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Host plant variation plastically impacts different traits of the immune system of a phytophagous insect

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Summary

1. Host plant quality affects herbivorous insect performance and consequently their susceptibility to natural enemies. Recently, it has been hypothesized that the immune function of herbivorous insects can be altered by their host plant, thus generating variation in their susceptibility to entomopathogens. Previous studies testing this hypothesis provided contradictory outcomes, mainly as a result of the differences in methodology such as measuring a single-immune parameter rather than considering trade-off-mediated interactions between immune defence systems of the insect. Here, we hypothesized that plant-mediated changes in insect immunity could result from the alteration of physiological immune effectors of the herbivore evident as trade-offs.

2. Larvae of an inbred strain of the European grape berry moth *Eupoecilia ambiguella* were reared on five artificial diets each based on a different grape variety (Chardonnay, Chasselas, Gewurztraminer, Merlot, Riesling) and tested for changes in the baseline concentration of haemocytes, activities of the prophenoloxidase (PPO) system and of antimicrobial peptides of their haemolymph. Immune responsiveness of larvae across diets was also assessed by measuring changes in haemocyte concentration and activity of the PPO system after a bacterial immune challenge.

3. We found that variation among diets significantly affected immune defences of larvae. The alteration of the *E. ambiguella* immune system appears plastic and partly mediated by existing physiological trade-offs between immune pathways, at least between induced antibacterial defences and the PPO system.

4. These results clearly show that host plant quality can affect immune defences and potentially disease resistance of *E. ambiguella* and that these changes in immunity may also result from intrinsic trade-offs between immune defence systems in insects.

Key-words: antimicrobial activity, enzymatic cascade of prophenoloxidase, *Eupoecilia ambiguella*, grapes, haemocytes, immune challenge, larval diet, larval immune defence

Introduction

In tritrophic systems between plants, phytophagous insects and natural enemies, host plant variation is often key to the effects of the relative performance of both the herbivore and its associated natural enemies (Awmack & Leather 2002; Teder & Tammaru 2002). The bottom-up effect of host plants could be positive for all upper trophic levels. For instance, highly nutritional and/or less-defended plants could increase the performance of both the herbivores and their natural enemies (Awmack & Leather 2002; Coley, Bateman & Kursar 2006). In contrast, the quality of the plant may differentially affect the performance of herbivorous insects and their natural enemies. For example, nutrient deficiencies and/or toxic defensive compounds of the plants could slow down the development of herbivorous insects, thus extending the window of vulnerability for attacks by natural enemies (Benrey & Denno 1997). Plant quality may also alter herbivorous insect condition (Singer *et al.* 2004; Coley, Bateman & Kursar 2006; Smilanich *et al.* 2009),

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making them more vulnerable to entomopathogens and parasitoids (Cory & Hoover 2006).

Recently, it has been proposed that plant-mediated resistance of herbivorous insects to pathogens and parasitoids could result from alterations of the insect immune function (Karimzadeh & Wright 2008). Insect immunity relies on a suite of systemic responses to combat a wide array of pathogens (Cherry & Silverman 2006) that involve constitutive and inducible mechanisms. Constitutive defences rely on the insect haemocytes and rapidly activated enzyme cascades such as the phenoloxidase (PO) (Cerenius & Söderhäll 2004; Siva-Jothy, Moret & Rolff 2005). The baseline of these immune effectors could be impaired during starvation periods (Siva-Jothy & Thompson 2002) or when exposed to protein deficient food sources (Lee, Simpson & Wilson 2008). Hence, phytophagous insects reared on host plants differing in nutritional values are expected to differ in their baseline levels of constitutive defences. Recent studies testing this hypothesis by directly measuring levels of some of these immune effectors or related resistance provided contrasting results, with some studies finding an effect of the host plant (Klemola *et al*. 2007; Bukovinszky *et al*. 2009; Alaux *et al*. 2010; Shikano *et al*. 2010) while other studies found no effect (Karimzadeh & Wright 2008; Klemola, Kapari & Klemola 2008). Associated with this constitutive line of defences is the induced response, which consists of a suite of antimicrobial peptides (Iwanaga & Lee 2005) produced within the first three hours following the immune challenge (Lavine, Chen & Strand 2005) and persisting for weeks in a variety of insects (see Haine *et al*. 2008 for review). This induced line of defence adds to the complexity of the insect immune system as its activation could potentially trade off with the maintenance of other immune pathways (Moret 2003), especially the PO activity (Moret & Schmid-Hempel 2009). Herbivorous insects make contact with the microbial community harboured by their host plants while feeding (Schoonhoven, van Loon & Dicke 2008), and induced antimicrobial activity may therefore be an important component during this process. Because of the trade-off between the induced response (i.e. antimicrobial activity) and the constitutive line of defences (i.e. phenoloxydase activity), we suggest that a high level of antimicrobial activity is associated with the down-regulation of the prophenoloxidase (PPO) system. As other studies (Klemola *et al*. 2007; Bukovinszky *et al*. 2009; Alaux *et al*. 2010; Shikano *et al*. 2010) did not consider the induced line of defences, the variability in the PPO system they found could be attributed to this trade-off mediated by the host plant. Therefore, we believe it is crucial to consider together these two lines of defence in one study.

Here, we investigated the influence of host plant variation on baseline levels of a large range of constitutive (haemocyte concentration, PPO system activity) and inducible immune defences (antimicrobial activity). We also studied the capacity of the immune system to respond against a pathogen attack. To do this, we measured the recruitment of haemocytes and PO activities 24 h after being artificially immunologically challenged with dead bacteria. To this purpose, we used the European grape berry moth *Eupoecilia ambiguella* (Lepidoptera, Tortricidae) (Fig. 1) as a target phytophagous insect. *E. ambiguella* is one of the most harmful pests of grapes in Europe. In this study, the effect of host plant quality variation on insect immunity was investigated by offering five different diets made of different cultivars of grapes to larvae of an inbred strain of *E. ambiguella*. We then searched for potential variation in the baseline concentration of haemocytes, activity of the PPO system and antimicrobial activity in the haemolymph of larvae achieved during their development across the five different diets. We also compared the immune responsiveness of larvae across diets by measuring changes in haemocyte concentration and activity of the PPO system after a bacterially based benign immune challenge. We expected that plant-induced variation in immune defences of *E. ambiguella* could be mediated by physiological trade-offs between immune effectors, especially between the induced production of antimicrobial peptides and the activity of the PPO system.

Materials and methods

EXPERIMENTAL PROCEDURES

Insects used in this study were from an inbred strain of *E. ambiguella* (Fig. 1) from INRA-Bordeaux Aquitaine (France) for several years. Larvae were cultured in groups and maintained under standard laboratory conditions (22 ± 1 °C, 70 ± 10% r.h., photoperiod: L16:D8) with *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) (see Thiéry & Moreau 2005).

The influence of different host plants on fitness related traits and immunity in *E. ambiguella* was tested by rearing larvae on artificial diets derived from different host plants. A total of 1466 newly hatched larvae (age < 24 h) were individually allocated at random to five experimental diets made of berries from the following grapevine vari-

Fig. 1. Adult female of our strain European grape berry moth (*Eupoecilia ambiguella*) (left) (Picture P. Goetgeluck & D. Thiéry) and fully grown (5th instar) larva (right) (Picture F. Vogelweith). Larva length is *c*. 1 cm.

eties: 'Chardonnay' (CHAR), 'Chasselas' (CHAS), 'Gewurztraminer' (GEW), 'Merlot' (MER) and 'Riesling' (RIE), and a semi-artificial diet (Control). Larvae were maintained until they had reached the fifth larval instar, during which they were checked every second day for survival. Within each treatment group, larvae that reached the fifth larval instar were randomly divided in two lots. In the first lot, individual larvae provided a sample of haemolymph to test for the concentration of haemocytes, the antibacterial activity and the maintenance and use of the PPO system when they were unchallenged. In the second lot, larvae were used to test for changes in the concentration of haemocytes and PO activities of their haemolymph 24 h after being immunologically challenged with dead bacteria. Body size of the fifth larval instar was measured from these two samples.

EXPERIMENTAL DIET

Experimental diets were made as described in Thiéry & Moreau (2005), Moreau, Benrey & Thiéry (2006a,b) and Moreau *et al.* (2006c). The recipe of the experimental diets was similar to the semi-artificial one described previously except that the amounts of maize flour and wheat germ were 16·93 g and 8·25 g, respectively. To the mixture was added 30 g of freeze-dried deseeded powder of berries (Moreau *et al.* 2006a) obtained from the five grapevine varieties described previously. Insecticide-free bunches of berries were all collected from the 'gene collection of grape plants' 'Domaine de la Grande Ferrade', INRA-Bordeaux Aquitaine at the pre-veraison during the last week of July 2009, which corresponds to the grape phenology on which the second annual generation of *E. ambiguella* occurs. All varieties are cultivated with similar practices in a small vineyard without insecticide in homogeneous pedological characteristics and climatic conditions.

Larvae were individually reared in centrifuge tubes filled with 1·5 mL of diet, which is enough for the larvae to complete development (Thiéry & Moreau 2005). Lids of the tubes were pierced with a needle to allow air circulation.

HAEMOLYMPH COLLECTION AND IMMUNE CHALLENGE

Individual larvae were chilled on ice for 20 min before a 3-μL sample of haemolymph was collected from a wound in the posterior part of the ventral side of the abdomen using a sterile glass capillary (Hirschmann®Laborgeräte, Eberstadt, Germany). One microlitre was flushed into a microcentrifuge tube containing 20 μL of cold sodium cacodylate/$CaCl_2$ buffer (0·01 M sodium cacodylate; 0·005 M $CaCl_2$; pH 6·5). Ten microlitres of this solution was immediately used to measure the concentration of haemocytes and the rest was stored at −27 °C for later measurement of the PO activity. The remaining haemolymph in the capillary (2 μL) was flushed into a *N*-Phenylthiourea (Sigma P7629)-coated microcentrifuge tube containing 4 μL of cold sodium cacodylate/$CaCl_2$ buffer and stored at −27 °C until later examination for antibacterial activity.

Twenty-four hours after being immunologically challenged with dead bacteria (second lot, see Experimental Procedures), only 1 μL of haemolymph was collected and flushed into a microcentrifuge tube containing 20 μL of cold sodium cacodylate/$CaCl_2$ buffer to measure the concentration of haemocytes and PO activity when unchallenged. This was to test for changes in the larval concentration of haemocytes and PO activities of the haemolymph. Then, they were immediately immune challenged with a sterile needle dipped into a concentrated suspension of heat-killed *Arthrobacter globiformis* (about 10^9 cells mL^{-1}) obtained from the Pasteur institute (CIP 105365) to mimic a bacterial infection. This bacterium is commonly used in the protocol testing antimicrobial activity (Moret and Siva-Jothy 2003, Moret 2006; Sadd & Schmid-Hempel 2007). Indeed, *A. globiformis* is sensitive enough to detect the antibacterial activity resulting from the production of antibacterial peptides upon an immune challenge. Larvae were then kept in their rearing tubes for 24 h before a second 1-μL sample of haemolymph was collected to measure again the concentration of haemocytes and PO activity when the individual is immune challenged. As our purpose was to compare immune activity after an experimental infection across diets, controlling for the effects of wounding was not necessary.

IMMUNE PARAMETERS

Concentration of haemocytes was measured using a Neubauer improved haemocytometer under a phase contrast microscope (magnification ×400). The activity of naturally activated PO enzymes only (hereafter PO activity) and the activity of the proenzymes (PPO) in addition to that of the PO (hereafter total-PO activity) were measured using a spectrophotometer following the method described in Cornet, Biard & Moret (2009). PO activity was quantified without further activation, while total activity required the activation of the PPO into PO with chymotrypsin. To this purpose, frozen haemolymph samples were thawed on ice and centrifuged (4000 *g*, 15 min, 4 °C). Five microlitres of supernatant was added to a microplate well containing 20 μL of PBS and either 140 μL of distilled water to measure PO activity only or 140 μL of chymotrypsin solution (Sigma C-7762, 0·07 mg mL^{-1} of distilled water; Sigma-Aldrich, St. Louis, MO, USA) to measure total-PO activity. Then, 20 μL of L-Dopa solution (Sigma D-9628, 4 mg mL^{-1} of distilled water) was added to each well. The reaction was allowed to proceed at 30 °C in a microplate reader (Versamax; Molecular Devices, Sunnyvale, CA, USA) for 40 min. Readings were taken every 15 s at 490 nm and analysed using the software SOFT-Max®Pro 4.0 (Molecular Devices). Enzyme activity was measured as the slope (Vmax value: change in absorbance unit per minute) of the reaction curve during the linear phase of the reaction and reported to the activity of 1 μL of pure haemolymph.

Antimicrobial activity in the haemolymph was measured using a standard zone of inhibition assay (Moret 2006). Samples were thawed on ice, and 2 μL of the sample solution were used to measure the antimicrobial activity on zone of inhibition plates seeded with *A. globiformis*. *Arthrobacter globiformis* from a single colony on a streak plate were incubated overnight at 30 °C in broth medium (10 g bacto-tryptone, 5 g yeast extract, 10 g NaCl in 1000 mL of distilled water, pH 7·0). From this culture, bacteria were added to broth medium containing 1% agar to achieve a final density of 10^5 cells mL^{-1}. Six millilitres of this seeded medium was then poured into a Petri dish and allowed to solidify. Sample wells were made using a Pasteur pipette fitted with a ball pump. Two microlitres of sample solution was added to each well, and a positive control (Tetracycline: sigma T3383) was included on each plate. Plates were then incubated for 48 h at 30 °C, after which the diameter of inhibition zones were measured for each sample.

BODY SIZE

Body size of the larvae was estimated by measuring the distance between the most distant lateral sides of the head capsule margins (Delbac, Lecharpentier & Thiéry 2010) using a Nikon SMZ-10A stereoscopic microscope and VTO 232 video analysis system (Linkam

Scientific Instruments). In *E. ambiguella*, body size is correlated with the age of the larvae (Delbac, Lecharpentier & Thiéry 2010). Therefore, head size is an estimator of larval instars.

STATISTICAL ANALYSIS

Survival of larvae across experimental diets was analysed using a Pearson's chi-square test. Data on basal concentration of haemocytes, basal PO, basal total-PO and basal antimicrobial activities could not be satisfactorily transformed to use parametric tests. They were consequently analysed using Wilcoxon's rank tests for potential difference between experimental diets. Significance levels for multiple comparisons were adjusted using Bonferroni's sequential correction. Immune responsiveness of larvae challenged with dead bacteria were analysed using analyses of variance for repeated measures (repeated ANOVA) with haemocyte concentration, PO and total-PO as dependent variables, experimental diet as factors and body size as covariate. For these latter analyses, the best statistical models were scanned using a stepwise backward procedure from initial models that included all main effects and interactions. All statistical tests were performed using the JMP software (Version 3.2.2; SAS institute Inc., Cary, NC, USA).

Results

SURVIVAL OF LARVAE

Larval survival to the fifth instar was significantly variable among experimental diets (Fig. 2; χ^2 Pearson: $\chi^2_5 = 471{\cdot}54$, $P < 0{\cdot}0001$). Larvae reared on Riesling and Merlot diets suffered the highest mortality whereas those reared on the control diet had the highest survival (Fig. 2). Survival on Chardonnay, Chasselas and Gewurztraminer diets was around 45% but did not differ from each other (Fig. 2).

IMMUNE PARAMETERS

Survival of the larvae on Riesling and Merlot diets was very low. Consequently, there were not enough larvae available to measure immune parameters and data on immune defences; therefore, results below only refer to larvae reared on control, Chardonnay, Chasselas and Gewurztraminer diets.

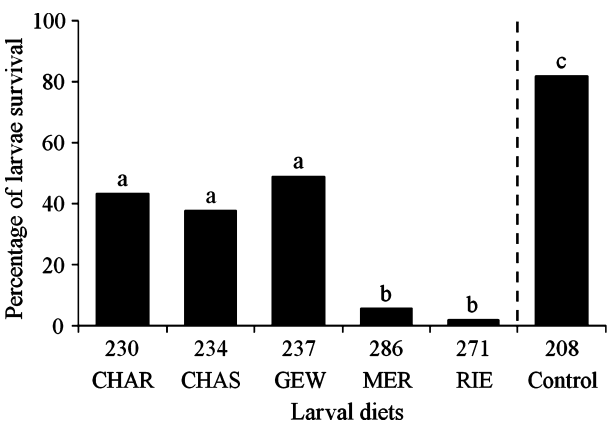

Fig. 2. Percentage of larvae survival from hatching to the fifth instar according to diets. Diets with the same lowercase letter are not significantly different ($P > 0.05$). The number of larvae tested on each diet appears below the *x*-axis.

Basal levels of haemocyte concentration, PO and total-PO, and antimicrobial activity

Although not significant, concentration of haemocytes of larvae from Chardonnay, Chasselas and Gewurztraminer diets tended to be dissimilar (Wilcoxon test: $\chi^2_2 = 5{\cdot}46$, $P = 0{\cdot}065$). When adding the control diet, variation became significant ($\chi^2_3 = 25{\cdot}69$, $P < 0{\cdot}0001$) mainly because larvae of the control diet exhibited the lowest concentration of haemocytes (Fig. 3a).

Phenoloxidase and total-PO activities of larvae were significantly different among experimental and control diets (PO: $\chi^2_3 = 27{\cdot}96$, $P < 0{\cdot}0001$; Total-PO: $\chi^2_3 = 23{\cdot}25$, $P < 0{\cdot}0001$). Both enzymatic activities were significantly higher in larvae from the Gewurztraminer diet than in those of the three other diets, which were not significantly different (Fig. 3b).

Antibacterial activity of larvae was significantly variable among the three experimental and the control diets (Wilcoxon test: $\chi^2_3 = 18{\cdot}2$, $P = 0{\cdot}0004$; Fig. 3c). Larvae reared on Gewurztraminer diet had the lowest antimicrobial activity and those reared on Chasselas diet had the highest one (Fig. 3c). Antibacterial activity of larvae of the control diet was similar to those of the Chasselas diet but was significantly lower than those of the two other experimental diets (Fig. 3c).

Immune challenge

Concentration of haemocytes was similar among larvae from experimental and control diets (Fig. 4; Table 1). Whatever the food treatment, concentration of haemocytes covaried positively with the larval age (Table 1). Interestingly, only larvae from the control and the Chardonnay diets exhibited an enhancement of their concentration of haemocytes in response to the immune challenge as suggested by the significant interaction term between diet and time in Table 1 (Fig. 4).

There were no significant temporal change in PO and total-PO within 24 h post-immune challenge and no significant variation among experimental and control diets (Table 1). Both enzyme activities positively covaried with age of the larvae (Table 1).

Discussion

Our study aimed to investigate the influence of host plant variation on immune defences of herbivorous insects. Because variation in host plant were previously shown to affect resistance of herbivorous insects to natural enemies (Moreau *et al.* 2010), we have hypothesized that variation in host plant could plastically affect the expression of their immune defences. Consistent with our prediction, we found that an inbred

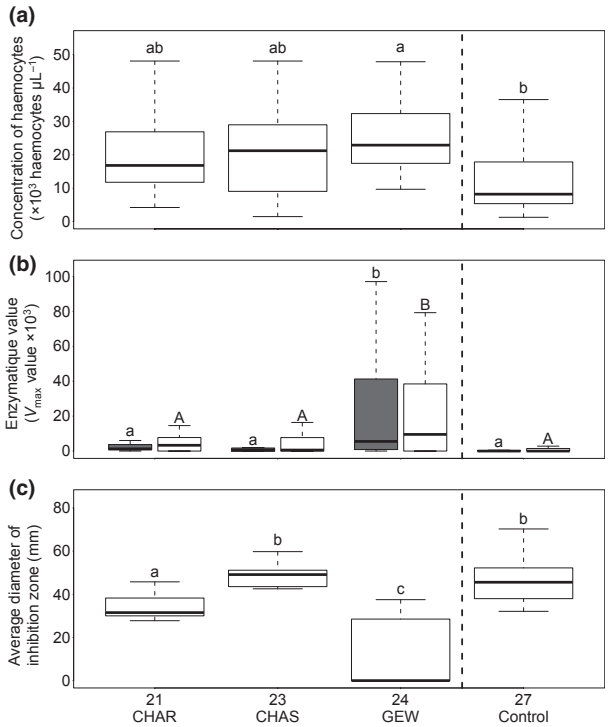

Fig. 3. (a) Concentration of haemocytes (haemocytes per microliter) ($\times 10^3$ + SE) of fifth-instar larvae, (b) Phenoloxidase (PO) (grey plot) and total-PO (white plot) activities of larvae haemolymph (V_{max} value) ($\times 10^3$ + SE), and (c) average diameter of inhibition zones (mm) (+SE), representative of antimicrobial activity in 1 µL of haemolymph according to diet on which larvae ware reared. The edges of the rectangles represent the first and third quartiles; the central features, the medians; and maxima and minima by dashed lines. Diets with the same letter are not significantly different ($P > 0.05$). The number of larvae tested on each diet appears below the *x*-axis.

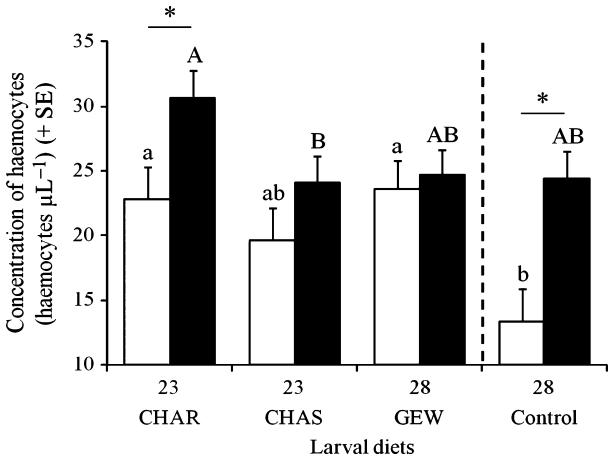

Fig. 4. Variation of concentration of haemocytes (haemocytes per microliter) ($\times 10^3$) immediately (white bars) and 24 h (black bars) after the immune challenge according to diet. Diets with the same letter are not significantly different ($P > 0.05$). Lowercase letters refer to the concentration of haemocytes immediately before the immune challenge and capital letters refer to the concentration of haemocytes 24 h after. An asterisk denotes a significant change in the concentration of haemocytes during the immune challenge ($P < 0.05$). The number of larvae tested on each diet appears below the *x*-axis.

strain of the moth *E. ambiguella* exhibited variable patterns of expression of immune defences when reared on different semi-artificial diets made of berries from different grapevine cultivars.

Here, all the diets used had similar physical characteristics (e.g. density, solubility and water contents), in contrast to having used entire berries. Therefore, variation in levels of immune defences of larvae reared on the different diets resulted only from chemical differences between the cultivars.

The control diet was the most favourable for larvae survival. Interestingly, those larvae exhibited the lowest levels of immune defences, except for antimicrobial activity. Maintaining and investing in immunity are known to be costly (Moret & Schmid-Hempel 2000) and such a cost could potentially explain the reduced survival of larvae in the experimental diets, which also exhibited relatively high levels of immune defences. Levels of immune defence were also variable among the experimental diets. Notably, larvae reared on the Gewurztraminer diets exhibited high PO activities but low antimicrobial activity, as opposed to those reared on Chardonnay and Chasselas diets. Antimicrobial activity in the haemolymph results from the inducible production of antimicrobial peptides after a microbial immune challenge (Hoffmann, Reichhart & Hetru 1996; Haine *et al.* 2008) and is associated with the down-regulation of the PPO system in other insect models (Moret & Schmid-Hempel 2009). Our results show that this immune pathway has been fully activated in larvae of the Chardonnay, Chasselas and control diets and was accompanied with a probable down-regulation of the PPO system as compared to larvae reared of the Gewurztraminer diet. Insects recognize bacteria through the presence of conserved molecules on their cell wall called 'Microbe-associated molecular patterns' (MAMPs). These molecules are recognized patterns recognition receptors (PPRs) expressed by host cells, thereby triggering either the PPO cascade or the synthesis of antimicrobial peptides (Yoshida, Kinoshita & Ashida 1996). The regulation of both immune pathways could thus be mediated by these PRRs. However, this would need further investigations. Antimicrobial activity in larvae might have been induced through the consumption of microorganisms that are likely growing in the food pellet. While the diets contain Scala® to limit fungal growth, they are not fully aseptic. Differences in larval antimicrobial activity among diets suggest that chemicals contained in the berries from the different cultivars act like antibiotics. Variation in these chemicals may differentially limit microbial growth in diets and therefore the activation of the antimicrobial activity in the haemolymph. Gewürztraminer berries are likely to contain more antibiotics than those of the other cultivars. Further study in comparing antibiotic activity of berries from different grapevine cultivars is needed to investigate their potential effects.

Antibiotic chemicals in host plants may indirectly provide protection against microbial pathogens to herbivorous insects, which could then maintain higher levels of immune defence against a range of other pathogens. It is probably the case for the larvae reared on the Gewurztraminer diet, which

Table 1. Results of the analysis for repeated measures of haemocytes, PO and total-PO activities

	Haemocytes		PO activity		Total-PO activity	
Source	*F*	*P*	*F*	*P*	*F*	*P*
Between subjects						
Diet	$F_{3,90} = 0{\cdot}07$	0·18	$F_{3,90} = 1{\cdot}15$	0·33	$F_{3,90} = 1{\cdot}54$	0·21
Age	$\mathbf{F_{1,90} = 0{\cdot}18}$	**0·0001**	$\mathbf{F_{1,90} = 9{\cdot}68}$	**0·002**	$\mathbf{F_{1,90} = 9{\cdot}37}$	**0·003**
Within subjects						
Time	$F_{1,90} = 0{\cdot}01$	0·28	$F_{1,90} = 0{\cdot}03$	0·86	$F_{1,90} = 0{\cdot}35$	0·55
Time × diet	$\mathbf{F_{4,90} = 0{\cdot}1}$	**0·04**	$F_{4,90} = 0{\cdot}64$	0·59	$F_{4,90} = 0{\cdot}84$	0·47
Time × age	$F_{1,90} = 0{\cdot}006$	0·46	$F_{1,90} = 0{\cdot}05$	0·81	$F_{1,90} = 0{\cdot}37$	0·54

PO, phenoloxidase.
Values $P \leq 0{\cdot}05$ are given in bold.

does not appear constrained by the use of their antimicrobial defences in favour of the PPO system. This latter immune pathway is highly involved in the immune protection against macro-parasites and parasitoids (Lavine & Strand 2002; Rolff & Reynolds 2009).

Larvae reared on different experimental diets also exhibited variable ability to develop an immune response through the recruitment of haemocytes after an immune challenge. Larvae grown on Chardonnay and control diets significantly enhanced the concentration of haemocytes in their haemolymph upon immune challenge whereas those grown on Chasselas and Gewurztraminer diets did not. The strong recruitment of haemocytes after an immune challenge has already been found in the cabbage lopper, *Trichoplusia ni* (Shikano *et al.* 2010). Either chemicals from Chardonnay and control diets had boosted larval immune responsiveness or, in contrast, chemicals from Chasselas and Gewurztraminer diets altered the recruitment of haemocytes in larvae upon challenge. Future work will examine the potential positive and negative effects of chemicals from the berries of the different cultivars on immunity of the European grape berry moth *E. ambiguella*. The outcome of such a study should illustrate whether the interactions between immunity of the herbivorous insect and its host plant result from an 'arms race'.

Toxic effects of the host plants look obvious when considering the high mortality of larvae on Merlot and Riesling diets. Such a toxic effect of Riesling berries may not come as a surprise because similar results were obtained on *L. botrana* (another grapevine pest) (Moreau *et al.* 2007). However, an eventual toxic effect of Merlot berries on this species has so far never been observed.

Finally, our results also highlight that older larvae have a higher immune potential (concentration of haemocytes, PO and total-PO activities) than younger larvae. Our result support previous results demonstrating that insect larvae become more immunocompetent with age, which may partly explain why parasitoids and parasites are more successful in infecting young host larvae (Vinson 1990; Brodeur & Vet 1995).

To conclude, we found that variation in host plant varieties within the same species grown on the same soil strongly affects the expression of immune defence levels in an inbred strain of the European grape berry moth *E. ambiguella*. Previous works found that varying host plant species affect levels of some immune defences in other herbivorous insects (Klemola *et al.* 2007; Karimzadeh & Wright 2008; Klemola, Kapari & Klemola 2008; Lee, Simpson & Wilson 2008; Bukovinszky *et al.* 2009; Smilanich *et al.* 2009). In our study, we consider within species variation of the host plant on several immune parameters that are, together, presumably relevant to the insect's immunocompetence to a large range of pathogens (from micro- to macro-parasites). Patterns of variation of immune defences across diets are likely plastic and were partly mediated by existing physiological trade-offs between immune pathways, at least between induced antibacterial defences and the PPO system (Moret & Schmid-Hempel 2009). Further analyses will be required to reveal the causes of this variation in levels of immune defences across host plants. Results of this study could have implications for the evolution of plant–herbivore–parasitoid interactions (Moreau *et al.* 2009, 2010). Indeed, they tend to emphasize an important role of the immune system and its variation, according to the host plant variation in bottom-up processes involving plants. For instance, alteration of herbivorous insect immunity by the host plant could be of major importance as it should determine the ability of natural enemies to control the herbivorous insect population and therefore the damage to the host plant.

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