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The Phloem as an Arena for Plant Pathogens

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Abstract

Although the phloem is a highly specialized tissue, certain pathogens, including phytoplasmas, spiroplasmas, and viruses, have evolved to access and live in this sequestered and protected environment, causing substantial economic harm. In particular, *Candidatus* Liberibacter spp. are devastating citrus in many parts of the world. Given that most phloem pathogens are vectored, they are not exposed to applied chemicals and are therefore difficult to control. Furthermore, pathogens use the phloem network to escape mounted defenses. Our review summarizes the current knowledge of phloem anatomy, physiology, and biochemistry relevant to phloem/pathogen interactions. We focus on aspects of anatomy specific to pathogen movement, including sieve plate structure and phloem-specific proteins. Phloem sampling techniques are discussed. Finally, pathogens that cause particular harm to the phloem of crop species are considered in detail.

INTRODUCTION

The phloem is a dwelling and a food source for several devastating pathogens. In many ways, it is an excellent place to live, providing pathogens with a replenishing supply of metabolic products in a sheltered domain, protected from the environment, other organisms, and pesticides (5, 52, 110). This relative inaccessibility means that the phloem is not easily invaded and, as a result, many phloem pathogens are vectored by insects. The plant responds to the invasion, of course, and defense mechanisms can be effective, but the phloem is itself an escape route; many mobile pathogens migrate along the sieve tubes, out of harm's way. As they do so they leave a trail of destruction, crippling the transport network and often leading to the demise of their hosts.

Phloem-inhabiting prokaryotes include wall-less Mollecutes, such as phytoplasmas, and walled bacteria, such as pernicious *Candidatus* Liberibacter spp. (45). Adaptation to the cloistered and privileged environments in the insect and plant has allowed pathogens to discard several genetic modules and live with pruned metabolism. Several of these organisms, in particular *Candidatus* Liberibacter spp., cause increasing damage to agricultural crops. In this review, we discuss phloem structure and physiology in the context of pathogen biology, with the hope that integrated knowledge will inspire new approaches to combating these destructive organisms.

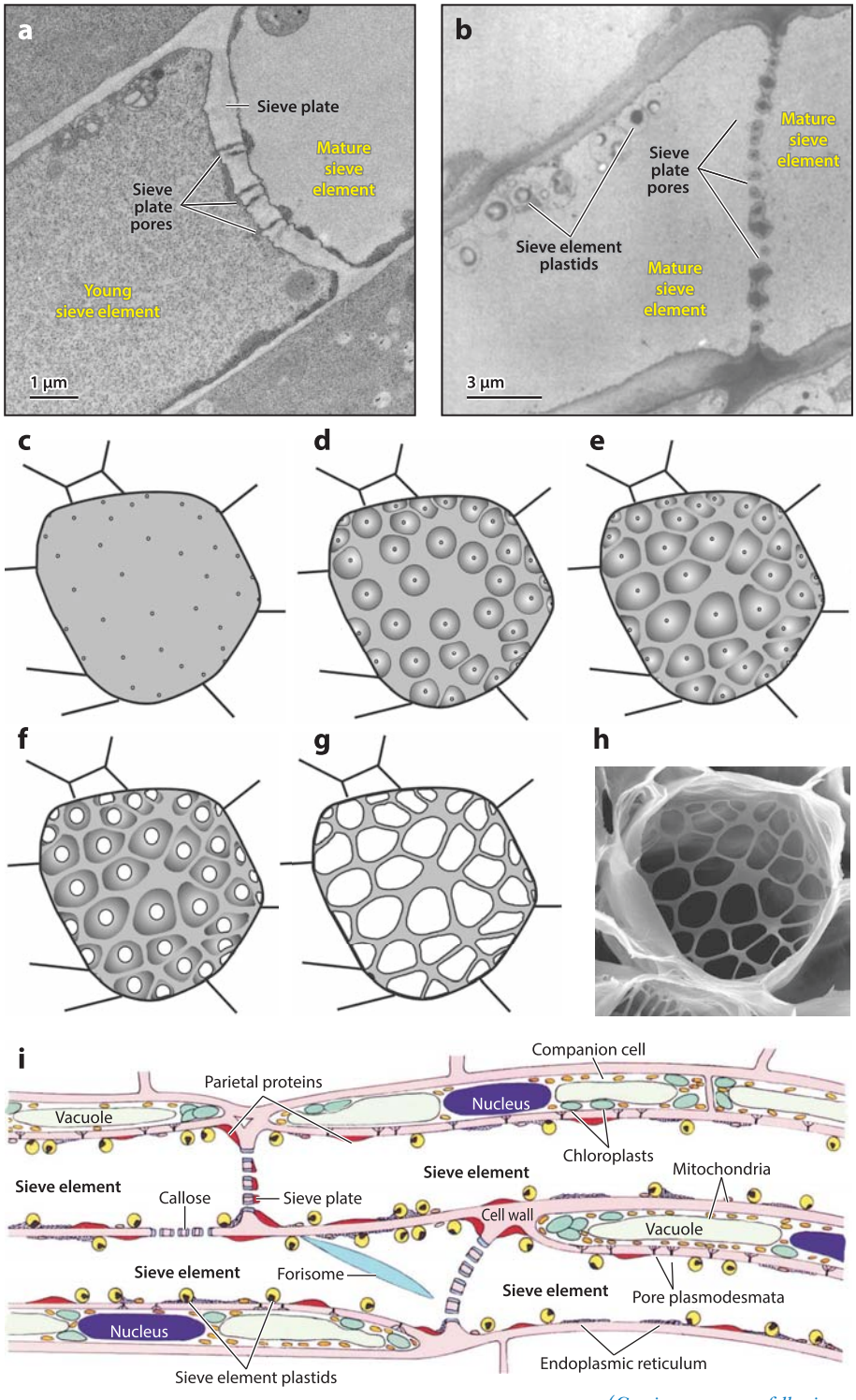
SIEVE ELEMENT ONTOGENY

Sieve elements are among the most sophisticated cells in nature. Although they lose critical organelles as they differentiate, including the nucleus and protein-synthesizing machinery, they nonetheless persist and continue to function, in some plants for many decades. Undoubtedly, they are supported in these physiological activities and the maintenance of structural integrity by the adjacent companion cell(s). Therefore, it is important to take into consideration the ontogeny of the sieve element/companion cell complex.

After an unequal division of a mother cell, the larger cell will generally differentiate into a sieve element and the smaller cell into its companion cell(s). The first ultrastructural indication that a cell will become a sieve element is the appearance of specific protein bodies (P-proteins) that quickly increase in size (17, 115). P-proteins are structural proteins and have been found in all dicot (31) and most monocot species. Only in the *Poaceae* and some palms are P-proteins absent (28).

Aside from the synthesis of P-proteins, the young sieve element appears similar to other cell types of the surrounding tissue until shortly before full maturation when they suddenly start to degrade many of their organelles. The sieve element fills with a dense mixture of organelle degradation products (**Figure 1*a***). The final step in sieve tube development is the opening of the sieve plate pores in the connecting end walls of two adjacent sieve elements (**Figure 1*b***). In this process, plasmodesmata, which are deposited across the cell plate when it is formed during cell division, play a key role. Developmental studies suggest that each plasmodesma develops into one pore in a future sieve plate (29). In developing pores, callose collars are deposited by callose synthases replacing the existing cell wall around the plasmodesmata (**Figure 1*c–b***). The size and shape of these collars appear to define the form of the future pore. Once the deposition is finalized, the cell starts to degrade the callose, which opens the pores. This process of callose deposition is, however, reversible and is part of an important defense mechanism that is discussed below. After pore opening, development is finalized and the mature sieve element becomes part of the sieve tube (**Figure 1*b,i***). The degraded organelle remnants are swept away with the translocation stream and can be found in significant concentrations in sieve tube exudates (61).

Although, to our current knowledge, the ontogeny of sieve elements is similar in different locations, their function may differ significantly. Terminal sieve elements of the protophloem, for example, mediate phloem unloading (94). They are often located in actively growing regions, are



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Sieve tube ontogeny. (*a*) Electron micrograph of the interface of a young sieve element on the left and a mature sieve element in the protophloem of *Arabidopsis*. The young sieve element is densely filled with organelle degradation products, and the mature sieve element has a lumen free of organelles or structural components, indicating active transport. The sieve plate contains several thin, developing sieve plate pores. (*b*) A mature sieve tube in tomato. The sieve plate pores are open and organelles such as sieve element plastids are located at the margins of the cells. (*c–h*) Schematic of sieve plate development. (*c*) Young sieve plate with plasmodesmata. (*d*) Callose collars develop around the plasmodesmata by repelling or removing cell wall material. (*e*) Collars extend and approach each other. (*f*) Callose formation is completed and callose removal starts in proximity to the plasmodesmata, leading to a widening of the pores. (*g*) Callose removal is completed and a mature sieve plate has developed. (*h*) Scanning electron micrograph of a mature *Cucurbita maxima* sieve plate. (*i*) Schematic of sieve tube structure in *Vicia faba* (broadbean). Panel *b* adapted with permission from Reference 27; panels *c–h* adapted from Reference 16; and panel *i* adapted from Reference 64.

in many cases active only for a few hours in unloading and die after one to a few days. As they do not contain a nucleus, they cannot follow growth and are passively stretched by several hundred percent. Secondary sieve elements deriving from fusiform initials in the cambium are fully grown when opening the pores. In palms, which do not have secondary growth, sieve elements may be active for years (e.g., 107).

Independent of their location, sieve elements contain a minimal set of cellular components at maturity. Those include the abovementioned P-proteins, some mitochondria, sieve element–specific plastids (SE-plastids), and a uniquely shaped, stacked smooth endoplasmic reticulum (ER) called the sieve element ER. All other organelles, including the nucleus, vacuole, cytoskeleton, and ribosomes, are degraded. The lack of the nucleus makes sieve element investigations especially challenging, as nucleic acid–based omics approaches are of little help in understanding sieve element cell biology. Because sieve elements are not self-supportive, the neighboring companion cells provide the majority of compounds necessary to keep the sieve element alive. Companion cells are metabolically highly active, which is reflected, at least in some species, in a dense cytoplasm with numerous mitochondria (66). Sieve elements are connected to companion cells via special plasmodesmata (79). On the sieve tube side, the plasmodesmata have a single large pore, and the cell wall on the companion cell side contains numerous branches that connect to the pore. The term pore-plasmodesmata has been adapted to reflect this anatomy. It has been shown that pore-plasmodesmata have a very large size exclusion limit of approximately 70 kDa, allowing larger proteins to enter the sieve elements (87).

SIEVE ELEMENT–SPECIFIC DEFENSE MECHANISMS

Mature sieve elements rely on other cells, such as their companion cells, for protein turnover, maintenance of cell integrity, and chemical defense. However, sieve elements themselves are not defenseless. Their structure/function relations are unique and so are their defense mechanisms. Mechanical defense strategies have evolved that would not work in other cell types but rival any biochemical approach.

Infection of plants may occur in various ways. For example, mechanical injury of the leaf surface may provide a pathway into the tissue. The infection of a single leaf by a pathogen is in many cases not an issue for the whole plant. But when specific pathogens are able to infect neighboring cells and ultimately reach the phloem for systemic infection, the organism is in particular danger. Mechanisms such as programmed cell death (20) target the ability of the pathogen to move to neighboring cells and ultimately prevent the pathogen from reaching the sieve tube system for systemic spread. However, pathogens transmitted by sieve tube feeding vectors are already in

the tube system before chemical defense mechanisms can be initiated. Therefore, sieve element–specific defense is crucially important, targeting flow stoppage and the isolation of the injured tube section. The two strategies that are usually applied to stop flow within seconds or less are a sudden increase in sap viscosity and/or a significant decrease in sieve tube or sieve pore radius.

There are two levels of sieve tube reaction to injury. Heavy injury resulting in the rupture of the sieve tube cell wall and plasma membrane leads to an immediate release of the high turgor pressure in the system. The drag of the fluid leaving the tube at high velocity results in the displacement of P-proteins and also of organelle fragments from disrupted ER and burst SE-plastids (1, 64). The structures are swept along until they reach the next downstream sieve plate, where they are strained out, resulting in the occlusion of the pores. Within a short time, a solid layer is formed that prevents further leakage. The mechanism can be compared to dumping a thick noodle soup down a drain. The strainer is quickly blocked and prevents further flow. This irreversible mechanism happens frequently when plants are mechanically injured by biotic or abiotic factors. The comparably strong injury leads to a file of multiple dead sieve elements within the sieve tube. To reconnect an injured tube system to neighboring files, parenchyma cells may remeristemize to form new sieve elements and bridge the area of injury. The reconnection process has been described in detail by Schulz (99). Most electron micrographs show sieve elements in this highly artificial, injured state because electron microscopy requires cutting small tissue samples, and the shock wave that induces artifacts transmits easily through the highly connected cells. Significant efforts have been made to develop protocols to capture sieve elements in their uninjured state (e.g., 27, 32, 36). Nevertheless, images of sieve elements that are considered mostly artifact free are sparse and available only for a few species.

The second level of sieve tube reaction to injury is a much more delicate interaction and may be triggered locally by, e.g., the careful insertion of an aphid stylet into the tube but may also be initiated by distant events that result in electropotential waves running along the tube and causing local reactions. Those stimuli may result in different forms of injury responses by the sieve tube, as outlined below.

P-Proteins and Forisomes

P-proteins are the most abundant proteins in sieve elements. A large variety of P-protein structures can be found in sieve elements of angiosperms (18). Historically, the first biochemically described P-proteins were PP1 and PP2 in *Cucurbita* (6, 13, 43, 92). PP1 is a protein filament covalently bound to PP2 by disulfide bridges, and it is predicted that PP2, a phloem lectin protein, anchors the filaments to either the plasma membrane or the sieve element ER via glycoproteins (13). Cucurbita sieve tube sap gels when exposed to air (3), and PP1 and PP2 are linked to this reaction. Burning leaf tips induces electropotential waves in the phloem that lead to distant formation of slime plugs formed by PP1 (39). Cucurbits have a unique and complex sieve tube system (102, 103, 123, 125), and, in line with this, PP1 turned out to be specific to this plant family.

The reaction of an unrelated group of P-proteins was initially discovered in *Vicia faba* (60, 62). Forisomes, specific to legumes, are spindle shaped, usually 10–30 μm–long P-protein bodies that quickly undergo a conformational change to a spherical high-volume state upon injury (**Figure 2**, upper left). The up to sixfold volumetric change can be completed within 0.15 seconds (90) and is sufficient to fill the lumen of sieve tubes, efficiently blocking flow (63). The reaction may be locally induced by membrane puncture or osmotic shock (62) or distantly induced electropotential waves (38).

Aphid saliva contains calcium-binding proteins, and application of the saliva to forisomes in vitro may reverse the high-volume state of forisomes into the low-volume state (117). Interestingly,

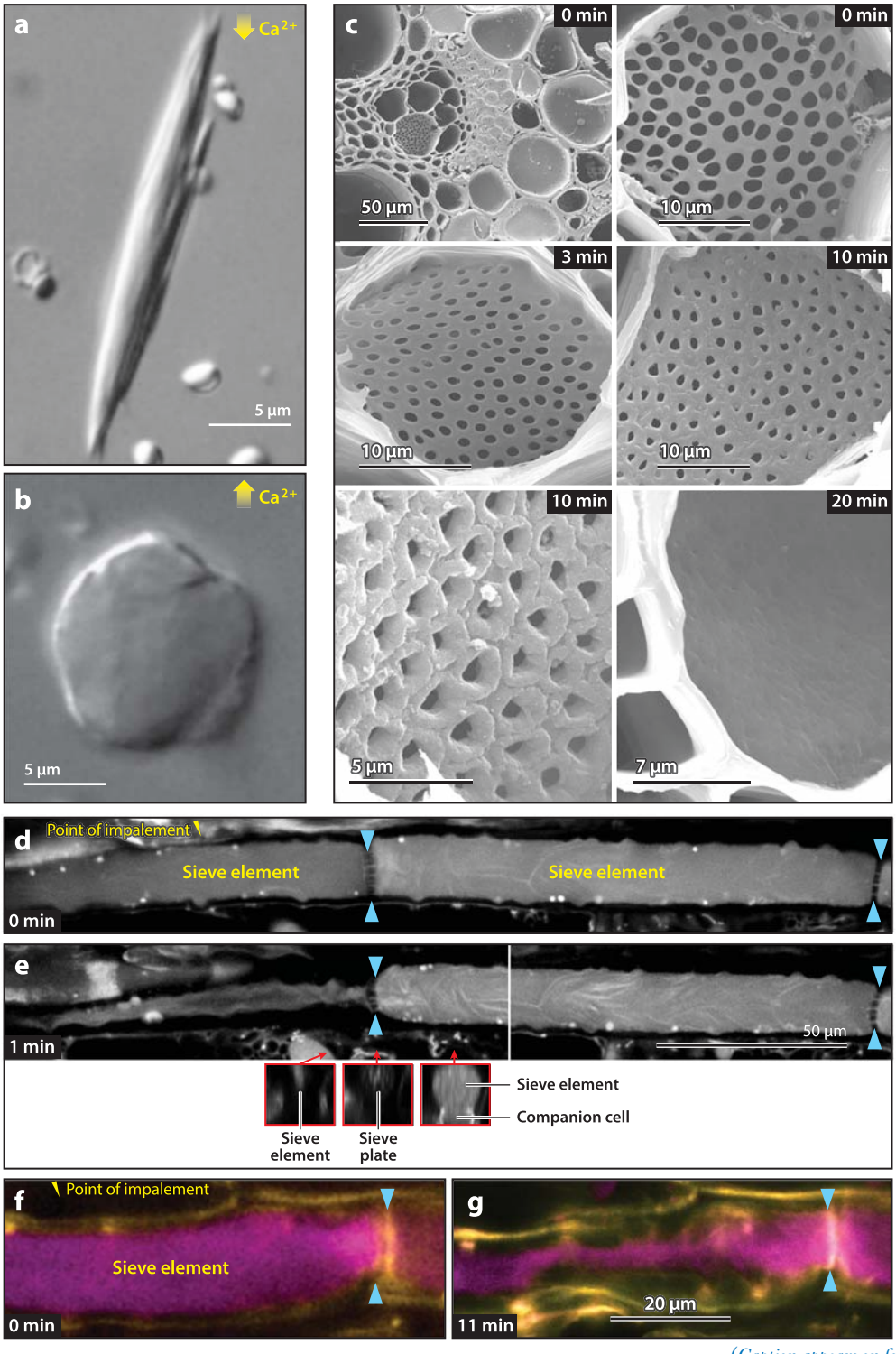


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Figure 2 (*Figure appears on preceding page*)

Mechanisms of sieve tube occlusion. Upper left: Forisome in vitro in the (*a*) low- and (*b*) high-volume state (63). Upper right: Progressive, time-dependent increase of callose deposition on sieve plates in bamboo (*Phyllostachys nuda*). (*c*) Low-magnification image of a vein and sieve plate pores after 0, 3, 10, and 20 min (76). Lower panels: Confocal laser-scanning micrographs of the formation of nacreous cell walls. (*d*) Transporting sieve tube just before impalement of one of the sieve elements by a micropipette (position indicated by a *yellow arrowhead*). Individual sieve pores are resolved in the sieve plates (between *blue arrowheads*). (*e*) At 1 min after impalement, the lumen of the punctured cell has collapsed. The different cross-sectional shapes of the punctured and unaffected sieve elements are shown in the optical sections below. (*f*) Transporting sieve tube before impalement. (*g*) The same cells 11 min after impalement. The space between the outer cell wall layer and the collapsed cytoplasmic compartment shows irregular cellulose-linked staining (58).

forisomes in faba bean function in defense against generalist aphids (72), but the pea aphid, a species specialized for striving on some legumes, can prevent forisome reaction (113), showing how specific and closely defense mechanisms and counteraction by pests have evolved.

Because forisomes are found only in legumes, one might expect that the proteins are specific to legume species. However, it has been found that the genes are widely abundant and exist in all angiosperm families, with very few exceptions (88, 95, 129). Since these genes were first identified in legumes and named sieve element occlusion (SEO) genes (88), homologous genes in other families are referred to as SEO related (SEOR). This nomenclature is not universally adopted, with some groups identifying all such genes as SEO.

Although there is no doubt that forisomes in legumes serve as a rapid first line of defense in preventing loss of assimilates, in situ studies revealed that SEOR proteins in *Arabidopsis* and *Populus* do not show a conformational change or direct detectable reaction to injury when observed in situ (36). Phloem exudation is increased in P-protein knockdown lines compared to wild-type tobacco plants (30), but this could be due to the reduced resistance in tubes lacking P-proteins. A study on phytoplasma-infected *Arabidopsis* SEOR knockout and knockdown lines reported that new P-protein filaments appear in sieve tubes after infection (83). As all investigated SEO and SEOR promoters, including those of *Arabidopsis*, are sieve tube specific, mature sieve elements (lacking a nucleus) are not capable of synthesizing new P-protein filaments. Because the neighboring cells lack promoter activity, it is as yet unclear how SEOR filaments can be newly synthesized unless there is an unknown unrelated gene family that also encodes P-protein filaments.

The structural diversity of P-proteins appears to reflect functional complexity, and we are far from understanding their function in the various plant families. Although it has been difficult to attribute P-protein function to plant defense against specific pathogens, this could be due to the fact that when a defense mechanism works efficiently there are no symptoms and therefore attribution is problematic. Here, we want to point out that some plant families have developed highly sophisticated P-protein-based occlusion mechanisms that react in a time range of milliseconds to seconds. The future might show whether P-proteins are also involved in other processes unrelated to sieve tube occlusion.

Callose

As outlined above, callose is involved in sieve plate pore formation by producing callose collars around plasmodesmata. Removing the collars opens the pores. This process is reversible, as injury triggers callose formation. Callose synthase 7 (CalS7) has been identified as the gene encoding the phloem-specific callose synthase (120). The enzyme, localized in the membrane, recruits sugars from the sieve tube lumen and produces callose to the extracellular space (112). The deposition of material onto the cell wall constricts the sieve plate pores, which results in a slowdown, and in some cases a complete halt, of sap flow. Obviously, the synthesis of new material is not as fast as a conformational change of existing proteins. Callose deposition on the sieve pore cell wall has

been reported to be in the range of 25–60 nm/s (79). Smaller pores in species such as bamboo or green bean can be fully occluded within 10–20 minutes (**Figure 2**, upper right). In contrast, full occlusion of the very large sieve plate pores in *Cucurbita maxima* was not observed even after 24 h (76). The wound signal may be local or distant (38, 39, 77) and may be induced by phloem-feeding insects (47, 65, 96).

CELL WALL SWELLING

Nacreous cell walls in sieve elements have been reported in a large number of plant species. The term describes very thick walls with a specific glistering appearance. Because of the wide distribution of such walls in sieve elements, they have historically been used as a diagnostic feature. Functionally, however, it was confusing that the cell walls obstruct a major part of the sieve tube lumen, which is required for low resistance flow. However, when conducting in situ studies, it turned out that in the turgescent, translocating state, the cell walls in sieve tubes of *Gerrardanthus macrorhizus* are actually thin and swell only when turgor is lost (**Figure 2**, lower). In the translocating turgescent state, the cell wall is compressed but quickly swells and constricts the tube lumen to reduce or stop flow in case of injury or osmotic-induced turgor loss (58). Cell wall swelling may be a very common defense mechanism in sieve elements of various taxa.

The three described mechanisms are not mutually exclusive, but a combination is very common. P-protein reaction is a quick, second-to-subsecond response. Cell wall swelling is a second-to-minute process, depending on the speed of turgor loss. Both are often followed by the slower but more robust and permanent sieve plate pore occlusion by callose.

SAMPLING THE PHLOEM

Many molecular signals and protective compounds that arise in response to pathogen infection are carried in the phloem stream. Identifying these compounds is an important goal in studying plant–pathogen interactions. Unfortunately, cataloging and quantifying substances in the phloem are a challenge (54). Unlike the extracellular circulatory system of animals, which is relatively easy to access, the sieve tube system of plants consists of highly differentiated, living cells embedded deep in tissue. The sieve tubes are difficult to reach in a specific manner and exuding sap is readily contaminated by fluid from surrounding cells. Various phloem sampling techniques have been devised, but none provide pure, unadulterated sieve tube sap. Recent overviews (53, 57) provide comprehensive analyses of long-distance signaling in plants. Here, we restrict our analysis to available sampling techniques, with cautions and suggestions as to how pitfalls may be avoided and data might be interpreted.

When $^{14}CO_2$ became available in the late 1950s, all the major translocated sugars (sucrose, raffinose, and stachyose) and sugar alcohols were identified as ^{14}C-labeled compounds downstream of the radiolabeled leaf blade. Exposing mature leaves to $^{14}CO_2$ is the best way to introduce a tracer naturally, although some caution is warranted in that a fraction of the transported material unloads into the tissue surrounding the phloem as it is carried away from the labeling site. These labeled compounds may be metabolized and the products inadvertently identified as phloem mobile. Also, different substances have different turnover times in the loading zone, so labeled compounds that enter the phloem slowly can be difficult to detect.

A more commonly used approach is tissue abrasion followed by the application of a radiolabeled compound of interest. However, this is not the native pathway into the phloem, so one cannot prove with certainty that labeled compounds that find their way into the phloem by this route are transported naturally.

Undoubtedly the best method for collecting authentic phloem sap is from cut stylets of insects in the Hemiptera (24, 116). This method confirms the conclusion that sieve tube sap is essentially free of monosaccharides. However, the samples are exceedingly small and cannot be obtained from all species. There is also the possibility that the sap will be altered by the rapid deployment of plant chemical defenses. Indeed, as shown by Mondal (75), effector proteins in the aphid's saliva induce a complex host response in an effort to stifle exudation.

One of the most common phloem sampling techniques takes advantage of the fact that certain plants "bleed" from the phloem when tissue is cut. This works well with many woody dicots (130) but not as successfully in herbaceous plants because wound healing quickly seals the phloem. When cut, the release of sieve tube pressure, which may exceed 1.5 MPa (59, 108), more than six times the pressure in a car tire, pulls phloem proteins into the sieve pores. Callose is also deposited at the edges of the pores and, as a result, exudation quickly ceases. Nonetheless, a small number of herbaceous species, including those in the genus *Lupinus*, do bleed from severed phloem (86), although one cannot conclude that the exudate is entirely free of contaminants, as Pate et al. (86) note.

Cucurbits are well known for bleeding from the vascular tissue when severed and have been used in many studies. The emerging fluid appears to come from the phloem, but it has been shown that it is seriously, indeed overwhelmingly, contaminated by the contents of the extrafascicular phloem, other cell types, and the xylem (69, 123, 125, 131). The extrafascicular phloem, specialized for producing defense compounds, is a unique cell type found only in the Cucurbitaceae (42). One common method to avoid sample contamination is to blot away the initial exudate. However, the sieve tubes seal quickly; within 2 min, the exudation of ^{14}C-labeled photoassimilates from cut pumpkin stems stops almost entirely (125). Therefore, fluid collected after this time point contains little, if any, material from sieve tubes.

Another simple method of obtaining exudate for long periods is to immerse the cut ends of the tissue in an EDTA solution. However, EDTA chelates Ca^{2+} in membranes and cell walls, softening the tissue in general and inducing leakage of soluble ions and compounds (69). Keeping the leaves in the dark reduces the amount of EDTA drawn into the petiole and lamina by transpiration, but even with this caution, the samples may still be overwhelmingly contaminated by leakage. In the original study, King & Zeevaart (56) exposed leaf blades to $^{14}CO_2$ and analyzed radiolabeled exudate, ensuring that the sampled compounds were in the transport stream. However, in subsequent studies this labeling technique is rarely used; instead, it is simply assumed that all captured compounds are phloem mobile. This is clearly not true, as profiles of radiolabeled and non-radiolabeled compounds exuded after $^{14}CO_2$ labeling differ considerably (69). In some studies, contamination has been estimated by measuring the concentrations of compounds assumed not to be present in sieve tubes, such as monosaccharides. However, this works only if the proportional differences in concentrations of these compounds in the various cell types surrounding the phloem are known. In many studies, damage induced by EDTA is reduced by preincubating the cut petiole in the EDTA, then transferring the leaves to an EDTA-free solution for the collection process. However, this may not effectively reduce damage and leakage (121).

Whereas the techniques discussed above sample fluid that may or may not derive from sieve tubes, simple dissection or laser capture techniques collect phloem tissue that obviously contains sieve elements, companion cells, phloem parenchyma, and, in some cases, additional cell types (70, 103). These analyses are limited in the sense that the compounds they record are clearly not all from the sieve tubes, but the origin of the derived data is at least unambiguous.

Functional analysis is the only way to demonstrate that a phloem-mobile compound is a true signal or has some other practical purpose. In this regard, identification of the flowering signal [Flowering Locus T (FT)] provides a cogent example (for review, see 119). FT is synthesized in

specific companion cells in the minor veins of leaves (9). Several advanced methods were used to prove that FT is the functional flowering protein, and many groups are now investigating the transport of specific long-distance signals (e.g., 10, 106, 109, 126). The field is growing rapidly (119), and the technology that drives it will undoubtedly be useful in the study of mobile signals associated with pathogens and plant defense strategies.

THE NATURE OF PHLOEM PATHOGENS

The phloem is an ideal niche for pathogens, providing sugars, micronutrients, and long-distance transport to the plant. Phloem-restricted bacteria typically have highly reduced genomes, lack core metabolic pathways, and depend on their plant hosts for nutrients. As a result, it is not yet possible to grow these pathogens in the lab, which has hindered the study of these important bacteria (73). They often grow very slowly in their plant hosts, which can make it difficult to tell if the plants are infected until they are very sick (100).

Pathogen infection substantially affects the RNA and protein composition of the phloem, although there are challenges associated with identifying phloem-specific molecules, as described in the section titled Sampling the Phloem. In susceptible infected hosts, the sieve elements become plugged by callose and phloem loading is impaired, as seen in citrus infected with *Candidatus* Liberibacter asiaticus (*C*Las), tomato infected with '*Candidatus* Phytoplasma solani' and *Arabidopsis* infected with '*Candidatus* Phytoplasma asteris' (22, 34, 55, 83). These effects are more severe in infected susceptible plants compared to infected tolerant or uninfected plants, suggesting that the plant's response leads to phloem dysfunction. Specific proteins, such as phloem protein 2 (PP2, a lectin) and forisomes, are induced by pathogen infection and are highly abundant in the phloem (2, 43, 122). PP2 has been proposed to contribute to defenses against pathogens, can interact with a wide variety of RNAs, and may be co-opted for viroid infection (80). Since PP2 can traffic between companion cells and sieve elements, viroids may interact with PP2 to facilitate their systemic movement (80). Forisomes are legume-specific calcium-responsive protein bodies that expand and occlude sieve plates (see above). Phytoplasmas are still able to colonize the host even when mass flow through the phloem is impaired (83), indicating that phytoplasma movement does not depend exclusively on mass flow.

Candidatus Liberibacter asiaticus: A Covert Suppressor of Plant Immunity

*C*Las is the most common cause of Huanglongbing (HLB) disease, also called citrus greening. *C*Las is vectored by the Asian citrus psyllid, *Diaphorina citri* (7). In the plant host, *C*Las migrates approximately 3 cm/day (91), 400 times slower than the rate of phloem transport, and is capable of moving into mature leaves against assimilate flow (84). *C*Las attaches to the plasma membrane at the sieve plate and moves through the phloem in an elongated form (1). Although *C*Las movement in the phloem is not mediated by flagella, bacteria have several other potential mechanisms of active movement, including twitching through type IV pili and slithering, known as gliding motility (51). Active lateral migration from the initial infection site to other sieve tubes probably explains why after a limited number of *C*Las-carrying psyllids feed on a citrus stem, the bacteria can be found in many of the numerous sieve tubes, ensuring that bacteria-free psyllids stand a good chance of becoming infected (25). HLB symptoms include blotchy mottle, leaf and shoot chlorosis, premature fruit drop, deformed and discolored fruit, and dieback and result in adverse fruit quality and quantity (114). Phosphate deficiency in infected plants is linked with symptoms (127).

Citrus species vary in their susceptibility or tolerance to *C*Las. The outcome depends on how quickly the host responds to infection and whether the pathogen suppresses host immunity (122).

Microcitrus such as lemon and lime are more tolerant to *C*Las infection, and lemon can regenerate its phloem after infection (23), as discussed in the section titled Sieve Element–Specific Defense Mechanisms. HLB-tolerant citrus show greater induction of immune-related genes, cell wall–associated genes, transcriptional regulators, signaling cascades, and hormone signaling compared to HLB-susceptible citrus (19, 122), although these studies did not look specifically at the phloem. Some tolerant citrus such as *Swingle citrumelo* display higher expression of pathogenesis-related genes such as PR1 as well as fewer impacts on hormone expression and sucrose content than susceptible varieties (118). Citrus can also be protected against infection by introducing master regulators of immunity such as NPR4 or by the application of antimicrobial compounds or immune-inducing peptides (11, 49, 78, 89). In contrast, sweet orange does not mount a rapid immune response after infection (2, 55). It is believed that the defense response is too slow to prevent infection in sweet orange and that P-proteins and/or callose deposition in sieve plates contribute to phloem dysfunction. PP2 is upregulated in HLB-infected sweet orange, which likely contributes to the plugging of sieve plates (55). HLB-infected sweet orange displays greater impairment of phloem loading and more callose deposition in sieve plates than HLB-tolerant rough lemon (34), which affects translocation through the phloem.

Many pathogens deliver effector proteins to their hosts to promote pathogenesis. Effectors are small proteins, encoded by the pathogen, that function in the host. Although many bacteria deliver effectors to hosts through the needle-like type III secretion system, *C*Las instead delivers Sec-dependent effectors (SDEs) through the Sec-dependent pathway. Many of these effector proteins suppress immune signaling pathways in the host. For example, CLIBASIA-04405, SDE15, and several other putative effectors are able to suppress cell death (26, 85, 124). Cell death can result from recognition of a specific effector protein and allows the host to kill the infected area, which protects the rest of the plant from infection. It is commonly used as a defense mechanism by plants. Suppression of cell death therefore promotes bacterial infection. Another effector, SahA, degrades salicylic acid (SA) and suppresses the plant immune response (68). SA is an important signaling molecule that induces systemic acquired resistance (SAR), a broad-spectrum defense response to pathogen infection after an initial exposure (37). SDE1 inhibits papain-like cysteine proteases, which normally contribute to plant immunity, and therefore promotes disease (14). These activities have been observed for other well-characterized bacterial effectors (98), indicating that cell death suppression and manipulation of SA are common strategies to promote bacterial virulence.

Since *C*Las is an insect-vectored pathogen, it is also likely that effector proteins modify host processes to promote insect vectoring, as seen with phytoplasmas (see next section). Effectors might affect plant or floral development, which could increase insect colonization or fecundity. A recent study examined effector proteins from *Candidatus* Liberibacter solanacearum, a bacterial pathogen related to *C*Las (93). Only a few effectors were able to suppress pattern-triggered immune (PTI) responses induced by the application of flagellin peptide (flg22) or fungal chitin. This is an intriguing result, as most effectors from foliar bacterial pathogens suppress PTI (98), and suggests that effectors of insect-vectored pathogens may have unique targets in plants.

One major question is how *C*Las manipulates host signaling when it is restricted to sieve elements that lack a nucleus. Effector proteins are likely key to this manipulation, as effectors from other bacterial and fungal pathogens are able to traffic through the plasmodesmata (4, 12). The effector proteins could then suppress immune signaling in nucleated cells and impact the production of systemic signals, which would create a conducive environment for disease. For example, multiple effectors from the bacterial pathogen *Pseudomonas syringae* suppress callose deposition (46) and some effectors suppress SAR (98).

Fatal Attraction: Phytoplasmas Manipulate Development to Attract Insect Vectors

Phytoplasmas are mycoplasma-like bacteria that lack cell walls. They are vectored by insects, primarily the leafhopper *Macrosteles quadrilineatus*, and cause disease in many crop plants. Similar to *C*Las, they have been observed in sieve cells and systemically infect plants through the vasculature. Common symptoms include leaf chlorosis, stunting, witches' broom (abnormal growth of stems, leaves, and branches), phyllody (flowers become leaves), and floral virescence (greening of flowers). Iron deficiency and aberrant distribution of iron are associated with symptoms (8).

Advances in transcriptomics and proteomics have facilitated analysis of vascular transcripts and proteins in plants infected with phytoplasma. Laser microdissection of grapevine phloem infected with *Candidatus* Phytoplasma solani, followed by expression analysis, found lower expression of sucrose transporters and higher expression of vacuolar sugar transporters, sugar synthases, and PR genes (97). Multiple mRNAs, proteins, and novel miRNAs are found in the phloem sap of mulberry infected with phytoplasma (40, 41). The mRNAs and proteins have gene ontology category annotations of metabolism, regulation, biotic stress, hormone responses, and signaling (40). In particular, the major latex protein-like 329 (MuMLPL329) gene is upregulated and the protein is more abundant in infected mulberry (40). Overexpression of MuMLPL329 in *Arabidopsis* results in less severe disease when plants are inoculated with an unrelated bacterial pathogen (*Pseudomonas syringae*) or phytoplasma (40). Consistent with this, defense gene expression is induced in transgenic MuMLPL329 plants, indicating that it enhances plant immunity (40). Similarly, the miRNAs have predicted targets whose gene ontology categories include metabolism, transcription, signaling, responses to the environment, hormone responses, etc. (41). However, it is not clear whether the miRNAs regulate other genes by trafficking through the plant or if they regulate the translation or movement of target RNAs.

Similar to *C*Las effectors, phytoplasma effectors are likely secreted through a Sec-dependent pathway. Although phytoplasmas are restricted to the phloem, effectors have been found in other tissues (48). Phytoplasma effectors are small and are believed to move cell-to-cell and unload from the phloem through the plasmodesmata. Many phytoplasma effectors induce development changes to the plant, which may help with insect vectoring. Transgenic or transient expression of specific effectors induces developmental changes similar to witches' broom. The effector protein TENGU from onion yellows phytoplasma suppresses auxin and jasmonic acid signaling pathways, which affects flower development (48, 74). Effector SAP54 from *Candidatus* Phytoplasma asteris promotes the degradation of MADS-box proteins, and their degradation suppresses flowering and increases insect colonization (71). The nuclear-localized protein SAP11 from *Candidatus* Phytoplasma asteris destabilizes TCP transcription factors, which promotes JA signaling and increases insect fecundity on *Arabidopsis* (101). *Candidatus* Phytoplasma asteris effector SAP05 mediates the degradation of SPL and GATA transcription factors through RPN10, the 26S ubiquitin receptor. SPL and GATA both control the transition to reproductive development. SAP05 causes the plants to remain in a vegetative state and leads to witches' broom and delayed senescence (50).

Viruses: Master Hijackers of Signaling

Viruses are typically introduced into plants through mechanical injury or by insects. Plant viruses tend to be encoded by very small genomes and therefore must depend heavily on their hosts for many functions. Infected host cells support replication and transcription of the viral genome and translation of viral proteins. Infection spreads locally from cell-to-cell by symplastic movement of the virus or viral replication complexes through the plasmodesmata using specialized viral proteins

called movement proteins (35). Viruses also encode suppressors of RNAi, which disrupt immune signaling and prevent activation of viral defense pathways. Viral RNAi suppressors help promote systemic infection. Once they reach the vasculature, viruses generally cause systemic infection by following source-to-sink movement of nutrients through the phloem. Systemic movement therefore requires that viruses cross several cell types: bundle sheath, vascular parenchyma, and companion cells, followed by sieve elements. Viruses can move through the vasculature as virions, where the coat protein coats the viral genome, or as viral replication complexes (35). Variation in the coat protein can determine whether a given virus can infect specific hosts. For example, mutation of key aspartic acid residues in the coat protein of *Wheat streak mosaic virus* affected systemic infection of maize but not wheat (105). It is not clear whether host specificity comes from differential recognition of the coat protein by the host immune system or the coat protein is better adapted to vascular uptake in one host versus another. However, the coat protein of *Tobacco mosaic virus* (TMV) was recently shown to suppress SA immune signaling to promote viral systemic movement (111). Interestingly, begomoviruses can reprogram chemical defenses to attract their whitefly vectors and deter non-vector insects (128).

The molecular mechanisms of systemic infection by viruses are still not well understood. A connection between TMV infection and auxin signaling was first suggested by phenotypic similarities between plants infected with TMV and plants impaired in auxin signaling. In fact, TMV co-opts Aux/IAA (auxin/indole acetic acid) transcriptional repressors to regulate phloem loading. IAA proteins typically function as negative regulators of auxin signaling. Auxin facilitates the interaction between the F-box protein TIR1 (Transport Inhibitor Response 1) and Aux/IAA proteins, which causes the degradation of Aux/IAA proteins and prevents AUXIN RESPONSE FACTOR transcriptional repression (67). Three IAA proteins (IAA26, IAA27, and IAA18) can interact with the replicase of TMV, and the strength of their interaction is correlated with their localization; stronger interactions between IAA proteins and TMV replicase resulted in cytoplasmic localization of the IAA protein (81). IAA26 appears to be the most biologically relevant IAA protein that is manipulated by TMV, as IAA26 is expressed in all vein classes in leaves as well as the phloem companion cells of stems and roots (15). Consistent with this, during TMV infection, the nuclear localization of IAA26 is strongly reduced (15). In addition, if IAA26 accumulates to high levels, TMV infection is impaired (15). The IAA26–replicase interaction and the age of the plants appear to be critical for loading TMV into the vasculature (15).

The movement protein [Triple Gene Block 1 (TGB1)] of *Potato mop-top virus* (PMTV) interacts with the stress sensor HIPP26 (heavy metal-associated isoprenylated plant protein) to facilitate long-distance movement (16). HIPP26 is a part of a family of proteins that contain heavy metal-binding domains and isoprenylation motifs. Some members of the family regulate transcriptional responses to abiotic and biotic stress (21, 132). HIPP26 is expressed in the vascular parenchyma and is induced during drought stress or PMTV infection (16). Infection induces similar responses as drought stress, as infected plants are more drought tolerant (16). When *HIPP26* is silenced in *Nicotiana benthamiana* plants, systemic infection by PMTV is impaired (16). Taken together, this suggests that HIPP26 regulates stress-responsive genes, including genes expressed in the vasculature, to promote drought tolerance, which is then co-opted by PMTV for systemic movement through the vasculature.

Some viruses such as *Citrus tristeza virus* (CTV) are restricted to the phloem. CTV can overcome this restriction if the host is suppressed for SA-mediated defenses and silencing pathways, the viral silencing suppressor (p23) is overexpressed, or a viral protein (p33) that induces reactive oxygen species is deleted (33, 44, 104). These data suggest that CTV is recognized in resistant hosts and is restricted by the plant immune system to the phloem.

CONCLUDING THOUGHTS AND REMAINING QUESTIONS

Diverse pathogens have substantial and often devastating physiological effects on the phloem, including callose deposition on the sieve plates, alteration in phloem loading capacity, effects on the composition of phloem mobile proteins and small molecules, and eventually necrosis. Even when restricted to the phloem, pathogens use effector proteins to manipulate host immune signaling and development in other parts of the plant. Effectors are typically small enough that they are able to unload from the phloem, traffic to other tissues, and modulate host expression. Effector mobility is therefore likely critical to manipulate host signaling even though the pathogen itself is found in non-nucleated cells of the phloem. Effectors from phytoplasmas and viruses (and likely those from *C*Las) co-opt developmental signaling to promote virulence. Some developmental changes can promote insect vectoring, for example, by enhancing flower formation or volatile production, and facilitate greater spread of the pathogen. Some effectors may have evolved to function in the plant host or insect host, and therefore may have novel targets compared to effectors in non-vectored pathogens. Effectors also suppress host immune signaling, which again promotes pathogen virulence.

Major challenges remain in controlling infection because many of these pathogens cannot be cultured or modified genetically. Insights from studies on *C*Las effectors and their movement from the site of infection allowed the development of serological assays for earlier diagnosis of infected citrus trees (82). Early diagnosis is important in preventing the spread of disease. Recent work on peptides or small molecules that boost plant immunity or act as antimicrobial agents (11, 49, 78) also holds promise for control of pathogens. Rational design of antimicrobial compounds that block key enzymatic activities in prokaryotic pathogens could be an effective strategy to block pathogen proliferation, particularly if the molecules can move systemically through the plant. New sources of resistance, potentially from wild relatives, or engineered resistance may also help protect plants from infection. A deeper understanding of phloem biology will undoubtedly lead to superior strategies for defeating pathogens in this remarkable tissue.

FUTURE ISSUES

1. Physiological coordination and information transfer between sieve elements and companion cells are fundamental and largely unexplored subjects.
2. Identification of host molecules that are manipulated by phloem pathogens will help better understand how the plant regulates immune responses.
3. Characterization of pathogen effectors will help dissect their roles in promoting disease, manipulating insect vectors, and modifying host development.
4. Dissecting the systemic movement of pathogen molecules will help better understand how the plant regulates traffic through the phloem.
5. Innovative and diverse approaches involving both pathogen and phloem biology will be needed to combat infection by these destructive pathogens.

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LITERATURE CITED

1. Achor D, Welker S, Ben-Mahmoud S, Wang C, Folimonova SY, et al. 2020. Dynamics of *Candidatus* Liberibacter asiaticus movement and sieve-pore plugging in citrus sink cells. *Plant Physiol*. 182:882–91
2. Albrecht U, Bowman KD. 2008. Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus* Liberibacter asiaticus causing Huanglongbing in Florida. *Plant Sci*. 175:291–306
3. Alosi MC, Melroy DL, Park RB. 1988. The regulation of gelation of phloem exudate from *Cucurbita* fruit by dilution, glutathione, and glutathione reductase. *Plant Physiol*. 86:1089–94
4. Aung K, Kim P, Li Z, Joe A, Kvitko B, et al. 2020. Pathogenic bacteria target plant plasmodesmata to colonize and invade surrounding tissues. *Plant Cell* 32:595–611
5. Bendix C, Lewis JD. 2018. The enemy within: phloem-limited pathogens. *Mol. Plant Pathol.* 19:238–54
6. Bostwick DE, Dannenhoffer JM, Skaggs MI, Lister RM, Larkins BA, Thompson GA. 1992. Pumpkin phloem lectin genes are specifically expressed in companion cells. *Plant Cell* 4:1539–48
7. Bové JM. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J. Plant Pathol.* 88:7–37
8. Buoso S, Pagliari L, Musetti R, Martini M, Marroni F, et al. 2019. '*Candidatus* Phytoplasma solani' interferes with the distribution and uptake of iron in tomato. *BMC Genom*. 20:703
9. Chen Q, Payyavula RS, Chen L, Zhang J, Zhang C, Turgeon R. 2018. *FLOWERING LOCUS T* mRNA is synthesized in specialized companion cells in *Arabidopsis* and Maryland Mammoth tobacco leaf veins. *PNAS* 115:2830–35
10. Chen X, Yao Q, Gao X, Jiang C, Harberd P, Fu X. 2016. Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. *Curr. Biol.* 26:640–46
11. Chen Y, Bendix C, Lewis JD. 2020. Comparative genomics screen identifies microbe-associated molecular patterns from *Candidatus* Liberibacter sp. that elicit immune responses in plants. *Mol. Plant-Microbe Interact.* 33:539–52
12. Cheval C, Faulkner C. 2018. Plasmodesmal regulation during plant-pathogen interactions. *New Phytol*. 217:62–67
13. Clark AM, Jacobsen KR, Bostwick DE, Dannenhoffer JM, Skaggs MI, Thompson GA. 1997. Molecular characterization of a phloem-specific gene encoding the filament protein, Phloem Protein 1 (PP1), from *Cucurbita maxima*. *Plant J.* 12:49–61
14. Clark K, Franco JY, Schwizer S, Pang Z, Hawara E, et al. 2018. An effector from the Huanglongbing-associated pathogen targets citrus proteases. *Nature Comm*. 9:1718
15. Collum TD, Padmanabhan MS, Hsieh Y-C, Culver JN. 2016. Tobacco mosaic virus-directed reprogramming of auxin/indole acetic acid protein transcriptional responses enhances virus phloem loading. *PNAS* 113:E2740–E49
16. Cowan GH, Roberts AG, Jones S, Kumar P, Kalyandurg PB, et al. 2018. Potato mop-top virus co-opts the stress sensor HIPP26 for long-distance movement. *Plant Physiol*. 176:2052–70
17. Cronshaw J. 1975. P-proteins. In *Phloem Transport*, ed. S Aronoff, J Dainty, PR Gorham, LM Srivastava, CA Swanson, pp. 79–115. Boston: Springer
18. Cronshaw J, Sabnis DD 1990. Phloem proteins. In *Sieve Elements*: *Comparative Structure*, *Induction and Development*, ed. HD Behnke, RD Sjolund, pp. 257–83. Berlin/Heidelberg: Springer
19. Curtolo M, Pacheco ID, Boava LP, Takita MA, Granato LM, et al. 2020. Wide-ranging transcriptomic analysis of *Poncirus trifoliata*, *Citrus sunki*, *Citrus sinensis* and contrasting hybrids reveals HLB tolerance mechanisms. *Sci. Rep.* 10:20865
20. Dalio RJD, Paschoal D, Arena GD, Magalhaes DM, Oliveira TS, et al. 2021. Hypersensitive response: from NLR pathogen recognition to cell death response. *Ann. Appl. Biol.* 178:268–80

21. de Abreu-Neto JB, Turchetto-Zolet AC, de Oliveira LFV, Zanettini MHB, Margis-Pinheiro M. 2013. Heavy metal-associated isoprenylated plant protein (HIPP): characterization of a family of proteins exclusive to plants. *FEBS J.* 280:1604–16
22. De Marco F, Pagliari L, Degola F, Buxa SV, Loschi A, et al. 2016. Combined microscopy and molecular analyses show phloem occlusions and cell wall modifications in tomato leaves in response to "*Candidatus* Phytoplasma solani." *J. Microsc.* 263:212–25
23. Deng H, Achor D, Exteberria E, Yu Q, Du D, et al. 2019. Phloem regeneration is a mechanism for Huanglongbing-tolerance of "Bearss" lemon and "LB8–9" Sugar Belle mandarin. *Front. Plant Sci.* 10:277
24. Dinant S, Bonnemain J-L, Girousse C, Kehr J. 2010. Phloem sap intricacy and interplay with aphid feeding. *C. R. Biol.* 333:504–15
25. Ding F, Duan Y, Paul C, Brlansky RH, Hartung JS. 2015. Localization and distribution of '*Candidatus* Liberibacter asiaticus' in citrus and periwinkle by direct tissue blot immuno assay with an anti-OmpA polyclonal antibody. *PLOS ONE* 10:e0123939
26. Du P, Zhang C, Zou X, Zhu Z, Yan H, Wuriyanghan H, Li W. 2021. "*Candidatus* Liberibacter asiaticus" secretes nonclassically secreted proteins that suppress host hypersensitive cell death and induce expression of plant pathogenesis-related proteins. *Appl. Environ. Microbiol.* 87:e00019-21
27. Ehlers K, Knoblauch M, van Bel AJE. 2000. Ultrastructural features of well-preserved and injured sieve elements: Minute clamps keep the phloem transport conduits free for mass flow. *Protoplasma* 214:80–92
28. Eleftheriou EP. 1990. Monocotyledons. In *Sieve Elements*: *Comparative Structure*, *Induction and Development*, ed. HD Behnke, RD Sjolund, pp. 139–59. Berlin/Heidelberg: Springer
29. Esau K, Thorsch J. 1985. Sieve plate pores and plasmodesmata, the communication channels of the symplast: ultrastructural aspects and developmental relations. *Am. J. Bot.* 72:1641–53
30. Ernst AM, Jekat SB, Zielonka S, Müller B, Neumann U, et al. 2012. *Sieve element occlusion* (*SEO*) genes encode structural phloem proteins involved in wound sealing of the phloem. *PNAS* 109:E1980–E89
31. Evert RF. 1990. Dicotyledons. In *Sieve Elements*: *Comparative Structure*, *Induction and Development*, ed. HD Behnke, RD Sjolund, pp. 103–37. Berlin: Springer
32. Evert RF, Eschrich W, Eichhorn SE. 1972. P-protein distribution in mature sieve elements of *Cucurbita maxima*. *Planta* 109:193–210
33. Fagoaga C, Pensabene-Bellavia G, Moreno P, Navarro L, Flores R, Pena L. 2011. Ectopic expression of the p23 silencing suppressor of *Citrus tristeza virus* differentially modifies viral accumulation and tropism in two transgenic woody hosts. *Mol. Plant Pathol.* 12:898–910
34. Fan J, Chen C, Yu Q, Khalaf A, Achor DS, et al. 2012. Comparative transcriptional and anatomical analyses of tolerant rough lemon and susceptible sweet orange in response to '*Candidatus* Liberibacter asiaticus' infection. *Mol. Plant-Microbe Interact.* 25:1396–407
35. Folimonova SY, Tilsner J. 2018. Hitchhikers, highway tolls and roadworks: the interactions of plant viruses with the phloem. *Curr. Opin. Plant Biol.* 43:82–88
36. Froelich DR, Mullendore DL, Jensen KH, Ross-Elliott TJ, Anstead JA, et al. 2011. Phloem ultrastructure and pressure flow: sieve-element-occlusion-related agglomerations do not affect translocation. *Plant Cell* 23:4428–45
37. Fu ZQ, Dong X. 2013. Systemic acquired resistance: turning local infection into global defense. *Annu. Rev. Plant Biol.* 64:839–63
38. Furch ACU, Hafke JB, Schulz A, van Bel AJE. 2007. Ca^{2+}-mediated remote control of reversible sieve tube occlusion in *Vicia faba*. *J. Exp. Bot.* 58:2827–38
39. Furch ACU, Zimmermann MR, Will T, Hafke JB, van Bel AJE. 2010. Remote-controlled stop of phloem mass flow by biphasic occlusion in *Cucurbita maxima*. *J. Exp. Bot.* 61:3697–708
40. Gai YP, Yuan SS, Liu ZY, Zhao HN, Liu Q, et al. 2018. Integrated phloem sap mRNA and protein expression analysis reveals phytoplasma-infection responses in mulberry. *Mol. Cell. Proteom.* 17:1702–19
41. Gai YP, Zhao HN, Zhao YN, Zhu BS, Yuan SS, et al. 2018. MiRNA-seq-based profiles of miRNAs in mulberry phloem sap provide insight into the pathogenic mechanisms of mulberry yellow dwarf disease. *Sci. Rep.* 8:812
42. Gaupels F, Ghirardo A. 2013. The extrafascicular phloem is made for fighting. *Front. Plant Sci.* 4:187
43. Golecki B, Schulz A, Thompson GA. 1999. Translocation of structural P proteins in the phloem. *Plant Cell* 11:127–40

44. Gómez-Muñoz N, Velazquez K, Vives MC, Ruiz-Ruiz S, Pina JA, et al. 2017. The resistance of sour orange to *Citrus tristeza virus* is mediated by both the salicylic acid and RNA silencing defence pathways. *Mol. Plant Pathol.* 18:1253–66
45. Gonella E, Tedeschi R, Crotti E, Alma A. 2019. Multiple guests in a single host: interactions across symbiotic and phytopathogenic bacteria in phloem-feeding vectors—a review. *Entomol. Exp. Appl.* 167:171–85
46. Guo M, Tian F, Wamboldt Y, Alfano JR. 2009. The majority of the type III effector inventory of *Pseudomonas syringae* pv. *tomato* DC3000 can suppress plant immunity. *Mol. Plant-Microbe Interact.* 22:1069–80
47. Hao P, Liu C, Wang Y, Chen R, Tang M, et al. 2008. Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. *Plant Physiol.* 146:1810–20
48. Hoshi A, Oshima K, Kakizawa S, Ishii Y, Ozeki J, et al. 2009. A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium. *PNAS* 106:6416–21
49. Huang C-Y, Araujo K, Sanchez JN, Kund G, Trumble J, et al. 2021. A stable antimicrobial peptide with dual functions of treating and preventing citrus Huanglongbing. *PNAS* 118(6):e2019628118
50. Huang W, MacLean AM, Sugio A, Maqbool A, Busscher M, et al. 2021. Parasitic modulation of host development by ubiquitin-independent protein degradation. *Cell* 184:5201–14
51. Jarrell KF, McBride MJ. 2008. The surprisingly diverse ways that prokaryotes move. *Nat. Rev. Microbiol.* 6:466–76
52. Jiang Y, Zhang C-X, Chen R, He SY. 2019. Challenging battles of plants with phloem-feeding insects and prokaryotic pathogens. *PNAS* 116:23390–97
53. Kappagantu M, Collum TD, Dardick C, Culver JN. 2020. Viral hacks of the plant vasculature: the role of phloem alterations in systemic virus infection. *Annu. Rev. Virol.* 7:351–70
54. Killiny N. 2019. Collection of the phloem sap, pros and cons. *Plant Signal. Behav.* 14:1618181
55. Kim J-S, Sagaram US, Burns JK, Li J-L, Wang N. 2009. Response of sweet orange (*Citrus sinensis*) to '*Candidatus* Liberibacter asiaticus' infection: microscopy and microarray analyses. *Phytopathology* 99:50–57
56. King RW, Zeevaart JAD. 1974. Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiol.* 53:96–103
57. Kondhare KR, Patil NS, Banerjee AK. 2021. A historical overview of long-distance signalling in plants. *J. Exp. Bot.* 72:4218–36
58. Knoblauch J, Knoblauch M, Vasina VV, Peters WS. 2020. Sieve elements rapidly develop 'nacreous walls' following injury—a common wounding response? *Plant J.* 102:797–808
59. Knoblauch M, Knoblauch J, Mullendore DL, Savage JA, Babst BA, et al. 2016. Testing the Münch hypothesis of long distance phloem transport in plants. *eLife* 5:e15341
60. Knoblauch M, Noll GA, Müller T, Prüfer D, Schneider-Hüther I, et al. 2003. ATP-independent contractile proteins from plants. *Nat. Mater.* 2:600–3
61. Knoblauch M, Peters WS, Bell K, Ross-Elliott TJ, Oparka KJ. 2018. Sieve-element differentiation and phloem sap contamination. *Curr. Opin. Plant Biol.* 43:43–49
62. Knoblauch M, Peters WS, Ehlers K, van Bel AJ. 2001. Reversible calcium-regulated stopcocks in legume sieve tubes. *Plant Cell* 13:1221–30
63. Knoblauch M, Stubenrauch M, van Bel AJ, Peters WS. 2012. Forisome performance in artificial sieve tubes. *Plant Cell Environ.* 35:1419–27
64. Knoblauch M, van Bel AJE. 1998. Sieve tubes in action. *Plant Cell* 10:35–50
65. Kuśnierczyk A, Winge P, Jørstad TS, Troczyńska J, Rossiter JT, Bones AM. 2008. Towards global understanding of plant defence against aphids—timing and dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. *Plant Cell Environ.* 31:1097–115
66. Lalonde S, Franceschi VR, Frommer WB. 2007. Companion cells. In *Handbook of Plant Sciences*, ed. K Roberts, pp. 190–95. Chichester, UK: Wiley
67. Lavy M, Estelle M. 2016. Mechanisms of auxin signaling. *Development* 143:3226–29
68. Li J, Pang Z, Trivedi P, Zhou X, Ying X, et al. 2017. '*Candidatus* Liberibacter asiaticus' encodes a functional salicylic acid (SA) hydroxylase that degrades SA to suppress plant defenses. *Mol. Plant-Microbe Interact.* 30:620–30

69. Liu DD, Chao WM, Turgeon R. 2012. Transport of sucrose, not hexose, in the phloem. *J. Exp. Bot.* 63:4315–20
70. Liu Y, Lin T, Valencia MV, Zhang C, Lv Z. 2021. Unraveling the roles of vascular proteins using proteomics. *Molecules* 26:667
71. MacLean AM, Orlovskis Z, Kowitwanich K, Zdziarska AM, Angenent GC, et al. 2014. Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. *PLOS Biol.* 12:e1001835
72. Medina-Ortega KJ, Walker GP. 2015. Faba bean forisomes can function in defence against generalist aphids. *Plant Cell Environ.* 38:1167–77
73. Merfa MV, Pérez-López E, Naranjo E, Jain M, Gabriel DW, De La Fuente L. 2019. Progress and obstacles in culturing '*Candidatus* Liberibacter asiaticus', the bacterium associated with Huanglongbing. *Phytopathology* 109:1092–101
74. Minato N, Himeno M, Hoshi A, Maejima K, Komatsu K, et al. 2014. The phytoplasmal virulence factor TENGU causes plant sterility by downregulating of the jasmonic acid and auxin pathways. *Sci. Rep.* 4:7399
75. Mondal HA. 2020. Aphid saliva: a powerful recipe for modulating host resistance towards aphid clonal propagation. *Arthropod-Plant Interact.* 14:547–58
76. Mullendore DL, Windt CW, van As H, Knoblauch M. 2010. Sieve tube geometry in relation to phloem flow. *Plant Cell* 22:579–93
77. Nakashima J, Laosinchai W, Cui X, Brown RM. 2003. New insight into the mechanism of cellulose and callose biosynthesis: proteases may regulate callose biosynthesis upon wounding. *Cellulose* 10:369–89
78. Nan J, Zhang S, Zhan P, Jiang L. 2021. Discovery of novel GMPS inhibitors of *Candidatus* Liberibacter asiaticus by structure based design and enzyme kinetic. *Biology* 10:594
79. Oparka KJ, Turgeon R, 1999. Sieve elements and companion cells—traffic control centers of the phloem. *Plant Cell* 11:739–50
80. Owens RA, Blackburn M, Ding B. 2001. Possible involvement of the phloem lectin in long-distance viroid movement. *Mol. Plant-Microbe Interact.* 14:905–09
81. Padmanabhan MS, Shiferaw H, Culver JN. 2006. The Tobacco mosaic virus replicase protein disrupts the localization and function of interacting Aux/IAA proteins. *Mol. Plant-Microbe Interact.* 19:864–73
82. Pagliaccia D, Shi JX, Pang ZQ, Hawara E, Clark K, et al. 2017. A pathogen secreted protein as a detection marker for citrus Huanglongbing. *Front. Microbiol.* 8:2041
83. Pagliari L, Buoso S, Santi S, Furch ACU, Martini M, et al. 2017. Filamentous sieve element proteins are able to limit phloem mass flow, but not phytoplasma spread. *J. Exp. Bot.* 68:3673–88
84. Pandy SS, Vasconcelos FNC, Wang N. 2021. Spatiotemporal dynamics of '*Candidatus* Liberibacter asiaticus' colonization inside citrus plant and Huanglongbing disease development. *Phytopathology* 10:1094
85. Pang ZQ, Zhang L, Coaker G, Ma WB, He SY, Wang N. 2020. Citrus CsACD2 is a target of *Candidatus* Liberibacter asiaticus in Huanglongbing disease. *Plant Physiol.* 184:792–805
86. Pate JS, Sharkey PJ, Lewis OAM. 1974. Phloem bleeding from legume fruits: a technique for study of fruit nutrition. *Planta* 120:229–43
87. Paultre DSG, Gustin MP, Molnar A, Oparka KJ. 2016. Lost in transit: long-distance trafficking and phloem unloading of protein signals in *Arabidopsis* homografts. *Plant Cell* 28:2016–25
88. Pelissier HC, Peters WS, Collier R, Bel AJ, Knoblauch M. 2008. GFP tagging of sieve element occlusion (SEO) proteins results in green fluorescent forisomes. *Plant Cell Physiol.* 49:1699–710
89. Peng AH, Zou XP, He YR, Chen SC, Liu XF, et al. 2021. Overexpressing a NPR1-like gene from *Citrus paradisi* enhanced Huanglongbing resistance in *C. sinensis*. *Plant Cell Rep.* 40:529–41
90. Peters WS, Knoblauch M, Warmann SA, Pickard WF, Shen AQ. 2008. Anisotropic contraction in forisomes: simple models won't fit. *Cell Motil. Cytoskelet.* 65:368–78
91. Raiol-Junior LL, Cifuentes-Arenas JC, Cunniffe NJ, Turgeon R, Lopes SA. 2021. Modelling '*Candidatus* Liberibacter asiaticus' movement within citrus plants. *Phytopathology* 111(10):1711–19
92. Read SM, Northcote DH. 1983. Subunit structure and interactions of the phloem proteins of *Cucurbita maxima* (pumpkin). *Eur. J. Biochem.* 134:561–69

93. Reyes-Caldas PA, Zhu J, Breakspear A, Thapa SP, Toruno T, et al. 2021. Effectors from a bacterial vector-borne pathogen exhibit diverse subcellular localization, expression profiles and manipulation of plant defense. bioRxiv 459857. **https://doi.org/10.1101/2021.09.10.459857**
94. Ross-Elliott TJ, Jensen KH, Haaning KS, Wager BM, Knoblauch J, et al. 2017. Phloem unloading in *Arabidopsis* roots is convective and regulated by the phloem-pole pericycle. *eLife* 6:e24125
95. Rüping B, Ernst AM, Jekat SB, Nordzieke S, Reineke AR, et al. 2010. Molecular and phylogenetic characterization of the sieve element occlusion gene family in Fabaceae and non-Fabaceae plants. *BMC Plant Biol.* 10:219
96. Saheed SA, Cierlik I, Larsson KA, Delp G, Bradley G, Jonsson LM, Botha CE. 2009. Stronger induction of callose deposition in barley by Russian wheat aphid than bird cherry-oat aphid is not associated with differences in callose synthase or β-1,3-glucanase transcript abundance. *Physiol. Plant.* 135:150–61
97. Santi S, Grisan S, Pierasco A, De Marco F, Musetti R. 2013. Laser microdissection of grapevine leaf phloem infected by stolbur reveals site-specific gene responses associated to sucrose transport and metabolism. *Plant Cell Environ.* 36:343–55
98. Schreiber KJ, Chau-Ly IJ, Lewis JD. 2021. What the wild things do: mechanisms of plant host manipulation by bacterial type III-secreted effector proteins. *Microorganisms* 9:48
99. Schulz A. 1990. Wound sieve elements. In *Sieve Elements*, ed. HD Behnke, RD Sjolund, pp. 199–217. Berlin/Heidelberg: Springer
100. Selvaraj V, Maheshwari Y, Hajeri S, Chen JC, McCollum TG, Yokomi R. 2018. Development of a duplex droplet digital PCR assay for absolute quantitative detection of "*Candidatus* Liberibacter asiaticus." *PLOS ONE* 13:e0197184
101. Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA. 2011. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. *PNAS* 108:E1254–E63
102. Sui X, Nie J, Li X, Scanlon MJ, Zjang C, Zheng Y, Ma S, et al. 2018. Transcriptomic and functional analysis of cucumber (*Cucumis sativus* L.) fruit phloem during early development. *Plant J.* 96:982–96
103. Sui X, Nie J, Lin T, Yao X, Turgeon R. 2021. Complexity untwined: the structure and function of cucumber (*Cucumis sativus* L.) shoot phloem. *Plant J.* 106:1163–76
104. Sun YD, Folimonova SY. 2019. The p33 protein of *Citrus tristeza virus* affects viral pathogenicity by modulating a host immune response. *New Phytol.* 221:2039–53
105. Tatineni S, French R. 2014. The C-terminus of *Wheat streak mosaic virus* coat protein is involved in differential infection of wheat and maize through host-specific long-distance transport. *Mol. Plant-Microbe Interact.* 27:150–62
106. Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang W, et al. 2015. Endogenous *Arabidopsis* messenger RNAs transported to distant tissues. *Nat. Plants* 1:15025
107. Tomlinson PB, Huggett BA. 2012. Cell longevity and sustained primary growth in palm stems. *Am. J. Bot.* 99:1891–902
108. Turgeon R. 2010. The puzzle of phloem pressure. *Plant Physiol.* 154:578–81
109. van Bel AJE, Furch ACU, Will T, Buxa SV, Musetti R, Hafke JB. 2014. Spread the news: systemic dissemination and local impact of Ca^{2+} signals along the phloem pathway. *J. Exp. Bot.* 65:1761–87
110. van Bel AJE, Musetti R. 2019. Sieve element biology provides leads for research on phytoplasma lifestyle in plant hosts. *J. Exp. Bot.* 70:3737–55
111. Venturuzzi AL, Rodriguez MC, Conti G, Leone M, Caro MD, et al. 2021. Negative modulation of SA signaling components by the capsid protein of tobacco mosaic virus is required for viral long-distance movement. *Plant J.* 106:896–912
112. Verma DP, Hong Z. 2001. Plant callose synthase complexes. *Plant Mol. Biol.* 47:693–701
113. Walker GP, Medina-Ortega KJ. 2012. Penetration of faba bean sieve elements by pea aphid does not trigger forisome dispersal. *Entomol. Exp. Appl.* 144:326–35
114. Wang N, Trivedi P. 2013. Citrus Huanglongbing: a newly relevant disease presents unprecedented challenges. *Phytopathology* 103:652–65
115. Wergin WP, Newcomb EH. 1970. Formation and dispersal of crystalline P-protein in sieve elements of soybean (*Glycine max* L.). *Protoplasma* 71:365–88

116. Will T, Furch ACU, Zimmermann MR. 2013. How phloem-feeding insects face the challenge of phloem-located defenses. *Front. Plant Sci.* 4:336
117. Will T, Tjallingii WF, Thönnessen A, van Bel AJE. 2007. Molecular sabotage of plant defense by aphid saliva. *PNAS* 104:10536–41
118. Wu J, Alferez FM, Johnson EG, Graham JH. 2018. Up-regulation of PR1 and less disruption of hormone and sucrose metabolism in roots is associated with lower susceptibility to "*Candidatus* Liberibacter asiaticus." *Plant Pathol.* 67:1426–35
119. Xia C, Zhang C. 2020. Long-distance movement of mRNAs in plants. *Plants* 9:731
120. Xie B, Wang X, Zhu M, Zhang Z, Hong Z. 2011. *CalS7* encodes a callose synthase responsible for callose deposition in the phloem. *Plant J.* 65:1–14
121. Xu Q, Ren Y, Liesche J. 2019. Studying phloem loading with EDTA-facilitated phloem exudate collection and analysis. *Methods Mol. Biol.* 2014:125–32
122. Yu QB, Chen CX, Du DL, Huang M, Yao JQ, et al. 2017. Reprogramming of a defense signaling pathway in rough lemon and sweet orange is a critical element of the early response to '*Candidatus* Liberibacter asiaticus'. *Hortic. Res.* 4:17063d
123. Zhang B, Tolstikov V, Turnbull C, Hicks LM, Fiehn O. 2010. Divergent metabolome and proteome suggest functional independence of dual phloem transport systems in cucurbits. *PNAS* 107:13532–37
124. Zhang C, Du PX, Yan HL, Zhu ZC, Wang XF, Li WM. 2020. A Sec-dependent secretory protein of the Huanglongbing-associated pathogen suppresses hypersensitive cell death in *Nicotiana benthamiana*. *Front. Microbiol.* 11:11
125. Zhang C, Yu X, Ayre BG, Turgeon R. 2012. The origin and composition of cucurbit "phloem" exudate. *Plant Physiol.* 158:1873–82
126. Zhang W, Thieme CJ, Kollwig G, Apelt F, Yang L, et al. 2016. tRNA-related sequences trigger systemic mRNA transport in plants. *Plant Cell* 28:1237–49
127. Zhao H, Sun R, Albrecht U, Padmanabhan C, Wang A, et al. 2013. Small RNA profiling reveals phosphorus deficiency as a contributing factor in symptom expression for citrus Huanglongbing disease. *Mol. Plant* 6:301–10
128. Zhao PZ, Yao XM, Cai CX, Li R, Du J, et al. 2019. Viruses mobilize plant immunity to deter nonvector insect herbivores. *Sci. Adv.* 5:14
129. Zielonka S, Ernst AM, Hawat S, Twyman RM, Prüfer D, et al. 2014. Characterization of five subgroups of the sieve element occlusion gene family in *Glycine max* reveals genes encoding non-forisome P-proteins, forisomes and forisome tails. *Plant Mol. Biol.* 86:51–67
130. Zimmermann MH, Ziegler H. 1975. List of sugars and sugar alcohols in sieve-tube exudates. In *Transport in Plants I. Phloem Transport*, eds. MH Zimmermann, JA Milburn, pp. 480–503. New York: Springer-Verlag
131. Zimmermann MR, Hafke JB, van Bel AJE, Furch ACU. 2013. Interaction of xylem and phloem during exudation and wound occlusion in *Cucurbita maxima*. *Plant Cell Environ.* 36:237–47
132. Zschiesche W, Barth O, Daniel K, Bohme S, Rausche J, Humbeck K. 2015. The zinc-binding nuclear protein HIPP3 acts as an upstream regulator of the salicylate-dependent plant immunity pathway and of flowering time in *Arabidopsis thaliana*. *New Phytol.* 207:1084–96

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