



**Szent István University**

**The role of mycorrhiza-springtails (Collembola) relationship in  
the nutrient uptake of maize**

Ph.D Thesis

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Gödöllő

2009

Biological Sciences PhD School

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## 1. INTRODUCTION

A mycorrhiza is a symbiotic association between a fungus and the roots of a plant. Almost 80-90% of terrestrial plants live in a symbiotic relationship with arbuscular mycorrhizal fungi (AMF). In a mycorrhizal association the fungus may colonize the roots of a host plant either in an intracellular or an extracellular way. This mutualistic association provides the fungus with relatively constant and direct access to mono- or dimeric carbohydrates, such as glucose and sucrose produced by the plant in photosynthesis. The carbohydrates are translocated from their source location (usually leaves) to the root tissues and then to the fungal partners. Mycorrhizal mycelia are much smaller in diameter than the smallest root, and can explore a greater volume of soil, providing a larger surface area for absorption. AMF can facilitate water and phosphorus uptake by plants, especially in stress situations.

There is an intriguing paradox in arbuscular mycorrhizal fungal ecology (Gange, 2000). Although there are numerous laboratory studies that have shown a variety of benefits to plants in forming a mycorrhizal association, we know of far less occasions when these benefits have also been demonstrated in natural situations (Klironomos és Kendrick, 1993). The symbiosis between host plant and AM-fungi is influenced by several biotic and abiotic parameters. First of all, among the biotic parameters, the soil fauna can influence this symbiosis. One of the important mezofaunal groups (0,1-2mm) is the springtails (Collembola). Collembola are abundant microarthropods in virtually all soils, feeding on a range of materials, including fungi, bacteria, lichens, decomposing vegetation and detritus. The Collembola feed on fungi and bacteria in the soil. So they play an important regulatory role through feeding on soil microbial biomass, but also take part in the decomposition consuming dead vegetation and hyphae (Hedlung és Ohrn, 2000).

Collembola often play an important role in the interrelationship of AMF and host plants. Collembola can influence the colonisation, the mycelium size and structure, the abundance of AMF and the nutrient uptake of plants through the AMF. It has been suggested that these animals reduce the functioning of the mycorrhiza by feeding on the hyphae and spores of AMF. But several controlled studies have shown a positive effect on plant growth as a consequence of collembolan feeding on mycorrhiza. One mechanism by which Collembola might positively affect AM fungi is dispersal, but there is only one known example from previous literature to demonstrate that Collembola are able to disperse AMF in the soil (Klironomos és Moutoglis, 1999).

## 1.1. Objectives

### 1.1.1. AMF consumption by Collembola species

Do *Folsomia candida* and *Sinella coeca* Collembola species feed on the spores of *Glomus mosseae* and *Glomus intraradices* AM fungus species in laboratory experiments?

Are there differences between the two collembolan species in terms of AMF consumption?

Does the spore-feeding influence the abundance of spores?

### 1.1.2. AMF dispersal by Collembola species

Are the two Collembola species able to spread the mycorrhiza in the soil?

Are there differences between the two collembolan species in terms of AMF dispersal?

Is there any connection between feeding and spreading behaviour of Collembola species?

### 1.1.3. The nitrogen-uptake of maize through the AM system in the presence of Collembola

In this experiment the question was addressed whether high density of Collembola (0.6 Collembola g soil<sup>-1</sup>) may decrease the nitrogen uptake of maize through the destruction of the AMF hyphal network?

### 1.1.4. Zn uptake by maize under the influence of AM-fungi and Collembola *Folsomia candida*

Is there any effect of two Collembola density levels on the growth and development of the AM-fungus *G. intraradices*?

Is *F. candida* able to influence Zn uptake of maize through the AMF in a Zn-polluted soil?

Do Collembola densities have any effect on the Zn uptake of the plant?

## 2. MATERIALS AND METHODS

### 2.1. Materials and methods common in all the experiments

The formation of mycorrhizae was quantified by measuring the arbuscular formation of the AMF hyphae after staining in 0.1% trypan blue and lactophenol. The percentage colonization was estimated by the grid-line intersect method (Giovanetti and Mosse, 1980).

Determination of AM fungal hyphal length in the soil was based on the methods of Bååth and Söderström (1979). The hyphal length was measured in the dried agar film with the intersection method (Tennant, 1975) under a binocular microscope (16 x magnification).

The method of Gerdemann and Nicholson (1963) was used for counting the AMF spores.

Two collembola species were used in the experiments: *Sinella coeca* (Schött) and *Folsomia candida* (Willem) (Collembola, Insecta). The animals were obtained from the culture of Department of Zoology and Ecology, Szent Istvan University.

The microbial biomass was measured by the method of Amato and Ladd (1988). STATISTICA V. 5. software was used to analyze the data.

### 2.2. The design of the experiments

#### 2.2.1. AMF consumption by Collembola species

The feeding of the two Collembola species, *Sinella coeca* and *Folsomia candida*, on spores of two AMF was studied in the feeding experiments. 25 adult individuals of each Collembola species were added to Petri-dishes in five replicates. Spores of the following two AMF species were placed on the agar surface (Bacto) in the Petri-dish: *Glomus mosseae* (BEG 12) (40 spores per dish) and *Glomus intraradices* (BEG 2) (25 spores per dish). Changes in the spore numbers were recorded after two days.

#### 2.2.2. AMF dispersal by Collembola species

In the dispersal experiment, four different treatments were set up in five replicates. The treatments were as follows: 1) no Collembola (negative control): maize planting in sterile soil and *Glomus mosseae* in a small plastic container; 2) *F. candida*: maize planting in a sterile soil, *Glomus mosseae* in a small plastic container and *F. candida* freely moving in the system; 3) *S. coeca*: maize

planting in sterile soil, *Glomus mosseae* in a small plastic container and *S. coeca* freely moving in the system; 4) infection (positive control): the maize infected with *G. mosseae*.

The experimental pot (12×8×5 cm) contained sterile soil placed between the maize seedlings and the container with AMF, located at a distance of 10 cm. Each pot received an equal amount of AMF inoculum (20g) placed in the small container (5×2×3 cm) which was penetrable for the Collembola. In treatment 4 (infection), the inoculum was mixed with the sterile soil. At the same time, 72 adult *F. candida* or *S. coeca* individuals were added to the pots. Collembola were kept on the soil surface for five days. After five days, the small container was taken out with the AMF spores and the animals were killed.

Plants were grown in a greenhouse for five weeks. Plants were watered with 20 ml of deionised water when a decrease in their turgor pressure was observed.

After five weeks, root and shoot biomass, water content of plants and soil, the degree of colonization and the spore number in the soil were measured.

### 2.2.3. The nitrogen-uptake of maize through the AM system in the presence of Collembola

A microcosm experiment was set up on the field. Each microcosm was separated by a screen of 42 µm mesh size into two compartments, A and B. The roots could not penetrate into the root-free compartment A. The AMF were able to spread to both compartments of the microcosms.

Microcosms were filled up with brown forest soil previously air-dried and insolarized for a week in order to reduce the density of meso- and macrofauna. Subsequently, 500 g of soil was filled into compartment A (length: 8 cm, diameter: 10.5 cm) and 1000 g of soil into compartment B (length:15 cm, diameter:10.5 cm).

Two seedlings were planted in each pot and after emergence thinned to one plant per pot. The soil surface of all pots was covered with a 2 cm layer of gravel sand (2mm size) to minimize evaporation.

Each pot received an equal amount of AMF inoculum (10% of soil volumes, respectively) into soil of compartment B. Microcosms were randomly placed near by near on a table of 1 m height. Plants were watered twice a week with 100 ml tap water.

Six weeks after planting the maize, 200 mg <sup>15</sup>N marked ammonium sulphate (labelled with <sup>15</sup>N at 49.8 atom percent excess) solved in distilled water was added to compartment A in each pot.

Three different treatments were set up, in three replicates. <sup>15</sup>N was added at a distance of 0.15 m from the roots of the maize with (C+) or without (C-) Collembola. In the third treatment, the <sup>15</sup>N was added directly to the root.

Mycorrhiza was allowed to grow for six weeks. In the same time adult individuals of the Collembola *Sinella coeca* was put in the maize free compartment of the microcosms in a density of 0.6 animal g<sup>-1</sup> soil. Supposing that most Collembola individuals live in the upper 5 cm of the soil (Larink, 1997) an approximate calculation shows that a density of 3x10<sup>-4</sup> individuals m<sup>-2</sup> refers to 0.6 individuals g<sup>-1</sup> soil. Microcosms were destructively sampled after two weeks. Total plant biomass, shoot biomass, root biomass, shoot/root ratio, plant water content, the total N content and <sup>15</sup>N content of the aboveground plant parts, length of the AMF hyphae and amount of microbial biomass were measured. N and <sup>15</sup>N concentrations were determined by VG Micromass 622 mass spectrometer.

#### 2.2.4. Zn uptake by maize under the influence of AM-fungi and Collembola *Folsomia candida*

The experiment consisted of eight treatments (Table 1). Five replicates were prepared for each treatment. *Folsomia candida* Collembola and *Glomus intraradices* AMF species were used.

Treatment	Zn (250 mg/kg)	<i>G.</i> <i>intraradices</i>	<i>F. candida</i> density
N0	-	-	-
N1	-	+	-
N2	-	+	low
N3	-	+	high
Z0	+	-	-
Z1	+	+	-
Z2	+	+	low
Z3	+	+	high

Soil was spiked with 100 ml of ZnSO<sub>4</sub>(H<sub>2</sub>O)<sub>7</sub> (Merck, GR for analysis) solution at a nominal concentration of 250 mg Zn kg<sup>-1</sup> dry soil which is the pollution threshold limit value in Hungary. Treatments without Zn received an equivalent quantity of distilled water. Zn was added four weeks after planting the maize. The aim of choosing this concentration was to demonstrate the effects of a moderate Zn pollution.

Microcosms with maize were destructively sampled 8 weeks after planting. Roots were carefully removed from the soil and gently washed in tap water. Shoots were cut at the upper part of the crown. Plant height was measured from crown to the highest end of the leaves. The weight of the shoots and the roots was determined after drying at 70 ° C for 72 h. The C and N contents of

thoroughly mixed dry plant roots and shoots were determined by a Carlo-Erba NA 1500 elemental analyser. Zn analyses were performed on soil, root and shoot samples. After digestion with cc. HNO<sub>3</sub>, the extracts were analysed for Zn with plasma emission spectrometry using a Jobin-Yvon JY24 ICP instrument. The microbial biomass, the percent of mycorrhiza colonisation, the length of hyphae and the spore number were measured.

### 3. RESULTS

#### 3.1. AMF consumption by Collembola species

*F. candida* did not consume the spores of *G. mosseae* and *G. intraradices* at all in laboratory experiments, while *Sinella coeca* consumed 45 % of *G. mosseae* spores and 71 % of *G. intraradices* spores.

#### 3.2. AMF dispersal by Collembola species

While the roots of the control plants from treatment 1. were not infected with AMF, the plants in the other three treatments were infected. Consequently, both species were able to spread mycorrhiza in the soil, but the efficiency of dispersal was different. *F. candida* carried the infection more effectively than *S. coeca*, in spite of the fact that *F. candida* did not consume the spores, as revealed by the food choice experiment.

#### 3.3. The nitrogen-uptake of maize through the AM system in the presence of Collembola

No significant difference was found among the treatments in terms of total plant biomass, shoot biomass, root biomass, shoot/root ratio, plant water content. The presence of the Collembola decreased both the <sup>15</sup>N atom percent excess and the total N of the maize. Collembola activity decreased the hyphal length in compartment A compared to the other treatment, while in compartment B the hyphal length increased. The presence of Collembola decreased the microbial biomass in compartment A while in compartment B there was no significance difference among the treatments in this respect.

### **3.4. Zn uptake by maize under the influence of AM-fungi and Collembola *Folsomia candida***

Collembola had significant effect on shoot dry weight and the total dry weight of the plant. The plants were greater when Collembola were present at low density than at high density. There were no significant differences in the root dry weight and the water content of the plant. The plants infected by AMF had higher shoot dry weight, than the plants without AMF, but the difference was not significant. The inoculated plants had significantly higher shoot/root ratio, than the not inoculated plants in the case of Zn pollution.

The percentage of the mycorrhizal colonization was under 5% in the uninoculated plants which is in the range of the methodological bias. Mycorrhizal inoculation had a great positive effect on mycorrhizal colonization, spore number and hyphal length. Both Zn and Collembola had a great effect on AMF colonization, spore number and hyphal length. High Collembola densities decreased mycorrhizal colonization compared to the treatments without Collembola and with Collembola at low density. In the case of hyphal length the results were similar. High Collembola densities had negative effect compared to the treatment without Collembola and the treatment with few Collembola. Low Collembola densities had no effect on AMF except that it decreased spore numbers in Zn-spiked soil. The presence of Collembola at both density levels decreased AMF spore numbers in the polluted soil. Spore number had a weak interaction between Collembola and Zn. Added Zn increased both hyphal length and the degree of the AMF colonization.

The application of Zn considerably enhanced plant Zn concentrations, both in the shoots and in the roots and had a greatly positive effect on the Zn concentration of the soil. Mycorrhizal infection increased shoot and root Zn concentrations, but did not influence the Zn content in the soil. Lower root Zn concentrations were found when Collembola were present, irrespective of the density. Root Zn concentration had interaction between Collembola and Zn. Soil Zn content and shoot Zn concentration was not affected by Collembola.

### 3.5. New scientific results

*F. candida* Collembola species was demonstrated not to feed on the spores of *G. mosseae* and *G. intraradices* AM-fungi in 48 hours.

*S. coeca* Collembolla species was the first time described to feed on the spores of *G. mosseae* and *G. intraradices* AM-fungi under laboratory conditions.

*F. candida* and *S. coeca* were proved be able to carry the mycorrhiza from *G. mosseae* inoculum to a sterile maize. However, the carrying capacity was different for the two species, *F. candida* carried the infection more effectively than *S. coeca*, in spite of the fact that *F. candida* did not consume the spores in the food choice experiment.

In our experiment, Collembola activity was demonstrated to destroy AMF hyphal network that led to a decrease in the nitrogen uptake of plants.

The results of our experiment demonstrate that the Collembola influence the growth and spread of AMF depending on the density of Collembola. High densities of Collembola (50000 individuals m<sup>2</sup>) decreased the degree of the AMF colonization on the plant roots and the hyphal length in the soil, but low densities (20000 individuals m<sup>2</sup>) did not influence these parameters.

In the presence of AMF, the Zn content of the plant shoots and roots was significantly higher than without AMF. This effect was decreased by Collembola at a low density and at a high density as well. Collembola seems to directly influence the Zn uptake by maize not only through the AMF-host plant system.

## 4. DISCUSSION

According to literature data (Moore et al., 1985 ), *F. candida* do not feed on the spores of *G. mosseae* and *G. intraradices*. In the contrary, *S. coeca* in our experiment consumed the spores of the two AMF species. We did not find any comparable date in literature. These results suggest that there are differences in the AMF feeding of the Collembola species.

In the AMF dispersal experiment, root biomass, total plant biomass and plant water content were found to be higher in the presence of both Collembola species, than in the control treatment without Collembola; however, the

differences were not statistically significant. Each parameter was significantly higher in the case of the infected plants (treatment 4), compared to the control plants. These results can be explained by Collembola activity. Collembola dispersed the AMF fungi, therefore they enhanced plant nutrient and water uptake. These results are in line with the finding of other authors, who also found that Collembola can enhance the growth and abundance of AMF (Gange and Ayres 1999; Bakonyi et al., 2002).

While the roots of the control plants from treatment 1) were not infected with AMF, the plants in the other three treatments were infected. Consequently, both species were able to spread mycorrhiza in the soil, but the efficiency of dispersal was different. *F. candida* carried the infection more effectively than *S. coeca*, in spite of the fact that *F. candida* did not consume the spores in the food choice experiment. Therefore, it may be assumed that dispersal of hypha fragments took place in the gut of the animals. It is known that *F. candida* is feeding on the hypha of different *Glomus* species (Moore, 1985 and personal observation). The difference of the degree of colonisation can be explained by the different activity and morphology of the two species. Klironomos and Moutoglis (1999) found that in the presence of Collembola the distance that the AMF can bridge between an inoculated and a nonmycorrhizal plant can be changed. Therefore, our experimental results have shown that the *F. candida* and *S. coeca* species were able to disperse the AMF from soil containing inoculum.

In the third experiment, AMF hyphal length were between 4.25 and 6.85 m g soil<sup>-1</sup>. Similar results were found in an other experiment with *G. intraradices*, *G. caledonium* and *G. invermaium* (Larsen, Jakobsen, 1996). These authors found that Collembola reduced the AMF hyphal length by 14, 37 and 39% depending on the AMF species. In our experiment a decrease of 20% in hyphal length was found in the presence of Collembola. These results are in line with the observations that Collembola at a higher density had negative effect on the growth of AMF (Bakonyi et al. 2002, Warnock et al. 1982) and the microbial biomass (Kaneda, Kaneko 2002). Other authors stress out the role of saprophytic fungi because evidence shows that Collembola prefer conidial fungi over AMF (Klironomos, Kendrick 1996). The <sup>15</sup>N uptake by maize plants was possible through AMF hyphal network under our experimental circumstances, because ammonium ions usually have low mobility in soil. Collembola destroyed hyphal network as it is proved by decreased hyphal length. As a consequence, the uptake of the marked ammonium nitrogen by plants decreased significantly. Cole et. al. (2004) found that microarthropods influenced the microbial community, but had no effect on the microbial or plant uptake of N. However one species (*Ceratophysella denticulata*) reduced root <sup>15</sup>N capture in monoculture in this experiment, too. Our data support the view that Collembola

can consume the AMF in the presence of saprophytic fungi and are able to influence the AMF hyphal length and function (Bakonyi et al. 2002).

Effects of the AMF on plant Zn uptake were found to be variable. Usually, AMF is supposed to be protecting plants against the toxic effects of Zn. In an experiment conducted with white clover, increasing Zn concentrations (0-400 mg kg<sup>-1</sup>) enhanced Zn uptake in the shoots and roots but this increase was greater without AMF (Zhu et al., 2001). Similar results were found in another experiment with ryegrass, where the metal concentrations were the following: 0, 30, 90, 270 mg kg<sup>-1</sup>. The presence of AMF was correlated with higher Zn content in the root, preventing Zn from penetrating the shoot, and thereby decreased the effects of metal toxicity (Takács and Vörös, 2003). In the case of maize the concentrations of Zn were lower in the mycorrhizal plants compared to the non-inoculated ones (Weissenhorn et al., 1995).

In other experiments more Zn was taken up by plants inoculated with AMF, both in the roots and shoots, than without AMF. Zn concentrations were higher in the roots than in the shoots (Jansa et al., 2003; Joner and Leyval 2001; Oudeh et al., 2002). Our results support these findings as mycorrhizal inoculation increased the Zn content of the roots and the shoots. Weissenhorn et al. (1995) suggested that the influence of AMF on metal uptake depends on the conditions of plant growth, on the fungal partner and on the metal. Besides, the Zn concentration applied and the host plant species are also important factors. This is why the results can not yet be generalized.

Zn contamination may decrease (Bi et al., 2003, Takács and Vörös, 2003), enhance (Zhu et al., 2001) or have no effect on AMF colonization (Li and Christie, 2001). Chen et al. (2001) demonstrated that Zn concentration in AM fungal mycelia is about 10 times higher than in the host plant tissues. In our experiment, Zn significantly increased the percentage of AMF colonization, and Zn had a positive effect on hyphal length in all treatments. In our case, probably the high Zn concentration in the soil enhanced the hyphal production of the AMF. In this way AMF help to keep the Zn in the root zone, as the AMF infection enhanced the Zn uptake to the root in greater ratio than to the shoot. This supports the hypothesis that AMF are able protect plants against heavy metal intoxicification. *F. candida* consumes the hyphae of certain *Glomus* species (Moore et al, 1985). According to our data (unpublished) *F. candida* fed on the hyphae of *Glomus intraradices* but did not consume the spores. The results of this experiment suggest that Collembola also fed on mycorrhiza. High densities of *F. candida* significantly decreased the degree of AMF colonization and hyphal length but low densities did not have any effect on the above parameters. A significant difference was observed between the two density levels concerning the degree of colonisation. Finlay (1985) obtained similar results studying the efficiency of mycorrhizal infection at different densities of the Collembola *Onychiurus ambulans*. Collembola at high density were found

to decrease the length of the external hyphae. However, at optimal density the dispersal of the AMF inoculum and the stimulation of hyphal growth compensated for the effect of feeding. In the present experiment, hyphal length correlated with the colonization rates; high Collembola densities decreased and low densities did not influence hyphal length. There was a significant difference between the hyphal lengths in the soil at different Collembola densities. Bakonyi et al. (2002) found that the degree of AMF colonization was the highest at a density of 0.2-0.4 *Sinella coeca* g soil<sup>-1</sup>. Higher or lower Collembola densities decreased the colonization rate. In this experiment the effect of different Collembola densities appeared also in terms of the plant biomass. In treatments applying lower Collembola densities plants grew higher and their dry weight was also greater.

Effects of Collembola on mycorrhizal development and growth also have an indirect effect on Zn uptake by the maize plants. Root Zn concentration had interaction between Collembola and Zn. This means that Collembola had greater effect on the root Zn concentration in the presence of Zn. At a high density, the Collembola decreased the AMF colonization and hyphal length in the soil, which resulted in a lower Zn concentration in the plant roots. Low Collembola densities had similar negative effect on the Zn concentration of the root; however this density did not have any effect on the AMF colonization and hyphal length. Therefore we have to underline the importance of the Zn uptake of the maize through the root, not through the AM-system. In this experiment the difference between the Zn uptake of non-inoculated and mycorrhizal plants was about 25% in the case of unpolluted soil and about 65% in the case of Zn spiked soil. Other authors found similar result concerning Zn uptake (Kothari et al., 1990). These results mean that the plants take up a great amount of Zn through the roots. Collembola may feed on the roots of the plants (Thimm and Larink, 1994; Petersen, 2002). We suppose that Collembola are able to influence the Zn uptake through not only AMF, but also the plant roots, however, the mechanism is not yet known.

We conclude that *F. candida* is able to influence the development of AMF and this effect depends on the density of Collembola. The presence of Collembola decreased root Zn concentrations of the maize plant both in the spiked and unspiked soil. However, Collembola densities were not an important factor in influencing the Zn uptake of plants through the plant-AMF fungi system. Collembola seems to directly influence the Zn uptake by maize not only through the AMF-host plant system.

Consequently, the Collembola consumption depend on the species, on the spores and hyphae of AM-fungi in the soil and can disrupt the mycelium network of the soil fungi. These effects cause an increase in the abundance of AMF. In the other side, Collembola are able to stimulate the growth of AMF by the mastication of the hyphae and they can help the colonization of new plants.

Because of these antagonistic processes, the effect of Collembola on the plant's production through the AMF may be positive, negative or neutral. An important conclusion of our experiments is that the density of Collembola is the principal factor to determine which way Collembola effect the nutrient uptake of plants through the soil – plant – AMF system.

## 5. PUBLICATIONS

**A. SERES, G. BAKONYI, K. POSTA (2006):** Zn uptake by maize under the influence of AM-fungi and Collembola *Folsomia candida*. Ecological Research 21 (5): 692-697. (IF: 1.012)

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**SERES A., BAKONYI G.**(2006) Az ugróvillások arbuskuláris mikorrhiza-gombára gyakorolt hatásainak mechanizmusa. 7. Magyar Ökológus Kongresszus, Budapest, p.188.