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Short research contribution

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COLLEMBOLA (INSECTA) DISPERSE THE ARBUSCULAR MYCORRHIZAL FUNGI IN THE SOIL: POT EXPERIMENT

ABSTRACT: Collembola often play an important controlling role in the interrelationship between arbuscular mycorrhizal fungi (AMF) and host plants. However, there are little data to prove AMF dispersing ability of Collembola. In our experiment *Folsomia candida* (Willem) did not consume the spores of *Glomus mossea* (Nicol. and Gerd.) and *G. intraradices* (Schenck and Smith), but *Sinella coeca* (Schött) consumed 45% of the *G. mossea* spores and 71% of *G. intraradices* spores.

Both species were able to disperse 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.

The total plant biomass was 23% higher in the presence of *F. candida* and 8.5% higher in the presence of *S. coeca* than in the control treatment without Collembola. The water content of the plants was also a little higher in the presence of both Collembola species (about 10%) than that of the control plants, but this difference was not statistically significant. Collembola improved the dispersion of the AM fungi, therefore enhanced the nutrient and water uptake of the plant.

KEY WORDS: Collembola, arbuscular mycorrhizal fungi, dispersal

Almost 80–90% of terrestrial plants live in a symbiotic relationship with arbuscular mycorrhizal fungi (AMF) (Malloch *et al.* 1980; Jakucs 1999). AMF can facilitate water and P uptake by plants, especially in stress situations (Posta and Füleky 1997, 2000). Collembola often play an important role in the interrelationship of AMF and host plants. Many species of Collembola graze on AMF (Larsen and Jakobsen 1996), and different Collembola species have a preference for different AMF species (Moore *et al.* 1985).

Several studies report that because of the AMF consumption by Collembola, the mycorrhizal colonization decreases, having a negative effect on nutrient uptake, growth and production of the plants (Warnock *et al.* 1982; Finlay 1985). In fact, this effect depends on Collembola density. At optimal densities Collembola may stimulate AMF growth and development by consumption (Gange and Ayres 1999; Bakonyi *et al.* 2002). It is also proved that although the AMF spores are too large to pass the Collembola gut in an intact way, the presence of Collembola help the AMF to colonize the plants (Klironomos and Moutoglis 1999). The mechanism of the dispersal of AMF by Collembola is still unknown. It is not clear

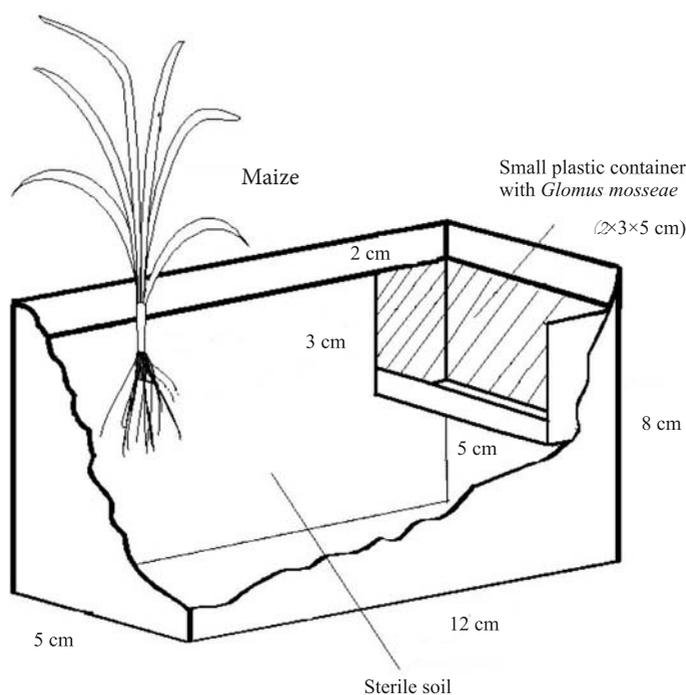


Fig. 1. The experimental set-up used in the study on the dispersal of AMF (*Glomus mosseae*, Nicol. & Gerd.) by Collembola (*Folsomia candida*, Willem and *Sinella coeca*, Schött).

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 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 which was placed between the maize seedlings and the container with AMF, located at a distance of 10 cm.

Table 1. Feeding of *S. coeca* and *F. candida* (Collembola) on spores of *G. mosseae* and *G. intraradices*. Average numbers of the consumed (by Collembola) spores ± SD and the result of t-test. Number of replications = 5.

	Initial numbers of spores per dish	Control (without Collembola)	<i>Folsomia candida</i>	t-test	<i>Sinella coeca</i>	t-test
<i>G. mosseae</i>	40	0.00	0.2 ± 0.2	n.s.	18.2 ± 6.18	***
<i>G. intraradices</i>	50	0.2 ± 0.2	1.4 ± 1.1	n.s.	35.6 ± 1.44	***

***: $P < 0.001$, n.s.: not significant

whether the hyphae, the spores in their intact or damaged form or another part of the AMF play a role in the spreading process.

The aim of our study was to examine whether 1) *Folsomia candida* (Willem) and *Sinella coeca* (Schött) Collembola species feed on the spores of *Glomus mosseae* (Nicol. and Gerd) and *Glomus intraradices* (Schenck and Smith) AM fungus species in laboratory experiments, 2) the two Collembola spe-

cies are able to spread the mycorrhiza in the soil, 3) this process has any influence on the height, biomass and water content of maize plants.

The feeding of the two Collembola species on spores of two AMF was studied in the feeding experiments. 25 adult individuals of two Collembola species *Sinella coeca* and *Folsomia candida* were added to a Petri-dish. Spores of the following two AMF spe-

cies were placed on the agar surface (Bacto) in the Petri-dish: *Glomus mosseae* (BEG 12) (40 spores per dish) and *Glomus intraradices* (BEG 2) (50 spores per dish). The number of replicates was five. Changes in the spore numbers were recorded after two days.

In the dispersal experiment four different treatments were set up in five replicates (Fig. 1.). 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 which was placed between the maize seedlings and the container with AMF, located at a distance of 10 cm. The soil was collected in the Botanical Garden of the Szent István University (Gödöllő, Hungary). The main chemical parameters of the soil were as follows: total C – 1.6%, total N – 0.15%, P – 0.0712%, K – 0.29%, Fe – 0.78%, Zn – 0.0043%, Mn – 0.0480%, pH_(H2O) – 7.5. Maize (*Zea mays* L.) seeds were sterilized on the surface using 30% H₂O₂ for 10 minutes and subsequently washed with sterile distilled water. Pre-germinated maize seedlings were planted in each pot and after germination thinned to one plant per pot. One week after planting, AMF and Collembola were added to the pots. Before the experiment, the inoculum of *Glomus mosseae* (BEG 12) was propagated on maize grown in a greenhouse for seven weeks. Roots with adhering soil were used as inoculum. Each pot received an equal amount of 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 and the degree of colonization were measured. After the trypan-blue staining of the roots (Giovanetti and Mosse 1980) four categories were set up for developmental state of the different AMF parts (external hyphae, apressorium, internal hyphae and arbusculum): 0=absent, 1=poor, 2=medium, 3=great. The results were analysed with paired t-test and Mann-Whitney u-test.

F. candida did not consume the spores of *G. mosseae* and *G. intraradices* at all in laboratory experiments, but *S. coeca* did (Table 1). *Sinella coeca* consumed 45% of *G. mosseae* spores and 71% of *G. intraradices* spores.

In the AMF dispersal experiment root biomass, total plant biomass and plant water content (Table 2) 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 AM 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 (Table 3), 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. Klirono-

Table 2. Growth parameters of maize plants, the water content of plants and soil in the different experiment treatments (average \pm SD). The explanations of the treatments see Fig 1.

	Treatments	Root dry mass (g plant ⁻¹)	Total dry mass (g plant ⁻¹)	Water content of plants (g plant ⁻¹)	Water content of soil (%)
1.	no Collembola	0.19 \pm 0.0	0.36 \pm 0.1	3.71 \pm 0.8	17.23 \pm 1.5
2.	<i>F. candida</i>	0.29 \pm 0.1	0.47 \pm 0.2	4.12 \pm 0.6	14.99 \pm 1.4
3.	<i>S. coeca</i>	0.19 \pm 0.1	0.40 \pm 0.2	4.13 \pm 0.2	16.66 \pm 1.5
4.	infection	0.39 \pm 0.1	0.50 \pm 0.3	5.04 \pm 0.4	14.42 \pm 1.7
Paired t-test					
1-2		n.s.	n.s.	n.s.	*
1-3		n.s.	n.s.	n.s.	n.s.
1-4		**	***	**	*
2-3		n.s.	n.s.	n.s.	n.s.
2-4		n.s.	*	*	n.s.
3-4		**	***	*	*

***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, n.s.: not significant

Table 3. The degree of AMF colonisation in the different treatments (average \pm SD). State of the development of the different AMF's parts (external hyphae, apressorium, internal hyphae, arbusculum): 0=absent, 1=poorly, 2=medium, 3=great. The explanations of the treatments see Fig 1.

	Treatments	External hyphae	Apressorium	Internal hyphae	Arbusculum
1.	no Collembola	0.2 \pm 0.1	0.0	0.0	0.0
2.	<i>F. candida</i>	2.8 \pm 0.2	2.4 \pm 0.3	2.4 \pm 0.3	0.4 \pm 0.3
3.	<i>S. coeca</i>	1.2 \pm 0.2	1.0 \pm 0.0	1.2 \pm 0.2	0.0
4.	infection	3.0 \pm 0.0	1.5 \pm 0.3	1.8 \pm 0.3	0.3 \pm 0.3
Mann-Whitney U-test					
1-2		**	**	**	n.s.
1-3		*	**	**	n.s.
1-4		*	*	*	n.s.
2-3		**	**	*	n.s.
2-4		n.s.	n.s.	n.s.	n.s.
3-4		*	n.s.	n.s.	n.s.

** : $P < 0.01$, * : $P < 0.05$, n.s.: not significant

mos 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.

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