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ORIGINAL PAPER

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The role of mycorrhizal infection in the resistance of *Vaccinium macrocarpon* to manganese

Abstract The role of mycorrhizal infection in the resistance of *Vaccinium macrocarpon* to manganese was investigated in perlite culture containing nutrient solution amended with Mn at 0, 250, 500 or 1000 μg/ml. Shoot and root dry weights of the mycorrhizal plants were higher than nonmycorrhizal plants. The mycorrhizal plants produced significantly longer main roots than the nonmycorrhizal plants. Differences between shoot and root Mn concentrations of mycorrhizal and nonmycorrhizal plants arose by reduction of Mn in the leaves of mycorrhizal plants and a corresponding increase in root tissues.

Key words *Vaccinium macrocarpon* *Hymenoscyphys ericae* · Manganese toxicity

Introduction

It is now well established that mycorrhizal infection can lead to increased efficiency of nutrient capture and that this in turn can lead to enhancement of growth in mycorrhizal plants (Harley 1969; Stribley and Read 1975; Read 1978; Carleton and Read 1990). Most studies of responses of mycorrhizal plants to metallic ions have examined either enhancement of uptake at low concentrations (Lambert et al. 1979; Burt 1984; Hashem 1987), or their exclusion at high concentrations (Bradley et al. 1981, 1982; Brown and Wilkins 1985; Hashem 1990). Previous work in ericoid mycorrhiza indicated that infection causes explusion of potentially toxic metals such as Cu and Zn. However except for the report by Medappa and Dana (1970), who worked with nonmycorrhizal plants, there have been no studies of Mn. Mn toxicity can be a problem in acid soils when levels exceed 350–2000 μg/ml (Kabata-Pendias and Pendias 1985). In the present study, the response of *Vaccinium macrocarpon* to different levels Mn was investigated both in the mycorrhizal and in the nonmycorrhizal condition.

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Materials and methods

Mycorrhizal and nonmycorrhizal seedlings of *V. macrocarpon* were grown from seeds sterilized by shaking for 10 min in a calcium hypochlorite solution (71 g/l), then washed in sterile distilled water and transferred into petri dishes containing 0.10% distilled water agar. After germination, seedlings were transferred to 500-ml flasks containing 40 g acid-washed perlite moistened with 50 ml of 1:5 diluted modified Melin liquid medium (10 g glucose, 0.2 g KH_2PO_4, 0.1 g NH_4Cl, 0.02 g $MgSO_4 \cdot 7H_2O$ per litre), with 5 seedlings per flask. Five disks were cut from the growing edge of a colony of the endophytic fungus *Hymenoscyphys ericae* (Read) Korf and Kernan [isolated from roots of *Calluna vulgaris* (L.) Hull, natural heath land, Mynydd Bodafon, Gwynedd, North Wales] and placed on the perlite. All flasks were placed in a controlled-environment growth room for 2 weeks (20° C) after which seedlings were examined to determine the level of mycorrhizal infection. High levels of infection were observed in all mycorrhizal seedlings while no infection was present in nonmycorrhizal seedlings. At this time Mn was added as $MnSO_4$ to 1:5 diluted modified Melin liquid medium to give the following treatments: 0, 250, 500 and 1000 μg/ml Mn (5 replicates per treatment). The solutions were adjusted to pH 5.0 and passed through a 0.2-μm Millipore filter; aliquots (50 ml) of thesee sterilized solutions were added to culture flasks. Seedlings were harvested after 6 weeks by removing them from the perlite under a jet of water. Shoot and root dry weights were determined. The Mn concentrations of shoots and roots were measured by placing shoots and roots into acid-washed 25-ml Pyrex beakers and heating at 80° C to constant weight. Tissues were then digested in 5 ml HNO_3 (Analar grade) until the solutions were clear. The solutions were boiled gently to reduce volume (120° C, 15 min) and then made up to 20 ml in volumetric flasks with deionised water. The solutions were analysed for Mn by atomic absorption spectrophotometry.

Results and discussion

Both mycorrhizal and nonmycorrhizal plants of *V. macrocarpon* survived and grew at Mn concentrations up to 1000 μg/ml, indicating considerable Mn resistance in this species. Shoot and root dry weights declined with

Table 1 Mean shoot and root dry weight (mg) of mycorrhizal and non-mycorrhizal *Vaccinium macrocarpon* seedlings after 6 weeks growth in perlite culture containing nutrient solution amended with Mn. Values given are means of 25 seedlings per Mn concentration ±SD. Mycorrhizal plants had significantly greater weights than nonmycorrhizal plants in all cases ($P \geq 0.99$)

Plant	Mn concentration (μg/ml)			
	0	250	500	1000
Mycorrhizal				
Shoot	38±1.1	34±1.0	30±0.8	25±0.6
Root	25±0.8	19±0.6	15±0.3	13±0.7
Nonmycorrhizal				
Shoot	25±0.9	22±0.7	18±1.0	13±0.8
Root	16±0.5	13±0.3	11±0.8	10±0.3

Table 2 Concentrations of Mn (μg/g) in shoot and root tissues of mycorrhizal and nonmycorrhizal *V. macrocarpon* seedlings after 6 weeks growth in perlite culture containing nutrient solution amended with Mn. Values given are means of 25 seedlings per Mn concentration ±SD. In all cases, mycorrhizal plants had significantly different tissue Mn concentrations than nonmycorrhizal plants at corresponding solution Mn concentrations ($P \geq 0.99$)

Plant	Mn concentration (μg/ml)			
	0	250	500	1000
Mycorrhizal				
Shoot	1.2±0.3	400±3.1	550±3.6	720±3.8
Root	1.0±0.1	600±3.2	800·3.8	1100±4.1
Nonmycorrhizal				
Shoot	1.5±0.1	600±4.0	900±3.8	1300±4.2
Root	1.2±0.3	350±2.1	560±2.6	700±3.0

Table 3 Mean lengths of the longest root (mm) on mycorrhizal and nonmycorrhizal *V. macrocarpon* seedlings after 6 weeks growth in perlite culture containing nutrient solution amended with Mn. Values given are means of 25 seedlings per Mn concentration ±SD. Mean values for mycorrhizal and nonmycorrhizal plants were significantly different at all Mn concentrations ($P \geq 0.99$)

Root	Mn concentration (μg/ml)			
	0	250	500	1000
Mycorrhizal	42±1.0	62±1.4	56±1.0	41±1.0
Nonmycorrhizal	50±1.3	40±1.2	35±1.5	28±1.2

Mn concentration, but at every level of treatment the weights of the mycorrhizal plants were higher than those of the nonmycorrhizal plants (Table 1). Moreover, the mycorrhizal plants showed a significantly reduced accumulation of Mn in the shoots (Table 2). Bradley et al. (1982) investigated the relationship between mycorrhizal infection and resistance to metal toxicity in three ericaceous plants, *Calluna vulgaris* (L.) Hull, *V. macrocarpon* Ait, and *Rhododendron ponticum* L., and found that ericoid mycorrhizas could reduce the accumulation of Cu and Zn in the shoots when the metals were present at potentially toxic concentrations, while simultaneously increasing the uptake of N and P. Medappa and Dana (1970) examined the patterns of uptake of Mn, Al and Fe by *V. macrocarpon* but they used cuttings as the test material which could not have been mycorrhizal. In the present study, at 250, 500 and 1000 μg/ml Mn the mycorrhizal plants produced significantly larger main roots than the nonmycorrhizal plants (Table 3).

The resistance of *H. ericae* to high concentrations of toxic metals (Burt et al. 1986; Hashem 1990, 1991) would be expected to provide particularly efficient exclusion mechanisms. Important in this respect, I found that Mn concentrations was significantly higher in mycorrhizal than in nonmycorrhizal roots. Thus, the lower tissue Mn concentration in mycorrhizal than in nonmycorrhizal shoots is probably due to reduced transport of Mn from roots to shoots. The mycorrhizal fungus in the present study clearly has the ability to detoxify the medium by accumulation of Mn in roots or in hyphae. In studies of Cu and Zn detoxification by ericoid mycorrhizas, Bradley et al. (1982) pointed out that the proliferation of hyphae inside cortical cells in the absorptive regions of ericaceous roots provides a large surface for adsorption of ions. All internal hyphae are covered with a sheath of host plasmalemma. This membrane is separated from the hyphal surface by an interfacial matrix which has been shown by Duddridge and Read (1982) to be made up of pectic material. Pectins are known to be very effective binders of metallic ions. Retention of Mn in roots, perhaps primarily in association with the mycorrhizal endophyte, protects the shoots and provides metal tolerance on the basis of exclusion rather than accumulation.

These results suggest that the success of ericaceous plants in some of the most extreme terrestrial environments can be partly attributed to avoidance of metal toxicity mediated by the mycorrhizal partner.

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