# Of moths, mites and microbes - The role of bacteria in the life history of two arthropod herbivores

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# Effects of immune challenge on the oviposition strategy of a noctuid moth

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***Abstract***

Infections can have detrimental effects on the fitness of an animal. Reproducing females may therefore be sensitive to cues of infection and be able to adaptively change their oviposition strategy in the face of infection. As one possibility, females could make a terminal investment and shift reproductive effort from future to current reproduction as life expectancy decreases. We hypothesized that females of the noctuid moth *Heliothis virescens* make a terminal investment and adapt their oviposition timing as well as their oviposition site selectivity in response to an immune challenge. We indeed found that females that were challenged with the bacterial entomopathogen *Serratia entomophila* laid more eggs than control females one night after the challenge. Additionally, bacteria-challenged females were less discriminating between oviposition sites than control females. Whereas control females preferred undamaged over damaged plants, immune-challenged females did not differentiate between the two. These results indicate that terminal investment is part of the life history of *H. virescens* females. Moreover, our results suggest that a strategy of terminal investment in *H. virescens* oviposition represents a fitness trade-off for females: in the face of infection, an increase in oviposition rate enhances female fitness, whereas low oviposition site selectivity reduces female fitness.

## INTRODUCTION

Pathogens are virtually always present in the environment of organisms and can have detrimental effects on their fitness when infection occurs (Grenfell & Dobson, 1995; Poulin, 2007; Schmid-Hempel, 2011). Under pathogen pressure, natural selection should favour adaptive changes in response to an immune challenge to minimize the costs of infection and maximize lifetime reproductive success (e.g., Adamo, 1999; Agnew *et al.*, 2000; Bonneaud *et al.*, 2004; Javoiš & Tammaru, 2004).

One possibility for organisms to adapt their life history strategy is terminal investment, that is the increase of current reproductive effort as life expectancy decreases (Clutton-Brock, 1984). Current reproductive output is expected to trade off with future reproductive output and is therefore in most cases not maximized (Williams, 1966). However, if life expectancy decreases, no resources need to be saved for future reproduction and investment in current reproduction is predicted to increase (Part *et al.*, 1992; Polak & Starmer, 1998; Velando *et al.*, 2006). Terminal investment may be apparent in increased courtship activity, a temporary increase in numbers of offspring and/or investment in offspring survival (Part *et al.*, 1992; Polak & Starmer, 1998; Adamo, 1999; Bonneaud *et al.*, 2004; Creighton *et al.*, 2009). For example, a temporary increase in numbers of eggs in response to an immune challenge has been found in crickets and freshwater snails (Minchella & Loverde, 1981; Adamo, 1999). Studies of several bird species showed an increase in parental care in response to an immune challenge and/or because of ageing (Part *et al.*, 1992; Hanssen, 2006; Velando *et al.*, 2006).

Nonsocial herbivorous insects do not generally provide parental care to their offspring (Janz, 2002). However, in many species, females show oviposition site choice that can be viewed as an investment in offspring survival, as well as a form of parental care (Wiklund & Persson, 1983; Janz, 2002; Lefèvre *et al.*, 2010). Oviposition site choice includes finding and selecting host plants on which offspring can obtain realized fitness and avoiding nonhost or herbivore-infested plants on which offspring cannot (Rothschild & Schoonhoven, 1977; Renwick, 1989; Nishida *et al.*, 1990; Tingle & Mitchell, 1991; De Moraes *et al.*, 2001; Kessler & Baldwin, 2001). Avoiding herbivore-infested plants may be especially important for offspring survival, and thus female fitness, because of enhanced intra- and interspecific competition on such plants (Denno *et al.*, 1995; Kaplan & Denno, 2007), indirect plant defences, such as the emission of volatiles that attract natural enemies of herbivores (Turlings *et al.*, 1990; McCall *et al.*, 1993; De Moraes *et al.*, 1998; Thaler, 1999) or the induced synthesis and enhanced accumulation of secondary plant metabolites that may render the plant unpalatable (e.g., Schoonhoven *et al.*, 2005).

It has long been suggested that when available oviposition time is limited, ovipositing females become less selective and more likely to accept lower-ranked hosts or even non-hosts (Jaenike, 1978; Courtney & Courtney, 1982). As infection can be a cue for shortened life expectancy, oviposition site selectivity and thus investment in offspring survival can be expected to decrease with infection. Moreover, if ovi-position rate actually increases in response to infection, ovipositing females become more time-limited. There is evidence that terminal investment behaviour in herbi-vorous insects in response to cues for shortened life expectancy indeed results in in-creased plant acceptance (Javoiš & Tammaru, 2004). However, to our knowledge, cues of shortened life expectancy have not yet been shown to lower oviposition site selectivity.

In this study, we investigated oviposition timing and oviposition site preference in response to an immune challenge in the generalist herbivore *Heliothis virescens* (Lepidoptera, Noctuidae). Females of this moth species can lay up to about 1500 eggs in their lifetime of about 30 days in the laboratory at 25 °C (Proshold *et al.*, 1982; Willers *et al.*, 1987; Fitt, 1989). Females oviposit throughout the night and lay their eggs singly on plants (Fitt, 1989; Ramaswamy, 1990). *Heliothis virescens* larvae are cannibalistic from their 3rd instar onwards (Gould *et al.*, 1980), so that avoiding to oviposit on plants that are infested with conspecific larvae is likely adaptive for H. virescens females. Accordingly, H. virescens females have been shown to avoid Nicotiana tabacum plants on which conspecific larvae had fed (De Moraes *et al.*, 2001).

Even though *H. virescens* can be regarded an r-strategist, oviposition site choice can be considered an investment into offspring survival, because females need to invest time and resources into finding and selecting an optimal site (Pianka, 1970; Wiklund & Persson, 1983; Willers *et al.*, 1987; Janz, 2002). As infection may limit the time that is available for oviposition, we hypothesized that *H. virescens* females would increase their oviposition rate after a bacterial challenge (H1). Secondly, we predicted that terminal investment would be apparent in oviposition site selectivity, such that bacteria-challenged females are less discriminating in their choice of oviposition site than control females (H2).

## MATERIAL AND METHODS

### *Insects and bacterial culture*

*Heliothis virescens* was collected in July 2011 in North Carolina, USA, and reared in climate chambers at 25 °C, 60% relative humidity and a light-dark cycle of 10L:14D with lights on at 11 am. Larvae were grown on artificial pinto bean diet (Burton, 1970). Adults were provided with a 10% (wt/vol) sugar-water solution.

To induce an immune response in *H. virescens* females, we used the entomopathogenic bacterium *Serratia entomophila*, which was obtained from the Department of Bioorganic Chemistry (MPICE, Jena, Germany). *Serratia entomophila* was grown overnight in CASO medium at 30 °C on a shaker set at 250 r.p.m. After one night, cultures were centrifuged and the supernatant was discarded. To investigate effects of immune challenge without the confounding effects of the dynamics of a living pathogen, it is common to use immune elicitors like lipopolysaccharides (LPS) (Moret & Schmid-Hempel, 2000; Korner & Schmid-Hempel, 2004) or dead bacterial cells (Haine *et al.*, 2008; Cotter *et al.*, 2010). We therefore killed the bacteria by freezing and drying the samples in a lyophilisator at -80 °C for 5 days. To confirm that bacteria were dead, we streaked them out on Luria-Bertani (LB) agar plates. Lyophilized cells of *S. entomophila* were stored at -20 °C until used in the experiments.

### *Immune activation via bacterial challenge in mated females*

In all experiments, two groups of females were tested; one group was injected with lyophilized cells of *S. entomophila* to induce an immune response [4 µl of a 1µg/µl solution of bacteria diluted in 1x phosphate buffered saline (PBS)]. This entomopathogenic bacterium was shown to be lethal for *H. virescens* larvae and to induce hemocyte apoptosis in *H. virescens* larvae (Barthel *et al.*, 2014). The other group of females was the control, which was injected with 4 µl of 1x PBS solution. All females were mated in single pair matings one night before they were used in the oviposition assay (night zero). Matings were observed to ensure that females were mated. At the onset of the photophase and between 15 and 17 h before the start of the experiments, the mated females were injected into their abdominal cavity with a 10-µl Hamilton syringe. Previously, we found that injecting lyophilized *S. entomophila* cells in this way elicits an immune response in *H. virescens* females (A. Barthel, H. Staudacher, A. Schmaltz, D.G. Heckel, A.T. Groot, unpublished data). Injecting bacteria has been commonly used to investigate the effects of an immune challenge and as such mimics the process of bacterial cells breaking through the cuticle (Shelby & Popham, 2008). Furthermore, injection ensures that equal amounts of bacterial and PBS solution are used for each female and in all experiments.

***H1: Females increase their oviposition rate after a bacterial challenge***
To test the hypothesis that females would make a terminal investment and increase their oviposition rate (that we define here as mean number of eggs/ female/ night) after a bacterial challenge, we conducted oviposition assays with two groups of 2- to 9-day-old *H. virescens* females. One group was injected with *S. entomophila* (n = 27), the other group of females served as the control and was PBS-injected (n = 25), as described above. Experimental females of different ages were distributed evenly between the two treatment groups. The mated and injected females were placed in paper cups (200 ml) at the beginning of the first night after mating (night one), and provided with one dental stick that was soaked in 10% sugar water, which was renewed every night. Cups were closed with transparent gauze. During this experiment, the females were kept without plants. For each female, eggs (all eggs in a cup) were counted at the end of each consecutive night until she died. In the course of the experiment females died, which was recorded daily. Females that did not lay eggs during the experiment were excluded. Also with these exclusions, the age distribution of females remained similar between the two treatment groups (W = 284.5, $P = 0.33$, Wilcoxon rank sum test with continuity correction) (see FIGURE S4.1 for age distribution of females in the two treatment groups).

*Statistical analysis.* Differences in number of oviposited eggs between *S. entomophila*-injected and control females for night one were tested with a linear model. Number of eggs at night one was used as response variable and treatment and female age at the start of the experiment as explanatory variables. Additionally, to test whether bacterial challenge had an influence on oviposition rate over time, we analysed the data from the first 13 nights. We chose the period of 13 nights, because after that period there were fewer than 10 surviving females in the *S. entomophila*-injected group left. We used a generalized linear mixed model with negative binomial distribution in the R package glmmADMB (version 0.8.0) (Fournier *et al.*, 2012; Skaug *et al.*, 2014). Number of eggs per female per night was used as response variable. Night and treatment as well as the interaction effect between night and treatment were used as explanatory variables. Female age at the start of the experiment was included as fixed effect in the model. To account for re-peated measurements, we added individual females as random effect to the model, because the eggs of each female were counted every night. To account for temporal autocorrelation, we added night as a random effect slope to the model (night | individual female). To improve the model fit, we included experimental night square and zero inflation in the model which lowered the AIC (Akaike information criterion).

We used a linear model to test for differences in total number of eggs (number of eggs that females laid after injection until they died) between *S. entomophila*-injected and PBS-injected females. Total number of eggs was used as response variable. Treatment and female age at the start of the experiment were used as explanatory variables. To analyse the survival of control and *S. entomophila*-injected females, we used weighted Cox regression in the package coxphw in R (Heinze *et al.*, 2014), with censoring applied to females that did not die by day 13, and using average hazard ratio (AHR) as template for the case of nonproportional hazards (Schemper, 1992; Schemper *et al.*, 2009; Heinze *et al.*, 2014). Treatment and age were used as explanatory variables. One female of the *S. entomophila* group escaped at night three and was excluded from the models of survival and number of total eggs. To test whether female age and treatment influenced the death of females two nights after injection (when many females were found dead), we constructed a generalized linear model with binomial distribution, using likelihood-ratio as test statistic. Survival [n(alive) = 41] or nonsurvival [n(dead) = 10] for longer than night two was used as response variable, female age and treatment served as explanatory variables.

To determine whether an actually shortened life span affected the number of eggs oviposited in night one after the bacterial challenge, we applied another linear model to the data of the *S. entomophila*-injected group. Number of eggs was used as response variable, and female age as well as survival [n(alive) = 16] or nonsurvival [n(dead) = 10] for longer than night two after injection were used as explanatory variables.

The response variables in the linear models were square-root-transformed when it improved the residual structure of the models. Interaction effects of treatment and female age were tested for all linear models, but were excluded when they were not significant.

***H2: Bacteria-challenged females are less discriminating in their choice of oviposition site than control females***

To test the hypothesis that immune system activation influences female oviposition preference, we conducted dual-choice oviposition assays with a different group of mated females, that were *S. entomophila*-injected or PBS-injected (control) as described above. The light-dark cycle of the moth rearing for this experiment was L16:D8 with lights on at 6 am. Mated females were given a choice between a damaged and an undamaged plant. We used *Nicotiana attenuata* plants, which is one of the natural host plants of *H. virescens.* To generate damaged plants, five *H. virescens* 3rd instar larvae were placed on the plants for 48 h and removed from the plants right before the start of the experiment. Undamaged plants were left untreated. All plants were eight to nine weeks old and in their flowering stage, as females mainly lay their eggs on tobacco buds and flowers. Experiments were conducted in cages (2.0 x 0.83 x 1.0 m), which contained one damaged plant, one undamaged plant and one mated female which was 2-8 days old at the night the oviposition experiment started. Age did not differ significantly between the treatments (W = 269, $P = 0.70$, Wilcoxon rank sum test with continuity correction; for age structure see FIGURE S4.2). Damaged and undamaged plants were placed at a distance of 1.5 m from each other. Each plant was only used once for testing one female. Positions of damaged and undamaged plants in the cages were switched every night to avoid directional effects. The experiment was conducted under natural light conditions during eight nights in May 2014 (sunrise ~5:35 and sunset ~21:40 hours) and two nights in June 2014 (sunrise 5:18 and sunset 22:06 hours) at 25 °C.

Each night, 2-8 females were tested and each female was tested only once, that is for one night. Each night we tested the same number of control and *S. entomophila*-injected females. Females were released in the middle of the cages 1 h before dusk and were removed from the cages on the next day. Eggs were counted on the undamaged and the damaged plant as well as on the sides, bottom and top of the cages, on the day after the experimental night. We will refer to eggs that were not found on plants but anywhere else in the cage as off-plant eggs.

*Statistical analysis.* To test differences in preference between control and *S. entomophila*-injected females for oviposition site (damaged plant, undamaged plant or off-plant), we performed mixed-design ANOVAs with one between variable (female age) and one within variable (oviposition site) to account for the paired character of oviposition site. The analysis was performed separately for control and *S. entomophila*-injected females. The interaction effect between female age and treatment was not significant for *S. entomophila*-injected females and thus excluded from the model for this group. To compare number of eggs between the three oviposition sites, we performed LS-means pairwise comparisons with Tukey correction based on the above-described ANOVA models. Differences in total number of eggs between control and *S. entomophila*-injected females were tested with a linear model, using number of eggs as response variable and treatment and female age as explanatory variables. All analyses were conducted with R version 3.0.2 (R Core Team, 2013).

## RESULTS

### *H1: Females increase their oviposition rate after a bacterial challenge*

*Serratia entomophila*-injected females laid on average significantly more eggs than control females one night after the immune challenge ($F_{1,49}$ = 7.66, $P$ = 0.0079, FIGURE 4.1a,b). Female age at the start of the experiment did not significantly affect the number of eggs one night after injection ($F_{1,49}$ = 2.48, $P$ = 0.12, FIGURE S4.3).

When we analysed the effect of treatment over time, we found an overall significant interaction effect of night and treatment on the number of eggs per female per night ($\chi^2$ = 4.56, df =1, $P$ = 0.033, FIGURE 4.1b). *Serratia entomophila*-injected females laid on average more eggs per night than control females from night one to five with an exception of night three. Control females laid on average more eggs per night from night six to night 13 (FIGURE 4.1b).

Total number of eggs laid by control females did not differ significantly from the total number of eggs laid by *S. entomophila*-injected females ($F_{1,48}$ = 2.10, $P$ = 0.15). Female age at the start of the experiment had a marginally significant effect on the total number of eggs of the females ($F_{1,48}$ = 3.95, $P$ = 0.053, FIGURE 4.2).

*Serratia entomophila*-injected females died significantly earlier than control females after injection (z = -2.25, $P$ = 0.024, FIGURE 4.3). Of the *S. entomophila*-injected females, 38.5% died two nights after the injection. Female age at the start of the experiment did not have a significant effect on the mortality of females (i.e., num-

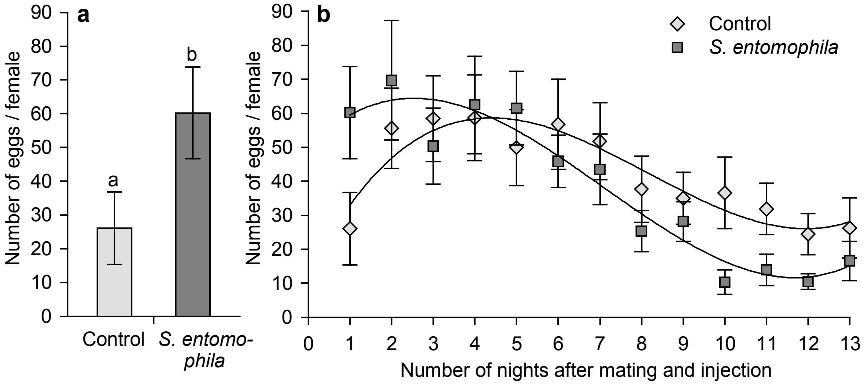


**FIGURE 4.1.** Mean (± SE) number of eggs oviposited per female in response to an immune challenge. a) Number per female 24h after immune challenge; different letters above the bars indicate significant differences at a level of alpha ≤ 0.01 (linear model). b) Daily number per female in the course of 13 nights with polynomial trendline (trendline is only for visualization and was not used for calculations); females were PBS-injected (n = 25 at night zero) or *Serratia entomophila*-injected (n = 27 at night zero).

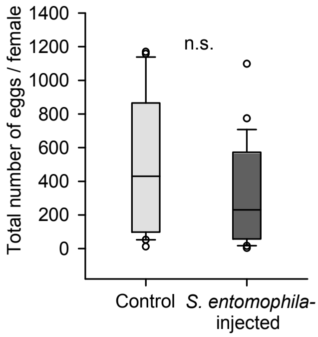


**FIGURE 4.2.** Total number of eggs laid by *Serratia entomophila*-injected (n = 26) and PBS-injected (n = 25) females after injection and mating. Boxes span the 25-75 percentiles, lines in the boxes represent the medians, whiskers span the 10-90 percentiles and circles represent data points outside this range; linear model, n.s. = not significant.

ber of days from injection to death) (z = 1.07, $P$ = 0.29). Female age at the start of the experiment also did not affect the number of females that died two nights after injection ($\chi^2$ = 0.69, df = 1, $P$ = 0.41). Treatment did have a significant effect on the number of females that were found dead at night two ($\chi^2$ = 16.5, df = 1, $P$ < 0.001, FIGURE 4.3). The number of eggs at night one that were laid by *S. entomophila*-injected females which survived longer than two nights after injection did not differ significantly from the number of eggs that were laid by nonsurvivors of *S. entomophila* injection ($F_{1,24}$ = 1.62, $P$ = 0.22, FIGURE 4.4).

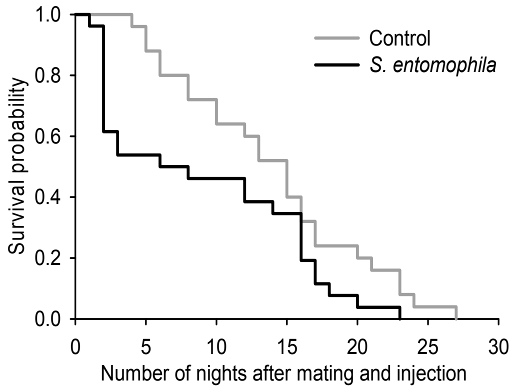


**FIGURE 4.3.** Survival probability of PBS-injected (n = 25) and *Serratia entomophila*-injected females (n = 26) on 27 consecutive nights after injection; weighted Cox regression.

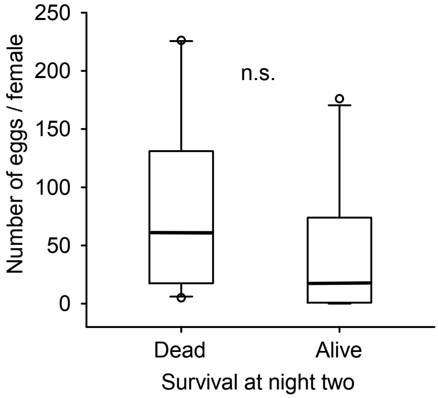


**FIGURE 4.4.** Number of eggs per *Serratia entomophila*-injected female one night after injection. Boxes span the 25-75 percentiles, lines in the boxes represent the medians, whiskers span the 10-90 percentiles and circles represent data points outside this range. Dead: females were dead at night two after injection (n = 10), alive: females survived longer than night two after injection (n = 16); n.s. = not significant.

***H2: Bacteria-challenged females are less discriminating in their choice of oviposition site than control females***

In control females, the number of eggs differed significantly between oviposition sites (undamaged, damaged plant or off-plant) ($F_{2,46}$ = 4.24, $P$ = 0.020, FIGURE 4.5a). We also detected a significant interaction effect between oviposition site and female age on the number of oviposited eggs ($F_{2,46}$ = 13.06, $P$ < 0.001, FIGURE S4.4). In the pairwise comparison of oviposition sites, control females oviposited more eggs on undamaged than on damaged plants ($t_{46}$ = 2.61, $P$ = 0.032) or off-plants ($t_{46}$ = 2.42, $P$ = 0.050), and a similar number of eggs on damaged plants and off-plant ($t_{46}$ = 0.191, *P* = 0.98, FIGURE 4.5a). In the control females, age did not have a significant effect on the number of eggs oviposited ($F_{1,23}$ = 1.38, *P* = 0.25). In *S. entomophila*-injected females, the number of oviposited eggs did not differ between oviposition sites ($F_{2,44}$ = 2.06, *P* = 0.14, FIGURE 4.5b), and there was no interaction effect between female age and oviposition site ($F_{2,42}$ =1.51, *P* = 0.23, FIGURE S4.5). The total number of eggs was not affected by female age when the two treatment groups were combined ($F_{1,45}$ = 0.014, *P* = 0.92).

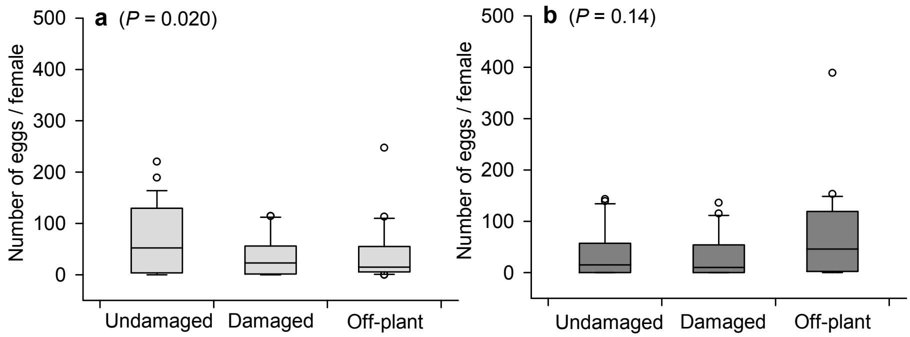


**FIGURE 4.5.** Oviposition site preference (undamaged plants, damaged plants and off-plant) in *Serratia entomophila*-injected and control *Heliothis virescens* females. a) Number of eggs per female on the three oviposition sites in control females (n = 25). Undamaged vs damaged plants: *P* = 0.032, undamaged plants vs off-plant: *P* = 0.050. b) Number of eggs per female on the three oviposition sites in *S. entomophila*-injected females (n = 23), overall effect: not significant. Boxes span the 25-75 percentiles, lines in the boxes represent the medians, whiskers span the 10-90 percentiles and circles represent data points outside this range.

## DISCUSSION

In this study, we found evidence for terminal investment in the oviposition behaviour of *H. virescens* females in response to immune challenge with the bacterial entomopathogen *S. entomophila*. We confirmed our hypotheses that (H1) *H. virescens* females increase their oviposition rate after bacterial challenge and (H2) bacteria-challenged *H. virescens* females are less discriminating in their choice of oviposition site than control females.

### *H1: Females increase their oviposition rate after a bacterial challenge*

The findings that *S. entomophila*-injected females oviposited more eggs earlier and especially laid more eggs on the first night after a bacterial challenge provide further evidence for the terminal investment hypothesis. Indication for terminal investment in nonsocial herbivorous insects has been found earlier in the cricket *Acheta domesticus*, which increased its egg output in response to immune challenge

(Adamo, 1999). Similarly, in the moth *Scotopteryx chenopodiata*, oviposition rate of injured females was also higher than that of females without injuries (Javoiš & Tammaru, 2004). In our study, the change in oviposition strategy in response to bacterial challenge was underlined by the fact that the total number of eggs laid by *S. entomophila*-injected and control females did not differ significantly, even though *S. entomophila*-injected females lived fewer days. This suggests that females compensate for a shortened lifetime with a shift of reproductive output from future to current reproduction in response to an immune challenge, which is in accordance with the terminal investment strategy (Williams, 1966; Clutton-Brock, 1984).

The fact that *S. entomophila* injection was deadly for many females in our study suggests a cost of immune response, which has been shown to reduce life span (Sheldon & Verhulst, 1996; Moret & Schmid-Hempel, 2000). Moreover, cytotoxic substances that are produced in the course of immune defence are possibly harmful for host tissue as well (Nappi & Vass, 1993; Zuk & Stoehr, 2002; Schmid-Hempel, 2005). Even though longevity was found to be affected by immune response in many studies (Moret & Schmid-Hempel, 2000; Armitage *et al.*, 2003; Javoiš & Tammaru, 2004), mortality is usually not as high as in our study. However, Krams *et al.* (2014) also found very high mortality in larvae of the moth *Galleria mellonella* in response to an immune challenge by nylon bead implantation: larvae which were grown on high energy food showed shorter developmental time, weaker encapsulation response and higher mortality in response to the challenge than larvae grown on low-energy food. As the shorter developmental time was associated with weak encapsulation response, the authors argue that low encapsulation response was likely responsible for high mortality in the high-energy food group (Krams *et al.*, 2014). Thus, possibly more complex relationships between life history traits and immune defence underlie the high mortality of *H. virescens* females in response to immune challenge.

### *H2: Bacteria-challenged females are less discriminating in their choice of oviposition site than control females*

As control females preferred undamaged over damaged plants and off-plant oviposition sites whereas *S. entomophila*-injected females did not differentiate between oviposition sites, we conclude that oviposition site selectivity can be lowered by cues of shortened life expectancy in herbivorous insects if females are given a choice between sites of different quality. Our results are in line with previous studies that investigated host plant acceptance and oviposition delay without providing a choice. For example, in the moth *S. chenopodiata*, survival of females was associated with oviposition latency on plants of different host quality (Javoiš & Tammaru, 2004): survivors of experimentally applied injuries showed a small oviposition latency on superior hosts, but a large oviposition latency on inferior hosts, whereas the reverse was found for moths that laid eggs but did not survive until the end of the experiment (Javoiš & Tammaru, 2004). We also confirmed the model prediction that oviposition site selectivity decreases when there is a cue that oviposition time is reduced (Jaenike, 1978; Courtney & Courtney, 1982).

***Trade-off between early oviposition and oviposition site preference***

The fact that a cue for shortened life expectancy provoked a temporary increase in oviposition rate in *H. virescens* females after a bacterial challenge indicates a change in oviposition strategy. This change likely optimizes female fitness when infected with pathogens (Minchella & Loverde, 1981; Adamo, 1999; Bonneaud *et al.*, 2004). However, a cue for shortened life expectancy also decreased female oviposition site selectivity, as we found differences in oviposition site choice between *S. entomophila*-injected and control females. A decrease in oviposition site selectivity is expected to reduce female fitness, because oviposition site selectivity has been shown to affect offspring survival in herbivorous insects (Singer, 1972; Rausher, 1982; Gripenberg *et al.*, 2010). The nonpreference for undamaged or damaged plants in *S. entomophila*-injected *H. virescens* females is possibly due to an increased pressure to oviposit early (before death) and indicates that increased egg output after an immune challenge may be linked to lowered oviposition site preference. Terminal investment in this moth may thus be characterized by a trade-off between early oviposition and oviposition site selectivity which likely translates into a fitness trade-off for the females.

The trade-off that we found in our experiments is not necessarily typical of all types of infections. In our experiments, we only used one strain of a pathogenic bacterium to induce an immune defence and one population of *H. virescens* as host. As genotype-specific host-parasite interactions are widespread in nature, it should be stressed that our results cannot be generalized for interactions of *H. virescens* with different parasites or even different strains of the same bacterium used in this study (Schmid-Hempel & Ebert, 2003; de Roode & Altizer, 2010). We chose to conduct the experiments with *S. entomophila*, because *S. entomophila* injection has been shown to induce the immune system of *H. virescens* in a previous study (Barthel *et al.*, 2014).

***The influence of female age on oviposition behaviour***

The finding that female age did not affect the number of eggs laid one night after mating could be explained by the fact that in *H. virescens* mating stimulates egg maturation and oviposition (Proshold *et al.*, 1982; Ramaswamy *et al.*, 1997; Zeng *et al.*, 1997). Virgin females lay far fewer eggs than mated females and mating has a particularly stimulating effect on oviposition one day after mating (Proshold *et al.* 1982). In our experiments, the effect of female age on total number of eggs was marginally significant, which is comparable to the results of Proshold *et al.* (1982) who found that the total number of eggs laid depended on the age at which females were mated. As in our experiments the number of females that were less than three and more than six days old was very small, we could not firmly test homogeneity in the number of eggs laid across age groups.

Interestingly, when we investigated oviposition site preference, we did not find an interaction effect between female age and oviposition site in *S. entomophila*-injected moths, but we did detect such an interaction in the control females. This result indicates that older females discriminate less between oviposition sites than young females. Since lifetime expectancy generally decreases with age, this age effect fits the prediction that oviposition site selectivity decreases with less time to oviposit (Jaenike, 1978). Hence, bacterial challenge and age seem to similarly induce terminal investment behaviour: possibly, age did not affect oviposition site choice in *S. entomophila*-injected females, because terminal investment was induced by bacterial challenge in females of all ages in this group. Since our experiments were not designed to measure the effect of age, future experiments are needed to investigate the effect of age on oviposition choice in *H. virescens*.

***Presence versus absence of plants in oviposition experiments***
When we tested oviposition preference between damaged and undamaged plants, both *S. entomophila*-injected and control females laid about three times as many eggs compared to *S. entomophila*-injected females in the first experiment, where plants were not involved. The presence of tobacco (*N. tabacum*) or tobacco leaf extracts is known to stimulate oviposition in mated *H. virescens* females (Jackson *et al.*, 1984; Mitchell *et al.*, 1990; Ramaswamy *et al.*, 1997): females were found to lay about three times as many eggs on cloth treated with tobacco leaf extract than on untreated cloth (Mitchell *et al.*, 1990). Conversely, Proshold *et al.* (1982) found that *H. virescens* females in mating cups without plant stimuli laid about 200 eggs per female one day after mating, which is more than three times the number that we encountered during oviposition without plant stimuli. These differences may be due to adaptation to host plant-free laboratory rearing conditions, which may inadvertently have selected for females that oviposit more readily on artificial substrate in the absence of plant stimuli: Proshold *et al*. (1982) used a *H. virescens* strain that had been reared in the laboratory for > 60 generations, whereas our oviposition timing experiment was performed with moths that were collected from the field as eggs in 2011 and reared in the laboratory for only 14 generations.

Interestingly, when we tested for an increase in the number of eggs after a bacterial challenge without plants, *S. entomophila*-injected females laid more eggs than control females one night after injection, whereas when we tested oviposition site preference, control and *S. entomophila*-injected females laid a similar total number of eggs. The absence of a suitable host plant can cause a delay in oviposition in moths (Leather & Burnand, 1987; Tammaru & Javoiš, 2000). Oviposition delay was also shown for *S. chenopodiata* on an inferior host plant for survivors of injury, but not for moths that laid eggs but did not survive until the end of the experiment (Javoiš & Tammaru, 2004). Hence, control females in our experiments possibly delayed oviposition only in the absence of a suitable host plant, whereas *S. entomophila*-injected *H. virescens* females laid high numbers of eggs one night after injection even in the absence of a suitable host. These findings are also in accordance with the terminal investment strategy.

### *Conclusions*

We conclude that *H. virescens* females are able to adapt their oviposition strategy by shifting their egg output from future to current reproduction, when survival prospects are compromised by infection. Moreover, by this shift, immune-challenged females seem to be able to compensate for their shorter lifetime by ovipositing more eggs earlier in life, as total number of eggs was not different between immune-challenged and control females. As immune-challenged females were less selective for oviposition site than control females, we show that oviposition site selectivity is another trait that can be affected by an immune challenge in herbivorous insects. As oviposition timing and plant selectivity are likely linked in herbivorous insects, we suggest that there is a fitness trade-off between making a terminal investment by laying more eggs early at the expense of oviposition site selectivity.

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## SUPPORTING INFORMATION

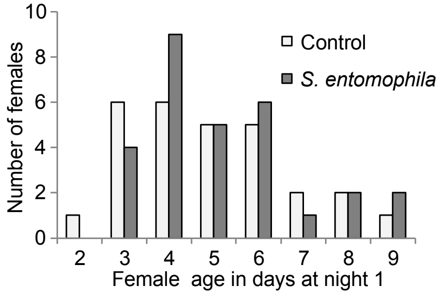


**FIGURE S4.1.** Age structure of control (n = 25) and *Serratia entomophila*-injected females (n = 27) at night one after injection and mating in the oviposition timing experiment.

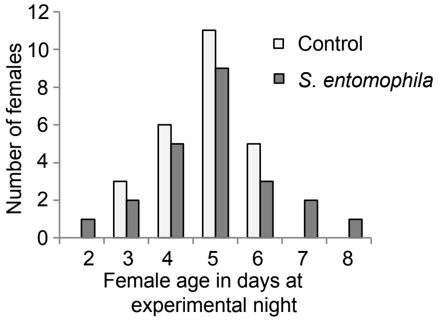


**FIGURE S4.2.** Age structure of control (n = 25) and *Serratia entomophila*-injected females (n = 23) in the experiment for oviposition site selectivity.

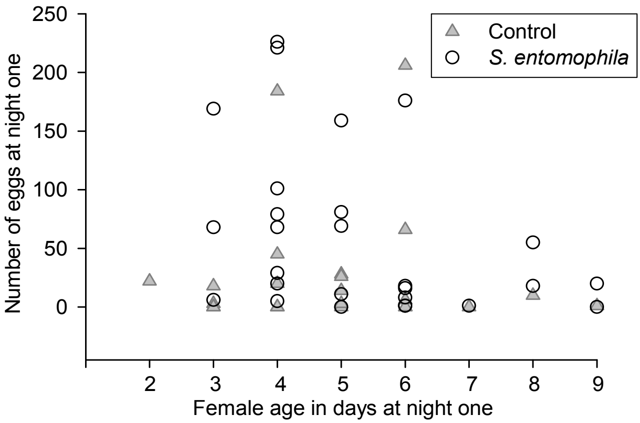


**FIGURE S4.3.** Effect of female age on the number of eggs at night one after injection and mating in the oviposition timing experiment in control (n = 25) and *Serratia entomophila*-injected (n = 27) females.

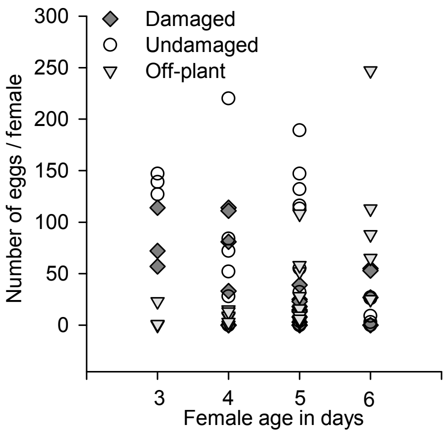


**FIGURE S4.4.** Oviposition site choice (damaged plants, undamaged plants, off-plant) in different age groups in control females (n = 25).

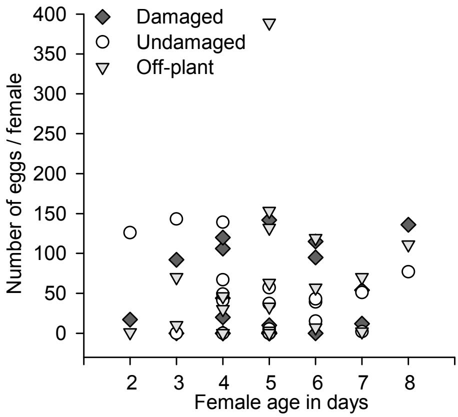


**FIGURE S4.5.** Oviposition site choice (damaged plants, undamaged plants, off-plant) in different age groups in *Serratia entomophila*-injected females (n = 23).