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Mycorrhizal infection of an *Agrostis capillaris* population on a copper contaminated soil

W.A.J. GRIFFIOEN, J.H. IETSWAART and W.H.O. ERNST
Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

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Abstract

To investigate the possible natural development of heavy metal tolerance in VA-mycorrhizal fungi, plants of *Agrostis capillaris* from an uncontaminated, a copper-contaminated and a zinc/cadmium-contaminated area were examined for VA-mycorrhizal infection. During a period of 5 years (1987 to 1991) the plants of the copper-tolerant population were hardly infected, whereas the population on the uncontaminated soil showed a mean infection of nearly 60% and the zinc/cadmium-tolerant population of 40%. A detailed analysis of the surroundings of the copper-enriched site revealed the presence of VA-mycorrhizal fungi and a negative correlation between the infection rate of *A. capillaris* and the copper content of the soil. In contrast to the copper-contaminated soil, the abundant presence of VA-mycorrhizal fungi in the area contaminated by zinc and cadmium indicates that these fungi have evolved a zinc and cadmium tolerance and that they may play a role in the zinc and cadmium tolerance of *A. capillaris*.

Introduction

Evolution of metal tolerance is well documented for a large number plant taxa. The mycorrhizal association between higher plants and ectomycorrhizal fungi (Brown and Wilkins, 1985a; Colpaert and Van Assche, 1993; Denny and Wilkins, 1987; Jones and Hutchinson, 1986; Jones et al., 1988) or ericoid mycorrhizal fungi (Bradley et al., 1982) can modify the toxicity of the heavy metal for higher plants. The role of the (vesicular-) arbuscular mycorrhizal fungi in the metal metabolism of higher plants is most restricted to metal-deficient soils (Li et al., 1991; Sharma et al., 1992). In the case of a high concentration of zinc an impact of VA-mycorrhiza on metal uptake could not be detected (Ietswaart et al., 1992). With regard to the interactions between VA-mycorrhizal associations and heavy metals there are three main problems to be solved:

1. The qualitative (taxa) and quantitative (infection rate) aspects of the occurrence of VA-mycorrhizal fungi in soils naturally enriched with or anthropogenically contaminated by heavy metals as an expression of the evolutionary potential of VA-mycorrhizal fungi (Gildon and Tinker, 1981; Ietswaart et al., 1992; Sambandan et al., 1992);
2. The physiological role of VA-mycorrhizal fungi in the uptake and translocation of heavy metals and other nutrients by host plants (e.g. Dueck et al., 1986; Faber et al., 1990; Kothari et al., 1991; Li et al., 1991);
3. The ecological implication of the absence/presence of VA-mycorrhizal fungi on the

selection and survival of the host population (Gange et al., 1990; Sanders and Fitter, 1992b).

The function of mycorrhizae (reviewed by Allen, 1991) in the first successional stage of colonizing bare or disturbed land seems to be of low importance: Colonizing plants of primary sand dunes (Nicolson, 1960) are nonmycotrophic or otherwise often nonmycorrhizal and colonizing plants of the zone with the highest metal concentration on ore outcrops (Ernst, 1990) are nonmycotrophic. After soil disturbance by mining activities mycorrhizal fungi in colonizing mycotrophic plants were absent, although present in the original soil (Allen and Allen, 1980; Khan, 1978). To investigate the evolutionary potential of VA-mycorrhizal fungi with regard to heavy metals we have examined the presence of VA-mycorrhizal associations in higher plants on heavy metal contaminated soils in a field study. Until now it appears to be impossible to grow VA-mycorrhizal fungi in aseptic culture, suggesting an obligate host dependence (Burggraaf and Beringer, 1989). Therefore research on the heavy metal tolerance of VA-mycorrhizal fungi can only be carried out in the presence of a host plant. Two heavy metal contaminated areas and one uncontaminated area in Central-Europe were investigated for quantitative aspects of the VA-mycorrhizal infection of *Agrostis capillaris*, a perennial grass and well known host (Harley and Harley, 1987 and 1990) occurring on various heavy metal contaminated sites throughout Europe (see reviews by Ernst, 1990 and Ernst et al., 1992). The data are correlated with soil concentrations of some elements and heavy metals.

Materials and methods

Sampling the field sites

From 1987 to 1991 tussocks of *Agrostis capillaris* L. (=*A. tenuis* Sibth. = *A. vulgaris* With.), containing soil and roots of mature plants with a sufficient biomass for analysis, were collected up to a depth of 10–15 cm from an old copper mine in an area with natural copper outcrops (Imsbach, Germany; 7°54′ E, 49°35′ N), a zinc refinery (Budel, The Netherlands; 5°36′ E, 51°14′ N) and a control area not contaminated with heavy metals (Schiermonnikoog, The Netherlands; 6°12′ E, 53°29′ N). In 1991 the copper mine area was sampled in detail as follows:

- Along a foot-path that runs from the highest part of the *A. capillaris* slope into a *Pinus sylvestris* wood; samples 1–5.
- From transect along brooklet towards small lake; samples 6–9.
- Waste site a few hundreds of metres away from the mine shaft; samples 10 and 11.
- Slope covered with *A. capillaris*; samples 12 and 13.

Soil analysis

Soil samples were examined for particle size, acidity, total C and N and the mineral elements Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P and Zn. For the general survey 4 duplicates research site were taken. In the study of the local variation at the surroundings of the copper mine soil samples were taken in duplicate. Analytical procedures were performed as mentioned below.

Soil structure was analysed by passing soil first through a 2 mm sieve, followed by sieves of 250 μm and 63 μm. Soil was divided in the 4 fraction: stones (greater than 2 mm), coarse sand (between 2.0 and 0.25 mm), fine sand (between 0.25 and 0.063 mm) and silt + clay (smaller than 0.063 mm). The analysis was carried out in duplicate.

The soil pH was determined after shaking the soil (ratio H_2O dest. : soil, 10 : 25 w/v) for 2 hours (75–100 rpm) and after filtration (Schleicher & Schüll, No. 1). For element analyses, samples consisting of soil particles less than 2 mm were shaken for 2 hours at 75–100 rpm, settled down over night and filtered (Schleicher & Schüll, No. 1). Element concentrations were measured by atomic absorption spectrophotometry (AAS, Perkin-Elmer 4000), Ca and Mg after addition of 1% lanthanium nitrate. P was determined colorimetrically by the molybdenum-blue method (Chen et al., 1956) and both C and N by column chromatography (Carlo Erba elemental analyzer; Kirsten, 1979). The following fractions were examined:

- Water soluble fraction: 10 g soil was added to 25 ml demineralized water.
- Ammonium acetate exchangeable chemical elements: resuspension of the soil after extraction of the water soluble fraction in 25 mL 1 *M* ammonium acetate pH 7.0.
- DTPA-extractable fraction: 10 g soil in 25 mL DTPA-solution pH 7.3, containing 5.0 m*M* DTPA, 0.1 *M* TEA and 0.01 *M* $CaCl_2$ (Lindsay and Norvell, 1978).
- Acid digestion for total amount: soil dried at 70°C for 24 hours, sieved and 0.5–1.0 g digested in 2.0 mL HNO_3:HCl (4:1 v/v; 6 hours at 140°C) in Teflon bombs. After digesting volume was made up to 10 mL with demineralized water.

Assessment of mycorrhizal infection

Roots were cleaned by washing and samples (existing of 3 specimens out of 1 root system) were preserved in formalic acetic acid alcohol (FAA) before clearing and staining or cleared and stained directly. At first the method of Phillips and Hayman (1970) was followed, excepting the stain solution; later on the method of Koske and Gemma (1989) was used. Instead of trypan blue the roots were stained with Chlorazol black E (Brundrett et al., 1984).

Roots were examined under a microscope at ×100 magnification. Infection was recorded using a line-intersect method (Giovannetti and Mosse, 1980) and expressed as percentage infected root length (the amount of infected root-line intersections divided by the total amount of root-line intersections found in the root sample, multiplied by 100). The presence of arbuscles and vesicles was recorded. The total amount of intersections between roots and lines varied between 400 and 1000 per sample, corresponding with a total root length of between 50 and 150 cm.

Statistics

Tests of significance were carried out using one-way analysis of variance, (comparison of field infection), Pearson's correlation (correlation between infection and soil elements) and a posteriori testing of differences between means by Tukey's multiple comparison test, all according to Sokal and Rohlf (1981).

Results

Overall situation

Nine out of 12 samples from the highly copper contaminated site at Imsbach did not show any infection at all, while 3 had a low percentage of infection (1, 3, and 10%). The mean infection for this part of the area was about 1%. No arbuscles and vesicles were found. Extensive VA-mycorrhizal infection was established for the plants from the Schiermonnikoog (uncontaminated site) and Budel (zinc/cadmium contaminated site) population, 58% and 41% respectively (Fig. 1). In the samples from both populations either vesicles or arbuscles or both were frequently observed.

The chemical soil analyses summarized in Table 1 indicated increased concentrations of Mn, Cu and Zn in the Imsbach soil and a Zn and Cd contamination in the Budel soil. Compared to the uncontaminated situation at Schiermonnikoog the concentration of Mg was also increased.

Local variation at the surroundings of the copper mine

In order to get a better understanding of the VA-mycorrhizal infection pattern in the sur-

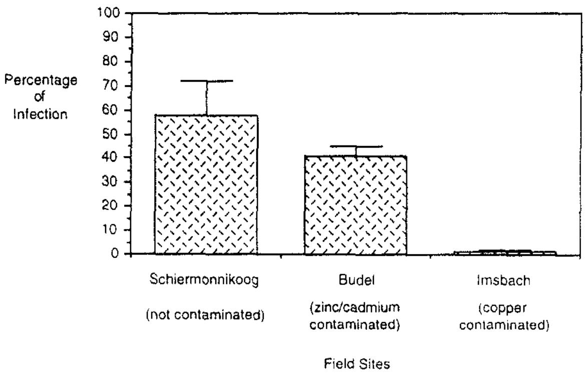

Fig. 1. Infection of *Agrostis capillaris* for the 3 different populations (based on 5, 12 and 14 samples respectively). Vertical *bar* represents standard error.

Table 1. Total concentrations of Mg, Mn, Cu, Zn and Cd and pH of the Schiermonnikoog Imsbach and Budel soil. All concentrations in μmol g^{-1} dry soil, except cadmium in nmol g^{-1} dry soil. Standard error between brackets

Site	pH	Mg	Mn	Cu	Zn	Cd
Schiermonnikoog	5.5	11.4 (0.71)	0.977 (0.062)	0.009 (0.0005)	0.077 (0.005)	3.66 (0.08)
Imsbach	6.2	87.6 (3.90)	7.33 (2.27)	11.9 (1.18)	4.49 (0.288)	4.12 (0.72)
Budel	5.6	26.3 (1.08)	4.19 (0.109)	2.56 (0.055)	15.6 (0.694)	120 (2.57)

Table 2. Percentage infection for 13 sites in the surroundings of a copper mine at Imsbach

Sample	Inf. perc.
1. Foot-path nr. 1, near mine	56.5
2. Foot-path nr. 2	40.6
3. Foot-path nr. 3	37.4
4. Foot-path nr. 4	20.3
5. Foot-path nr. 5, into the wood	67.6
6. Between mine and lake	7.8
7. Lake, upstream 1	3.1
8. Lake, upstream 2	0.0
9. Lake, downstream	0.0
10. Waste slope 1	—
11. Waste slope 2	48.0
12. Slope with *A. capillaris* 1	3.1
13. Slope with *A. capillaris* 2	1.3

roundings of the copper mine, the area was examined in more detail (Table 2). Contrary to the low VA-mycorrhizal infection in plants from along the brooklet and the slope near the mine, the infection of plants from the foot-path (20–68%) and on the waste slope (48%) approximates the values found for the plants of the Budel and the Schiermonnikoog population. Significant correlations between VA-mycorrhizal infection and a specific element concentration in soil were restricted to the negative correlations between infection and the total contents of Zn, Cd and Cu (Table 3), together representing 44% of the variation. The metals were positive correlated among themselves. Figure 2 illustrates the correlations between VA-mycorrhizal infection and total metal content of the soil for each site.

Discussion

In the vicinity of refineries away from ore outcrops, as at Budel (Dueck et al., 1984) or Prescot (Wales: Wu et al., 1975), the pioneer vegetation is built up by perennial grasses of the genus *Agrostis* which have evolved heavy metal tolerance. The abundant mycorrhizal infection found at the Budel area is in accordance with earlier research at zinc contaminated sites (Díaz and Honrubia, 1990; Gildon and Tinker, 1981, 1983; Ietswaart et al., 1992; Sambandan et al., 1992; Weissenhorn et al., 1991) and maybe a result of selection from a dormant spore bank or from an input of spores together with the import of metal ores. The examination of a unique arbuscular mycorrhizal fungal species, i.e. *Scutellospora dipurpurascens*, at the Budel site (Griffioen, pers. observation) support the last hypoth-

Table 3. Mean, range of values and correlation matrix for VA-mycorrhizal infection and some soil element concentrations for the copper mine region at Imsbach. Means and range (minimum-maximum) in μmol/g dry soil (Cd in nmol/g dry soil). Correlation between VA-mycorrhizal infection and nutrients based on 12 values and a one-tailed test, other correlations based on 13 values and a two-tailed test

			Pearson correlation matrix					
	Mean	range	infection	Mg	Mn	Zn	Cu	Cd
Infection	23.8	0–67.6						
Mg	59.5	17–122	-0.493^{ns}					
Mn	15.0	2.2–61	-0.132^{ns}	0.211^{ns}				
Zn	1.52	0.30–5.2	-0.584^{*}	0.576^{*}	-0.145^{ns}			
Cu	7.91	1.3–38	-0.505^{*}	0.503^{ns}	0.286^{ns}	0.414^{ns}		
Cd	2.87	0.42–7.9	-0.588^{*}	0.750^{**}	0.280^{ns}	0.651^{*}	0.590^{*}	
P	14.2	3.2–26	-0.281^{ns}	0.374^{ns}	0.046^{ns}	0.797^{**}	0.551^{ns}	0.618^{*}

Significance: ns: $\alpha > 0.05$ (not significant); $^{*}0.05 > \alpha > 0.01$; $^{**}\alpha < 0.01$.

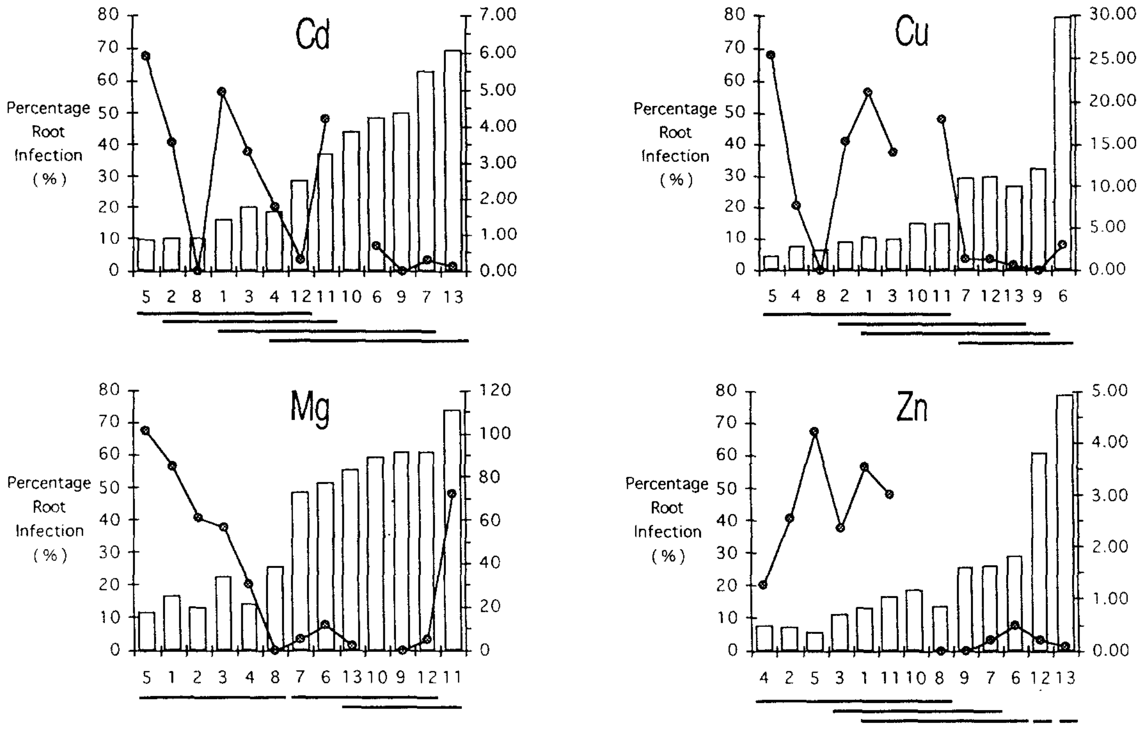

Fig. 2. VA-mycorrhizal infection (-●-) and resp. Cd, Cu, Mg or Zn concentration in the soil (☐) for the 13 sample sites in the surroundings of the copper mine at Imsbach. Numbering as in Table 2. Differences between means are tested for significance using Tukey's multiple comparison ($\alpha = 0.05$), given in horizontal bars beneath the site numbers. Sites linked by the same bar are statistically not significantly different. Arrangement of sites first based on groups with increasing metal concentration and second on infection level of plants at the site. Differences for copper and cadmium are based on log-transformed values.

esis. Unfortunately, at the other above mentioned site, Prescot, the existing grass species have not been examined for mycorrhizal associations.

The absence of VA-mycorrhizal infection in roots of *A. capillaris* at the Imsbach mine site, may be a direct fungitoxic effect of copper on the mycorrhizal fungi. The increase of VA-mycorrhizal infection in plants in a wider region around the copper outcrop coincides with a decrease of soil copper, thus supporting the Cu-toxicity hypothesis. As mycorrhizal associations were established at zinc and cadmium contaminated sites (Budel, as described here) and in soils near ore outcrops at Breinigerberg (Ietswaart et al., 1992), the copper status of the Imsbach soil causes a more severe selection than Zn and Cd. This finding coincides with the different physiological pattern of zinc and copper tolerant plants (Ernst et al., 1992). It seems unlikely that an indirect negative effect on the presence of the VA-mycorrhizal fungi is caused by the copper metabolism of the host plant (Graham, 1983; Mench et al., 1988).

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