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Collembola, soil fungi, wheat plants: images of their interactions

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Why do we publish these images?

Trophic interactions of Collembola with some soil fungi and their effects on wheat plants health and animal life have been our field study for about twenty years. The collaboration between a zoologist and a plant pathologist, both passionate about their organisms, has allowed to explore this field with different eyes.

The results have been published along the way, and we hope that they have been useful for those working in this field of study. We also hope that they will be useful in the future for all the possible applications the interactions Collembola–fungi might generate.

We have documented our works with images that have been only partially used for oral presentations or posters.

We want to share and provide free access to these images. The Italian Journal of Mycology makes it possible. The images are introduced by a brief text.

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The soil is a reservoir of organisms with the same aim: feeding !

Collembola, commonly known as springtails, are among the most important groups of soil mesofauna as they live in all climatic environments from Arctic and Antarctic to tropical areas, and are largely dependent upon fungal food sources.

Collembola interact with propagules of soil fungi, and in particular with those of the following fungi that influence plant growth and health:

- pathogenic fungi which are responsible of diseases

- antagonistic fungi which act against pathogenic fungi by different mechanisms reducing their inoculum and activity

- arbuscular mycorrhizal (AM) fungi which promote plant growth through their role in nutrition and protection against pathogens.

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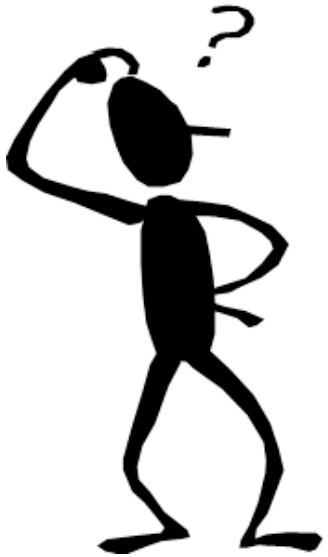
Since the disease is the result of the competiton between pathogenic, antagonistic and AM fungi for rhizoplane/rhizosphere colonisation (competence) the question is :



can trophic activity of Collembola modulate plant-fungi interactions by reinforcing or switching the existing relationship between fungi?

We are going to exam these interactions separately

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Interactions between Collembola and soil borne plant pathogenic fungi: *in vivo* and *in vitro* tests

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Collembola used in *in vitro* tests

In the image specimens of *P. armata* feeding on *F. culmorum* hyphae and sporodochia

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Fungal pathogens and symptoms they cause on wheat plants

Gaeumannomyces graminis var. *tritici*

Fusarium culmorum
Bipolaris sorokiniana

Rhizoctonia cerealis

These fungi are responsible for important diseases of winter cereals indicated with the generic name of foot and root rot. They usually coexist in the same soil portion, even though one is the prevalent depending on edaphic conditions. Severe infections cause white-headed plants as shown in the image.

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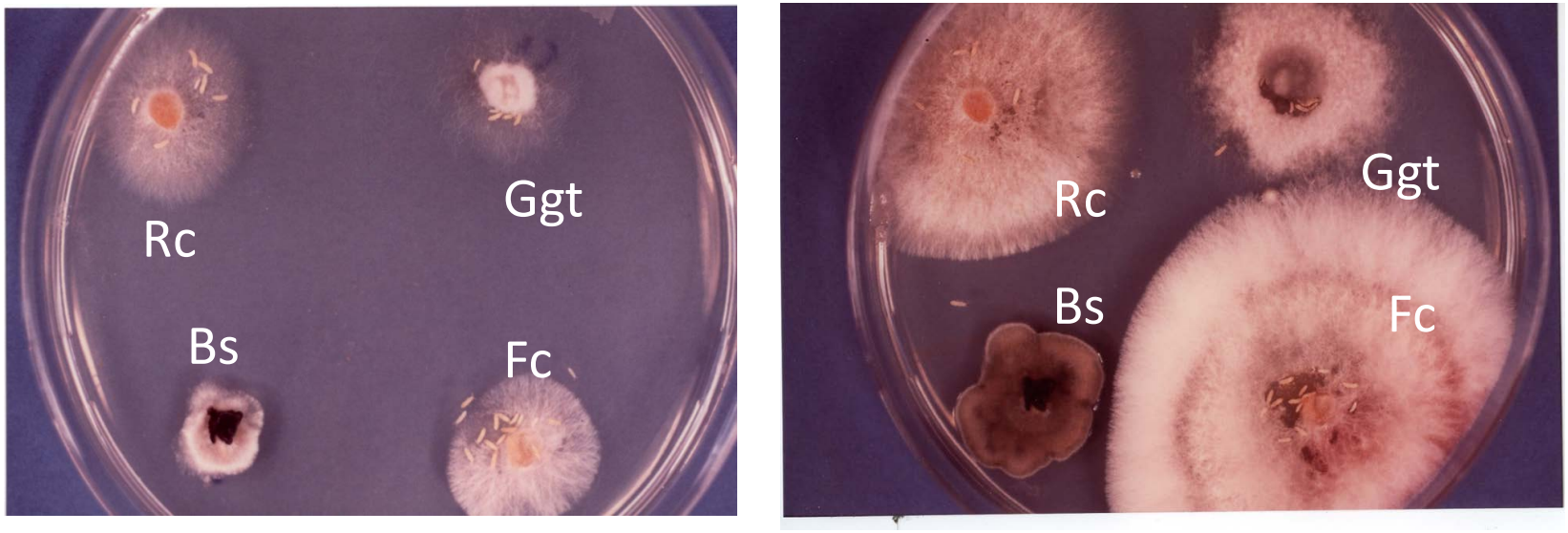
In *in vitro* multiple choice tests, Collembola were able to interact contemporaneously with *R. cerealis* (Rc), *G. graminis tritici* (Ggt), *B. sorokiniana* (Bs) and *F. culmorum* (Fc) inoculated at different times to have colonies with similar size when animals were introduced into the Petri dishes.



In the images distribution of *P. armata* specimens 3 h (left) and 50 h (right) after their introduction into the Petri dish with the fungal colonies on agarised medium. No animals are feeding on Bs colony in both images.

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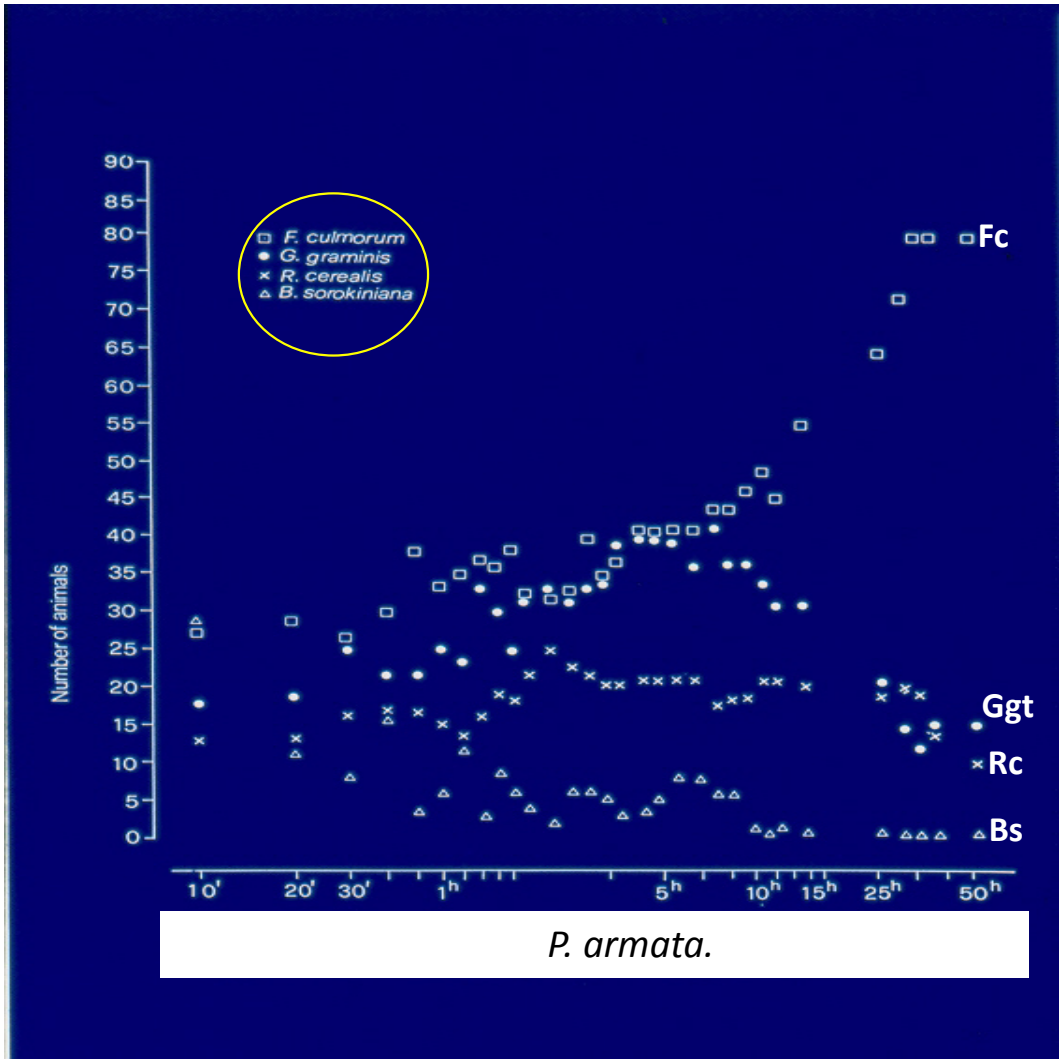
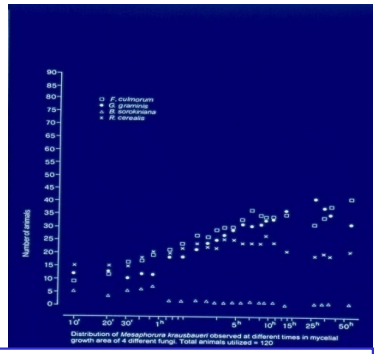
Italian Journal of Mycology vol. 47 (2018) ISSN 2531-7342

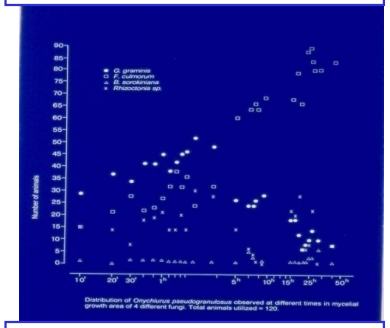
Distribution of Collembola at different times. Total animas = 120



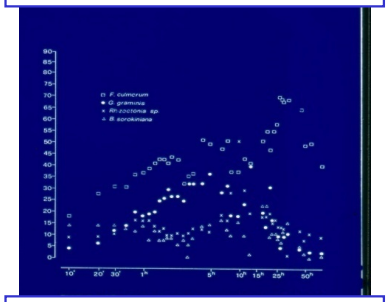
P. armata.



M. krausbaueri



O. pseudogranulosus



K. tuberculata

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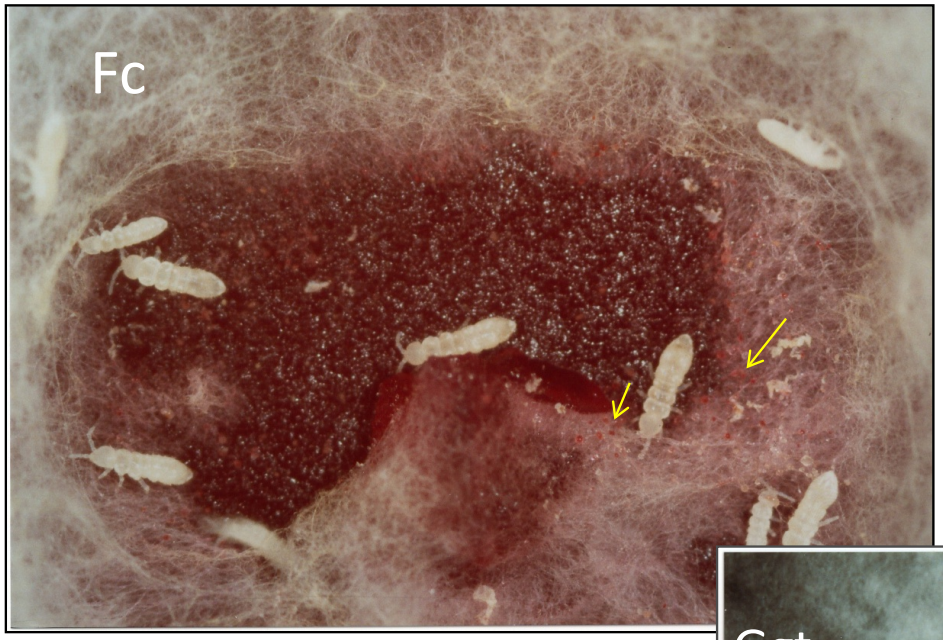
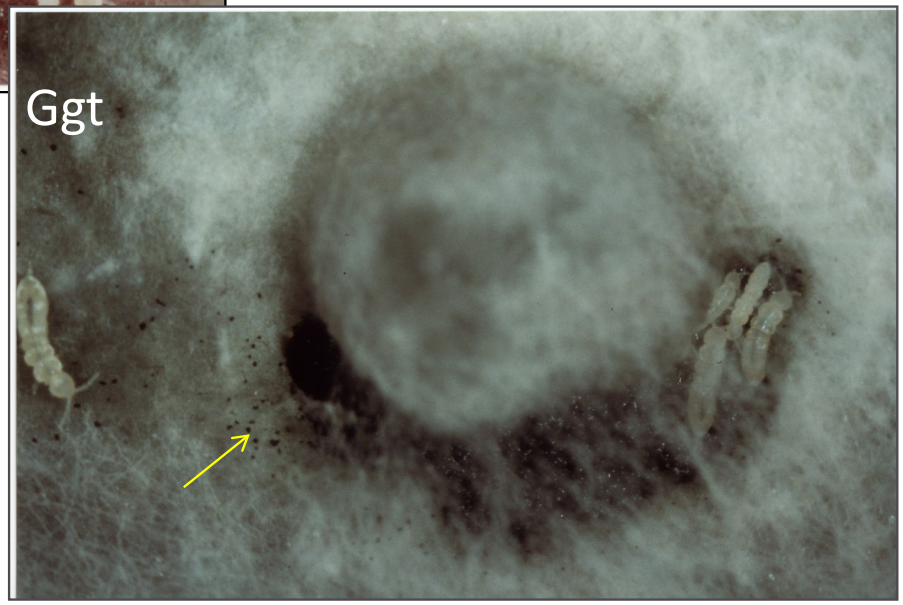
Italian Journal of Mycology vol. 47 (2018) ISSN 2531-7342



Specimens of *P. armata* feeding on *F. culmorum* (Fc) and *G. graminis tritici* (Ggt) colonies. Note the absence of hyphae in the animal feeding area.

Note also that the gut color depends on the hyphal color, carmine red for Fc, and black for Ggt. Faecal pellets of the same color of hyphae are also present as indicated by arrows.



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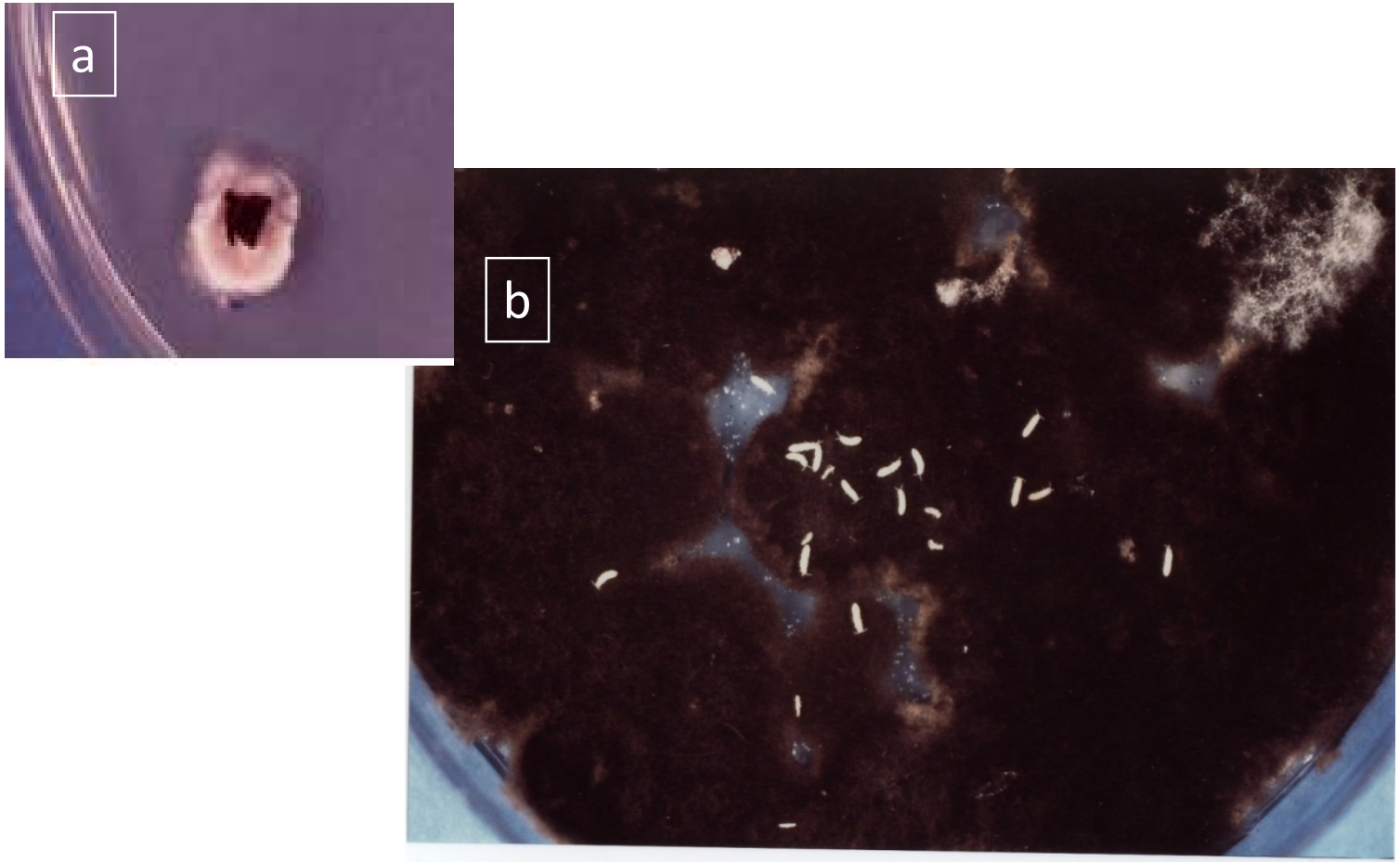
No animals are on young non- sporulating colony area of *B. sorokiniana* (a) , whereas collembolans feed on mature colonies of the fungus, characterised by an abundant presence of melanised conidia (b).



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Subsequent long term (150 d) experiments with the parthenogenetic collembolan *M. krausbaueri* showed that:

. mycelia of *F. culmorum*, G*. graminis tritici* and *R. cerealis* were appropriate food for reproduction, all eggs were viable, all eggs developed up to hatching, and all newborns reproduced by thelitoky

. a diet of *G. graminis tritici* determined early maturation, oviposition of more eggs over shorter time, early loss in fertility and early death

. a diet of *F. culmorum* determined maximum eggs production later

. a diet of *R. cerealis* determined the production of the highest total number of eggs, but late maturation, oviposition of more eggs very late, and very late death

. a diet of *B. sorokiniana* conidia determined the lowest fertility.

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Interactions between Collembola and antagonistic fungi belonging to *Trichoderma* spp.

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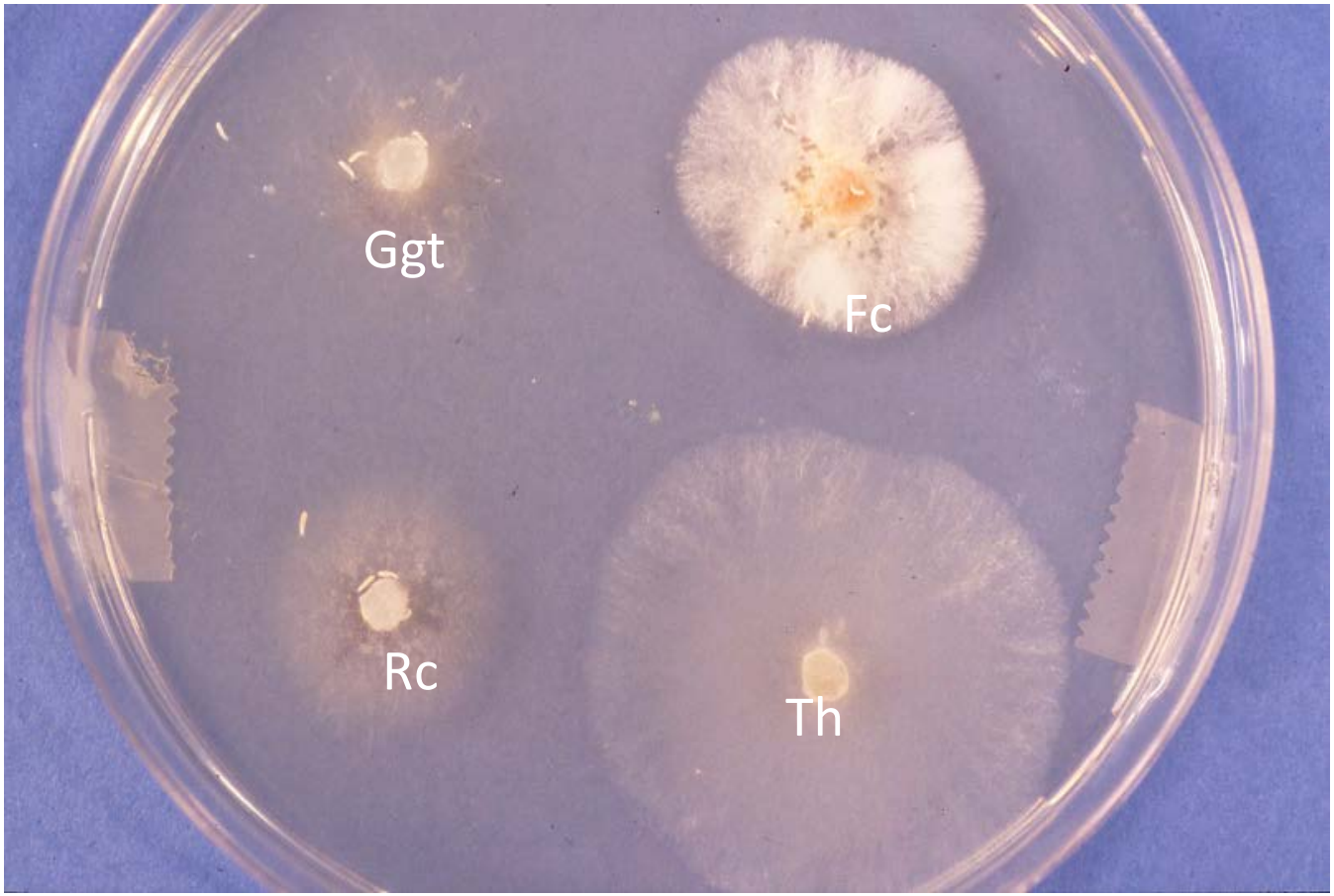
In *in vitro* multiple choice tests, in the presence of *G. graminis tritici* (Ggt), *F. culmorum* (Fc), *R. cerealis* (Rc) and the non sporulating young *Trichoderma harzianum* (Th) colonies, *P. armata* specimens avoided the Th colony, even if more developed than others.



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Distribution of *Protaphorura armata* specimens at different times on the mycelia of pathogenic fungi and of antagonistic fungus *Trichoderma atroviride* present contemporaneously in the same Petri dish.

	Hours after animals introduction						
	1	2	6	12	24	36	48
F. culmorum	8.7 a	10.0 a	11.2 a	18.2 a	17.7 a	18.7 a	17.7 a
G. graminis	8.0 a	6.0 a	6.2 a	6.2 b	6.2 b	8.0 b	7.5 b
R. cerealis	9.2 a	10.2 a	10.0 a	3.5 bc	4.2 bc	1.0 c	2.2 c
T. atroviride	1.2 b	0.5 b	0.2 b	0.2 c	0 c	0 c	0 c

In the same column, values followed by the same letter are not statistically different (DMS, $P < 0.05$)

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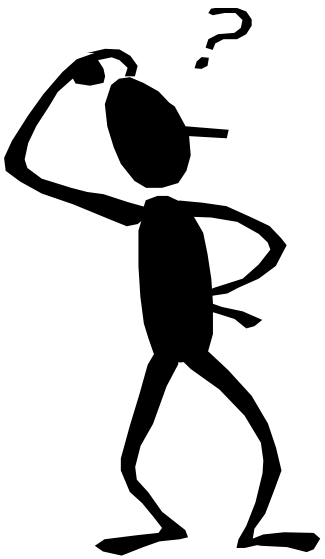

Can the Collembola spread fungal propagules?

Are conidia and hyphae viable after gut transit?

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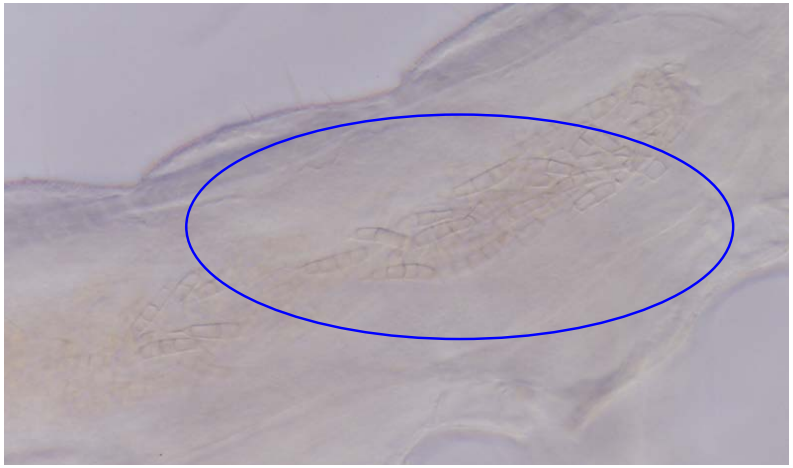
Conidia of *F. culmorum* (Fc) were palatable and no toxic for Collembola. They were damaged during the gut transit, indeed very few faecal pellets produced by animals fed by the fungus, gave rise to colonies of the fungus when transferred on agar medium.

Conidia of Fc produced in pure colture.



Gut of a *P. armata* specimen with Fc conidia.

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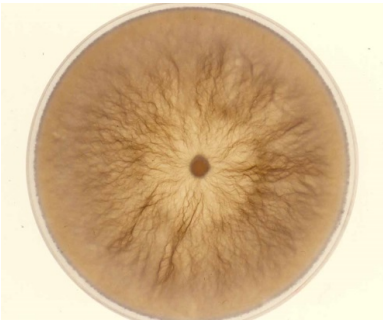
Melanized macrohyphae of *G. graminis tritici* (Ggt) were fed by Collembola. No fungal colonies raised from faecal pellets produced by animals fed with the fungus when these were inoculated on agarised medium.



Colony of Ggt in Petri dish on agarised medium with the typical melanised macrohyphae.

Gut containing portions of Ggt macrohyphae.

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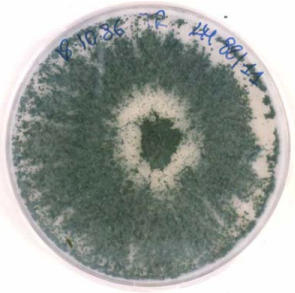
Conidia of *Trichoderma* were palatable and no toxic for Collembola



Colony of *Trichoderma* sp. in Petri dish on agarised medium, with masses of mature conidia green in colour.

Collembola feeding on highly sprulating *Trichoderma* colony. Note the green colour of gut and faecal pellets (a). Particular of gut with masses of conidia (b, c).

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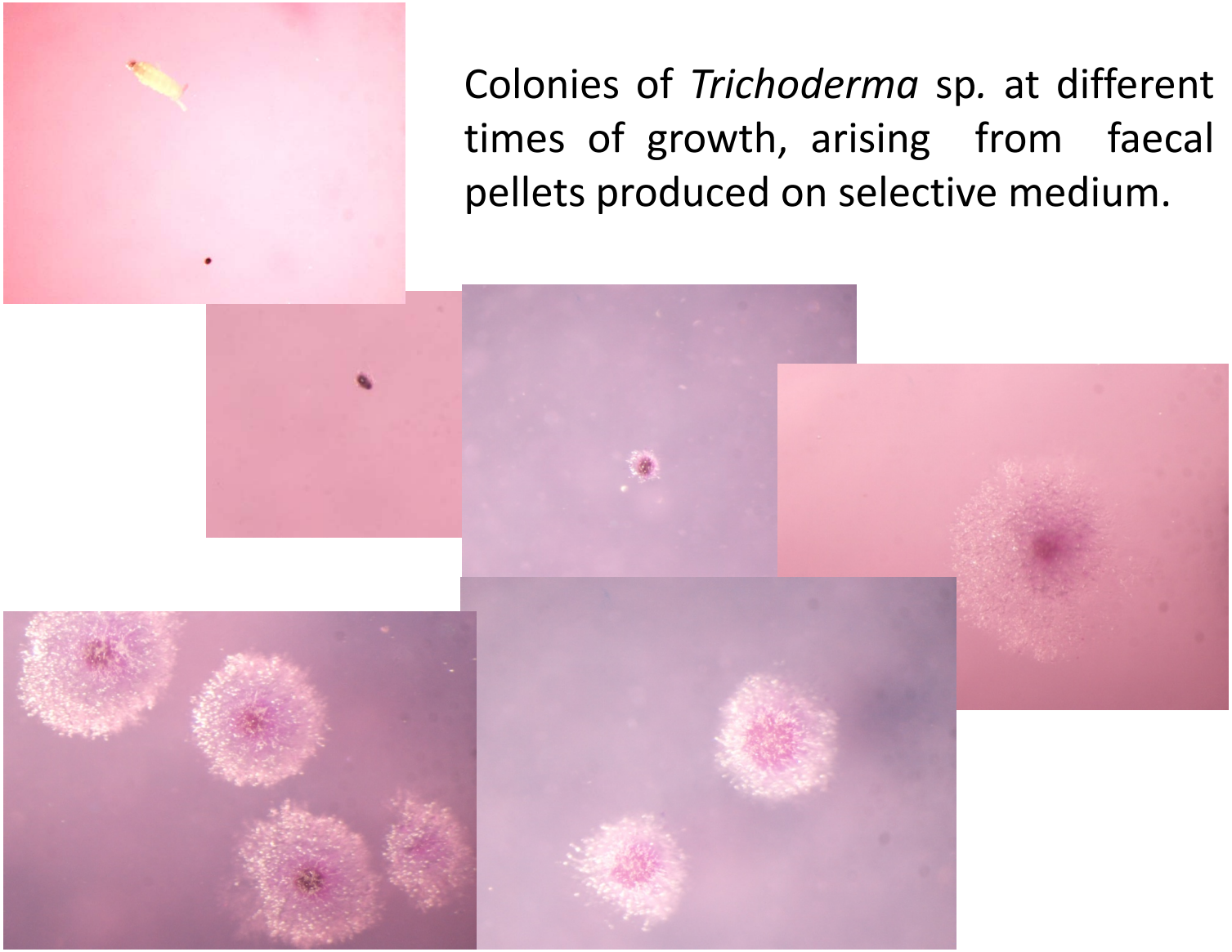

Colonies of *Trichoderma* sp. at different times of growth, arising from faecal pellets produced on selective medium.

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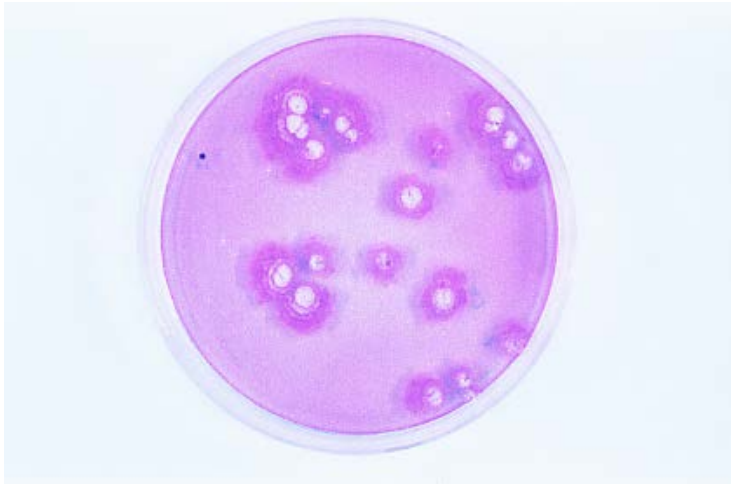
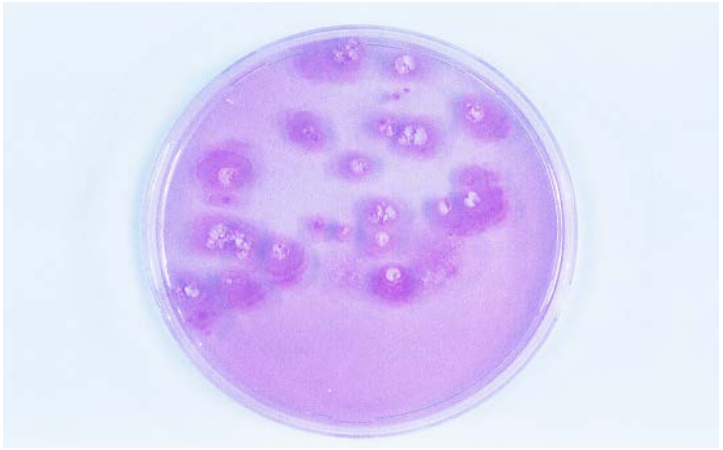
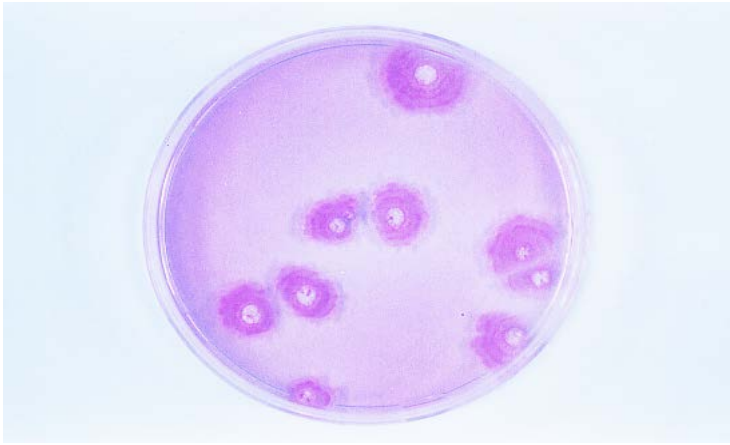


Protaphorura armata



Folsomia candida

Back Petri dishes with *Trichoderma* colonies from faecal pellets produced by different species of Collembola fed with conidia of the fungus.



Onychiuroides pseudogranulosus

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From Petri dish to microcosm: effect of interactions between fungi and Collembola on wheat plant disease

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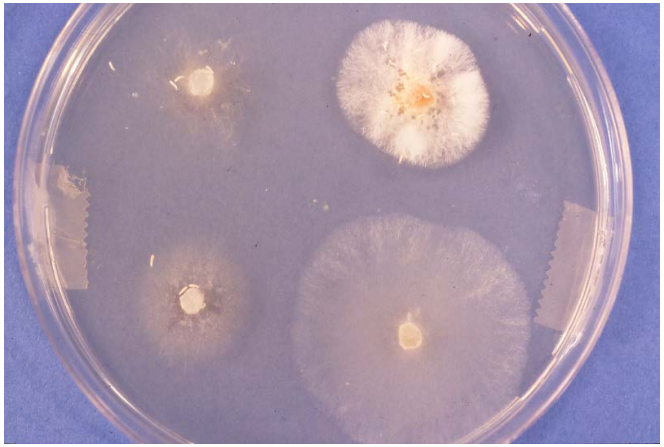
Petri dish



Microcosms: glass tubes (35 mm in diameter by 300 mm high)

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Each microcosm

- contains 150 g sterile sand mixed with 1.5 g sterile millet kernels colonised by *F. culmorum* or *G. graminis tritici* + 3 seeds of wheat cv Creso + 50 specimens of *P. armata*

- was maintained for 4-5 weeks under controlled conditions, then seedlings were removed and animals collected by floating and counted

The density of springtails corresponds to about 40,000 individuals per square meter, comparable to the density of Collembola in the Po Valley cultivated soils.

The presence of Collembola did not damage plants, on the contrary increased plant dry weight, and reduced the disease severity caused by both pathogens.

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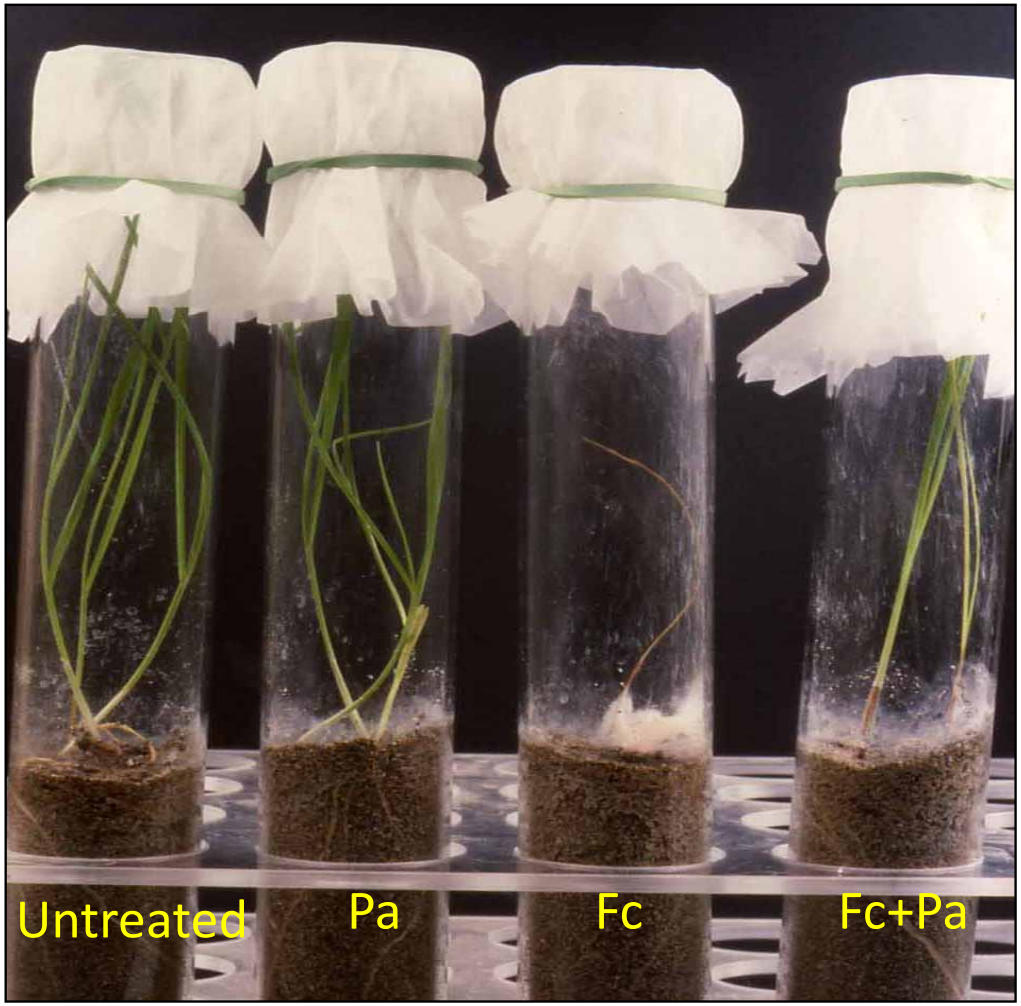
Effect of *F. culmorum* (Fc), *P. armata (*Pa) and their interactions (Fc+ Pa) on wheat seedlings compared with untreated plants.



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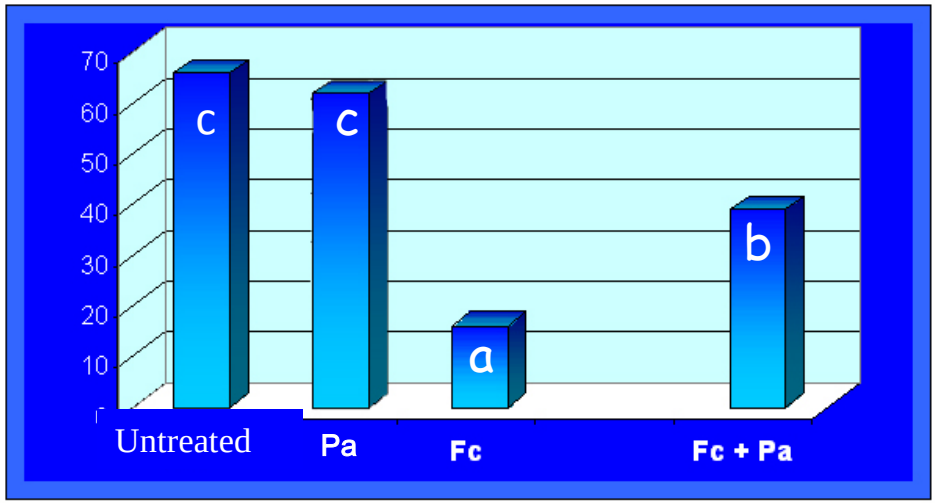
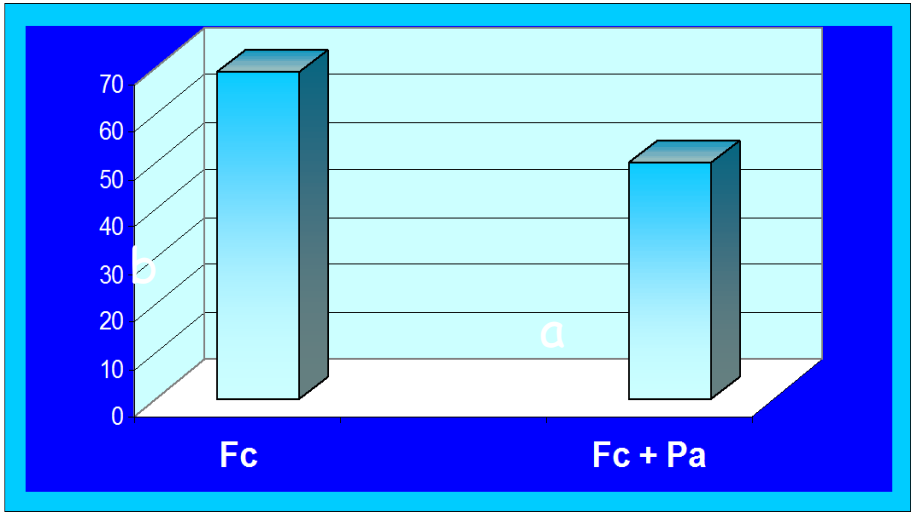


Plant total dry weight (mg)

Disease index (%)



Bars with the same letter are not statistically different (DMS, $P < 0.05$)

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Effect of *G. graminis tritici* (Ggt), *P. armata* (Pa) and their interactions (Ggt+Pa) on wheat seedlings compared with untreated plants.

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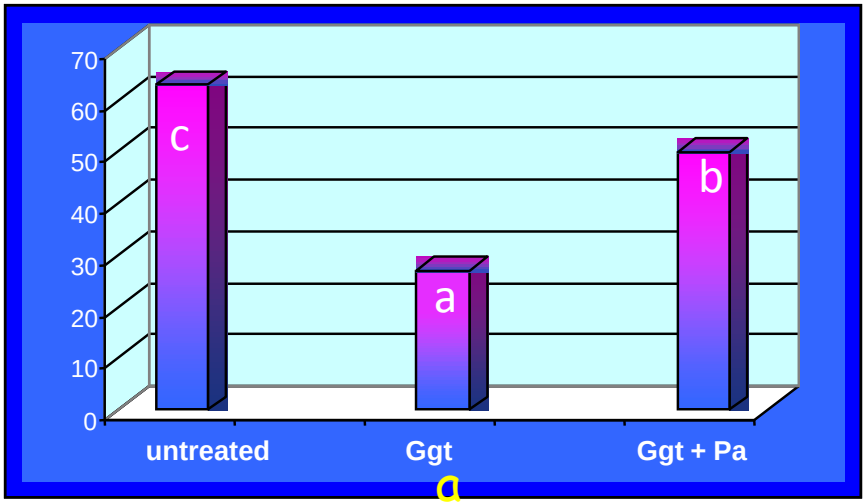
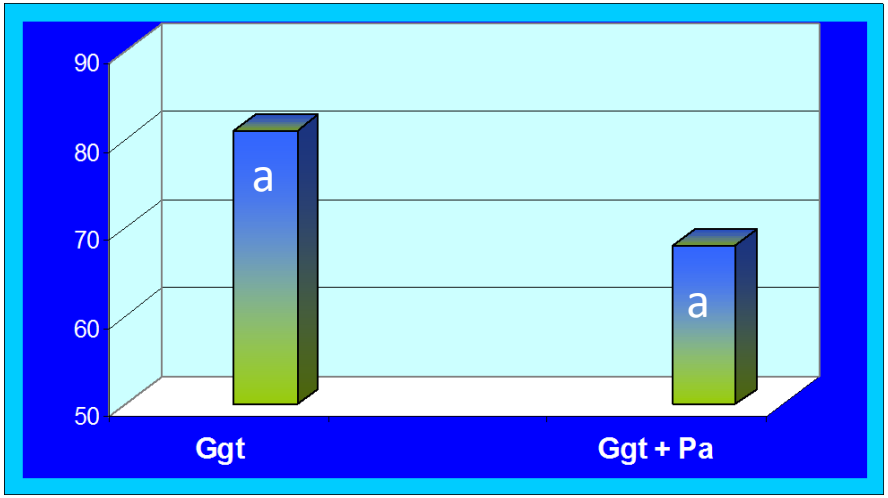


Plant total dry weight (mg)

Disease index (%)



Bars with the same letter are not statistically different (DMS, $P < 0.05$)

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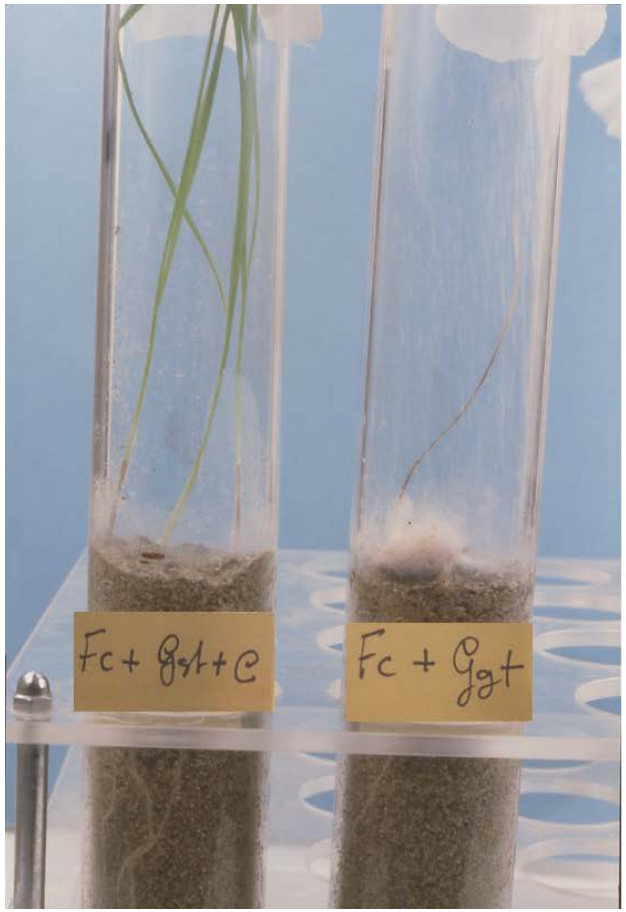
What is the effect on plant health when *F. culmorum* (Fc) , *G. graminis tritici* (Ggt), and Collembola (C) were in the same substrate?



√ Collembolans reduced disease severity.

√ Ggt was the prevalent pathogen.

√ The number of animals containing only or mainly Fc conidia in their gut was significantly higher than that of specimens containing only or mainly portions of Ggt macrohyphae.

√ The preference of collembolans for Fc showed in Petri dish was confirmed.

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Do Collembola spread the disease?

When springtails collected from microcosms containing propagules of *F.culmorum* (Fc) or *G. graminis tritici* (Ggt) were released into microcosms containing sterile sand and wheat seeds, no disease symptoms were observed on wheat seedlings.

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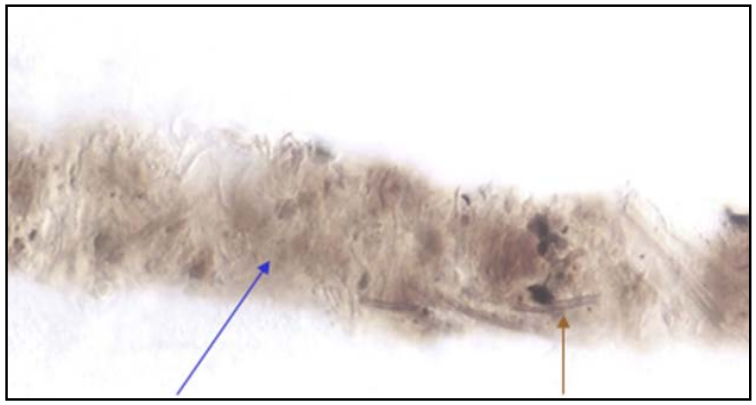
Effect of Collembola-*Trichoderma* interaction on plant health

Trichoderma harzianum (Th) inoculum, consisting of power formulation of conidia + ground rice kernels, was coated to seeds (c), or mixed with substrate (s) as Ggt inoculum just before wheat sowing and animals addition.



Gut with Th conidial masses (blu arrow) and Ggt macrohyhae portions (red arrow).

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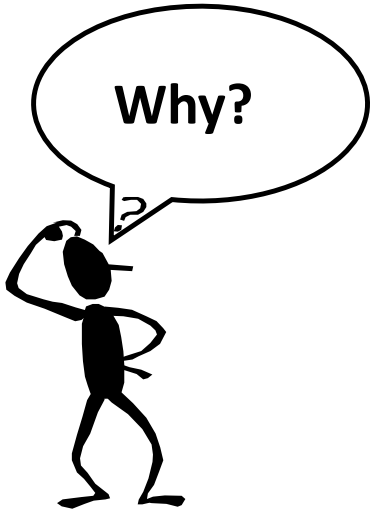
Collembola: yes biocontrol

Collembola + *Trichoderma* in soil: no biocontrol

Collembola + *Trichoderma* coated to seeds: yes biocontrol



.....probably when *Trichoderma* conidia were mixed with substrate, animals that fed on them were attracted not only by conidia, but also by ground rice kernels used to produce the inoculum. Their feeding activity on Ggt was not enough to control the disease. When *Trichoderma* was coated to seeds, it was present in a much smaller amount than Ggt and more localized, therefore collembolans turned mainly to Ggt as food source, reducing disease.

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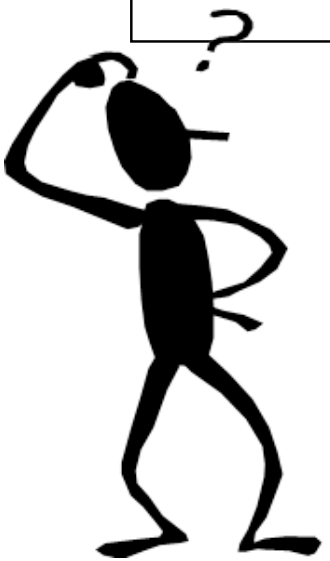
What is the effect of Collembola, arbuscular mycorrhizal and pathogenic fungus interaction on plant health?



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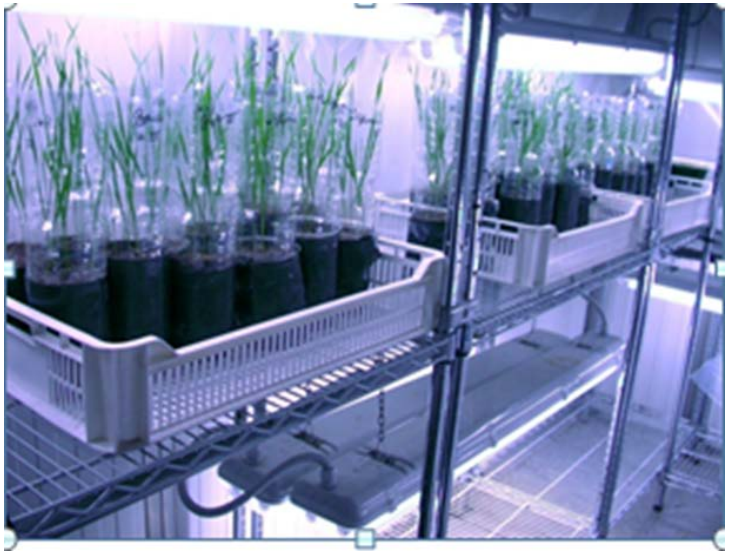
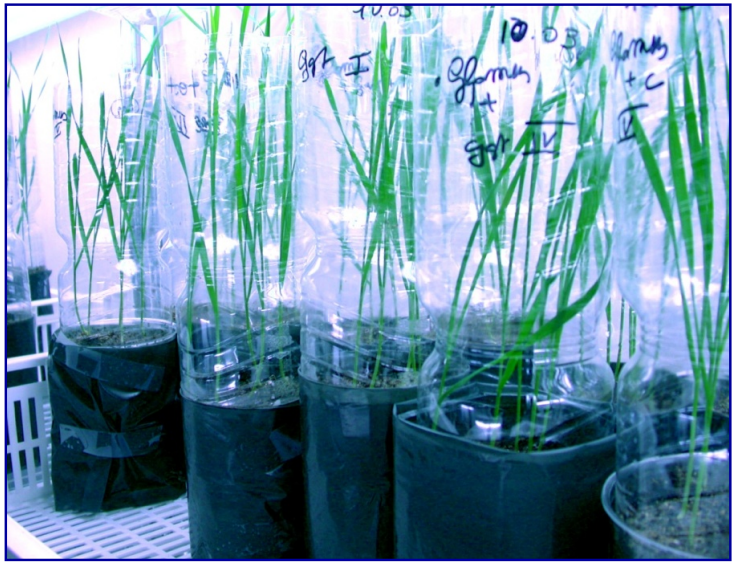
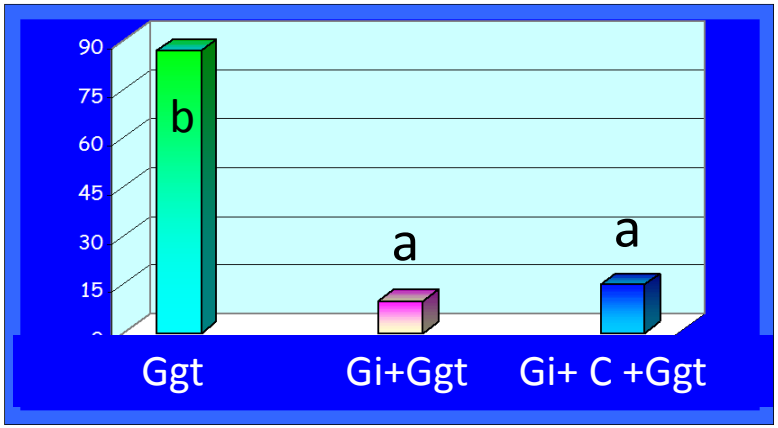
Wheat plants in plastic bottles containing sterile sand with *P. armata*, *G. graminis tritici* (Ggt), and the AM fungus *Glomus intraradices* (Gi) inocula in growth chamber.





Studies indicated compatibility between *P. armata* and *Glomus intraradices* to reduce the disease index (%).

Disease index (%)



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From microcosm to mesocosm

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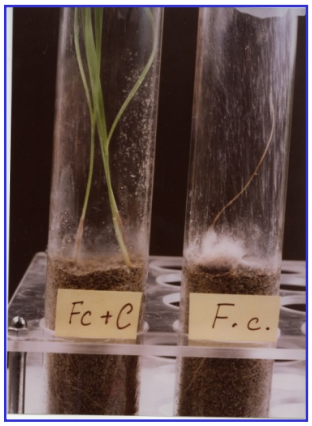

Microcosms

Mesocosms: polypropilene boxes (200 x 400 x 300 mm) with containers, each consisting in metallic frame enveloped by a wrap to allow passage of hyphae but not of animals (100 x 100 x 400 mm) in growth chamber.

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Each container was filled with 500 g of soil inoculated with *G. graminis tritici* (Ggt) propagules, then wheat seeds were sown and 350 specimens of *P. armata* were introduced to the surface. Three containers were placed in each microcosm. After three weeks plants and animals were collected, disease index and dry biomass were determined.

Also under these conditions, Collembola showed biocontrol effect *vs* the disease caused by Ggt.

Containers with wheat plants grown in the substrate inoculated with Ggt in presence (fig. 1) or in absence of Collembola (fig. 2), untreated plants (fig. 3).

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Concluding remarks

It has been found that Collembola feed preferably on pathogenic rather than on antagonistic or AM fungal propagules, and they don't spread pathogenic fungal inoculum, thus springtails may reduce the inoculum of pathogens without counteracting the activity of beneficial fungi.

Agronomists could study the effect of cultural practices to these animals, particularly for glasshouse crops.

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Could Collembola modulate the plant disease by their feeding activity on fungal propagules?

WE THINK SO!