Oecologia (2008) 157:399–407
DOI 10.1007/s00442-008-1094-y

PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Trophic cascades initiated by fungal plant endosymbionts impair reproductive performance of parasitoids in the second generation

Simone A. Härri · Jochen Krauss · Christine B. Müller

Received: 27 November 2007 / Accepted: 4 June 2008 / Published online: 18 July 2008

Abstract Variation in plant quality can transmit up the food chain and may affect herbivores and their antagonists in the same direction. Fungal endosymbionts of grasses change the resource quality by producing toxins. We used an aphid-parasitoid model system to explore how endophyte effects cascade up the food chain and influence individual parasitoid performance. We show that the presence of an endophyte in the grass *Lolium perenne* has a much stronger negative impact on the performance of the parasitoid *Aphidius ervi* than on its aphid host *Metopolophium festucae*. Although the presence of endophytes did not influence the parasitism rate of endophyte-naïve parasitoids or their offspring's survival to adulthood, most parasitoids developing within aphids from endophyte-infected plants did not reproduce at all. This indicates a delayed but very strong effect of endophytes on parasitoid performance, which should ultimately affect plant performance negatively by releasing endophyte-tolerant herbivores from top-down limitations.

Keywords Bottom-up cascades · Endophyte · Indirect effects · Multi-trophic interactions · *Neotyphodium lolii*

C. B. Müller deceased 7 March 2008.

Communicated by Jay Rosenheim.

S. A. Härri · J. Krauss · C. B. Müller
Institute of Environmental Sciences, University of Zürich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

J. Krauss
e-mail: jochen.krauss@uni-bayreuth.de

J. Krauss
Department of Animal Ecology I, Population Ecology, University of Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany

S. A. Härri (✉)
Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1
e-mail: shaerri@uoguelph.ca

Introduction

Natural communities and ecosystems have a huge diversity of species, which corresponds with an enormous diversity of interactions among species. To understand the nature and magnitude of these interactions among species within whole communities it is important to estimate the impact of species loss on ecosystem functioning (McCann 2007). Such biotic interactions can be direct, involving only two species (e.g. plant–herbivore interactions), or they can be indirect, involving more than two species (e.g. trophic cascades, apparent competition; Strauss 1991). Indirect effects are common and important for structuring natural communities but are only detectable by experimentation (Holt and Lawton 1993; Müller and Godfray 1999; Werner and Peacor 2003). Indirect effects propagating upward or downward through a food chain are called trophic cascades and may be common in many ecological communities (Carpenter et al. 1985; Pace et al. 1999; Schmitz et al. 2000). Classically, trophic cascades are defined as "top-down" when the removal of predators results in decreased plant abundance through release and increase in herbivore numbers (Hairston et al. 1960; Pace et al. 1999). For example, the removal of largemouth bass, a top predator in prairie streams, leads to a decrease in algal abundance caused by an increase in herbivorous minnows (Power et al. 1985). However, cascading indirect effects can also propagate upwards or "bottom-up".

Bottom-up cascades are generally less well studied, but are common in terrestrial systems as a structuring force (Hunter and Price 1992; Denno et al. 2002). In contrast to top-down cascades, most studies on bottom-up cascades show directional responses that are the same for herbivores and their natural enemies, i.e. poor plant quality decreases herbivore population density as well as natural enemy performance and good plant quality enhances herbivore abundance as well as natural enemy performance (Hunter and Price 1992; Nakamura et al. 2005; Kagata and Ohgushi 2006). For example, Nakamura et al. (2005) showed that increased foliage sprouting after a flood increased the abundance of leaf beetles and their natural enemies. It has also been shown that bottom-up cascades can be triggered by allelochemical compounds of plants, but here the cascading effects on natural enemies are reported to be weak or absent (Kagata and Ohgushi 2006; but see Soler et al. 2005; Ode 2006). This interruption of cascading effects by allelochemicals can be caused by herbivorous insects that are resistant to the plant's defensive toxins or by herbivores accumulating plant secondary compounds within their own body tissue and using them for their own defence against natural enemies (Barbosa et al. 1991; Francis et al. 2001). Chemical defences are not produced exclusively by plants themselves, but also by symbiotic associations of plants with microbes, as is the case for many grasses that are poorly protected by secondary plant compounds but frequently associate with systemic, seed-borne endophytic fungi that produce herbivore-toxic compounds (Clay 1990; Bush et al. 1997).

Endophytic fungi (= endophytes) live intercellularly in leaf and stem tissue (Clay 1990; Schardl et al. 2004). The consequences of this grass–fungus alliance for herbivores are fairly well understood, especially for endophytes of the genus *Neotyphodium* found in association with cool-season grasses, where they reduce herbivore performance in most cases (Faeth and Bultman 2002; Hunt and Newman 2005; Meister et al. 2006). A few studies have shown that grass symbionts can trigger bottom-up cascades and alter the performance of and the interactions among consumers and their natural enemies at an individual, population or community level (Omacini et al. 2001; Finkes et al. 2006; de Sassi et al. 2006). However, experimental studies on endophytes and their effects on higher trophic levels are still rare (Faeth and Bultman 2002; Müller and Krauss 2005). Existing studies have generally found negative effects on herbivores and no or negative effects on the associated natural enemies (Barker and Addison 1996; Bultman et al. 1997, 2003; Goldson et al. 2000; de Sassi et al. 2006). However, no study so far has investigated the effects of endophytes on the reproductive ability of parasitoids developing within herbivores feeding on endophyte-infected plants. Other studies on endophytes and parasitoids have mainly concentrated on the parasitism rate (= attack rate) of endophyte-naïve individuals and on developmental time and survival to adulthood of their offspring (Barker and Addison 1996; Bultman et al. 1997, 2003). Here, we additionally examine fecundity traits (i.e. fitness) of parasitoids because changes in fecundity traits of individuals will ultimately determine the dynamics of whole populations. In addition, we do not restrict the study to simple parasitoid responses but follow the offspring generation through its development and its own reproductive performance. This allows for the detection of possibly delayed effects of endophytes on parasitoid fitness. The effects of endophytes on natural enemies may be: (1) direct (fungal-derived toxins accumulate in the prey or host tissue and directly harm predators and parasitoids); (2) indirect via changes in herbivore densities (density-mediated); or (3) indirect via changes in life histories, body size and/or the behaviour of herbivores (trait-mediated; Wootton 1993; Abrams 1995; van Veen et al. 2006).

By manipulating endophyte presence in the basal resource, we can compare multi-trophic interactions among consumers and their enemies on grasses with and without endophytes. This approach allows studying changes in basal resources and the associated cascading effects upwards in the food chain. The aphid-parasitoid system proves to be a good model system to test the effects of endophytes on several trophic levels. Aphids feed directly on the phloem sap of the plants and are thus intimately affected by the host plant quality (Dixon 1998) and possibly by its infection status. Parasitoids are also intimately linked to their aphid host's metabolism as their whole development occurs within the host's body (Godfray 1994) with potentially close contact to accumulating toxins in host tissue and haemolymph. Measurements of attack rates, longevity and reproductive success of parasitoids allow a direct link to be made between life history traits and expected population densities and dynamics.

Here, we tested for the effects of the endophytic fungi *Neotyphodium lolii* Glen, Bacon and Hanlin, a fungal endosymbiont of the pasture grass *Lolium perenne* L., on individual life history traits of the cereal aphid *Metopolophium festucae* Theobald and on fitness estimates of its primary parasitoid *Aphidius ervi* Haliday (Braconidae: Aphidiinae). This food chain occurs naturally on wild grasses, even though the tested grass-endophyte association is of agricultural origin. We followed parasitoids over two generations; the endophyte-naïve parental generation and the first offspring generation that developed within aphids feeding on either endophyte-free or on endophyte-infected grass. For the parental generation, we measured differences in attack rates, and in the first offspring generation we tested several life history traits including fecundity (= lifetime reproductive success). To separate the effects of endophytes

on parasitoid fecundity from those on oviposition decision behaviour per se, females of the first offspring generation were all offered aphids from endophyte-free plants. We hypothesized that herbivores reared on endophyte-infected grass and primary parasitoids emerging from these herbivores both have fitness disadvantages that are reflected in life history traits. We found that endophyte infection had a much stronger impact on parasitoids than on aphid hosts. Parasitoids developing within hosts from the endophyte-infected environment suffered from strongly reduced reproductive performance.

Materials and methods

L. perenne seeds were provided by Brian Tapper (AgResearch, New Zealand). All seeds were the Grassland Samson cultivar and were either uninfected (E−; identity number, 11104; <0.01% infection) or infected with the common wildtype *N. lolii* endophyte (E+; identity number, A12038; 89% infection). The infection status of the seed batches was checked with a combination of microscopic examination of stained seeds and immunoblotting of stems (see Härri et al. 2007). The stock culture of *M. festucae* was started in summer 2005 with a few individuals collected from *L. perenne* near the University of Zürich, Switzerland. This aphid culture was maintained on commercially available endophyte-free fodder grass, *L. perenne* Arion (Fenaco, Winterthur, Switzerland; staining of 30 seeds, 0% infection).

A. ervi belongs to the subfamily Aphidiinae, which are solitary koinobionts that attack the nymphal stages of aphids and develop within the still growing hosts. The killing of the aphid and the formation of the mummy, in which the parasitoid larvae pupates, occur after the aphids reach adulthood or in a late nymphal stage (Godfray 1994). The stock culture of *A. ervi* was started with 250 individuals bought from Andermatt Biocontrol (Grossdietwil, Switzerland). *A. ervi* was kept on *M. festucae* feeding on endophyte-free *L. perenne* Arion. All insect cages and experiments were kept in controlled environment chambers at 22°C with a light:dark 16:8-h light regime.

Life history of the aphid *M. festucae*

To test for the effects of *N. lolii* on individual life history traits of *M. festucae*, single first instar nymphs were each followed for their whole life on cuttings of either E− or E+ *L. perenne*. Cuttings were used instead of potted plants to follow individual aphids over their entire lifespan, as individual aphids are easily lost on potted plants. Additionally, grass cuttings are easier to handle and allow for more uniform conditions for each aphid. We assume that the effects of the cuttings on aphid performance are relatively similar for E− and E+ plants. We replicated each treatment 20 times and cuttings were exchanged every second day. The measured life history traits were: (1) developmental time (time from first instar nymph to a mature adult); (2) fecundity (total number of offspring produced during an adult's life); (3) daily fecundity (mean number of nymphs produced per day during the period of adult life); and (4) lifespan (here defined as reproductive lifespan, i.e. the number of days as adult). After death, the hind tibia length was measured for each mother to assess possible effects on body size. Body length and hind tibia length have been shown to be highly correlated for a related aphid species (Meister et al. 2006). Tibia length measurements were also taken for the first ten nymphs of each mother, at the age of maximally 1 day after birth.

Life histories of the parasitoid *A. ervi*

The duration of the experiment covered three parasitoid generations: the parental generation and the first and second offspring generation. This allowed us to measure lifetime reproductive success of parasitoids and possible delayed effects on parasitoid fitness developing on aphids from E− and E+ *L. perenne*. Parasitoids of the parental generation originated from a stock culture that had no previous experience with aphids from E+ grasses. The parental generation parasitoids were offered aphids feeding on either E− or E+ plants. The resulting first offspring generation parasitoids thus developed either in aphids feeding on E− (E− offspring) or aphids feeding on E+ (E+ offspring). The first offspring generation parasitoids (E− and E+ offspring) were offered aphids feeding on endophyte-free *L. perenne* Arion. Their progeny is referred to as the second offspring generation. All the potted plants used in the experiments were covered with an inverted polyethylene bottle that had two windows covered with mesh for ventilation.

Parental generation

The parental generation was collected as parasitized aphids (= mummies) from the stock culture. After emergence, females were allowed to mate for 12 h with two males of the same age in a plastic vial sealed with a foam stopper (5 cm × 2 cm). The mating individuals were provided with a piece of apple. This procedure was repeated 40 times to obtain 20 females for each of the two endophyte treatments (E− and E+). The female together with the two males were then placed on a pot (E− or E+; pot diameter = 10 cm; 100 seeds per pot; 6 days old) with an ad libitum number of aphids (approximately 80–120 aphids) and left for 24 h. After parasitoid removal, the pots were left for 7 days after which aphids were transferred to a fresh pot of grass of the

respective treatment (E− or E+; pot diameter = 10 cm; 100 seeds per pot; 6 days old). After another 7 days, all mummies were removed and placed singly into gelatine capsules. The mummies in the gelatine capsules were checked twice a day for emergence of the first offspring generation.

The recorded life history traits of the parental generation were: (1) the proportion of females (mothers) producing mummies, (2) the number of mummies produced per mother, (3) the proportion of mothers producing viable offspring, (4) the number of offspring produced per mother, and (5) the sex ratio of their offspring (proportion of males).

First offspring generation

The fecundity of all female parasitoids produced by the parental generation was tested on an ad libitum number of aphids (approximately 80–120 aphids) feeding on endophyte-free *L. perenne* Arion (pot diameter = 10 cm; 100 seeds per pot; 6 days old). The parental generation produced 42 E− and 25 E+ females. These females were mated with one similarly aged, preferably unrelated male from the same endophyte treatment, avoiding brother–sister matings. There were not enough males of the same age and treatment to match all the females, so only 32 E− and 15 E+ females were mated. Pairs or single females were kept for half a day in a plastic vial without the addition of sugar-rich food to ensure mating. Thereafter the females were transferred singly onto the pots with the aphids. After 24 h, the female parasitoids were transferred to a fresh pot with ad libitum *M. festucae*. This was repeated every 24 h until the female died. Most of the females died within the first 24 h. This resulted in too little variation in the data on longevity to allow for statistical analysis of these data. The short lifetime was probably caused by the absence of sugar-rich food in the experimental pots and the relatively small size of *M. festucae*. Sugar-rich food was not provided as we would not have been able to control for the amount of sugar-rich food each parasitoid consumed.

The pots with the potentially parasitized aphids were left for 7 days before transferring all aphids onto a fresh pot of endophyte-free *L. perenne* Arion (pot diameter = 10 cm; 100 seeds, E− or E+; 5 days old). Aphids were left to form mummies for another 7 days before these mummies were collected and individually placed into gelatine capsules. The capsules were checked daily for emerged parasitoids. The sex of the emerged parasitoids was determined and they were placed into alcohol.

The fitness estimates obtained for the first offspring generation were the same as for the parental generation (life history traits, nos. 1–5), but in contrast to the parental generation, these estimates are more meaningful as the individuals were followed and experienced our treatment over their entire lifespan. Therefore, the measurements on the number of viable offspring refer to total fecundity (= lifetime reproductive success). Additionally, for the first offspring generation we measured: (6) emergence rate (= survival to adulthood), and (7) days to emergence (= developmental time).

Second offspring generation

The offspring produced by the E− and E+ female parasitoids from the first offspring generation are referred to as the second offspring generation. For the second offspring generation: (6) survival to adulthood and (7) developmental time were measured as life history traits.

Data analysis

All statistical analyses were performed using R (version 2.5.0 for MacOS X). All mean values are presented as mean ± 1 SE. Aphid life history traits were either analysed with ANOVAs with endophyte treatment as explanatory variable or linear mixed effects (LME) model with endophyte treatment as fixed effects and mother identity as random effect. Developmental time and fecundity of aphids were ln-transformed to meet assumptions of normality and heteroscedasticity of the model residuals. Replication number differed between treatments because in one replicate the nymph did not reach adulthood and one replication was lost after the nymph reached adulthood. For the analysis of development time, replicates where the nymphs were second instars at the beginning of the experiment instead of first instar were excluded (E+, 5; E−, 2).

In all the analyses on *A. ervi*, endophyte treatment was included as a fixed factor. The measured life history traits were either analysed with ANOVAs, LME models, including mother identity as random effect, generalized linear models (GLM) with quasibinomial error structure to correct for the overdispersion, or generalized mixed effects models (GLMMPQL) with mother identity as random effect and a quasibinomial error structure (Venables and Ripley 2002). The use of each model is indicated directly in the Results.

For the number of mummies (life history trait no. 2) and the number of viable offspring (life history trait no. 4) only females producing at least one mummy or one viable offspring respectively, were included in the analyses. These numbers were ln-transformed. For the analyses of life history traits nos. 1–5 of the first offspring generation, including the mating status (yes/no) did not explain much of the variance and was therefore neglected. The sex ratios (life history trait no. 5) were only analysed for replicates where at least one female emerged, as only in these cases was it certain that the female had been mated. Including all replicates did not make a difference for calculating significance levels. Sex ratios were analysed only for the parental

generation, as the lack of offspring produced by the first generation did not allow for analysis of sex ratios (see Results). The sex ratios and the survival to adulthood (life history trait no. 6) were arcsine-square-root-transformed. For the analyses of developmental time (life history trait no. 7), the sex of the offspring was included as a fixed effect.

Results

Life histories of the aphid *M. festucae*

The presence of the endophyte in the grass had no clear negative effects on *M. festucae*. The developmental time was not significantly influenced by the presence of endophytes (ANOVA: $F_{1,30} = 0.12$, $P = 0.733$; Fig. 1a). Fecundity tended to be slightly lower on E+ than on E− plants (ANOVA: $F_{1,36} = 3.87$, $P = 0.057$; Fig. 1b). However, daily fecundity (ANOVA: $F_{1,36} = 1.95$, $P = 0.172$; Fig. 1c) and lifespan (ANOVA: $F_{1,36} = 2.36$, $P = 0.133$; Fig. 1d) were not significantly affected by the presence of endophytes. The body size of the mothers (E−, 770.53 ± 31.61 µm; E+, 746.32 ± 46.37 µm; ANOVA: $F_{1,36} = 0.94$, $P = 0.340$) and the body size of the nymphs (E−, 299.84 ± 4.98 µm; E+, 283.36 ± 8.42 µm; LME: $F_{1,37} = 1.50$, $P = 0.228$) were not significantly affected by the presence of endophytes.

Life histories of the parasitoid *A. ervi*

Parental generation

From the parental generation, 14 out of the 20 females on E− (70%) and 15 out of the 20 females on E+ (75%) produced at least one mummy; this proportion of females producing mummies did not differ between the endophyte treatments (GLM: $F_{1,38} = 0.12$, $P = 0.732$). Also the number of mummies resulting from each female that produced at least one mummy did not differ significantly between the endophyte treatments (E−, 12.50 ± 2.39; E+, 9.27 ± 1.98; ANOVA: $F_{1,27} = 0.85$, $P = 0.365$). The proportion of parental

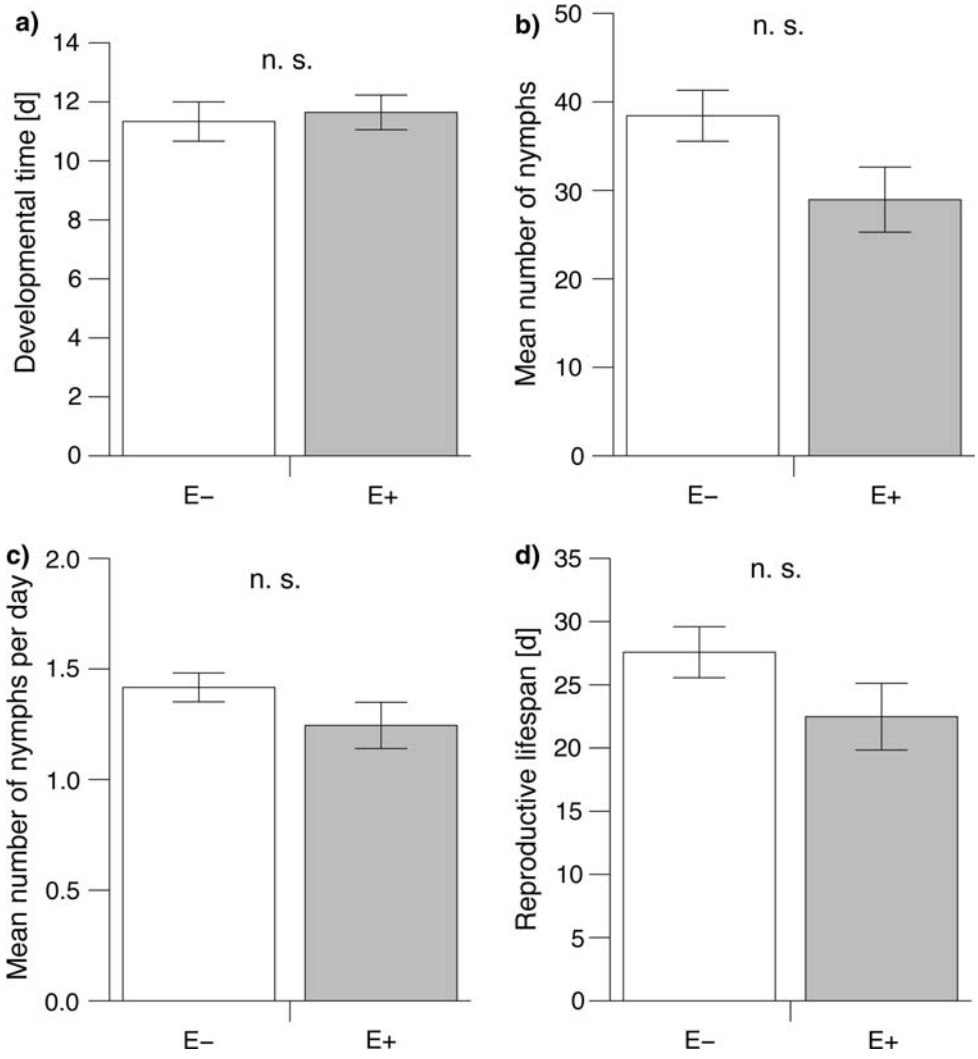

Fig. 1 Aphid performance. Developmental time (**a**), fecundity (**b**), daily fecundity (**c**) and adult lifespan (**d**) for the aphid species *Metopolophium festucae* feeding on endophyte-free (*E*−) or endophyte-infected (*E*+) *Lolium perenne*. Error bars show ± 1 SE. None of the measured life history traits were significantly affected by endophyte presence. However, fecundity tended to be slightly reduced on E+ ($P = 0.057$). *n.s.* Not significant ($\alpha = 0.05$)

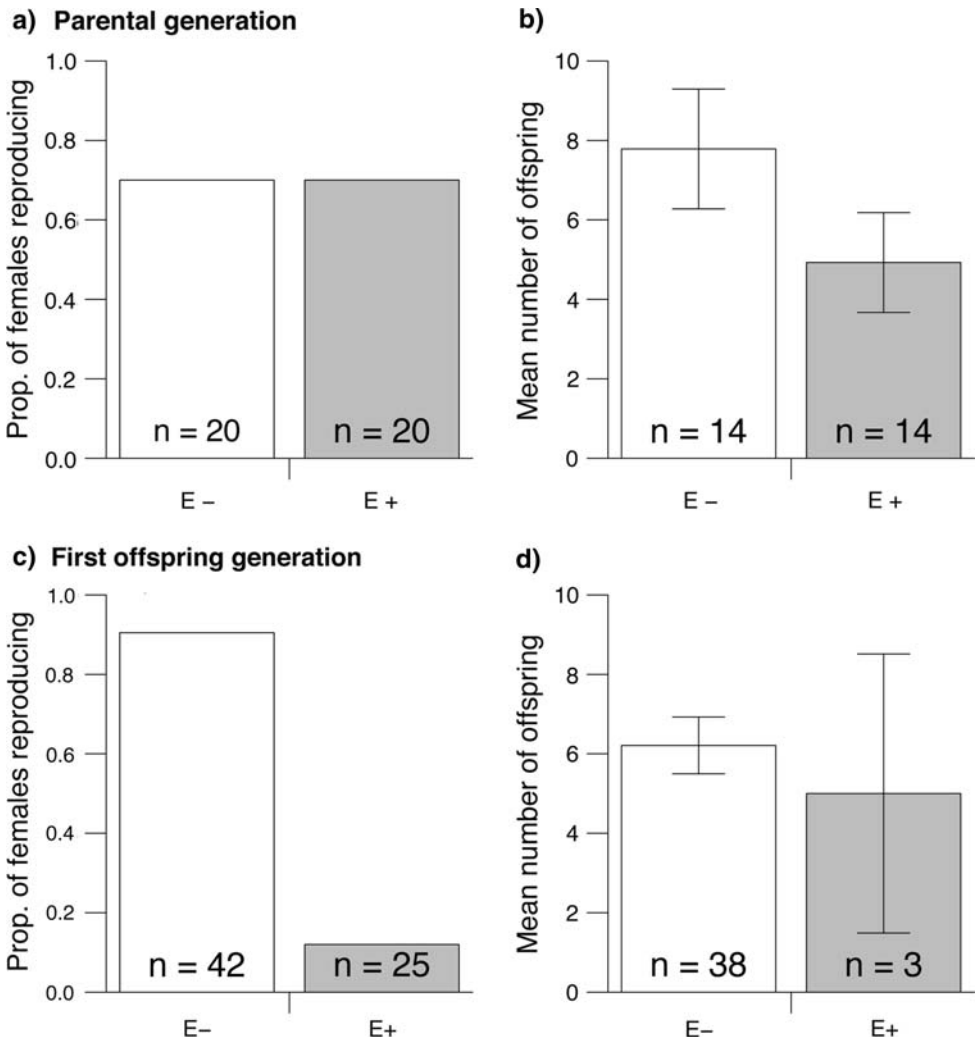

Fig. 2 Parasitoid performance. The proportion of parasitoid females reproducing on E− or E+ *L. perenne* in **a** the parental generation and **c** the first offspring generation. Mean (±1 SE) number of offspring produced by **b** the parental generation and **d** the first offspring generation. For the first offspring generation, the difference in replication between E− and E+ and the start of the experiment is caused by the fact that all females produced by the parental generation were used for the test of fecundity and an unequal number of first offspring generation females were produced on E− and the E+ treatments. For the number of offspring produced (**b**, **d**), only females which produced at least one viable offspring were included in the analyses. *n* Number of replicates; for other abbreviations, see Fig. 1

generation females producing at least one viable offspring (GLM: $F_{1,38} = 0.00$, $P = 1.00$; Fig. 2a) and the number of viable offspring produced by these females did not differ between the endophyte treatments (ANOVA: $F_{1,26} = 2.20$, $P = 0.150$; Fig. 2b). Also the sex ratio of the first offspring generation with 31.10 ± 9.42% on E− and 46.59 ± 6.04% on E+ was not significantly influenced by the presence of endophytes (ANOVA: $F_{1,26} = 2.01$, $P = 0.177$).

First offspring generation

Survival to adulthood (proportion of emerged first offspring individuals out of the mummies produced by the parental generation) was not significantly influenced by the presence of endophytes, with 66.06 ± 5.58% on E− and 52.04 ± 6.99% on E+ (ANOVA: $F_{1,27} = 2.01$, $P = 0.168$). Similarly, the developmental time of the first offspring generation was not significantly influenced by the presence of endophytes (E−, 18.39 ± 0.24 days; E+, 18.59 ± 0.39 days; LME: $F_{1,26} = 0.39$, $P = 0.540$), but as expected for parasitoids, developmental time was approximately 1 day longer for females than for males (females, 18.59 ± 0.17 days; males, 17.79 ± 0.21 days; LME: $F_{1,138} = 10.03$, $P = 0.002$).

All of the 42 E− and 25 E+ female offspring emerging in the first generation were tested for their fecundity. Out of these females, 40 E− females produced at least one mummy whereas only six E+ females produced at least one mummy (proportion of females producing mummies, GLMMPQL: $F_{1,18} = 22.44$, $P < 0.001$). The E− females producing mummies also produced significantly more mummies than E+ females (E−, 11.45 ± 1.13; E+, 5.33 ± 3.94; LME: $F_{1,13} = 12.36$, $P = 0.004$). For the proportion of first offspring generation females producing viable offspring, this difference was even more pronounced (GLMMPQL: $F_{1,18} = 22.76$, $P < 0.001$; Fig. 2c). However, the three E+ females that had viable offspring produced a similar number to the 38 E− females (LME: $F_{1,10} = 0.84$, $P = 0.380$; Fig. 2d). The sex ratio of the offspring from the E+ females was not analysed, as only one female out of the

three reproducing E+ female parasitoids was previously mated. The one mated E+ female parasitoid produced 25 mummies of which 12 were females. The sex ratio of the offspring from the E− females was 40.22 ± 5.76%.

Second offspring generation

The survival to adulthood (E−, 49.38 ± 3.50%; E+, 41.33 ± 20.04%; LME: $F_{1,13} = 0.71$, $P = 0.413$) and developmental time (E−, 17.24 ± 0.12 days; E+, 16.92 ± 0.96; LME: $F_{1,10} = 0.001$, $P = 0.975$) were not significantly influenced by the presence of endophytes. As in the first generation, the developmental time was longer for females than for males (females, 17.76 ± 0.12 days; males, 16.79 ± 0.09 days; LME: $F_{1,239} = 42.31$, $P < 0.001$).

Discussion

The presence of a fungal endosymbiont in the basal resource of an insect food chain reduced the reproductive performance of parasitoids even though the associated herbivore species showed no clear negative effect. The detrimental effect of the endophyte on the parasitoids was not visible in the parasitism rate of endophyte-naïve females, which suggests that they attack E+ and E− hosts equally. However, parasitoids that developed within hosts from endophyte-infected plants had a highly impaired reproduction with only 12% of females from E+ compared to 90% from E− producing any offspring. Endophyte infection did not affect survival to adulthood but had a strong detrimental effect on the reproductive ability of these females. The lack of an effect on endophyte-naïve females coupled with the strong reduction in reproductive ability of their offspring represents a delayed effect.

Even though the overall fecundity of the aphid host *M. festucae* tended to be reduced on E+ plants ($P = 0.057$), we argue that this species is rather insensitive to endophyte presence. This is mainly because none of the other measured traits showed any differences, but also because in a population-level experiment the numbers of *M. festucae* were similar on E− and E+ (Härri 2007). This lack of a clear negative effect on *M. festucae* is also in accordance with field studies based on the same and on a different grass-fungus association (Omacini et al. 2001; Krauss et al. 2007). This relative insensitivity of the host species towards the presence of endophytes associated with the strong detrimental effects on the parasitoids contrasts with typical bottom-up cascades, where plant and herbivore consumer levels are affected in the same direction and at a similar magnitude by changes in the quality of the basal resource (e.g. Teder and Tammaru 2002; Soler et al. 2005). However, the presence of allelochemicals in host plants may weaken or interrupt trophic cascades (Kagata and Ohgushi 2006). For example, in tobacco plants with high concentrations of nicotine, little or no effect on the herbivore was detected, although the toxins caused mortality of parasitoids (Barbosa et al. 1991). This phenomenon is referred to as "toxic environmental effect" (Hunter 2003) but is presumed to be rare (Hunter 2003; Kagata and Ohgushi 2006). The toxic environmental effect occurs when herbivores insensitive to certain plant allelochemicals use these substances to defend themselves against attacks by predators and parasitoids (Campbell and Duffey 1979; Kazana et al. 2007).

With our experimental set-up, we were able to distinguish between detrimental effects by endophytes caused during the developmental time and those caused by differences in attack rates. Had we not studied the reproductive performance of the first generation, we would have concluded that the parasitoids show no difference in behaviour on E+ and E− hosts. By following the offspring performance, we demonstrated that parasitoids developing within herbivores feeding on endophyte-infected plants were negatively affected and most were unable to reproduce at all. The reduction in reproductive success could have been due to several reasons, which we were unable to identify in our experiment. It is possible that the exposure to toxins during larval development weakened the adult parasitoid females in a way that they were unable to find or attack the aphids, or they attacked the aphids successfully but the eggs may not have been viable and/or not protected enough to evade destruction by the aphid immune system. Herbivorous insects do not have as sophisticated immune responses as vertebrates, but they can encapsulate parasitoid eggs and thus prevent them from developing (Henter and Via 1995; Kraaijeveld and Godfray 1997). A reduction in the fitness of parasitoids could also be explained by a small host size (Godfray 1994). However, as the body size of *M. festucae* was not influenced by the presence of endophytes, we can exclude such an indirect endophyte effect caused by smaller sized hosts. Sex determination of *A. ervi* occurs through haplodiploidy, with fertilized eggs resulting in females and unfertilized eggs in males (Godfray 1994). The impact of the endophytes on the male parasitoids of the first offspring generation was not measurable, as too few offspring were born to analyse the sex ratio.

The few studies that have investigated whether endophytic fungi can interact with natural enemies used systems where the herbivore shows a strong reduction in performance on endophyte-infected plants (Barker and Addison 1996; Bultman et al. 1997, 2003; de Sassi et al. 2006). Ours is the first study to show that an herbivore species that is relatively tolerant to the presence of endophytes propagates the endophyte effects to its enemy and thus receives protection from parasitoids. Ultimately, the stronger negative

effects of endophytes on parasitoids than on herbivores could result in a release from top-down limitation by parasitoids. In combination with the absence of bottom-up limitation of herbivores by endophytes this could lead to pest outbreaks.

In a multi-trophic setting, a plant may be protected from damage by some herbivorous species when entering an alliance with fungal endosymbionts. However, if there are species that can withstand the mycotoxin and additionally transfer toxic effects to their natural enemies, the plant pays a three-fold cost: it provides resources for the endophyte, it is still attacked and consumed by the particular herbivore that is tolerant to the mycotoxins, and it suffers from an indirect density-mediated effect of reduced biological control by parasitoids. The proposed view of endophytes as defensive mutualists of plants may therefore not hold when the third trophic level is considered. This provides further evidence that the nature of the relationship between plants and fungal endophytes depends on the biotic and abiotic environment and may range from mutualistic to parasitic.

Acknowledgements We thank Julia Nüscheler and Virginie Boreux for practical help in the laboratory, Brian Tapper (AgResearch, New Zealand) for contributing the seeds, Adrian Leuchtmann (ETHZ) for help with endophyte detection, Swiss Caps (Kirchberg, Switzerland) for providing the gelatine capsules and Dennis Hansen, Tom Jones, Geraldine Ryan, Jay Rosenheim and two anonymous reviewers for helpful discussions and comments on the manuscript. The project was funded by a SNSF grant to Christine B. Müller (631-065950).

References

Abrams PA (1995) Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. Am Nat 146:112–134

Barbosa P, Gross P, Kemper J (1991) Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. Ecology 72:1567–1575

Barker GM, Addison PJ (1996) Influence of clavicipitaceous endophyte infection in ryegrass on development of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) in *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae). Biol Control 7:281–287

Bultman TL, Borowicz KL, Schneble RM, Coudron TA, Bush LP (1997) Effect of a fungal endophyte on the growth and survival of two *Euplectrus* parasitoids. Oikos 78:170–176

Bultman TL, McNeill MR, Goldson SL (2003) Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. Oikos 102:491–496

Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1–7

Campbell BC, Duffey SS (1979) Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. Science 205:700–702

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interactions and lake productivity. Bioscience 35:634–639

Clay K (1990) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275–297

Denno RF, Gratton C, Peterson MA, Langellotto GA, Finke DL, Huberty AF (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. Ecology 83:1443–1458

Dixon AFG (1998) Aphid ecology, 2nd edn. Chapman & Hall, London

Faeth SH, Bultman TL (2002) Endophytic fungi and interactions among host plants, herbivores and natural enemies. In: Tscharntke T, Hawkins BA (eds) Multitrophic level interactions. Cambridge University Press, Cambridge, pp 89–123

Finkes LK, Cady AB, Mulroy JC, Clay K, Rudgers JA (2006) Plant-fungus mutualism affects spider composition in successional fields. Ecol Lett 9:347–356

Francis F, Lognay G, Wathelet JP, Haubruge E (2001) Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. J Chem Ecol 27:243–256

Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton

Goldson SL, Proffitt JR, Fletcher LR, Baird DB (2000) Multitrophic interaction between the ryegrass *Lolium perenne*, its endophyte *Neotyphodium lolii*, the weevil pest *Listronotus bonariensis*, and its parasitoid *Microctonus hyperodae*. N Z J Agric Res 43:227–233

Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. Am Nat 94:421–425

Härri SA (2007) Fungal endosymbionts of grasses, and their effects on multitrophic interactions. PhD thesis, University of Zürich, Switzerland

Härri SA, Krauss J, Müller CB (2007) Can aphids learn to cope with the presence of endopyhtic fungi in their food plants? In: Popay AJ, Thom ER (eds) Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses. New Zealand Grassland Association, Dunedin

Henter HJ, Via S (1995) The potential for coevolution in a host-parasitoid system. 1. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49:427–438

Holt RD, Lawton JH (1993) Apparent competition and enemy-free space in insect host-parasitoid communities. Am Nat 142:623–645

Hunt MG, Newman JA (2005) Reduced herbivore resistance from a novel grass-endophyte association. J Appl Ecol 42:762–769

Hunter MD (2003) Effects of plant quality on the population ecology of parasitoids. Agric For Entomol 5:1–8

Hunter MD, Price PW (1992) Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology 73:724–732

Kagata H, Ohgushi T (2006) Bottom-up trophic cascades and material transfer in terrestrial food webs. Ecol Res 21:26–34

Kazana E et al (2007) The cabbage aphid: a walking mustard oil bomb. Proc R Soc Lond B Biol Sci 274:2271-2277

Kraaijeveld AR, Godfray HCJ (1997) Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Nature 389:278–280

Krauss J et al (2007) Effects of fertilizer, fungal endophytes and plant cultivar on the performance of insect herbivores and their natural enemies. Funct Ecol 21:107–116

McCann K (2007) Protecting biostructure. Nature 446:29

Meister B, Krauss J, Härri SA, Schneider MV, Müller CB (2006) Fungal endosymbionts affect aphid population size by reduction of adult life span and fecundity. Basic Appl Ecol 7:244–252

Müller CB, Godfray HCJ (1999) Indirect interactions in aphid-parasitoid communities. Res Popul Ecol 41:93–106

Müller CB, Krauss J (2005) Symbiosis between grasses and asexual fungal endophytes. Curr Opin Plant Biol 8:450–456

Nakamura M, Utsumi S, Miki T, Ohgushi T (2005) Flood initiates bottom-up cascades in a tri-trophic system: host plant regrowth in-

creases densities of a leaf beetle and its predators. J Anim Ecol 74:683–691
Ode PJ (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Annu Rev Entomol 51:163–185
Omacini M, Chaneton EJ, Ghersa CM, Müller CB (2001) Symbiotic fungal endophytes control insect host-parasite interaction webs. Nature 409:78–81
Pace ML, Cole JJ, Carpenter SR, Kitchell JF (1999) Trophic cascades revealed in diverse ecosystems. Trends Ecol Evol 14:483–488
Power ME, Matthews WJ, Stewart AJ (1985) Grazing minnows, piscivorous bass, and stream algae—dynamics of a strong interaction. Ecology 66:1448–1456
de Sassi C, Müller CB, Krauss J (2006) Fungal plant endosymbionts alter life history and reproductive success of aphid predators. Proc R Soc Lond B Biol Sci 273:1301–1306
Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340
Schmitz OJ, Hambäck PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. Am Nat 155:141–153
Soler R, Bezemer TM, van der Putten WH, Vet LEM, Harvey JA (2005) Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. J Anim Ecol 74:1121–1130
Strauss SY (1991) Indirect effects in community ecology: their definition, study and importance. Trends Ecol Evol 6:206–210
Teder T, Tammaru T (2002) Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids. Ecol Entomol 27:94–104
van Veen FJF, Morris RJ, Godfray HCJ (2006) Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annu Rev Entomol 51:187–208
Venables WN, Ripley BD (2002) Modern applied statistics with S. Springer, New York
Werner EE, Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100
Wootton JT (1993) Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. Am Nat 141:71–89