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ORIGINAL PAPER

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON THE CONTENT OF ZINC IN LETTUCE GROWN AT TWO PHOSPHORUS LEVELS AND AN ELEVATED ZINC LEVEL IN A NUTRIENT SOLUTION*

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Abstract

Zinc (Zn) is an important element for the proper plant growth and development. However, Zn is also a metal commonly used in the industry, which may cause its excessive accumulation in soil. High soil Zn content leads to its increased uptake by plants growing near industrialized areas. Arbuscular mycorrhizal fungi (AMF) live in symbiosis with the majority of plant species. These fungi have the ability to reduce Zn uptake by plants, when this element is present in an increased concentration in the plant root zone. An experiment was carried out to determine the effect of AMF on Zn uptake by lettuce plants grown in rockwool at increasing levels of Zn in the nutrient solution. This effect was investigated at two P levels in the nutrient solution, i.e. 40 mg P (optimal for lettuce) and 10 mg P dm^{-3}. Mycorrhizal parameters, yield, content of dry matter, ascorbic acid, glucose, fructose and sucrose as well as the plant's nutritional status in P, Cu, Mn, Mo, Fe and Zn were determined in the study. The effectiveness of mycorrhization of lettuce grown in rockwool was proven in the study. AMF reduced the Zn content in lettuce grown under excessive Zn concentrations in the root zone. Moreover, this decrease was greater in plants receiving the nutrient solution with a lower concentration of P. Additionally, mycorrhiza decreased the content of Cu, Fe, Mn and Mo in lettuce, despite an optimal (not increased) concentration of these micronutrients in the nutrient solution supplied to plants. Arbuscular mycorrhiza did not affect the plant yield, content of ascorbic acid, glucose, fructose, sucrose or the content of P in lettuce leaves.

Keywords: arbuscular mycorrhiza, heavy metal, plant nutrition, yield.

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INTRODUCTION

Increasing industrialisation contributes to the progressive environmental pollution with heavy metals. Zinc (Zn) is one of the metals commonly used in industry. It is a waste product from mines, smelters, incinerators, the chemical or galvanizing industry. Intensification of industrial production may lead to excessive accumulation of Zn in soil, resulting in an increased uptake of this nutrient by plants growing near industrialized areas (WUANA, OKIEIMEN 2011). Although Zn is essential micronutrient for plants, its high concentration in tissues may affect negatively the plant's growth and development (SAGARDOY et al. 2009).

Arbuscular mycorrhizal fungi (AMF) live in symbiosis with the majority of plant species. These fungi increase the absorption surface of roots, resulting in an increased uptake of nutrients, including Zn by plants (SMITH, READ 2008). However, in the plant Zn nutrition, AMF have a dual mode of action (WATTS-WILLIAMS et al. 2013). On the one hand, they stimulate the uptake of Zn by plants grown with an insufficient amount of this micronutrient in the soil (THOMPSON et al. 2013). On the other hand, they inhibit the uptake of Zn when it is present in an excessive concentration in the root zone (LI, CHRISTIE 2001, CHEN et al. 2003). According to CHRISTIE et al. (2004), the limiting effect of AMF on Zn uptake by plants may depend on the quantity of both internal and external fungal hyphae. Many environmental factors have an influence on the development of mycorrhizal structures (SMITH, READ 2008). One of the factors, which reduces the growth of AMF is a high concentration of phosphorus (P) in the plant root zone (KOWALSKA et al. 2015*b*).

The hypothesis of the study assumes that mycorrhizal colonisation of lettuce grown at excessive Zn concentrations in the root zone contributes to the reduction of the Zn content in lettuce. The main objective of the experiment was to determine the effect of mycorrhizal colonisation on the Zn accumulation in plants grown under conditions of elevated Zn concentrations in the root zone. The effect of the P concentration in the plant root zone on mycorrhizal colonisation as well as the effects of all experimental factors on the nutritional status of lettuce and yield quantity and quality were also determined in the study. The experiment was carried out with the use of rockwool, which belongs to the group of inert media. Therefore, it was possible to investigate interactions between experimental factors regardless of impacts of physico-chemical soil properties, e.g. sorption processes and general soil microbial activity.

MATERIAL AND METHODS

The two-year experiment was conducted in the autumn of 2013 and 2015, in a high tunnel. Lettuce plants (*Lactuca sativa*) cv. Melodion were grown in cultivation rows filled with rockwool mats. The experiment was carried out as a factorial design with three factors and triple replication, each replication consisting of ten plants. The first factor included two P levels in the nutrient solution (10 or 40 mg dm^{-3}), the second factor represented inoculation or no inoculation of rockwool with arbuscular mycorrhizal fungi (+AMF – inoculated, -AMF – un-inoculated) and the third one consisted of three Zn levels in the nutrient solution (0.1, 5 or 15 mg dm^{-3}). In sum: the experiment consisted of 12 treatments, each in triplicate.

Untreated lettuce seeds were sown in rockwool multiblocks. At the time of sowing, half of the seeds were inoculated with multistrain inoculum containing *Funneliformis mosseae*, *Rhizophagus intraradices, Claroideoglomus claroideum*, *Claroideoglomus etunicatum, Glomus microaggregatum* and *Funneliformis geosporum,* mixed in a proportion as follows 4.5:4.5:1:1:1:1 (Symbiom®, 720 propagules g^{-1}). Inoculation was performed by introducing the inoculum into the holes for seeds in the rockwool multiblocks. Plants received a nutrient solution containing the macronutrient concentration recommended for lettuce seedling production (in mg dm^{-3}): N – 90, P – 10, K – 70, Mg – 15, Ca – 70 (Adamicki et al. 2015). At the stage of four leaves (16 days after sowing), the seedlings were planted into the rockwool mats and placed in the cultivation rows. Plants were divided into two sub-blocks: the first sub-block received a nutrient solution with 40 mg P dm^{-3} (optimal level), the second one a solution containing 10 mg P dm^{-3} (reduced level). Each sub-block was divided into two parts. A lettuce seedling not inoculated during sowing was planted into the first part of each sub-block, and an inoculated seedling was planted to the second part. The second inoculation of plants inoculated during sowing was performed at the time of planting the seedling into mats, using an amount of 5 g of inoculum per plant. Both inoculated and non-inoculated plants received a nutrient solution with one of three Zn concentrations: 0.1 (optimal for lettuce), 5 or 15 mg dm^{-3}. Except for P and Zn, all plants received the nutrient solution containing the same concentration of macro- and micronutrients, i.e. (in mg dm^{-3}): N – 150, K – 200, Mg – 40, Ca – 170, Fe – 2.0, Mn – 0.5, B – 0.3, Cu – 0.10, Mo – 0.05 (Adamicki et al. 2015).

Lettuce plants were harvested once at the readiness for harvest (48 days after planting seedlings into the mats) and the plant material was then analysed.

At the end of the cultivation period, we isolated the root systems of three plants from each replication. Samples of 10 g were collected from the isolated root system and were used to prepare microscopic slides according to a modified method described by Phillips and Hayman (1970). Microscopic slides were observed using an Axio Imager N2 (Carl Zeiss) microscope via Nomarsky optics. AMF root colonisation was assessed according to the method of Trouvelot et al. (1986) by determining the following parameters: mycorrhizal frequency (F), relative mycorrhizal intensity (M) and relative abundance of arbuscules (A). These parameters were calculated using the Mycocalc software (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.htmlhttp://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Lettuce yield was expressed as the weight of a head. Plants were collected separately from each replication, weighed, counted and the mean weight of a head was calculated for each replication.

The five representative plants from each replication were selected for chemical analysis of plant material. One quarter of each lettuce head was chopped in a blender. The dry matter content was measured by drying at 105°C until the constant weight. Glucose, fructose and sucrose were analysed after fresh leaves sample preservation by boiling in 96% ethanol by capillary electrophoresis (Beckman Coulter PA 800plus Pharmaceutical Analysis System) with the DAD detection. Another quarter of each lettuce head was cut with a porcelain knife and L-ascorbic acid was determined after extraction of 20 g sample in 80 ml of 2% oxalic acid using capillary electrophoresis. The remaining half of each lettuce was dried at 65°C for 24 h in a laboratory dryer with forced air circulation. Dried samples were ground and P, Cu, Fe, Mn, Mo and Zn contents were determined after mineralisation in 65% extra pure HNO_3 in a CEM MARS-5 Xpress microwave system (Pasławski, Migaszewski 2006), using a high-dispersion spectrometer ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry, Prodigy Teledyne Leeman Labs).

The data were subjected to three-way analysis of variance using the ANOVA module of Statistica 10.PL (StatSoft Inc., USA). We used the HSD Tukey test to determine differences between the means. Results were considered significant at a probability level below 0.05 ($p < 0.05$). Similar results were obtained in both years of the experiment and are therefore presented as the means of the data obtained in two years of cultivation.

RESULTS AND DISCUSSION

In this experiment, despite the cultivation in rockwool, we could prove the efficacy of lettuce inoculation with AMF. The average mycorrhizal frequency (F) of inoculated plants was 33.85% (Table 1). This value was lower than the degrees of AMF colonisation obtained in a few experiments with soil-less cultivation of lettuce. However it was higher than the effectiveness of mycorrhization observed inan experiment with the cultivation of lettuce in peat (Konieczny and Kowalska 2016). Baslam et al. (2011*a*, *b*) in their experiments on lettuce grown in a mixture of vermiculite, peat and sand, found AMF colonisation levels in a range of 56-61% and 64-74%. The lower coloni-

Table 1

Effect of P and Zn concentrations in the nutrient solution (mg dm^{-3}) and inoculation of the growth medium with AMF on the mycorrhizal frequency (F), relative mycorrhizal intensity (M) and relative abundance of arbuscules (A) in lettuce roots

Treatment	F	M	A
	(%)		
P (mg dm^{-3})			
10	19.74 ± 4.834	1.593 ± 0.444	0.417 ± 0.192
40	14.10 ± 3.511	0.396 ± 0.126	0.000 ± 0.000
	*	*	*
Inoculation			
-AMF	0.00 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
+AMF	33.85 ± 1.716	1.989 ± 0.372	0.418 ± 0.192
	*	*	*
Zn (mg dm^{-3})			
0.1	18.04 ± 5.491	1.330 ± 0.545*b*	0.331 ± 0.250
5	17.01 ± 5.322	1.078 ± 0.460*ab*	0.266 ± 0.173
15	15.71 ± 5.120	0.575 ± 0.234*a*	0.030 ± 0.020
	n.s.	*	n.s.
Interaction P × inoculation × Zn	n.s.	n.s.	n.s.

* – means are significantly different, n.s. – differences are not significant, *a*,*b* – means followed by different letters differ at $p < 0.05$, ± – standard error, -AMF – plants not inoculated with arbuscular mycorrhizal fungi, +AMF – plants inoculated with arbuscular mycorrhizal fungi

sation level detected in our experiment, compared to Baslam et al. (2011*a*, *b*), might be related to the characteristics of the growing medium as well as the predisposition of a particular variety of lettuce to form symbiosis with AMF. Our experiment showed that the P concentration in the nutrient solution had an impact on all the determined mycorrhizal parameters. Higher F, M and A values were found in the roots of lettuce receiving lower P level in the nutrient solution, i.e. 10 mg dm^{-3} (Table 1). These results are similar to those obtained by Schmidt et al. (2010) and Kowalska et al. (2015*b*), who observed a higher degree of mycorrhizal colonisation in roots of plants grown under the conditions of a lower P content in the nutrient solution, respectively in the cultivation of marigold and tomato. According to Amijee et al. (1989), a negative effect of a high P level on the development of AMF may be associated with changes in the physiology of both the plant and the fungus. The creation of mycorrhizal symbiosis requires an exchange of molecular signals between the fungus and plant (Bécard et al. 2004). Recently, it has been suggested that strigolactones are signalling molecules, which positively affect the germination of fungal spores and branching of extraradical hyphae (Akiyama et al. 2005). Yoneyama et al. (2012) proved that exudation of strigolactones by plant roots is increased in the presence of low P and N concentrations in the root zone. Both optimal and increased concentrations of these nutrients restrain strigolactone exudation. A low level of strigolactone exudation may inhibit the development of hyphae and therefore limit the probability of the formation of symbiosis between the plant and fungus.

Inoculation of the growing medium increased the dry matter content compared to plants growing without AMF (Table 2). However, besides the

Table 2

Effect of P and Zn concentrations in the nutrient solution (mg dm^{-3}) and inoculation of the growth medium with AMF on the content of dry matter, ascorbic acid, glucose, fructose and sucrose in leaves of lettuce

Treatment	Dry matter	Ascorbic acid	Glucose	Fructose	Sucrose
	(%)	(mg 100 g^{-1} f.m.)			
P (mg dm^{-3})					
10	6.985 ± 0.063	14.23 ± 0.280	151.3 ± 4.432	200.7 ± 7.339	213.6 ± 8.299
40	5.146 ± 0.039	11.27 ± 0.262	215.2 ± 9.913	277.0 ± 12.44	97.03 ± 4.957
	*	*	*	*	*
Inoculation					
-AMF	5.996 ± 0.210	12.88 ± 0.285	191.7 ± 13.21	247.7 ± 16.19	150.8 ± 12.38
+AMF	6.134 ± 0.246	12.62 ± 0.567	174.8 ± 7.401	230.0 ± 10.44	159.8 ± 18.38
	*	n.s.	n.s.	n.s.	n.s.
Zn (mg dm^{-3})					
0.1	6.095 ± 0.275	12.58 ± 0.654	179.1 ± 8.778	225.7 ± 10.10	154.6 ± 21.66
5	5.967 ± 0.269	12.89 ± 0.347	185.5 ± 11.69	250.4 ± 17.46	155.1 ± 19.24
15	6.133 ± 0.307	12.79 ± 0.621	185.2 ± 18.31	240.5 ± 21.12	156.2 ± 14.43
	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction P × inoculation × Zn	n.s.	n.s.	n.s.	n.s.	n.s.

* – means are significantly different, n.s. – differences are not significant, ± – standard error, -AMF – plants not inoculated with arbuscular mycorrhizal fungi, +AMF – plants inoculated with arbuscular mycorrhizal fungi

content of dry matter, this experiment showed no effect of AMF on plant yield (Table 3), content of ascorbic acid, glucose, fructose, sucrose (Table 2) and P (Table 4) in lettuce leaves. Likewise, neither Maboko et al. (2013) in the cultivation of tomato in sawdust and coir nor Michałojć et al. (2015) in an experiment with tomato grown in rockwool and straw confirmed any beneficial effect of AMF on plant yield. The colonisation of plants with AMF contributes to an increase of the absorption surface of the plant root system, which results in the enhancement of nutrient uptake, including P (Smith, Read 2008). As it was mentioned above, we did not observe any improvement of the plant's P nutritional status in the presence of AMF (Table 4), which may be connected with a relatively low abundance of arbuscules obtained in our experiment, i.e. 0.418% (Table 1). Arbuscules are bushy branched ends of AMF hyphae, which participate in the exchange of nutrients between the fungus and plant (Smith, Read 2008). The low level of arbuscule development means the lack of nutrient exchange between symbionts, and therefore the improvement of the plant's nutritional status is not observed. In our previous

Table 3

Effect of P and Zn concentrations in the nutrient solution (mg dm^{-3}) and inoculation of the growth medium with AMF on the yield of lettuce

Treatment	Yield (g/plant)
P (mg dm^{-3})	
10	100.3 ± 5.175
40	174.4 ± 1.245
	*
Inoculation	
-AMF	137.7 ± 9.697
+AMF	137.1 ± 9.776
	n.s.
Zn (mg dm^{-3})	
0.1	146.9 ± 8.619*b*
5	143.3 ± 9.815*b*
15	121.9 ± 15.35*a*
	*
Interaction P × inoculation × Zn	n.s.

* – means are significantly different, n.s. – differences are not significant, ± – standard error, *a*,*b* – means followed by different letters differ at $p < 0.05$, -AMF – plants not inoculated with arbuscular mycorrhizal fungi, +AMF – plants inoculated with arbuscular mycorrhizal fungi

studies on tomato (Kowalska et al. 2015*b*) and lettuce (Kowalska et al. 2015*a*), we did not find beneficial effects of AMF on the plant's P nutritional status. Cwala et al. (2010) suggested that in hydroponic conditions, the absence of any improvement of the plant's nutritional status, despite the presence of AMF, is caused by a relatively high concentration of nutrients in the plant root zone.

We demonstrated that the inoculation of a medium with AMF led to a decrease in the Zn content in lettuce leaves compared to non-inoculated plants (95.75 and 127.0 mg Zn kg^{-1} dry matter, respectively) – Table 4. It is well known that colonisation of plant roots by AMF contributes to the increased uptake of nutrients, including Zn, by plants (Thompson et al. 2013). However, in the presence of a high Zn content in the root zone, AMF can reduce the accumulation of this micronutrient in aerial parts of plants (Christie et al. 2004). Watts-Williams et al. (2013) cultivated tomato at low and high levels of Zn in soil and proved that inoculated plants accumulated more Zn when the element was deficient in the soil but less when the content of this micronutrient in the soil was close to being toxic. The mechanism which inhibits the Zn uptake by mycorrhizal plants grown under condition of high and toxic Zn concentration in root zone has not been described yet, although it differs from the mechanism that stimulates the Zn uptake in a situation of Zn deficit (Smith, Read 2008). The differences may be due to the

Table 4

Effect of P and Zn concentration in the nutrient solution (mg dm^{-3}) and inoculation of the growth medium with AMF on the content of P, Cu, Fe, Mn, Mo and Zn, in leaves of lettuce

Treatment	P	Cu	Fe	Mn	Mo	Zn
	(g kg^{-1} d.m.)	(mg kg^{-1} d.m.)				
P (mg dm^{-3})						
10	1.966 ± 0.011	4.297 ± 0.285	85.37 ± 3.337	73.88 ± 3.848	0.389 ± 0.020	159.4 ± 31.12
40	5.147 ± 0.008	4.860 ± 0.209	97.44 ± 4.488	48.53 ± 0.917	0.557 ± 0.017	63.29 ± 9.179
	*	*	*	*	*	*
Inoculation						
-AMF	3.476 ± 0.041	5.038 ± 0.220	95.46 ± 4.863	65.34 ± 4.925	0.499 ± 0.024	127.0 ± 29.25
+AMF	3.538 ± 0.041	4.119 ± 0.247	87.26 ± 3.160	57.07 ± 2.879	0.447 ± 0.029	95.75 ± 20.99
	n.s.	*	*	*	*	*
Zn (mg dm^{-3})						
0.1	3.289 ± 0.047*a*	5.573 ± 0.123*c*	87.72 ± 4.799*a*	53.07 ± 1.908*a*	0.415 ± 0.034*a*	22.42 ± 1.839*a*
5	3.513 ± 0.047*ab*	4.639 ± 0.234*b*	84.66 ± 3.211*a*	60.31 ± 4.367*b*	0.472 ± 0.029*ab*	95.49 ± 10.08*b*
15	3.719 ± 0.050*b*	3.524 ± 0.223*a*	101.7 ± 5.845*b*	70.23 ± 6.626*c*	0.532 ± 0.031*b*	216.2 ± 34.99*c*
	*	*	*	*	*	*
Interaction P × inoculation × Zn	n.s.	n.s.	n.s.	n.s.	n.s.	*

* – means are significantly different, n.s. – differences are not significant, *a*,*b*,*c* – means followed by different letters differ at $p < 0.05$, ± – standard error, -AMF – plants not inoculated with arbuscular mycorrhizal fungi, +AMF – plants inoculated with arbuscular mycorrhizal fungi

expression of Zn transporter genes, characteristic for mycorrhizal fungi, or to the direct nutrient uptake by roots (WATTS-WILLIAMS et al. 2015). JONER et al. (2000) proved that fungal mycelium is highly capable of binding heavy metals. Therefore, immobilisation of Zn in the fungal hyphae may contribute to the lower content of this micronutrient in aerial parts of plants grown with a high Zn concentration in the root zone (CHEN et al. 2003).

Surprisingly, in our experiment, despite optimal concentrations of Cu, Fe, Mn and Mo in the nutrient solution, the content of these micronutrients was lower in inoculated plants than in non-inoculated ones (Table 4). These results are in agreement with our previous studies (KOWALSKA et al. 2015*a*, *b*). We found lower levels of Cu, Zn and Mn in inoculated plants in the cultivation of lettuce in rockwool or coconut (KOWALSKA et al. 2015*a*). Similarly, in the cultivation of tomato in the same growing media, the Zn and Cu content was lower in mycorrhized plants than in plants grown without AMF (KOWALSKA et al. 2015*b*). In a hydroponic culture, although the plants were supplied optimal concentrations of macro- and micronutrients, an increased concentration of cations and anions in the root zone is a common finding (JAROSZ et al. 2011). We suppose that the reducing effect of AMF on the Cu, Fe, Mn and Mo uptake in inoculated plants might be the result of an elevated concentration of these micronutrients in the plant root zone.

We observed a significant interaction among all the three experimental factors with respect to the Zn content in lettuce (Figure 1). The content of Zn in lettuce increased with the increasing Zn concentration in the nutrient solution. At optimal Zn doses (0.1 mg dm^3), the P level did not affect the Zn

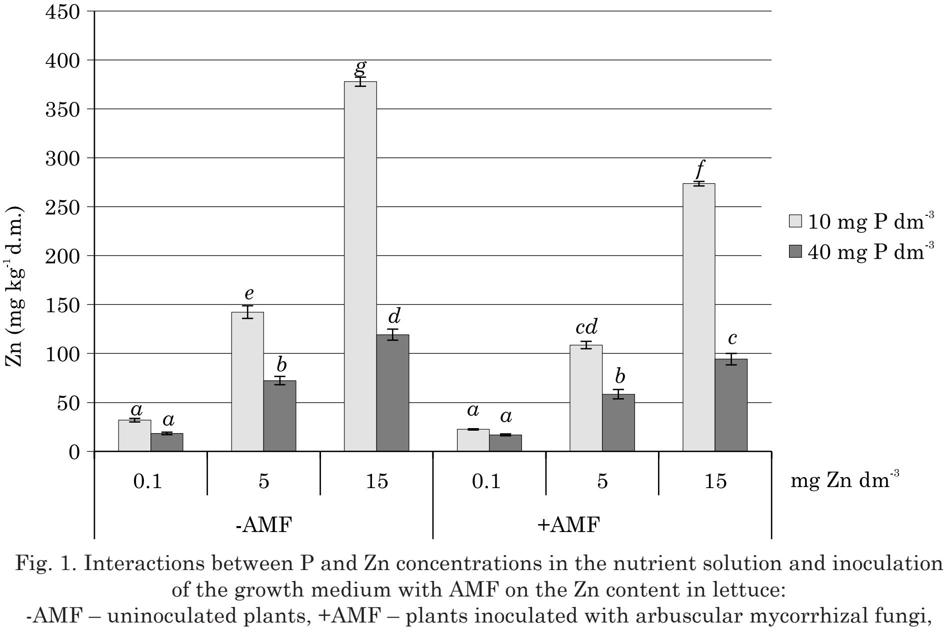

Fig. 1. Interactions between P and Zn concentrations in the nutrient solution and inoculation of the growth medium with AMF on the Zn content in lettuce:
-AMF – uninoculated plants, +AMF – plants inoculated with arbuscular mycorrhizal fungi, *a*,*b*,*c*,...– means followed by different letters differ at $p < 0.05$, bars indicate standard error

content in lettuce. However, at higher Zn doses, plants receiving a nutrient solution with the optimal P concentration contained significantly less Zn than plants grown at 10 mg P dm^{-3}. Nevertheless, the effect of the P concentration on the Zn content in mycorrhized lettuce plants was lower than in non-mycorrhized plants. The Zn uptake by plants is also affected by the interaction of Zn with other elements in the soil, including P (Christie et al. 2004). According to Shetty et al. (1994), P and Zn are mutually antagonistic, especially when one of these elements exceeds its optimal content in the root zone and this interaction may have an influence on the content of these two nutrients in plants. Moreover, the authors suggest that through phytic acid molecules involved in sequestration of Zn in plants, P can have an effect of the Zn detoxification in plant tissues. The interaction between P and Zn is probably more complicated in the presence of AMF. When P and Zn are deficient, AMF stimulates the uptake of both nutrients, whereas in a situation of excessive concentrations of these element, it is only the Zn uptake that is limited by fungi (Christie et al. 2004). In our experiment, we did not find any increased uptake of P by inoculated plants; however, we observed a limited Zn uptake. It can be assumed that the better development of mycorrhizal structures at a lower P level in the root zone might have improved the efficiency of mycorrhizal plants in reducing the Zn uptake. On the one hand, in our experiment, all mycorrhizal parameters were significantly higher in roots of plants receiving a reduced P concentration in the nutrient solution. On the other hand, despite the weaker development of mycorrhizal structures in plants grown at the optimal P concentration, AMF decreased the Zn content of lettuce grown at 15 mg Zn dm^{-3}. Contrary, in our previous study (Konieczny, Kowalska 2016) regarding the effect of arbuscular mycorrhiza on the zinc uptake in lettuce grown in a peat substrate, we did not verify any significant interaction between P and Zn on their uptake by plants. The level of P did not affect the Zn content in lettuce and the level of Zn did not affect the P content in plants. This lack of interaction was probably connected with the fact that the Zn dose used in that experiment was not high enough to reveal the antagonism between P and Zn or it may have been caused by the peat sorption complex, which reduced the contact between these two nutrients (Konieczny, Kowalska 2016). In their experiment on red clover grown at four Zn levels in soil, Chen et al. (2003) proved that there was a Zn concentration under which AMF increased the uptake of Zn by plants, while above this concentration fungi inhibited the Zn transfer from roots to shoots. According to Chrisite et al. (2005), this concentration may be different for different soil types and plant species. Moreover, in the presence of AMF, the plant's response to a high Zn concentration in the root zone may also depend on the type of a substrate and on a fungus species. We can conclude that at the optimal P level in this experiment, the dose of 15 mg Zn dm^{-3} in the nutrient solution was excessively high for plants and induced protective mechanisms of AMF, despite the relatively low degree of colonisation.

CONCLUSIONS

In conclusion, we showed the effect of AMF on reducing the Zn uptake in lettuce grown at a high concentration of Zn in the root zone. This decrease was greater in plants grown under the conditions of a reduced P content. Further studies investigating the mechanism which stimulates the Zn uptake by AMF plants in a situation of Zn deficit and which inhibits uptake under condition of its excess are needed. Another question worth investigating is the interaction between P and Zn in the presence of AMF.

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