Reduction of viability of soil borne inoculum of common bunt (*Tilletia tritici*) by collembolans

Karsten M. Dromph[a*], Anders Borgen[b]

[a] Department of Ecology, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.
[b] Department of Agricultural Sciences, Royal Veterinary and Agricultural University, Agrovej 10, DK-2630 Taastrup, Denmark

*Corresponding address:
Karsten M. Dromph
Department of Biological Sciences, Institute of Environmental and Natural Sciences, Lancaster University, Lancaster LA1 4YQ, U.K.

Tel: (+44) 0 1524 592 278, Fax: (+44) 0 1524 843 854
Email: K.Dromph@lancaster.ac.uk

Abstract

The aim of the study was to test the effect of collembolans on the viability of soil borne inoculum of *Tilletia tritici* (syn. *T. caries*). Teliospores were fed to five species of collembolans: *Onychiurus cebennarius*, *Mesaphorura macrochaeta*, *Folsomia fimetaria*, *Proisotoma minuta* and *Orchesella villosa*. Faeces were collected from all species and only faeces from *M. macrochaeta* did not contain teliospores. Ingestion by the four other collembolan species reduced germination rates of *T. tritici* teliospores from 76.5% in the uningested control to below 3% after ingestion. There were no significant differences between the germination rates after ingestion by the different collembolan species. The effect of collembolans on the viability of teliospores in the soil was tested in two soil types, a sphagnum based potting medium and a coarse sandy field soil. 5 ml of soil was inoculated with 0.1, 0.2 or 0.3 mg of teliospores and incubated with 15 *F. fimetaria* or *P. minuta* for 3 days. In both soil types *F. fimetaria* significantly reduced the proportion of viable spores in the two lowest inoculation levels, while *P.minuta* only had a significant effect at the lowest inoculation level. The effect of collembolan density on the infection of wheat was tested by inoculating field

soil in 5-l pots with 2.0 mg of teliospores and collembolan densities equivalent to between 0 and 199,200 *P. minuta* m^{-2}. Incubation with densities of 59,800 *P. minuta* m^{-2} or higher for 10 days before sowing, reduced the infection of wheat from 30% in the control to 3.5%.

Keywords: *Tilletia tritici*; spore viability; Collembola; feeding

1. Introduction

Common bunt (*Tilletia tritici* (Berk.) Wint., syn. *T. caries* (DC.) Tul.) is one of the most important fungal diseases of cereals. Seed transmission is the predominant source of inoculum, but teliospores can survive for several years in the soil and still cause infection of a wheat crop (Johnsson, 1990; Borgen, 2000). When the spores are kept in the topsoil they lose viability within a few months (Kühnel, 1960), but the factors affecting the survival of spores in soil are unclear.

Invertebrates such as earthworms and grasshoppers ingest bunt spores, but the ingestion does not reduce their viability significantly (Hoffmann and Purdy, 1964; Smilanick et al., 1986). Therefore, it is not likely that they play an important role in the reduction of spore viability in soil. On the contrary they may promote the dispersal of bunt diseases by carrying viable spores in their guts (Hoffmann and Purdy, 1964; Smilanick et al., 1986). None of these studies have included collembolans, although they constitute a very large proportion of the soil fauna (Hale, 1967).

Most collembolans are considered to feed mainly on dead organic matter and micro-organisms (Christiansen, 1964; Hale, 1967). Studies of the gut contents of several species have shown that fungal material, including spores, often forms a substantial part of their diet (Bödvarsson, 1970). Through their feeding collembolans interact with soil-borne microfungi in several ways; by suppressing some fungal species by grazing selectively on them (Curl, 1988; Lartey et al., 1991) and by dispersing inoculum by carrying viable spores on the cuticle or in the gut (Wiggins and Curl, 1979; Whipps and Budge, 1993; Williams et al., 1998).

Our objective was to investigate the effect of collembolans on the viability of soil borne inoculum of *T. tritici*. To test the effect of ingestion on the viability, teliospores were fed to five species of collembolans representing three families: Onychiuridae (*Onychiurus cebennarius* Gisin and *Mesaphorura macrochaeta* Rusek), Isotomidae (*Folsomia fimetaria* (L.) and *Proisotoma minuta* (Tullberg)) and Entomobryidae (*Orchesella villosa* (Geoffroy)), all five collembolan species are common in arable soils in Northern Europe (Fjellberg, 1998; P.H. Krogh, unpublished). The effect of collembolans on the viability of teliospores in soil was tested by incubating teliospores in two soil types, a sphagnum based potting medium and a coarse sandy field soil in combination with either *F. fimetaria* or *P. minuta*. Finally the effect of collembolan density on infection of wheat (*Triticum aestrivum* L.) was tested by adding different numbers of *P. minuta* to soil inoculated with *T. tritici* before the wheat was sown.

2. Materials and methods

2.1 Collembolans.

The collembolans originated from laboratory cultures derived from specimens collected in a clover-grass field at an experimental farm situated 20 km west of Copenhagen, Denmark. The cultures were maintained on a mixture of soil and finely chopped barley straw (v:v 4:1) with the addition of baker's yeast and kept at 20°C. Samples taken at several times from the cultures were cleared and mounted in 80% lactic acid and identified according to Gisin (1960) and Fjellberg (1980; 1998).

2.2 Teliospores.

For the ingestion experiment spores of *T. tritici* were obtained from infected heads of the susceptible winter wheat variety "Husar" in August 1998 and kept at 5°C until the start of the experiment in February in the following year. Bunt sori were ground and sieved through a 1.5 mm mesh. The teliospores used for testing the effect of collembolans on the viability of *T. tritici* in soil were obtained from the winter wheat variety "Hereward" in August 1999, and treated the same way as the spores from the previous year.

2.3.1 Effect of ingestion on viability of teliospores.

In the experiment reproductive adults of the five collembolan species were selected by size. Five *O. villosa* and 15 of each of the four other species were transferred to 30-ml plastic vials with 5 ml of 1.5% water agar at the bottom and 0.2 mg of dry teliospores of *T. tritici* on top of the agar. Teliospores of the control group were treated in the same way except that no collembolans were added.
After incubation for 3 days at 17°C in darkness, the collembolans were transferred to vials with clean 1.5% water agar and bakers yeast as food supply. One day later faecal pellets were collected under a stereo microscope and suspended in 0.5 ml sterile deionised water. The spore suspension was centrifuged at 1000 G for 5 min, and the spores were surface sterilised for 1 min in 0.1% $NaHClO_3$, centrifuged at 1000 G for 5 min, and rinsed twice in sterile deionised water before they were resuspended in 0.1 ml sterile deionized water. Spores of the control groups were surface sterilised and rinsed in the same way as the spores ingested by collembolans.
The spore viability was examined by adjusting the teliospore concentration to approximately 1000 spores ml^{-1}, 0.5 ml of the suspensions was transferred to Petri-dishes with 3% water agar and incubated for 7 days at 17°C in darkness. Because teliospores of *T. tritici* are easily distinguished from other fungal spores by their morphology (Line, 1993), spore germination of *T. tritici* was examined directly under a light microscope. For each replicate two 2.25 cm^2 pieces were cut out of the agar and stained with lactophenol-cotton blue before 100 teliospores were observed on each of the pieces. Spores were recorded as germinated if the germ-tube was visible. The experiment was repeated five times.

2.4 Teliospores carried on the cuticle.

To assess the quantity of teliospores carried on the cuticle, collembolans were incubated for 1 h in 30-ml plastic vials with 5 ml of 1.5% water agar at the bottom and 0.2 mg of dry teliospores of *T. tritici* added on top of the agar. The short incubation time was chosen to allow the collembolans to move around on the spores, but not ingested fungal material to pass through the gut causing contamination from faeces potentially containing conida. After the incubation 10 of each collembolan species were immersed singly in 0.2 ml 0.05% Tween-20 and stirred twice for 30 s each at 2000 rev min^{-1}. The spores in the suspensions were then counted under a microscope.

2.5 Reduction of viability of teliospores in soil

The effect of collembolans on the viability of soil borne teliospores was tested in two different soil types, a coarse sand soil, originating from a farm at Jyndevad situated 1.5 km north of the Danish-German border (Hansen, 1976) and a commercial fine grained sphagnum based growth medium, limed and fertilised to pH 5.6-6.6 and conductivity 1.5-3.0 msiemens produced by Pinstrup Mosebrug A/S, Denmark. The Jyndevad soil was first air dried and sieved through a 5 mm mesh. 5 ml of soil was added each to 30-ml plastic vials with perforated bottoms. These were placed in 0.5 cm of deionised water for 24 h and thereafter allowed to drain for 24 h, to allow the soil to reach its maximum water holding capacity. 0.1 mg, 0.2 mg or 0.3 mg of dry teliospores of *T. tritici* was added to the soil surface and 15 adult *P. minuta* or *F. fimetaria* were transferred to each vial. No collembolans were added to the control vials. The vials were incubated for 3 days in darkness at 17ºC, after which the soil was transferred to glass vials and suspended in 10 ml of 0.05% Tween-20. The suspension was stirred for 30 s at 2000 rev m^{-1} and filtered through a paper towel (Kleenex®) allowing the teliospores to pass through. The spores were surface sterilised and rinsed as described in section 2.3 before they were resuspended in 0.1 ml of sterile deionized water. The viability of the teliospores was assessed as described under the ingestion experiment, section 2.3. The experiment was repeated five times.

2.6 Effect of density of *P. minuta* on the infection of wheat by *T. tritici*

The effect of density of *P. minuta* in soil on the infection of wheat was tested in 5-l pots containing a commercial fine grained sphagnum based growth medium in the bottom covered by a 10 cm thick layer of a non-sterile field soil, resulting in a surface area of 502 cm^2 (see section 2.5 for descriptions of the soils used). Each pot was inoculated by dispersing 2.0 mg of dry teliospores of *T. tritici* mixed into 10 ml of dry soil in an even layer on the surface, and 0, 500, 2000, 3000, 5000 or 10,000 *P. minuta* were added per pot, the range being equivalent to 0, 10,000, 39,800, 59,800, 99,600 and 199,200 collembolans m^{-2}. In cereal fields the density normally reaches 10-20,000 collembolans m^{-2} (Lagerlöf and Andrén, 1991; P.H. Krogh, unpublished), but densities of 90.000 collembolans m^{-2} have been recorded after application of green manure (Axelsen and Kristensen, 2000). A negative control not inoculated with *T. tritici* was included. After incubation for 10 days in darkness at 10 C, 29 seeds of the susceptible wheat variety

"Apogee" were planted at 1 cm depth in each pot, and the night temperature was lowered to 5 C. When the seedlings started to emerge after 9 days, the light was set to L:D 12:12 and the day temperature was gradually lowered by 1 C a week down to 5 C. After the second leaf had emerged the pots were transferred to a greenhouse at L:D 14:10 and a day temperature of 15 C and night temperature of 10 C, where the infection level was recorded three and a half month later as formation of sori in the ear. The experiment included four replicates of each treatment.

2.7 Statistical analysis

The data, except the number of spores carried on the cuticle, were logit transformed and analysed using the GENMOD procedure in SAS 6.12, the treatment means were compared using the CONTRAST procedure for pairwise comparison ($P<0.05$) (SAS Institute, 1990). The number of spores carried on the cuticle was analysed using the ANOVA procedure, and the mean number of spores carried on each collembolan species was compared using least significant differences ($P<0.05$) (SAS Institute, 1990).

3. Results

3.1. Effect of ingestion by collembolans on viability of teliospores.

Faecal pellets containing teliospores were obtained from all species except *M. macrochaeta*. Microscopy of 10 individuals of *M. macrochaeta* cleared and mounted in 80% lactic acid showed no teliospores in the gut. The germination rates of teliospores recovered from faeces of the four other species are shown in Table 1. Ingestion by these four collembolan species inhibited germination of the teliospores, as germination rates were reduced from 77.2% in the control to below 3% after ingestion, although there were no visible signs of damage to the spores. There were no significant differences between the germination rates after ingestion by the different collembolan species.

3.2. Teliospores carried on the cuticle

The number of teliospores carried on the cuticle per individual are shown in Table 2. The numbers were very low, below 10 spores per collembolan, for all species and only the number of spores carried on *O. villosa* differed significantly ($P<0.05$) from the others.

3.3. Effect of collembolans on viability of teliospores in soil.

Germination rates of teliospores recovered from the two soil types, with or without the two species of collembolans added are shown in Fig. 1. Both *F. fimetaria* and *P. minuta* significantly ($P<0.05$) reduced the viability of teliospores, the effect being most pronounced for *F. fimetaria* and at the lowest spore concentration.

3.4 Effect of *P. minuta* on infection of wheat by *T. tritici*

The effect of density of *P. minuta* on the infection of wheat with *T. tritici* is shown in Fig. 2. There was no effect on the infection level at densities of *P. minuta* of 2000 per pot or less, while densities of 3000 *P. minuta* per pot, equivalent to 59,800 collembolans m^{-2}, or more nearly prevented infection completely. There was no effect of *P. minuta* on the germination of wheat (data not shown).

4. Discussion

Four of the five collembolan species used in this experiment fed readily on teliospores of *T. tritici*; the only exception being *M. macrochaeta*. Other studies have found fungal spores in the gut of *Mesaphorura* species (Bödvarsson, 1970). This species is with its size of less than 0.7 mm (Fjellberg, 1998), by far, the smallest of the five species used in this study. Therefore a possible explanation for the lack of feeding on *T. tritici* teliospores could be that fully hydrated bunt spores, being 19-25 m in diameter (Ettel and Halbsguth 1963), are too large to be ingested by *M. macrochaeta*.
The present study shows that ingestion by collembolans almost completely inhibits germination of teliospores of *T. tritici*, and that collembolans can reduce the amount of viable teliospores of *T. tritici* in the soil, and thereby reduce the infection of wheat. This result is not consistent with previous studies with invertebrates showing that ingestion by earthworms and grasshoppers does not have any effect on the viability of teliospores of *Tilletia species* (Hoffmann and Purdy, 1964; Smilanick et al., 1986).
When spores of *T. tritici* are kept in the moist topsoil after harvest, they lose their ability to infect after a few months (Kühnel, 1960; Weltzien, 1957), but when kept under the ploughing layer, they can survive for at least 5-10 years and still infect wheat after reploughing of the field (Johnsson, 1990; Borgen, 2000). One explanation for this difference might be that spores in the topsoil readily germinate, and being an obligate pathogen they die in the absence of a host. The present study shows that collembolans can play an important role in reducing spore viability by ingesting the spores. This effect will be most pronounced in the uppermost layers of the soil, because most collembolans, including four of the species used in this study, are restricted to the uppermost 5 cm of the soil. Here 50-70% of the total collembolan population is found, while only very low densities of small euedaphic species like *Mesaphorura* spp. are found down to 20 cm depth (Marshall, 1974; Persson and Lohm, 1977; Lagerlöf and Andrén, 1991).
In the present study exposure of the spores for 10 days to grazing by a density equivalent to 59,800 *P. minuta* m^{-2} reduced the infection of wheat with *T. tritici* from 30% to 3.5%. In cereal fields the density normally only reaches 10-20,000 collembolans m^{-2} (Lagerlöf and Andrén, 1991; P.H. Krogh, unpublished), but densities of up to 90.000 m^{-2} have been recorded when green manure had been applied the previous autumn (Axelsen and Kristensen, 2000). Even at relatively low densities collembolans are still likely to have an effect under field conditions, because the spores will be exposed to grazing by collembolans for a much longer time than in the present experiment.

Soil dwelling collembolans rarely damage crops and never cereals (Hopkin, 1997). They can therefore be considered beneficials, as they both play an important role in soil formation (Hale, 1967), and in reducing the infection potential of plant pathogenic fungi. Because this effect is dependent on the density of collembolans, it will probably be more pronounced in farming systems which favour the soil fauna, for example in organically grown fields (P.H. Krogh, unpublished).

Acknowledgements

The authors wish to thank Susanne Olsen for technical assistance, Lars Kristensen for good advice concerning *T. tritici*. Peter Esbjerg and David Coleman are thanked for their invaluable comments to the manuscript.

References

Axelsen, J.A., Kristensen, K.T., 2000. Collembola and mites in plots fertilised with different types of green manure. Pedobiologia 44, 556-566.
Borgen, A., 2000. Perennial survival of common bunt (Tilletia tritici) in soil under modern farming practice. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 107, 182-188.
Bödvarsson, H., 1970. Alimentary studies of seven common soil-inhabiting Collembola of Southern Sweden. Entomologica Scandinavica 1, 74-80.
Christiansen, K., 1964. Bionomics of Collembola. Annual Review of Entomology 9, 147-178.
Curl, E.A., 1988. The role of soil microfauna in plant-disease suppresion. CRC Critical Reviews in Plant Sciences 7, 175-196.
Ettel, G.E., Halbsguth, W., 1963. Über die Wirkung van Trimethylamin, Calciumnitrat und Licht bei der Keimung der Brandsporen von Tilletia tritici (Bjerk.) Winter. Beiträge zur Biologi der Pflanzen. 39, 451-488.
Fjellberg, A., 1980. Identification Keys to Norwegian Collembola. Norsk entomologisk forening. Ås-NLH. 152 pp.
Fjellberg, A., 1998. The Collembola of Fennoscandia and Denmark. Part 1: Poduromorpha. Brill, Leiden. 184 pp.
Gisin, H., 1960. Collembolenfauna Europas. Museum d'Histoire Naturelle, Genève. 312 pp.
Hale, W.G., 1967. Collembola. In: Burges, A., Raw, F. (Eds) Soil Biology. Academic Press. London, New York, pp. 397-411.
Hansen, L., 1976. Jordtyper ved Statens Forsøgsstationer. Danish Journal of Plant and Soil Science 80, 742-758.
Hoffmann, J.A., Purdy, L.H., 1964. Germination of dwarf bunt teliospores after ingestion by earthworms. Phytopathology 54, 878-879.
Hopkin, S.P., 1997. Biology of the springtails (Insecta: Collembola). Oxford University Press, Oxford New York, 330 pp.
Johnsson, L., 1990. Survival of common bunt (Tilletia caries (DC) Tul.) in soil and

manure. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 97, 502-507.
Kühnel, W., 1960. Der Einfluss der Faktoren Bodenart, Bodenfeuchtigkeit und Bodentemperatur af die Pathogenitätsabnahme des Steinbranderregers des Weizens (Tilletia caries, DC.Tul.) im Boden. Nachrichtenblatt für den Deutschen Pflanzenschutzdienst, Berlin 14, 21-26.
Lagerlöf, J., Andrén, O., 1991. Abundance and activity of Collembola, Protura and Diplura (Insecta, Apterygota) in four cropping systems. Pedobiologia 35, 337-350.
Lartey, R.T., Curl, E.A., Peterson, C.M., Williams, J.C., 1991. Control of Rhizoctonia solani and Cotton Seedling Disease by Laetisaria arvalis and a mycophagous insect Proisotoma minuta (Collembola). Journal of Phytopathology 133, 89-98.
Line, R.F., 1993. Common bunt (Tilletia tritici and Tilletia laevis) In: Mathur, S. B., Cunfer, B. M. (Eds) Seed-borne diseases and seed health testing of wheat. Jordbrugsforlaget. Copenhagen, pp. 45-52.
Marshall, V.G., 1974. Seasonal and vertical distribution of soil fauna in a thinned and urea-fertilized Douglas fir forest. Canadian Journal of Soil Science 54, 491-500.
Persson, T., Lohm, U., 1977. Energetical significance of the annelids and arthropods in a Swedish granssland soil. Ecological Bulletins/NFR 23, 1-211.
SAS Institute, 1990. SAS/STAT(R) User's Guide, version 6, 4th edition, vol. 1-2. Cary, North Carolina. 1848 pp.
Smilanick, J.L., Dupler, M., Goates, B.J., Hoffmann, J.A., 1986. Germination of teliospores of Karnal, Dwaf, and Common Bunt fungi after ingestion by animals. Plant Disease 70, 242-244.
Weltzien, H.C., 1957. Unterschungen über den Befall von Winterweizen durh Tilletia tritici (Bjerk.) Winter unter besonderer Berücksichtigung der Frage der Beizmittelresistenz. Phytopathologische Zeitschrift 29, 121-150.
Whipps, J.M., Budge, S.P., 1993. Transmission of the mycoparasite Coniothyrium minitans by collembolan Folsomia candida (Collembola: Entomobryidae) and glasshouse sciarid Bradysia sp. (Diptera: Sciaridae). Annals of Applied Biology 123, 165-171.
Wiggins, E.A., Curl, E.A., 1979. Interactions of Collembola and microflora of cotton rhizosphere. Phytopathology 69, 244-249.
Williams, R.H., Whipps, J.M., Cooke, R.C., 1998. Role of soil mesofauna in dispersal of Coniothyrium minitans: mechanisms of transmission. Soil Biology & Biochemistry 30, 1937-1945.

Table 1.
Effect of ingestion by four species of collembolans on the germination of *Tilletia tritici* teliospores. Percentage germination shown as means of five replicates back-transformed after logit transformation. Rows followed by different letters are significantly different ($P<0.05$).

Collembolan species	Percentage germination	S.E.	Pairwise comparison
Control	77.2	0.8	A
Onychiurus cebenarius	0.9	0.2	B
Folsomia fimetaria	2.0	0.3	B
Proisotoma minuta	2.2	0.3	B
Orchesella villosa	1.7	0.2	B

Table 2.

Number of teliospores carried on the cuticle of five species of collembolans. Numbers are means of ten individuals. Rows followed by different letters are significantly different ($P<0.05$).

Collembolan species	Number of teliospores per specimen	S.E.	Pairwise comparison
Onychiurus cebenarius	0.6	0.6	A
Mesaphorura macrochaeta	0.2	0.3	A
Folsomia fimetaria	1.6	2.5	A
Proisotoma minuta	1.4	1.5	A
Orchesella villosa	8.5	7.8	B

a)

b)

Fig. 1. Effect of collembolans on the germination percentage of teliospores of Tilletia tritici incubated for three days in either a) a sphagnum based growth medium or b) a coarse sand field soil. Figures are means of five replicates ± S.E back-transformed after logit transformation. Columns headed by different letters with in each level of T. tritici are significantly different ($P<0.05$) in pairwise comparison.

Fig. 2. Effect of density of Proisotoma minuta in soil on the infection of wheat by Tilletia tritici. The control was not inoculated with T. tritici nor P. minuta. Numbers are means of four replicates ± S.E back-transformed after logit transformation. Columns headed by different letters are significantly different ($P<0.05$) in pairwise comparison.