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Food-mediated modulation of immunity in a phytophagous insect: An effect of nutrition rather than parasitic contamination

Fanny Vogelweith [a,b,c,*], Jérôme Moreau [a], Denis Thiéry [b,c], Yannick Moret [a]

[a] Université de Bourgogne, Equipe Ecologie-Evolution, UMR 6282 Biogéosciences, 6 Bd Gabriel, F-21000 Dijon, France
[b] INRA, UMR 1065 Save ISVV, B.P.81, F-33883 Villenave d'Ornon Cedex, France
[c] Université de Bordeaux, Bordeaux Sciences Agro, INRA, UMR 1065 Save ISVV, B.P.81, F-33883 Villenave d'Ornon Cedex, France

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ABSTRACT

Inherent to the cost of immunity, the immune system itself can exhibit tradeoffs between its arms. Phytophagous insects face a wide range of microbial and eukaryotic parasites, each activating different immune pathways that could compromise the activity of the others. Feeding larvae are primarily exposed to microbes, which growth is controlled by antibiotic secondary metabolites produced by the host plant. The resulting variation in abundance of microbes on plants is expected to differentially stimulate the insect antimicrobial immune defenses. Under the above tradeoff hypothesis, stimulation of the insect antimicrobial defenses is expected to compromise immune activity against eukaryote parasites. In the European grape berry moth, *Eupoecilia ambiguella*, immune effectors directed towards microbes are negatively correlated to those directed towards eukaryotic parasites among host plants. Here, we hypothesize this relationship is caused by a variable control of the microbial community among host plants by their antibiotic metabolites. To test this hypothesis, we first quantified antimicrobial activity in berries of several grape varieties. We then measured immune defenses of *E. ambiguella* larvae raised on artificial diets in which we mimicked levels of antimicrobial activity of grape berries using tetracycline to control the abundance of growing microbes. Another group of larvae was raised on artificial diets made of berry extracts only to control for the effect of nutrition. We found that controlling microbe abundance with tetracycline in diets did not explain variation in the immune function whereas the presence of berry extracts did. This suggests that variation in immune defenses of *E. ambiguella* among grape varieties is caused by nutritional difference among host plants rather than microbe abundance. Further study of the effects of berry compounds on larval immune parameters will be needed to explain the observed tradeoff among immune system components.

1. Introduction

Immune defense is an important trait in biology with profound implications for the fitness of organisms. Because immunity is costly to maintain and use, its activation can affect other important physiological processes, and vice versa (Armitage et al., 2003). In addition, as the immune system is multifaceted, tradeoffs can also occur between pathways of the immune system itself. Organisms are therefore expected to invest in defense based on the risk of infection, or allocate defense in response to the most fitness-threatening parasite (Graham, 2001; Moret, 2003).

The invertebrate immune system involves humoral antimicrobial peptides used to combat microbial infection (Imler and Bulet, 2005), and the phenoloxidase–prophenoloxidase (PO–PPO) system, which is a component of the oxidative and melanization defenses used against eukaryotic parasites (Cerenius and Soderhall, 2004). Negative correlations between these two pathways of the immune system have been observed in bumblebees (Moret and Schmid-Hempel, 2009), the cabbage looper *Trichoplusia ni* (Freitak et al., 2007) and the European grape berry moth *Eupoecilia ambiguella* (Vogelweith et al., 2011). Changes in relative expression among immune effectors may result from selection across host generations, but might also result from plastic modulations of the immune system within individuals either in response to external cues predicting the presence of parasites (Wilson and Reeson, 1998) or upon infection. In the latter case, as hosts are facing a wide range of different parasite types, each

* Corresponding author at: Institut National de la Recherche Agronomique (INRA), UMR 1065 Save ISVV, B.P.81, F-33883 Villenave d'Ornon Cedex, France. Tel.: +33 05 57 12 26 12; fax: +33 05 57 12 26 21.
E-mail address: fanny.vogelweith@gmail.com (F. Vogelweith).

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activating different immune effectors, the infection by one parasite type may compromise the activity of other immune effectors that are competent against other parasite types (Freitak et al., 2007; Moret and Schmid-Hempel, 2009; Vogelweith et al., 2011).

As many invertebrates, phytophagous insects are exposed to a large range of microbial and eukaryotic parasites. Feeding larvae are primarily exposed to variable abundance and diversity of microbes on host plant structures (Renouf et al., 2005). Consumption of microbes is known to affect the expression of immune effectors in insects (Brown et al., 2003; Freitak et al., 2007). Hence, the relative expression of immune effectors might be affected by the ability of the host plant to control the microbial community on its surfaces. Some secondary metabolites of host plants have antibiotic activity that controls microbial development in the plant (Visser, 2011). Variation in plant antibiotic chemicals can differentially affect microbial growth on host plants, and feeding larvae would then be subject to variable amount of microbes, activating the antimicrobial immune response of phytophagous insect larvae. Assuming tradeoffs between the inducible productions antimicrobial peptides and the constitutive activity of the phenoloxidase (Moret and Schmid-Hempel, 2009), variation in the antibiotic activity of plants should indirectly affect how phytophagous insects allocate resource between these two immune pathways.

Besides being sources of microbial infection, host plants are also sources of nutrients of variable quantity and quality for phytophagous insects. Nutrition appears to be an important modulator of the host immune response. For instance, food deprivation leads to reduced immune responsiveness (Siva-Jothy and Thompson, 2002), lower resistance to pathogenic infection (Feder et al., 1997), and sometimes greater tolerance to pathogenic infection (Ayres and Schneider, 2009). Furthermore, variation in the intake of macronutrients in the food, including proteins and carbohydrates, could markedly affect immune traits (Cotter et al., 2011; Povey et al., 2009). Other dietary substances, including carotenoids, have also been found to influence the immune response of both vertebrates (Chew and Park, 2004) and invertebrates (Babin et al., 2010).

Therefore among phytophagous insects, natural variation in immune defense and resistance to pathogens could be partly host-plant dependent. On the one hand, expression of immune defense might be indirectly affected by the host plant antibiotic activity, controlling microbial pathogens consumed by larvae. On the other hand, changes in immune defense could directly result from variation in the nutritional quality of the host plant. Testing the relative importance of these non-exclusive factors should therefore improve our understanding of how host plants affect the immune response of phytophagous insects, and ultimately their fitness.

E. ambiguella (Lepidoptera: Tortricidae) is one of the two major moth pests of several grape vine cultivars in Europe. Antimicrobial activity in the hemolymph of *E. ambiguella* larvae was found negatively associated with the concentration of circulating hemocytes, and the activity of the PO–PPO system among vine cultivars (Vogelweith et al., 2011). This pattern of covariation between immune defenses is consistently observed in *Lobesia botrana* collected on grapes in the field (Vogelweith et al., 2014) and in *E. ambiguella* fed on diets made of grape extracts (Vogelweith et al., 2011). The grape surface is a phyllosphere habitat containing yeasts, bacteria and fungi (Boe, 2005), and varies with factors including climatic conditions and viticultural practices (Barata et al., 2012). To control these microbes, grape berries produce several antimicrobial peptides constitutively or in response to infection (Visser, 2011). Grape varieties vary in their susceptibility to microbial infection. For instance, Chardonnay vines in South Africa have been shown to be highly susceptible to crown gall (Burr et al., 1998), whereas the Pinot Noir variety appears to be protected through the production of a berry-specific defensin that has antifungal activity (de Beer and Vivier, 2008).

This study tested whether microbe abundance regulated by the antibiotic activity of grape berries affects the relative expression of the different immune effectors in the phytophagous insect, *E. ambiguella*. To this purpose, we first measured antimicrobial activity from berries of different grape variety and estimated it as a concentration of tetracycline, an antibiotic substance with a large spectrum of antimicrobial activity. Then, we raised larvae on six artificial diets that varied in their concentration of tetracycline to mimic natural levels of antimicrobial activity found in the different grape varieties. One of these diets did not contained tetracycline but was supplemented with bacteria. These diets enabled to assess the influence of host plant microbial control on the phytophagous insect immune system, independently of the plant nutritional quality. We also raised larvae on 2 artificial diets that did not contain tetracycline but berry extracts, which were used to test the direct effect of grape berries and variable plant nutritional quality. At the end of the larval stage, we measured constitutive (concentration of hemocytes, and PO and total-PO activities) and induced (antimicrobial activity) immune defenses. We previously found that induced immune defenses in the hemolymph of *E. ambiguella* larvae were negatively associated with constitutive ones among vine cultivars (Vogelweith et al., 2011). If the presence of microbes in the diet is important, we expect that diets containing tetracycline that control microbe growth will favor the maintenance of high levels of PPO activity in the hemolymph of larvae. Conversely, the absence of tetracycline should favor the development of microbes in the diet, which should stimulate antimicrobial activity in the hemolymph, at the expense of the PO–PPO system.

2. Materials and methods

2.1. Insect model

The insects used in this study were from an inbred stock maintained for several years at the INRA-Bordeaux Aquitaine (France). The stock culture is based on a large number of caged adults (several thousand per week) to which wild adults are periodically added. This laboratory strain has conserved genetic variability as considerable variation is found in immune parameters among larvae (Vogelweith et al., 2011). Larvae were maintained in boxes ($18 \times 11.5 \times 7$ cm) under standard laboratory conditions (22 ± 1 °C; 70 ± 10% rh; photoperiod: L16:D8), and provided with an *ad libitum* supply of a semi-artificial diet (1000 ml water, 15 g agar, 84.63 g maize flour, 41.25 g wheat germ, 45.48 g yeast, 6.03 g ascorbic acid, 0.32 g Scala®, 3.35 g mineral salt, 5 ml ethanol, 2.65 g benzoic acid and 2.76 g Nipagine) (Thiery and Moreau, 2005). The density of individuals was 100 per box (300 ml of diet).

2.2. Experimental design

2.2.1. Antibiotic activity in grape berries

The first part of this study involved measures of the levels of antimicrobial activity in berries from six grape varieties, and equating them to standard tetracycline concentrations. During the last week of July 2010 (which corresponds to the grape stage on which the second annual generation of *E. ambiguella* occurs), bunches of berries at the pre-veraison stage were collected from the 'gene collection of grape plants', Domaine de la Grande Ferrade (INRA-Bordeaux Aquitaine). To avoid a potential plant effect, we took 10 bunches per grape variety on different plant and at different place in the plant. All grape varieties were cultivated using similar practices and without insecticide application, in a small vineyard having homogeneous soil characteristics and