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# Should grape moth larval immunity help explaining resistance against natural enemies?

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**Abstract:** In tritrophic systems (plants, phytophagous insects and natural enemies), host plant variation often keys the relative performance of both the herbivore and its associated natural enemies. In bottom-up effects, host plants could affect the fitness of phytophagous insects including growth rate and adult fertility. These effects are indirectly reflected in parasitoids whose success depends on their host quality. For instance, nutrient deficiency or/and toxic defensive compounds of the plants could slow-down the development of herbivorous insects, thus extending the window of vulnerability of attacks by natural enemies.

The immune system is arguably the most common resistance mechanisms used by phytophagous insects against natural enemies such as parasites and parasitoids. However, only a limited number of studies have really linked tritrophic interactions and immune defenses of phytophagous insects. Our work considers two grape moths, the European grapevine moth, *Lobesia botrana,* and the European grapeberry moth *Eupoecilia ambiguella.*

In this study, we have tested the influence of different grapevine varieties on the baseline level of three immune parameters (concentration of haemocytes, activity of the prophenoloxidase system and antimicrobial activity) of larvae of the European grapevine moth. In this presentation we discuss the results obtained in this experiment and their effects and importance in tritrophic.

**Keys words:** grapevine, grapevine moth, insect immune system, tritrophic interactions

## Introduction

### *The problem of pest and the method of control*

Larvae of lepidopterans, and especially those of moths, are exclusively phytophagous and often cause damages to crops and forests. The Tortricid moths or grapevine moths (the European grapevine moth *Lobesia botrana* (EGVM) and the grapeberry moth *Eupoecilia ambiguella* (GBM) are major grapevine pests in Europe. They mainly feed on grape berries, but they can also feed on alternative host plants (see a review in Thiéry, 2008).

Originally from central Europe, the European grapeberry moth (*E. ambiguella*) has been taxonomically described for the first time in 1796 (Thiéry, 2008). However, this species was already described as a harmful pest of vineyards in the bible (Thiéry, 2005). In the early twentieth century, *E. ambiguella* was devasting on French vineyards and more particularly on those of Bordeaux (Thiéry, 2008). Its geographical range is wide, extending from the Mediterranean to southern Britain, but also in Scandinavia, the Balkans, the Caucasus and China (Charmillot *et al*., 1996). The European grapevine moth (*L. botrana*) has colonized later the French vineyards and probably came from South Eastern Europe. The first damages in French vineyards were recorded in 1890 in Alpes-Maritimes (Thiéry, 2005) and currently, *L. botrana* is the major depredator in European vineyards and several diseases occurring to berries are related to this pest (e.g. *Botrytis cinerea*, *Aspergillus niger* or *carbonarius*). It is located in vineyards rather warm and dry while *E. ambiguella* prefers cold and moist vineyards (Thiéry, 2008). These two species are polyvoltin and have between two (GBM) and four generations (EGVM) per year.

There are different ways to control the population density of grapevine moth: pesticide, mating disruption and biological control (Thiéry, 2011). The mating disruption is species specific and non-target organisms are not affected (Tasin, 2005). In recent years, this method has been increasingly applied throughout Europe (Hoffman & Thiéry, 2010), but in France less than 3% of the vineyards are treated with this technique (Thiéry & Delbac, 2010). In some vineyards, it has partially or completely replaced the use of pesticides, which have represented the main control strategy until present (Charmillot & Pasquier, 2001). However, the mating disruption technique against *L. botrana* is not efficient at high population densities (Tasin, 2005).

The bio-insecticide *Bacillus thuringiensis* is an effective microbial control agent, which contributes to *L. botrana* control, limiting the use of toxic chemicals. It is a ubiquitous gram-positive spore-forming bacterium that forms a parasporal crystal during the stationary phase of its growth cycle. *B. thuringiensis* was initially characterized as an insect pathogen, and its insecticidal activity was attributed largely to the parasporal crystals. This observation led to the development of bioinsecticides based on *B. thuringiensis* for the control of certain insect species (Schnepf *et al.*, 1998). But the use of *B. thuringiensis* has led the development of resistance.

In future, the regulation of populations of Tortricid moths will probably use more and more biological control methods involving natural enemies and *Trichogramma* releases at large scale. *Trichogramma* species are solitary egg endoparasitoids, host-specific and widely use in biological control (Ibrahim, 2004). These are very important in preventing crop damage because they kill their hosts during the egg stage before the insect can cause grape damage. For instance, releases of the parasitoid in Canada, China, Switzerland and the former USSR were associated with consistent elevated levels of parasitism (60% to 80%) and reduced damages by 77% to 92% on crops such as sugarcane, wheat and corn (Ibrahim, 2004). However, the use of *Trichogramma* release in pest control of vineyards was not always efficient.

This is the reason why biological control needs extended knowledge of the biology and ecology of the three main protagonists of the tritrophic system: the host plant, the pest and the agent. Grape variety was shown to affect the performances of both larvae and adult grapevine moth. For instance, grape variety affects the larval development time, female fecundity and the size of the eggs (Thiéry & Moreau, 2005; Moreau *et al.*, 2006). In addition, egg parasitism rate by *Trichogramma* species varied depending on the host plant consumed by the moth during the larval stage (Moreau *et al.*, 2009). The same result was shown for different species of larval parasitoids (Moreau *et al.*, 2010).

Several hypotheses have already been issued to explain the difference of parasitism rate. For example, several grape varieties slow down the development time of larvae, increasing their window of vulnerability to natural enemies (Benrey & Denno, 1997). Until now, no hypothesis integrates the involvement of the immune system of the pest in tritrophic interactions. However, the implication of immune system of the pest could be an interesting parameter to explain differences in rate of parasitism according the host plant on which larvae fed on.

***Contribution of immunoecology to biological control***

The immune system of a phytophagous insect may represent the last line of defenses against parasitic natural enemies (nematode, fungi, parasitoid…). Indeed, endoparasitoids have evolved an intimate relationship with their hosts. As a consequence, herbivore physiological defenses may respond to parasitoid attack in complex ways (Godfray, 1994). To defend itself against parasitoid attacks, a host may mount various immune responses such as encapsulation of the intruder (Kraaijeveld *et al.*, 2001). After the recognition of non-self (e.g. parasitoid egg), blood cells (haemocytes), circulating in the haemolymph, aggregate in several layers on the surface of the foreign object (Figure 1). This cellular response is often accompanied by a deposit of melanin to the surface of the foreign object, forming a melanotic capsule (Cerenius & Söderhäll, 2004; Siva-Jothy *et al.*, 2005) (Figure 1). The synthesis of melanin is catalyzed by the phenoloxidase (PO) enzyme (Figure 1). This active enzyme is produced from its inactive precursor, the prophenoloxidase (PPO), which is stored in the hemolymph and haemocytes (Figure 1). It appears that the basal number of haemocytes circulating is important in determining ability of *Drosophila* to encapsulate a parasitoid (Fellowes & Godfray, 2000). For example, Eslin & Prevost (1998) showed that *Drosophila* having high concentration of haemocytes were also more resistant to the braconid parasitoid, *Asobara tabida*. Recognition of microorganisms such as fungi or bacteria also induces the production of antibacterial peptides in the haemolymph (Hoffmann *et al.*, 1996). This immune pathway is more specific than the PPO system against microbial infection but has the disadvantage of being effective after relatively long lag-phase following the infection (Haine *et al.*, 2008).

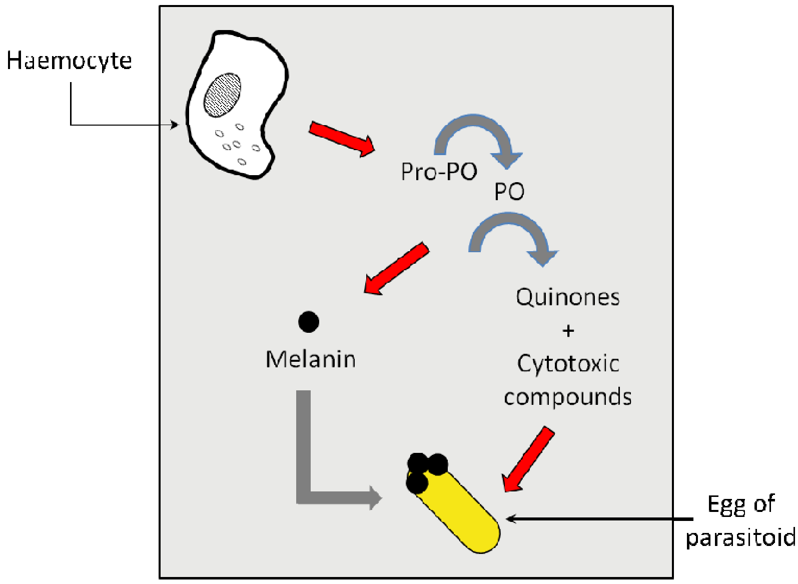


Figure 1. Summary diagram of the immune response of an insect after the injection of a parasitoid egg.

Currently, only a limited number of studies really linked tritrophic interactions and immune defenses of phytophagous insects (Klemola *et al.*, 2007; Bukovinszky *et al.*, 2009; Shikano *et al.*, 2010). Because of the relations between these three protagonists (grape varieties, pest and its parasitoid), we could hypothesis that grape variety may affect the expression of immune defenses of the pest and, therefore, its ability to resist against parasitoid infection. In other words, the success of the method used to biologically control pests will depend on the actual effect of the host plant on the immunity of the phytophagous insects. To examine this possibility, we tested whether grape variety affects the pest immune system of the European grapeberry moth, *E. ambiguella*.

## Material and methods

***An example: study of the influence of grapevine on the immune system of E. ambiguella***

In this study, we have investigated the effect of grape variety on the immune system of the European grapeberry moth (*E. ambiguella*). To this purpose, we offered five different diets of lyophilized berries (Chardonnay, Chasselas, Gewürztraminer, Merlot and Riesling) to larvae of an inbred strain of the *E. ambiguella* (Lepidoptera, Tortricidae) (see the standard protocol in Thiéry & Moreau, 2005). We measured mortality from the 1st to the 5th larval stage. We also measured the basal level of three key immune parameters: haemocytes concentration, prophenoloxidase activity (by spectrophotometry) and antimicrobial activity (using a zone of inhibition assay with *Arthrobacter globiformis*) in the haemolymph of 5th instar larvae (see Vogelweith *et al.*, 2011 for a detailed protocol).

## Results

Larval mortality was strongly affected by diets (Figure 2; $\chi^2_5$ Pearson = 471.54, P < 0.0001). Larvae reared on Merlot and Riesling suffered high mortality whereas larvae reared on others grape varieties exhibited a mortality rate close to 50% (Figure 2). In parallel, mortality of larvae from the control diet was below 20% (Figure 2).

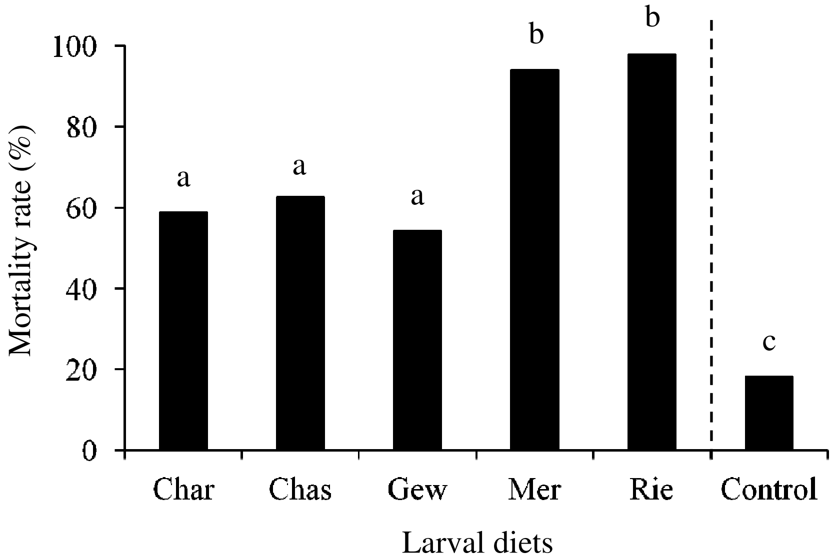


Figure 2. Mortality rate (%) of *E. ambiguella* during the larval stage (from hatching to the 5th instar) according to diets. The diet "Char" represents Chardonnay, "Chas" Chasselas, "Gew" Gewürztraminer, "Mer" Merlot and "Rie" Riesling. Diets with the same lowercase letter are not significantly different (P > 0.05).

Because of the strong mortality on Riesling and Merlot diets, we did not have enough surviving larvae for measurements of their immune defenses. The three immune parameters measured, haemocyte concentration, PPO activity and antimicrobial activity were affected by diets (Figure 3; respectively, Wilcoxon's rank tests: $\chi^2_4$ = 25.69, P < 0.0001; $\chi^2_4$ = 23.25,

$P < 0.0001$ and $\chi^2_4 = 18.2$, $P < 0.0004$). Larvae reared on the Gewürztraminer diet had more haemocytes and higher PPO activity than larvae from the control diet. They also have more PPO activity than larvae from Chardonnay and Chasselas diets. In contrast, larvae from the Gewürztraminer diet have less antimicrobial activity than those from the other diets (Figure 3).

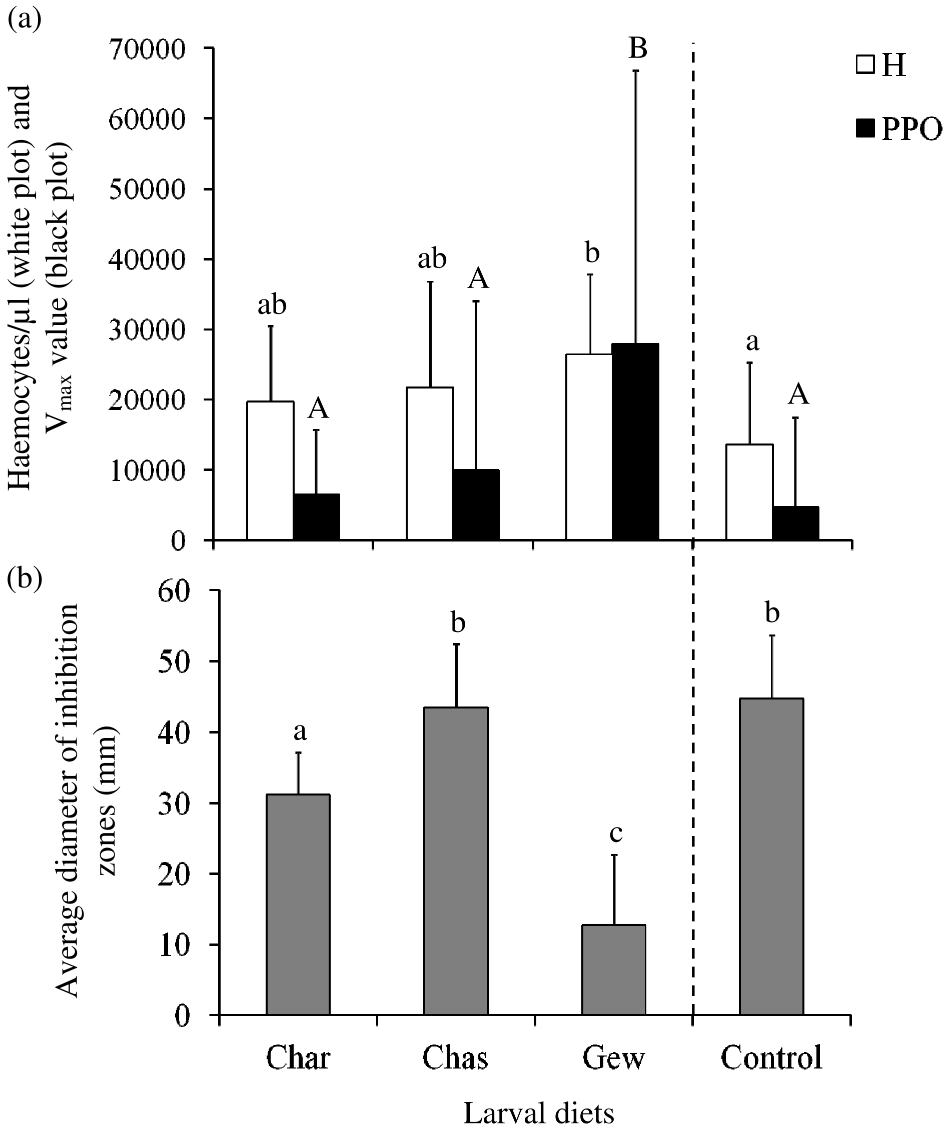


Figure 3. (a) Concentration of haemocytes (haemocytes/µl) (white bars) and PPO activity ($V_{max}$ value) (black bars) in haemolymph of fifth instar larvae according to the experimental diets; and (b) average diameter of inhibition zones (mm), representative of antimicrobial activity in 1µl of haemolymph according to diets on which larvae were reared. The diet "Char" represents Chardonnay, "Chas" Chasselas, "Gew" Gewürztraminer, "Mer" Merlot and "Rie" Riesling. Diets with the same letter on top of error bars are not significantly different ($P > 0.05$).

## Discussion

A previous study (Moreau *et al*., 2010) showed that grape variety affects resistance of the grapeberry moth to natural enemies. For instance, larvae collected on some grape varieties are more susceptible to parasitoid attacks than larvae collected on others. Based on these results, we have hypothesized that variation in grape variety could plastically affect the expression of immune defenses of the grapeberry moth. Consistent with our prediction, we found that an inbred strain of the moth *E. ambiguella* exhibited variable mortality rate and patterns of expression of immune defenses when reared on different semi-artificial diets made of berries from different grapevine cultivars.

Several grape varieties, like Riesling and Merlot, were not favorable or toxic to the moth. In contrast, the control diet was the most favorable in terms of larval survival. Interestingly, larvae from control diet exhibited the lowest levels of immune defenses, except for antimicrobial activity. Basal levels of immune defenses were also variable among the experimental diets. Notably, larvae reared on the Gewürztraminer diets exhibited high PPO activity but low antimicrobial activity, as opposed to those reared on Chardonnay and Chasselas diets. These results are consistent with previous studies showing the existence of a physiological trade-off between immune pathways, at least between induced antibacterial defenses and the PPO system (Moret & Schmid-Hempel, 2009). This trade-off appears important because antimicrobial activity in the haemolymph results from the inducible production of antimicrobial peptides after a microbial immune challenge (Hoffmann *et al*., 1996; Haine *et al*., 2008). Antimicrobial activity in the haemolymph of larvae must result from the presence of bacteria in the diets, which, by activating the synthesis of antibacterial peptides may also have down regulated the activity of PPO system. Differences in larval antimicrobial activity among diets suggest that chemicals contained in the berries from the different cultivars might have acted as antibiotics (Vogelweith *et al*., 2011). Variation in these chemicals may differentially limit microbial growth in the 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.

## Conclusion

Different cultivars differentially affected the immune system of the grapeberry moth, *E. ambiguella,* which may lead to different relative levels of resistance against natural enemies. Larvae grown on the Gewürztraminer diet exhibited higher concentration of haemocytes and levels of the PPO system, suggesting they should be more resistant to natural enemies like parasitoids (Fellowes & Godfray, 2000). In contrast, larvae reared on control and Chasselas diets exhibited low concentration of haemocytes and levels of PPO activity, whereas the antibacterial activity of their haemolymph was relatively high. These larvae are therefore expected to be more resistant to microbial infections (Haine *et al*., 2008).

This study highlights the importance of knowing the effect of host plant on the pest immune system to customize biological control programs and improve their success, especially by choosing the right biological agent. In the case of the grapeberry moth, *E. ambiguella*, the method used to biologically control the population of the pest will depend on the grape variety present in the vineyard. From our results, we may infer that the release of parasitoids in a vineyard containing the Chasselas grape variety should be more efficient to control the population of the pest than in a vineyard containing the Gewürztraminer grape variety. In contrast, the use of a microbial agent should be more efficient to control population of *E. ambiguella* infesting Gewürztraminer grapes than Chasselas ones. Therefore, it becomes clear that extending our knowledge on the specificity of the interactions between host plants and the immune system of phytophagous insects in the context of tritrophic systems should provide important insights that should help us to propose solutions for successful biological control of pests.

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