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Effects of Collembola on plant-pathogenic fungus interactions in simple experimental systems

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Abstract Take-all and brown foot rot, caused respectively by *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum,* are two important components of the foot and root fungal disease complex of winter cereals world-wide. These fungi persist in soil and in crop debris in the same layer of agricultural soil as Collembola, a well represented taxon of soil animals. Previous *in vitro* tests showed that these fungi grown on agarised medium were readily consumed by springtails. In a simplified experimental system with wheat plants and the pathogenic fungi grown on millet and wheat kernels, the severity of disease was significantly reduced by collembolan feeding activity.

Keywords *Onychiurus armatus* · Biocontrol · Foot and root disease complex · *Gaeumannomyces graminis* var. *tritici* · *Fusarium culmorum*

Introduction

The foot and root disease complex of cereals represents a serious problem in cereal growing areas world-wide. The disease can affect cereal plants at virtually any stage during plant growth, and its major impact is the limitation of the number of heads and kernel weight (Cook and Veseth 1991). Several pathogenic fungi, all living in the top 10–15 cm of soil, are responsible for this disease complex. These fungi have different morphological and physiological features and are thus able to adapt to different edaphic and climatic conditions. The fungal propagules (spores, sclerotia and mycelium) located on plant residues in the soil constitute the primary inoculum. Fungi use the plant remains as a food base and energy source to attack the crop. The relationship between the primary inoculum and disease is very strong: the more abundant the primary pathogen population, the greater the severity of the disease. Many biotic and abiotic factors influence the switch of fungi from the saprotrophic to parasitic stage. These factors and their interactions are very complex and still, for the most part, not exhaustively known.

Previous works carried out *in vitro* (Sabatini and Innocenti 1995, 2000a, 2000b; Innocenti et al. 1997) indicate that *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker and *Fusarium culmorum* (W.G. Smith) Sacc., two of the most important components of the foot and root disease complex, are fed on by Collembola that live in the same layer of agricultural soil as the propagules of these fungi. The feeding pattern and consumption rates varied with each fungal species. In tests where the collembolan *Onychiurus armatus* was given a choice, the preferred fungus was *F. culmorum*, but *G. graminis* var. *tritici* was also grazed heavily (Sabatini and Innocenti 2000b). However, no data directly confirm that collembolan feeding activity can exert a beneficial effect with respect to the control of disease caused by these fungi.

Some promising effects of collembolan grazing have been shown for other pathogenic fungi. In experiments carried out in growth chambers a reduction in the severity of disease caused by *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum* f. sp. *cucumerinum* associated with the feeding activity of Collembola was reported by Curl et al. (1985); Bollen et al. (1991); El Titi and Ulber (1991); Nakamura et al. (1992); Lartey et al. (1994); Lootsma and Scholte (1997a, 1997b; 1998); Scholte and Lootsma (1998).

The aim of this study was to investigate the interactions between Collembola and soil-borne cereal pathogenic fungi with the goal of identifying and optimis-

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ing the natural biological control agents already resident in soil.

Materials and methods

Test organisms

Fungi

The fungal isolates considered were *G. graminis* var. *tritici* (LM 14.95) and *F. culmorum* (LM 20.91). Code numbers of isolates (LM) are accession numbers of cultures from the collection of the Department of Protezione Valorizzazione Agroalimentare, University of Bologna. They were isolated from wheat plants in local fields and stored in 10 × 150-mm tubes on Difco potato dextrose agar (PDA) under mineral oil at 5 °C in the dark. From these sources of prepared inoculum, each fungus was transferred onto plates of PDA and cultured at 23 °C; mycelial plugs (6-mm diameter) were cut from the edge of actively growing cultures and used for the preparation of inocula**.** Before the experiments isolates were tested for pathogenicity.

Collembola

The Collembola utilised belonged to the species *Onychiurus armatus* (Tullberg). The specimens were reared in the laboratory for several generations. They were maintained in glass jars containing a clay bottom saturated with distilled water and kept in a thermostatic chamber at 20 °C. Animals were fed on brewer's yeast. Under these conditions the first oviposition occurs on average 20–21 days after hatching, and the eggs hatch on average 12 days after oviposition. The original stock of *O. armatus* was obtained from a cultivated cereal field of the University of Bologna located in the Po Valley near Carpi (Modena, Italy). Tests were performed utilising sexually mature animals of the same age which had been starved for 48 h.

Plants

Creso durum wheat (*Triticum durum* Desf.) cultivar susceptible to *F. culmorum* and *G. graminis* var. *tritici* was used in the experiments. Seeds were inspected under a stereomicroscope, and those showing damage were discarded.

Fungal inoculum preparation

The pathogen inoculum was prepared using a modified method of Simon and Sivasithamparam (1988). Twenty grams of millet and wheat kernels (1:1, v:v) steeped in tap water for 12 h, and then drained by pressing with gauze, were dispensed in a 250-ml Erlenmeyer flask and autoclaved for 1 h at 120 °C on each of 2 consecutive days. Each flask was then inoculated with five discs of agar (6-mm diameter) cut from the edge of an actively growing colony of *G. graminis* var. *tritici.* The inoculated kernels were maintained at 20 °C (± 2 °C) in the dark for 3 weeks. Seeds colonised by fungi were then air dried and stored at 5 °C until the beginning of the experiment. The same procedure was followed for the *F. culmorum* inoculum.

Tube assay preparation

Glass tubes (35 mm diameter × 300 mm height) were used as experimental containers. Three untreated kernels were sown in each tube containing 1.5 g of the pathogen inoculum mixed with 150 g sand that had been sterilised twice at 121 °C for 1 h on each of 2 consecutive days. The kernels were previously surface sterilised with 1% sodium hypochlorite for 10 min, rinsed several times in sterile demineralised water and dried at room temperature on sterile filter paper. After planting, 50 springtails were released onto the substratum surface of each tube. The final animal density was about 40,000 individuals m^{-2}, considering a depth of 13 cm, corresponding to the height of the substrate in the tube assay.

Control tubes were prepared by adding animals in non-inoculated substratum or planting kernels on soil with or without fungal inoculum. Eight replicates for each combination were prepared for a total of 32 tubes for each pathogenic fungus. The lower part of each tube was covered with a black plastic wrap to keep roots in darkness; the top of the tube was covered with a cloth to block the movement of animals between tubes.

Tube assays were placed upright in racks in a randomised complete block design and incubated in a growth chamber at 20–25 °C under a 12-h day/night photoperiod and a relative humidity of 70–80%. Each tube was watered with 5 ml tap water at the start of the trial and weekly thereafter.

Evaluation of the treatments

Three weeks after planting the substratum, plants and springtails were carefully extracted from each tube.

Plants

Roots of wheat seedlings were washed clean of adhering substrate. The severity of take-all caused by *G. graminis* var. *tritici* was rated on a scale of 0–8, as described by Ownley et al. (1992), where 0 = no visible symptoms; 1 < 10% root black; 2 = 10–25% root black; 3 = 25–50% root black, 4 = 50–100% root black; 5 = all roots with lesions and lesions at the stem base; 6 = lesions to the stem; 7 = plant severely stunted and 8 = plant dead or nearly so. The severity of brown foot rot caused by *F. culmorum* was rated on a scale of 0–4 as described by Ledingham (1981) with modifications, where 0 = no lesions; 1 < 25% of basal stem with brown stripes or browning; 2 = 25–50% basal stem browning; 3 = basal stem diffusely browning; 4 = plant dead or nearly so.

The disease severity index was then calculated by the following formula (Jones and Clifford 1978):

$$DI\% = [(\Sigma n\, i)/N]100/imax,$$

where n = number of tillers in each class, i = numerical value (code) of the class, N = total tillers in the sample, $imax$ = value of the highest class.

After disease assessment, the roots and shoots were separated and dried at 80 °C for 24 h before being weighed. The weight of the roots and shoots was evaluated for the plants of each tube.

Collembola

Springtails were hand sorted from the substratum extracted from each tube. The animals were grouped into adults and juveniles and counted. A few animals were fixed in Gisin's fluid (Gisin 1970) to determine their gut contents. The remaining sample of springtails from the fungal treatments were utilised to verify whether Collembola could transport fungal propagules capable of inducing plant disease. These Collembola were released on the surface of a set of tubes containing sterile sand previously seeded with untreated wheat kernels. The plants were analysed for disease symptoms 5 weeks after planting.

Statistical analysis

Percentage values of the disease index were arc-sin transformed before statistical analysis (Snedecor and Cochran 1980). The data were then subjected to ANOVA. Effects of the different treatments on disease index and on the number of springtails were

analysed by Student's *t*-test. Effects of the tested combinations on dry weights were analysed using the Student-Newman-Keuls' test.

Results

The values of the disease index (Fig. 1) show that wheat seedlings grown in the substratum containing fungal inoculum and springtails had significantly lower disease severity than plants grown in infected substratum without animals. Disease indexes were significantly different in trials performed with *G. graminis* var. *tritici* ($P \leq 0.01$) and in those with *F. culmorum* ($P \leq 0.05$). Accordingly, shoot and root dry weight was significantly higher where springtails and fungal pathogen were both present as compared to where the pathogen was present alone (Figs. 2, 3).

Wheat plants grown in tubes containing Collembola in the absence of the fungal inoculum did not show any visible symptoms of distress. However, the dry root and shoot weights of plants grown in the presence of springtails were slightly lower than those of control plants: the difference was significant only for shoot weight in the experiment carried out with *G. graminis* var. *tritici* (Figs. 2, 3).

In both trials, all springtails collected at the end of the experiments from the tube-assay substratum were alive and similar in number to the animals initially introduced. In addition to adults, live juveniles were found in all tubes. The number of animals in tubes containing only plants was not significantly different from that in tubes inoculated with fungal pathogens (Fig. 4). Wheat plants grown in tubes where springtails had

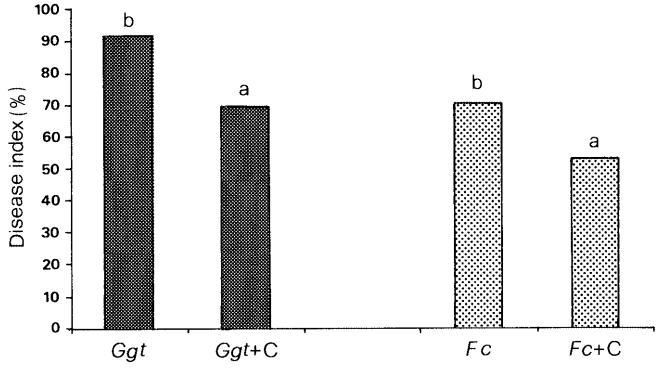

Fig. 1 Disease suppression of wheat seedlings using *Onychiurus armatus* in tube assays: effect on take-all caused by *Gaeumannomyces graminis* var. *tritici* (*left*); effect on brown foot rot caused by *Fusarium culmorum* (*right*). Bars indicate the disease index when only pathogen was present (*Ggt*) or (*Fc*) and when the pathogen and springtail were present together (*Ggt*+C) or (*Fc*+C). Values of disease index (%) represent the mean number of eight replicates. Data were arc-sin transformed as given by Bliss before statistical analysis (Snedecor and Cochran 1980). Different letters indicate significantly different means at $P \leq 0.01$ in trials performed with *G. graminis* var. *tritici* and significantly different ($P \leq 0.05$) in those with *F. culmorum* according to the Student's *t*-test

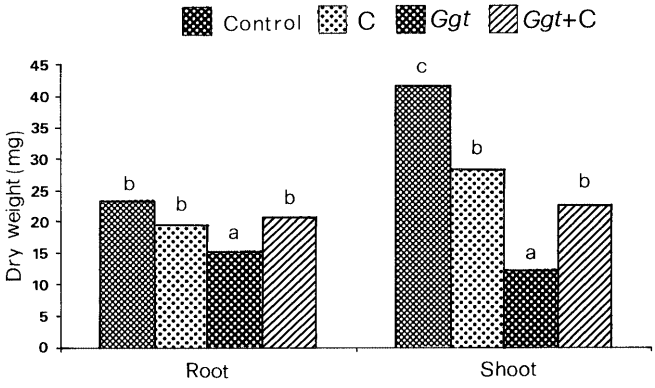

Fig. 2 Dry weight of wheat seedlings grown in uninoculated substratum (*Control*), in substratum inoculated with *G. graminis* var. *tritici* (*Ggt*), only Collembola (C), fungal inoculum and Collembola (*Ggt*+C). Values represent the mean number of eight replicates. Data were arc-sin transformed before statistical analysis. Different letters indicate significantly different means at $P \leq 0.05$ according to the Student-Newman-Keuls' test

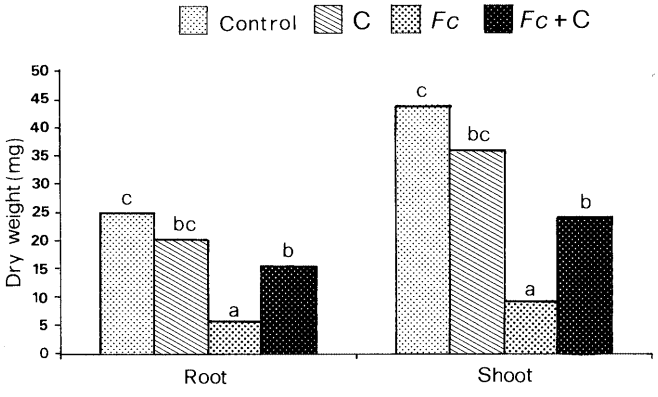

Fig. 3 Dry weight of wheat plants seedlings grown in uninoculated substratum (*Control*), in substratum inoculated with *F. culmorum* (*Fc*), only Collembola (C), fungal inoculum and Collembola (*Fc*+C). Values represent the mean number of eight replicates. Data were arc-sin transformed before statistical analysis. Different letters indicate significantly different means at $P \leq 0.05$ according to the Student-Newman-Keuls' test

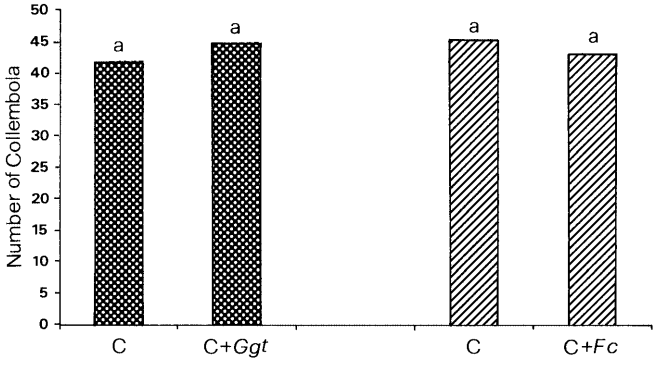

Fig. 4 Abundance of *O. armatus* adults collected from tube assays containing: wheat seedlings grown in uninoculated substratum (C); substratum inoculated with *G. graminis* var. *tritici* (C+*Ggt*) (*left*); wheat seedlings grown in uninoculated substratum (C); substratum inoculated with *F. culmorum* (C+*Fc*) (*right*) same letters indicate no significant difference according to the student's *t*-test

been transferred from substratum containing fungal pathogen had no visible symptoms of disease.

Analysis of animals collected from substratum containing fungi demonstrated the presence of mycelial fragments in the gut, whereas only amorphous material was found in the gut of springtails extracted from tubes with plants alone.

Discussion

Disease control in soils naturally suppressive to pathogens is not based on the effect of a single antagonist but on optimisation and integration of different control organisms that are already resident in these soils. The results from this study indicate that Collembola living in the same portion of agricultural soils as plant pathogenic fungi are part of a complex of antagonistic and competitor organisms that can influence plant-pathogenic fungus interactions in order to obtain healthier plants. Indeed, this study revealed that the presence of Collembola can be effective in limiting the severity of take-all and brown foot rot.

Although the study was carried out under laboratory conditions the results were remarkable since the pathogens were very aggressive and the number of springtails used in the experiments was of the same order of magnitude as that in the upper 12.5 cm of soil in a cultivated wheat field in the Po Valley (Italy) (Sabatini et al. 1997).

These results must be verified under field conditions where the interactions of physical, chemical and biological factors are much more complex than in laboratory experiments.

In the simplified system utilised, the reduced severity of take-all and brown foot rot may be attributed to collembolan activity that lowered the fungal inoculum density or decreased the spread of mycelia due to their consumption of fungal propagules. This has been previously demonstrated in numerous *in vitro* tests performed with these pathogens and springtails (Sabatini and Innocenti 1995, 2000b; Innocenti et al. 1997), and was also confirmed in this study by a gut content analysis. An additional hypothesis that does not exclude the former one is that in the rhizosphere Collembola might control disease by interfering with interactions between root exudates and pathogens.

Leonard (1984) points out that the culture substratum may influence the palatability of a fungus as food source for Collembola. In our experiments the palatability to the collembolan *O. armatus* of *G. graminis* var. *tritici* and *F. culmorum,* demonstrated *in vitro* on artificial agarised medium (PDA) (Sabatini and Innocenti 1995, 2000b; Innocenti et al. 1997), was confirmed when the same fungi were grown on millet and wheat kernels; this substratum resembles the plant remains used by pathogens as a food base to survive in the absence of the host crop more closely than agarised medium.

It is known that Collembola can carry mycelial fragments and spores on their bodies (Anderson and Healey 1972; Visser et al. 1987) or in their gut (Vannier 1979). Nonetheless, our data revealed no evidence that springtails spread fungal pathogens, in line with the results for other pathogenic fungi and different species of Collembola (Wiggins and Curl 1979; Nakamura et al. 1992). Thus, we hypothesise that the amount of pathogen that springtails can transport is not sufficient to induce disease or that their feeding activity can limit the growth and spread of fungal mycelia.

In this study wheat plants grown in a substratum containing springtails in the absence of fungal inoculum showed a reduction in biomass as compared to control plants. Since visible damage to the root system was not detected, it is not at present possible to identify the cause of the reduced biomass.

As mentioned above, for an optimal control of disease it is desirable that more than one biocontrol agent is present in the same soil portion, and that the activity of each agent is effective against the pathogen and does not contrast with that of other antagonists. Preliminary results of glasshouse trials (work in progress) showed good disease control when wheat seeds dressed with *Trichoderma harzianum,* a well-known biocontrol fungus, were sown in a substratum containing specimens of *O. armatus* and propagules of *G. graminis* var. *tritici*. These results are in line with those of previous works (Curl 1979; Lartey et al. 1994; Innocenti et al. 1997) and support a condition of coexistence or minimal interference between Collembola and *T. harzianum* activity.

References

Anderson JM, Healey IN (1972) Seasonal and interspecific variation in major components of the gut contents of some woodland Collembola. J Anim Ecol 41:359–368

Bollen GJ, Middelkoop J, Hofman TW (1991) Effects of soil fauna on infection of potato sprouts by *Rhizoctonia solani.* In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schippers B, Tempel A (eds) Biotic interactions and soil-borne diseases. Elsevier, Amsterdam, pp 27–34

Cook RJ, Veseth RJ (1991) Wheat health management. American Phytopathological Society, St. Paul, Minn.

Curl EA (1979) Effects of mycophagous Collembola on *Rhizoctonia solani* and cotton-seedling disease. In: Schippers B, Gams W (eds) Soil-borne plant pathogens. Academic Press, London, pp 253–269

Curl EA, Gudauskas RT, Harper JD, Peterson CM (1985) Effects of soil insects on populations and germination of fungal propagules. In: Parker CA, Rovira AD, Moore KJ, Wong PTW, Kollmorgen KF (eds) Ecology and management of soil-borne plant pathogens. American Phytopathological Society, St. Paul, Minn., pp 20–23

El Titi A, Ulber B (1991) Significance of biotic interactions between soil fauna and microflora in integrated arable farming. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schippers B, Tempel A (eds) Biotic interactions and soil-borne diseases. Elsevier, Amsterdam, pp 1–19

Gisin H (1970) Liquides pour la fixation, l'étude, le montage et la conservation des Collemboles. Rev Ecol Biol Sol 7:45–49

Innocenti G, Cappi C, Sabatini MA (1997) Interactions of Collembola with soil-borne cereal pathogen and biological control fungi. In: Tvaruzek L (ed) Proceedings of the International Conference Protection of cereal crops against harmful organisms. Kromeriz, July 1–4, 1997. Agricultural Research Institute Kromeriz, Kromeriz, Czech Republic, pp 72–75

Jones GD, Clifford BC (1978) Cereal diseases. Their pathology and control. Wiley, Chichester

Lartey RT, Curl EA, Peterson CM (1994) Interactions of mycophagous Collembola and biological control fungi in the suppression of *Rhizoctonia solani*. Soil Biol Biochem 26:81–88

Ledingham RJ (1981) Special methods: common rot on wheat. In: FAO (ed) Crop loss assessment methods. FAO manual on the evaluation and prevention of losses by pests disease and weeds. CAB International, London, p 29

Leonard MA (1984) Observations on the influence of culture conditions of the fungal feeding preferences of *Folsomia candida* (Collembola: Isotomidae). Pedobiologia 26:361–367

Lootsma M, Scholte K (1997a) Effects of the springtail *Folsomia fimetaria* and the nematode *Aphelenchus avenae* on *Rhizoctonia solani* stem infection of potato at temperatures of 10 and 15 °C. Plant Pathol 46:203–208

Lootsma M, Scholte K (1997b) Effects of soil moisture content on the suppression of *Rhizoctonia* stem canker on potato by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimetaria*. Plant Pathol 46:209–215

Lootsma M, Scholte K (1998) Effects of soil pH and amendments with dried fodder rape on mycophagous soil animals and *Rhizoctonia* stem canker of potato. Pedobiologia 42:215–222

Nakamura Y, Matsuzaki I, Itakura J (1992) Effect of grazing by *Sinella curviseta* (Collembola) on *Fusarium oxysporum* f. sp. *cucumerinum* causing cucumber disease. Pedobiologia 36:168–171

Ownley BH, Weller DM, Thomashow M (1992) Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2–79. Phytopathology 82:178–144

Sabatini MA, Innocenti G (1995) Interactions between Collembola and soil-borne cereal pathogen fungi. Pol Pismo Entomol 64:191–197

Sabatini MA, Innocenti G (2000a) Functional relationships between Collembola and plant pathogenic fungi of agricultural soils. Pedobiologia 44:467–475

Sabatini MA, Innocenti G (2000b) Soil-borne plant pathogenic fungi in relation to some collembolan species under laboratory conditions. Mycol Res 104 (in press)

Sabatini MA, Rebecchi L, Cappi C, Bertolani R, Fratello B (1997) Long-term effects of three different continuous tillage practices on collembolan populations. Pedobiologia 41:185–193

Scholte K, Lootsma M (1998) Effect of farmyard manure and green manure crops on populations of mycophagous soil fauna and *Rhizoctonia* stem canker of potato. Pedobiologia 42:223–231

Simon A, Sivasithamparam K (1988) Interactions among *Gaeumannomyces graminis* var. *tritici* and soil bacteria. Can J Microbiol 34:871–876

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa State University Press, Ames, Iowa

Vannier G (1979) Relations trophiques entre la microfaune et la microflore du sol; aspects qualitatifs et quantitatifs. Boll Zool 46:343–361

Visser S, Parkinson D, Hassal M (1987) Fungi associated with *Onychiurus subtenuis* (Collembola) in an aspen woodland. Can J Bot 65:635–642

Wiggins EA, Curl EA (1979) Interaction of Collembola and microflora of cotton rhizosphere. Phytopathology 69:244–249