Mycorrhiza (2006) 16: 277–283
DOI 10.1007/s00572-006-0045-x

ORIGINAL PAPER

Patrick Audet · Christiane Charest

Effects of AM colonization on "wild tobacco" plants grown in zinc-contaminated soil

Received: 20 July 2005 / Accepted: 17 January 2006 / Published online: 1 March 2006
© Springer-Verlag 2006

Abstract This greenhouse study aimed to determine the effect of colonization by the arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck & Smith) on the "wild" tobacco (*Nicotiana rustica* L. var. Azteca), under soil–zinc (Zn) conditions. Plants of *N. rustica* were grown in AM or non-AM inoculated substrate and subjected to four soil–[Zn] concentrations (0, 50, 100, and 250 mg Zn kg^{-1} dry soil). The AM root colonization increased markedly from 14 to 81% with the increasing soil–[Zn] and the mycorrhizal structures were significantly more abundant at the highest soil–[Zn], suggesting that Zn may be involved directly or indirectly in AM root colonization. In addition, total Zn content or Zn concentrations in shoots and roots were shown to increase as soil–[Zn] increased in both AM and non-AM plants. As for the growth parameters studied, there were no significant differences between treatments despite the increase in Zn content or concentration. The AM roots subjected to the highest soil–[Zn] had a significant reduction by about 50% of total Zn content and Zn concentration compared to non-AM roots. Still, the relative extracted Zn percentage decreased dramatically as soil–[Zn] increased. Soil pH was significantly lower in non-AM than AM treatments at the highest soil–[Zn]. In summary, AM plants (particularly roots) showed lower Zn content and concentration than non-AM plants. In this regard, the AM fungi have a protective role for the host plant, thus playing an important role in soil-contaminant immobilization processes; and, therefore, are of value in phytoremediation, especially when heavy metals approach toxic levels in the soil.

Keywords *Glomus intraradices* · Heavy metals · *Nicotiana rustica* · Phytoremediation

P. Audet · C. Charest (✉)
Department of Biology, University of Ottawa,
30 Marie.Curie St.,
Ottawa, ON, K1N 6N5, Canada
e-mail: ccharest@science.uottawa.ca
Tel.: +1-613-5625800
Fax: +1-613-5625486

Introduction

Phytoremediation is defined as the use of plant systems to clean up contaminated environments (Meagher 2000). The ultimate goal of phytoremediation research, whether under laboratory or field conditions, is the restoration of polluted ecosystems, although it is equally important to examine the environmental fate and direct effects of contaminants on plant systems (Schutzendubel and Polle 2002). Contaminants reviewed thus far have been classified as halogenated solvents, polycyclic aromatic hydrocarbons, or heavy metals. In particular, heavy metals have reached phytotoxic concentrations in some ecosystems as a result of metal galvanization, rubber vulcanization, and agricultural feed additives or fertilizers (Barceloux 1999; Wenger et al. 2002). Zinc (Zn), an essential micronutrient, has been shown to reach critical toxicity levels when leaf concentrations approach and/or surpass 400 mg kg^{-1} dry tissue, at which point leaf necrosis and ultimately plant death occurs (Chaney 1993; Marschner 1986). This is of concern considering that soil–Zn concentrations have been found beyond 1,000 mg Zn kg^{-1} dry soil in some areas near industrial sites and agricultural fields.

Arbuscular mycorrhizal (AM) symbiosis is a very ancient interaction between plant roots and zygomycetous fungi (Morton and Benny 1990). It is believed that AM symbiosis occurs in more than 90% of terrestrial plants; it significantly increases tolerance to drought, nutrient deficiency, cold or warm temperature, and, in some cases, heavy metal contamination (Charest et al. 1997; Davies et al. 2001; Paradis et al. 1995; Subramanian et al. 1997; Subramanian and Charest 1998). Recent studies, having examined AM symbiosis in the context of phytoremediation, have shown mixed findings. Some studies have shown that AM colonization increases the uptake and accumulation of heavy metals in host plants (Davies et al. 2001, 2002; Hovsepyan and Greipsson 2004; Rufyikiri et al. 2002, 2003), while others suggested that AM fungi exude enzymes that participate in the immobilization process of soil contaminants, in which case accumulation in plants is reduced (Joner et al. 2000; Leyval et al. 1997;

Weissenhorn et al. 1993). The AM fungi might be beneficial for plants under excess-nutrient conditions as they are known to help plants under various stressful conditions. The "wild" tobacco, *Nicotiana rustica* L. var. Azteca, was selected because some species of the Solanaceae family, including tomato and tobacco cultivars, are known as phytoaccumulators (Wenger et al. 2002). The main objective was to evaluate the role of an AM fungus, *Glomus intraradices* Schenck & Smith, as well as the efficacy of "wild" tobacco in the phytoremediation process. Our working hypotheses were that AM root colonization in host plants leads to (1) their enhanced tolerance to increasing soil–[Zn] and (2) an increased uptake and content in their tissues. The derived predictions were that physiological parameters (e.g., biomass and solute concentrations) and Zn content would be greater in AM than non-AM plants.

Materials and methods

Growth conditions

The factorial block design (one plant *sp.* ×2 AM ×4 Zn) used in this greenhouse experiment consisted of AM and non-AM plants subjected to four soil–Zn concentrations (0, 50, 100, and 250 mg Zn kg^{-1} dry soil) with seven blocks per treatment for a total of 56 plants (one plant per pot). Plants of *N. rustica* L. var. Azteca were grown from seeds (Ethnogens Seeds, Wichita, KS, USA), for 10 weeks in a soil mixture (sand:potting soil, 1:1 v/v) (Table 1). This soil mixture was thoroughly homogenized with an industrial mixer, autoclaved (20 min at 121°C), and allowed to cool at ambient temperature before potting, with approximately 4.75 kg per pot (7.5 l). Half of the pots (AM) were inoculated with *G. intraradices* Schenck & Smith as a 3 cm-thick layer of inoculum substrate containing 15 propagules g^{-1} dry substrate (Myke Pro Endo, Rivière-du-Loup, QC, Canada), whereas an equivalent volume of control substrate (without propagules) was incorporated in the control pots (non-AM). The greenhouse conditions consisted of 22°C (day)/35°C (night), a 16-h photoperiod, and 40% relative humidity. The average light intensity measured at five different locations in the greenhouse with a light meter (LICOR LI-250 A quantum sensor, Lincoln, NE, USA) was 364.2 μmol m^{-2} s^{-1}. All the plants were watered as needed and fertilized biweekly (400 ml per week), avoiding any leaching, from week 4 to 10 using a modified Long-Ashton nutrient solution (K_2SO_4 2.0 mM, $CaCl_2$ anhydride 4.0 mM, $MgSO._4{\cdot}7H_2O$ 1.5 mM, $NaH_2PO._4{\cdot}H_2O$ 1.5 mM, NH_4NO_3 5.0 μM, $MnSO._4{\cdot}4H_2O$ 0.01 mM, $CuSO._4{\cdot}5H_2O$ 1.0 μM, $ZnSO._4{\cdot}7H_2O$ 1.0 μM, H_3BO_3 0.05 mM, NaCl 0.09 mM, $Na_2MoO._4{\cdot}2H_2O$ 0.5 μM, and EDTA-Fe 0.1 mM). A total of 0.84 mg Zn was added to each pot, including the 0-[Zn] treatment, over the growth period due to fertilization. The Zn was added between the 7th and 8th week in 100 ml doses from a $ZnSO._4{\cdot}7H_2O$ stock solution (45 mM) until the desired soil–[Zn] was reached; except for the 0-[Zn] control treatment, the 50-[Zn] was applied in one dose, the 100-[Zn] in two doses, and the 250-[Zn] in five doses.

Table 1 Soil properties for pre-experimental soil

Parameters	
Total Kjedahl nitrogen	0.12%
Total organic carbon	2.67%
Organic matter	6%
Sand (>0.050 mm)	89%
Silt (0.002–0.050 mm)	<1%
Clay (<0.002 mm)	4%
Zn	22 μg g^{-1}
P	7 ppm
Mn	137 μg g^{-1}

Harvest

All the plants were harvested 10 weeks after seeding. The fresh plant tissues were measured separately as shoots and roots. All the roots were thoroughly cleaned with tap water. Plants from three blocks were randomly selected for the determination of percent AM root colonization. Plant tissues from the four remaining blocks were oven dried at 70°C for 72 h, then later weighed and sampled for mineral analyses. The soil pH measurements were done before and after harvesting; the pH was read in dH_2O with a 1:2.5 soil: water ratio (Jackson 1973).

Leaf protein and chlorophyll analyses

One gram of fresh leaf tissue from each replicate (three per treatment) was used for soluble protein analysis according to Bradford (1976). Soluble proteins were extracted with 10 ml of a 25 mM Tris–HCl buffer solution (pH 8.0) and 2 ml 2% polyvinylpyrollidone (PVP) using a mortar and pestle on ice. The extracts were centrifuged at 13,000 g for 20 min at 4°C. The readings were done at 595 nm and the protein concentrations measured from a BSA (bovine serum albumin, 99% protein) standard curve.

Chlorophyll concentrations from each replicate (three per treatment) were determined according to Bruinsma (1963). One gram of fresh leaf tissue was submerged into 100 ml of 95% ethanol and kept in dark at room temperature until the tissue was discolored; the readings were taken at 649 and 665 nm.

Soil and plant mineral analyses

Before the experiment, soil minerals were extracted using a H_2SO_4 solution (e.g., B, Ca, Co, I, Mn, Mo, Na, Ni, Zn), $NaHCO_3$ solution (e.g., P), and $C_2H_7NO_2$ solution (e.g., K and Mg) and analyzed via Atomic Absorbtion Spectrometry (AAS) (Accutest Laboratories, ON, Canada).

Minerals (e.g., Al, Ca, Cu, Fe, K, Mn, Mo, P, Zn) extracted from a H_2SO_4 solution for both dry shoots and roots (n=4) were analyzed via AAS ("Laboratoire de Chimie Organique et Inorganique, Direction de la Recherche Forestière", MRN, QC, Canada). The Zn content was calculated from the Zn concentration multiplied by the shoot and root dry mass, separately. The relative extracted Zn percentage (%) was determined by dividing the total Zn content in plant tissue by the total Zn added to the soil and multiplying by 100. The extracted Zn% for the 0-[Zn] treatments were calculated using the values for Zn amended through fertilization.

Root colonization

Roots were cleaned and stained using aniline blue 0.02% dye solution (6.78 mM aniline blue; 500 ml glycerol; 450 ml H_2Od; 50 ml 1% HCl) according to Dalpé (1993). Fifty 1-cm long root segments per replicate (n=3) were examined at ×100 and ×400 under a compound microscope for the presence of vesicles, arbuscules, or hyphae (and possibly spores). Mycorrhizal colonization was estimated as the percentage of the total root segments containing vesicles, arbuscules, and hyphae individually, as well as the percentage of roots containing at least one of these AM fungal structures.

Statistical analyses

One- and two-tailed parametric analyses of variance (ANOVA) were performed, coupled with Bonferonni studentized range tests for mean comparison analyses. Kolmogorov–Schmirnoff and Levene's tests were used, respectively, to verify both normality and evenness of variability (Zar 1999). All of the p-values were determined using S-Plus 6.2 (Insightful 2003). A block design was used to reduce the statistical error of the tests attributed to the greenhouse variability.

Results

In summary, showing no significant differences, shoot and root DM ranged from 31.2 to 37.3 g and 11.2 to 13.9 g, respectively, and shoot height varied from 75 to 92 cm. Leaf protein and chlorophyll concentrations ranged from 2.45 to 2.92 mg g^{-1} fresh mass (FM) and 13.1 to 17.2 mg g^{-1} FM, respectively. No visual stress (e.g., leaf necrosis, chlorosis, wilting, or senescence) was observed over the course of the 10-week growth period. The soil pH, ranging from 5.83 to 4.97, was significantly ($p<0.05$) the lowest for the non-AM treatment at 250-[Zn].

The total Zn content significantly ($p<0.001$) increased as soil–[Zn] increased in the shoots (Fig. 1a) and in the roots (Fig. 1b) of both AM and non-AM plants. The same result was found for Zn concentrations in the shoots (Table 2) and in the roots (Table 3). More specifically, the Zn content and Zn concentration were significantly lower in the roots of AM (24.5 mg Zn per total root mass and 2.07 mg g^{-1} DM) than non-AM plants (43.1 and 3.13) at the highest soil–[Zn], respectively. In the shoots, the P and Mn concentrations significantly decreased as soil–[Zn] increased (Table 2). In the roots, Mn was significantly lower in AM than non-AM treatments at 100- and 250-[Zn] (Table 3). All the other minerals tested (e.g., K, Ca, Mg, Cu, Al, Fe, Mo) showed no significant differences among the treatments. In the shoots, the mean concentrations (mg g^{-1} DM±SE) for K, Ca, and Mg were 39.8 (1.35), 24.7 (0.99), and 8.25 (0.42), respectively; in the roots, these were of 20.7 (0.74), 4.16 (0.29), and 4.69 (0.30), respectively. The Cu, Al, Fe, and Mo were found in trace amounts in shoot and root tissues.

The relative extracted Zn% significantly decreased in both shoots (from 79.9% to 6.57%) and roots (from 49.1 to

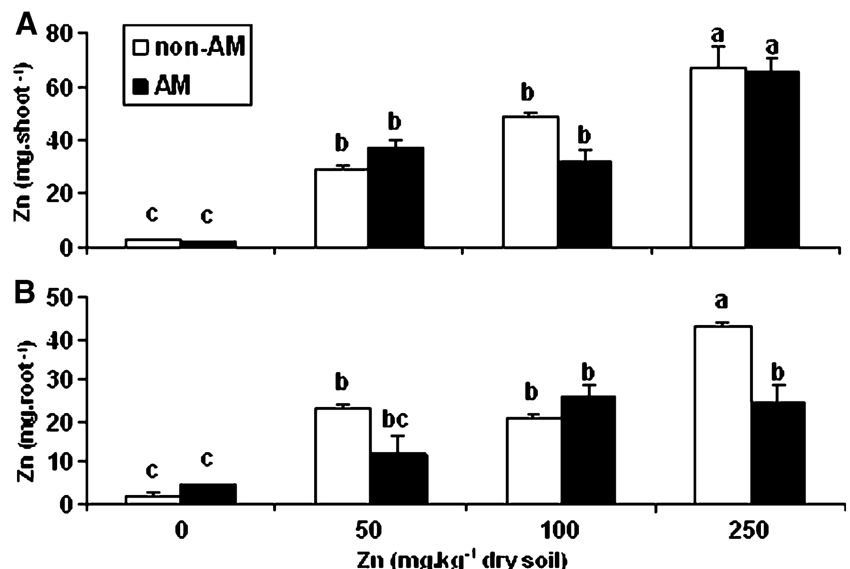

Fig. 1 Total Zn content (mg $plant^{-1}$) in both shoots (**a**) and roots (**b**) of *N. rustica* under increasing soil–[Zn]. Means (n=4) and SE for non-AM (*empty bars*) and AM (*solid bars*) treatments are shown. Different *letters* designate treatments that are significantly different according to Bonferonni mean comparison test ($p<0.05$)

Table 2 Zn, P, and Mn concentrations (mg g^{-1} DM) in shoots of *N. rustica* under increasing soil–[Zn]

Zn (mg kg^{-1} dry soil)	Inoculum	Zn	P	Mn
0	non-AM	0.10^{d} (0.01)	7.24^{a} (0.87)	0.61^{a} (0.15)
	AM	0.12^{d} (0.0)	7.13^{a} (0.80)	0.67^{a} (0.15)
50	non-AM	1.70^{b} (0.09)	6.51^{ab} (0.22)	0.48^{ab} (0.18)
	AM	1.32^{b} (0.13)	6.09^{ab} (0.78)	0.67^{a} (0.06)
100	non-AM	1.61^{b} (0.09)	5.64^{b} (0.65)	0.63^{a} (0.15)
	AM	2.30^{b} (0.17)	6.68^{a} (0.35)	0.31^{b} (0.08)
250	non-AM	3.41^{a} (0.41)	5.47^{b} (0.35)	0.40^{b} (0.12)
	AM	3.20^{a} (0.17)	5.39^{b} (0.46)	0.30^{b} (0.09)
F-values and level of significance	Zn	123**	3.56*	2.82*
	Inoculum	0.05ns	0.05ns	0.27ns
	Zn × inoculum	4.34**	0.60ns	2.55ns
	Block	3.42ns	2.05ns	6.82ns

Means (*n*=4) and SE (inside parentheses) are shown
Different letters in columns designate treatments that are significantly different according to Bonferonni test
ns not significant
*$p<0.05$
**$p<0.001$

2.44%) as soil–[Zn] increased (Table 4). In the shoots, at 100-[Zn] the extracted-Zn% was significantly higher in non-AM than AM treatments. In the roots, at 50-[Zn] the extracted-Zn% was significantly higher in non-AM than AM treatments. The 250-[Zn] treatment showed the lowest extracted-Zn% in the AM roots.

Root AM colonization significantly increased from 14 to 81% as soil–[Zn] increased (Table 5). The hyphae (extraradical or intraradical), arbuscules, and vesicles were all observed in the AM roots at all the Zn concentrations. Each of these mycorrhizal structures was significantly more abundant at 250-[Zn] than any other soil–[Zn] level.

Discussion

Of particular interest in this study, the AM colonization level was observed to increase by five-fold as soil–Zn concentration reached the highest level, thus suggesting that Zn affects directly the root colonization, and/or indirectly the mycorrhizospheric conditions. In other words, Zn may have affected the host plant, the AM fungus, or both, and therefore induced AM root colonization. To our knowledge, this is the first time that the effect of Zn contamination inducing AM root colonization has been shown. The increase in AM root colonization is likely the result of numerous factors including increases in soil–Zn concentration and the subsequent decrease in soil pH. Rufyikiri et al. (2003), who assessed root- and hypha-induced substrate-pH modifications under in vitro conditions, suggested that roots lead to an acidification while mycelium to an alkalinization of the growth medium. This phenomenon may explain the significantly lower soil pH in non-AM than AM treatments at 250-[Zn]. Yet, the differences in soil pH could also be attributed to the metal-binding capacity of AM fungi, as well as active pH modification by the hyphal exudates (Rufyikiri et al. 2003). In our study, the significant increase in the abundance of AM structures, especially hyphae and arbuscules, may imply that the root–fungal symbiosis was the most active under the highest soil–Zn concentration. Thus, the host plant seemed to invest more in the AM symbiosis than in heavy metal sequestration under soil–Zn excess; this finding is interesting as lower total Zn content and concentration were measured in the AM than non-AM roots at the highest soil–[Zn]. These findings agree with Janoušková and Vosatká (2005), who reported increased colonization of in vitro carrot roots by *G. intraradices* when Cd concentration increased in the medium, with lower Cd concentration or content in AM than non-AM roots. It is possible that *G. intraradices* is tolerant to Zn and Cd soil excess, a property that has been described for Cd-tolerant *G. mosseae* found at heavy metal polluted sites

Table 3 Zn, P, and Mn concentrations (mg g^{-1} DM) in roots of *N. rustica* under increasing soil–[Zn]

Zn (mg kg^{-1} dry soil)	Inoculum	Zn	P	Mn
0	non-AM	0.12^{d} (0.02)	2.97^{a} (0.53)	0.25^{a} (0.03)
	AM	0.38^{d} (0.25)	3.60^{a} (0.15)	0.28^{a} (0.04)
50	non-AM	1.71^{bc} (0.20)	3.36^{a} (0.37)	0.21^{a} (0.03)
	AM	0.98^{cd} (0.27)	3.02^{a} (0.14)	0.20^{a} (0.02)
100	non-AM	1.84^{b} (0.18)	3.26^{a} (0.36)	0.20^{a} (0.04)
	AM	2.23^{b} (0.25)	2.87^{a} (0.50)	0.15^{ab} (0.04)
250	non-AM	3.13^{a} (0.16)	3.16^{a} (0.32)	0.20^{a} (0.04)
	AM	2.07^{b} (0.20)	2.85^{a} (0.23)	0.11^{b} (0.02)
F-values and level of significance	Zn	43.6***	0.40ns	3.42*
	Inoculum	2.47ns	0.35ns	1.92ns
	Zn × inoculum	4.59**	1.14ns	1.83ns
	Block	4.59ns	2.47ns	2.28ns

Means (*n*=4) and SE (inside parentheses) are shown
Different letters in columns designate treatments that are significantly different according to Bonferonni test
ns not significant
*$p<0.05$
**$p<0.01$
***$p<0.001$

Table 4 Relative extracted Zn percentage in shoots and roots of *N. rustica* under increasing soil–[Zn]

Zn (mg.kg^{-1} dry soil)	Inoculum	Shoots	Roots
0	non-AM	79.9^{a} (3.4)	49.1^{a} (5.2)
	AM	63.5^{a} (2.5)	45.5^{a} (6.0)
50	non-AM	14.7^{bc} (1.6)	11.6^{b} (2.4)
	AM	18.5^{b} (0.7)	6.0^{c} (1.6)
100	non-AM	12.2^{c} (1.1)	5.2^{c} (0.7)
	AM	8.1^{d} (0.4)	6.5^{c} (0.6)
250	non-AM	6.6^{d} (0.7)	4.3^{cd} (0.4)
	AM	6.8^{d} (0.5)	2.4^{d} (0.2)
F-values and level of significance	Zn	403***	70.3***
	Inoculum	4.1*	9.9**
	Zn x Inoculum	7.1**	3.0*
	Block	2.4ns	0.7ns

Percent values (n=4) and SE (inside parentheses) are shown

Different letters in columns designate treatments that are significantly different according to Bonferonni test

ns not significant

*p<0.05

**p<0.01

***p<0.001

(Weissenhorn et al. 1993). Moreover, Gildon and Tinker (1981) found a 35% colonization of *G. mosseae* in clover roots from a contaminated site. However, in a pot study, Gildon and Tinker (1983) did not observe any colonization of onion roots by *G. mosseae* at a soil–Zn concentration of 75 mg Zn kg^{-1} dry soil, that is a concentration three times lower than the highest soil–[Zn] used in the present study. These differences between field and greenhouse experiments may be attributed to differences in plant species, plant species mycorrhizal dependency, plant nutrient requirements, and/or AM fungus strain susceptibility or resistance under heavy metal stress.

Our hypothesis that Zn uptake is more enhanced in AM than non-AM plants must be rejected on the basis that total Zn content and concentration in roots at the highest soil–[Zn] level were lower in AM than non-AM roots. Likewise, the shoots and roots for some soil–[Zn] treatments showed lower extracted Zn% than non-AM plants, a difference of 25 to 50%. These trends are in agreement with the study of Bradley et al. (1981, 1982) who reported that plants of *Calluna vulgaris* L. grown in soil–Zn and –Cu excess showed significantly lower total Zn and Cu contents in the shoots of mycorrhizal than nonmycorrhizal plants. It was also shown that Zn immobilization in the fungal mycelium of *G. mosseae* and *G. versiforme* led to lower Zn uptake in clover plants, using a compartmental system; in fact, ten times higher Zn concentration was measured in the mycelium than in the host plant (Chen et al. 2001). As found in the present study, Li and Christie (2001) observed lower Zn concentration in AM than non-AM plants, particularly as soil–Zn application rate increased. Accordingly, the mycorrhizal fungus may have immobilized soil contaminants and prevented these from being taken up by the host plant, especially under increasingly toxic soil–Zn concentrations (Leyval et al. 1997, Weissenhorn et al. 1995). Recent molecular analyses have identified Zn transporters in *Medicago truncatula* (Burleigh et al. 2003) and in *G. intraradices* (González-Guerrero et al. 2005), which may be important in explaining the lower Zn level in AM than non-AM roots found in the present study. Other nutrients of interest are P and Mn, particularly in the context of Phosphorus–Zn interactions. The P concentrations we have measured in “wild tobacco” shoots decreased as soil–[Zn] increased, whereas Li et al. (2004) have shown that Zn concentrations in barley decreased as soil–[P] increased; this suggested a reciprocal antagonism between Zn and P (Shetty et al. 1994). The lower Mn concentrations in AM than non-AM plants, measured in our study, are consistent with the results of Kothari et al. (1991), who suggested the reduction of Mn by soil-microorganisms.

Contrary to our predictions, the results from all the physiological parameters indicated that both AM and non-AM “wild” tobacco plants are as tolerant to the soil–Zn levels used in the present study. In this case, the hypothesis that tolerance to increasing soil–[Zn] is enhanced in AM than non-AM plants must be rejected. Because Zn was added late in the growth phase, this likely accounts for the lack of significant differences among the treatments at the physiological level. The increases in percentage AM colonization must have transpired as a rapid burst only after the addition of Zn. Also, it may be that the Zn toxicity level is greater than our highest soil–Zn concentration. In

Table 5 Percent of total roots colonized, arbuscules, vesicles, and hyphae in *N. rustica* under increasing soil–[Zn]

Zn (mg kg^{-1} dry soil)	Total Roots	Arbuscules	Vesicles	Hyphae
0	14.2^{c} (3.7)	6.7^{b} (4.2)	4.2^{b} (2.2)	10.0^{b} (0)
50	29.4^{b} (0.8)	15.0^{b} (5.2)	5.0^{b} (1.4)	15.8^{b} (5.5)
100	47.5^{b} (7.7)	13.6^{b} (3.3)	15.0^{b} (1.8)	23.0^{b} (4.4)
250	81.6^{a} (4.6)	58.9^{a} (3.9)	37.9^{a} (8.8)	74.2^{a} (12)
F-values and level of significance				
Zn	62.3***	57.1***	53.6***	65.3***
Block	1.11ns	0.62ns	2.81ns	0.87ns

The percent values and SE (inside parentheses) are indicated and represent the number of roots having AM root structures in relation to the total number of roots sampled ×100 per treatment (n=3)

Different letters in columns designate treatments that are significantly different according to Bonferonni test

ns not significant

***p<0.001

this regard, Wenger et al. (2002) reported plant mass decreases in cultivated tobacco and maize when grown in 750 mg Zn kg^{-1} dry soil, a concentration three times higher than the highest concentration used in the present study. However, as Zn was applied to the soil under different conditions, this limits the breadth of comparison with our study. Despite this, it has been suggested that AM fungi may eliminate the bioavailability of otherwise toxic contaminants and consequently buffer the soil for plants and other microorganisms (Joner et al. 2000; Leyval et al. 1997). By contrast, Davies et al. (2001, 2002) and Rufyikiri et al. (2002, 2003) showed, respectively, that colonization by *G. intraradices* enhanced uptake and accumulation of chromium in sunflower and of uranium in cultivated in vitro carrot roots. Although AM fungi may enhance heavy metal phytoextraction for some plant species, our findings agree with the hypothesis of metal-binding AM fungal capacity as suggested by others (Chen et al. 2001; Joner et al. 2000; Leyval et al. 1997; Li and Christie 2001). The dramatic decrease in the relative extracted Zn% may indicate that the efficacy of Zn removal from the soil declined as soil–[Zn] increased, an effect having implications for the role of phytoextraction in soil remediation. Furthermore, it can be presumed that AM colonization benefits host plants by increasing the uptake of limiting trace minerals, and possibly decreasing their uptake when in excess. Chen et al. (2003) have suggested that this effect occurs beyond a critical soil–[Zn] level which, in our study, was between 100 and 250 soil–[Zn] for the "wild" tobacco species.

In summary, this study has contributed significant evidence with regard to the effects of an AM fungus on the "wild" tobacco plants subjected to soil–[Zn] excess. Further investigation on the effects of heavy metals on AM fungi in interaction with host plant species will be of interest and should contribute to a better understanding of the mycorrhizospheric network.

Acknowledgement This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to C.C.

References

Barceloux DG (1999) Zinc. J Toxicol Clin Toxicol 37:279–292

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye-binding. Ann Biochem 72:248–254

Bradley R, Burt AJ, Read DJ (1981) Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. Nature 292:335–337

Bradley R, Burt AJ, Read DJ (1982) The biology of mycorrhiza in the Ericaceae: VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytol 91:197–209

Bruinsma J (1963) The quantitative analysis of chlorophylls a and b in plant extracts. Photochem Photobiol 72:241–249

Burleigh SH, Kristensen BK, Bechmann IE (2003) A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol Biol 52:10–77–1088

Chaney RL (1993) Zinc Phytotoxicity. In: Robson AD (ed) Zinc in soils and plants. Kluwer, Dordrecht, The Netherlands, pp 135–150

Charest C, Clark G, Dalpé Y (1997) The impact of arbuscular mycorrhizae and phosphorus on the growth of two turfgrass species. J Turfgrass Manag 2:1–14

Chen BD, Christie P, Li XL (2001) A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. Chemosphere 42:185–192

Chen BD, Li XL, Tao HQ, Christie P, Wong MH (2003) The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere 50:839–846

Dalpé Y (1993) Vesicular–arbuscular mycorrhizae. In: Carter MR (ed) Soil sampling and methods of analysis, 3rd edn. Can Soc Soil Sci, CRC, Boca Raton, Fla, pp 287–301

Davies FT, Puryear JD, Newton RJ, Egilla JN, Saraiva Grossi JA (2001) Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). J Plant Physiol 158:777–786

Davies FT, Puryear JD, Newton RJ, Egilla JN, Saraiva Grossi JA (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth, and gas exchange. J Plant Nutr 25:2389–2407

Gildon A, Tinker PB (1981) A heavy metal tolerant strain of mycorrhizal fungus. Trans Br Mycol Soc 77:648–649

Gildon A, Tinker PB (1983) Interactions of vesicular–arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular–arbuscular mycorrhizae. New Phytol 95:147–161

González-Guerrero M, Azcón-Aguilar C, Mooney M (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130–140

Hovsepyan A, Greipsson S (2004) Effect of arbuscular mycorrhizal fungi on phytoextraction by corn (*Zea mays*) of lead-contaminated soil. Int J Phytoremediation 6:305–321

Jackson ML (1973) In: Soil chemical analysis. Prentice Hall, New York, USA

Janoušková M, Vosatká M (2005) Response to cadmium of *Daucus carota* hairy roots dual cultures with *Glomus intraradices* or *Gigaspora margarita*. Mycorrhiza 15:217–224

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227–234

Kothari SK, Marschner H, Romheld V (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil 131:177–185

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological, and applied aspects. Mycorrhiza 7:139–153

Li XL, Christie P (2001) Changes in soil solution Zn and pH and uptake of Zn by an arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42:201–207

Li HY, Zhu YG, Smith SE, Smith FA (2004) Phosphorus–zinc interactions in two barley cultivars differing in phosphorus and zinc efficiencies. J Plant Nutr 26:1085–1099

Marschner H (1986) Mineral nutrition of higher plants, 2nd edn. Academic, Orlando, FL

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162

Morton JB, Benny GL (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae, with an emendation of Glomaceae. Mycotaxon 37:471–491

Paradis R, Dalpé Y, Charest C (1995) The effects of arbuscular mycorrhizae and low temperature on wheat. New Phytol 129:637–642

Rufyikiri G, Thiry Y, Wang L, Delvaux B, Declerck S (2002) Uranium uptake and translocation by the arbuscular mycorrhizal fungus, *Glomus intraradices*, under root-organ culture conditions. New Phytol 156:275–281

Rufyikiri G, Thiry Y, Declerck S (2003) Contribution of hyphae and roots to uranium uptake and translocation by arbuscular mycorrhizal carrot roots under root-organ culture conditions. New Phytol 158:391–399

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Shetty KG, Hetrick BAD, Schwab AP (1994) Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ Pollut 86:181–188

Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought recovery. Physiol Plant 102:285–296

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1997) Effects of arbuscular-mycorrhizas on leaf water potential, sugar and P contents during drought and recovery of maize. Can J Bot 75:1582–1591

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant AM fungi from heavy-metal polluted soils. Plant Soil 157:247–256

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza 5:245–251

Wenger K, Gupta SK, Furrer G, Schulin R (2002) Zinc extraction potential of two common crop plants, *Nicotiana tabacum* and *Zea mays*. New Phytol 242:217–225

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Upper-Saddle River, NJ