MPMI Vol. 37, No. 3, 2024, pp. 211–219, https://doi.org/10.1094/MPMI-10-23-0167-FI

CURRENT REVIEW

Unveiling the Slippery Secrets of Saliva: Effector Proteins of Phloem-Feeding Insects

Jade R. Bleau,[1] Namami Gaur,[1] Yao Fu,[1] and Jorunn I. B. Bos[1,2,†]

[1] Division of Plant Sciences, School of Life Sciences, University of Dundee, Dundee, DD2 5DA, U.K.
[2] Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, U.K.

Accepted for publication 19 December 2023.

Phloem-feeding insects include many important agricultural pests that cause crop damage globally, either through feeding-related damage or upon transmission of viruses and microbes that cause plant diseases. With genetic crop resistances being limited to most of these pests, control relies on insecticides, which are costly and damaging to the environment and to which insects can develop resistance. Like other plant parasites, phloem-feeding insects deliver effectors inside their host plants to promote susceptibility, most likely by a combination of suppressing immunity and promoting nutrient availability. The recent emergence of the effector paradigm in plant–insect interactions is highlighted by increasing availability of effector repertoires for a range of species and a broadening of our knowledge concerning effector functions. Here, we focus on recent progress made toward identification of effector repertoires from phloem-feeding insects and developments in effector biology that will advance functional characterization studies. Importantly, identification of effector activities from herbivorous insects promises to provide new avenues toward development of crop protection strategies.

Keywords: effector, hemipterans, insect–plant interactions, phloem-feeding insects, tritrophic interactions

Phloem-feeding insect pests pose a major threat to agriculture and include species within the order Hemiptera, such as aphids, whiteflies, leafhoppers, plant hoppers, and spittlebugs. These insect pests not only can cause direct feeding damage but also include some of the major vectors of plant viruses and phytopathogenic bacteria. With only limited and, in many cases, no genetic crop resistances available to phloem-feeding insect pests, control relies on environmentally damaging pesticides to which insects can develop resistance.

While phloem-feeding insects feature a variety of life cycles, these insects share similar infestation strategies that rely on the delivery of molecules into the host environment using specialized mouthparts, called stylets (Fig. 1A). Like plant pathogenic microbe effectors, these molecules, including proteins, RNAs, and potentially secondary metabolites, target biological processes in the host environment to promote susceptibility and enhance feeding and reproduction (as recently reviewed by Naalden et al. 2021; Wang et al. 2023). The most obvious sites for effector production in phloem-feeding insects are the salivary glands, allowing effector delivery in saliva, which is secreted into the host environment via the stylets during probing and feeding (Tjallingii 2006), although effector/elicitor activity has also been reported in other insect secretions such as, for example, honeydew, oviposition fluids, and frass (Reymond 2013; Schwartzberg and Tumlinson 2014). Early studies on saliva from phloem-feeding insects, such as aphids, provided evidence for enzymatic activities and highlighted the importance of saliva proteins in suppressing host defenses (Miles 1999). With the recent emergence of the effector paradigm in plant–insect interactions, advances in genomics and proteomics unveiled effector repertoires of an increasing number of species, paving the way for functional and comparative analyses. Though challenging, identification of effector activities from herbivorous insects promises to provide new avenues toward development of crop protection strategies. In this review, we will highlight recent advances in effector biology of phloem-feeding insects, the current knowledge gaps, as well as future directions in the context of translational research. Herein, we will focus on proteinaceous salivary effectors, which have been more extensively studied than other molecules that function as effectors, with "effector" being defined as a molecule that affects any biological host cell process to the benefit of the plant parasite.

Identification of Effectors from Phloem-Feeding Insects: The Omics Route

Although phloem-feeding insects share similar mouthparts called stylets, there are differences in probing and feeding behavior across species, as reviewed by Stafford et al. (2012). For example, while both aphid and whitefly stylets form a mostly intercellular pathway, less intracellular probing takes place before the phloem-feeding phase by whiteflies versus aphids (Jiang et al. 1999; Walker and Perring 1994). A common theme is that saliva, which is produced in insect salivary glands that consist of multiple lobes, is secreted during both probing and feeding (Dai et al. 2019; Ghanim et al. 2001; Rao et al. 2013). This saliva is the vehicle for delivery of effectors inside host plant cells and the apoplast (Naalden et al. 2021; Wang et al. 2023), as well as being involved in virus transmission (Stafford et al. 2012). It has long been hypothesized that phloem feeders produce two types of saliva, gelling saliva, which forms a protective sheath around the insect stylets, and watery saliva, which is delivered inside

[†]Corresponding author: J. I. B. Bos; j.bos@dundee.ac.uk

Funding: This work was supported by the European Union (H2020 European Research Council Consolidator grant, project number 101000997, APHIDTRAP) and by the Chinese Scholarship Council.

The author(s) declare no conflict of interest.

plant cells and the phloem (as reviewed for aphids by Tjallingii 2006). This hypothesis is supported by the observation of stylet tracks in leaf tissues and formation of stylet sheaths when insects are fed an artificial diet. The production and secretion of two distinct types of saliva would require remarkably rapid regulation when switching between the extracellular pathway to intracellular probing. One alternative explanation for formation of stylet sheaths could be that certain components within the insect saliva may form a matrix surrounding the stylets, perhaps upon binding to the cuticle, which is visible as gelling saliva, whereas other components, the watery saliva, are secreted into the host cells or apoplast. A recent study by Deshoux et al. (2022) suggested that a specific structure in the region of the maxillary stylets, called the acrostyle, may bind aphid effectors, as shown for Mp10, and thereby contributes to regulation of effector delivery. Interestingly, effector LsSP1 from *Laodelphax striatellus* (small brown planthopper) was shown to bind to a protein within the salivary sheath, salivary sheath mucin-like protein (LsMLP), to potentially suppress LsMLP-triggered defenses, pointing to further effector-stylet/stylet-bound protein interaction (Huang et al. 2023).

Functional genomic and transcriptomic approaches have been used to predict transcripts encoding effector proteins, largely based on these being both upregulated in the heads (containing the salivary gland) and/or salivary glands of phloem-feeding insects and the presence of a signal peptide and no transmembrane domains. Their application in, for example, various aphid species, whiteflies, psyllids, and planthoppers has led to the identification of effector repertoires predicted to regulate host susceptibility and promote feeding (examples: Bos et al. 2010; Carolan et al. 2009; Ji et al. 2013; Pacheco et al. 2020; Peng et al. 2023; Shangguan et al. 2018; Thorpe et al. 2016; Zhang et al. 2017). Transcriptomic approaches to select candidate effectors based on enrichment in salivary glands and/or heads often iden-

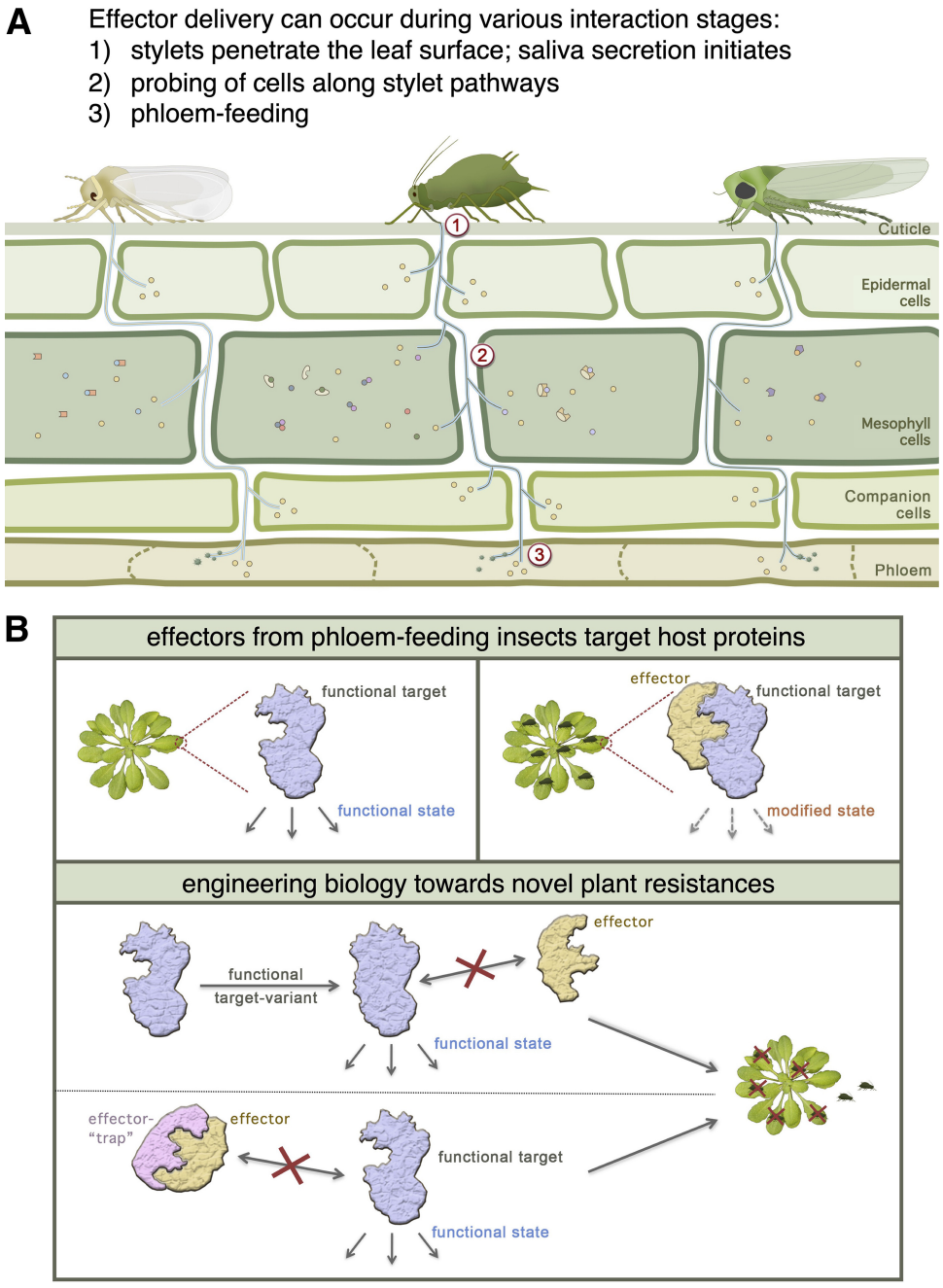

Fig. 1. Model for preventing effector-mediated susceptibility in plant–phloem-feeding insect interactions. **A,** Phloem-feeding insects use specialized mouthparts, called stylets, to feed from the phloem. Saliva secretion starts upon probing the leaf surface (1). During feeding and probing, saliva containing a variety of effector molecules is secreted into different host cellular compartments and the apoplast (2). Sustained saliva secretion into the phloem takes place during feeding (3). In addition, salivary proteins may contribute to the transmission of viruses and microbes. **B,** Salivary effector proteins interact with host proteins to modify their activity and promote susceptibility. With limited or no genetic crop resistance to most phloem-feeding insect pests available, an alternative protection strategy may rely on preventing effector-mediated susceptibility. One part of this strategy is based on gene editing or using natural variants of host target genes to produce variants that are not targeted by effectors, while maintaining the function of the host protein in plant biological processes. Another part of this strategy is based on generating effector "traps" that function to prevent effector activity, thereby protecting the functional host target. Such effector traps could be small peptides that inactivate the effectors or high-affinity binding domains that outcompete binding to host proteins and can be expressed in plants or externally applied. By preventing the activity of multiple effectors at once, susceptibility is predicted to be strongly reduced, leading to a decrease in crop infestation. This figure was created using BioRender.com.

tify large numbers of differentially expressed genes (DEGs) that encode predicted secreted proteins, some of which may not necessarily play a role outside the insect but rather may be secreted into the hemolymph to regulate growth and development (Z. Li et al. 2022).

Additional approaches for effector identification from phloem-feeding insects rely on either saliva or salivary gland proteomics. Salivary gland proteomics is challenging, in that it requires the dissection of salivary glands, and obtaining sufficient protein sample may not be feasible for smaller species. Many of the proteomic studies identifying effectors have used a similar workflow wherein proteins are detected from saliva of phloem-feeding insects fed on artificial diets, and effectors are predicted from the identified saliva proteins based on the presence of a putative signal peptide (Boulain et al. 2018; Carolan et al. 2009; Harmel et al. 2008; Huang et al. 2018, 2021; Liu et al. 2016; Thorpe et al. 2016). Typically, fewer predicted effectors are identified using saliva proteomics compared with bioinformatics pipelines using transcriptome data, and there are varying but overall low degrees of overlap between transcriptome and proteome datasets (Boulain et al. 2018; Huang et al. 2021; Liu et al. 2016; Thorpe et al. 2016). For example, Huang et al. (2021) reported that *Bemisia tabaci* (whitefly) effector Bt56, which is shown to be secreted into plants during feeding using Bt56-specific antibodies and promotes susceptibility in tomato as shown by RNAi experiments (Xu et al. 2019), was not identified in any of their saliva proteomics replicates. However, the corresponding *Bt56* transcript was highly upregulated in the salivary gland transcriptome (Huang et al. 2021). Saliva proteomics may only allow detection of proteins that are stable in an artificial diet environment for 24 to 48 h, which are typical collection time points, and/or effectors may be in low abundance in saliva in the absence of host plant cues. Proteomics-based detection of insect saliva proteins upon secretion into host plants is challenging and yet to be achieved. There is a need to identify effector repertoires from phloem-feeding insect more accurately and comprehensively. This may be achieved through development of novel approaches, for example, based on spatial proteomics and increased mass spectrometry capabilities. Altogether, combining bioinformatics pipelines based on transcriptome data with proteomics-based approaches on insect saliva for effector identification can overcome limitations associated with a single method and is a powerful strategy to generate high-confidence lists of candidate effector proteins that may aid infestation.

Diversity and Evolution of Effector Repertoires

Comparative studies of phloem-feeding insects have identified highly conserved candidate effector repertoires predicted to be involved in common virulence strategies, as well more diverse candidate effectors that may be linked to host specificity (Boulain et al. 2018; Huang et al. 2021; Thorpe et al. 2016). Comparison of effector repertoires across different aphids, both generalist and specialist species, showed that many conserved candidate effectors are predicted enzymes that may be associated with detoxification and digestion (Chaudhary et al. 2015; Thorpe et al. 2016; Vandermoten et al. 2014). Similarly, many of the whitefly *B. tabaci* candidate effectors that are conserved among 22 other arthropod species are predicted enzymes, including oxidoreductases and hydrolytic enzymes (Huang et al. 2021). Part of this "core" set of conserved putative effectors that frequently appear in phloem-feeder effector datasets also include apolipophorin, chemosensory proteins, and vitellogenin. To what extent the functions of these conserved putative effector proteins are similar across different plant–phloem feeder interactions remains to be investigated.

While some proteins are conserved across effector repertoires of diverse phloem-feeding insects, many effectors are specific to insect families or species, which may reflect their long evolutionary history and the diversification of insects. For example, comparisons between effectors identified by saliva proteomics from three different leafhopper species (*Nilaparvata lugens* [brown planthopper], *Sogatella furcifera* [whitebacked planthopper], *L. striatellus* [small brown planthopper]) that are pests of rice showed more overlap between *S. furcifera,* and *L. striatellus*, the two species that can adapt to hosts other than rice, compared with *N. lugens*, which is monophagous (Huang et al. 2018). Indeed, comparative effector studies in several aphid species showed that effectors may be fast evolving, with evidence for positive selection (Boulain et al. 2018; Carolan et al. 2011; Thorpe et al. 2016, 2018). For example, Thorpe et al. (2016) identified 49 effectors as being under positive selection, based on having the ratio of the number of nonsynonymous substitutions to the number of synonymous substitutions per synonymous site (DN/DS) > 1. These included several well-characterized effectors such as MpC002 and Me10-like. Moreover, some of these candidate effectors showed variation within *Myzus persicae* (green peach aphid), based on transcriptome data from three different genotypes. An extensive analyses of the *Acyrthosiphon pisum* (pea aphid) effector repertoire, based on genotype LSR1, showed that higher dN/dS ratios were associated with upregulated salivary gland genes predicted to encode effectors and pointed to positive selection in effector genes *Ap1* (putative ortholog of *Mp1*) and *Me10-like* (Boulain et al. 2018). While diversification of effectors is likely driven by host plant adaptation, it is worth considering that many phloem-feeding insects are effective vectors of viruses and bacteria, and that some of these proteins may contribute to transmission, either directly or indirectly, adding another layer of complexity to plant–insect coevolution (see "Beyond Host–Plant Interactions: Salivary Effectors in Vector Transmission").

Analyzing patterns in effector genome organization can provide insights into the evolutionary history of aphid effectors and the mechanisms that control them. Comparison of the genomes of five different aphid species, *M. persicae*, *A. pisum*, *Myzus cerasi* (black cherry aphid), *Rhopalosiphum padi* (bird cherry-oat aphid), and *Diuraphis noxia* (Russian wheat aphid) identified unique characteristics in the genome organization of aphid effectors (Thorpe et al. 2018). Like fungal and oomycete plant pathogen effectors, aphid effectors are present in less gene-dense regions of the genome, which may correspond with regions of high mutability, enabling rapid evolution of genes under strong positive selection. In addition, many aphid effectors are part of larger gene families, with evidence of gene duplication (Boulain et al. 2018; Thorpe et al. 2018). However, often only one member within these gene families within a certain aphid species is predicted to be a secreted effector, pointing to potential neofunctionalization upon gene duplication (Thorpe et al. 2018). Levels of gene duplication are likely different across insect species. While around half of *A. pisum* predicted effectors belong to one of three major gene families, members of two of these families in *M. persicae* are present as singletons (Boulain et al. 2018), in line with reduced rates of observed genome expansion in the latter species.

A family of cysteine-rich secreted proteins initially identified in a gall-forming aphid, *Hormaphis cornu* (Korgaonkar et al. 2021), features a widely spaced cysteine-tyrosine-cysteine (CYC) motif and is encoded by *bicycle* genes, including *determinant of gall color* (*dgc*). Sequence homology searches only identified few potential homologs of *bicycle* genes in other aphid species. However, similarity searches at the gene-structure level, such as searches based on exon length and number of internal exons, identified putative *bicycle* gene orthologs in other

gall-inducing aphids, non-gall inducing aphids, and non-aphid phloem-feeding insects, such as mealybugs (Stern and Han 2022). In addition, computational prediction of effector protein structures from plant pathogenic microbes has unveiled new families of sequence-unrelated proteins, demonstrating that novel effectors have evolved from conserved ancestral folds (Seong and Krasileva 2021, 2023). Similarly, structure prediction of 71 *M. persicae* effectors identified potential common folds and pointed to significant intrinsic disorder among proteins (Waksman et al. 2023). As more effectors are identified from phloem-feeding insects, similar approaches can be applied to explore structural features of effector repertoires and potentially enhance our understanding of their evolutionary history.

Transcriptional Plasticity and Gene Expression Regulation

Transcriptional plasticity in phloem-feeding insects has been implicated in host adaptation, but the extent to which it plays a role may vary across species. In aphids, transcriptional responses upon host switching are limited, with only a relatively small subset of genes showing differential gene expression upon host transfer. For example, in *A. pisum* lineages switched to different host plant species, between 164 and 554 genes were found to be differentially expressed (Eyres et al. 2016), and in *M. persicae* genotype O transferred from *Brassica rapa* to *Nicotiana benthamiana*, only 171 genes showed differential expression (Mathers et al. 2017). While there was no apparent enrichment in differentially expressed candidate effector genes among these 171 *M. persicae* genes, a predicted secreted protease, cathepsin B, contributed to aphid fitness on *Arabidopsis* but not on *N. benthamiana* (Mathers et al. 2017). More recently, a transcriptome comparison of two *M. persicae* lineages adapted and non-adapted to *Nicotiana tabacum* identified 600 DEGs, which in part may be linked to aphid fitness, and pointed to an important role of CathB3 in adaptation to *N. tabacum* (Guo et al. 2020). Comparison of *M. persicae* and *R. padi* transcriptional responses on host versus non/poor-host plant species and artificial diet showed only up to 162 DEGs expressed across multiple comparisons with no enrichment for DE candidate effectors (Thorpe et al. 2018). Similarly, once adapted to secondary hosts, *M. cerasi* showed only limited gene expression variation when transferred from one secondary host species to another (Thorpe et al. 2019). In stark contrast, 20% of greenhouse whitefly *Trialeurodes vaporariorum* genes were differentially expressed when exposed to different hosts, with the strongest response observed in insects on plant species within the *Solanaceae* (Pym et al. 2019). Moreover, in the whitefly *B. tabaci*, 13 genes encoding for salivary secreted proteins showed differential expression when the insects were exposed to different host species (Huang et al. 2021).

Despite the apparent lack of transcriptional plasticity in aphid effector gene expression, analyses of multiple transcriptomic datasets revealed tight co-regulation for a subset of candidate effectors. Interestingly, six pairs of effectors were colocated across all five aphid genomes, including the pair *Mp1* and *Me10-like* (*Me10-like* is called *Mp58* in Elzinga et al. [2014] and Thorpe et al. [2018]). While *Mp1* and *Mp58* and their putative orthologs are physically linked in the genome, the genes and transposons adjacent to this pair are different in each aphid species. The expression of *Mp1* and *Mp58* and of the putative orthologous pair in *R. padi* is tightly co-regulated and under shared transcriptional control with a subset of candidate effectors (Thorpe et al. 2018), raising new questions about how effector gene expression is regulated within insects.

An interesting and less-studied route toward diversification of effector repertoires is through alternative splicing (AS). Indeed, AS of effector genes has been widely reported in predicted effector repertoires of plant pathogens, but its potential impact on functional diversification is not well understood (as reviewed by Betz et al. 2016). High rates of AS have been observed in phloem-feeding insects, which can contribute to their ability to colonize different hosts, but also may enable them to evade plant defense responses (Dommel et al. 2020; Liu et al. 2021). Single-molecule real-time sequencing of the brown planthopper *N. lugens* transcriptome provided evidence for novel fusion genes and extensive AS (Zhang et al. 2019). Moreover, AS events have contributed to expansion and variation of *N. lugens* effector family *Nl40* (Rao et al. 2019). In the aphid species *M. cerasi*, differential exon usage is associated with insect feeding on different host plant species (Thorpe et al. 2019). The widespread contribution of AS to insect host adaptation and virulence largely remains to be explored and will require further exploration of the functions of alternatively spliced effector and non-effector isoforms in plant–insect interactions.

Effector Activities Revealed: Evidence for Common Virulence Strategies

While enzymatic activities have been described in phloem-feeder insect saliva for many decades (as reviewed by Miles 1999), the identification of proteins in saliva using mass spectrometry- and transcriptome-based approaches took off only around 15 years ago (Carolan et al. 2009; Harmel et al. 2008). Over the past decade, these salivary protein discovery studies have expanded from aphids to other hemipterans, including whiteflies, planthoppers, leaf hoppers, and psyllids. Functional assays to characterize effector activities remain challenging, because of challenges associated with RNAi, genetic modification, and gene editing in these insects, and therefore typically rely on ectopic expression in plants, either transiently through *Agrobacterium*-mediated expression or in transgenic lines. Current methods to study predicted effectors from hemipteran pests, their limitations, and an overview of identified effector activities were recently extensively described elsewhere (Naalden et al. 2021; Wang et al. 2023) and will therefore not be covered in this review. Instead, we will highlight some emerging areas and concepts relevant to the future of hemipteran effector biology.

It is well established that plant pathogens from different kingdoms target common proteins in host plants to suppress immunity (Mukhtar et al. 2011; Weßling et al. 2014). The recent identification of effector targets of hemipteran pests provided evidence that herbivorous insects also target similar proteins and/or biological processes in plants compared with plant pathogens, pointing to overlap in infestation and infection strategies despite differences in lifestyle.

The tomato 14-3-3 protein TFT7 positively regulates programmed cell death associated with plant immunity and interacts with an effector from the aphid species *Macrosiphum euphorbiae* called Me10 (Chaudhary et al. 2019). Isoforms of the 14-3-3 protein are targeted by bacterial effector XopQ to suppress effector-triggered immunity, and this effector also weakly associates with TFT7 in yeast (Teper et al. 2014). OsWRKY71 is targeted by effector VgC (C-terminal peptide of vitellogenin) from *L. striatellus* to suppress H_2O_2-mediated defenses (Ji et al. 2021). Another member of the WRKY family, AtWRKY33, is targeted by *B. tabaci* effector Bsp9 and may disrupt signal transduction to promote host susceptibility (Wang et al. 2019). WRKY33 is a regulator of MAPK signaling (Qiu et al. 2008) and in *Arabidopsis* contributes to defenses against a necrotrophic pathogen and camalexin production upon infection by *Pseudomonas syringae* (Birkenbihl et al. 2012). Moreover, effectors PopP2 from *Ralstonia solanacearum* and AvrRPS4 from *P. sy-*

ringae interact with the WRKY domain of *Arabidopsis* RRS1 and weakly associate with WRKY33 in co-immunoprecipitation assays, suggesting that multiple effectors target WRKY host proteins (Sarris et al. 2015). Another effector from *L. striatellus*, LsSP1, associates with multiple papain-like cysteine proteases (PLCPs) from different subfamilies in rice (Huang et al. 2023). However, exactly how this association impacts insect performance remains to be further explored. Apoplastic PLCPs are known hubs of host immunity to a range of pathogens, including oomycetes, fungi, bacteria, and nematodes, and common targets of effectors (Misas-Villamil et al. 2016). Other common targets of pathogen effectors are components of plant membrane trafficking pathways that contribute to plant immunity (as reviewed by Yuen et al. 2023). *M. persicae* effector Mp1 associates with host protein Vacuolar-Protein-Sorting-associated protein 52 (VPS52), which is part of the GARP complex and involved in endosome retrograde trafficking (Rodriguez et al. 2017). Overexpression of VPS52 reduces host susceptibility to *M. persicae*, and VPS52 is degraded during infestation, but the exact mechanisms by which aphids target VPS52 and how Mp1 is involved remains to be explored. An interesting hypothesis proposed by Yuen et al. (2023) is that aphids may perturb pathogen recognition receptors (PRRs) by promoting vacuolar degradation of VPS52 to suppress PRR-mediated immunity. Finally, the E3 SUMO ligase SIZ1, an important regulator of plant immunity, is targeted by both an oomycete and aphid effector, most likely through different mechanisms, to enhance host susceptibility to pathogens and pests (Liu et al. 2022). Aphid effector Mp64 and *Phytophthora capsici* effector CRN83_152 both associate with SIZ1. While expression of Mp64 in plants increases SIZ1 protein levels, CRN83_152 enhances its E3 SUMO ligase activity. Knockout/silencing of SIZ1 reduces host susceptibility, indicating that this E3 SUMO ligase may function as a susceptibility factor. Interestingly, SIZ1 is also targeted by an effector from the nematode *Globodera pallida*, GpRbp1 (Diaz-Granados et al. 2019), and also seems to act as a factor in susceptibility to nematodes. Interestingly, the effectors Mp64, CRN83_152, and GapRbp1 do not share sequence similarity. Either these proteins may share structural features that similarly target SIZ1, or they may have evolved independent strategies for modulating its role in plant immunity. The convergence of such virulence strategies on common targets may provide opportunities to provide broad-spectrum resistance to a range of pests and pathogens, for example, based on host target gene editing. More insights into how exactly targeting of common host proteins takes place, including on structural features required for interaction and the downstream consequences, will help to inform on such strategies.

Effector Recognition: Tripping the Wire

Plants feature multilayered defense strategies to protect themselves against phloem-feeding insects. These include preformed barriers; induced and constitutive chemical defenses, such as volatiles; and plant immune responses activated upon recognition of herbivore-associated molecular patterns (HAMPs) and/or effectors by PRRs or nucleotide-binding domain leucine-rich repeat proteins (NLRs) (Snoeck et al. 2022; Wang et al. 2023). However, there are limited genetic crop resistances to most hemipteran pests, and where such resistances are available, these tend to be overcome by evolution of new pest genotypes.

Only few PRRs involved in recognition of phloem-feeding insects have been identified to date. The *Bph3* locus in rice harbors three lectin receptor kinases that confer broad-spectrum resistance to *N. lugens* (Liu et al. 2015). In addition, the common PRR co-receptor BRI1-Associated Kinase 1 (BAK1) reduces *Arabidopsis* nonhost resistance to *A. pisum* and is required for immune responses triggered by total aphid extract (Prince et al. 2014), which may contain insect- but also bacteria-derived pathogen-associated molecular patterns (PAMPs) that trigger PRR-mediated immunity. It is important to note that HAMPs are not necessarily derived from the insects themselves but may also be produced by insect-associated organisms, such as bacteria and viruses, including endosymbionts. For example, chaperone proteins GroEL and GroES from the aphid endosymbiont *Buchnera aphidicola* are secreted in saliva by *M. persicae* and *Sitobion miscanthi* (Indian grain aphid), respectively, and were found to activate defense responses in *Arabidopsis* and wheat, leading to reduced aphid performance (Chaudhary et al. 2014; Q. Li et al. 2022).

Some phloem-feeding insect salivary proteins may function both as HAMPs and as effectors that promote virulence (Snoeck et al. 2022). However, many assays used to characterize salivary protein activities to date rely on ectopic expression in host plants, and the phenotypic outcomes of such experiments may not be directly linked to endogenous protein function. For example, ectopic expression of salivary proteins in plants may lead to mistargeting of host proteins because of high levels of expression and/or expression in the "wrong" location (e.g., cell type, tissue) and therefore induce a phenotype indicative of defense response activation. Though HAMP activity has been reported for several phloem-feeding insect molecules (Snoeck et al. 2022), further research is needed to better understand the biological function of these throughout the infestation process.

In addition to PRR-mediated recognition of HAMPs, recognition of effectors by NLRs may activate defense responses that provide protection against hemipteran insects. While NLRs have been identified that provide (partial) resistance to phloem-feeding insects (e.g., Dogimont et al. 2014; Rossi et al. 1998; Zhao et al. 2016), only one NLR–effector pair has been reported to date. The rice CC-NB-LRR receptor BPH14 interacts with and recognizes the *N. lugens* salivary protein BPH14-Interacting Salivary Protein 1 (BISP1) leading to plant resistance (J. Guo et al. 2023). BPH14 likely mediates immunity by associating with and stabilizing WRKY transcription factors in the host nucleus to promote defense gene expression; however, this response does not involve a hypersensitive response (HR)-like cell death (Hu et al. 2017). Importantly, the BPH14-BISP1 interaction activates Neighbor of BRCA1 (NBR1)-mediated selective autophagy, which leads to degradation of BISP1 to limit defense hyperactivation and restore cell homeostasis (J. Guo et al. 2023). A lack of HR-like cell death has also been reported for several other cloned NLRs that mediate resistance to phloem-feeding insects, and therefore this response may not be a common feature of NLR-mediated immunity to hemipterans (Kanvil et al. 2015; Martinez de Ilarduya et al. 2003; Stewart et al. 2009), which contrasts with NLR-mediated immunity in plant–microbe interactions.

An emerging concept in NLR biology and function in plant–microbe interactions is that NLRs function in pairs and networks to mediate resistance. Such networked NLRs may either function as sensor NLRs to detect pathogen molecules or as helper NLRs that enable translation of detection into a defense response (Adachi et al. 2019). Characterization of one class of NLRs in the *Solanaceae*, called NLRs Required for Cell death (NRCs), showed these proteins are paired with many different sensor NLRs and mediate immune responses to a range of pests and pathogens (Wu et al. 2017). Moreover, effectors from distinct pathogens and pests can suppress HR cell death mediated by sensor NLRs that are networked with helper NRCs (Derevnina et al. 2021). However, none of the 47 *M. persicae* (aphid) effectors screened showed evidence for suppression of HR cell death mediated by NRC2, NRC3, and NRC4. The effector repertoire of *M. persicae* is likely significantly larger than the 47 selected effectors, so if aphids feature effectors that

act as suppressors of these NRCs, they may have been missed in this screen. Alternatively, other NLR classes and networks may be involved in recognition of phloem-feeding insects such as aphids. Another consideration is that non-NLR-mediated resistances also can provide protection against infestation and may be constitutive rather than induced upon recognition of insect HAMPS/effectors. Several rice resistances to *N. lugens* involve non-NLR proteins. For example, rice *Bph6* encodes a protein with no similarity to proteins of known function that localizes to the host exocyst complex to promote exocytosis and alters cell wall development and hormone signaling (Guo et al. 2018). In addition, *Bph30*, which confers dominant resistance to *N. lugens*, encodes a protein with two LRR domains that fortifies the sclerenchyma to prevent stylets from reaching the phloem (Shi et al. 2021). Overall, plant resistance to phloem-feeding insects is complex and likely involves a combination of induced and constitutive defenses. Further exploration of germplasm collections, including wild crop species, is needed to find novel resistances, which will help elucidate underlying mechanisms and enable development of insect-resistant crop varieties.

Beyond Host–Plant Interactions: Salivary Effectors in Vector Transmission

When identifying effectors from phloem-feeding insects, it is important to consider that they are hosts to plant pathogenic microbes and viruses. The evolution of salivary insect effectors may not only be driven by the interaction with plant species, but also by the interaction with the microbes and viruses they transmit. There is extensive evidence for vector effectors impacting plant host responses toward viruses and vice versa for virus effectors impacting plant host responses to vectors (as reviewed by Ray and Casteel 2022). In addition, salivary proteins, some of which function as effectors, also play a more direct role in transmission of viruses. For example, during the rice–*L. striatellus*–rice stripe virus (RSV) interaction, a salivary carbonic anhydrase named LssaCA interacts directly with the RSV nucleocapsid protein in the insect salivary glands (Zhao et al. 2023). The LssaCA-RSV nucleocapsid protein complex can bind rice Thaumatin-Like Protein OsTLP and activates its β-1,3-glucanase activity, which is thought to lead to reduced callose deposition. In contrast, a salivary carbonic anhydrase highly expressed in winged morphs of the aphid *M. persicae* facilitates virus spread of turnip mosaic virus and cucumber mosaic virus in *N. tabacum*, while suppressing aphid performance (H. Guo et al. 2023). Viruses can also alter the saliva composition of their vectors to enhance transmission. The rice gall dwarf virus (RGDV) infecting the leafhopper *Recilia dorsalis* inhibits the expression of a salivary calcium-binding protein (CBP) to compete for transmission into the salivary cavities before secretion into host cells in an exocytosis-like manner (Wu et al. 2022). The reduction of RdCBP is associated with an increase in cytosolic Ca^{2+} in host cells, callose deposition, and callose-associated sieve-cell occlusion. These increased plant defenses are thought to lead to increased probing and saliva secretion by *R. dorsalis* and subsequent enhanced virus transmission. However, the mechanism underlying RGDV inhibition of RdCBP expression and secretion and how this affects insect performance remains unknown.

As mentioned earlier, there is evidence that some effectors may bind to a specific region of the insect stylets. Similarly, stylets are implicated in binding virus proteins (Uzest et al. 2007). The P2 protein from cauliflower mosaic virus binds to the tip of aphid maxillary stylets, the acrostyle, and this interaction likely involves RR1 cuticular protein Stylin01 (Webster et al. 2018).

Generating further insights into the dynamics between phloem-feeding insects as vectors of microbes and viruses is critical for understanding complex coevolution of tripartite interactions and ultimately engineering resistances that combat multiple pests and diseases simultaneously.

Outlook

With the effector biology paradigm extended to herbivorous insects, including phloem feeders, and the increasing number of effector repertoires for these being identified, one of the most challenging areas of progress is toward understanding effector functions. With genetic modification of most insect species being either not possible or difficult, current assays mostly rely on ectopic expression of individual proteins to test for induction of phenotypes associated with immune suppression/activation and insect performance and protein–protein interaction experiments, which require extensive follow-up validation and characterization. These effector studies tend to be time-consuming and focused on one protein at a time, and therefore prioritization of candidates is essential. However, most effector proteins do not show homology to proteins of known functions based on sequence similarity, making it difficult to form and test hypotheses. The recent revolution in artificial intelligence-based prediction of protein structures is a groundbreaking development that can facilitate effector protein structure and function prediction and thereby prioritization. In addition, it is now possible undertake large-scale comparative effector structure analyses among species to gain insights into evolutionary history of effector repertoires. Such structural biology approaches have successfully been applied to the secretomes of plant pathogenic fungal species and unveiled new effector families with common ancestry between proteins that have greatly diverged in sequence (Derbyshire and Raffaele 2023; Seong and Krasileva 2021, 2023). Computational structure prediction of 71 aphid salivary effectors pointed to potential functions and common folds, but also highlighted a large set of proteins with disorder spanning most of the protein primary sequence. Intrinsic disorder is common among pathogen effectors and is hypothesized to play a role in effector translocation, targeting of host proteins, and evasion of plant immunity (Marín et al. 2013). In addition, disordered regions may facilitate effector evolution via domain fusions (Seong and Krasileva 2023). Although intrinsically disordered proteins are challenging to study, being prevalent across plant parasites, these proteins are likely involved in promoting host susceptibility, and dissecting their role in doing so will likely uncover novel protein functions.

As detailed in this review, salivary proteins may function not only in host plant manipulation but may also contribute to infection success of the microbes and viruses they transmit, either directly or indirectly. This highlights the importance of considering the function of insect salivary effectors in a broader context, as well as coevolution with other plant pathogenic organisms and the wider ecological context of protein functions. In addition, tool development is needed to be able to expand salivary effector work, while taking into account the spatial–temporal and biological context of interactions. Effectors of phloem-feeding insects may function in specific cellular compartments (e.g., apoplast or cytoplasm), in specific tissues (e.g., phloem or mesophyll cells), and in pairs or groups rather than alone, as suggested by extensive co-regulation of the genes encoding salivary effectors. Development of cell-specific reporter lines and spatial transcriptomics and proteomics approaches (Yin et al. 2023; Zhang et al. 2023), coupled with state-of-the-art imaging, will help bring a more integrated view on plant–insect interactions and the role of salivary proteins in mediating these.

While effectors from plant pathogenic microbes initially were discovered through their avirulence activities, upon recognition by plant NLRs, only one effector with such known avirulence

activity has been described for a phloem-feeding insect (J. Guo et al. 2023), and only a few NLRs have been identified to confer resistance. Perhaps phloem-feeding insects are extremely effective at suppressing NLR-mediated immunity, and/or dominant resistances have been lost in selective breeding for other traits, leading to limited or even no available genetic crop resistances to agriculturally important pests. More work is needed to understand how different mechanisms underlying plant immunity contribute to defense against phloem-feeding insects, and again this would need to take into consideration the spatial–temporal context and biology of interactions. Comprehensive knowledge on salivary effector structure and function will support new directions toward crop protection strategies. Engineering biology approaches aimed at, for example, modifying the susceptibility targets of salivary effectors to prevent binding or aimed at inactivating effectors (Fig. 1B) may provide alternative and durable means to protect crops against insect pests in the long term and will require knowledge on effector structure, function, and interaction in a more integrated context.

Acknowledgments

We thank our colleagues for helpful discussion on the topics covered in this review.

Literature Cited

Adachi, H., Derevnina, L., and Kamoun, S. 2019. NLR singletons, pairs, and networks: Evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. Curr. Opin. Plant Biol. 50:121-131.

Betz, R., Walter, S., and Requena, N. 2016. Alternative splicing – an elegant way to diversify the function of repeat-containing effector proteins? New Phytol. 212:306-309.

Birkenbihl, R. P., Diezel, C., and Somssich, I. E. 2012. Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. Plant Physiol. 159:266-285.

Bos, J. I. B., Prince, D., Pitino, M., Maffei, M. E., Win, J., and Hogenhout, S. A. 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PLoS Genet. 6:e1001216.

Boulain, H., Legeai, F., Guy, E., Morlière, S., Douglas, N. E., Oh, J., Murugan, M., Smith, M., Jaquiéry, J., Peccoud, J., White, F. F., Carolan, J. C., Simon, J.-C., and Sugio, A. 2018. Fast evolution and lineage-specific gene family expansions of aphid salivary effectors driven by interactions with host-plants. Genome Biol. Evol. 10:1554-1572.

Carolan, J. C., Caragea, D., Reardon, K. T., Mutti, N. S., Dittmer, N., Pappan, K., Cui, F., Castaneto, M., Poulain, J., Dossat, C., Tagu, D., Reese, J. C., Reeck, G. R., Wilkinson, T. L., and Edwards, O. R. 2011. Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): A dual transcriptomic/proteomic approach. J. Proteome Res. 10:1505-1518.

Carolan, J. C., Fitzroy, C. I. J., Ashton, P. D., Douglas, A. E., and Wilkinson, T. L. 2009. The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterised by mass spectrometry. Proteomics 9:2457-2467.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., and Kaloshian, I. 2014. GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense. Proc. Natl. Acad. Sci. U.S.A. 111:8919-8924.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., and Kaloshian, I. 2015. Potato aphid salivary proteome: Enhanced salivation using resorcinol and identification of aphid phosphoproteins. J. Proteome Res. 14:1762-1778.

Chaudhary, R., Peng, H.-C., He, J., MacWilliams, J., Teixeira, M., Tsuchiya, T., Chesnais, Q., Mudgett, M. B., and Kaloshian, I. 2019. Aphid effector Me10 interacts with tomato TFT7, a 14-3-3 isoform involved in aphid resistance. New Phytol. 221:1518-1528.

Dai, L., Yang, B., Wang, J., Zhang, Z., Yang, R., Zhang, T., Ren, Z., and Lin, C. 2019. The anatomy and ultrastructure of the digestive tract and salivary glands of *Hishimonus lamellatus* (Hemiptera: Cicadellidae). J. Insect Sci. 19:3.

Derbyshire, M. C., and Raffaele, S. 2023. Surface frustration re-patterning underlies the structural landscape and evolvability of fungal orphan candidate effectors. Nat. Commun. 14:5244.

Derevnina, L., Contreras, M. P., Adachi, H., Upson, J., Vergara Cruces, A., Xie, R., Sklenar, J., Menke, F. L. H., Mugford, S. T., MacLean, D., Ma, W., Hogenhout, S. A., Goverse, A., Maqbool, A., Wu, C.-H., and Kamoun, S. 2021. Plant pathogens convergently evolved to counteract redundant nodes of an NLR immune receptor network. PLoS Biol. 19:e3001136.

Deshoux, M., Monsion, B., Pichon, E., Jiménez, J., Moreno, A., Cayrol, B., Thébaud, G., Mugford, S. T., Hogenhout, S. A., Blanc, S., Fereres, A., and Uzest, M. 2022. Role of acrostyle cuticular proteins in the retention of an aphid salivary effector. Int. J. Mol. Sci. 23:15337.

Diaz-Granados, A., Sterken, M. G., Persoon, J., Overmars, H., Pokhare, S. S., Mazur, M. J., Martin-Ramirez, S., Holterman, M., Martin, E. C., Pomp, R., Finkers-Tomczak, A., Roosien, J., Elashry, A., Grundler, F., Petrescu, A. J., Smant, G., and Goverse, A. 2019. SIZ1 is a nuclear target of the nematode effector GpRbp1 from *Globodera pallida* that acts as a negative regulator of basal plant defense to cyst nematodes. bioRxiv 725697.

Dogimont, C., Chovelon, V., Pauquet, J., Boualem, A., and Bendahmane, A. 2014. The *Vat* locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance. Plant J. 80:993-1004.

Dommel, M., Oh, J., Huguet-Tapia, J. C., Guy, E., Boulain, H., Sugio, A., Murugan, M., Legeai, F., Heck, M., Smith, C. M., and White, F. F. 2020. Big genes, small effectors: Pea aphid cassette effector families composed from miniature exons. Front. Plant Sci. 11:1230.

Elzinga, D. A., De Vos, M., and Jander, G. 2014. Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. Mol. Plant-Microbe Interact. 27:747-756.

Eyres, I., Jaquiéry, J., Sugio, A., Duvaux, L., Gharbi, K., Zhou, J.-J., Legeai, F., Nelson, M., Simon, J.-C., Smadja, C. M., Butlin, R., and Ferrari, J. 2016. Differential gene expression according to race and host plant in the pea aphid. Mol. Ecol. 25:4197-4215.

Ghanim, M., Rosell, R. C., Campbell, L. R., Czosnek, H., Brown, J. K., and Ullman, D. E. 2001. Digestive, salivary, and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B type. J. Morphol. 248:22-40.

Guo, H., Zhang, Y., Li, B., Li, C., Shi, Q., Zhu-Salzman, K., Ge, F., and Sun, Y. 2023. Salivary carbonic anhydrase II in winged aphid morph facilitates plant infection by viruses. Proc. Natl. Acad. Sci. U.S.A. 120:e2222040120.

Guo, H., Zhang, Y., Tong, J., Ge, P., Wang, Q., Zhao, Z., Zhu-Salzman, K., Hogenhout, S. A., Ge, F., and Sun, Y. 2020. An aphid-secreted salivary protease activates plant defense in phloem. Curr. Biol. 30:4826-4836.e7.

Guo, J., Wang, H., Guan, W., Guo, Q., Wang, J., Yang, J., Peng, Y., Shan, J., Gao, M., Shi, S., Shangguan, X., Liu, B., Jing, S., Zhang, J., Xu, C., Huang, J., Rao, W., Zheng, X., Wu, D., Zhou, C., Du, B., Chen, R., Zhu, L., Zhu, Y., Walling, L. L., Zhang, Q., and He, G. 2023. A tripartite rheostat controls self-regulated host plant resistance to insects. Nature 618:799-807.

Guo, J., Xu, C., Wu, D., Zhao, Y., Qiu, Y., Wang, X., Ouyang, Y., Cai, B., Liu, X., Jing, S., Shangguan, X., Wang, H., Ma, Y., Hu, L., Wu, Y., Shi, S., Wang, W., Zhu, L., Xu, X., Chen, R., Feng, Y., Du, B., and He, G. 2018. Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. Nat. Genet. 50:297-306.

Harmel, N., Létocart, E., Cherqui, A., Giordanengo, P., Mazzucchelli, G., Guillonneau, F., De Pauw, E., Haubruge, E., and Francis, F. 2008. Identification of aphid salivary proteins: A proteomic investigation of *Myzus persicae*. Insect Mol. Biol. 17:165-174.

Hu, L., Wu, Y., Wu, D., Rao, W., Guo, J., Ma, Y., Wang, Z., Shangguan, X., Wang, H., Xu, C., Huang, J., Shi, S., Chen, R., Du, B., Zhu, L., and He, G. 2017. The coiled-coil and nucleotide binding domains of BROWN PLANTHOPPER RESISTANCE14 function in signaling and resistance against planthopper in rice. Plant Cell 29:3157-3185.

Huang, H.-J., Lu, J.-B., Li, Q., Bao, Y.-Y., and Zhang, C.-X. 2018. Combined transcriptomic/proteomic analysis of salivary gland and secreted saliva in three planthopper species. J. Proteomics 172:25-35.

Huang, H.-J., Wang, Y.-Z., Li, L.-L., Lu, H.-B., Lu, J.-B., Wang, X., Ye, Z.-X., Zhang, Z.-L., He, Y.-J., Lu, G., Zhuo, J.-C., Mao, Q.-Z., Sun, Z.-T., Chen, J.-P., Li, J.-M., and Zhang, C.-X. 2023. Planthopper salivary sheath protein LsSP1 contributes to manipulation of rice plant defenses. Nat. Commun. 14:737.

Huang, H.-J., Ye, Z.-X., Lu, G., Zhang, C.-X., Chen, J.-P., and Li, J.-M. 2021. Identification of salivary proteins in the whitefly *Bemisia tabaci* by transcriptomic and LC-MS/MS analyses. Insect Sci. 28:1369-1381.

Ji, R., Fu, J., Shi, Y., Li, J., Jing, M., Wang, L., Yang, S., Tian, T., Wang, L., Ju, J., Guo, H., Liu, B., Dou, D., Hoffmann, A. A., Zhu-Salzman, K., and Fang, J. 2021. Vitellogenin from planthopper oral secretion acts as a novel effector to impair plant defenses. New Phytol. 232:802-817.

Ji, R., Yu, H., Fu, Q., Chen, H., Ye, W., Li, S., and Lou, Y. 2013. Comparative transcriptome analysis of salivary glands of two populations of rice brown planthopper, *Nilaparvata lugens*, that differ in virulence. PLoS One 8:e79612.

Jiang, Y. X., Lei, H., Collar, J. L., Martin, B., Muñiz, M., and Fereres, A. 1999. Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. J. Econ. Entomol. 92:357-366.

Kanvil, S., Collins, C. M., Powell, G., and Turnbull, C. G. N. 2015. Cryptic virulence and avirulence alleles revealed by controlled sexual recombination in pea aphids. Genetics 199:581-593.

Korgaonkar, A., Han, C., Lemire, A. L., Siwanowicz, I., Bennouna, D., Kopec, R. E., Andolfatto, P., Shigenobu, S., and Stern, D. L. 2021. A novel family of secreted insect proteins linked to plant gall development. Curr. Biol. 31:1836-1849.e12.

Li, Q., Fu, Y., Liu, X., Sun, J., Hou, M., Zhang, Y., and Chen, J. 2022. Activation of wheat defense response by *Buchnera aphidicola*-derived small chaperone protein GroES in wheat aphid saliva. J. Agric. Food Chem. 70:1058-1067.

Li, Z., Qian, W., Song, W., Zhao, T., Yang, Y., Wang, W., Wei, L., Zhao, D., Li, Y., Perrimon, N., Xia, Q., and Cheng, D. 2022. A salivary gland-secreted peptide regulates insect systemic growth. Cell Rep. 38: 110397.

Liu, K., Su, Q., Kang, K., Chen, M., Wang, W.-X., Zhang, W.-Q., and Pang, R. 2021. Genome-wide analysis of alternative gene splicing associated with virulence in the brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae). J. Econ. Entomol. 114:2512-2523.

Liu, S., Lenoir, C. J. G., Amaro, T. M. M. M., Rodriguez, P. A., Huitema, E., and Bos, J. I. B. 2022. Virulence strategies of an insect herbivore and oomycete plant pathogen converge on host E3 SUMO ligase SIZ1. New Phytol. 235:1599-1614.

Liu, X., Zhou, H., Zhao, J., Hua, H., and He, Y. 2016. Identification of the secreted watery saliva proteins of the rice brown planthopper, *Nilaparvata lugens* (Stål) by transcriptome and Shotgun LC-MS/MS approach. J. Insect Physiol. 89:60-69.

Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., Sun, Z., Pan, G., Wang, Q., Hu, J., Zhou, F., Zhou, K., Zheng, X., Ren, Y., Chen, L., Wang, Y., Zhao, Z., Lin, Q., Wu, F., Zhang, X., Guo, X., Cheng, X., Jiang, L., Wu, C., Wang, H., and Wan, J. 2015. A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nat. Biotechnol. 33:301-305.

Marín, M., Uversky, V. N., and Ott, T. 2013. Intrinsic disorder in pathogen effectors: Protein flexibility as an evolutionary hallmark in a molecular arms race. Plant Cell 25:3153-3157.

Martinez de Ilarduya, O., Xie, Q., and Kaloshian, I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. Mol. Plant-Microbe Interact. 16:699-708.

Mathers, T. C., Chen, Y., Kaithakottil, G., Legeai, F., Mugford, S. T., Baa-Puyoulet, P., Bretaudeau, A., Clavijo, B., Colella, S., Collin, O., Dalmay, T., Derrien, T., Feng, H., Gabaldón, T., Jordan, A., Julca, I., Kettles, G. J., Kowitwanich, K., Lavenier, D., Lenzi, P., Lopez-Gomollon, S., Loska, D., Mapleson, D., Maumus, F., Moxon, S., Price, D. R. G., Sugio, A., van Munster, M., Uzest, M., Waite, D., Jander, G., Tagu, D., Wilson, A. C. C., van Oosterhout, C., Swarbreck, D., and Hogenhout, S. A. 2017. Rapid transcriptional plasticity of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species. Genome Biol. 18:27.

Miles, P. W. 1999. Aphid saliva. Biol. Rev. 74:41-85.

Misas-Villamil, J. C., van der Hoorn, R. A. L., and Doehlemann, G. 2016. Papain-like cysteine proteases as hubs in plant immunity. New Phytol. 212:902-907.

Mukhtar, M. S., Carvunis, A.-R., Dreze, M., Epple, P., Steinbrenner, J., Moore, J., Tasan, M., Galli, M., Hao, T., Nishimura, M. T., Pevzner, S. J., Donovan, S. E., Ghamsari, L., Santhanam, B., Romero, V., Poulin, M. M., Gebreab, F., Gutierrez, B. J., Tam, S., Monachello, D., Boxem, M., Harbort, C. J., McDonald, N., Gai, L., Chen, H., He, Y., European Union Effectoromics Consortium, Vandenhaute, J., Roth, F. P., Hill, D. E., Ecker, J. R., Vidal, M., Beynon, J., Braun, P., and Dangl, J. L. 2011. Independently evolved virulence effectors converge onto hubs in a plant immune system network. Science 333:596-601.

Naalden, D., van Kleeff, P. J. M., Dangol, S., Mastop, M., Corkill, R., Hogenhout, S. A., Kant, M. R., and Schuurink, R. C. 2021. Spotlight on the roles of whitefly effectors in insect-plant interactions. Front. Plant Sci. 12:661141.

Pacheco, I. D. S., Galdeano, D. M., Maluta, N. K. P., Lopes, J. R. S., and Machado, M. A. 2020. Gene silencing of *Diaphorina citri* candidate effectors promotes changes in feeding behaviors. Sci. Rep. 10:5992.

Peng, Z., Su, Q., Ren, J., Tian, L., Zeng, Y., Yang, Y., Wang, S., Xie, W., Wu, Q., Li, Z., and Zhang, Y. 2023. A novel salivary effector, BtE3, is essential for whitefly performance on host plants. J. Exp. Bot. 74:2146-2159.

Prince, D. C., Drurey, C., Zipfel, C., and Hogenhout, S. A. 2014. The leucine-rich repeat receptor-like kinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 and the cytochrome P450 PHYTOALEXIN DEFICIENT3 contribute to innate immunity to aphids in Arabidopsis. Plant Physiol. 164:2207-2219.

Pym, A., Singh, K. S., Nordgren, Å., Davies, T. G. E., Zimmer, C. T., Elias, J., Slater, R., and Bass, C. 2019. Host plant adaptation in the polyphagous whitefly, *Trialeurodes vaporariorum*, is associated with transcriptional plasticity and altered sensitivity to insecticides. BMC Genomics 20: 996.

Qiu, J.-L., Fiil, B. K., Petersen, K., Nielsen, H. B., Botanga, C. J., Thorgrimsen, S., Palma, K., Suarez-Rodriguez, M. C., Sandbech-Clausen, S., Lichota, J., Brodersen, P., Grasser, K. D., Mattsson, O., Glazebrook, J., Mundy, J., and Petersen, M. 2008. *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J. 27:2214-2221.

Rao, S. A. K., Carolan, J. C., and Wilkinson, T. L. 2013. Proteomic profiling of cereal aphid saliva reveals both ubiquitous and adaptive secreted proteins. PLoS One 8:e57413.

Rao, W., Zheng, X., Liu, B., Guo, Q., Guo, J., Wu, Y., Shangguan, X., Wang, H., Wu, D., Wang, Z., Hu, L., Xu, C., Jiang, W., Huang, J., Shi, S., and He, G. 2019. Secretome analysis and in planta expression of salivary proteins identify candidate effectors from the brown planthopper *Nilaparvata lugens*. Mol. Plant-Microbe Interact. 32:227-239.

Ray, S., and Casteel, C. L. 2022. Effector-mediated plant-virus-vector interactions. Plant Cell 34:1514-1531.

Reymond, P. 2013. Perception, signaling and molecular basis of oviposition-mediated plant responses. Planta 238:247-258.

Rodriguez, P. A., Escudero-Martinez, C., and Bos, J. I. B. 2017. An aphid effector targets trafficking protein VPS52 in a host-specific manner to promote virulence. Plant Physiol. 173:1892-1903.

Rossi, M., Goggin, F. L., Milligan, S. B., Kaloshian, I., Ullman, D. E., and Williamson, V. M. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc. Natl. Acad. Sci. U.S.A. 95:9750-9754.

Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S. B., Wirthmueller, L., Menke, F. L. H., Sohn, K. H., and Jones, J. D. G. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. Cell 161:1089-1100.

Schwartzberg, E. G., and Tumlinson, J. H. 2014. Aphid honeydew alters plant defence responses. Funct. Ecol. 28:386-394.

Seong, K., and Krasileva, K. V. 2021. Computational structural genomics unravels common folds and novel families in the secretome of fungal phytopathogen *Magnaporthe oryzae*. Mol. Plant-Microbe Interact. 34:1267-1280.

Seong, K., and Krasileva, K. V. 2023. Prediction of effector protein structures from fungal phytopathogens enables evolutionary analyses. Nat. Microbiol. 8:174-187.

Shangguan, X., Zhang, J., Liu, B., Zhao, Y., Wang, H., Wang, Z., Guo, J., Rao, W., Jing, S., Guan, W., Ma, Y., Wu, Y., Hu, L., Chen, R., Du, B., Zhu, L., Yu, D., and He, G. 2018. A mucin-like protein of planthopper is required for feeding and induces immunity response in plants. Plant Physiol. 176:552-565.

Shi, S., Wang, H., Nie, L., Tan, D., Zhou, C., Zhang, Q., Li, Y., Du, B., Guo, J., Huang, J., Wu, D., Zheng, X., Guan, W., Shan, J., Zhu, L., Chen, R., Xue, L., Walling, L. L., and He, G. 2021. Bph30 confers resistance to brown planthopper by fortifying sclerenchyma in rice leaf sheaths. Mol. Plant 14:1714-1732.

Snoeck, S., Guayazán-Palacios, N., and Steinbrenner, A. D. 2022. Molecular tug-of-war: Plant immune recognition of herbivory. Plant Cell 34:1497-1513.

Stafford, C. A., Walker, G. P., and Ullman, D. E. 2012. Hitching a ride: Vector feeding and virus transmission. Commun. Integr. Biol. 5:43-49.

Stern, D. L., and Han, C. 2022. Gene structure-based homology search identifies highly divergent putative effector gene family. Genome Biol. Evol. 14:evac069.

Stewart, S. A., Hodge, S., Ismail, N., Mansfield, J. W., Feys, B. J., Prospéri, J.-M., Huguet, T., Ben, C., Gentzbittel, L., and Powell, G. 2009. The *RAP1* gene confers effective, race-specific resistance to the pea aphid in *Medicago truncatula* independent of the hypersensitive reaction. Mol. Plant-Microbe Interact. 22:1645-1655.

Teper, D., Salomon, D., Sunitha, S., Kim, J.-G., Mudgett, M. B., and Sessa, G. 2014. *Xanthomonas euvesicatoria* type III effector XopQ interacts with tomato and pepper 14-3-3 isoforms to suppress effector-triggered immunity. Plant J. 77:297-309.

Thorpe, P., Cock, P. J. A., and Bos, J. 2016. Comparative transcriptomics and proteomics of three different aphid species identifies core and diverse effector sets. BMC Genomics 17:172.

Thorpe, P., Escudero-Martinez, C. M., Cock, P. J. A., Eves-van den Akker, S., and Bos, J. I. B. 2018. Shared transcriptional control and disparate

gain and loss of aphid parasitism genes. Genome Biol. Evol. 10:2716-2733.

Thorpe, P., Escudero-Martinez, C. M., Eves-van den Akker, S., and Bos, J. I. B. 2019. Transcriptional changes in the aphid species *Myzus cerasi* under different host and environmental conditions. Insect Mol. Biol. 29:271-282.

Tjallingii, W. F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. J. Exp. Bot. 57:739-745.

Uzest, M., Gargani, D., Drucker, M., Hébrard, E., Garzo, E., Candresse, T., Fereres, A., and Blanc, S. 2007. A protein key to plant virus transmission at the tip of the insect vector stylet. Proc. Natl. Acad. Sci. U.S.A. 104:17959-17964.

Vandermoten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubruge, E., and Francis, F. 2014. Comparative analyses of salivary proteins from three aphid species. Insect Mol. Biol. 23:67-77.

Waksman, T., Astin, E., Fisher, S. R., Hunter, W. N., and Bos, J. I. B. 2023. Computational prediction of structure, function and interaction of aphid salivary effector proteins. bioRxiv 560451.

Walker, G. P., and Perring, T. M. 1994. Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. Ann. Entomol. Soc. Am. 87:363-374.

Wang, H., Shi, S., and Hua, W. 2023. Advances of herbivore-secreted elicitors and effectors in plant-insect interactions. Front. Plant Sci. 14:1176048.

Wang, N., Zhao, P., Ma, Y., Yao, X., Sun, Y., Huang, X., Jin, J., Zhang, Y., Zhu, C., Fang, R., and Ye, J. 2019. A whitefly effector Bsp9 targets host immunity regulator WRKY33 to promote performance. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374:20180313.

Webster, C. G., Pichon, E., van Munster, M., Monsion, B., Deshoux, M., Gargani, D., Calevro, F., Jimenez, J., Moreno, A., Krenz, B., Thompson, J. R., Perry, K. L., Fereres, A., Blanc, S., and Uzest, M. 2018. Identification of plant virus receptor candidates in the stylets of their aphid vectors. J. Virol. 92:e00432-18.

Weßling, R., Epple, P., Altmann, S., He, Y., Yang, L., Henz, S. R., McDonald, N., Wiley, K., Bader, K. C., Gläßer, C., Mukhtar, M. S., Haigis, S., Ghamsari, L., Stephens, A. E., Ecker, J. R., Vidal, M., Jones, J. D. G., Mayer, K. F. X., Ver Loren van Themaat, E., Weigel, D., Schulze-Lefert, P., Dangl, J. L., Panstruga, R., and Braun, P. 2014. Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. Cell Host Microbe 16:364-375.

Wu, C.-H., Abd-El-Haliem, A., Bozkurt, T. O., Belhaj, K., Terauchi, R., Vossen, J. H., and Kamoun, S. 2017. NLR network mediates immunity to diverse plant pathogens. Proc. Natl. Acad. Sci. U.S.A. 114:8113-8118.

Wu, W., Yi, G., Lv, X., Mao, Q., and Wei, T. 2022. A leafhopper saliva protein mediates horizontal transmission of viral pathogens from insect vectors into rice phloem. Commun. Biol. 5:204.

Xu, H.-X., Qian, L.-X., Wang, X.-W., Shao, R.-X., Hong, Y., Liu, S.-S., and Wang, X.-W. 2019. A salivary effector enables whitefly to feed on host plants by eliciting salicylic acid-signaling pathway. Proc. Natl. Acad. Sci. U.S.A. 116:490-495.

Yin, R., Xia, K., and Xu, X. 2023. Spatial transcriptomics drives a new era in plant research. Plant J. 116:1571-1581.

Yuen, E. L. H., Shepherd, S., and Bozkurt, T. O. 2023. Traffic control: Subversion of plant membrane trafficking by pathogens. Annu. Rev. Phytopathol. 61:325-350.

Zhang, J., Guan, W., Huang, C., Hu, Y., Chen, Y., Guo, J., Zhou, C., Chen, R., Du, B., Zhu, L., Huanhan, D., and He, G. 2019. Combining next-generation sequencing and single-molecule sequencing to explore brown plant hopper responses to contrasting genotypes of japonica rice. BMC Genomics 20:682.

Zhang, L., Liang, X., Takáč, T., Komis, G., Li, X., Zhang, Y., Ovečka, M., Chen, Y., and Šamaj, J. 2023. Spatial proteomics of vesicular trafficking: Coupling mass spectrometry and imaging approaches in membrane biology. Plant Biotechnol. J. 21:250-269.

Zhang, Y., Fan, J., Sun, J., Francis, F., and Chen, J. 2017. Transcriptome analysis of the salivary glands of the grain aphid, *Sitobion avenae*. Sci. Rep. 7:15911.

Zhao, J., Yang, J., Meng, X., Fang, R., Huo, Y., and Zhang, L. 2023. Plant arbovirus mobilizes a vector salivary protein to initiate plant infection. bioRxiv 535507.

Zhao, Y., Huang, J., Wang, Z., Jing, S., Wang, Y., Ouyang, Y., Cai, B., Xin, X.-F., Liu, X., Zhang, C., Pan, Y., Ma, R., Li, Q., Jiang, W., Zeng, Y., Shangguan, X., Wang, H., Du, B., Zhu, L., Xu, X., Feng, Y.-Q., He, S. Y., Chen, R., Zhang, Q., and He, G. 2016. Allelic diversity in an NLR gene *BPH9* enables rice to combat planthopper variation. Proc. Natl. Acad. Sci. U.S.A. 113:12850-12855.