmotic adjustment and salt uptake. Aust J Plant Physiol 4:37–646

Shemakhanova NM (1967) Mycotrophy of woody plants. Israel Program for Scientific Translations, Jerusalem

Tresner HD, Hayes JA (1971) Sodium chloride tolerance of terrestrial fungi. Appl Microbiol 22:210–213

Wilson JM, Griffin DM (1979) The effect of water potential on the growth of some solid basidiomycetes. Soil Biol Biochem 11:211–212

Yeo AR (1983) Salinity resistance: physiologies and prices. Physiol Plant 58:214–222

Van der Moezel PG, Watson LE, Bell DT (1989) Gas exchange properties of two *Eucalyptus* species to salinity and water logging. Tree Physiol 5:251–258