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Insect egg-induced innate immunity: Who benefits?

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Abstract

Plants perceive the presence of insect eggs deposited on leaves as a cue of imminent herbivore attack. Consequential plant signaling events include the accumulation of salicylic acid and reactive oxygen species, transcriptional reprogramming, and cell death. Interestingly, egg-induced innate immunity shows similarities with immune responses triggered upon recognition of microbial pathogens, and in recent years, it became apparent that egg perception affects plant–microbe interactions. Here, we highlight recent findings on insect egg-induced innate immunity and how egg-mediated signaling impacts plant–microbe interactions. Ecological considerations beg the question: Who benefits from egg perception in these complex interactions?

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Perception of insect eggs

Throughout their life cycle, plants interact with a multitude of biotic stressors and have therefore evolved an elaborated immune system to counteract such threats. Initiation of plant immune signaling involves recognition of conserved molecular patterns of the aggressor by plasma membrane-localized pattern recognition receptors, a process called pattern triggered immunity (PTI). Similarly, activation of insect egg-induced immune signaling depends on the recognition of specific egg-associated molecular patterns (EAMPs) and not on microbial patterns associated with insect eggs [1,2]. Initiation of plant immune signaling upon egg recognition was reported for insects from different orders, including butterflies and moths (Lepidoptera), planthoppers (Hemiptera), bugs (Hemiptera and Heteroptera), beetles (Coloptera), and sawflies (Hymenoptera) [1]. Known EAMPs include small molecules, such as indole, benzyl cyanide, bruchins, and phosphatidylcholines (PCs), as well as the annexin-like protein diprionin [3,4]. Although several EAMPs and their physiological effects on plants have been described, their cognate receptors remain to be identified and no direct receptor–ligand interaction has been demonstrated so far. However, in the model plant *Arabidopsis thaliana* (hereafter *Arabidopsis*), the L-TYPE LECTIN RECEPTOR KINASE I.8 (LecRK-I.8) has been reported as a crucial component of egg perception from different insect species, such as the large white butterfly *Pieris brassicae*, the Egyptian cotton worm *Spodoptera littoralis*, and the cabbage looper *Trichoplusia ni* [5–7]. Induction of PTI in response to egg-derived PCs depends partially on LecRK-I.8, suggesting a role for this putative receptor in PC-induced immune signaling [8]. Intriguingly, the same receptor has been described as a sensor for

extracellular NAD^+ [6]. However, a relation of NAD^+-mediated signaling in insect egg-induced immunity has not been described yet and will be an interesting topic for further investigations. A genome-wide association study has recently discovered that the L-TYPE LECTIN RECEPTOR KINASE I.1 (LecRK-I.1), a close paralog of LecRK-I.8, is involved in egg-mediated cell death in *Arabidopsis* [9]. Additionally, an ortholog of LecRK-I.1 may potentially regulate egg-induced cell death in the Chinese cabbage *Brassica rapa* [10]. Together, these data indicate that responsiveness to insect eggs is controlled by multiple LecRKs in cruciferous plants.

Perception of EAMPs induces typical immune responses in *Arabidopsis*, including the accumulation of reactive oxygen species (ROS), immune regulatory signals salicylic acid (SA) and pipecolic acid (Pip), and indolic metabolites [4,11,12]. Moreover, egg recognition triggers extensive transcriptional reprogramming with an up-regulation of immunoregulatory and defense-related genes at the expense of photosynthesis and development [13–15]. Hypersensitive response-like (HR-like) lesions develop on leaf tissue underneath the eggs. This programmed cell death is SA dependent, requires sphingolipid metabolism, and impedes egg survival [1,16]. Interestingly, the strength of HR-like in the crucifer family depends on the plant and insect species considered. A particularly strong HR-like is observed in a clade including several crops, such as cabbage (*Brassica oleracea*) and close relatives. In addition, this response is more pronounced in response to eggs from crucifer-specialized pierids, which have the capacity to detoxify glucosinolates, the main defense compounds from in this plant family. These observations suggest that egg killing by HR-like cell death is a defense trait which cruciferous plants have evolved against specialized herbivore species [17]. Although HR-like necrosis is a local phenomenon that is limited to the site of egg deposition, the activation of other immune responses is not limited to the perception site. Accumulation of SA, Pip, specific indoles, and *PR1* transcript levels were also measured in leaves distal to the site of initial egg recognition [12,18], indicating an activation of systemic immunity upon local egg perception. In addition to direct immune responses, plants also employ indirect defense responses against insect eggs. For instance, plants emit a bouquet of volatile compounds in response to oviposition that attract egg parasitoids, such as the wasp *Trichogramma brassicae*, whose offspring in turn kills the herbivore eggs (Fig 1) [1,4]. However, information on the signaling pathways leading to volatile emission upon oviposition is scarce and an interesting aspect for future studies.

Insect eggs trigger plant resistance against microbial pathogens

Local pathogen exposure and activation of immunity lead to an increased broad-spectrum resistance against microbial pathogens in the whole plant foliage, a complex phenomenon called systemic acquired resistance (SAR) [19,20]. Strikingly, egg-induced PTI also increases plant resistance against different strains of the hemibiotrophic bacterial pathogen *Pseudomonas syringae* (*Pst*), the necrotrophic fungal pathogen *Botrytis cinerea*, and the biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis*, both at the site of oviposition and in distal leaves, indicating that egg perception triggers SAR (Fig 1) [12,18]. In *Arabidopsis*, microbial-induced SAR is (1) regulated by N-hydroxypipecolic acid (NHP) and SA signals [21]; (2) tightly associated with priming of defenses, a status in which a plant responds more quickly and vigorously to a subsequent pathogen infection [22,23]; and (3) accompanied by an activation of indolic metabolism [24]. Egg-induced SAR displays the same characteristics, demonstrating that oviposition triggers a SAR that is highly similar to that induced by microbial pathogens [12,18].

Intriguingly, egg-induced SAR in *Arabidopsis* is not limited to the oviposited plant. Indeed, plants growing next to egg-laden neighbors show increased resistance against *Pst* and *B*.

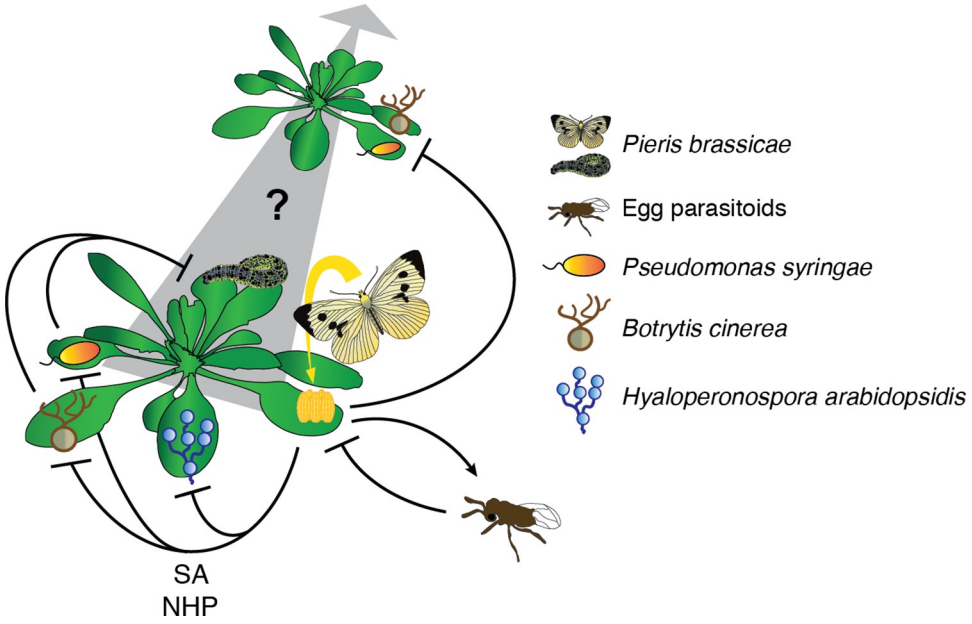

Fig 1. Insect egg-triggered immunity affects plant–microbe interactions in *Arabidopsis*. *Arabidopsis* plants perceive egg deposition and respond with a local induction of innate immunity. Emission of leaf volatiles leads to the attraction of egg parasitoids, which impedes egg survival. Activated signaling events lead to an increased resistance in the full plant foliage against microbial pathogens with different lifestyles including the bacterial plant pathogen *Pseudomonas syringae*, the fungal plant pathogen *Botrytis cinerea*, and the oomycete pathogen *Hyaloperonospora arabidopsidis*. Induction of this egg-induced SAR fully depends on SA and NHP signals and, surprisingly, also takes place in neighboring plants via yet unknown root-derived signal(s) (grey arrow). Interestingly, larvae of the specialist herbivore *Pieris brassicae* perform less well when feeding on plants infected with *P. syringae* and *B. cinerea*, indicating that activation of SAR in response to oviposition could be a strategy to ensure a healthy host plant to feed hatching larvae. NHP, N-hydroxypipecolic acid; SA, salicylic acid; SAR, systemic acquired resistance.

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cinerea (Fig 1) [12,25]. Like the intraplant SAR, this interplant SAR depends on functional NHP and SA signaling pathways. Moreover, egg-treated plants produce a belowground signal to trigger defenses in receiving plants [24]. However, the chemical nature of the root-derived compound is not known and remains to be elucidated. Although it was shown that distinct immune responses, such as the accumulation of SA, are induced in plants neighboring egg-laden plants, the full extent of immune activation has not been investigated yet. For instance, whether emission of parasitoid-attracting volatiles is triggered in receiver plants is an intriguing hypothesis that will deserve further investigation.

Who benefits?

The ecological role of insect egg-induced intra- and interplant SAR is an intriguing, yet not fully resolved, question. The activation of SA in response to egg recognition was initially investigated for its impact on plant defense against chewing larvae. Indeed, it is well established that increased SA levels repress jasmonic acid (JA)-mediated signaling, which mainly orchestrates plant immunity against chewing herbivores [26,27]. Accordingly, larvae of the generalist *S. littoralis* performed better on *Arabidopsis* when plants were previously exposed to *S. littoralis* or *P. brassicae* eggs, and this effect was dependent on functional SA biosynthesis [28]. This finding suggests that generalist herbivorous insects may benefit from activating the SA pathway, although this could be at the cost of reduced egg survival. The potential fitness cost for plants incurred by enhancing future generalist larval performance through SA/JA crosstalk may be less pricey compared to the opportunity to decrease the total imminent herbivore load by

impeding egg development. In contrast, biomass gain of larvae of the specialist *P. brassicae* was not affected, or decreased, in response to *P. brassicae* eggs, indicating that that the outcome of egg-induced signaling on larval fitness may depend on the insect species considered [28,29]. Moreover, SA and NHP signaling pathways have been previously reported to be involved in the regulation of stress-inducible emission of volatiles in *Arabidopsis* and treatment with SA leads to the emission of a bouquet of volatiles in tomato [30–32]. Therefore, activation of SA and NHP signaling pathways might be additionally involved in regulating oviposition-induced volatile emission, which constitutes a crucial indirect plant defense against insect eggs.

Alternatively, as wounding occurs during herbivory, activation of SA signaling and SAR in response to eggs may protect plants against potential infections from opportunistic pathogens. Bacterial plant pathogens, such as *Pst*, enter host leaves through natural openings or wounds [33,34]. Moreover, the microbial community of the phyllosphere is modulated by herbivory, and the bacterial load of pathogenic bacteria, such as *P. syringae*, increases [35]. Thus, egg-induced immunity may be the evolutionary outcome of a trade-off between enhancing larval performance through SA/JA crosstalk, impeding egg development via an HR-like, and reducing the threat of wound-related infection via SAR. However, an increase in pathogen load may be detrimental not only to the plant but also to the attacking herbivore. *P. brassicae* larvae grow slower when feeding on *Arabidopsis* plants infected with *Pst* or *B. cinerea* (Fig 1) [12,18], pointing to the additional hypothesis that, from an insect-centric point of view, egg-induced SAR creates a healthy and nutrient-rich plant environment for feeding larvae. This implies that, by releasing egg-derived EAMPs, insects may have evolved a strategy to hijack the SA pathway to protect host plants against microbial pathogens and therefore to benefit survival of their progeny. This idea blurs the boundaries surrounding the concept of EAMPs/PAMPs being only elicitors of plant defenses and adds a putative function as defense suppressing molecules. However, since they are not mutually exclusive, these hypotheses illustrate a situation in where both insects and plants may profit from activation of the same signaling pathway. It is well established that SA/JA crosstalk is exploited by microbial pathogens to support their virulence. Indeed, necrotrophic pathogens evolved mechanisms to modulate SA signaling to suppress JA-mediated immunity, whereupon biotrophs hijack the JA pathway for suppression of the SA pathway [36]. It will be interesting for future studies to investigate how microbe-mediated modulation of defense signaling pathways affects insect herbivore performance and if microbes benefit from such processes in a natural setting, where microbial plant colonization and herbivore attack happen simultaneously.

The role of egg-induced interplant SAR is an even more complex question. Although the release of volatile or belowground signals by plants and their perception by neighbors has been clearly documented in the context of plant defense [37–39], the biological relevance of such phenomenon is not clear. Whereas alerting a neighbor of an incoming threat may appear favorable if plants are genetically related, as postulated by the kin selection theory [40], this could be counterproductive in case the neighbor is a competitor for limited resources. Plants seldom grow in monocultures in nature and as the effect of egg-induced SAR diminishes with distance from the emitter plant, it is likely to alarm species other than the emitter. Therefore, alerting neighbors altruistically and regardless of kinship will strengthen the preexisting competition between species but may help to increase plant resistance on a community level in the field [41].

In conclusion, the question whether insects, plants, or both benefit from egg-triggered immune signaling in the dynamic interaction between plants, insects, and microbes is fascinating and not fully understood yet. Findings on how plants respond to EAMPs and how the consequential signaling events affect plant–herbivore–microbe interactions open the way for future investigations under more realistic natural conditions where plant communities are constantly challenged by multiple attackers.

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Vectorized plant, egg parasitoid, butterfly, and caterpillar icons used in Fig 1 were designed by Olivier Hilfiker (University of Lausanne). We apologize to authors whose work was not mentioned in this article due to required brevity of the article.

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