Extended larval development time for aphid parasitoids in the presence of plant endosymbionts

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Abstract. 1. Variation in plant chemistry does not only mediate interactions between plants and herbivores but also those between herbivores and their natural enemies, and plants and natural enemies.
2. Endophytic fungi complete their whole life cycle within the host plant’s tissue and are associated with a large diversity of plant species. Endophytes of the genus Neotyphodium alter the chemistry of the host plant by producing herbivore toxic alkaloids.
3. Here we asked whether the endophyte-tolerant aphid species Metopolophium festucae could be defended against its parasitoid Aphidius ervi when feeding on endophyte-infected plants. In a laboratory experiment, we compared life-history traits of A. ervi when exposed to hosts on endophyte-infected or endophyte-free Lolium perenne.
4. The presence of endophytes significantly increased larval and pupal development times, but did not affect the mortality of immature parasitoids or the longevity of the adults. Although the number of parasitoid mummies tended to be reduced on endophyte-infected plants, the number of emerging parasitoids did not differ significantly between the two treatments.
5. This shows that the metabolism of individual aphids feeding on infected plants may be changed and help in the defence against parasitoids. An increase in parasitoid development time should ultimately reduce the population growth of A. ervi. Therefore, endophyte presence may represent an advantage for endophyte-tolerant aphid species through extended parasitoid development and its effect on parasitoid population dynamics.

Key words. Aphidius ervi, development time, host-parasitoid interactions, larval mortality, life-history traits, longevity, Metopolophium festucae, multitrophic interactions, Neotyphodium lolii, sex ratio.

Introduction

Variation in plant quality and plant chemistry can mediate interactions between herbivores and their natural enemies (Price et al., 1980). Generally, effects of plant allelochemicals on natural enemies are assumed to be positive. An example is the herbivore-induced plant volatiles that attract natural enemies (e.g. Turlings et al., 1990). However, some studies showed that plant allelochemicals reduce the fitness of natural enemies by rendering their hosts toxic (Campbell & Duffey, 1979; Thaler, 2002; Harvey et al., 2005; Ode, 2006; Kazana et al., 2007). In situations in which herbivores use allelochemicals as an acquired defence against natural enemies and are themselves only negligibly affected by the toxins (Campbell & Duffey, 1979; Barbosa et al., 1991), the plants are losing the indirect benefits from the action of natural enemies on herbivores.

Most studies investigating the mediating effects of plants on herbivore-natural enemy interactions focus on plant secondary
Theobald and its parasitoid an endophyte-tolerant grass aphid from the presence of the fungal endosymbionts. Acquired defence against their natural enemies, they will benefit phyto-tolerant herbivores can use the mycotoxins as a kind of direct contact with toxins accumulating within the herbivorous natural enemies may be either direct (e.g. parasitoids getting in direct contact with toxins accumulating within the herbivorous host tissue) or indirect (e.g. via changes in herbivore host density or size). However, not all herbivorous insects react the same in the presence of endophytes (Omacini et al., 2001; Faeth & Bultman, 2002; Saikkonen et al., 2006). If endophyte-tolerant herbivores can use the mycotoxins as a kind of acquired defence against their natural enemies, they will benefit from the presence of the fungal endosymbionts.

In this study, we used an aphid-parasitoid system consisting of an endophyte-tolerant grass aphid Metopolophium festucae Theobald and its parasitoid Aphidius ervi Haliday (Hymenoptera: Braconidae), both of which are commonly found on Perennial ryegrass Lolium perenne L. (Krauss et al., 2007). Parasitoids are responsible for drastic reductions of aphid densities in the field and thus play a central role in the biological control of pest aphids (Schmidt et al., 2003; Brewer & Elliott, 2004). Aphidius 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, occurs after the aphids reach adulthood or during the late nymphal stage (Godfray, 1994). During the larval growth period, the parasitoid larva feeds on host aphid haemolymph and tissue and may therefore be directly exposed to toxic substances ingested by aphids through the plant sap. Endoparasitoids such as A. ervi cannot excrete waste before reaching the final larval instar, as it is only then that the midgut and hindgut fuse (Quicke, 1997). As a consequence of this waste product accumulation, the larval stages of parasitoids could be particularly affected by the presence of endophytes.

Sex determination of A. ervi, as for all hymenopterans, occurs through haplodiploidy, with fertilised eggs resulting in females and unfertilised eggs becoming males. The mated female decides at oviposition whether the egg gets fertilised or not, and has thus control over the sex of her offspring (Godfray, 1994). Theory predicts that unfertilised male eggs are placed into lower quality hosts, whereas fertilised female eggs are laid into higher quality hosts (Charnov et al., 1981). Aphids feeding on endophyte-infected plants may represent lower quality hosts for the parasitoids and females may decide to place unfertilised, male eggs into such aphids if she can perceive the presence of the endosymbiont in the plants.

We compared a range of life-history traits of A. ervi when offered host aphids fed either on endophyte-free (E−) or endophyte-infected L. perenne (E+). We questioned whether the endophyte-tolerant aphid species M. festucae can use the mycotoxins that are produced by the plant-endophyte symbiosis, as acquired defence against A. ervi. Based on the biology of the parasitoid A. ervi, our main predictions were that the presence of endophytes in the host’s food plant: (i) reduces the rate of parasitism, (ii) results in a sex ratio biased towards males, (iii) increases developmental time, (iv) increases larval mortality, and (v) reduces longevity of adult parasitoids.

Material and methods

Material

The seeds of L. perenne were provided by Brian Tapper (AgResearch, NZ) and all belonged to the same cultivar (Samson). They were either uninfected (E−; identity number: 11104, < 0.01% infection) or infected with the common wild-type endophyte Neotyphodium lolii Glen, Bacon and Hanlin specific to L. perenne (E+; identity number: A12038, 89% infection). The infection status of the seed batches was confirmed by a combination of seed staining and microscopic examination, and immunoblotting of stems.

The stock culture of M. festucae was started in summer 2005 with a few individuals collected near the University of Zürich, Switzerland and was maintained on commercially available endophyte-free fodder grass L. perenne ARION (staining of 30 seeds: 0% infection), provided by FAL Reckenholz, Switzerland. The stock culture of A. ervi was started with 250 individuals bought from Andermatt Biocontrol AG, Switzerland and maintained on M. festucae feeding on L. perenne ARION. All stock cultures and experiments were conducted under controlled climatic conditions at 22 °C with a LD 16:8h light regime.

Experiment

Fifty 1-day-old, mated female parasitoids from the stock culture were used for the experiment. For mating, each female was kept in a vial (Ø 5 cm × 2 cm height) for 8 h together with two males of the same age. A piece of apple was added after 4 h to prevent starvation. After this mating period, each of the 50 females was offered a mixture of 20 second and third instar M. festucae nympha on E− or E+ cuttings in a Petri dish. These nymphs were the progeny of adult M. festucae from the stock culture that had been placed on either E− or E+ cuttings in individual Petri dishes. Therefore, these nymphs fed on either E− or E+ plants since their birth.

The 50 mated parasitoid females were left in their Petri dish with the nymphs for a 13–15 h oviposition period overnight. This time span was considered long enough for oviposition to take place but short enough to avoid super-parasitism (S. A. Härri, pers. obs.). After this oviposition period, the parasitoids were removed and placed in alcohol for later measurements of
The dry weight. The dry weight of the A. ervi female mothers in the experiment did not differ between the endophyte treatments ($F_{1,6} = 0.21, P = 0.648$) and was therefore not added as an explaining co-factor for statistical analyses. The aphids were removed from the 50 Petri dishes and transferred onto 50 potted plants with either E− or E+ L. perenne (50 seeds, 6 days old). The pots were then covered with a cage built from an empty PET – bottle with ventilation slits.

Nine days later, all 50 pots were checked once a day for the presence of mummies. The exact time until mummy formation (larval development time) in number of days was recorded. The mummies were collected and transferred singly into gelatine capsules. This was repeated for eight consecutive days until all parasitised aphids had mummified. All aphids that did not turn into mummies after 17 days were dissected to assess the cause of parasitoid larval mortality. Dissected aphids were classified as unparasitised or parasitised adults (= surviving adult aphid with a dead parasitoid larva). The gelatine capsules containing the mummies were checked twice daily for parasitoid emergence. Time from mummy formation to emergence was recorded (= pupal development time) and the total development time was calculated as number of days from oviposition to adult emergence. Emerged parasitoids were sexed and categorized as healthy or crippled. Individuals were assigned to the crippled category when they had, for example, one wing deformed or when they were so weak that they died shortly after emergence within the gelatine capsules. None of the crippled individuals lived long enough to be assessed for longevity (see below). The proportion of healthy parasitoids emerging from mummies was calculated (= emergence rate). The proportion of parasitoids that died during the pupal stage (= pupal mortality rate) is the counterpart of the emergence rate. The sex ratio was calculated as the proportion of male offspring of all offspring per female. Some randomly selected individuals of the emerged females were used for a different experiment. Therefore, for the measurements of longevity and offspring weight, not all emerged healthy female parasitoids were tested. On E−, 34 out of 53 emerged female parasitoids and on E+ 29 out of 39 emerged female parasitoids were included in the measurements. All the emerged healthy males were tested for their longevity. Longevity was measured by placing each individual singly in a plastic vial sealed with a piece of foam. Fresh apple was supplied every day and survival was recorded twice a day. After the death of the parasitoids, the dry weight, after drying them in an oven for 72 h at 80 °C, was recorded. At the end of the experiment, the size of the aphid mummies was recorded by measuring its length taken from the front of the head to the end of the abdomen, not including the cauda.

Statistical analyses

All analyses were performed with R (version 2.4.0 for Mac OS X). For the endophyte-infected treatment, one replicate was lost at the beginning of the experiment. Three females on E− and three females on E+ did not produce any mummies and these replicates had to be omitted from the analyses. For all analyses, endophyte infection (E−/E+) was included as a fixed effect. The recorded life-history traits were either analysed with one-way ANOVA’s (ANOVA), generalised linear model with quasipoisson error structure (GLM, poisson), generalised linear model with quasibinomial error structure (GLM, binomial) or linear mixed effects model. This included the mother identity as a random effect as well as sex and its interaction with endophyte presence being additional fixed effects (LME). The use of each model is indicated in the Result section. To meet model assumptions of residual normality and heteroscedasticity, pupal survival rate was arcsin-square root-transformed.

Results

The presence of endophytes showed a trend towards lower numbers of mummies produced by parasitoids on E+ plants and thus possibly fewer aphids attacked (Fig. 1). Dissection of the aphids that did not turn into mummies showed only very few cases where the larvae died within the aphid hosts and there was no significant difference in larval mortality between the two treatments (Fig. 1). The larval development time and the pupal development time were significantly extended in host aphids feeding on endophyte-infected plants, resulting in an increase in total development time on E+ (Fig. 2). Furthermore, female parasitoids took longer to develop than male parasitoids (LME: larval stage: $F_{1,235} = 14.78, P < 0.001$, pupal stage: $F_{1,235} = 5.62, P = 0.019$, total development time: $F_{1,235} = 17.00, P < 0.001$). However, the effect of gender was statistically independent of endophyte infection for larval and pupal development times (LME: larval stage: $F_{1,259} = 0.36, P = 0.546$, pupal stage: $F_{1,235} = 0.61, P = 0.437$, total development time: $F_{1,235} = 1.32, P = 0.252$). The final emergence rate and also the resulting number of emerged and healthy parasitoids were not significantly influenced by the endophyte treatment (Fig. 3).

Following from this, the pupal mortality rate was not affected by endophyte presence. The sex ratio of the emerged parasitoids did not differ significantly between the endophyte treatments.

Fig. 1. Mean (± SE) of number of mummies (white), number of dead parasitoid larvae within aphid hosts (= larval mortality, wide-dashed) and unparasitised aphids (narrow-dashed) on endophyte-free (E−) or endophyte-infected aphid food plants (E+). Number of mummies produced tended to be lower on E+ (ANOVA: $F_{1,4} = 3.27, P = 0.078$) whereas larval mortality did not differ between the treatments (GLM, poisson: $F_{1,4} = 0.14, P = 0.709$).
resulting in an overall increase in total development time on E, developmental time were prolonged on endophyte-infected plants, be lower on E. Even though the number of mummies tended to parasitoids were not significantly affected by the endophyte presence. Unexpectedly, all other measured life-history traits significantly increased the larval and pupal development time of endophyte-infected plants, resulting in an overall increase in total development time on E (LME: $F_{1,41} = 10.53, P = 0.002$). For additional effects of gender on development times, see text.

Discussion

The presence of endophytes in the food plant of the aphid hosts significantly increased the larval and pupal development time of parasitoids. Unexpectedly, all other measured life-history traits of the parasitoids were not significantly affected by the endophyte presence. Even though the number of mummies tended to be lower on E+ ($P = 0.078$), the resulting number of emerged offspring was not significantly different between the endophyte treatments. The number of mummies does not stringently reflect the oviposition rate, as parasitoid eggs can get destroyed by the aphids' immune system. Generally, insects can defend themselves against parasitoid attacks by encapsulating the eggs (Kraaijeveld & Godfray, 1997). For aphids, the mechanism by which the eggs are destroyed remains unclear, but it has been observed that in resistant aphid hosts the eggs do not develop and eventually disappear (Henter & Via, 1995; Ferrari et al., 2001). As we did not directly observe oviposition, and successful placing of eggs can only be detected by dissection shortly after oviposition, we do not know whether attack rates differed between the endophyte treatments.

Comparisons of attack behaviour of different aphid parasitoid species show that oviposition and handling time of less than 0.5 s for A. ervi is relatively short (Völkl & Mackauer, 2000). Short handling times suggest that A. ervi does not assess hosts carefully, but attacks all available hosts independent of their quality. Therefore, a tendency of a reduced number of mummies formed may reflect differences in the aphids' ability to defend a parasitoid egg before the larva hatches. Other laboratory studies of endophyte effects on parasitoids generally found no differences in attack rates (Barker & Addison, 1997; Bultman et al., 2003; but see Barker & Addison, 1996). However, in field experiments, parasitism rate of Microtus hyperodae on endophyte-infected L. perenne (Goldson et al., 2000) and of Phyllonorycter sp. on endophyte-infected Quercus gambelii (Preszler et al., 1996) are reduced. Under field conditions, in which insects have to choose between hosts from endophyte-free or endophyte-infected plants, such differences in attack rate may be caused by preferences of parasitoids for hosts on uninfected plants.

The slow growth – high mortality hypothesis predicts that development time is increased for herbivores feeding on low nutritional plants or plants defended by allelochemicals and that this extension in development time increases the window of exposure to parasitoids of larval herbivores (Clancy & Price, 1987). An extended development time on low-quality hosts has also been proposed and shown for parasitoids (Vinson & Ivantsch, 1980; Damman, 1987). For example, M. hyperodae, a parasitoid of the Argentine stem weevil, has a longer developmental time when developing within hosts feeding on endophyte-infected plants (Barker & Addison, 1996; Bultman et al., 2003). The larvae of koinobiont parasitoids are intimately associated with and caged in the developing host organism without an option of defence against natural enemies. An extended larval parasitoid development time is thus disadvantageous (Price et al., 1980), because it results in an extension of the life stage which is most vulnerable to attack by secondary parasitoids and other predatory arthropods (Müller & Godfray, 1997, 1999; Brodeur & Rosenheim, 2000).

Host size and host age are often assumed to be important determinants of the parasitoid’s development time (Vinson &
Iwantsch, 1980; Charnov et al., 1981; Sequeira & Mackauer, 1992; Godfray, 1994). Nymphs of the aphid species *M. festucae* that served as hosts for *A. ervi* in our experiment do not show any size differences when feeding on endophyte-free or endophyte-infected grasses (Härri, 2007). Therefore, it is likely that at the time of oviposition, the size of *M. festucae* did not differ between the treatments. At the end of the larval development when mummy formation occurred, the size of the hosts was again not different between the treatments, as indicated by the non-significant differences for mummy size. Therefore, we speculate that host size at oviposition is not the prime determinant for the observed differences in development time, but rather the lower nutritional quality of the host tissue caused by the presence of endophytes. Thus, the observed extended development time on E+ might be a direct effect caused by the direct contact of the parasitoid larvae with toxins that possibly accumulated within the host tissue, and not an indirect effect via host size differences at the time of oviposition.

Overall, the effects of endophytes on *A. ervi* were not very strong in our experiment, because endophyte presence did not significantly affect oviposition behaviour, rate of parasitism or longevity of emerging parasitoids. However, we found a significant increase in larval and pupal development time for parasitoids from aphid hosts feeding on endophyte-infected *L. perenne*. Significant extensions of parasitoid development time could ultimately reduce population growth rates of parasitoids. Additionally, parasitoids developing in endophyte-tolerant aphids may only experience a fitness disadvantage after developing within the endophyte environment (Härri, 2007).

In conclusion, in the special case where the herbivore was tolerant to the fungal-derived toxins, these endophyte effects proliferate only weakly up the food chain to the aphid parasitoids. In particular, the developing larva is the stage that is most exposed to the mycotoxins and as a consequence, the development time of the parasitoids was extended. Other life-history traits, such as longevity and sex ratios, showed no effects from the endophyte presence in the basal resource plant. Whether this extension in larval development is caused by a host that uses toxins to defend a parasitoid larva, by the parasitoid to force its host to acquire the correct size before pupation, or as a side-effect of the accumulated toxins within endophyte-tolerant aphids feeding on infected plants, remains to be elucidated by experimentation, focusing more precisely on parasitoid larval development.

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