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Collembola and seed germination: relevance of substrate quality and evidence for seed attack

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Abstract

Besides species-specific characteristics and abiotic conditions, germination rate of seeds depends on biotic interactions such as seed predation and fungal-induced mortality.

We investigated the influence of *Folsomia candida* Willem, 1902 on the germination rate of four grassland plant species (*Agrostis capillaris* L., *Hypericum perforatum* L., *Plantago major* L. and *Vicia cracca* L.) in grassland soils and in artificial soil under laboratory conditions. Just one of the selected plant species, *H. perforatum*, showed a significant positive increase in germination rate in presence of the euedaphic *F. candida* in the artificial soil substrate whereas no effect was found in natural soils. The influence of Collembola on seed germination therefore appears highly dependent both on the experimental conditions and on the Collembola species studied.

However, the study at the same time revealed an unexpected observation which opens another aspect under which Collembola might directly influence seed mortality: Pictures made during the germination experiment gave evidence that individuals of *F. candida* fed on the mucilaginous seed coat as well as on the seed embryo of *P. major* under the prevailing conditions. This effect was confirmed in a second experiment in the natural soil Lufa 2.2.

The overall impact on seed germination was only small, but significant. Besides one hidden hint in a book, so far it has not been reported in the literature that Collembola use seeds as a food source and therefore can potentially act as seed predators.

Keywords: *Folsomia candida*, *Hypericum perforatum*, *Plantago major*, seed germination, soil fungi

1. Introduction

How successfully a seedling can establish is predominantly determined by the physiological and biochemical constitution of the seeds (Bewley & Black 1994). However post-dispersal hazards such as senescence, germination in depth, seed predation and fungal attack decide over the proportion of seeds that stay viable in soil until germination is induced (Fenner & Thompson 2005). The importance of these hazards varies greatly between seed species and years and with environmental conditions (Crawley 1992, Blaney & Kotanen 2001, Clark & Wilson 2003). In seed mortality especially moisture conditions have been shown

to be a central factor for the relevance of fungal pathogens (Leishman et al. 2000, Schafer & Kotanen 2003). But selectivity, varying pathogenicity of fungi and sensitivity of seeds make the impact of pathogens strongly dependent on plant species (Crist & Friese 1993, Leishman et al. 2000, Schafer & Kotanen 2004, Wagner & Mitschunas 2008). Additionally also biotic interactions have the potential to alter the impact of fungal pathogens in seed mortality: Previous studies in our group brought evidence that, by influencing fungal growth, Collembola (*Protaphorura fimata* Gisin, 1952) can reduce seed mortality in grassland plant species *Centaurea nigra* L. and *Origanum vulgare* L. under laboratory (Mitschunas et al. 2006) and field conditions (Mitschunas et al. 2008).

On the basis of the experimental set-up of Mitschunas et al. (2006) we wanted to test the influence of another Collembolan species, *Folsomia candida*, and another set of grassland plant species in this study. Our main questions were: Does *Folsomia candida* influence germination rates of the selected plant species? Does substrate quality alter the outcome? Due to an unexpected observation during the course of our experiment, we performed an additional experiment in which we studied if *F. candida* might act as a potential seed predator of *Plantago major.*

2. Material and Methods

2.1. Organisms

We selected four grassland species representing three functional groups – herbs, grasses and legumes – and differing soil requirements: *Agrostis capillaris*, *Hypericum perforatum*, *Plantago major* and *Vicia cracca.* Seeds were provided by the group of Markus Fischer, University of Potsdam. They had been collected on grassland sites belonging to the biodiversity exploratory Schorfheide in the eastern part of Germany (www.biodiversity-exploratories.de).

Originally we had intended to use Collembola species extracted from grassland sites in the same area (near the village of Biesenbrow, 53° 07' 10.32'' N, 14° 01' 51.55'' O, 18 m above sea level), but our attempts to establish lab cultures from those extracted individuals failed. As a substitute we chose the euedaphic species *Folsomia candida* which could be withdrawn from our continuous lab culture. We used synchronized individuals which had reached an age of twelve days at the beginning of our experiments.

In one additional treatment we used a species mixture of the remaining extracted Isotomidae from grassland (consisting of at least three species including *Parisotoma notabilis* (Schäffer, 1896), *Cryptopygus* spec. Willem, 1901 and *Hemisotoma* spec. Bagnall, 1949) instead of *F. candida.* Identification of taxa followed the key of Potapov (2001); for *Hemisotoma* we referred to Rusek (2002). We distributed the single taxa as even as possible among the single replicates, ending up with a total of 15 individuals per dish.

2.2. Soils

Three substrates which differed in soil characteristics such as pH, water holding capacity and organic matter content (see Tab. 1) were used during the experiment. One of them, **Lab**, was an artificial soil mixture composed of sand and a standardised unfertilised soil ('Nullerde', mixed of clay and white peat, Einheitserde ® Werkverband, Sinntal-Jossa) in equal parts. The other two substrates were natural soils withdrawn from meadow land (**Field1**) and pasture land (**Field2**) in the aforementioned area in October 2007.

All substrates were sieved, defaunated and watered by soakage before we started the experiment.

Tab. 1 Estimated soil parameters of the different substrates. Mean values and standard errors are shown (n = 3), SOM = Soil organic matter, WHC_{max} = maximum water holding capacity.

	Substrate type					
	Lab		Field1		Field2	
	Mean	s.e.	Mean	s.e.	Mean	s.e.
Water content [1] [% WHC_{max}]	77	-	57	-	57	-
WHC_{max} [%]	45	0.0	151	0.0	124	0.3
SOM [%]	6.6	0.28	49.2	0.40	17.1	0.08
pH	6.6	0.01	5.5	0.04	7.3	0.04

[1] Calculated values at the beginning of the germination experiment after five weeks of incubation.

For **Lab** both substrate components were sieved to exclude particles larger than 2 mm in diameter and then mixed in equal parts in volume. In contrast, **Field1** and **Field2** were sieved using a larger mesh size of 6.3 mm to prevent severe destruction of the soil structure.

For defaunation a procedure with two freeze-thaw cycles (for 24 hours each step) at -20 °C and room temperature, respectively, was carried out with all substrates (modified after Mitschunas et al. 2006). This method is effective in eliminating microarthropods without affecting microorganisms on a long term (Huhta et al. 1989).

Before the experiment started, the two natural soils were additionally treated with heat in order to suppress germination of remaining seeds. This was done by heating the spread soil for 24 hours at 60 °C. Temperatures in the 50–121 °C range can lead to mortality of microorganisms depending on species (Neary et al. 1999). Thus heat treatment will have affected soil fungal community both by reducing species diversity and by favouring heat resistant fungi and bacteria. After incubation for 5 weeks in sealed petri dishes during the experiment, seeds in treatments without Collembola were covered with fungal hyphae, which proved the presence of some fungi.

Just before setting up the experiments the substrates were also watered with tap water by soakage. For this, boxes were filled with a definite amount of soil, closed with a cloth and laid top-down in a water bath. **Lab** was saturated to 62% of the maximal water holding capacity (WHC_{max}). **Field1** and **Field2** had a water content of 48% and 49% of the WHC_{max}, respectively.

2.3. Fungicide treatment of seeds

For the fungicide coating, seeds were sprayed with 0.075% Chitosan solution with an aerosol can and dried on soft paper (modified after Mitschunas et al. 2006). The solution was prepared with distilled water and chitosan powder (ChitoPlant ®, ChiPro GmbH, Bremen). We decided for Chitosan because it had been shown to have an antifungal effect while being non-toxic to other organisms and biologically degradable (Bautista-Baños et al. 2006).

2.4. Experimental set-up

Two experiments were conducted. In the first, we addressed the question if *F. candida* has an effect on germination of the four plant species and if this is due to soil fungal influence on seeds. The artificial soil served as substrate and treatments were control (Co), addition of *Folsomia candida* (Fc) and fungicide treated seeds (Fn). The latter treatment was set up to find out whether an increase in germination rate was due to a reduction of fungal attack on seeds or due to another mechanism.

In the second experiment we investigated if a possible influence of the collembolans differed with respect to soil quality. Here the two grassland soils **Field1** and **Field2** and two plant species, *Agrostis capillaris* and *Hypericum perforatum*, were used. The treatments were control (Co) and addition of *Folsomia candida* (Fc), both on heat-treated soil. To control for the effectiveness of the heat treatment, untreated soil (-HCo) was taken as a third treatment (but excluded from statistical analysis since this was not part of our main questions). Additionally one variant with *Agrostis capillaris* and 15 individuals drawn from the species mixture of extracted Isotomidae (Mx) was set up on **Field1** (Tab. 2).

Both experiments were performed in a balanced design with five replicates per treatment.

Tab. 2 Experimental set up. Treatments were: Co = control, Fn = fungicide treated seeds, Fc = variants with added *F. candida*, of soils, Mx = variant with a mixture of Isotomidae added. All substrates were heat-treated except for -Hco. *Ac = A. capillaris*, *Hp = H. perforatum*, *Pm = P. major*, *Vc = V. cracca*.

Substrate type	Plant species			
	Ac	*Hp*	*Pm*	*Vc*
Lab	Co, Fn, Fc	Co, Fn ,Fc	Co, Fn, Fc	Co, Fn, Fc
Field1	-HCo, Co, Fc, Mx	-HCo, Co, Fc	-	-
Field2	-HCo, Co, Fc	-HCo, Co, Fc	-	-

2.5. Procedure

The watered substrate was weighed in petri dishes (7.5 cm in diameter) up to a level of 9 mm which corresponded to 37 g of **Lab,** 25 g of **Field1** or 30 g of **Field2**. After slightly flattening the surface with a spoon, 25 seeds of one of the selected plant species were placed in the middle of the dish. For Collembola treatments, 15 individuals were added, which corresponds to 3,400 individuals/m². Petersen and Luxton (1982) found densities of 1,000,000 - 670,000 Ind/m² for *F. candida* in natural soils. Chosen start densities were much lower in our experiment since Collembola were restricted to an almost two-dimensional habitat and densities would increase during the course of the experiment.

Closed petri dishes were sealed with Parafilm® and randomly distributed in light-proof boxes. Only variants with and without added Collembola were separated to prevent contamination. The microcosms were incubated for five weeks in darkness with a constant temperature of 14 °C to favour fungal growth without stimulating germination (Mitschunas et al. 2006). After this period we counted the seedlings and removed them. Subsequently 3 ml of water were added and the petri dishes were transferred to germination conditions (light-dark-cycle 16:8 hours, with analogous changing temperatures of 25 °C and 15 °C) in a climate chamber (MLR-350H, Sanyo Electric Biomedical CO Ltd. Japan). During the following two weeks the microcosms were randomized each day and germinated seeds were counted and removed weekly (Mitschunas et al. 2006).

2.6. Seed predation experiment

This experiment was designed to reveal a potential seed predation effect of *F. candida* on seeds of *P. major*. Basically it was performed as described above, with the following specifications: Substrates were again **Lab** and a new field soil, in the following referred to as **Lufa**. Lufa 2.2, a standard arable field soil used in many ecotoxicological experiments, was obtained from Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany.

According to the supplier, this is a loamy sand soil with 1.9% organic carbon and 0.17% nitrogen. The pH ($CaCl_2$) was 5.3 and the maximum water holding capacity 48%, very close to **Lab**. Both soils were adjusted to 50% WHC_{max} for the experiment. The substrates were defaunated, but without additional heat treatment. Seeds of *P. major* had been collected few weeks before the experiment in the surroundings of Grasberg near Bremen, Germany. Per Petri dish 25 seeds of *P. major* were added, and treatments with Collembola were supplied with 15 individuals of *F. candida* each. This design contained four treatments, Lab (Co), Lab (Fc), Lufa (Co) and Lufa (Fc) with ten replicates each. Compared to the first experiments, the germination conditions were slightly modified, with a light-dark cycle 15:9 hours at 25 °C and 15 °C, respectively. Since nothing had germinated after five weeks, germination rates were only recorded after six and seven weeks.

2.7. Data analysis

For statistical data analysis we estimated the germination rates after 5, 6 and 7 weeks for every species and treatment on the different substrates. We performed arcsin square root transformations from the original values to render normality of data (Quinn & Keough 2002). The transformed data set was divided to groups by plant species and substrate (or by +/- Collembola and substrate in the seed predation experiment). We tested normal distribution after Shapiro-Wilk and checked for homogeneity of variances with the Levene test for every data subset. If none of these assumptions was violated we performed a general linear model with repeated measurements in SPSS©13.0 (2004) to test for treatment effects. For *Hypericum perforatum* and *Plantago major* only germination rates after the sixth and the seventh week were included into the analysis, as after the fifth week almost no seedlings occurred. For pair-wise comparisons Tukey's HSD test was used. In the case normal distribution of the data or equality of variances could not be achieved by further transformations data were analysed with Friedman's non-parametric ANOVA with time as a block factor in GenStat © 8.1 (2005). Since this was not possible in the experiment on seed attack (only two factor levels), here separate H tests were done and corrected for multiple comparisons after Holm-Bonferroni (Holm 1979). To test for differences between species on the same substrate and between substrates in *Hypericum perforatum* and *Agrostis capillaris*, only the final germination rates in the control treatments on all substrates were included into the statistical analysis. If all assumptions were met, a one-way ANOVA was used; otherwise the Kruskal-Wallis test was performed. Pairwise comparisons were made after Tukey's HSD or Mann-Whitney, respectively.

3. Results

3.1. Germination and treatment effects on artificial substrate Lab1:1

The species differed significantly in final germination rates (after two weeks under germination conditions), with the highest percentage in *V. cracca* (81.3%) and the lowest in *A. capillaris* (11.7%). Final germination rates for *P. major* and *H. perforatum* were 24.3% and 62.4% respectively (see Fig. 1).

Only for *H. perforatum* the statistical analysis revealed a significant treatment effect on germination rate (see Tab. 3). This was due to a significant difference between control treatment and Collembola treatment (Tukey's HSD, $p = 0.033$, $n = 5$), whereas there was no difference between fungicide treated seeds and the control (Tukey's HSD, $p = 0.093$, $n = 5$).

After 7 weeks, 55% of the seeds had germinated in the control which increased to 73% in treatments where *F. candida* had been present (Fig. 1). Our fungicide treatment with chitosan must be judged ineffective to influence fungal attack: no visual reduction of fungal hyphae compared to control treatment could be detected in microcosms where seeds had been treated with chitosan.

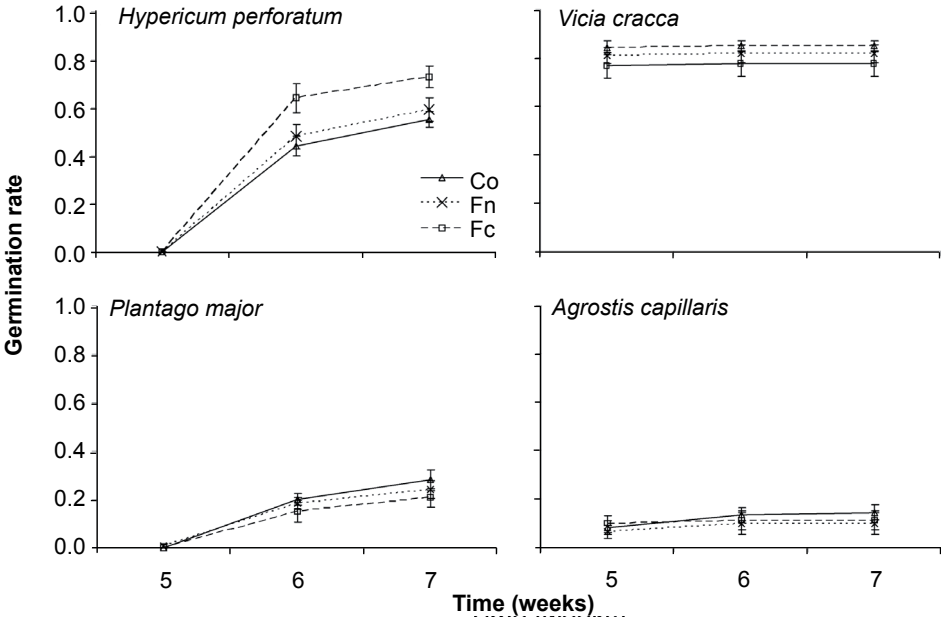

Fig. 1 Treatment effects on germination rates of the four selected plant species in **Lab** (Mean ± SE, n = 5). Co = control, Fn = fungicide treated seeds, Fc = variant with *F. candida* added.

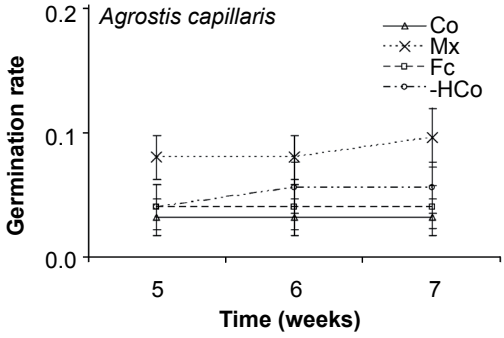

Fig. 2 Treatment effects on Field1 in *A. capillaris* (Mean ± SE, n = 5). Co = control, Mx = variant with a mixture of Isotomidae added, Fc = variant with added *F. candida*. All substrates were heat-treated except for -HCo. Note that scale is smaller compared to Fig. 1.

Tab. 3 Treatment effects on germination rate. Probability values and statistical tests are shown, n = 5 in all analyses. Significant results are marked with *. GLM rep. = General Linear Model with repeated measurements, Friedman = Non-parametric-Friedman-ANOVA, *Ac* = *A. capillaris*, *Hp* = *H. perforatum*, *Pm* = *P. major*, *Vc* = *V. cracca*.

Substrate type	Plant species			
	Ac	*Hp*	*Pm*	*Vc*
Lab	p = 0.731 (GLM rep.)	p = 0.031 * (GLM rep.)	p = 0.417 (GLM rep.)	p = 0.605 (GLM rep.)
Field1	p = 0.149 (GLM rep.)	p = 0.223 (Friedman)	-	-
Field2	p = 0.060 (Friedman)	p = 0.223 (GLM rep.)	-	-

Tab. 4 Effects of soil properties on germination rates (matrix of pairwise comparisons). Probability values and statistical tests are shown, n = 5 in all analyses. Significant results are marked with *. *Ac* = *A. capillaris*, *Hp* = *H. perforatum*.

Substrate type	Plant species			
	Ac		*Hp*	
	Lab	**Field1**	**Lab**	**Field1**
Field1	p = 0.032 * (Mann-Whitney)		p = 0.016 * (Mann-Whitney)	
Field2	p = 0.095 (Mann-Whitney)	p = 0.151 (Mann-Whitney)	p = 0.032 * (Mann-Whitney)	p = 0.690 (Mann-Whitney)

3.2. Germination and treatment effects on natural soils / relevance of soil type

Unlike on artificial substrate, no significant treatment effect on germination could be shown either for *A. capillaris* or for *H. perforatum* in the two grassland soils (Tab. 3). But when treatments -HCo and Fc were excluded from the analysis of *A. capillaris* on **Field1**, the germination rate tended to be higher in treatment Mx than in the control (GLM repeated measurements, p = 0.062, see Fig. 2).

Combining the data of the two experiments, there were significant effects of substrate type (including associated water content) on the final germination rate of *A. capillaris* and *H. perforatum* (Kruskal-Wallis test, p = 0.029 for *Ac* and p = 0.020 for *Hp*). Pairwise comparisons revealed a divergent outcome for the two species: *A. capillaris* had its highest germination rate on the artificial soil (11.7 % compared to 5.6 % on **Field1** and 6.4 % on **Field2**). These differences were significant between **Lab**/**Field1** but not between **Lab**/**Field2** and **Field1**/**Field2** in *A. capillaris* (Tab. 4). In contrast *H. perforatum* had its lowest germination rates on the artificial substrate **Lab** (62.4 % compared to 72.0 % on **Field1** and 65.9 % on **Field2**). Those differences were significant for **Lab** compared to **Field1** and **Field2** (see Tab. 4).

3.3. Seed attack

In addition, one really unexpected incidence could be observed on the artificial substrate during the experiment: In variants with seeds of *P. major*, already after five weeks of incubation, *F. candida* showed clear feeding activity on the mucilaginous cover of the seed coat as well as on the embryo of single seeds (Fig. 3). This corresponds to germination rates which were slightly, though not significantly, lower in presence of *F. candida* (Fig. 1).

The subsequent experiment revealed, first of all, a much higher germination rate of *P. major* than in the first experiment: almost 100% of the seeds had germinated (note that Fig. 4 is therefore shown in a different scaling of the y axis). Since data could not be transformed to normal distribution and Friedman's ANOVA requires at least three factor levels, the following statistical test results refer to separately performed Kruskal Wallis' H tests. After six weeks,

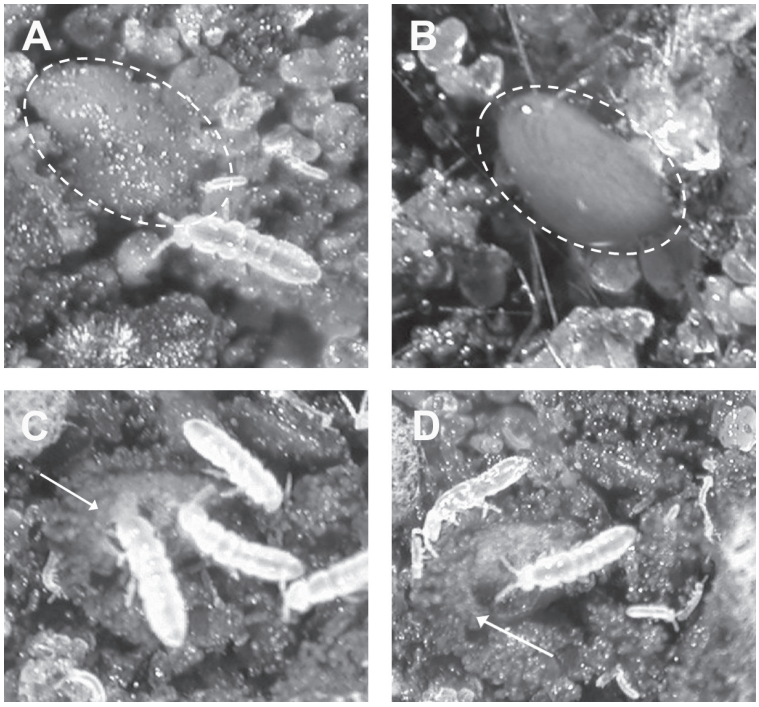

Fig. 3 Photographs of *F. candida* feeding on seeds of *P. major*. **A** shows the changes of the seed surface in presence of Collembola compared to seeds in **B** in control treatments. Seeds are framed with dotted lines. **C** and **D** show individuals of *F. candida* feeding on seeds of *P. major*. Arrows point to holes in the seed coat due to feeding activity.

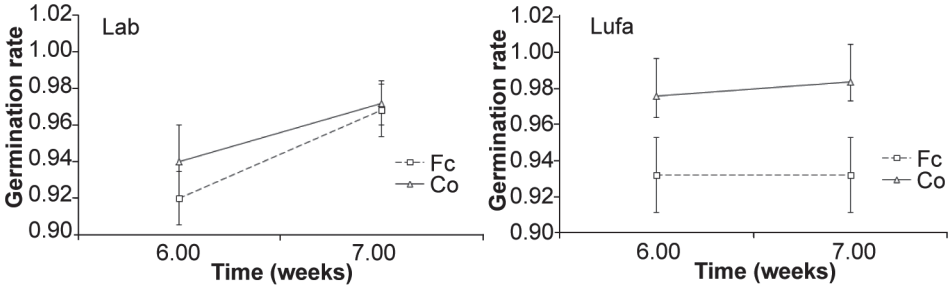

Fig. 4 Treatment effects on germination rates of *Plantago major* in **Lab** and **Lufa** (Mean ± SE, n = 10). Co = control, Fc = variant with *F. candida* added. Note that scale is smaller compared to Fig. 1 and 2.

the substrate type had no influence on germination rate ($p = 0.128$, $n = 20$), yet in presence of Collembola germination rates of *P. major* were slightly, but significantly lower than in the control ($p = 0.047$, $n = 20$, Fig. 4). After 7 weeks, the Collembola effect had disappeared ($p = 0.798$, $n = 20$), yet by now somewhat more seeds had germinated in **Lab** than in **Lufa** soil ($p < 0.001$, $n = 20$, Fig. 4). Following Holm-Bonferroni correction, the soil effect remains significant whereas the Collembola effect after six weeks drops below the significance threshold.

4. Discussion

4.1. Influence of Collembola on germination rates

Due to the fact that our fungicide treatment was apparently ineffective in reducing fungal growth on the seeds and in their surroundings it cannot be used as comparative measure to reveal the relevance of fungal attack in seed germination. Mitschunas et al. (2006) had shown in a similar experimental set-up that alterations in germination rates could be explained by reduced fungal-induced seed mortality in presence of Collembola, although they encountered the same problem with the fungicide. If we claim the equivalent for our results, a reduced detrimental effect of fungi could have led to higher seedling emergence in *H. perforatum* in artificial substrate. For *Vicia cracca* the early and fast germination in our experiment might have prevented a detrimental effect of fungi to the seeds. Schafer and Kotanen (2003) reported similar observations with the fast germinating species *Bromus inermis* in an experiment investigating differences in fungicide effects between habitats.

Small seeds are generally believed to be more susceptible to fungal attack (Crist & Friese 1993) and we observed an apparent reduction in fungal hyphae in the surroundings of the seeds in presence of *F. candida*. In our experiment only a very small percentage of *Agrostis capillaris* seeds germinated in all treatments, which is probably the reason why the higher germination rate in Fc treatments had only weak statistical support.

Plantago major was the only seed species where germination rates in presence of *F. candida* were lower than in the control (Fig. 1, 4). Again, in experiment 1 overall germination rates were rather low and dormancy or inadequate germination conditions possibly hindered the detection of significant differences.

In our study substrate quality proved to be important for total germination rates of *Hypericum perforatum* and *Agrostis capillaris* as well as for the extent to which Collembola affected germination rates of plants, both positively and negatively.

The importance of seed mortality due to fungal pathogens varies between different habitats (Blaney & Kotanen 2001). Especially moisture seems to be a prominent factor for the relevance of fungal attack to seed mortality (Leishman et al. 2000, Schafer & Kotanen 2003, Wagner & Mitschunas 2008). This is partly corroborated by our results in *Hypericum perforatum*: On **Field1**, which had a lower water content as **Lab**, the highest proportion of seeds germinated but the presence of *F. candida* did not affect the outcome. On **Lab** germination rates were lower but increased in presence of *F. candida*. This reduction of detrimental effects of fungi on seeds of *H. perforatum* may be due to more unfavourable conditions for microbiota in general or due to the presence of another subset of the microbial community, which is less harmful to the selected seeds. Different studies dealing with the susceptibility of seeds to fungi revealed that fungal attack differed strongly with respect to the seed species (Crist & Friese 1993, Leishman et al. 2000). The decomposition and pathogenesis of specific seeds was highly dependent on the fungi attacking the seeds (Schafer & Kotanen 2004). In contrast to

Lab, grazing by *F. candida* did not affect survival and germination of *Hypericum perforatum* in natural soils. If this indirect effect disappeared because the fungi in the natural soils were less relevant for seed mortality or because other factors were involved (e.g. changes in local nitrogen concentrations on a small scale via collembolan feeding activity) requires further investigation. Moreover, selectivity of fungi and specificity of fungal attack basically decide over quality and quantity of food for potential grazers.

The slightly positive effect on germination rate in presence of the mixture of Isotomidae (Mx) showed that under more adequate germination conditions for seeds Collembola might foster the germination rate of *A. capillaris* and possibly could be more effective in species mixtures. However, due to the weak data support in our study, more studies are necessary to support this proposition.

4.2. Seed attack

Seeds are highly abundant even in farmland soil, especially in the upper 30 cm (Koch 1969). But so far it has not been reported in the literature that Collembola also use seeds as a food source. Hopkin (1997), Rusek (1998), Filser (2002) and Petersen (2002) reviewed a wide range of literature on Collembolans. Although they came to the conclusion that, taken as a heterogeneous group, Collembola are omnivorous, none of them mentioned that seeds could possibly be a part of the diet of Collembolans. Furthermore 'web of science' does not give any results for searches with the keywords 'granivory + Collembola', 'seed predation + Collembola', 'seed + Collembola' and similiar. The only existing report is hidden in an Australian book and was indicated to us by the author herself after presenting these results on a conference: Greenslade (2006) described significant damage by *Protaphorura fimata* on poppy seeds in Australia, 'sometimes completely preventing emergence by feeding on the germinating seeds'.

Even if predation by invertebrates usually plays a minor role in post dispersal seed predation compared to rodents (Hulme 1998), it seems possible that due to the common methods (cages to exclude predators of specific sizes, as in Clark & Wilson 2003) the impact of soil-inhabiting invertebrates might be underestimated (Hulme 1998). *Plantago major* produces myxospermous seeds that develop a mucilaginous layer outside the seed coat when moistened. This layer primarily consists of polysaccharides with high molecular weight (Morton 1990). In the case of *P. major*, these are mainly heteroxylanes (Samuelsen et al. 1999). As energy-rich polysaccharides are known to be a high quality food for microorganisms in the rhizosphere (Whipps & Lynch 1983), this opens also the opportunity that *F. candida* actually fed on the adherent microorganisms rather than on the mucilage itself. The same has been proposed for Collembola feeding on plant detritus (Hopkin 1997). However, the pictures show that at times also the seed embryo of *P. major* had been consumed by *F. candida*. *P fimata* seemed unable to gnaw through the thick coat of the poppy seeds but once the shell cracked with moisture they would completely demolish the seed inside (P. Greenslade, pers. comm.). In respect to the high energy content of myxospermous seeds and the seed itself it seems persuasive that Collembola which are supposed to 'chose the best preferred food present in immediate vicinity and adjust the time spent to a given spot to the amount and quality of that food' (Petersen 2002) also use those seeds as a food source.

Also here the substrate quality influenced the effect of Collembola: it was more pronounced in **Lufa** than in **Lab** soil (Fig. 4). Since the water holding capacity of both substrates was comparable and the natural **Lufa** contains more alternative food sources than **Lab** one would rather expect the opposite. It is possible that pH differences (5.3 in **Lufa** vs. 6.5 in **Lab**) played

a role, perhaps by softening the seed shell under more acidic conditions, thus making the embryo itself easier accessible to the Collembola.

4.3. Conclusions

Our data give additional support to the results of Mitschunas et al. (2006, 2008), again showing that Collembola have the potential to increase the proportion of germinating seeds in specific grassland plants – but this time we showed that they can also have a negative impact. At the same time our study adds soil quality as an important factor in the relationship between seed germination and collembolan feeding activity. The set of organisms in terms of Collembola species, plant species and the microbial community which is associated with soil characteristics seems to be of high importance for the strength and the possibility of those interactions. Studies which investigate aspects as nitrate concentration, water content and fungal community are now necessary to extract main influencing variables. Approaches with species mixtures of Collembola should be preferred over those with single species to understand such interactions and their relevance under real outside conditions.

Finally our observation of feeding activity of *Folsomia candida* on seeds opens a new chapter about how Collembola have the potential to take influence on the aboveground plant community. In the present study the impact of *F. candida* on *P. major* emergence was almost negligible, yet Greenslade's (2006) observations on *P. fimata* – which is also known as a pest in sugar beet seedlings (Ulber 1980) – hint at a quite drastic potential of Collembola to affect seedling emergence. Further research must clarify if this occurs only under laboratory conditions and at exceptional situations (e.g. particular seeds, crops, monocultures) or if it can be *de facto* of ecological importance.

5. Acknowledgements

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6. References

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