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Seasonality of VAM infection in three populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal enrichment

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Abstract

Three populations of the perennial grass *Agrostis capillaris*, growing on limestone derived clay with and without natural enrichment of the heavy metals cadmium, lead, and zinc, and on a sandy soil polluted by a metal smelter have been investigated with regard to the percentage and seasonality of infection with vesicular-arbuscular mycorrhizal (VAM) fungi and its impact on mineral nutrition.

In all populations VAM infection was lowest during winter, and highest during late summer and autumn. The population at the metal smelter site was less infected by VAM fungi than both other populations. The concentration of mineral nutrients for the three populations was clearly related to the soil concentration, but hardly modified by the degree of VAM infection.

Introduction

Numerous experiments with young plants under optimum conditions have emphasized the importance of VAM fungi for the improvement of a plant's access to nutrients, especially phosphorus, and water (for a review: Smith and Gianazzi-Pearson, 1988; Baas, 1989). Under natural conditions often a low effectiveness of VAM fungi on plant phosphate uptake has been demonstrated (Fitter, 1985; McGonigle, 1988). Seasonality of the degree of VAM infection (Boerner, 1986; Khan, 1974; Read et al., 1976; Van Duin et al., 1990) may modify the role of VAM fungi for established perennial plants under field conditions, perhaps in relation to the efficiency of the VAM species (Ernst et al., 1984; Jensen, 1982).

Only a few studies, however, have considered the impact of VAM infection on the broad scale of essential plant nutrients (Ernst et al., 1984; Jensen, 1982). The present study will integrate the effect of seasonality of VAM infection and its impact on mineral nutrition in three populations of the perennial grass *Agrostis capillaris* on three different soil types.

Materials and methods

In October 1987 and in January, March and August 1988 samples were taken from three *Agrostis capillaris* L. populations, growing at Bemelerberg (5°46′E, 50°51′N), Breinigerberg (6°12′E, 50°44′N) and Budel (5°36′E, 51°14′N), in order to gain an insight into the degree of infection during the season and the possible role of VAM in the uptake of nutrients in these natural populations. The Bemelerberg is a marly hill (Upper Cretaceous) just east of Maastricht, with a soil pH of 6.5 in the top layer and no exceptionally high concentrations of heavy metals. The Breinigerberg is situated to the southeast of Aachen and is composed of hard lime-

stone (Lower Devonian; more or less dolomite-sized) in which mineral veins occur, containing in particular zinc and lead; the pH of the soil is here slightly above 7 (Ernst, 1974). The *A. capillaris* population of Budel grows in the vicinity (northeastern side) of a zinc refinery (formerly smelter) at Budel-Dorplein which lies to the south of Eindhoven (Dueck et al., 1984). The original top soil layer here consists of sand (Pleistocene), and has a pH of about 5.5.

Ten complete plants were collected at each sampling time at the 3 sites, from an area of about 20 m^2. The plant roots were cleaned by washing them several times with demineralized water. They were partly fixed with FAA (formaldehyde acetic acid) and afterwards stained with Chlorazol Black E, following Phillips and Hayman (1970). Percentages of VAM infection were established by estimating about 50 microscope fields for each sample. The rest of the roots and the shoots of each plant were dried at 80°C, and both were analysed for their element concentration. This was carried out for 3 plants with the lowest and 3 plants with the highest VAM infection from each March and August sample of 10 plants. Nitrogen content was determined by burning the sample with pure oxygen and separating the gaseous compounds by column chromatography (Carlo Erba Elemental Analyzer 1106, Kirsten, 1979). All other elements were analysed after wet-ashing with a mixture of nitric and perchloric acid (7:1, v/v). Phosphorus was determined by spectrophotometry after formation of a blue ascorbic acid-phosphorus complex (Chen et al., 1956), and all other elements by atomic absorption spectrophotometry (Perkin-Elmer 4000), i.e. calcium and magnesium after addition of 1% (v/v) lanthanum nitrate. Plants were not different in biomass so that we gave all elements as concentrations.

Zinc tolerance experiments were carried out with *A. capillaris* plants from each population. Cuttings were grown for one week on a Hoagland solution (1/10 of the standard strength). After that, the roots formed in this period were stained with a suspension of charcoal powder, and the cuttings were grown further on 1/10 Hoagland to which was added 0, 200, 800 and 2400 μM zinc sulfate. For each zinc concentration 10 cuttings were used.

Significance calculations were carried out using a two-way anova analysis.

Results

Most VAM was formed in all 3 populations by *Glomus fasciculatum* Gerdemann & Trappe and *G. tenue* Hall; in the Budel population a *Scutello spora* sp. was also found to be fairly frequent. The Breinigerberg population usually shows the highest infection percentages and the Budel population the lowest, but the differences between the populations are not significant at $p < 0.05$.

VAM infection (Table 1) is high in October, varying from 42% (Budel) to 63% (Breinigerberg) and nearly the same infection percentages were present in August. The lowest values were established for the January samples (mean 27%), many old fungal hyphae being present. In March the *Agrostis capillaris* plants had started to grow and a slight rise of the percentage of infection was found. The differences between the mean infection percentages of the 3 plants with the lowest and the 3 plants with the highest infection in March, for the Bemelerberg, Breinigerberg and Budel population respectively, are 17, 13 and 8%. For the August samples these values are 28, 25 and 18% respectively.

From the element analyses of shoots and roots it can be established (Tables 2, 3 and 4) that shoots and roots of the plants with a high VAM

Table 1. Seasonal variation of VAM-infections (% of total roots) in 3 populations of *Agrostis capillaris* at 4 sampling dates. For each site and time the roots of 10 plants were counted (mean ± 1 S.D.)

	October 1987	January 1988	March 1988	August 1988
Bemelerberg	50 ± 11	29 ± 5	32 ± 8	51 ± 11
Breinigerberg	63 ± 23	28 ± 6	35 ± 8	61 ± 12
Budel	42 ± 7	25 ± 4	23 ± 4	47 ± 6

Table 2. Elements in shoots and roots for the 3 *Agrostis capillaris* populations, given for low and high VAM-infected plants harvested in March (μmol g dw^{-1}; each value is based on 3 plants)

VAM-infection (%)	Bemelerberg				Breinigerberg				Budel			
	Shoot		Root		Shoot		Root		Shoot		Root	
	Low: 23	High: 40	Low: 23	High: 40	Low: 29	High: 42	Low: 29	High: 42	Low: 20	High: 28	Low: 20	High: 28
N	2660	2560	819	1015	2865	3450	1070	1050	3315	2685	957	915
P	73.5	64.6	17.0	18.2	145	94.1	23.2	20.0	101	97.8	21.0	18.1
K	615	538	113	127	894	732	185	146	707	815	106	76.4
Ca	90.2	103	35.1	48.7	68.4	92.3	72.0	68.7	28.5	31.3	18.7	19.7
Mg	61.1	55.0	15.2	18.6	66.1	77.3	28.6	24.7	53.2	50.0	11.2	9.6
Na	13.1	18.5	16.9	12.2	15.6	18.6	32.9	14.8	24.6	26.0	24.7	27.5
Fe	5.83	4.95	5.78	8.76	7.23	5.66	21.2	16.5	3.86	5.93	7.22	4.32
Mn	7.63	6.60	2.33	2.51	0.87	1.00	2.35	1.78	2.97	3.53	1.54	1.51
Zn	3.54	3.93	3.85	4.85	14.4	6.38	19.4	15.1	8.85	6.78	14.2	13.6
Cu	0.18	0.19	0.14	0.15	0.88	0.46	0.43	0.35	0.43	0.43	2.33	1.96
Cd	0.007	0.01	0.10	0.25	0.003	0.003	1.61	1.08	0	0.003	0.14	0.15
Pb	0	0	0.10	0.13	0.24	0.13	1.40	1.15	0.32	0.36	6.20	5.56

Table 3. Elements in shoots and roots for the Breinigerberg *Agrostis capillaris* population, given for low and high VAM-infected plants harvested in March and August (μmol g dw^{-1}; each value is based on 3 plants)

VAM-injection (%)	Shoot				Root			
	March Low: 29	August Low: 48	March High: 42	August High: 73	March Low: 29	August Low: 48	March High: 42	August High: 73
N	2865	879	3450	1135	1070	812	1050	616
P	145	13.0	94.1	21.5	23.2	14.3	20.0	11.7
K	894	288	732	285	185	134	146	119
Ca	68.4	36.5	92.3	50.7	72.0	58.6	68.7	52.0
Mg	66.1	25.3	77.3	31.9	28.6	21.5	24.7	18.6
Na	15.6	15.4	18.6	18.5	32.9	20.6	14.8	17.0
Fe	7.23	2.17	5.66	1.79	21.2	18.6	16.5	22.1
Mn	0.87	0.71	1.00	0.43	2.35	2.02	1.78	1.26
Zn	14.4	3.23	6.38	2.66	19.4	12.7	15.1	12.0
Cu	0.88	0.10	0.46	0.15	0.43	0.38	0.35	0.19
Cd	0.003	0.02	0.003	0.02	1.61	0.46	1.08	0.42
Pb	0.24	0	0.13	0	1.40	0.87	1.15	0.66

Table 4. Significance calculations for the elements analysed in shoots and roots, comparing the data for the two time points (March and August), of the three populations (Bemelerberg, Breinigerberg, Budel), two VAM-infection levels (high, low), and their interactions. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant. Results above the line are for the shoots, those below for roots

	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Cd	Pb
Time (t)	***	***	***	***	***	*	***	*	***	***	***	***
	***	***	*	n.s.	**	n.s.	*	n.s.	**	n.s.	***	n.s.
Population (p)	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.
	n.s.	n.s.	n.s.	*	*	*	n.s.	n.s.	*	*	*	*
Infection (i)	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
t × p	n.s.	***	***	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	n.s.	***
	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	*
t × i	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p × i	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
t × p × i	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

infection generally do not show significantly higher nutrient contents than those of plants with a low infection, independent of the season and the population. Roots and particularly shoots harvested in March usually contain higher concentrations than those harvested in August. Shoots mostly contain more macro-nutrients than roots of the same sample, and roots more sodium and heavy metals than shoots. The populations differ between each other as follows.

More nitrogen is found in the August shoots and roots of the Breinigerberg and the Budel population compared with those of the Bemelerberg. Calcium is significantly greater in concentration in all Bemelerberg and Breinigerberg shoots and roots than in those of Budel. The magnesium values differ slightly, but consistently, between the 3 populations. Iron concentration is comparatively greater in Breinigerberg roots. The 3 populations differ significantly from each other in their shoot manganese content. More zinc is found in the Breinigerberg and

Budel shoots and roots (partly significant). The Breinigerberg March shoots from plants with a low VAM infection have a significantly higher zinc content than shoots from plants with a high infection, while the same tendency is present in all other Budel and Breinigerberg shoots. Significant differences, are found in the copper content of the roots of the 3 populations. Cadmium concentration is significantly greater in the roots of the Breinigerberg population. The amount of lead differs significantly for the roots of the 3 populations and the same trend is found for the shoots.

The results of the zinc tolerance experiments are given in Figure 1. From this it can be concluded that the Bemelerberg population possesses almost no tolerance, the Breinigerberg population a moderate and the Budel population a relatively high zinc tolerance.

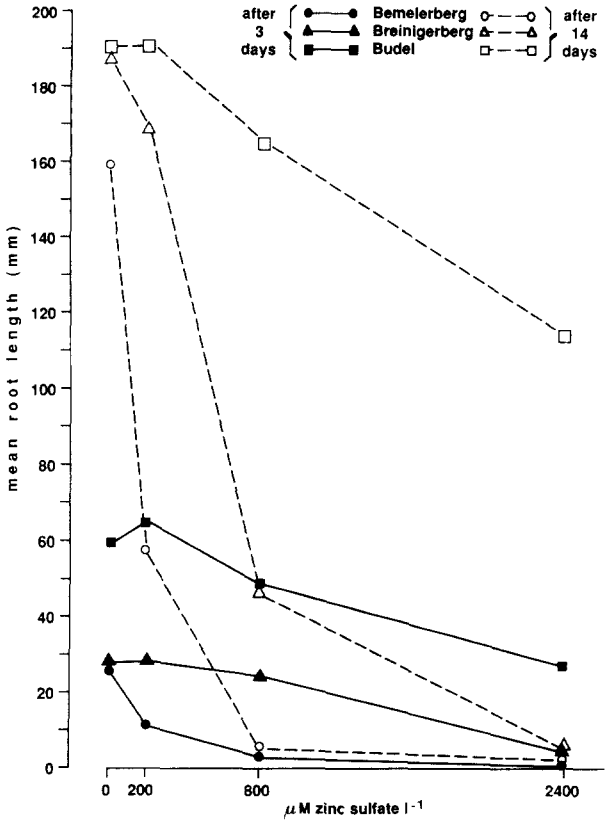

Fig. 1. Increased root length for *Agrostis capillaris* cuttings grown for 3 and 14 days on 1/10 Hoagland solution, to which was added respectively 0, 200, 800 and 2400 μM zinc sulfate L^{-1} (each value is based on 10 cuttings).

Discussion

The impact of VAM infection on the nutrient concentration of *Agrostis capillaris* under field conditions was found to be small. McGonigle and Fitter (1988) reached the same conclusion for *Trifolium repens*, while Bryla and Koide (1990) found a generally lower response to VAM infection for wild accessions of *Lycopersicon esculentum* than for its cultivars. Nevertheless, there are population specific nutrient patterns. *Agrostis capillaris* shows a strong VAM infection, which is the common situation for the Poaceae in the temperate zone (e.g. Ernst et al., 1984; Hopkins, 1987; Nicolson and Johnston, 1979; Read et al., 1976; Van Duin et al., 1990). Under some extreme ecological conditions, however, low VAM infection percentages have been found in grasses: in autumn and winter annuals, that grow naturally at low temperatures (Ernst et al., 1984), for high concentrations of sodium (Kim and Weber, 1985), for frequently flooded (hypoxic) soil (Van Duin et al., 1991), and for strongly copper contaminated areas (Griffioen, 1989).

The general tendency that high percentages of VAM infection correlate with active growth of the host (e.g. Allan, 1983; Ernst et al., 1984; Nicolson and Johnston, 1979; Saif, 1986; Van Duin et al., 1990), is consistent with our results, i.e. low infection in winter, increasing infection in spring and high infection in summer and autumn.

A somewhat lower VAM infection at the site with the lowest soil pH (Budel, pH of 5.5) does agree with the findings of, amongst others, Read et al. (1976). In general this effect is attributed to a higher availability of (heavy) metals at a lower pH (Killham and Firestone, 1983; Kuo and Baker, 1980; Wang et al., 1985). At the Budel site, for which this is true (see below), these effects are reinforced by a disturbed decomposition of the organic material (Denneman, 1986; Van Capelleveen, 1987). A low calcium concentration alone can also cause a reduced VAM infection (Hepper and O'Shea, 1984; Saif, 1986).

VAM infection is high in the *A. capillaris* populations that grow at the zinc contaminated sites: Breinigerberg (0.2–10 μmol, ammonium acetate extractable, zinc gdw $soil^{-1}$, Ernst 1976) and Budel (0.2–3 μmol, ammonium acetate ex-

tractable, zinc gdw soil^{-1}, Dueck, 1986). From this it can be concluded that coevolution of Agrostis and Glomus towards a high degree of zinc tolerance has taken place. The same has been established for cadmium (Gildon and Tinker, 1981). Griffioen (1989), however, found nearly no VAM infection for *A. capillaris* growing in a copper mining area in West Germany (about 5.75 μmol DTPA extractable, copper gdw soil^{-1}). This is most probably due to the fact that the fungicidal effect of copper also holds for species of the Endogonaceae. For another grass, *Lolium perenne* (probably without VAM), it has been shown that copper was the most toxic of a whole range of (heavy) metals (Wong and Bradshaw, 1982).

The higher nitrogen levels in the August shoots and roots of the Breinigerberg and Budel population, compared with those of the Bemelerberg population, may be connected with the possible role that nitrogen compounds play in the detoxification processes of (heavy) metals, found by Weigel et al. (1982) for *A. capillaris* plants from populations with different zinc tolerance. The differences between our populations for magnesium content fit with the conclusion made by Veltrup (1982) that zinc tolerant plants of *A. capillaris* need more magnesium for optimum ATPase activities than sensitive plants. The calcium/magnesium ratio is below or just 1 in the zinc tolerant population (Budel, Breinigerberg respectively) and above 1.5 in the sensitive population (Bemelerberg). Little difference was found in iron content between the leaves of our Agrostis populations, while De Neeling and Ernst (1986) found a threefold increase in iron for a calcareous population of *Chamaenerion angustifolium* compared with an acidic one, which disparity is consistent with the difference in iron uptake between dicots and monocots (Bienfait 1989). From the manganese and the iron data it can be concluded that the 3 *Agrostis capillaris* populations are adapted in quite different ecophysiological ways.

The August shoots of Breinigerberg and Budel show a much lower zinc content than those harvested in March, this is most probably due to metal dilution consequent on rapid growth. The Breinigerberg and Budel roots, however, show a consistently high zinc content, that may point to a partial withholding of zinc in the roots (see Mathys, 1973). In favour of this is the fact that the zinc content of shoots from Breinigerberg and Budel plants with a low VAM infection is always higher than that of counterparts with a high infection, thus indicating that VAM fungi do not operate as an additional sink for an element in high concentrations. Another part of the absorbed zinc is transported to the shoots (Dueck et al., 1986). Zinc, copper, cadmium and lead show low values for the Bemelerberg population, compared with the Breinigerberg and Budel population, which is mainly due to differences in soil composition.

In conclusion, infection has only a slight or no relationship with mineral composition (Table 4) supporting Fitter's (1991) conclusion that under natural conditions the benefits to plants of VA mycorrhizal infection are difficult to demonstrate.

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