

# Does substrate water content influence the effect of Collembola-pathogenic fungus interaction on plant health? A mesocosm study

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## Abstract

The effect of interaction between the springtail *Protaphorura armata* (Tullberg) (Collembola Onychiuridae), and the foot and root pathogenic fungus *Gaeumannomyces graminis* Von Arx et Olivier var. *tritici* Walker on number, dry biomass and health of wheat seedlings under two substrate water content levels was studied in a mesocosm experiment. Adult specimens of *P. armata* were added to each container consisting in metallic frame enveloped by a wrap to prevent the passage of animals, filled with sand previously inoculated with *G. graminis* var. *tritici* propagules, where, immediately before the springtails addition, wheat kernels were sown. Containers were placed in plastic boxes (mesocosms) provided by a system of watering regime regulation. The sand moisture content was set up at 5 or 15%, which are the lowest and the highest level respectively of available water for plants in a 100% sandy substrate. Mesocosms were maintained in a growth chamber at 12 hours light, 22 °C temperature, and 60% RH for three weeks. Then wheat seedlings were collected, counted, and disease index and dry biomass determined. At 15% water content, in presence of *P. armata* the disease severity was lower than that of plants grown in presence of the pathogenic fungus and in absence of animals. At 5% water content, no differences between plant parameters in presence or absence of Collembola were found.

**Key words:** *Protaphorura armata*, *Gaeumannomyces graminis* var. *tritici*, substrate moisture, wheat seedlings, plant health.

## Introduction

Recent studies on global climatic change scenario for the period 2030-2050 (Intergovernmental Panel on Climate Change - IPCC, 2007) have predicted for the Mediterranean area, indicated as "Climate Change Hot Spot Zone" a drastic elongation of the drought period and a shift in the precipitation pattern towards more intense and fewer moderate rains. The soil moisture and temperature are very important factors for plants and soil organisms. In particular the distribution, abundance and life cycle of soil mesofauna are affected by soil moisture and temperature directly through desiccation, and indirectly through changes in food resources and microhabitat modifications (Tsiafouli *et al.*, 2005).

Some studies showed a negative impact of drought, even in a short-term, on various taxa of the soil fauna, comprising Collembola (Bengtsson, 1994). In particular, the soil humidity is known to be critical factor in population dynamic and distribution of Collembola (Verhoef and van Selm, 1983). The response of Collembola to different soil water content conditions has been investigated in agricultural and forest ecosystems with contrasting results. Frampton *et al.* (2000a; 2000b) showed a negative effect of drought on Collembola in a field of spring peas in UK and found that irrigation had the opposite effect. Tsiafouli *et al.* (2005) in a short-term experiment carried in a Mediterranean pine forest found that collembolan community showed higher species evenness and diversity in the frequently irrigated plots than in the infrequently irrigated ones. Bardgett *et al.* (1993) however, observed that Collembola numbers decreased under high moisture conditions in upland grasslands. Dombos (2001) found after heavy sheep grazing,

a reduction in soil water, but an increase of Collembola number. However, the physiological resistance of Collembola to desiccation varies between species (Joose, 1981). Others studies carried out under controlled conditions have also shown contrasting results about the effect of moisture on Collembola. Bayley and Holmstrup (1999) showed in a Petri dish experiment, an adaptive mechanism that allowed to collembolan *Folsomia candida* (Willem) to remain active in the same range of drought that plants are capable of surviving. Choi *et al.* (2002) observed, in a jar experiment carried out with artificial soil, that the moisture significantly affected the reproduction of the collembolan *Paronychiurus kimi* (Lee). The *P. kimi* population was unable to increase at 10% soil moisture content except when provided by a large amount of yeast. Sinka *et al.* (2007) in a mesocosm study, showed that *F. candida* have a higher tolerance for dry than for wet conditions.

Many studies carried out under controlled conditions, showed the ability of Collembola to control the severity of some plant diseases caused by soil-borne pathogenic fungi (Curl, 1979; Lartey *et al.*, 1994; Nakamura *et al.*, 1992; Sabatini and Innocenti, 2001; Shiraishi *et al.*, 2003). Therefore it could be interesting to study the plant-Collembola-fungi interaction under different soil moisture conditions. There is very limited knowledge about this. Shiraishi *et al.* (2005) studying in a greenhouse experiment the ability of *Folsomia hidakana* Uchida et Tamura to decrease the damping-off of brassicaceous vegetables caused by the fungus *Rhizoctonia solani* Kuhn under different soil water content conditions, showed that changes in soil moisture from 30 to 70% on dry basis, had no effect on the biocontrol ability of *F. hidakana* against the disease.

The aim of this study was to investigate the effect of the collembolan *Protaphorura armata* - *Gaeumannomyces graminis* var. *tritici* interaction on wheat plant health under two levels of soil moisture content. *G. graminis* var. *tritici* is a soil-borne fungus responsible of take-all, one of the most important diseases of winter cereals in temperate growing areas. The fungus persists in infected host plants and soil debris, and roots became infected as they grow through soil near infected debris; root necrosis can determine the premature death or the early maturation of plants with bleached and sterile heads (Wiese, 1987). Studies carried out in Petri dishes on agarised medium (Sabatini and Innocenti, 1995; 2000), showed that *P. armata* fed on propagules of *G. graminis* var. *tritici*. A subsequent experiment carried out in microcosms under controlled conditions showed that i) no effects on root biomass were observed when wheat seedlings were grown in the presence of Collembola alone; ii) disease severity caused by this fungus decreased in presence of *P. armata* (Sabatini and Innocenti, 2001).

## Materials and methods

### Test organisms

Sexually mature specimens of *Protaphorura armata* (Tullberg) sensu Gisin (Collembola Onychiuridae) (Pa) were used in the test. The original stock was obtained from a wheat field located in the Po Valley (northern Italy). Animals were reared for several generations in glass jars containing a clay bottom saturated with distilled water and feed with brewer yeast at the Soil Biology Laboratory of the Department of Biology, University of Modena and Reggio Emilia. Springtails had been starved for 48 h before the beginning of the experiment.

The isolate LM 06 of *Gaeumannomyces graminis* Von Arx et Olivier var. *tritici* Walker (Ggt) was used. The fungus was isolated from wheat plants collected in a local field and stored in tubes on Potato Dextrose Agar (PDA; Difco) under mineral oil at 5 °C in darkness at the Mycology Laboratory, University of Bologna. From these sources the fungus was transferred onto plates of PDA and cultured at 20 °C. Sterile wheat and millet kernels contained in Erlenmeyer flasks, were inoculated with agar discs (0.5 × 0.5 cm) from the edge of actively growing colonies of Ggt and maintained at 20 °C in the dark for three weeks. Then kernels colonised by hyphae of the fungus were air-dried and used as inoculum.

Soft wheat (*Triticum aestivum* L.) cv. Serio was employed. Kernels were surface sterilised by immersion in NaClO (1%) for 10 minutes, then washed several times in sterile distilled water before sowing.

### Mesocosm assay

Twelve containers (10 × 10 × 40 cm), consisting in metallic frame enveloped by a wrap to allow the passage of water and fungal hyphae, but to prevent the passage of animals, were filled with sand (1500 g each) to a depth of 10 cm. Sand was previously inoculated with Ggt (1% w:w). Then 21 kernels of wheat were sown in each container. Immediately after sowing, 350 specimens of

*P. armata* were transferred to the surface substrate of each of six of these containers (+Ggt +Pa). The remaining six containers being left without animals constituted the fungal infected and no Collembola treatment (+Ggt -Pa). Others six containers where wheat kernels were sown in substrate without fungal propagules and animals, constituted the untreated controls (-Ggt -Pa). Containers were placed in plastic boxes (mesocosms; 20 × 40 × 30 cm each) containing the same sandy substrate as containers and provided by a system of watering regime regulation. In each mesocosm, two TDR feelers (Time Domain Reflectometry; 200 mm length and 3 mm diameter) were inserted into the sand around containers and connected with an electronic valve, to regulate the passage of the irrigation water. A system of micro-irrigation distributed the water on the substrate surface. Three containers of the same treatment were placed in each mesocosm. In an half of mesocosms the substrate moisture content was set up at 5%, in the remaining half at 15% water content on dry basis, which are the lowest and the highest level respectively of available water for plants in a 100% sandy substrate (Giardini, 2002).

Mesocosms were maintained in a growth chamber at 12/12h day/night regime, 22 °C temperature and 60% RH for three weeks. Then wheat seedlings were collected, kindly washed under running water, counted, and divided in 0-3 classes (where 0 = no symptoms; 1 = light infection of roots; 2 = infection of roots and stem base; 3 = plant dead or nearly) and disease index (DI) was calculated for each container from the following formula by Jones and Clifford (1983) (plants in class 1) + 2 (plants in class 2) + 3 (plants in class 3) / total plants × 100/3. Subsequently dry biomass of plants grown in each container were determined after drying at 80 °C for 24 h. The gut content of some animals extracted by floating from +Pa +Ggt treatments at 5 and 15% water content substrate were analysed. Springtails were fixed in Gisin's fluid, mounted on slides in Gisin's medium (Gisin, 1970), then the gut content was observed under the light microscope using differential interference contrast.

### Statistical analysis

Plant number and biomass data were analysed by one-way ANOVA and compared by LSD multiple range test calculated at  $P < 0.05$  significance level. Percentage disease index data were arc-sin transformed for analysis and compared by Student *t* test calculated at  $P \leq 0.05$  significance level. Back transformed data are presented in the table. Statistical procedures were carried out with the software Statgraphic plus version 2.1 (Statistical Graphics Corp., USA 1996).

## Results

The effects of substrate moisture on number, dry weight and disease severity of wheat seedlings grown for three weeks in a 100% sandy substrate inoculated with the pathogenic fungus *G. graminis* var. *tritici* in presence or absence of *P. armata* specimens, are reported in tables 1 and 2. At 15% moisture substrate level the number and total biomass of wheat seedlings were higher than those

**Table 1.** Effect of 5 and 15% water content on dry basis on number and dry weight of wheat seedlings grown in a 100% sandy substrate inoculated with the pathogenic fungus *G. graminis* var. *tritici* (Ggt; 1% w:w) in presence or absence of specimens of *P. armata* (Pa). Data were collected three weeks after the sowing of wheat kernels and Pa addition.

Treatments	5% Water content		15% Water content	
	Plant number	Total dry mass (mg)	Plant number	Total dry mass (mg)
-Ggt -Pa	16.0 a ( $\pm$ 2.00)	510 a ( $\pm$ 2.93)	19.3 b ( $\pm$ 2.08)	720 b ( $\pm$ 3.49)
+Ggt -Pa	14.3 a ( $\pm$ 1.15)	390 a ( $\pm$ 2.50)	16.7 a ( $\pm$ 1.52)	570 a ( $\pm$ 2.81)
+Ggt +Pa	13.7 a ( $\pm$ 3.21)	420 a ( $\pm$ 4.88)	16.0 a ( $\pm$ 1.00)	690 b ( $\pm$ 1.27)

Each value represents the mean of 3 containers. In each container 21 kernels of soft wheat cv. Serio were sown and 350 specimens of Pa were added immediately after sowing. Mean values in the same column followed by different letters are significantly different at  $P < 0.05$  significance level according to LSD test. In parentheses are reported  $\pm$  SD values.

**Table 2.** Effect of 5 and 15% water content on dry basis, on disease severity index (0-100).

Treatments	5% Water content	15% Water content
+Ggt -Pa	21.9 a ( $\pm$ 7.74)	35.1 b ( $\pm$ 4.51)
+Ggt +Pa	21.2 a ( $\pm$ 9.99)	22.5 a ( $\pm$ 9.92)

Each value represents the mean of three containers. Mean values in the same column followed by different letters are significantly different at  $P < 0.05$  significance level according to Student *t* test. In parentheses are reported  $\pm$  SD values.

of plants grown at 5% water content level. The mean number and dry weight of plants from moister substrate treatments were respectively 17.3 and 660 mg. The values of the same parameters of plants from drier substrate were 14.7 and 440 mg respectively. The pathogen was also favoured by the higher moisture of substrate; the mean disease index (DI) values were 21.5 and 28.8 % at 5 and 15% water content respectively. In drier conditions (5% substrate water content) plant parameters were similar in presence or in absence of *P. armata*. In particular the disease index values were 21.2 and 21.9% for +Ggt +Pa and +Ggt -Pa treatments respectively. At 15% soil moisture, the plant number was similar in +Ggt -Pa (16.0) and +Ggt +Pa (16.7) treatments, and both numbers were significantly lower than that of control treatment -Ggt -Pa (19.3). In wetter conditions (15% substrate water content), the disease severity was significantly reduced by 35.7% in the +Ggt +Pa treatment compared to that of +Ggt -Pa treatment, being DI = 22.5 and 35.1% in presence or absence of Collembola respectively. Mature and young specimens of Pa were observed in both 5 and 15% water content substrate, and portions of blackened macro-hyphae typical of extra-root growth of Ggt, were observed in their gut together with mineral particles, plant debris, and amorphous material.

## Discussion and conclusions

Considering the climate changes predicted for the near future reported above, the interaction plant - Collembola - pathogenic fungus was studied in conditions of abundance or scarcity of water available for plants. It is important to underline that this study was carried out with

a true soil inhabiting collembolan species of Italian agricultural soils, and with a fungal pathogen responsible of a serious disease of wheat plants in Italy. Against this disease no chemical control is, at the moment, available. Moreover being the density of springtails an important factor in these interactions, Collembola specimens were used at the same order of magnitude as that found in a cereal field in the Po Valley, Northern Italy (Sabatini *et al.*, 1997).

The results obtained in this study in wet conditions (at 15% substrate water content), confirm the finding of a previous smaller-scale experiment carried out with the same collembolan, fungal and plant species, and the same substrate under optimal water content conditions for wheat plants (Sabatini and Innocenti, 2001). In both these experiments the disease severity caused by Ggt was significantly controlled in presence of *P. armata* specimens. The reduced Ggt disease activity in wet soil is not related to water content, since it is well-known the preference of Ggt for soils where the moisture is plentiful (Wiese, 1987). The analysis of gut content confirmed the feeding of Collembola on *G. graminis tritici* hyphae. In the present study no effect on disease severity was observed when plants were grown in presence of Collembola in dry conditions (at 5% substrate water content). These results are in line with studies which showed the sensibility of Collembola to dry conditions. Bayley and Holmstrup (1999) have demonstrated that Collembola survival was reduced when the relative humidity of soil pores falls below 90%. Frampton *et al.* (2000a; 2000b) showed also a negative effect of drought on Collembola, and Tsiafouli *et al.* (2005), found that frequently irrigated plots were more favourable to collembolan community than infrequently irrigated ones. As known, the soil moisture could influence Collembola not only directly by desiccation (Tsiafouli *et al.*, 2005), but also indirectly by microhabitat modifications. The reduction of soil water content results in decreasing of plant growth and root exudation process (Cook and Papendick, 1970), and also in Ggt growth (Wiese, 1987). Root exudates and fungal propagules were the only food sources available for animals in the very poor nutritional substrate used in this experiment and they were both more scarce at lower level of water content. In wetter substrate, the habitat was more favourable for plants, pathogenic fungus, and therefore to collembolans which were able to control the disease caused by Ggt on wheat seedlings.

In conclusions, the soil moisture seems, on the basis of our data, to be a factor able to influence the biocontrol ability of *P. armata* against *G. graminis* var. *tritici* disease. Another study showed the compatibility of *P. armata* and the beneficial effect of the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck et Smith on wheat plants (Innocenti *et al.*, 2009). It could be interesting to verify also this result in relation to soil moisture. Therefore, generalisations about the effect of moisture on Collembola - fungi interactions should be made with caution since the complexity of these interactions.

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