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Response of plant growth to Collembola, arbuscular mycorrhizal and plant pathogenic fungi interactions

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Abstract

We studied the effects of interactions among the springtail *Protaphorura armata* (Tulberg 1869) sensu Gisin 1952 (Collembola Onychiuridae), the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck et Smith, and the foot and root pathogenic fungus *Fusarium culmorum* (W.G.Sm.) Sacc. on the growth and health of durum wheat plants cv. Creso in modified Leonard plastic bottle-jars containing sterile soil added with peat and sand. Five weeks old mycorrhizal plants were grown in the presence of *F. culmorum* propagules and specimens of *P. armata* for five weeks under controlled conditions. The control plants consisted in non treated plants, non mycorrhizal plants infected with *F. culmorum*, and mycorrhizal plants with and without Collembola. The mycorrhizal colonisation, the root and shoot dry weight, and the disease index were determined at the end of the experiment. Also the number of Collembola was determined, and their gut content analysed. Under the experimental conditions considered, the presence of Collembola did not decrease the positive effect of *G. intraradices* on the plant biomass and did not reduce the biocontrol capacity of the arbuscular mycorrhizal fungus.

Key words: *Fusarium culmorum*, *Glomus intraradices*, *Protaphorura armata*, wheat, compatibility.

Introduction

The soil is a reservoir of organisms ranging from beneficial to deleterious for plants. The interactions among these organisms are very important for plant growth and health, but they are complex and difficult to study, and therefore, they are for the most part not exhaustively known. The arbuscular mycorrhizal (AM) fungi are important soil borne micro-organisms, and 90% of all vegetal taxa are colonised by these fungi (Smith and Read, 1997). The external root mycelium explores the soil more efficiently than plant root hairs, up-taking and transporting water and mineral nutrients. In this context, phosphate and nitrogen nutrition, and water uptake are especially facilitated by AM fungi (Smith and Read, 1997) and AM colonization become essential for the optimal growth of plants. AM fungi can also show a biocontrol effect against diseases caused by soil-borne fungi**;** however, this effect seems related to the AM and pathogenic fungus species and to environmental conditions (Azcón-Aguilar and Barea, 1996). The external mycelium of AM fungi can be an important food source for fungivorous soil animals such as Collembola living in the same soil layers as mycorrhizal fungi. Studies on effects of interactions between springtails and AM fungi on plant growth have shown contrasting results. It has been observed that the feeding activity of springtails could reduce fungal biomass (Larsen and Jacobsen, 1996), disrupt the contact of AM fungal hyphae with root host (Tiunov and Scheu, 2005), and restrict the mycorrhizal functioning in the field (Fitter and Garbaye, 1994). However Schreiner and Bethlenfalvay (2003) showed that the grazing activity of *Isotoma* sp. specimens on the mycelium of AM fungi was detrimental to plant growth only when other fungal food sources were limited, but grazing on mycorrhizal fungal hyphae did not occur when saprotrophic fungi and organic residues were present. Gormsen *et al.* (2004) confirmed the preference of *Folsomia candida* Willem 1902 for saprotrophic fungi over AM fungi. Tiunov and Scheu (2005) also observed that in spite of the large amount of mycorrhizal mycelium in soil, it contributed little to Collembola nutrition. They suggest that Collembola might help AM fungal activities through their feeding on saprotrophic fungi which compete for nutrients with AM fungi. Endlweber and Scheu (2007) observed that in the presence of Collembola, changes in plant biomass and root structure were not associated with a reduction in mycorrhizal formation. Larsen and Jakobsen (1996), in an experiment carried out without the root interference, observed that the interaction between *F. candida* and the external mycelium of *Glomus caledonium* (Nicolson et Gerdemann) Trappe et Gerdemann was really scanty. Kaiser and Lussenhop (1991) and Lussenhop (1996) showed that *F. candida* had positive, neutral or negative effects on AM colonisation in soybean plants depending on Collembola density and mycorrhizal level at the moment of springtails addition. When Collembola were added to substrate containing the AM inoculum immediately after the transplanting of soybean seedlings, the number of infection sites of mycorrhizal fungus was significantly lower than that observed when springtails were added two weeks after transplanting. In addition, Bakonyi *et al.* (2002) found evidence that Collembola depending on their density, could differently affect mycorrhizal colonization and growth of *Zea mays* L. and *Festuca rubra* L.; clearly when more specimens were present, more hyphae were damaged. In a study carried out by Harris and Boerner (1990), low density of *F. candida* enhanced the growth of *Geranium robertianum* L., whereas high density decreased plant growth because of Collembola grazing on AM hyphae.

Different Collembola species were found to graze also

on AM fungal spores (Bakonyi *et al*., 2002), and different Collembola species showed a preference for different AM fungal species (Moore *et al*., 1985). In a soil experiment, Hishi and Takeda (2008) observed that Collembola decreased the density of small-sized spores of saprotrophic fungi, and did not decrease that of AM fungi generally much larger than those of saprotrophic fungal species. It has been also shown that spores of AM fungi can adhere to the body of Collembola surface, thus they are potential carriers for the dispersal of spores in the soil (Visser *et al*., 1987).

Up to now, the effects of springtails and AM fungus interactions on plant were studied using a plant, AM fungus, and collembolan combination without any plant pathogenic fungus. In this study, we included *Fusarium culmorum* (W.G.Sm.) Sacc., one of the most important soil borne fungal pathogen of winter cereals worldwide, which is a high-quality food source for Collembola (Sabatini and Innocenti, 2000a; 2000b; 2001; Larsen *et al*., 2008). Preliminary observations seem to indicate compatibility between springtails and an AM fungus in presence of a plant pathogenic fungus (Innocenti *et al*., 2006).

Materials and methods

Test organisms

We used specimens of *Protaphorura armata* (Tulberg 1869) sensu Gisin 1952 (Pa). The original stock of Pa was obtained from a cultivated wheat field located in the Po Valley (Northern Italy). Collembolans were reared for several generations in a laboratory of the "Dipartimento di Biologia Animale" (University of Modena and Reggio Emilia). They were maintained in glass jars containing a clay bottom saturated with distilled water and feed with brewer's yeast (*Saccharomyces cerevisiae*). Jars were kept in a thermostatic chamber at 20 °C. Under these conditions the first oviposition occurs on average 20–21d after hatching, and eggs hatch on average 12 d after oviposition. Test was performed with sexually mature springtails of the same age which had been starved for 48 hours before the beginning of the experiment.

The AM fungus was obtained from the commercial inoculum of *Glomus intraradices* Schenck et Smith (Gi) (ENDORIZE Sol; Biorize, Dijon, France) consisting in spores and hyphae.

We used the isolate *F. culmorum* LM Fc02 (Fc) from the collection of the "Dipartimento di Protezione Valorizzazione Agroalimentare" (University of Bologna). The fungus was isolated from wheat plants in a local field and stored in tubes on Potato Dextrose Agar (PDA; Difco) under mineral oil at 5 °C in darkness. From these sources, the fungus was transferred onto plates of PDA and cultured at 23 °C for preparation of inoculum. Before the experiment, the isolate was tested for pathogenicity. It showed a high pathogenicity against wheat seedlings.

Creso durum wheat (*Triticum durum* Desf.) cultivar susceptible to Fc was employed.

Bioassay

Modified Leonard bottle-jars (Vincent, 1970) made by polyethylene terephthalate mineral water bottles (90-mm diameter by 450-mm height), were used as experimental containers. Each bottle was cut in the basal part and used as support and reservoir of water for the upper inverted part. This upper part contained the plant growing substrate: 500 g of sterile sieved field soil, sand and peat (1:1:0.5, v:v:v) mixed to AM fungus inoculum (1% w:w). A pressed filter paper plug was located in the place of the bottle plastic cup. The soil was collected from the upper 30 cm of a set-aside agricultural field (table 1). The basal part of each container was covered by a black plastic wrap to maintain roots in darkness. Surface-sterilised seeds of wheat were germinated on wet filter paper for 7 days, and seven healthy seedlings were transplanted into each bottle. The bottles were placed in a growth chamber (12:12 L:D, 20 ± 3 °C, 60-70% RH and a photosynthetic photon flux density of 300 μmol $m^{-2}s^{-1}$) and watered weekly by adding deionised water to the reservoir without any fertilisation treatment. Control treatment consisted in plants grown in absence of AM fungus. After 5 weeks, one plant was randomly collected from each bottle. Fine roots from mycorrhizal plants were used to estimate the AM infection level (MI%) by the method of Trouvelot *et al*. (1986) after Trypan blue coloration (Phillips and Hayman, 1970). Therefore the Fc inoculum consisting of sterile wheat and millet kernels colonised by the fungus, was mixed (1% w:w) to the plant growing substrate Thereafter, 110 specimens of Pa twenty days old (corresponding to about 40,000 individuals m^2 considering a depth of 13 cm) were released by a funnel onto the substrate surface. The top of each bottle was covered with a transparent cloth to prevent the escape of springtails. The six established treatments and their symbols are reported in table 2. Eight replicates for each treatment were made; bottles were maintained in a growth chamber following a complete randomised design at the same climatic conditions indicated above. Five weeks after Fc and Pa addition, substrate, plants and Collembola were carefully extracted from each container. Springtails were sorted by floating and only adults were counted, fixed in Gisin's fluid, then mounted on slides in Gisin's medium (Gisin, 1970) to analyse their gut content under the light microscope using differential interference contrast (DIC). The severity of wheat disease was rated on a 0-3 visual scale (where 0 = no symptoms; 1 = light infection; 2 = severe infection; 3 = plant dead or nearly so),

Table 1. Characteristics of soil utilised in the experiment.

Texture (USDA)	Sandy-loam
pH (H_2O)	7.8
Organic matter (Lotti) (%)	2.24
N (Kjeldahl) (%)	1.6
C/N	8.1
Total P (ppm)	1097.6
P ass (Olsen) (ppm)	30.6
P_2O_5 (ppm)	70.1

Table 2. Description and symbols of treatments.

Treatments	*G. intraradices* (Gi)	*F. culmorum* (Fc)	*P. armata* (Pa)	Symbols
1 Untreated control	−	−	−	−Gi−Fc−Pa
2 Mycorrhizal control	+	−	−	+Gi−Fc−Pa
3 Infected control	−	+	−	−Gi+Fc−Pa
4 Mycorrhizal fungus + Collembola	+	−	+	+Gi−Fc+Pa
5 Mycorrhizal fungus + Pathogenic fungus	+	+	−	+Gi+Fc−Pa
6 Mycorrhizal fungus + Pathogenic fungus + Collembola	+	+	+	+Gi+Fc+Pa

and the disease severity index (DI) was then calculated for each bottle from the following formula (Jones and Clifford, 1978): (plants in class 1) + 2 (plants in class 2) + 3 (plants in class 3) / total plants in sample x 100 / 3. The same aliquot of roots was cut from each container, and roots from +Gi−Fc−Pa and +Gi−Fc+Pa treatments used to calculate MI%. Thereafter, plants were dried at 80 °C for 24 h before being weighted. Plant mass was evaluated for each bottle.

Statistical analysis

After checking the normality of data, one-way ANOVA was used for analysis of variance, and comparisons among weight and disease index data were performed by LSD multiple range test calculated at $P \leq 0.05$. Percentage values were arcsine transformed for analysis; back transformed means are presented in the table. Effect of treatments on the number of Collembola was analysed by Student's *t* test at $P \leq 0.05$. Statistical procedures were carried out with the software package Statgraphic plus 2.1 (1996).

Results

Five weeks after wheat seedlings transplanting into a substrate containing AM inoculum, the mean MI% value was 37.0. Five weeks later, the level of mycorrhizal colonisation was not statistically different for plants with (+Gi−Fc+Pa; MI% = 37.4) and without (+Gi−Fc−Pa; MI% = 38.2) Collembola.

The effects of Collembola-fungi interactions on wheat plant growth are reported in table 3. The wheat plant biomass was increased in the presence of AM fungus. The dry root, shoot and total weights of mycorrhizal plants (treatment: +Gi−Fc−Pa) were significantly higher (133 mg, 587 mg, and 720 mg respectively) than those of control plants (treatment: −Gi−Fc−Pa; 90 mg, 533 mg, 623 mg respectively). The presence of Collembola in the growing substrate (treatment: +Gi−Fc+Pa) did not decrease the biomass stimulation effect of AM fungus (120 mg, 670 mg, and 790 mg for root, shoot and total weights respectively).

The pathogenic fungus significantly reduced the wheat plant growth (treatment: −Gi+Fc−Pa; 73 mg, 423 mg, and 496 mg for root, shoot and total dry weights respectively) and determined the highest disease index (table 4; DI% = 86.7). Mycorrhizal plants grown in presence of pathogenic fungus (treatment: +Gi+Fc−Pa) showed a disease index significantly lower (DI% = 10.0) than that of infected control plants, and dry weights (110 mg, 570 mg, and 680 mg) were similar to those of mycorrhizal control plants. Mycorrhizal plants grown in presence of Collembola and Fc (treatment: +Gi+Fc+Pa), showed dry weights and disease index (116 mg, 526 mg, and 643 mg; DI% = 15.2) significantly lower than those of infected control plants with and without springtails.

Live adult and juvenile specimens of Pa were extracted from all containers. The mean number of adult

Table 3. Effects of interactions among *P. armata*, *G. intraradices* and *F. culmorum* on dry weights of durum wheat plants cv. Creso ten weeks after AM fungus inoculation, and five weeks after pathogenic fungus inoculation and Collembola addition.

Treatments	Root dry mass bottle^{-1} (mg)	Shoot dry mass bottle^{-1} (mg)	Total dry mass bottle^{-1} (mg)
−Gi−Fc−Pa	90 b	533 b	623 b
+Gi−Fc−Pa	133 c	587 bc	720 c
+Gi−Fc+Pa	120 bc	670 c	790 c
−Gi+Fc−Pa	73 a	423 a	496 a
+Gi+Fc−Pa	110 b	570 b	680 b
+Gi+Fc+Pa	116 b	526 b	643 b

Each value represents the mean number of 8 replicates, in each bottle six wheat seedlings 7 days old were transplanted. Mean values in the same column followed by different letters are significantly different at $P \leq 0.05$ significance level according LSD test.

Table 4. Effects of interactions among *P. armata*, *G. intraradices* and *F. culmorum* on disease index of durum wheat plants cv Creso ten weeks after AM fungus inoculation, and five weeks after pathogenic fungus inoculation and Collembola addition.

Treatments	Disease index bottle^{-1} (0-100)
−Gi+Fc−Pa	86.7 b
+Gi+Fc−Pa	10.0 a
+Gi+Fc+Pa	15.2 a

Each value represents the mean number of 8 replicates, in each bottle six wheat seedlings 7 days old were transplanted. Percent values were arcsine transformed for analysis, with the presented data being back transformed means. Mean values followed by different letters are significantly different at $P \leq 0.05$ significance level according LSD test.

individuals counted in the treatment +Gi−Fc+Pa (n = 108) was significantly higher than that counted in the treatment +Gi+Fc+Pa (n = 95) as reported in table 5. Analysis of the gut content of all *P. armata* showed that 47.6% of springtails from +Gi−Fc+Pa, and 49.5% from +Gi+Fc+Pa had an empty gut. In the gut of remaining collembolans fungal materials were well represented. These materials were for the most part constituted by propagules not attributable to the two fungi artificially inoculated to plant growing substrate; in contrast spores of Fc and/or Gi were very scarce. Plant debris, exuviae, and other organic and mineral particles also were well represented in collembolan gut.

Table 5. Effects of interactions among *P. armata*, *G. intraradices* and *F. culmorum* on springtail number, ten weeks after AM fungus inoculation, and five weeks after pathogenic fungus inoculation and Collembola addition.

Treatments	Adult springtail number/110 bottle^{-1}
+Gi−Fc+Pa	108 b
+Gi+Fc+Pa	95 a

Each value represents the mean number of 8 replicates, in each bottle six wheat seedlings 7 days old were transplanted. Mean values followed by different letters are significantly different at $P \leq 0.05$ significance level according Student's *t*-test.

Discussion and conclusions

Gange (2000) examines results of studies carried out to investigate the effect of AM fungi and Collembola interactions on plants and concludes that Collembola might be beneficial, rather than detrimental to mycorrhizal functioning. Our results support that finding, suggesting that Collembola are compatible with the positive effect of AM fungus on plant growth. Under the experimental conditions considered, the presence of Collembola did not reduce the beneficial effect of AM fungus on plant biomass and health. It is important to underline that the study was carried out with a true soil inhabiting collembolan species present in Italian agricultural soils, and that Collembola were used at the same order of magnitude as that of a wheat field soil in the Po Valley (Sabatini *et al.*, 1997). The springtails density is a very important factor in Collembola-AM fungus interaction (Klironomos and Ursic, 1998; Bakonyi *et al.*, 2002) and to set up a more realistic system, we used a density similar to that of the Po Valley field. As observed by numerous authors, Collembola are capable of grazing on AM hyphae and spores, but these are not probably their preferred food when other food sources are available (Schreiner and Bethlenfalvay, 2003; Gormsen *et al.*, 2004; Tiunov and Scheu, 2005; Larsen *et al*., 2008). This is confirmed in the present study, where a substrate containing organic matter and/or plant pathogenic fungal propagules was used. At the present, it is not possible to know why AM fungi appear to be less palatable to collembolans compared with other fungi. The mechanism which determines the palatability of a fungus is still unclear. It may be due to hyphal architecture (Friese and Allen, 1991), reproductive and nutritive value (Sabatini and Innocenti, 2000a; Larsen *et al*., 2008), or biochemical or metabolic features (Hiol Hiol *et al*., 1994), hyphal dark pigmentation (Maraun *et al*., 2003). Furthermore, our data showed that the addition of Collembola to the plant growing substrate some weeks after the AM fungus inoculation and plant transplanting, did not negatively affect the colonisation of root sites by mycorrhizal fungus compared to that of mycorrhizal control plants. This, confirm the finding of Kaiser and Lussenhop (1991) and Lussenhop (1996).

To have more realistic information, the gut content analysis might be repeated at different times during the bioassay. It is difficult to be sure that an analysis made only five weeks after springtails addition is really representative of their feeding preferences. In this study we observed that collembolans feed scarcely on propagules of Fc, which is a high-quality food source for Collembola (Sabatini and Innocenti, 2000a; 2000b; 2001; Larsen *et al*., 2008). Important effects of grazing would have occurred previously, and it is also possible that some fungal propagules, which are not easy to be observed by microscopic examinations, are also grazed.

In conclusion, the present study indicates a condition of compatibility between Collembola and the beneficial effect of an AM fungus on plants. However, more experiments should be carried out in more complex systems with different species of Collembola, fungi and plant growing substrates.

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