Challenging battles of plants with phloem-feeding insects and prokaryotic pathogens

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For the past 4 decades, intensive molecular studies of mostly leaf mesophyll cell-infecting pathogens and chewing insects have led to compelling models of plant–pathogen and plant–insect interactions. Yet, some of the most devastating pathogens and insect pests live in or feed on the phloem, a systemic tissue belonging to the plant vascular system. Phloem tissues are difficult to study, and phloem-inhabiting pathogens are often impossible to culture, thus limiting our understanding of phloem–insect/pathogen interactions at a molecular level. In this Perspective, we highlight recent literature that reports significant advances in the understanding of phloem interactions with insects and prokaryotic pathogens and attempt to identify critical questions that need attention for future research. It is clear that study of phloem–insect/pathogen interactions represents an exciting frontier of plant science, and influx of new scientific expertise and funding is crucial to achieve faster progress in this important area of research that is integral to global food security.

plant immunity | insect pest | plant pathogen | planthopper | citrus greening

Numerous insects and pathogens extract nutrients from the phloem tissue buried deeply inside the plant. Being part of the plant vascular system, phloem is essential for long-distance transportation of photosynthates and other molecules from source tissues (e.g., mature leaves) to sink (e.g., buds, flowers, seeds and roots; Fig. 1*A*). Nutritionally rich, phloem tissue represents a unique ecological niche for a variety of pathogens and insects that have evolved mechanisms to gain access to it. In fact, some of the most devastating insects and pathogens feed on or live in the phloem (*SI Appendix*, Table S1) and cause significant economic losses in major crops plants. For instance, citrus greening disease (aka "huanglongbin" or HLB) is estimated to cause an economic loss of as much as 418 million dollars per year in Florida alone (1). Likewise, the brown planthopper (*Nilaparvata lugens* Stål; BPH) can cause a loss of more than 300 million dollars annually in Asia (2). However, because phloem tissues are difficult to study and phloem-infecting pathogens are often impossible to culture, our understanding of phloem–insect/pathogen interactions has trailed significantly behind that of chewing insects and leaf mesophyll cell-infecting pathogens. How does the phloem tissue ward off invading pests and pathogens? How do phloem-adapted insects and pathogens subvert and exploit the phloem tissue? Are there common and unique features between phloem-adapted insects/pathogens compared to insects and pathogens that attack other plant tissues? These are fundamental questions, and answering them may substantially improve our ability to develop innovative and environmentally safe methods to control some of the most serious pests and diseases in agriculture.

Despite technical challenges, the past 2 decades have witnessed steady progress in the understanding of phloem–insect/pathogen interactions at the molecular level (3–7). In this Perspective, we highlight studies across phloem-feeding insects and prokaryotic pathogens, with the hope to stimulate future efforts and influx of new scientific expertise to address outstanding questions in this important area of research.

Major Phloem-Feeding Organisms

Phloem is a highly evolved vascular tissue and is comprised of sieve elements, companion cells, and parenchyma cells (8). Sieve elements are the major conducting cells. In flowering plants, elongated sieve elements form continuous channels that are crucial for long-distance translocation of molecules. These sieve elements have developed specialized end walls (called sieve plates) that have pores (called sieve pores). Moreover, numerous pores are also found on the side walls of sieve elements, which allow cytoplasmic connections to adjacent companion cells and movement of photosynthates and other molecules. These connections are crucial for sieve element function, as sieve elements lack nuclei and many other organelles and thus rely on proteins and other molecules synthesized in companion cells to be translocated into the sieve elements. In adaptation, companion cells, which contain a large number of organelles and highly active mitochondria to produce energy, are a "power supplier" for sieve elements. Finally, surrounding parenchyma cells are distributed in a radial fashion in the phloem tissue. These radial parenchyma cells make physical

Significance

Piercing-sucking insects and phloem-inhabiting prokaryotic pathogens contribute to some of the world's most disastrous crop losses, including those caused by the brown plant hopper in rice or citrus greening in citrus. For historic and technical reasons, our knowledge of phloem–insect/pathogen interactions remains fragmentary. We highlight recent progress and areas of deficiency in the study of phloem-associated prokaryotic pathogens and insects. Increased efforts are needed to make major advances in this historically understudied area of research.

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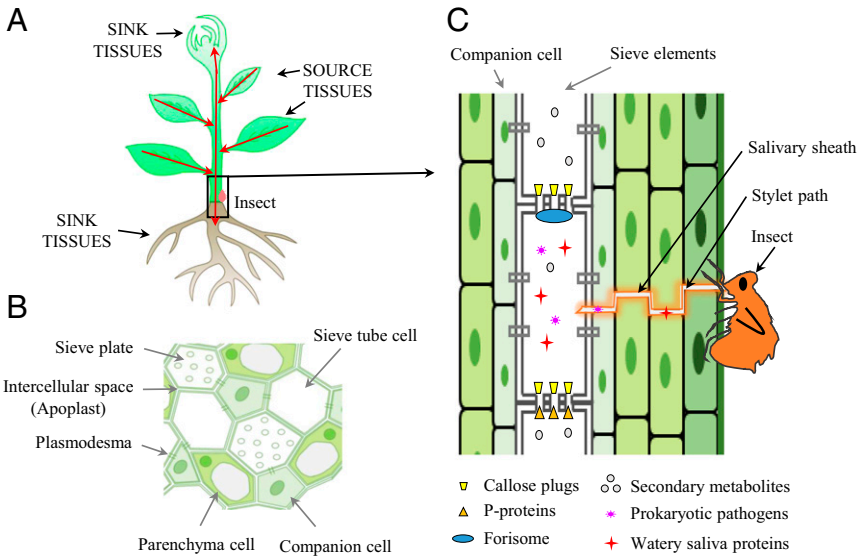

Fig. 1. Phloem as a long-distance transport system and a habitat for prokaryotic pathogens and piercing-sucking insects. (*A*) A schematic source-to-sink translocation of metabolite and signaling molecules through the phloem, indicated by red arrows. (*B*) A cross section of phloem, indicating spatial relationships of various cell types. (*C*) A longitudinal diagram of phloem–insect/pathogen interactions. An insect is shown with its stylet piercing the plant tissue in order to reach a phloem sieve cell. The stylet often takes a long route in the intercellular spaces (apoplast) to reach the phloem. During this process, the stylet encounters defenses in the plant apoplastic space. As part of its counter defense strategy, the insect secretes a gelling saliva to form a hard sheath, which seals off plant cell leaks caused by the penetration process and provides a path to facilitate the stylet movement. When it has reached the phloem, the insect secretes watery saliva proteins (effector molecules) into phloem cells to interfere with, among other host processes, defense-associated callose deposition and protein plugging (P-proteins and forisome) at sieve plates. Insect vectors deliver prokaryotic pathogens into the sieve cells.

contacts with companion cells and sieve elements via intercellular spaces (collectively called the apoplast; Fig. 1*B*), and therefore have potential to modify the composition of the apoplastic phloem sap (9). Overall, phloem contains a fascinating collection of unique and interactive cell types that will need to be considered in the context of interpreting plant interactions with phloem-feeding insects and pathogens.

Many insects and pathogens have evolved to gain access to the phloem as a rich source of nutrients and/or a living habitat (*SI Appendix*, Table S1). Most phloem-feeding insects belong to the insect order Hemiptera, including aphids, planthoppers, leafhoppers, treehoppers, whiteflies, cicadas, spittlebugs, scale insects, and shield bugs, and represent some of the most important pests of crop plants (*SI Appendix*, Table S1). As a defining evolutionary trait, all herbivorous hemipteran insects have developed specialized stylets for piercing the plant tissue to access the nutrient-rich phloem sap (Fig. 1*C*). Unlike phloem-feeding insects, prokaryotes cannot actively enter the phloem; therefore, all known phloem-associated prokaryotes are passively delivered into the phloem by phloem-feeding insects. The genomic sizes of most phloem-limited pathogenic prokaryotes are very small, and they do not encode all core metabolic pathways. Thus, many phloem-associated prokaryotes are obligates and rely on this tissue to obtain essential nutrients and signals.

Challenges and Thoughts for Future Research.

New strategies are needed to culture phloem-inhabiting prokaryotic pathogens. Phloem-inhabiting prokaryotes, notably phytoplasmas, spiroplasmas, *Candidatus* (*Ca.*) liberibacters, and *Ca.* phlomobacters, cause devastating disease outbreaks in citrus and other crop plants. However, advances in research into phloem–pathogen interactions have been limited by one major obstacle over the past few decades: the difficulty of culturing these pathogens in a laboratory setting. The inability to establish pure cultures of most phloem-associated pathogens makes it difficult to test Koch's postulate for establishing the causality of a phloem-inhabiting pathogen in disease. Of phloem-limited prokaryotic pathogens, only genus *Spiroplasma* (10) and a nonpathogenic strain, *Liberibacter crescens* (Lcr) BT-1, which is also a phloem-inhabiting prokaryotic organism, could be cultured in vitro (11). The inability to culture other phloem-inhabiting prokaryotic pathogens is likely attributed to the reduced genomes of almost all phloem-inhabiting pathogens, thus relying on many host phloem constitutes for survival and multiplication. In the case of culturing pathogenic *Ca.* Liberibacter asiaticus (CLas), the presumed causal agent of the devastating citrus green disease, only transient cultures have been reported (12–14), including a recent study reporting a biofilm-based mixed culture, in which CLas is a minor component (15). Nevertheless, current efforts appear to have reached a point that indicates little hope that robust in vitro media will be found to culture the majority of phloem-inhabiting prokaryotic pathogens (16). Yet, establishment of successful in vitro pure cultures remains a priority to allow breakthrough advances in the study of disease etiology, disease mechanisms, and effective screening of antibacterial compounds. Innovative culturing approaches, including synthetic prokaryotic genomics and phloem cell culture systems (17), need to be considered in future research to remove one of the most formidable barriers to the study of phloem–pathogen interactions.

How many phloem-inhabiting microbes are associated with healthy vs. diseased phloem tissues? The inability to culture many of the phloem-associated pathogens without contamination from xylem and other cell types also raises a related fundamental question with respect to whether a single microbe or a microbial consortium consisting of 2 or more interacting microbes causes disease. Indeed, progression of citrus HLB appears to be associated with certain compositions of citrus microbiome (18, 19), and it is conceivable that obligate pathogens with greatly reduced genomes may require "assistance" from other microbes. Thus, large-scale surveys of phloem-associated microbiota in phloem diseases may be an important future research area. With the advent of culture-independent bacterial profiling methods, it will be feasible to determine a potentially core microbiota that occupies the healthy phloem across plant taxa. Conversely, future

research should address whether diseased plant phloem is associated with the presumed pathogens only, or with pathogens together with specific "disease-associated" microbiota. This line of research could lead to new insight into the etiology and potential biocontrol of phloem-inhabiting pathogens by targeting disease-associated microbiota.

Plant Defense against Phloem-Feeding Insects and Pathogens

Molecular studies of mostly leaf mesophyll cell-infecting pathogens and chewing insects have advanced our understanding of many aspects of the plant immune system (20–22). In particular, we now have a quite advanced understanding of plant interactions with leaf mesophyll cell-infecting pathogens. It appears that individual plant leaf cells can broadly respond to microbial signals to initiate 2 branches of immunity, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is activated by PAMPs following detection by plasma membrane (PM)-localized pattern recognition receptors (PRRs) (21). Virulent pathogens have evolved various virulence-promoting molecules, called effectors, to suppress plant PTI defenses (23, 24). To antagonize pathogen effectors, plants evolved ETI, which is triggered by recognition of effectors by disease-resistance proteins (mostly nucleotide-binding, leucine-rich repeat [NLR] proteins) directly or indirectly (22). For defense against chewing insects, plants respond to both insect-derived signals and physical damage (wounding) associated with insect feeding. Recognition of these signals partially relies on a PTI-like mechanism through PRRs (25, 26). For example, insect chewing releases herbivore-associated molecular patterns (HAMPs) (27) and plant-derived damage-associated molecular patterns (DAMPs) such as AtPeps in *Arabidopsis*, which are signals for triggering PTI-like immune responses against insects (28). In the case of AtPeps, 2 PRRs, PEP-Receptors 1 and 2 (PEPR1 and PEPR2), recognize AtPeps and amplify defense signaling through activation of jasmonate (JA) signaling pathway (29), a major regulator of plant defense responses against chewing insects (26). It should be pointed out that DAMPs may be generated during pathogen infections and therefore may operate during plant–pathogen interactions.

It appears that some aspects of the aforementioned "leaf mesophyll cell-pathogen interaction" model are applicable to plant interactions with phloem-infecting pathogens. For example, an aphid endosymbiont bacterium, *Buchnera aphidicola*, also triggers PTI responses via its chaperonin protein, GroEL, in tomato and *Arabidopsis* (30). GroEL-triggered PTI responses are dependent on BAK1, a pattern recognition coreceptor, as those responses were greatly decreased in the *Arabidopsis bak1-5* mutant. In the case of CLas, it has been reported that this bacterium encodes a flagellin (Fla_{Las}) with a flg22-like 22-amino acid sequence ($flg22_{Las}$). Transient expression of Fla_{Las} protein triggered plant defenses, including up-regulation of defense gene expression, callose deposition, and plant cell death, in heterologous *Nicotiana benthamiana* plants. Application of $flg22_{Las}$ also induced differential expression of a number of citrus genes between tolerant and susceptible citrus cultivars (31), suggesting that Fla_{Las} could potentially trigger PTI against CLas bacteria (32). Phytoplasmas, on the contrary, lack cell walls and flagella and therefore do not possess typical cell wall- and flagellum-derived PAMPs, such as peptidoglycans or the flg22 epitope. However, some intracellular PAMPs, including cold shock proteins and translation elongation factor Tu, are encoded by phytoplasma genomes (3) and may trigger PTI, a possibility that needs to be determined in the future. Notably, ETI has not been reported for any phloem-inhabiting pathogens. Whether this is due to insufficient research or is inherent to phloem–pathogen interactions remains to be investigated.

For plant defense against phloem-feeding insects, both PTI and ETI seem to be required for resistance. For instance, a protein fraction of 3 to 10 kDa derived from Myzus persicae aphid extract triggered PTI-like responses in Arabidopsis (33, 34) 3 cell surface receptor kinases, LecRKs 1 to 3, encoded by a rice brown planthopper resistance locus, *Bph3*, function as putative PRRs to perceive unidentified HAMPs or DAMPs and confer broad-spectrum and durable resistance against planthoppers (Fig. 2*A* and ref. 35). Likewise, BAK1, a coreceptor for multiple PRRs in detecting leaf mesophyll cell-infecting pathogens, is also required for recognition of aphids in *Arabidopsis* (Fig. 2*A* and ref. 34). Other insect resistance genes encode classical NLR proteins involved in ETI against pathogens, including *Mi-1.2* for aphid resistance and *BPH9*/*14* for BPH resistance, respectively (Fig. 2*A* and refs. 36–38). Beyond canonical PTI and ETI, *Bph6* encodes a novel protein that localizes to secretory exocysts and interacts with the rice exocyst subunit OsEXO70E1. Bph6 appears to participate in plant cell wall maintenance and reinforcement and confers broad resistance to all tested BPH biotypes as well as to white-backed planthopper (Fig. 2*B* and ref. 39). In addition, a plant cell wall pectin-modifying enzyme, PECTIN ACETYLESTERASE 9, was recently shown to reduce aphid feeding in *Arabidopsis* (40). Finally, an *Arabidopsis* small heat shock-like protein, which may be involved in modulating the firmness and thickness of the parietal layer of the sieve cell, is found to affect aphid feeding (41).

Challenges and Thoughts for Future Research. A comprehensive understanding of phloem defense against pathogens and insects is crucial for the development of innovative long-term control measures. However, current knowledge on phloem-based defense is limited. There are many questions that need to be addressed as follows.

What aspects of leaf mesophyll cell-based plant immunity are applicable to phloem-inhibiting pathogens and insects? A crucial aspect of current models of PTI is based on studies of extracellular bacterial, fungal, and oomycete pathogens of leaf mesophyll cells (21, 42). This raises a fundamental question with respect to the applicability of current PTI models to phloem-feeding insects or phloem-inhabiting prokaryotic pathogens, as these insects and pathogens are in direct contact with the phloem cell cytoplasm. Although there is evidence for *PRR* expression in the phloem (43), and PRRs can dynamically traffic between the PM and intracellular vesicles (44, 45), it is not clear whether PRR recognition could occur inside the cytoplasm of phloem cells. Therefore, future research should examine how intracellular phloem-infecting pathogens and phloem-feeding insects could be recognized by phloem sieve cells to activate PTI. Of note, PAMP recognition can occur intracellularly in animals (46). In contrast to PTI, most ETI pathways in plants are activated intracellularly upon NLR recognition of effector proteins inside the cell (22). However, as mentioned earlier, to date, no ETI pathway has been identified for phloem-infecting pathogens, a puzzle that needs further investigation.

A related important question is which cell type(s) in the phloem recognizes and responds to pathogen/insect infection. Phloem-feeding insects and phloem-infecting pathogens appear to feed or live mostly on sieve cells (47–49). However, can sieve cells alone, which lack critical organelles (50), mount an effective immune response? What roles do companion cells and/or parenchyma cells play in phloem–insect/pathogen interactions? Major efforts are needed to develop a set of well characterized phloem cell type-specific biosensor lines that will allow researchers to track in vivo spatiotemporal kinetics of immune gene expression, reactive oxygen species, subcellular pH and/or Ca^{2+} changes, and plasmodesmatal and sieve plate pore sizes in various cell types within the phloem tissue.

Are there unique phloem defense responses? Whether phloem cells have evolved unique defense responses is uncertain because of the difficulty of studying phloem-unique processes. Although callose

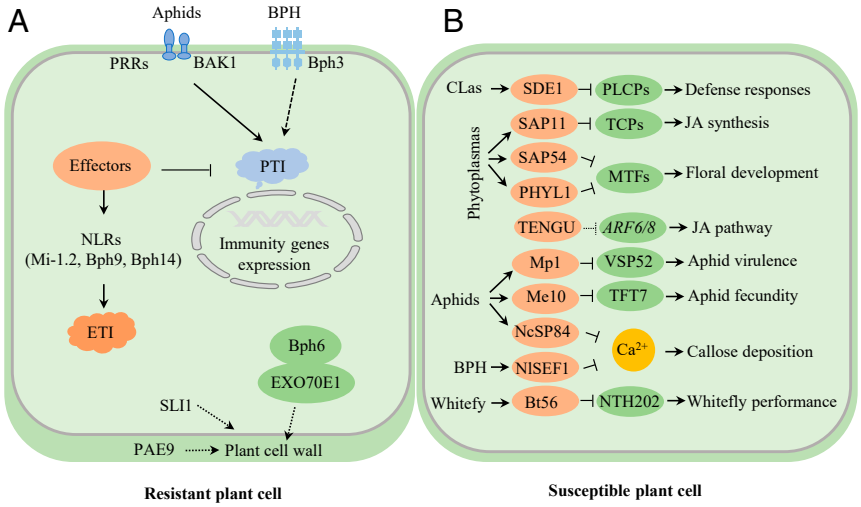

Fig. 2. A schematic of plant cellular responses to phloem-feeding insects and prokaryotic pathogens. (*A*) In a resistant plant cell, the membrane-localized pattern recognition receptor (PRR) Bph3 and a coreceptor, BAK1, recognize insect- and pathogen-derived elicitors (e.g., PAMPs and HAMPs) to trigger pattern-triggered immunity (PTI). Some plants evolved disease resistant proteins, such as Mi-1.2, Bph9, and Bph14, to recognize specific "effectors" from insects and pathogens, resulting in the activation of effector-triggered immunity (ETI). Bph6 interacts with EXO70E1, correlated with strengthening plant cell walls against BPH feeding. EXO70E1, EXOCYST70E1; PAE9, PECTIN ACETYLESTERASE 9; SLI1, SIEVE ELEMENT-LINING CHAPERONE1. (*B*) In a susceptible plant cell lacking disease-resistant proteins and ETI, effectors from insects and pathogens target a variety of phloem cellular processes to facilitate pathogen multiplication and insect fecundity. Effectors from insects and pathogens are shown in orange color, and the plant targets are shown in green and yellow colors. NLRs, nucleotide-binding, leucine-rich repeat proteins; CLas, *Ca.* Liberibacter asiaticus; SDE1, Sec-delivered effector 1; PLCPs, papain-like cysteine proteases; SAP11, secretes AY-WB protein 11; TCPs, TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR; JA, jasmonic acid; SAP54, secreted AY-WB protein54; PHYL1, phytoplasma-secreted protein 1; MTFs, MADS domain transcription factors; TENGU, tengu-su inducer; ARF6/8, *AUXIN RESPONSIVE FACTOR 6/8*; Mp1, saliva protein 1 of *M. persicae*; VPS52, Vacuolar Protein Sorting Associated Protein 52; Me10, saliva protein 10 of *M. euphorbiae*; TFT7, tomato 14–3-3 isoform 7; NcSP84, 84-kDa calcium-binding effector protein of *N. cincticeps*; BPH, brown plant hopper (*N. lugens*); NISEF1, an EF-hand calcium-binding motif of *N. lugens*; Ca^{2+}, calcium; Bt56, a whitefly *B. tabaci* salivary protein; NTH202, a tobacco class II KNOTTED 1-like homeobox (KNOX) transcription factor.

deposition is a well-documented plant defense response to pathogen and insect attacks in various plant tissues, this defense response may be particularly relevant to phloem-based defense against pathogens and piercing-sucking insects. This is because callose deposition may obstruct pores at sieve cell plates or between sieve cells and companion cells, which are believed to be vital for cell-to-cell movement of signaling and metabolic molecules as well as pathogens (Fig. 1*C*). In response to BPH feeding, for example, plants up-regulate the expression of callose synthase genes and induce callose deposition precisely in the sieve tubes where BPHs insert their stylets, which has been interpreted as a physical defense method to prevent BPH from ingesting the phloem sap (51). Moreover, plugged sieve pores and inhibited phloem transportation occur in sweet orange leaves exhibiting severe HLB symptoms caused by CLas infection (52, 53). Similarly, forisomes in *Fabaceae* and phloem proteins (P-proteins) are well-known phloem-localized proteins that seal sieve plates rapidly after damage (54, 55) and could constitute part of sieve cell-unique defense responses (Fig. 1*C*). However, the physiological functions of forisomes and P-proteins still requires further characterization. In particular, mutational analysis of forisome- and P-protein–encoding genes should be performed. Likewise, the role of callose in plugging plasmodesmata and sieve plate pores needs to be tested using callose synthetase mutants.

Can new technologies be developed to advance the study of phloem defense responses? It is clear that future studies of phloem–insect/pathogen interactions will rely increasingly on cell type-specific technologies in order to achieve a next-level understanding of how various phloem cell types respond to phloem-associated pathogens and insects. In addition to developing phloem cell type-specific biosensor lines that could monitor immune responses in situ, as mentioned earlier, alternative techniques need to be developed to advance the study of phloem defense responses. For instance, developing in vitro phloem cell cultures (13) and transient transformation/expression methods may provide a facile strategy for rapid analysis of phloem responses to insect/pathogen-derived molecules. Single-cell transcriptome analysis may be particularly useful in analyzing phloem-specific responses to insect and pathogen attacks in intact plants. Micro-computed tomography (micro-CT) combined with microscopy is a promising technique to visualize fine structural changes of phloem tissues (55) and may be used to study phloem cells responding to pathogens/insects. Finally, the "laser-ablated stylet" technique allows pure phloem sap collection (56) and can be utilized to analyze not only the effectoromes of pathogens/insects, but also potential plant defense proteins and metabolites.

Subversion of Phloem Functions by Phloem-Feeding Insects and Prokaryotic Pathogens

A fascinating area of research to watch in the coming decade is how phloem-feeding insects and pathogens overcome plant defense and subvert other phloem cellular functions. Not only can such studies further reveal mechanisms of insect infestation and pathogen infection, but they could also lead to new insight into certain structural and functional aspects of fundamental phloem biology that may not have been appreciated without considering millions of years of phloem interaction (and possibly coevolution) with pathogens and insects.

Pathogens. All phloem-inhabiting pathogens have a common lifestyle: they invade and multiply in both insect vectors and plants. These pathogens secrete effectors into plant and insect cells as a major mechanism of manipulating host plants and insects to their advantage. A remarkable example of interkingdom manipulation during tritrophic (pathogen–plant–insect) interactions is accomplished by Aster Yellows phytoplasma strain Witches' Broom (AY-WB). This pathogen secretes AY-WB protein 11 (SAP11) to destabilize *Arabidopsis* CINCINNATA (CIN)-related

TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP) transcription factors. Destabilization of these transcription factors alters plant development and morphological characteristics to attract insect vectors and also inhibits JA-mediated defense responses to increase fecundity of its leafhopper vector (57). Likewise, secreted AY-WB protein 54 (SAP54) and its homolog phytoplasma-secreted protein 1 (PHYL1) from the same pathogen modulate floral developmental features by interacting with and degrading the floral homeotic MADS domain transcription factors (MTFs) to attract its leafhopper vector (58, 59). Finally, tengu-su inducer (TENGU), a small effector of Onion Yellows phytoplasma, down-regulates *AUXIN RESPONSIVE FACTOR* (*ARF*) genes *ARF6* and *ARF8* to repress JA response, presumably to favor insect vectors, and induce plant sterility, which is a feature of plants infected by Onion Yellows phytoplasma (60). Collectively, these examples illustrate the concept that some of the secreted phytoplasma effectors are devoted to facilitate the fitness of their insect vectors as a unique adaptation of phloem-infecting pathogens that rely on these vectors for completing their infection cycles (Fig. 2*B* and ref. 61).

Although phytoplasmas are limited to phloem sieve cells (3), phytoplasma effectors can be detected in tissues beyond the phloem (62, 63), which leads to the fundamental questions of how these effectors move from phloem sieve cells into other plant cells. Plasmodesmata likely play a key role in this process. In *Arabidopsis*, the size exclusion limits (SELs) of plasmodesmata connecting sieve cells and adjoining companion cells are up to 67 kDa (64), whereas the SELs of plasmodesmata involved in nonphloem cells are about 27 kDa (65). Notably, the molecular sizes of reported phytoplasma effector proteins are relatively small. SAP11 is a 9-kDa protein, and TENGU, SAP54, and PHYL1 are 5-kDa, 10.7-kDa, and 10.6-kDa proteins, respectively (57, 58, 60). Hence, the majority of phytoplasma effectors may freely move between phloem and other cells through plasmodesmata. It remains to be determined whether there is specificity in plasmodesmata-mediated effector movements and, if yes, how specificity is controlled.

Progress has also been made in the study of molecular actions of effector proteins of the bacterial pathogen CLas. A particularly notable recent finding is the identification of the host targets of an effector called Sec-delivered effector 1 (SDE1). SDE1 interacts with and inhibits immune-related papain-like cysteine proteases (PLCPs) to attenuate plant defense responses against CLas infection (Fig. 2*B* and ref. 66). CLas produces many potential effectors (*SI Appendix*, Table S2 and ref. 31), but how these effectors facilitate pathogen multiplication and survival in both the phloem and the insect vector to facilitate disease development and vector transmission remains enigmatic.

Insects. Planthoppers, aphids, and whiteflies have developed a specialized mouthpart, the stylet, to access nutrition in the phloem (Fig. 1*C*). During the feeding process, insects secrete both gelling and watery saliva from their salivary glands into plant cells. Gelling saliva solidifies quickly and forms a continuous salivary sheath along the stylet, providing mechanical stability, lubrication, and protection of the insect against plant defense chemicals. The sheath is essential to the survival and reproduction of aphids, as silencing the expression of salivary sheath protein (SHP)-encoding genes results in abnormal sheath formation and disrupted aphid and BPH feeding (67–69). Watery saliva, on the contrary, contains putative "effector molecules," including digestive, hydrolyzing, and cell wall-degrading enzymes and other bioactive components that facilitate insect infestation and suppress plant defenses (*SI Appendix*, Table S2). Of these, some cell wall-degrading enzymes, represented by endo-β-1,4-glucanase (EG1) produced by BPH, facilitate insect ingestion by degrading celluloses in plant cell walls (Fig. 2*B* and ref. 70). Protein C002, an aphid salivary effector, facilitates feeding behaviors and infestation of aphids via an unknown mechanism. *C002*-silenced aphids spend little time taking up phloem sap (71). In contrast, overexpression of *C002* in *N. benthamiana* significantly enhances aphid fecundity (72). Similarly, phloem-expressed *Arabidopsis* actin-depolymerizing factor 3 (ADF3) appears to have an important role in controlling green peach aphid feeding of the phloem. In the *adf3-1* mutant, which lacks a functional *ADF3*, green peach aphids spend significantly less time searching for a sieve element and feed for longer periods of time once a sieve element is found (73). It will be interesting in the future to determine whether aphids produce effectors that target ADF3.

Other salivary effectors, such as Me10, Me23, and Me47 from potato aphids (*Macrosiphum euphorbiae*), as well as Mp1, Mp2, and Mp55 from green peach aphids (GPA; *M. persicae*), promote aphid infestation via suppression of a variety of molecular pathways in the plant (74–76). Me10 interacts with tomato 14–3-3 isoform 7 (TFT7) protein, and silencing *TFT7* expression in tomato leaves enhanced the longevity and fecundity of *Aphis gossypii* (Fig. 2*B* and ref. 77). Mp1 targets a plant trafficking pathway protein, Vacuolar Protein Sorting Associated Protein52 (VPS52), to promote infestation (Fig. 2*B* and ref. 78). Recently, a whitefly *Bemisia tabaci* salivary protein, Bt56, was reported to interact with a tobacco class II KNOTTED 1-like homeobox (KNOX) transcription factor (NTH202) to facilitate insect performance by eliciting the salicylic acid (SA)-signaling pathway, which likely leads to down-regulation of the JA defense pathway via SA-JA antagonism, required for resistance against whitefly (Fig. 2*B* and ref. 79).

As described earlier, sieve plate occlusion caused by callose, forisomes, and P-proteins represents a potentially unique phloem defense strategy against pathogens and insects. Sieve plates occlusion by aggregates formed by P-proteins and callose sealing is likely dependent on accumulation of free Ca^{2+} in sieve cells (80, 81). Interestingly, aphids secrete Ca^{2+}-binding effector proteins in watery saliva to prevent sieve cell occlusion (82, 83). Likewise, in the saliva of the green rice leafhopper (*Nephotettix cincticeps*), an 84-kDa calcium-binding effector protein (NcSP84) has been identified (Fig. 2*B* and ref. 84). In the saliva of BPH, a protein with an EF-hand calcium-binding motif (NlSEF1) is secreted into rice cells for calcium scavenging (Fig. 2*B* and ref. 85). NlSEF1 is able to capture Ca^{2+} and decrease rice cytosolic Ca^{2+} accumulation in rice cells. This decrease of Ca^{2+} possibly relieves phloem plugging to allow BPH to continuously obtain sap from rice phloem (85).

Challenges and Thoughts for Future Research. Understanding how pathogen and insect effectors modulate phloem cell functions will be a key step toward a conceptual framework of phloem–pathogen/insect interactions. In the past decade, several studies have focused on identifying the secretomes of phloem-limited insects and pathogens (*SI Appendix*, Table S2). Progress has been made toward understanding how some effector proteins of phloem-associated insects facilitate feeding behavior and performance of insects in plants; however, research to understand how they influence plant and insect physiology is still at an early stage, and many questions await answer. We highlight several such questions as follows.

Are there common themes of effector actions from phloem-attacking insects and pathogens? Although biologically diverse, phloem-infesting insects and phloem-infecting pathogens appear to deliver effectors into the phloem cells as a common mechanism of infection/infestation. This raises 2 related questions. Are there common effector molecules from pathogens and insects? Do different effectors from pathogens and insects attack similar phloem processes or even the same phloem components? Currently, answers to these questions are not clear. Nevertheless, we can anticipate identification of at least 2 phloem cellular processes/structures as common targets of diverse pathogen and insect effectors. One such process would likely be related to immunity (i.e., PTI, ETI,

SA, and JA pathways), as a number of effectors from phloem-adapted pathogens and insects have already been shown to target components of plant immunity, as discussed earlier. Another process targeted by effectors is likely related to regulation of sieve plate pores and plasmodesmatal aperture between sieve tubes and companion cells, as they are crucial for cell-to-cell movement of pathogens and continuous insect feeding. Again, there has been some evidence supporting this mechanism, as mentioned earlier. Future research will test these predictions and possibly reveal additional effector-targeted processes that are fundamental and/or unique to pathogen and insect adaptation to phloem infection/infestation.

Can new technologies be developed to examine physiologically relevant mechanisms of phloem-infecting pathogens and insects? A unique challenge for analyzing the in vivo functions of effectors from phloem-attacking pathogens and insects is the lack of a facile system to track the molecular action of effectors inside phloem cells. Current research relies heavily on heterologous expression, which may or may not reflect the true functions of effectors in phloem cells. As mentioned earlier, development of plant lines with phloem cell-specific biosensors detecting real-time changes of immunity, pH, Ca^{2+}, and sieve plate and plasmodesmatal pore sizes in living phloem cells, together with single-cell sequencing technology, should greatly facilitate future noninvasive analysis of physiologically relevant mechanisms of pathogen/insect manipulation of phloem functions. An additional resource to develop would be phloem cell cultures, which may enable both phloem–pathogen propagation in a laboratory setting and transient expression of effectors in phloem cells.

Conclusion and Outlook

Phloem-feeding insects and pathogens cause tremendous economic losses worldwide and represent some of the most difficult pests to understand due to their specialized feeding strategies. They are also among the most costly infestations and diseases to manage in agriculture. Current control strategies for many of these diseases and infestations rely heavily on insecticide and antimicrobial sprays (86, 87). Alternative control strategies exist, ranging from resistant cultivars to biological control to cultural practices, but they are specific to certain insects and pathogens. In particular, although natural resistant cultivars can control against phloem-feeding insects (88–90), this has not been found against particular phloem-infecting bacteria, including CLas. Current control measures against CLas rely on chemical-mediated suppression of vectors and pathogens, planting disease-free nursery stock, removing infected trees, and promoting root health (91). Unfortunately, these management strategies are inadequate in stopping HLB epidemics, which are spreading. As pointed out in a recent US National Academy of Sciences, Engineering, and Medicine review of research on HLB since the devastating outbreak in Florida in 2005, ". . .there have been no breakthroughs in HLB management. The reasons for the lack of breakthroughs in HLB management, despite the investments in research, are complex" (1).

Hopefully, fundamental research into the biology of phloem-inhabiting insects and pathogens can lead to breakthrough solutions that will dramatically change the outcomes of phloem-associated insect infestations and plant diseases. Indeed, one could envision many new cellular processes in plants, insects, and pathogens for future manipulations to improve the outcome of phloem-associated insect infestations and plant diseases. Most importantly, identifying host genes that underlie plant interactions with phloem-feeding insects and pathogens would be attractive targets to improve host resistance via introgression of naturally occurring variants or genome editing. In addition, researchers may explore the potential utility of HLB-antagonistic phloem microbiota as a control method, because development of HLB seems to require "ecological services" provided by CLas-associated microbiota (92). In parallel, innovative chemical genetic screens, such as those reported recently (87, 93), may yield more effective and safer antimicrobials. Clearly, a new wave of studies is needed to build up the foundational knowledge necessary for the development of a new generation of innovative techniques to stop the global destruction caused by phloem-feeding pests and diseases.

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