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Effects of arbuscular mycorrhizal inoculation on cadmium accumulation by different tobacco (*Nicotiana tabacum* L.) types

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Abstract

The effect of arbuscular mycorrhiza (AM) on cadmium (Cd) uptake by tobacco (*Nicotiana tabacum* L.) was studied in a pot experiment. Three commercial varieties, Basma BEK, K326 and TN90, representing three distinct tobacco types, were each grown in a different soil with nutritional conditions matching as closely as possible their requirements for field production. Cd concentrations in these soils were within the background range. Each variety was either non-mycorrhizal or inoculated with one of five AM fungal isolates. Cd concentration in leaves was decreased by inoculation with selected isolates in the K326 and TN90 variety grown in acidic soils. In contrast, it was increased by inoculation with most isolates in the Basma BEK variety grown in a basic soil with low Cd availability. Besides, plants of all three varieties had significantly higher leaf concentrations of phosphorus and nitrogen in some inoculated treatments. The percentage of root colonisation was mostly low in the inoculated treatments. In the Basma BEK and TN90 variety, the tested AM fungal isolates differed in their ability to colonise roots, but no correlation was found between the root colonisation of an isolate and its effects on the Cd concentrations in tobacco leaves. One isolate influenced most pronouncedly Cd concentrations and improved mineral nutrition in all the three combinations of variety and soil despite its low colonisation levels. AM symbiosis probably affected Cd uptake of tobacco by indirect mechanisms such as stimulation of root growth or mycorrhizal plant mediated changes in chemical or biological soil properties.

Keywords: Agriculture; *Glomus*; Heavy metals; Nitrogen; Phosphorus

1. Introduction

The non-essential element cadmium (Cd) is relatively mobile in soils and potentially highly toxic in comparison with other "heavy metals" (Schachtschabel et al., 1992; see Duffus, 2002, for a discussion of the term heavy metal). It is classified as a known human carcinogen (Class 1) by the International Agency for Research on Cancer (IARC, 1993). In many plants, a high proportion of the Cd uptake is effectively bound in roots. In contrast, *Nicotiana tabacum* (tobacco) usually accumulates more Cd per unit biomass in leaves than in roots and may display relatively high Cd concentrations in leaves (Lugon-Moulin et al., 2006). Food is the major source of Cd exposure for the general non-smoking population in most areas, but heavy smoking may represent another important source of exposure to this metal (WHO, 1992). Indeed, during smoking, some Cd is transferred to the mainstream smoke, which is directly inhaled by the smoker (Pappas et al., 2006; Smith et al., 1997).

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Several strategies may be followed to decrease the Cd concentration in plant leaves. For example immobilisation of soil Cd by amendments or genetic modifications of plants to restrict their Cd uptake (reviewed in Lugon-Moulin et al., 2004). Among other options, the formation of arbuscular mycorrhiza (AM) with obligatory symbiotic fungi of the order Glomeromycota may decrease Cd concentration in the shoots of plants exposed to Cd contamination in soil (Heggo et al., 1990; Weissenhorn et al., 1995; Chen et al., 2004), but the opposite effect of mycorrhiza has been reported as well (Rivera-Becerril et al., 2002). Several factors seem to play a role, especially the plant species (Joner and Leyval, 2001) or even variety (Rivera-Becerril et al., 2002), the AM fungal isolate involved (Liao et al., 2003; Wang et al., 2005) and the Cd concentration in soil (Heggo et al., 1990; Chen et al., 2004). In addition, cultivation conditions such as pot volume, light intensity or the length of the cultivation period may constitute important factors for the heavy metal uptake of plants in pot cultivation experiments (Weissenhorn et al., 1995; Joner and Leyval, 2001).

The plant partner primarily benefits from AM symbiosis by improved phosphorus (P) nutrition, but acquisition of other macro- and micro-nutrients can be enhanced as well under certain conditions (Smith and Read, 1997). This is often accompanied by a positive growth response to AM symbiosis, which may account for decreased heavy metal concentrations in plant shoots due to the biomass dilution effect (Jarrell and Beverly, 1981; Meharg and Cairney, 2000). Receptivity to infection by AM fungi and the AM effects on plant nutrient status and growth depend on similar factors as described for heavy metal uptake: plant genotype to the variety level (Vierheilig and Ocampo, 1991; Martensson and Rydberg, 1994; Eason et al., 2001) and the colonising AM fungal isolate (e.g. Bethlenfalvay et al., 1989). Important external factors are especially P availability in soil and light supply (Graham et al., 1982; Son and Smith, 1988).

Recent studies using the tobacco variety Wisconsin 38 have shown that this tobacco readily forms AM symbiosis and responds to mycorrhiza by decreased Cd concentrations in shoots when grown in Cd contaminated substrates under a broad range of cultivation conditions (Janoušková et al., 2005a,b). However, AM symbiosis of commercial tobacco varieties has apparently been little studied. As tobacco is subdivided in many different varieties, regrouped in several types, and is cultivated in a wide range of soils under different agroclimatic conditions, it is not clear to what extent AM may impact Cd uptake in commercially produced tobacco.

The principal aim of this study was to test the effect of AM symbiosis on the Cd concentration in tobacco leaves. We approached the topic by testing three distinct commercial tobacco varieties representing three major types. They were either grown non-mycorrhizal or inoculated with one of five different AM fungal isolates originating from a wide range of soil conditions including heavy metal-contaminated soils. Besides Cd accumulation in leaves, the development of AM symbiosis and its effects on tobacco growth and mineral nutrition (nitrogen, phosphorus and potassium) were also followed.

2. Material and methods

2.1. Plant material and soils

Three tobacco (*Nicotiana tabacum* L.) varieties, Basma BEK, K326 and TN90, were selected for the experiment. Each variety belongs to a distinct tobacco type important in commercial tobacco production, Oriental, flue-cured and Burley (respectively). These three tobacco types differ markedly in terms of morphology, soil requirements, cultivation and curing practices (Layten Davis and Nielsen, 1999). For instance, Oriental tobacco is typically produced on low fertility soils without fertilisation, and produces smaller leaves than the other two types. Flue-cured is usually grown on sandy soils while Burley tobaccos are grown on heavier-textured soils. The latter receive higher N fertilisation inputs than flue-cured tobacco. Consequently, the three cultivars (types) were not compared under the same growth conditions (i.e. soil and fertilisation). The goal of this study was to use soil and fertilisation conditions matching as closely as possible each type's requirements. Therefore, we studied the response to AM inoculation of each type independently.

Each variety was cultivated in a soil obtained from a field where it had been produced previously (for origin and characteristics of the soils, see Table 1). The total amounts of Cd in these soils were similar. However, Cd bioavailabilities, estimated here by extraction with 0.01 M $CaCl_2$, were different, most likely due to large pH differences (Table 1). Indeed, soil acidity appears as an important factor increasing Cd bioavailability to tobacco (Tsadilas et al., 2005; review in Lugon-Moulin et al., 2004).

2.2. Fungal material

Each tobacco variety was either left uninoculated or inoculated with one of the following five AM fungal

Table 1
Characterisation of the soils used for cultivation of three tobacco varieties (Basma BEK, K326, TN90) and concentrations of nutrients in the applied fertilisation solutions

	Basma BEK	K326	TN90
Soil characteristics			
Origin	Elbasan (Albania)	Rio Grande do Sul (Brazil)	Rio Grande do Sul (Brazil)
pH (H_2O)	7.6	4.9	5.3
pH (KCl)	7.2	4.3	4.7
C (%)	1.36	0.85	1.76
N (%)	0.12	0.11	0.16
P (Olsen) (mg kg^{-1})	34.7	37.0	9.8
CEC	281.5	133.6	182.4
Cd total (mg kg^{-1})	0.13	0.10	0.20
Cd (0.01 M $CaCl_2$) (mg kg^{-1})	0.004	0.058	0.023
Fertilisation solution			
N (g l^{-1}) as $Ca(NO_3)_2$	0.06	0.12	0.18
P (g l^{-1}) as KH_2PO_4	0.03	0.06	0.09
K (g l^{-1}) as KCl, KH_2PO_4	0.69	1.38	2.07
Mg (g l^{-1}) as $MgSO_4$	0.12	0.25	0.37
Ca (g l^{-1}) as $Ca(NO_3)_2$	0.08	0.17	0.25
Cl (g l^{-1}) as KCl	0.59	1.19	1.78
S (g l^{-1}) as $MgSO_4$	0.16	0.33	0.49

isolates (isolate name and origin in parentheses): *Glomus intraradices* Schenck & Smith (PH5; Pb contaminated waste-disposal site also containing elevated concentrations of Cd, Czech Republic), *Glomus intraradices* Schenck & Smith (BEG75; agricultural site, Switzerland), *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (BEG25; agricultural site, United Kingdom), *Glomus claroideum* Schenck & Smith (Kb1; Cd contaminated agricultural site, Czech Republic), *Glomus geosporum* (Nicol. & Gerd.) Walker (Madag; agricultural site, Madagascar). The isolates were selected to represent different AM fungal species and soils of origin in order to obtain information on the isolate-dependent variability in the reaction of the tobacco varieties to inoculation.

The fungal inoculum originated from 4-month-old sporulating maize-clover cultures of the AM fungi maintained in greenhouse conditions in inert substrate (sand and/or zeolite). The isolates originating from contaminated soils (PH5, Kb1) were kept in their soils of origin prior to the establishment of the inoculum cultures in inert substrate.

2.3. Establishment of the experiment and cultivation conditions

Tobacco was seeded into commercial peat-based seeding substrate (Agro CZ) sterilised by autoclaving (20 min at 120 °C). At cross stage (with four leaves), the plantlets were transplanted into 2.8 l plastic pots (one plant per pot) filled with 2.5 kg of dry soil, previously sterilised by γ-irradiation (50 kGy).

The pots of the inoculated treatments received 10 ml of inoculum suspension of the AM fungus consisting of colonised root segments, extraradical mycelium (ERM) and spores in water. The quality of the inocula had been checked under a binocular microscope prior to application to ensure high density of the AM fungal propagules. The non-inoculated treatments received the same amount of inoculum sterilised by autoclaving (twice for 30 min on two consecutive days). In order to re-establish microbial conditions of the original soils, all pots were irrigated with 10 ml of microbial filtrate obtained by passing non-sterile soil suspension from the corresponding soil through a filter paper (Whatman No. 1). Similarly, microbial filtrates were prepared from the AM fungal cultures used for inoculation, mixed and supplied in the same amount to all pots in order to equalise the microbial conditions of the inoculation treatments. Each treatment consisted of six replicates.

The experiment was grown in a greenhouse with light supplement (12 h, metalhalide lamps, 400 W) for 16 weeks. The plants were supplied once a week with 100 ml (the first 4 weeks) or 200 ml (from the fifth week till the end of the experiment) of a fertilisation solution (see Table 1). The concentrations of macronutrients in the fertilisation solution were adjusted to the differences in nutrient demand of the three tobacco varieties.

2.4. Parameters determined at harvest

Shoot biomass was determined after drying at 80 °C for 24 h, separated into leaf biomass and the biomass of stalks and flowers. For the Basma BEK variety, which formed suckers (regenerants on stalks), the biomass of the suckers was recorded separately. Middle leaves (No. 8-14 in Basma BEK, No. 7-12 in K326 and TN90) were ground and analyzed for their contents of Cd. Moreover, the three major nutrients typically used for tobacco fertilisation were quantified: nitrogen (N), phosphorus (P) and potassium (K). Cd concentrations in roots were also determined in the inoculated treatments (and the corresponding controls) in which decreased Cd concentration in leaves was determined, to find out whether the decrease in Cd leaf concentration was accompanied by a modification of the Cd concentration in roots.

For the analysis of Cd, P and K, 500 mg of oven-dried (80 °C) tobacco leaf biomass was weighed and digested in 10 ml of concentrated nitric acid (HNO_3) in a microwave accelerated reaction system (MARS 5; CEM Corp., Matthews, NC). The element concentrations were assessed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500A; Agilent Technologies, Palo Alto, CA). Blanks were introduced regularly and Certified Virginia Tobacco Leaves (CTA-VTL-2) were used as reference material (Dybczynski et al., 1997). The N concentration in leaves was determined by the combustion method with the Elemental Analyzer Carlo Erba NC 2500 using a constant flow of helium enriched with oxygen and reduction of the combustion gases with copper.

Mycorrhizal colonisation was evaluated on root samples stained with 0.05% trypan blue in lactoglycerol (Koske and Gemma, 1989) using the grid-line intersect method (Giovannetti and Mosse, 1980). A minimum of 200 intersections were counted in every sample. The ERM length in soil was estimated by a modified membrane filtration technique (Jakobsen et al., 1992) and calculated to meters of hyphae in 1 g of air-dried substrate. The average length of background mycelium was assessed on samples from the six non-inoculated pots of each tobacco variety. The values (0.15, 0.07 and 0.3 m g^{-1} for the Basma BEK, K326 and TN90 variety, respectively) were subtracted from the ERM length values obtained in the corresponding inoculated treatments.

2.5. Statistical analysis

Data on root colonisation and ERM length were logarithmically transformed in order to meet the requirements of ANOVA and differences between treatments were evaluated by ANOVA and Tukey's HSD test at the significance level $P < 0.05$. Data on shoot biomass and element concentrations were treated by one-way ANOVA with the factor inoculation and Dunnett's multiple range test for each tobacco variety separately.

3. Results

3.1. Mycorrhizal development

No root colonisation with AM fungi was found in the non-inoculated treatments. Percentage of root colonisation in the inoculated treatments was significantly influenced by the factors variety x soil combination ($F = 77.21$, $P < 0.001$) and AM fungal isolate

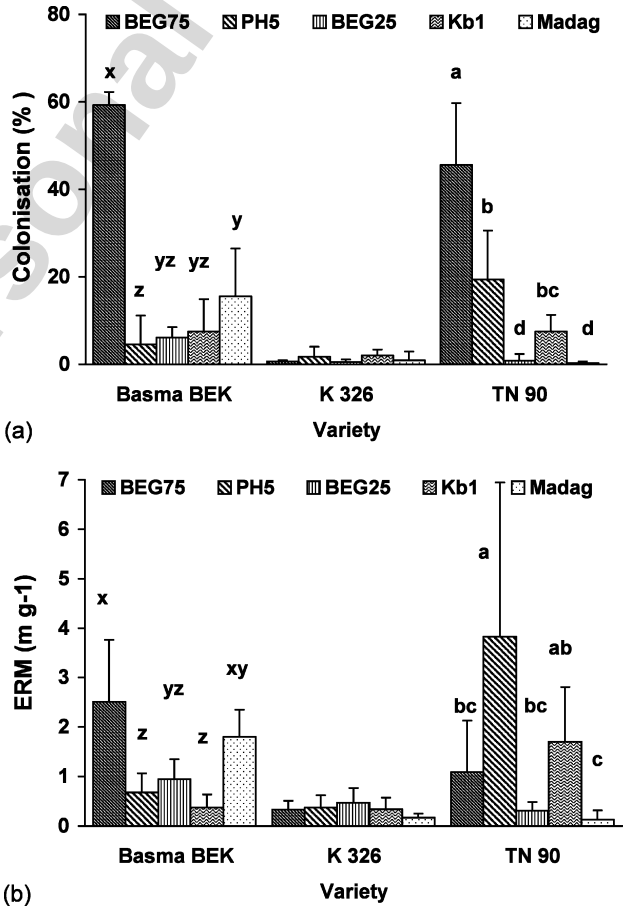

Fig. 1. Root colonisation of three tobacco varieties (Basma BEK, K326 and TN90) by five AM fungal isolates (BEG75, PH5, BEG25, Kb1, Madag) (a) and the ERM length of the isolates in soil (b). Each variety was grown in a different soil and with different fertilisation conditions to better match their requirements for field production. The values are given as means of six replicates (+S.D.). Letters indicate significant differences between the inoculation treatments within the Basma BEK and TN90 varieties according to one-way ANOVA and Tukey's HSD test at the level $P < 0.05$. Columns indicated with the same letter are not significantly different.

($F = 30.73$, $P < 0.001$) as well as by their interaction ($F = 18.83$, $P < 0.001$). Generally, root colonisation was very low (<2%) in the K326 variety without significant differences between isolates ($F = 1.37$, $P > 0.05$) whereas colonisation was higher and significantly dependent upon isolate in the Basma BEK ($F = 16.77$, $P < 0.001$) and TN90 ($F = 59.93$, $P < 0.001$) variety (Fig. 1a). In both varieties, *G. intraradices* BEG75 reached the highest root colonisation whereas *G. mosseae* BEG25 and *G. claroideum* Kb1 reached only low colonisation levels (<10%). The ERM length was in linear relationship with root colonisation (constant ± 95% confidence interval = 0.19 ± 0.12, slope ± 95% confidence interval = 0.23 ± 0.06, $R^2 = 0.409$, $P < 0.001$). The differences in ERM length between treatments therefore followed a similar pattern as described for root colonisation (Fig. 1b).

3.2. Effect of AM on Cd concentrations in leaves

Cd concentration in the middle leaves was affected by inoculation in all the three tobacco varieties (Fig. 2). Leaves of non-mycorrhizal Basma BEK plants contained only 0.24 μg g^{-1} Cd, and inoculation with four out of the five tested isolates significantly increased their Cd concentration ($F = 24.01$, $P < 0.001$). Cd concentrations in the leaves of non-mycorrhizal K326 and TN90 plants were about 10-fold higher (2.53 and 3.15 μg g^{-1}, respectively) than in the Basma BEK variety. Two and three isolates decreased the Cd concentrations in the leaves of K326 and TN90, respectively, when compared with the corresponding controls (Fig. 2). However, these AM isolates did not affect root Cd concentrations with the exception of

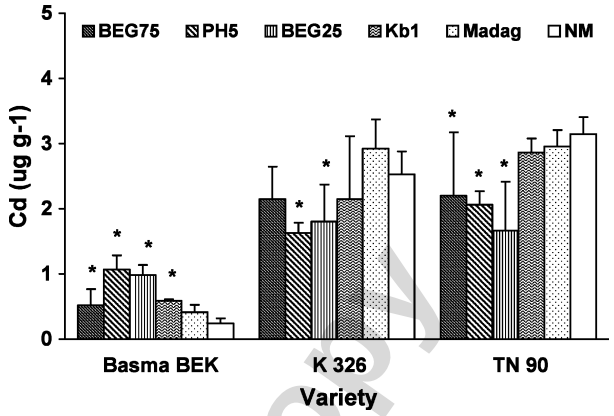

Fig. 2. Cadmium concentration in the middle leaves of three tobacco varieties (Basma BEK, K326 and TN90) as affected by inoculation with five AM fungal isolates. Each variety was grown in a different soil and with different fertilisation conditions to better match their requirements for field production. The values are given as means of six replicates (+S.D.). Columns indicated by an asterisk significantly differ from the NM treatment within tobacco variety according to Dunnett's multiple range test at the level $P < 0.05$. *Abbreviations*: BEG75, PH5, BEG25, Kb1, Madag: treatments inoculated with the corresponding isolate; NM: non-mycorrhizal treatment.

BEG25 in TN90 (see Table 2). Higher leaf to root concentration ratios suggested that Cd translocation from roots to shoots was higher in TN90 than in K326. Inoculation tended to decrease the ratio in the TN90 variety, although it did not significantly influence this parameter in either variety (Table 2).

3.3. Effect of AM on tobacco biomass and NPK uptake

Inoculation with none of the AM isolates significantly increased tobacco leaf biomass in any variety

Table 2
Cadmium concentrations in the middle leaves and roots of two tobacco varieties (K326 and TN90) and the ratio of leaf to root concentration, as affected by inoculation with selected AM fungal isolates

		Cd leaf concentration (μg g^{-1})	Cd root concentration (μg g^{-1})	Concentration ratio (leaves/roots)
K326	PH5	1.6# (0.16)	1.0 (0.28)	1.7 (0.30)
	BEG25	1.9# (0.54)	0.8 (0.19)	2.5 (0.93)
	NM	2.5 (0.35)	1.1 (0.18)	2.3 (0.47)
Significance		**	n.s.	n.s.
F-value		9.31	2.41	2.45
TN90	BEG75	2.2# (0.97)	0.7 (0.05)	3.0 (1.36)
	PH5	2.1# (0.21)	0.6 (0.13)	3.3 (0.56)
	BEG25	1.7# (0.74)	0.6# (0.13)	2.9 (1.17)
	NM	3.1 (0.26)	0.8 (0.11)	4.2 (0.93)
Significance		**	*	n.s.
F-value		5.84	3.55	2.04

The values are given as means of six replicates (S.D.). Significant effects according to one-way ANOVA: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$; n.s. non-significant effect. Means indicated by the symbol "#" are significantly lower than the corresponding NM treatment according to Dunnett's multiple range test at significance level $P < 0.05$. For abbreviations see Table 3.

Table 3
The dry weight (DW) of total shoot biomass, leaves and suckers and concentrations of the nutrients N, P and K in the middle leaves of three tobacco varieties (Basma BEK, K326 and TN90) as affected by inoculation with five AM fungal isolates. Each variety was grown in a different soil and with different fertilisation conditions to better match their requirements for field production

		BEG75	PH5	BEG25	Kb1	Madag	NM	*F*-value	Significance
Basma BEK	Shoot DW (g)	32 (2.6)	36* (1.3)	36* (1.8)	33 (1.7)	31 (1.7)	32 (2.1)	8.05	***
	Leaves DW (g)	12 (1.6)	12 (1.2)	13 (0.8)	13 (0.8)	13 (1.0)	13 (0.9)	1.82	n.s.
	Suckers DW (g)	0.4 (0.5)	4.3* (2.3)	2.7* (1.2)	0.5* (0.3)	0.4 (0.7)	0.1 (0.1)	15.87	***
	N (%)	1.2 (0.1)	2.1* (0.4)	4.8* (0.3)	1.6* (0.3)	0.9 (0.1)	0.9 (0.1)	266.79	***
	P (mg g^{-1})	2.1* (0.5)	3.4* (0.2)	5.5* (0.6)	2.3* (0.5)	1.4 (0.1)	1.3 (0.1)	90.75	***
	K (mg g^{-1})	12.6* (1.7)	19.0* (1.6)	24.3* (1.0)	14.2* (1.6)	9.5 (0.8)	9.0 (0.7)	124.27	***
K326	Shoot DW (g)	27 (2.8)	30 (1.4)	33* (3.8)	29 (3.8)	30 (3.4)	26 (2.4)	3.66	*
	Leaves DW (g)	15 (2.4)	15 (1.3)	17 (2.0)	14 (2.3)	17 (3.3)	15 (2.3)	1.34	n.s.
	N (%)	0.8 (0.03)	1.1 (0.4)	2.0* (1.2)	1.5 (0.4)	0.8 (0.05)	0.8 (0.1)	4.90	**
	P (mg g^{-1})	1.1 (0.2)	1.1 (0.6)	2.7* (1.6)	0.9 (0.1)	1.3 (0.3)	1.2 (0.1)	6.37	***
	K (mg g^{-1})	15.0 (2.2)	17.4 (3.5)	19.8 (5.5)	18.8 (1.7)	15.0 (1.9)	15.5 (1.5)	2.74	*
TN90	Shoot DW (g)	26 (1.7)	25 (3.2)	29 (3.8)	28 (2.0)	24# (1.3)	28 (1.9)	4.03	**
	Leaves DW (g)	15 (1.2)	14 (1.2)	15 (1.9)	16 (0.9)	13# (0.7)	15 (0.5)	3.62	*
	N (%)	1.3 (0.1)	1.5 (0.1)	3.4* (1.6)	1.3 (0.1)	1.6 (0.1)	1.4 (0.1)	8.12	***
	P (mg g^{-1})	1.4 (0.2)	1.3 (0.1)	2.6* (1.0)	1.4 (0.2)	1.4 (0.1)	1.3 (0.2)	7.08	***
	K (mg g^{-1})	27.4 (2.8)	26.6 (4.5)	37.2* (7.2)	26.1 (1.7)	28.3 (1.5)	26.7 (3.2)	6.73	***

Suckers were formed only by the Basma BEK variety. The values are given as means of six replicates (S.D.). Significant effects according to one-way ANOVA: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$; n.s. non-significant effect. Means indicated by an asterisk (*) are significantly higher than the corresponding NM treatment, means indicated by the symbol "#" are significantly lower than the corresponding NM treatment according to Dunnett's multiple range test at significance level $P < 0.05$. *Abbreviations*: BEG75, PH5, BEG25, Kb1, Madag: treatments inoculated with the corresponding isolate; NM: non-mycorrhizal treatment.

(Table 3). However, inoculation with three isolates increased the regeneration capacity of Basma BEK (determined as biomass of suckers) resulting in increased total biomass in two inoculated treatments, and inoculation with one isolate increased the total shoot biomass of K326.

In the Basma BEK variety, concentrations of all three nutrients were significantly higher in three or four inoculated treatments when compared with the non-inoculated control (Table 3). Plants inoculated with *G. mosseae* BEG25 had the highest leaf concentrations of the three nutrients from all inoculation treatments. In the TN90 variety, higher concentrations of N, P, K were measured only with the BEG25 isolate. In K326, the same isolate led to significantly increased concentrations of N and P (Table 3). Therefore, *G. mosseae* BEG25 was clearly the most effective isolate in improving leaf concentrations of N, P, K in the three "variety × soil" combinations tested.

4. Discussion

Inoculation with AM fungi resulted only in low levels of root colonisation in most treatments. This result was unexpected, as tobacco had been previously found to become extensively colonised with AM fungi: the *Glomus intraradices* isolate PH5 colonised 59% (in sand) or 95% (in soil) of the roots of the tobacco variety Wisconsin 38 under very similar inoculation and cultivation conditions (Janoušková et al., 2005a). The experimental design does not allow conclusions on whether the low colonisation levels were caused by low affinity of the tested tobacco varieties to mycorrhiza, as reported, e.g. for some cultivars of wheat (Vierheilig and Ocampo, 1991), or by the soil or fertilisation conditions. The overall low root colonisation of the K326 variety suggests the former cause, whereas the differential development of the isolates in the roots of the other two varieties indicates rather inhibition by some external factors, which may have been better tolerated by some isolates than by others.

Root colonisation by AM fungi can also decrease during cultivation period as a result of fast root growth. However, evaluation of colonisation in some randomly selected replicates after 8 weeks (data not shown) revealed similarly low values as at harvest. Therefore, our results show that significant changes in plant Cd uptake and nutrient acquisition may be achieved by AM even at very low colonisation levels. In fact, the differential effects of the isolates were not related to their development in the roots or soil. In contrast to the report of Diaz et al. (1996), no clear relationship was found between the effect of an isolate (on Cd uptake by tobacco) and its origin (i.e. from contaminated or

non-contaminated soil). These results illustrate that the factors responsible for isolate-dependent variability in the effects of AM symbiosis are far from being clear.

Interestingly, the effect of inoculation on the Cd concentration in tobacco leaves differed between the cultivar–soil combinations. Inoculation with some isolates decreased Cd concentrations in the TN90 and K326 variety by up to 47% and 36%, respectively. This result was consistent with the decreased Cd concentration in the shoots of mycorrhizal tobacco of the variety Wisconsin 38 grown in substrates with high Cd availability (Janoušková et al., 2005b). The effect of mycorrhiza on the Cd concentration in Basma BEK leaves, however, sharply contrasted with the results obtained for the other two varieties: inoculation with four out of the five tested isolates increased the Cd concentration in the leaves of this variety. The Basma BEK variety was grown on a soil with low Cd availability, probably due to the high soil pH (Schachtschabel et al., 1992). This is reflected in the Cd concentration in the leaves of non-mycorrhizal Basma BEK plants, which was 11- or 13-fold lower than that of non-mycorrhizal K326 and TN90, respectively. It is noted that different tobacco varieties may show differences in Cd uptake (review in Lugon-Moulin et al., 2004). However, it is unlikely that the above >10-fold differences in Cd concentration between Basma BEK and the other two varieties would have been found if the three control varieties had been grown in the same soil conditions, although we cannot rule out that some of the difference is cultivar-based.

Our results are therefore in accordance with an assumption that mycorrhiza may increase Cd concentrations in plant shoots on soils with low Cd availability and decrease them on soils with higher Cd availability (Heggo et al., 1990). The critical Cd leaf concentration, at which the effect of mycorrhiza on Cd concentration in leaves changed from positive to negative, was between 8 and 18 $\mu g\ g^{-1}$ for soybeans in the study of Heggo et al. (1990), whereas it was much lower (below 2.5 $\mu g\ g^{-1}$) for tobacco in the present study. Soil Cd bioavailability was estimated using $CaCl_2$. It is noted that chemical extractants may not always be good indicators for Cd availability to tobacco (e.g. Keller et al., 2005; Tsadilas, 2000), but the bioavailability values estimated by $CaCl_2$ extraction were consistent with the data on soil pH and Cd concentrations in the biomass of tobacco in this study if the Basma BEK variety is compared with the other two varieties.

The mechanisms behind the decreased Cd concentrations in the leaves of mycorrhizal TN90 and K326 tobacco plants are not clear. Inoculation did not increase the shoot biomass of these two varieties so that biomass dilution of Cd, observed by Weissenhorn et al. (1995), cannot be assumed. Data on Cd concentrations in roots were not in support of enhanced Cd retention in mycorrhizal roots as described, e.g. by Loth and Höfner (1994), although the biomass of roots was not assessed (it was difficult to isolate a representative proportion of the root system from the soils used because of the abundancy of fine, easily broken roots). Lower Cd availability in the rhizosphere of mycorrhizal plants due to Cd sorption on ERM has been proposed for contaminated soils as a mechanism of lower total uptake by mycorrhizal plants (Joner et al., 2000). However, the decreased Cd concentrations in some inoculated treatments were not consistently associated with higher ERM density in soil as could have been expected if ERM had played a role in our results.

It is therefore probable that the lower Cd concentration in the leaves of tobacco in some inoculated treatments was a result of indirect, plant-mediated mechanisms, which were also suggested to play a role in AM effects on Zn uptake by plants (Christie et al., 2004). Mycorrhizal plants may have higher rhizosphere pH (Marschner and Baumann, 2003; Chen et al., 2004) and an altered pattern of carbohydrate release (Jones et al., 2004) in comparison with non-mycorrhizal plants. Such differences between mycorrhizal and non-mycorrhizal rhizosphere may also result in differences in Cd availability.

In addition to altered Cd concentrations, improved P acquisition has been observed in all the three tobacco varieties after inoculation with at least one isolate despite low colonisation levels. Of particular interest is the finding that improved P nutrition was accompanied by enhanced acquisition of N, a nutrient especially important in Burley tobacco (represented in our study by TN90). Despite improved mineral nutrition, there were only small positive growth effects. They can be partly related to pot size, as suggested by Zhu et al. (2001). Indeed, the available soil volume was relatively small for the large root system of tobacco towards the end of the cultivation.

In the K326 and TN90 varieties, nutrient acquisition was only improved by inoculation with the BEG25 isolate. This isolate had low root colonisation levels below 1% and an ERM length of 0.5 and 0.3 $m\ g^{-1}$ soil, which is at the lowest limit of the range reported for the density of AM fungal ERM in soil (as summarised by Smith and Read, 1997). This suggests that indirect, plant-mediated effects were involved both in the improved nutrient acquisition and in the decreased Cd uptake of mycorrhizal plants. van der Heijden

(2001) observed functional AM symbiosis in *Salix repens* plants with less than 5% of their root length colonised by *G. mosseae*. They concluded that the mycorrhizal effects were related to the higher root length of AM plants. Stimulation of root growth by mycorrhiza has been also observed for oat (Loth and Höfner, 1994) or *Citrus* seedlings (Fidelibus et al., 2001). This mechanism may also have been responsible for the observed effects of BEG25 on the nutrient uptake of tobacco.

5. Conclusion

Our results show that under certain conditions the same AM isolate can decrease Cd uptake by tobacco, while in other conditions, it may increase it. While this may be accounted for by different fertilisation regimes or soil characteristics, it appears that Cd bioavailability played a role in this result. Another important and intriguing result is the significant responses in terms of Cd and nutrients uptake even when the symbiosis was based on very low root colonisation levels. The small percentage of colonised roots and low amounts of ERM in most inoculated treatments suggest that plant-mediated mechanisms, such as enhanced root growth or changes in root exudation, were involved in the effects of AM symbiosis. As differences in efficiency between isolates were to a certain degree consistent among the three combinations of variety × soil, the selection of one or more effective isolates for a broader range of tobacco genotypes and soil conditions may be possible. However, there is a need to better understand the precise mechanisms by which different AM and tobacco varieties respond to each other under different environments.

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