Resource quality impacts the immune system of the Indianmeal moth

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ABSTRACT

Invertebrates are exposed to many pathogens and parasites during their life cycle. Host immune responses are sensitive to changes in resource quality due to trade-offs between life-history traits and immune defenses. However, we lack a full understanding of how trade-offs play out in the presence of pathogens and varying resource environments. In this study, we investigated whether resource quality in conjunction with bacterial infection alters the immune system of the insect species, *Plodia interpunctella*, commonly known as the Indian meal moth. We used a factorial design with food quality and pathogen presence varied for a total of eight treatment combinations. Larvae in the infected condition were infected by Bacillus thuringiensis, a soil-dwelling, natural pathogen of *Plodia Interpunctella*. Total haemocyte count was determined across all treatments conditions. We found significant differences in the total number of haemocyte cells between larvae reared in different food quality conditions, in both infected and uninfected larvae. Moreover, we found a significant difference in total haemocyte counts between infected and uninfected larvae, throughout food quality conditions. Finally, our results show a significant difference in the body mass of the larvae reared under varying food quality conditions. This work provides a deeper understanding of how insect immunity responds to bacterial pathogen infection across food-quality environments. Specifically, how food quality and pathogen conditions interact to affect host immune responses. Furthermore, this information helps us understand and predict host-pathogen dynamics under changing environments.

INTRODUCTION

In this study, we used the Lepidopteran species, *Plodia Interpunctella,* also known as the Indianmeal moth, and the bacterial species Bacillus thuringiensis to explore trade-offs between immunity and developmental interactions in varying food quality environments.

<u>OBJECTIVE:</u> How does exposure to varying food qualities and pathogen infection impact immune resistance in *P. interpunctella* populations?

HOST AND PATHOGEN

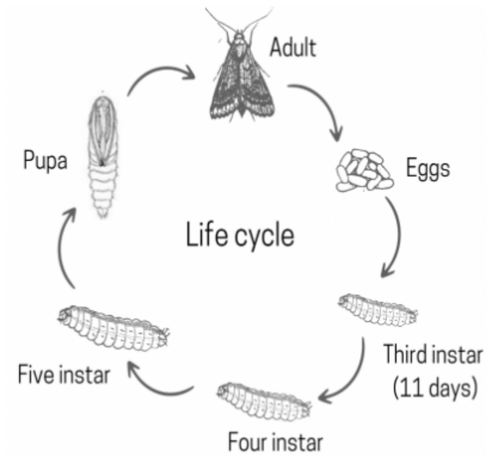
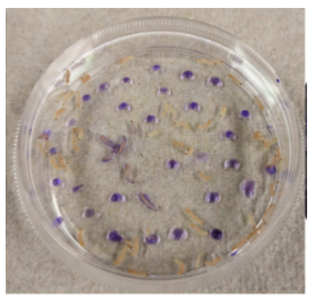


Figure 1: Life cycle of Plodia interpunctella
Source: ARS USDA

Figure 2: *Bacillus thuringiensis*
Source: *Koppert n.d Bacillus thuringiensis*, 2019

METHODS



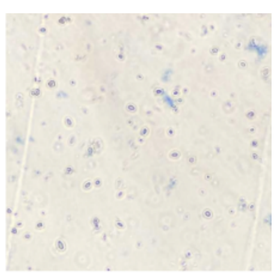


Figure 3: *P. interpunctella* infection by *Bt*
Figure 4: Haemocytes under microscope

Food media culturing
Various qualities of food (figure 5) were produced by replacing part of the standard food recipe with methylcellulose, an indigestible bulking agent. 0%, 20%, 40%, and 60% of larva's food was replaced, making 0% the highest quality and 60% the lowest quality of food. All larvae were reared in their respective food quality treatment.
Bioassays: lethal dosage
Bt infections (figure 3) were carried out on third instar larvae to determine lethal dosage of 50% (LD 50).*Bt* was diluted and quantified and number of colonies per ml at each density was found. Infection experiment results indicated that ~3,000 colonies/mL resulted in LD 50.
Haemocyte counts
Hemolymph from 5-7 larvae were extracted to produce a 1 microliter sample for each food treatment. Samples were collected by piercing the larvae and then adding the larvae to a new syringe and squeezing to allow hemolymph to escape through the needle onto a petri dish. Hemolymph was combined with buffer and a hemocytometer was used to count cells (figure 4).
Data analysis
The mean of each condition was calculated and analyzed with a general linear model. Weights of the larvae were also measured and analyzed with an ANOVA. A post-hoc test was performed to observe parings between the conditions with statistical significance.

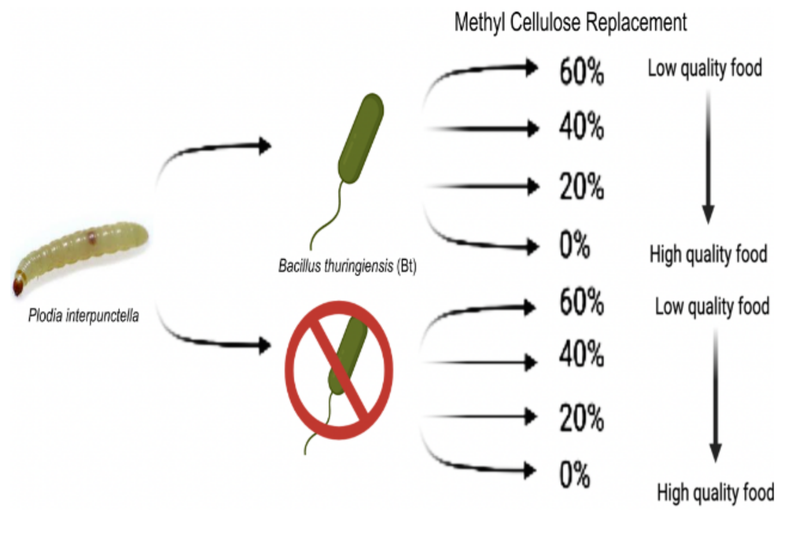


Figure 5: Dispersal experiment setup
Created with BioRender.com.

RESULTS

Food quality was found to impact immune system functioning of *P. interpunctella* with significant differences in haemocyte/ml being found between 0% and the rest of the food groups (*p* = <0.0001, <0.0001, <0.0001) and between 20% and 40% (*p = 0.0003).*

Infection by Bt was found to strongly impact immunity of *P. interpunctella* across food quality conditions (*p = <0.001)*.

Food quality was found to strongly impact body mass of *P. interpunctella,* with a *significant* difference in mass between all except 0% - 20% and 40% - 20% (*p = <0.001, 0.001, 0.000055, 0.001).*

Bt infection did not significantly impact body mass of P. interpunctella.

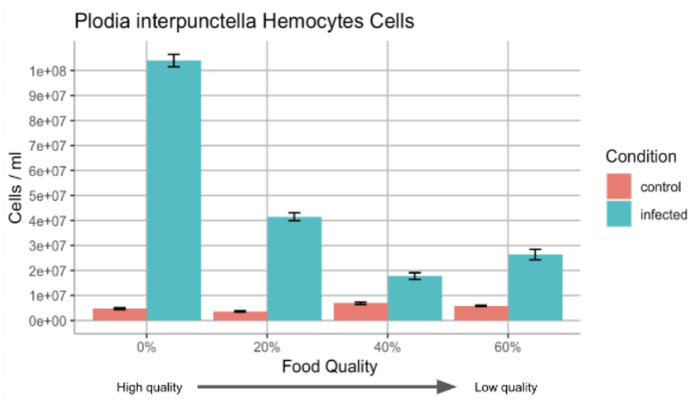


Figure 6: Food quality versus cells/mL, with uninfected and infected larvae across food quality conditions.

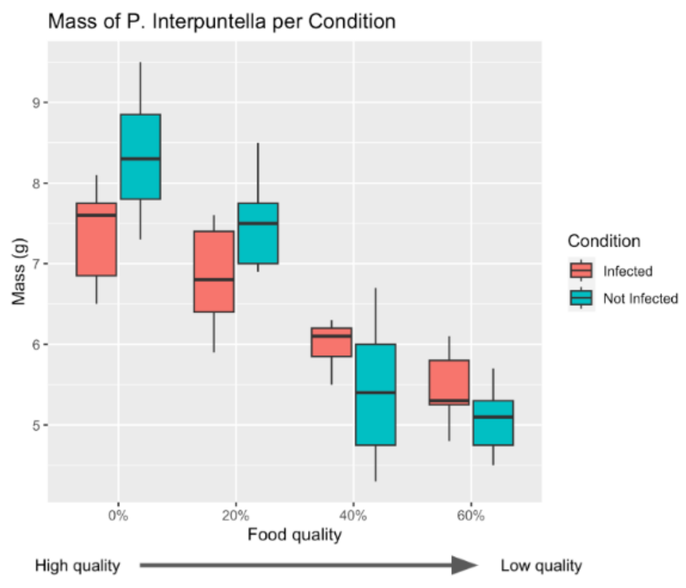


Figure 7: Food quality versus mass (g) of uninfected and infected larvae across food quality conditions.

DISCUSSION

Here, we showed that there is a significant impact of food quality on host immunity. The comparison between the 0% food condition and the other three food conditions was strongly statistically significant, as well as between the 20% and 40% food conditions. Moreover, we show here an increased immune investment in *P. interpunctella* when challenged with the *Bt* pathogen, irrespective of the food quality environment the larvae experienced. These results are consistent with previous studies showing similar trends when *P. interpunctella* was challenged with a naturally occurring viral pathogen (Boots, 2011).

Furthermore, significant differences in larvae body masses were detected between food conditions. In general, average body mass declined in lower-quality resource environments. These results show that the food quality environment impacts larval weight, similar to previous studies (Boots, 2011). Our results potentially indicate a trade-off between development and pathogen virulence, although more research is needed to explore this relationship further.

This study successfully shows how the interaction between the *Bt* pathogen and food quality can elicit a host immune response. Furthermore, under certain environmental conditions larvae may undergo a trade-off between body growth and immune defense. These findings can shed light on host-pathogen dynamics in natural environments. Understanding these dynamics is becoming increasingly relevant, as insect pests continue to expand their natural host ranges under global change scenarios.

ACKNOWLEDGEMENTS

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Boots, M. (2011). The Evolution of Resistance to a Parasite Is Determined by Resources. *The American Naturalist, 178*(2), 214–220. https://doi.org/10.1086/660833