Systemic signaling in response to wounding and pathogens

Richard Hilleary and Simon Gilroy

Department of Botany, University of Wisconsin, Birge Hall, 430 Lincoln Drive,
Madison, WI 53706, USA

Author for correspondence: Simon Gilroy
Department of Botany,
University of Wisconsin,
Birge Hall,
430 Lincoln Drive,
Madison,
WI 53706, USA

Email: sgilroy@wisc.edu
Tel: (608) 262 4009

Abstract

Plants possess systemic signaling networks that allow the perception of local stresses to be translated into plant-wide responses. Although information can be propagated via a variety of molecules such as hormones and RNAs moving within the bulk flow of the phloem or in the transpiration stream, the vasculature also appears to be a major pathway whereby extremely rapid signals move bi-directionally throughout the plant. In these cases, the movement mechanisms are not dependent on redistribution through bulk flow. For example, self-reinforcing systems based around changes in Ca^{2+} and reactive oxygen species, coupled to parallel electrical signaling events appear able to generate waves of information that can propagate at hundreds of µm/sec, These signals then elicit distant responses that prime the plant for a more effective defense or stress response in unchallenged tissues. Although ion channels, Ca^{2+}, reactive oxygen species and associated molecular machineries, such as the NADPH oxidases, have been identified as likely important players in this propagation system, the precise nature of these signaling networks remains to be defined. Critically, whether different stimuli are using the same rapid, systemic signaling network, or whether multiple, parallel pathways for signal propagation are operating to trigger specific systemic outputs remains a key open question.

Highlights

- The vascular system provides a conduit for rapid systemic signaling of local biotic and abiotic stresses.
- Self-reinforcing cascades of regulators such as ROS and Ca^{2+} have the potential to traverse the vasculature in directions not dictated by source-sink or transpiration stream relationships.
- Electrical signals may provide a rapid signal propagation system operating in parallel to a ROS-based network.

Introduction

Biologists have long known that plants show the ability to perceive events locally and then translate them into distant, often plant-wide reactions. Such systemic plant responses are classified into broad categories based on the triggering stimulus: systemic acquired resistance (SAR) to pathogens (reviewed in [1]), systemic wound responses (SWR) to damage and herbivory (reviewed in [2]) and systemic acquired acclimation (SAA) to abiotic stresses (reviewed in [3]). The theme behind these plant-wide response networks is that the signals generated by a local stimulus lead to distant parts adapting more efficiently to subsequent stresses of the same (or often of different) nature. For SAR, this effect can translate into the mounting of a more rapid and efficient defense response [4][5] and for SAA, local stressing of one leaf can lead to the ability to withstand high levels of e.g., light and heat stress in other leaves (e.g., [6,7]). Such changes can even be passed from generation to generation, providing a significant advantage to the “primed” offspring [8-10] and potentially even to those offspring’s own progeny [8]. In the case of SAR, these transgenerational effects are likely being mediated via a salicylic acid (SA) -dependent epigenetic mechanism centered on RNA-directed DNA methylation [11].

For a localized stress to trigger such long-range responses requires signaling networks that integrate activities across the entire plant body. The cells of the vascular system are emerging as likely central conduits for such rapid, long-distance information exchange. Indeed, the vasculature has long been recognized as an information superhighway, providing systemic interconnection between distant organs to coordinate physiology and development. This long-range signaling is thought to be accomplished through the exchange of a host of signals ranging from proteins and RNAs to hormones and metabolites carried by the flow of the transpiration and translocation streams [12,13]. However, we are only now just beginning to understand the makeup of a parallel machinery that uses signals such as reactive oxygen species (ROS), Ca^{2+} and electrical changes that is also likely using the vasculature to propagate stress-related information throughout the plant at speeds of hundreds of μm/sec (Figure 1).

Information flow: RNAs, proteins and metabolites

A wide spectrum of potential signaling molecules have been seen moving in the vasculature ranging from hormones and metabolites to proteins and RNAs (reviewed in [12,14]) that all have the potential to be carrying systemic information. The challenge now is to define specific information-carrying molecules from those caught up in the mass transit from, e.g., source to sink [15] and from those that may represent the cellular debris of the developmental transition from proto-phloem, with its full array of cellular components, to the much reduced architecture of the mature sieve element. Recently, for RNAs, a structural motif that may define motility within the vasculature has been reported, providing hints towards a mechanism for specificity and potentially for specific information flow. Thus, endogenous plant mRNAs containing tRNA-like structures (TLS) have been seen to be preferentially trafficked through the phloem [16]. mRNAs that are not normally seen in the phloem could be engineered to be transported once this TLS “stem-bulge-stem-loop” structure had been added. Positive stranded RNA viruses also appear to use TLS-like motifs to potentiate their transit through the phloem. At first glance, this type of movement seems like it should be limited to the source to sink bulk flow of the translocation stream. Yet, grafting experiments have shown long-distance RNA movement that does not simply mirror these source-sink relationships (e.g., [17]), implying an alternative motility machinery. The possibility that plants possess such a multi-directional macromolecule transport system that permeates the plant body and carries a specific subset of information carrying molecules may sound unlikely. However, the PIN, AUX/LAX, ABCB system driving polar auxin movements (reviewed [18]) provides one example of this kind of system being hardwired throughout plant tissues.

Multidirectional information flow: from small molecules to electrical signals and self-reinforcing waves

For SAR, many, structurally diverse small molecules have been proposed to underlie the systemic nature of induced resistance including: methyl salicylate, glycerol-3-phosphate, azelaic acid, pipecolic acid, dehydroabietenal, nitric oxide and S-nitrosoglutathione (Figure 1; reviewed in [19]). However, despite much investigation, the precise identity of the transmitted signal still remains elusive. Likewise, for SWR, jasmonate-related molecules were initial candidates for potential systemic, phloem mobile signals, but *de-novo* synthesis is now thought to be a key source of jasmonates in the systemic leaves [20]. Indeed, signal transmission (as judged by systemic induction of jasmonic acid synthesis and related defense gene induction) occurred in < 1 min in Arabidopsis (e.g., [21,22]) and in patterns that although largely mapping to the immediate direct vascular connections between leaves also show responses between leaves where there is not thought to be a directly connected vasculature (e.g., [23]), implying a possibly alternative underlying machinery to small molecule exchange via the sieve tubes.

In the case of SWR, electrical coupling has emerged as a likely core component of the systemic signaling system (reviewed in [24]). Propagation of electrical signals circumvents the limitation on delivering chemical messengers in the phloem translocation stream where, e.g., signaling of herbivore attack must often propagate from sink tissues (e.g., [25]). Similarly, a self-reinforcing/regenerating signaling system such as ROS production or Ca^{2+} influx (reviewed in [26]) does hold the potential to propagate signals in directions not dictated by the mass flow of vascular contents. For example, the electrical signals that are transmitted through the phloem in response to herbivory of Arabidopsis plants travel at hundreds of μm/s in directions that are not simply dominated by the source/sink relationships between leaves [27]. Thus, fast and slow depolarizations propagated via the phloem to immediate neighbor leaves of the wounded leaf, whereas more distant leaves only received slow depolarizations. Two isoforms of the glutamate receptor-like (GLR) ion channel family, GLR3.3 and 3.6 are known to be key elements of the systemic component of electrical wound signaling in Arabidopsis [23], including the propagation of both fast and slow phloem depolarizations induced by the wounding of caterpillar feeding [27]. These observations are consistent with a feed-forward amplification system of ion influx propagating systemic information. In contrast, GLR3.5 appears to be required to limit the spread of these electrical signals to non-neighbor leaves [28] suggesting a complex interplay of ion fluxes acting to both amplify and inhibit the propagation machinery. How such interactions play out at the cellular level is a key unanswered question. In addition, wound-triggered action potentials can propagate in the phloem even in the *glr3.3 glr3.6* double knockout [24] hinting at the potential richness of the landscape of the electrical signals that can move within this tissue.

In Arabidopsis, GLRs are a 20-member family of ligand (possibly amino acid) gated, most likely Ca^{2+}-permeable ion channels [29-32]. Application of exogenous amino acids has also been shown trigger elevations in cytosolic Ca^{2+} and alterations in membrane potentials that are dependent on GLR function (e.g. [30,33]), consistent with a close relationship between the GLRs and the Ca^{2+} and electrical components of systemic signaling. Defining the endogenous ligands of these channels could therefore provide important insight into the signal propagation mechanism. For instance, GLR1.2 is thought to be gated by the non-structural amino acid D-serine [34] as part of a pollen tube guidance mechanism, suggesting not only the classic 20 proteinergic amino acids, but also related molecules could be playing roles in these signaling events. For example, pipecolic acid is an unnatural amino acid linked to phloem-related triggering of SAR but any potential relationship to GLR action is as yet poorly defined.

The machinery decoding these signaling events can be extremely fast. Thus, local application of high light or heat stress can trigger RNA transcript abundance changes in 60 sec or less [35]. These rapid changes in transcript levels are dependent in part on ABA and ROS signaling cascades that are closely linked to the systemic element of SAA [35]. However, they are not altered in mutants in *RBOHD*. RBOHD has been shown to be a critical player in systemic Ca^{2+} and ROS waves (e.g., [36,37]) suggesting that although the propagation and response elements of this particular systemic signaling network may share components such as Ca^{2+} and ROS, the precise molecular machinery underlying each part of the network is likely distinct. One key question arising from such studies is how far this model for a self-reinforcing ROS and Ca^{2+} wave-based transmission machinery, and especially the molecular elements that support it, applies to the phloem. The phloem is known to propagate wound-related electrical signals (as described above) and e.g., wounding triggers Ca^{2+} changes in the vasculature [38,39], likely the phloem [40]. However, not all attacks on the plant leaf trigger propagating Ca^{2+} signals [41] and whether a systemic ROS wave [37] is traversing the sieve tubes, companion cells or other tissues has yet to be precisely defined

Signal transmission via the xylem

While the living cells of the phloem present obvious candidates for the rapid propagation of signals, what of the transpiration stream of the xylem? These cells are dead at functional maturity and the mass flow of liquid is occurring in the apoplastic space. It is clear that, much like the bulk flow of translocation in the phloem, the transpiration stream does carry many bioactive molecules from nutrients to hormones. However, mature xylem elements present a barrier to propagating electrical or ROS/Ca^{2+}-based signals as these cells are dead at functional maturity. A physical pressure-related signal has been proposed to propagate in the xylem, where e.g., wounding would cause a break in the tension of the transpiration stream that would rapidly propagate throughout the vessel system [42,43]. Such mechanical signaling could trigger stretch sensors in the membranes of adjacent cells, the so-called “squeeze cell hypothesis” [44]. This systemic, mechanically-based signaling provides an attractively plant-oriented signaling network based on the unique characteristic of the transpiration stream but has proven difficult to design experiments to directly test. Recent work modeling the propagation of variation potentials (electrical signals that propagate systemically in response to wounding, reviewed in [26]) also suggests that it may be bulk flow of a chemical messenger rather than a hydraulic signal that can carry the long-distance wound signal to distant tissues via the xylem [13]. It is also important to remember that vascular bundles incorporate not only the transmitting cells of the xylem and phloem but an array of associated living parenchyma cells that could play key roles in signaling. For example, LOX6 is known to be a key enzyme in systemic induction of the jasmonic acid production that is triggered by long-range electrical signaling in response to wounding. *LOX6* is specifically expressed in a subset of xylem parenchyma cells called xylem contact cells [44], suggesting an interplay between the phloem-based and xylem associated signaling networks. These xylem-associated cells would also be ideally suited to receive a mechanical signal from the loss of transpirational tension or a chemical signal carried in the transpiration stream as outlined above. Similarly, although in response to local salt stress of the root systemic changes in Ca^{2+} are visible in the veins (i.e. vasculature) of the aerial parts of the plant [45], it is the cortex and endodermis around the vasculature that show root-wide Ca^{2+} changes [46]. Thus, it is possible that multiple parallel signals could be being transmitted through distinct tissue conduits, offering the possibility to greatly enhance the specificity of any subsequent response.

The possibility of functional coupling between xylem- and phloem-based long-range signaling systems is hinted at in some other systemic phenomena. For example, nitrogen status and response is integrated by mobile polypeptide signals (C-TERMINALLY ENCODED PEPTIDE; CEP) and CEP DOWNSTREAM 1 (CEPD1) and CEPD2 respectively [47,48]. CEP travels from the root to shoot in the translocation stream to signal nitrogen deprivation and CEPDs travel down from the shoot to the root in the phloem to upregulate the plant's soil nitrogen scavenging systems. Although CEP peptide signals are carried through the xylem they are perceived by leucine-rich repeat transmembrane receptors that are preferentially expressed in the phloem of the shoot [47,49-51]. These receptors then trigger responses, such as producing CEPDs and potentially hormones that are redistributed by the phloem (reviewed in [52]). Thus, in this case, the long-range transmission of information and integration of root and shoot activities involves close interactions between both xylem and phloem.

VI. Conclusions and future perspectives

There seems a high likelihood that there are multiple signals (chemical, ionic, electrical, mechanical) traversing the vasculature, possibly through multiple cellular pathways and conveying systemic information about local stress perception within the plant (Figure 1). The degree to which these reflect multiple parallel signaling networks or are acting in concert to deliver an information-rich single signal to distal tissues remains a key question to be answered. The associated challenge will be to map these signals with high spatial and temporal resolution as they move through the plant. Here, the development of a toolkit of increasingly sensitive fluorescent biosensors that are also now being developed for plant-related signaling components, holds great promise to be able to simultaneously visualize multiple signaling components as they propagate systemic signals in real time. For example, engineering plants expressing both a red shifted Ca^{2+} biosensor and the ABAleon ABA sensor [53] has allowed simultaneous visualization of these two regulatory elements already closely linked to the rapid transmission of signals associated with SAA. As a wider range of such sensors is developed and deployed, we can expect them to begin to reveal just how extensive and integrated the information flow within the plant truly is.

Acknowledgements

The work in the authors' laboratory is supported by grants from the National Science Foundation (MCB1329723 and IOS1557899), The National Aeronautics and Space Administration (NNX17AD52G and NNX14AT25G) and the Graduate School of the University of Wisconsin-Madison. RH was supported by NSF GRF and the Graduate School of the University of Wisconsin-Madison. We apologize to our many colleagues whose work could not be included due to length restrictions of this manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Fu ZQ, Dong X: **Systemic acquired resistance: turning local infection into global defense**. *Annu Rev Plant Biol* 2013, **64**:839-863.
2. Savatin DV, Gramegna G, Modesti V, Cervone F: **Wounding in the plant tissue: the defense of a dangerous passage**. *Front Plant Sci* 2014, **5**:470.
3. Mittler R, Blumwald E: **The roles of ROS and ABA in systemic acquired acclimation**. *Plant Cell* 2015, **27**:64-70.
4. Traw MB, Kniskern JM, Bergelson J: **SAR increases fitness of Arabidopsis thaliana in the presence of natural bacterial pathogens**. *Evolution* 2007, **61**:2444-2449.
5. Mauch-Mani B, Baccelli I, Luna E, Flors V: **Defense Priming: An Adaptive Part of Induced Resistance**. *Annu Rev Plant Biol* 2017, **68**:485-512.
6. Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P: **Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis**. *Science* 1999, **284**:654-657.
7. Suzuki N, Miller G, Salazar C, Mondal HA, Shulaev E, Cortes DF, Shuman JL, Luo X, Shah J, Schlauch K, et al.: **Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants**. *Plant Cell* 2013, **25**:3553-3569.
8. Luna E, Bruce TJ, Roberts MR, Flors V, Ton J: **Next-generation systemic acquired resistance**. *Plant Physiol* 2012, **158**:844-853.
9. Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G: **Herbivory in the previous generation primes plants for enhanced insect resistance**. *Plant Physiol* 2012, **158**:854-863.
10. Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B: **Descendants of primed Arabidopsis plants exhibit resistance to biotic stress**. *Plant Physiol* 2012, **158**:835-843.
11. Luna E, Ton J: **The epigenetic machinery controlling transgenerational systemic acquired resistance**. *Plant Signal Behav* 2012, **7**:615-618.
12. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav SR, Helariutta Y, He XQ, Fukuda H, Kang J, Brady SM, et al.: **The plant vascular system: evolution, development and functions**. *J Integr Plant Biol* 2013, **55**:294-388.
13. Evans MJ, Morris RJ: **Chemical agents transported by xylem mass flow propagate variation potentials**. *Plant J* 2017, **91**:1029-1037.
14. Ham BK, Lucas WJ: **Phloem-Mobile RNAs as Systemic Signaling Agents**. *Annu Rev Plant Biol* 2017, **68**:173-195.
15. Calderwood A, Kopriva S, Morris RJ: **Transcript Abundance Explains mRNA Mobility Data in Arabidopsis thaliana**. *Plant Cell* 2016, **28**:610-615.
    - This paper highlights that because a molecule is being translocated through the plant, it does not directly identify it as part of functional signaling or regulatory network.

16. Zhang W, Thieme CJ, Kollwig G, Apelt F, Yang L, Winter N, Andresen N, Walther D, Kragler F: **tRNA-Related Sequences Trigger Systemic mRNA Transport in Plants**. *Plant Cell* 2016, **28**:1237-1249.
    - This paper reveals a tRNA-like structure that appears to act as a signal for RNAs to become phloem mobile.

17. Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang W, Yang L, Minambres M, Walther D, Schulze WX, Paz-Ares J, et al.: **Endogenous Arabidopsis messenger RNAs transported to distant tissues**. *Nat Plants* 2015, **1**:15025.
18. Grones P, Friml J: **Auxin transporters and binding proteins at a glance**. *J Cell Sci* 2015, **128**:1-7.
19. Gaupels F, Durner J, Kogel KH: **Production, amplification and systemic propagation of redox messengers in plants? The phloem can do it all!** *New Phytol* 2017, **214**:554-560.
20. Koo AJ, Howe GA: **The wound hormone jasmonate**. *Phytochemistry* 2009, **70**:1571-1580.
21. Glauser G, Dubugnon L, Mousavi SA, Rudaz S, Wolfender JL, Farmer EE: **Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded Arabidopsis**. *J Biol Chem* 2009, **284**:34506-34513.
22. Glauser G, Grata E, Dubugnon L, Rudaz S, Farmer EE, Wolfender JL: **Spatial and temporal dynamics of jasmonate synthesis and accumulation in Arabidopsis in response to wounding**. *J Biol Chem* 2008, **283**:16400-16407.
23. Mousavi SA, Chauvin A, Pascaud F, Kellenberger S, Farmer EE: **GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling**. *Nature* 2013, **500**:422-426.

●● This paper reveals a key role for the glutamate receptor-like channels in the propagation of electrical wound signaling in Arabidopsis. It provides one of the first insights into the molecular basis of this rapid signaling system.

24. Hedrich R, Salvador-Recatala V, Dreyer I: **Electrical Wiring and Long-Distance Plant Communication**. *Trends Plant Sci* 2016, **21**:376-387.
25. Ferrieri AP, Appel HM, Schultz JC: **Plant vascular architecture determines the pattern of herbivore-induced systemic responses in Arabidopsis thaliana**. *PLoS One* 2015, **10**:e0123899.
26. Choi WG, Hilleary R, Swanson SJ, Kim SH, Gilroy S: **Rapid, Long-Distance Electrical and Calcium Signaling in Plants**. *Annu Rev Plant Biol* 2016, **67**:287-307.
27. Salvador-Recatala V, Tjallingii WF, Farmer EE: **Real-time, in vivo intracellular recordings of caterpillar-induced depolarization waves in sieve elements using aphid electrodes**. *New Phytol* 2014, **203**:674-684.
28. Salvador-Recatala V: **New roles for the GLUTAMATE RECEPTOR-LIKE 3.3, 3.5, and 3.6 genes as on/off switches of wound-induced systemic electrical signals**. *Plant Signal Behav* 2016, **11**:e1161879.
29. Kong D, Hu HC, Okuma E, Lee Y, Lee HS, Munemasa S, Cho D, Ju C, Pedoeim L, Rodriguez B, et al.: **L-Met Activates Arabidopsis GLR Ca(2+) Channels Upstream of ROS Production and Regulates Stomatal Movement**. *Cell Rep* 2016, **17**:2553-2561.
30. Stephens NR, Qi Z, Spalding EP: **Glutamate receptor subtypes evidenced by differences in desensitization and dependence on the GLR3.3 and GLR3.4 genes**. *Plant Physiol* 2008, **146**:529-538.
31. Tapken D, Anschutz U, Liu LH, Huelsken T, Seebohm G, Becker D, Hollmann M: **A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids**. *Sci Signal* 2013, **6**:ra47.
32. Vincill ED, Bieck AM, Spalding EP: **Ca(2+) conduction by an amino acid-gated ion channel related to glutamate receptors**. *Plant Physiol* 2012, **159**:40-46.

33. Qi Z, Stephens NR, Spalding EP: **Calcium entry mediated by GLR3.3, an Arabidopsis glutamate receptor with a broad agonist profile**. *Plant Physiol* 2006, **142**:963-971.
34. Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliham M, Liu LH, Obermeyer G, Feijo JA: **Glutamate receptor-like genes form Ca2+ channels in pollen tubes and are regulated by pistil D-serine**. *Science* 2011, **332**:434-437.
35. Suzuki N, Devireddy AR, Inupakutika MA, Baxter A, Miller G, Song L, Shulaev E, Azad RK, Shulaev V, Mittler R: **Ultra-fast alterations in mRNA levels uncover multiple players in light stress acclimation in plants**. *Plant J* 2015, **84**:760-772.
   - These authors reveal a remarkably rapid response system linked to SAA, with abiotic stresses triggering changes in transcript levels within seconds of applying the stimulus.

36. Evans MJ, Choi WG, Gilroy S, Morris RJ: **A ROS-Assisted Calcium Wave Dependent on the AtRBOHD NADPH Oxidase and TPC1 Cation Channel Propagates the Systemic Response to Salt Stress**. *Plant Physiol* 2016, **171**:1771-1784.
37. Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R: **The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli**. *Sci Signal* 2009, **2**:ra45.
   - This is a key paper in the development of our understanding of the molecular machinery behind systemic signals, highlighting the NADPH oxidases and waves of increased ROS as likely important players in propagating information about abiotic stresses throughout the plant.

38. Beneloujaephajri E, Costa A, L'Haridon F, Metraux JP, Binda M: **Production of reactive oxygen species and wound-induced resistance in Arabidopsis thaliana against Botrytis cinerea are preceded and depend on a burst of calcium**. *BMC Plant Biol* 2013, **13**:160.
39. Kiep V, Vadassery J, Lattke J, Maass JP, Boland W, Peiter E, Mithofer A: **Systemic cytosolic Ca(2+) elevation is activated upon wounding and herbivory in Arabidopsis**. *New Phytol* 2015, **207**:996-1004.
40. Furch AC, van Bel AJ, Fricker MD, Felle HH, Fuchs M, Hafke JB: **Sieve element Ca2+ channels as relay stations between remote stimuli and sieve tube occlusion in Vicia faba**. *Plant Cell* 2009, **21**:2118-2132.
41. Vincent TR, Avramova M, Canham J, Higgins P, Bilkey N, Mugford ST, Pitino M, Toyota M, Gilroy S, Miller AJ, et al.: **Interplay of Plasma Membrane and Vacuolar Ion Channels, Together with BAK1, Elicits Rapid Cytosolic Calcium Elevations in Arabidopsis during Aphid Feeding**. *Plant Cell* 2017, **29**:1460-1479.
42. Malone M, Stankovic B: **Surface-Potentials and Hydraulic Signals in Wheat Leaves Following Localized Wounding by Heat**. *Plant Cell and Environment* 1991, **14**:431-436.
43. Stahlberg R, Cosgrove DJ: **Rapid Alterations in Growth-Rate and Electrical Potentials Upon Stem Excision in Pea-Seedlings**. *Planta* 1992, **187**:523-531.
44. Farmer EE, Gasperini D, Acosta IF: **The squeeze cell hypothesis for the activation of jasmonate synthesis in response to wounding**. *New Phytol* 2014, **204**:282-288.

45. Xiong TC, Ronzier E, Sanchez F, Corratge-Faillie C, Mazars C, Thibaud JB: **Imaging long distance propagating calcium signals in intact plant leaves with the BRET-based GFP-aequorin reporter**. *Front Plant Sci* 2014, **5**:43.
46. Choi WG, Toyota M, Kim SH, Hilleary R, Gilroy S: **Salt stress-induced Ca2+ waves are associated with rapid, long-distance root-to-shoot signaling in plants**. *Proc Natl Acad Sci U S A* 2014, **111**:6497-6502.
47. Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y: **Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling**. *Science* 2014, **346**:343-346.
48. Ohkubo Y, Tanaka M, Tabata R, Ogawa-Ohnishi M, Matsubayashi Y: **Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition**. *Nat Plants* 2017, **3**:17029.
49. Okamoto S, Shinohara H, Mori T, Matsubayashi Y, Kawaguchi M: **Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase**. *Nat Commun* 2013, **4**:2191.
50. Bryan AC, Obaidi A, Wierzba M, Tax FE: **XYLEM INTERMIXED WITH PHLOEM1, a leucine-rich repeat receptor-like kinase required for stem growth and vascular development in Arabidopsis thaliana**. *Planta* 2012, **235**:111-122.
51. Nontachaiyapoom S, Scott PT, Men AE, Kinkema M, Schenk PM, Gresshoff PM: **Promoters of orthologous Glycine max and Lotus japonicus nodulation autoregulation genes interchangeably drive phloem-specific expression in transgenic plants**. *Mol Plant Microbe Interact* 2007, **20**:769-780.
52. Notaguchi M, Okamoto S: **Dynamics of long-distance signaling via plant vascular tissues**. *Front Plant Sci* 2015, **6**:161.
53. Waadt R, Krebs M, Kudla J, Schumacher K: **Multiparameter imaging of calcium and abscisic acid and high-resolution quantitative calcium measurements using R-GECO1-mTurquoise in Arabidopsis**. *New Phytol* 2017, **216**:303-320.

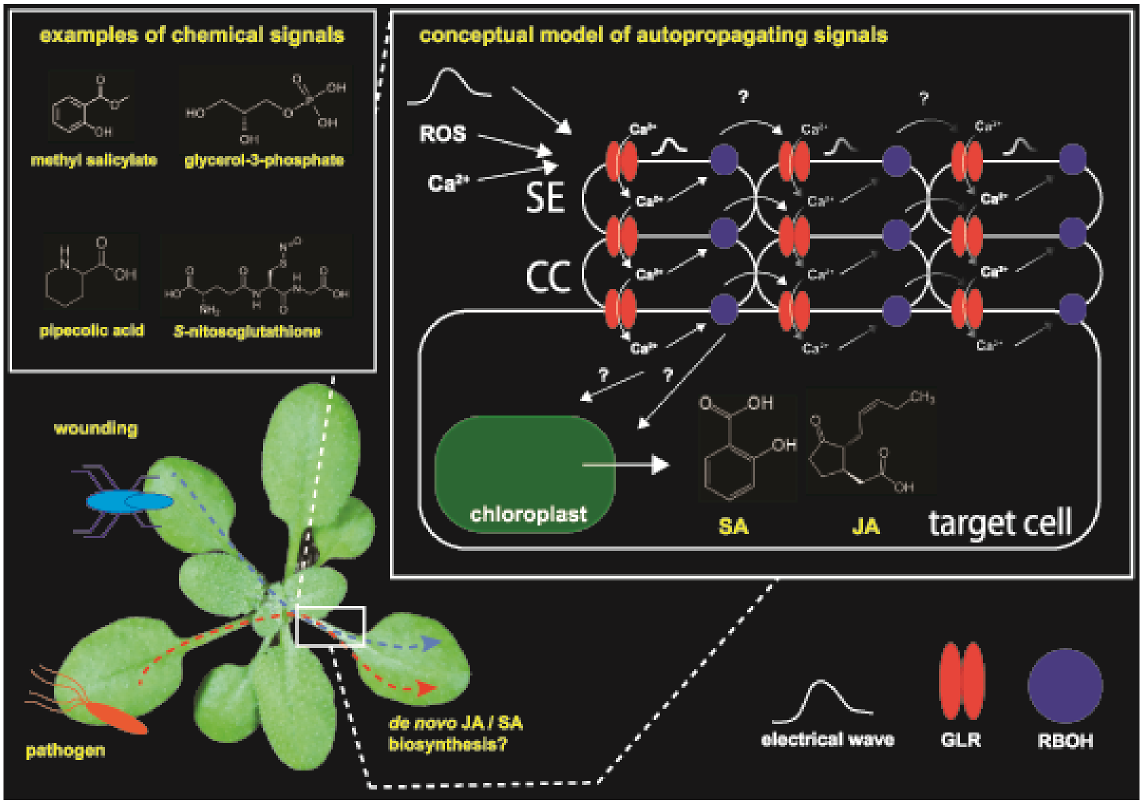

Figure 1. Local stresses such as leaf wounding or pathogen infection can elicit long-distance signals that propagate through the vasculature to affect defense responses in distal leaves. In local leaves, production of a structurally diverse set of chemical signals (e.g. methyl salicylate, G3P, pipecolic acid, *S*-nitrosoglutathione, jasmonic acid, salicylic acid) occurs. These signals can then potentially be: (1) transmitted to distal leaves through the vasculature, or other symplastic transport mechanisms, or (2) trigger downstream long-range signaling networks. Auto-propagating signals (e.g. electrical waves, calcium waves, and ROS waves) can rapidly move to distal leaves to induce systemic response such as *de novo* synthesis of defense compounds (e.g. jasmonic acid for wounding/herbivory and salicylic acid for biotrophic pathogen infection). These waves of information likely work in a self-reinforcing manner, by which membrane depolarization through gating of plasma membrane ion channels may activate further

plasma membrane calcium channels in a feed-forward loop. The GLR family of channels appears important in this network but their precise role(s) remain to be defined. Increased cytosolic calcium can in turn activate RBOHs through binding to the N-terminal EF hands on this enzyme, increasing ROS, leading to further ion channel gating and so wave propagation. A major unanswered question at present, is how far this model can be applied to the conducting cells of the phloem.