Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field

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Abstract

Effects of foliar and soil insecticide applications on collembolan density and community structure were investigated in an early set-aside arable field. Insecticides were applied separately and in combination to the soil surface (chlorpyrifos) and vegetation (dimethoate). The treatments were established to investigate effects of above- and below-ground insects on plant succession. Starting in 1997, the insecticides were applied from April to November at 2-week (dimethoate) or monthly intervals (chlorpyrifos). Samples were taken in 2000 prior to and after insecticide application in March and June, respectively. Both insecticides are lethal to Collembola and insecticide applications resulted in a strong decline in the density of total Collembola. Application of chlorpyrifos reduced collembolan density to a greater extent than dimethoate; the effect of the combined application on total collembolan numbers was similar to that of chlorpyrifos only. Collembolan numbers recovered after the insecticide applications in 1999, but in the treated plots populations were still reduced in March 2000 before the re-application of insecticide treatments in that year. The insecticide applications changed the dominance structure of the collembolan community, but had no effects on species composition. The results may be of relevance for the interpretation of studies on plant–insect herbivore interactions using insecticides.

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1. Introduction

Plant roots and soil invertebrates are intimately linked as components of the below-ground food web. Single plants and plant communities may suffer from herbivorous insects feeding on roots. In contrast to herbivores, the effects of decomposer invertebrates on plant growth are less apparent. Decomposers affect plant growth and the structure of plant communities by a variety of mechanisms including mineralization of nutrients, grazing on rhizosphere microorganisms and by modifying soil structure, i.e. by forming the root environment (Scheu and Setälä, 2002; Wardle, 2002). Collembola are among the most abundant soil arthropods and play an important role in decomposer
food webs (Butcher et al., 1971; Petersen and Luxton, 1982; Petersen, 2002).

Collembola are known to be food generalists (Hopkin, 1997; Scheu and Folger, 2004). The diet of most species is composed of a mixture of detritus, algae, bacteria and fungi, and varies with season (Wolters, 1985). Due to their feeding activity, Collembola affect decomposition processes and the microstructure of the soil (Seastedt, 1984; Cragg and Bardgett, 2001). The modification of decomposition processes results in changes in nutrient mineralization and may ultimately affect plant growth (Moore et al., 1987; McGonigle, 1995; Gange, 2000). Bardgett and Chan (1999) and Scheu et al. (1999) showed that the presence of Collembola resulted in changes in plant growth and plant shoot N contents. The effects of Collembola on decomposition processes and nutrient mineralization may depend on species composition and dominance structure of collembolan communities, since different species of Collembola have different feeding preferences (Mebes and Filser, 1998; Cole et al., 2004; Chahartaghi et al., 2005).

Collembola influence plant growth not only by altering decomposition processes and nutrient cycling, but also by feeding on fungi and modifying microbial community structure and function. Feeding on mycorrhizal fungi may detrimentally affect plant growth as documented by McGonigle and Fitter (1988). However, Klironomos et al. (1999) and Gange (2000) assumed that Collembola prefer to feed on saprophytic rather than mycorrhizal fungi and moderate feeding on mycorrhiza has been shown to stimulate mycorrhizal activity resulting in an increase in plant growth (Ek et al., 1994; Lussenhop, 1996). Similarly, moderate grazing on saprophytic fungi by Collembola has been found to stimulate fungal growth (Hanlon and Anderson, 1979).

If arable land is left uncultivated, plant community composition changes rapidly (Horn, 1974; Van Andel et al., 1993). Parallel to plant secondary succession, collembolan density, biomass and community composition change (Parr, 1978; Petersen, 1995) with biomass reaching up to 10% of total soil animal biomass in early successional stages (Petersen, 1994). Therefore, Collembola may affect the succession of plant species. In fact, it has been documented that excluding soil insects alters plant succession by fostering annual forbs and retarding colonisation by grasses (Brown and Gange, 1989). However, this has been uniformly ascribed to a reduction in below-ground insect herbivores, neglecting the potential effects of decomposer insects, such as Collembola (Ganade and Brown, 1997; Mortimer et al., 1999).

This study formed part of an experiment that focused on the effects of herbivorous insects on secondary plant succession (Schädler et al., 2004). The experiment was established on an arable field near Halle (Germany) in 1998. To investigate the effect of herbivores on plant succession, insects were excluded by insecticide application. Soil invertebrates, particularly Collembola, were investigated to evaluate whether the effects of insecticide applications may feed back to plants via changes in the decomposer community.

Collembola are known to be vulnerable to insecticides (Frampton, 1994). Dimethoate used to exclude above-ground herbivores in the experiment of Schädler et al. (2004) is known to detrimentally affect collembolan species (Martikainen et al., 1998). Chlorpyrifos used to exclude root-feeding herbivores is also known to be toxic for Collembola (Frampton, 1997).

2. Materials and methods

The experiment was established on a set-aside arable field near Halle (Thuringia, Germany). Within an area of 98 m × 28 m, a total of 96 experimental plots of 3 m × 3 m were set up. Above- and below-ground insects were reduced by applying dimethoate (Perfekthion, 40% w/w; 2 ml in 1 l water, 170 ml per plot; BASF, Ludwigshafen, Germany) to the vegetation and chlorpyrifos (Hortex, 2% w/w; 45 g in 1 l water, 1 l per plot; Celaflor, Dow Agrociences LCC, USA) to the soil surface, respectively. Both insecticides function as contact poisons. A combined treatment of insecticides was applied to exclude both above- and below-ground insects. Twelve replicate plots of each treatment were arranged in a randomised complete block design. Starting in 1997 the insecticides were applied every 2 weeks to the vegetation and every month to the soil from April until November. Control plots were sprinkled with the same amount of water at the times of dimethoate application. Further details of experimental methods are given in Schädler et al. (2004).
The fauna were sampled in March 2000 prior to the first insecticide application of that year and 2 months later in June 2000. The first sampling date was assumed to reflect the chronic response of the collembolan community including recovery from insecticide application in winter. The second sampling was assumed to reflect in addition the immediate (short-term) effect of the insecticides. One soil core per plot of 21 cm diameter was taken and divided into a litter layer, 0–3 cm and 3–6 cm soil depth in the field. In the laboratory, soil invertebrates were extracted by heat (Kempson et al., 1963), collected into dilute picric acid and transferred into ethanol (70%). Collembola were separated from sample debris by addition of n-heptane; the animals concentrating in the n-heptane phase due to their hydrophobic surface while soil particles sank into the ethanol phase. The samples were spread evenly over a filter paper marked into nine sections of 1 cm width. The Collembola in two central sections were counted and the total number in the sample was estimated according to sub-sample ratios established in preliminary experiments. Collembola in the counted sub-sample were determined to genus level, except for sminthurids that were determined to family level. Collembola were identified according to Fjellberg (1980). The genus Parisotoma was not distinguished from Isotoma in the study.

Data on the numbers of total Collembola and individual genera were log-transformed and analysed by two-factor ANOVA and MANOVA, respectively, with foliar insecticide (with, without) and soil insecticide (with, without) as factors. Data on numbers of Collembola on the first and the second sampling date were compared by repeated measures MANOVA.

Data on the density of individual genera were analysed by two-factor ANOVA if the MANOVA suggested that the response varied between taxa. Differences between means were analysed using Tukey’s honestly significant difference for balanced data and the Scheffé-test for unbalanced data (Sokal and Rohlf, 1995). A discriminant function analysis (DFA) was carried out with the abundance data of all collembolan species to investigate if the community structure differed between insecticide treatments. Squared Mahalanobis distances between group centroids were calculated to evaluate significant differences among insecticide treatments. ANOVAs and DFA were implemented in SAS 8.1 (Cary, Florida, USA) and STATISTICA (Version 5.0, StatSoft GmbH, Hamburg), respectively. Genera with low abundance (less than 10 individuals per sampling date) were not considered in the statistical analysis.

3. Results

Sixteen genera of Collembola were identified from the study site. The genera Isotomiella and Anurophorus were only found on the first sampling date, while the genera Onychiurus, Cryptopygus, Proisotoma and Desoria were only found at the second sampling. The genus with the highest density on both sampling dates was Isotoma, including the most common species Isotoma notabilis Schäfer.

Insecticide application significantly affected collembolan numbers on the first ($F_{3,44} = 5.68, P < 0.01; \text{Fig. 1a}$) and second sampling dates ($F_{3,44} = 26.07, P < 0.001; \text{Fig. 1b}$). Overall density of Collembola was reduced by insecticides. Comparing total numbers

![Fig. 1. Separation of experimental treatments based on the density of Collembola on the first (a) and second (b) sampling date; foliar insecticide (FI), soil insecticide (SI), combined foliar and soil insecticide (SFI) and control (C). Backtransformed means of log-transformed data. Bars with the same letter are not significantly different (Tukey’s honestly significant difference, $P < 0.05$).]
of Collembola, there was no significant difference between the two sampling dates, but an interaction between time and treatment ($F_{3,44} = 5.12, P < 0.01$). Reduction of collembolan density by insecticide application was more pronounced on the second sampling date.

The results from the first sampling date indicated that collembolan densities had been strongly affected by the foliar and soil insecticides applied in the previous years. On that sampling date the total abundance of Collembola was not significantly affected by application of the foliar insecticide but application of the soil insecticide alone, the combined application of both insecticides had not resulted in a further decrease in total collembolan density. MANOVA suggested that the reduction in collembolan numbers varied between taxa ($F_{9,34} = 6.00, P < 0.001$). Separate ANOVAs indicated that Isotoma ($F_{3,44} = 4.45, P = 0.042$) and Lepidocyrtus ($F_{3,44} = 4.29, P < 0.048$) contributed most to the overall reduction in numbers (Fig. 2); the numbers of Sminthuridae and Entomobrya were also reduced but the reduction was not significant. Compared to the foliar insecticide, the effects of the soil insecticide were more pronounced (Fig. 3).

Application of the soil insecticide resulted in the exclusion of Sminthuridae from samples ($F_{3,44} = 22.55, P < 0.001$), whereas the density of Isotoma ($F_{3,44} = 28.88, P < 0.001$), Lepidocyrtus ($F_{3,44} = 21.07, P < 0.001$) and Entomobrya ($F_{3,44} = 22.69, P < 0.001$) declined significantly in soil insecticide treatments. Other genera were not affected significantly. The decline in density of Sminthuridae and Entomobrya was most pronounced in plots receiving both insecticides (significant soil x foliar insecticide interaction; $F_{3,44} = 4.95, P = 0.039$ and $F_{3,44} = 4.47, P = 0.042$, respectively).

At the second sampling date, after the soil insecticide had been applied twice and the foliar insecticide four times, the overall density of total Collembola was further reduced. Overall, collembolan abundance was significantly reduced after application of both the foliar ($F_{3,44} = 4.70, P = 0.041$) and soil insecticide ($F_{3,44} = 67.77, P < 0.001$) (Fig. 4). The densities of Isotoma ($F_{3,44} = 6.34, P = 0.021$), Friesea ($F_{3,44} = 13.04, P = 0.002$), Entomobrya ($F_{3,44} = 6.53, P = 0.020$) and Sminthuridae ($F_{3,44} = 30.10, P < 0.001$) were significantly reduced by foliar insecticide application (Fig. 5). Furthermore, the density of Lepidocyrtus spp. tended to be reduced ($F_{3,44} = 3.87, P = 0.061$). The application of the soil insecticide resulted in a decrease in most collembolan genera,
and appeared to have eliminated species of the genera *Pseudosinella*, *Ceratophysella* and of *Sminthuridae* (Fig. 6). The densities of the genera *Lepidocyrtus* ($F_{3,44} = 185.16, P < 0.001$), *Isotoma* ($F_{3,44} = 163.89, P < 0.001$) and *Entomobrya* ($F_{3,44} = 65.82, P < 0.001$) were strongly reduced, and also the decreases of the genera *Friesea* ($F_{3,44} = 11.33, P = 0.002$) and *Folsomia* ($F_{3,44} = 9.09, P = 0.003$) were significant, but the effect was less pronounced. In contrast, application of

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**Fig. 3.** Response to soil insecticide application (SI) of the density of *Lepidocyrtus, Isotoma, Entomobrya, Sminthuridae* and of total density of Collembola on the first sampling date. Backtransformed means of log-transformed data. Bars with the same letter are not significantly different (Tukey’s honestly significant difference, $P < 0.05$).

**Fig. 4.** Response to soil (SI) and foliar (FI) insecticide application of the density of Collembola on the second sampling date. Backtransformed means of log-transformed data. Bars with the same letter are not significantly different (Tukey’s honestly significant difference, $P < 0.05$).
Fig. 5. Response to foliar insecticide application (FI) of the density of *Isotoma*, *Friesea*, *Entomobrya*, Sminthuridae and *Lepidocyrtus* on the second sampling date. Backtransformed means of log-transformed data. Bars with the same letter are not significantly different (Tukey’s honestly significant difference, $P < 0.05$).

Fig. 6. Response to soil insecticide application (SI) of the density of *Isotoma* (Isot.), *Lepidocyrtus* (Lepi.), *Entomobrya* (Ento.), *Friesea* (Frie.), *Folsomia* (Fols.), Sminthuridae (Smin.), *Pseudosinella* (Pseu.), *Ceratophyssella* (Cera.) and *Protaphorura* (Prot.) on the second sampling date. Backtransformed means of log-transformed data. Bars with the same letter are not significantly different (Tukey’s honestly significant difference, $P < 0.05$).
the soil insecticide resulted in an increase in the density of species of the genus *Protaphorura* ($F_{3,44} = 11.37, P = 0.002$).

The collembolan communities in the treatments were separated by discriminant function analysis (DFA; $F_{3,44} = 1.88, P = 0.04$; Fig. 7a, Table 1). On the first sampling date, collembolan communities in the control plots differed significantly from those in the soil and combined insecticide plots but were not different from those in the foliar insecticide treatment. At the second sampling the DFA separated all four treatments ($F_{3,44} = 9.41, P < 0.001$; Fig. 7b, Table 1). The collembolan community in the control treatment differed significantly from that in the insecticide treatments. The foliar insecticide treatment differed significantly from the control, the soil insecticide and

![Fig. 7. Separation of experimental treatments based on the structure of collembolan communities by discriminant function analysis on the first (a) and second (b) sampling date; foliar insecticide (FI; ▲), soil insecticide (SI; ■), combined foliar and soil insecticide (SFI; ●) and control (C; ●). Ellipses represent 95% confidence ranges. For statistical analysis see Table 1.](image-url)
the combined insecticide treatments. Application of the soil insecticide significantly changed the species composition, and the soil insecticide treatment and the combined insecticide treatment were separated from the control and the foliar insecticide treatment but not from each other.

4. Discussion

Soil samples were taken on the first sampling date in 2000 before insecticide applications were resumed in that year, in order to investigate the chronic response and recovery of the collembolan community following previous insecticide application in 1999. Insecticide application decreased collembolan densities in the treated plots on both sampling dates. As expected, the impact of insecticide application was stronger following resumed application of foliar and soil insecticides. Comparing total densities of Collembola on the two sampling dates, the insecticide applications led to a disproportionate decrease in collembolan numbers on the second sampling date.

4.1. Chronic response to insecticide application

Soil and foliar insecticide application decreased the density of Collembola, but the effects differed between the two sampling dates. Generally, the foliar insecticide, dimethoate, significantly decreased the density of epigeic Collembola (cf. Christiansen, 1964), including the genera Isotoma and Lepidocyrtus, and also tended to decrease the density of Entomobrya and Sminkthridae. In contrast, the density of endogeic genera remained little affected. Probably, the effect of the application of dimethoate, which functions as a contact pesticide, was restricted to surface living species and its effect penetrated little into the soil. Joy and Chakravorty (1991) reported that dimethoate is lethal to many species of Collembola; however, its effect in the field is likely to vary between species depending on exposure. Similar to the present study, Martikainen et al. (1998) reported that Tullbergia spp. are little affected by dimethoate whereas the density of Isotoma notabilis is strongly reduced. As indicated by Krog (1995), even very similar species, such as Folsomia fimetaria L. and E. candida Willem, may differ strongly in sensitivity to insecticides.

Chlorpyrifos also functions as a contact pesticide and is lethal to Collembola (Stark, 1992; Wilson et al., 1995; Frampton, 1999; Wiktelius et al., 1999). However, like dimethoate, the sensitivity of different collembolan species to chlorpyrifos varies considerably (Wiles and Frampton, 1996). In the present study, chlorpyrifos differentially affected epigeic collembolan species of the genera Isotoma, Entomobrya and Lepidocyrtus. Similarly, Frampton (1997) also found a strong decline in density of epigeic species, such as Sminttharinus elegans Fitch., Entomobrya multifasciata Tullberg and Lepidocyrtus spp. Following the application of chlorpyrifos, however, S. elegans in particular recovered quickly. In the present study, the density of Sminkthridae was still significantly reduced even 4 months after the last insecticide application. In contrast, Isotoma spp. recovered quickly, which most likely is related to the fact that the most abundant species at the study site, I. notabilis, is a species that reproduces by parthenogenesis and therefore might have a high reproduction rate and recovery potential (Petersen, 1971).

In addition to reducing overall collembolan abundance, the application of insecticides changed the dominance structure of the collembolan community. Genera such as Isotoma and Lepidocyrtus that were dominant in control plots were reduced by 70–90% in chlorpyrifos treatments. In contrast, species of the genus Protaphorura reached their highest density in chlorpyrifos plots. Despite the marked differences in dominance structure, there was no effect on species composition of insecticide application, in contrast to findings of Martikainen et al. (1998) and Frampton (2000) which suggested that application of both

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** P < 0.005.
*** P < 0.001.
dimethoate and chlorpyrifos may alter species composition of Collembola. The lack of evidence for such effects in the current study might be due to the fact that the insecticide treated plots were open to recolonization by Collembola whereas the experiment by Martikainen et al. (1998) was conducted in microcosms in the laboratory which precluded recolonization. Recolonization depends on the size of the disturbed area and the mobility and life cycle of the collembolan species involved (Duffield and Aebischer, 1994). Due to their mobility, epigeic species have a high recolonization potential; in fact, epigeic species generally had reached densities comparable to the control on the first sampling date. Compared to epigeic species, the migration rate of endogeic species is probably lower (Hertzberg, 1997), and consequently the effects of insecticides on them were more persistent. Density recovery of all collembolan genera in the treated plots is more likely due to immigration than a result of reproduction.

In contrast to other Collembola, the density of *Friesea* and *Protaphorura* in chlorpyrifos treatments exceeded that of the control and species of these genera dominated in the insecticide plots. Presumably, by living deeper in the soil *Protaphorura* spp. escaped contact with the insecticides used and in addition could have benefited through release from competition with other species of Collembola. Information on competitive interactions among collembolan species is scarce but Hägvar and Abrahamsen (1980) and Hägvar (1990) documented that in disturbed areas a few species can reach high densities, which in part may be due to lack of competition.

4.2. Immediate response to insecticide application

In June, shortly after the second application of the foliar and the fourth application of the soil insecticide in 2000, the density of Collembola was strongly reduced compared to the first sampling in March. Generally, similar species were affected, but in contrast to the first sampling the foliar insecticide also reduced the density of *Friesea* spp. As in the first sampling, the densities of *Protaphorura* spp. were significantly increased by the soil insecticide treatment, supporting our conclusion that these species benefited from release from competition with other species of Collembola.

Overall, the soil insecticide caused such a strong change in the composition of the collembolan community that the additional application of foliar insecticide had no further effect. In fact, the soil insecticide application virtually eradicated most species of Collembola.

In addition to direct toxicity, both insecticides may have affected the density of Collembola indirectly. Some predators that were not affected by insecticide application might have profited from a release from competition and reduced collembolan densities. Furthermore, densities of competitors for food sources might have increased and caused a further decline in collembolan numbers. Collembola are able to perceive insecticides, such as dimethoate, and it has been documented that *F. fimetaria* avoids contact by retreating from insecticide contaminated sites (Fabian and Petersen, 1994; Petersen and Gjelstrup, 1998). Therefore, emigration of Collembola out of the insecticide plots may have contributed to the observed decline in density after insecticide application.

The study showed that insecticide applications strongly affect the structure of the decomposer community. Results from studies using insecticides to manipulate above- and below-ground herbivores (Masters et al., 1993; Gange and Brown, 2002; Schädler et al., 2004) therefore have to be interpreted with caution. Changes in plant growth, plant competition and plant succession may not exclusively be due to reduced damage caused by herbivores, but also may be affected by decomposer-mediated changes in decomposition processes and nutrient cycling.

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