Butterfly-plant interaction – a dicey ménage à trois?

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Abstract

Commentary based on Paniagua Voirol et al. (2020) Plant responses to insect eggs are not induced by egg-associated microbes, but by a secretion attached to the eggs

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A hungry caterpillar is making its way across a leaf, unsuspiciously munching on the fresh plant tissue. Little does the insect know that its nibbling has initiated avalanche-like effects underneath its tiny feet. It is unaware that its food is not willing to resign to the fate of being eaten without fighting back. And it is oblivious about the fact that it is being meticulously observed and recorded - observed and recorded by scientists who aim to reveal the very details of the complex plant-herbivore interactions. The paper by Paniagua Voirol et al. in this Issue focusses on the interface between plant and insect – an area that is far less investigated than the plant's internal defence response.

One of the internal plant signals set off by chewing insects has recently been demonstrated by Toyota et al. (2018). Using fluorescent reporter plants, they visualized the rapid spread of the second messenger Ca^{2+}. The Ca^{2+} wave originated from the site of attack, and travelled mainly through the leaf vasculature to activate systemic responses throughout the entire plant. This Ca^{2+}-related signalling is triggered by a release of the wound signal glutamate (Toyota et al., 2018), and is further transduced and propagated by ion channels of the GLUTAMATE-RECEPTOR-LIKE family (Mousavi et al., 2013). The systemic defence responses include, for example, increased synthesis of jasmonic acid, and accumulation of toxic and repellent compounds to limit the amount of herbivore damage (Wu & Baldwin, 2010).

Reacting directly to larval herbivory is only one of the manifold mechanisms that plants have developed in the course of the never-ending evolutionary arms race with insect herbivores. Many species can 'anticipate' larval attacks and respond to the deposition of eggs before the insects are hatching. Anticipatory defence mechanisms include the release of volatile compounds to ward off adult insects or to attract parasitoids (M. Hilker et al., 2002), production of harmful defence compounds (Austel et al., 2016; Bandoly et al., 2016), mechanical egg removal or destruction (Balbyshev & Lorenzen, 1997; Desurmont & Weston, 2011), and locally restricted necrosis that increases egg mortality (Geuss et al., 2017). The latter is a typical response to egg deposition by *Pieris* butterflies. The processes involved strikingly resemble the pathogen-induced hypersensitive response, in which cells immediately surrounding the infection site are killed to prevent the pathogen spread. The hypersensitive response is initiated by membrane-bound receptors such as FLAGELLIN-SENSITIVE 2 (FLS2) and BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) that perceive pathogen-associated molecular patterns (Chinchilla et al., 2007), and induce complex cellular signalling pathways, resulting in the accumulation of reactive oxygen species and nitric oxide (Balint-Kurti, 2019; Clarke et al., 2000). Similarly, egg deposition by *Pieris* butterflies and other herbivorous insects causes accumulation of reactive oxygen species and salicylic acid, callose deposition, up-regulation of pathogenesis-related genes, and localized programmed cell death (Figure 1) (Bittner et al., 2017; Little et al., 2007).

With this in mind, Paniagua Voirol et al. hypothesized that the defence responses to eggs of the large white butterfly *Pierisbrassicae* are in fact mediated and induced by egg-associated microorganisms and not by the eggs themselves. The authors found that butterfly larvae grew significantly less and developed more slowly on egg-primed leaves than on un-primed ones. The egg-induced up-regulation of defence mechanisms in the plant was reflected in increased expression levels of defence marker genes such as PATHOGENESIS-RELATED GENE 1 (PR1) and PATHOGENESIS-RELATED GENE 5 (PR5). Depositing surface-sterile eggs from antibiotics-treated butterflies onto leaves, however, resulted in significantly lower marker gene transcript levels, both in*Arabidopsis thaliana* and in the natural butterfly host*Brassica nigra* . Larvae feeding on these plants put on more weight compared to plants that were treated with non-sterile eggs, and they developed faster into pupae. Do these findings already solve the case and prove a tripartite insect-microbe-plant relationship? As usual in biology, the answer is more complex. Surprisingly, the authors found that also ‘unsterile’ egg clutches of *P. brassicae* harboured only negligible amounts of bacteria. Furthermore, treatment of Arabidopsis leaves with egg-associated bacterial mixtures, cultivated*in situ* , did not prime the plant anti-herbivore defence. Larvae feeding on these leaves grew and developed similarly well as on untreated leaves, making bacteria unlikely culprits for inducing the hypersensitive response-like plant defence mechanisms.

The authors concluded that the eliciting factor had to be an attribute of the eggs themselves. *Pieris brassicae* deposits its eggs in clutches that get ‘glued’ onto the leaf surface by secretory substances produced by the butterfly female’s accessory reproductive gland (ARG). Application of various amounts of the ARG secretion product onto leaves, and following larvae feeding experiments revealed a dose-dependent relationship with the plant defence response and larvae performance.

But how then explain the initial findings with surface-sterile eggs? Further analysis revealed that antibiotics treatment of the female butterflies significantly decreased the biomass of the butterflies’ accessory reproductive gland, and consequently reduced the secretion quantity. Less ARG secretion resulted in poor plant defence response and consequently prosperous larvae growth (Figure 2). The causes for the reduced ARG biomass in the treated butterflies remain unknown, as do(es) the specific elicitor(s) contained in the secretion mix and the mechanism for its perception on the plant epidermis.

This study by Paniagua Voirol et al. shows that plants seem to have gained the upper hand in this specific insect-plant relationship. High amounts of ARG secretion are necessary for proper attachment of gregariously laid butterfly egg clutches, and therefore indispensable for successful insect recreation. This type of egg deposition contrasts with single laid eggs, which in turn are more prone to being killed by the hypersensitive response-like leaf necrosis. What appears to be an advantageous adaptation conferring a fitness benefit to the butterflies has been turned into a payoff by the evolutionary co-adaptation of the plant. Such an imbalance in “armaments” between interacting species pairs can occur if the strength of selection imposed by the species on one another is not perfectly equal. Natural selection is frequently acting more strongly on species with more at stake in terms of evolutionary performance, i.e. reproductive success, leading to a faster evolution of fitness-enhancing adaptations for species under stronger selective pressure (Anderson et al., 2010; Humphreys & Ruxton, 2020).

These latest findings by the Hilker group come at a significant time for research on plant biotic interactions. The year 2020 has been declared as "The International Year of Plant Health" by the General Assembly of the United Nations to raise global awareness for the devastating damage caused by plant pests and diseases. Not only crops but whole ecosystems are more threatened than ever by biotic stressors. International trade and travel, monocultural farming systems, and cultivation of non-endemic species have accelerated the fast spread of transboundary pests and diseases with dramatic consequences. The Food and Agricultural Organization of the United Nations estimates that the global annual crop loss caused by phytopathogens and herbivores to be up to 40% leading to trading losses of over $220 billion (FAO, 2019). The herbivorous caterpillars of the large white cabbage butterfly *Pieris brassicae* investigated by Paniagua Voirol et al. are considered a serious and highly destructive pest that can cause significant economic losses in brassicaceous crops (CABI, 2020). The caterpillars are specialist herbivores feeding only on plants containing glucosinolates.*Pieris brassicae* is mainly distributed in Europe, Asia, and Northern Africa, but can also be found in America and Australia due to the migratory nature of the adult insect. Many of the host plants such as cabbage, rape seed, and cauliflower are of economic importance in these regions, and caterpillar infestations can cause severe yield losses (Hasan & Ansari, 2011) with financial and social consequences on all levels of food production.

The United Nations emphasize that protecting crops against phytopathogens and herbivores holds the potential to reduce poverty and hunger, and they urge researchers to develop innovative practices and technologies for environmentally friendly methods such as integrated pest management. The adverse environmental impacts of conventional synthetic broadband pesticides have been flagged up in recent years because of the concerning decline in the global bee population. Integrated pest management aims to avoid poisonous substances when dealing with pests. Biological control practices are promoting insectivores, or usage of 'biological insecticides' derived from microorganisms or plants that have less severe side effects. The findings by Paniagua Voirol et al. therefore have practical value, because the application of the secretory substances from female butterflies have potential to strengthen the plant's natural defence, and consequently reduce the herbivore damage. Identifying the active component of the ARG secretion is the obvious next challenge, but this study already represents a fundamental stepping-stone that paves the way for an environmentally friendly crop pest control in the future.

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Figure 1 : Cellular responses that are part of both the pathogen-induced hypersensitive (HR) response (solid black arrows) and the oviposition-induced HR-like response (dashed grey arrows). Pathogen-associated molecular patterns (PAMPs) bind to membrane receptors (e.g. flg22 binding to the FLS2-BAK1 complex) and trigger ROS production, callose deposition, SA synthesis, and activation of pathogenesis-related genes (PR1, PR5). All of these metabolic adaptations are also elicited by the deposition of herbivore eggs, but details about the perception pathway are missing so far (Monika Hilker & Fatouros, 2015, 2016; Reymond, 2013; Smith et al., 2014).

Figure 2 : Arabidopsis infestation by *Pieris brassicae* . Left: A female butterfly that was not treated with antibiotics has large accessory reproductive glands (ARGs), and produces ample secretion during egg deposition. The secretion elicits the plant's defence response, which leads to poor larvae growth and development. Right: The ARGs of an antibiotics-treated butterfly are smaller, and less secretion is released for egg attachment. The plant does not up-regulate defence mechanisms, and the larvae can successfully infest the leaf (after Monika Hilker and Fatouros (2015)).

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