Plant Archives Vol. 26, Supplement 1, 2026 pp. 1384-1393 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.183

DECODING PLANT VASCULAR SAP RESPONSES TO APHID FEEDING: A REVIEW

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(Date of Receiving : 14-09-2025; Date of Acceptance : 25-11-2025)

ABSTRACT

Vascular sap has emerged as an important interface in plant aphid interactions, acting as not only a nutrient bank but also as a long-distance conduit for signaling plant defensive responses. In this review we summarize up to date research on how aphid feeding alters the composition of sap (the solution of sugars, amino acids, and other metabolites being transported by the plant) and how plants utilize these networks, including the phloem and xylem, to evoke systemic responses. We discuss the role of reactive oxygen species (ROS) mediated enzymes, and hormone-involved cross-talk, including salicylic acid, jasmonic acid, ethylene, and abscisic acid, as central to networking cascades for plant-mediated defenses. We also show transportation of small RNAs, peptides, and secondary metabolites as messengers of systemic potential, as well as impacted sap profiles via aphid effectors, endophytes, and plant associated viruses or others. Research advances in proteomics, metabolomics, and transcriptomics have changed the way sap can be studied and enabled us to make comparative observations between resistant and susceptible genotypes for important food crops, including *Brassica juncea*, *Arabidopsis thaliana*, wheat and legumes. We also investigated the potential of sap-based biomarkers used for resistance screening, how places for sap-based phenotyping could be established into breeding programs, and strategies could be employed to re-engineer phloem composition using biotechnology to engender durable aphid resistance. Overall, vascular sap appears to be a both the theatre of plant aphid conflict and a promising frontier for sustainable pest management.

***Keywords*: Plant Vascular Sap, Aphid Feeding.**

Introduction

Vascular sap is an important resource for plant physiology because it integrates nutrient transport, osmoregulation, and long-distance organ communication. Phloem and xylem sap transports the bulk of mass flow of photosynthates, mineral ions, amino acids, and a wide pool of small metabolites, which determine sink strength and nutritional status; therefore, the sap composition has strong impacts on growth, development, and herbivore performance (Dinant, 2010; Broussard, 2023). In addition to nutrition, vascular fluids also contain proteins, peptides, hormones, and mobile RNAs, which act in systemic signaling during development and stresses; hence sap composition analysis provides direct insight into metabolic status and whole-plant signaling networks (Carella *et al*., 2016; Dinant, 2010). As an immediate medium of interaction for phloem feeding insects, aspects of the biochemical alterations to sap composition reflect host plant responses to attack, and shape aphid behaviour, fecundity, and population dynamics, rendering vascular sap an important biological and agronomic avenue of inquiry in aphid plant interactions.

Phloem and xylem are distinct in their primary transport responsibilities but together compose the vascular "highway" for long distance signaling. The major role of phloem is to translocate photo assimilates (sucrose along with other transport sugars), amino acids, and an array of signaling molecules (phytohormones, small RNAs, and peptides), thus providing a channel for the redistribution of nutrients and systemic defense signals, xylem primarily translocates water and mineral nutrients, but also provides channels for the translocation of drought and root derived signals that affect shoot physiology (Notaguchi and Okamoto, 2015; Ham and Lucas, 2017). Modern metabolomics and proteomics studies have demonstrated that both phloem sap and xylem sap have a complex and dynamic chemical composition, including stress-responsive proteins and metabolites that can change rapidly in response to biotic challenge (Lohaus, 2022; Carella *et al*., 2016). Notably, many signaling molecules are phloem mobile (i.e. small RNA's, peptides, and hormone conjugates) and disturbances to sap composition due to aphid feeding could carry systemic defense or susceptibility cues through the plant (Ham and Lucas, 2017; Notaguchi and Okamoto, 2015).

Aphids are just one of the most vital phloem-feeding insects, and they can cause significant yield losses to cereals, legumes, Brassicaceae, and many other crops around the world. In aphids, specialized stylets allow them direct access to the phloem sap, and in return, they remove nutrient rich phloem sap (Douglas, 2006; Powell *et al*., 2006). Since the amount and number of wounds caused by aphids is minimal, they are very effective as feeders. In addition to sap removal, aphids additionally imposed salivary secretions into the plant that contained proteins and effectors to disrupt the cellular and metabolic processes, favouring sustained feeding (Hogenhout & Bos, 2011). The economic importance of aphids is further underscored by the numerous plant viruses they can transmit, facilitating major disease epidemics in numerous major cropping systems (Ng & Falk, 2006). Our knowledge of identifying resistance and susceptibility mechanisms remain incomplete, and while aphids are exclusively dependent on phloem sap for nutrition, our understanding of if and how plants modulate the chemical and signaling properties of sap as a function of infestation is critical to revealing these mechanisms.

Following the aphid's interaction with vascular sap, it is the first biochemical interface that aphids experience with their host, that will change continuously and that strongly influences aphid performance, plant defenses and downstream systemic signaling. Any change to the chemical composition of vascular sap (sugar, amino acid, phytohormones, proteins, or defence metabolites) signifies local metabolic reprogramming and communiques the potential for some long distance communication and whole plant response (Dinant *et al*., 2010; Carella *et al*., 2016). Typically, if sap related responses can be decoded, it could provide important information's about biochemical markers associated resistance traits, thus uncoupling the molecular cross talk potentially promoting host resistance against pest influences, but equally showing how aphids exploit plant physiological activity (Louis & Shah, 2013). A similar rationale of applied sap analysis for developing durable resistance strategies could be channelled during breeding for modified sap composition, through metabolic engineering programs, or in integrated pest management systems. Here, sap analysis represents a diagnostic view for understanding plant defence and a way of systemizing preventative approaches for sustainable aphid control.

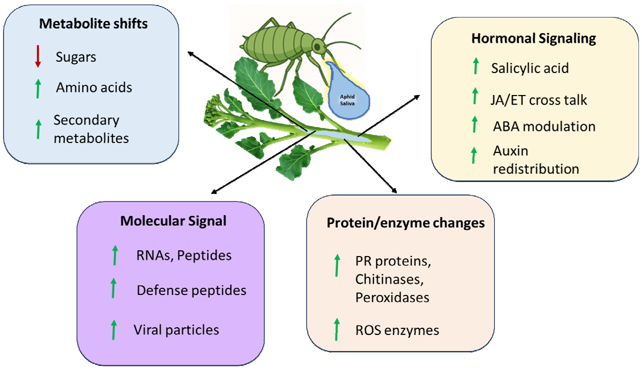

Fig. 1 : Dynamic Changes in Plant Vascular Sap During Aphid Saliva Release.

Aphid Feeding Mechanisms and Plant Invasion

Aphid Stylet Penetration and EPG Studies

Aphids employ long stylets, which enable them to move intercellularly through plant tissues until they arrive at the sieve elements of the phloem, where they feed most readily. This process is done stealthily, and it causes minimal mechanical damage, facilitated by the combination of repeated puncturing and testing of surrounding cells (Tjallingii & Hogen Esch, 1993). Tjallingii (2006) and Walker (2000) were the first to utilize Electrical Penetration Graph (EPG) technology to observe this feeding behaviour and found each wave form corresponded to period of stylet pathway actions, namely puncturing cells, phloem salivating, and prolonged. These studies help demonstrate how aphids explore their surroundings by probing while quickly transitioning into a prolonged feeding period. This action implies evolutionary efficiency.

Saliva Secretion and Interaction with Phloem Elements

Aphids produce two different types of saliva when they feed, one is gelling saliva, which forms a sheath around the stylet, and watery saliva which is injected into sieve elements (Will *et al*., 2007). Watery saliva contains a mixture of proteins and effectors that can suppress plant defenses, regulate calcium influx, and sustain phloem conductivity (Mutti *et al*., 2006; Hogenhout & Bos, 2011). In abundant amounts, watery saliva can coat the sieve pores of the sieve plates, occlude the sieve plates and prevent the flow of nutrients, using saliva to continue feeding before occlusion occurs. Furthermore, some salivary proteins are recognized by host plants as elicitors of defense responses; thus, aphid saliva can serve for both feeding and immunity (Rodriguez & Bos, 2013).

Key Differences between Resistant and Susceptible Plants in Sap-Level Responses

The composition of sap and the associated defensive responses following aphid feeding can differ substantially between resistant and susceptible host plants. In resistant plants, sap boring as a result of stylet insertion usually initiates defense reactions including sieve element occlusion and deposition of callose, as well as sap concentrations of detention secondary metabolites (Kempema *et al*., 2007; Louis *et al*., 2012). These responses could either reduce the efficiency of aphids feeding or fecundity. They often exhibit shorter and limited sap ingestion in susceptible plants; such as changes in the sugar/amino acid ratio associated with reduced concentration of defensive compounds and cause the sap to be advantageous (Guo *et al*., 2020; Walling *et al*., 2008). Composing the differences in resistant/susceptible responses, aphid success or doom seems to be solely based on colonization and manipulation of phloem sap qualities as well as avoiding or disarming plant defenses, demonstrating that responses at the sap level could be contributing factors to resistance (and performance).

Vascular Sap as a Window into Plant-Aphid Interactions

Methods of Sap Collection

Accessing vascular sap for biochemical and molecular analyses has long posed a methodological challenge as it is low in abundance and easily contaminated. Many methods have developed to provide vascular sap access. EDTA-facilitated exudation utilizes chelation of calcium to prevent callose from blocking the sieve-plates, allowing sap to flow from excised tissues, but sap will be contaminated by surrounding cells (King & Zeevaart, 1974; Gururani *et al*., 2015). Aphid stylectomy, where the stylets of feeding aphids are severed by microcautery of high frequency, provides one method for direct access to pure phloem sap, however, it is highly technical, and produces only trace volumes (Fisher & Frame, 1984; Tjallingii, 2006). Centrifugation methods utilize the gentle force of centrifugal separation to collect exudates from cut tissues while pressure chambers use positive pressure to excised petioles or roots to access sap; both methods will not influence or lose (as much) exudate but you do risk contamination by xylem or apoplastic fluids (Hijaz & Killiny, 2014). Using any method, you are working with a trade-off among contamination, yield and ease of conducting the experiment.

Analytical Tools

New technology has greatly enhanced our capability to profile the composition of sap. High-performance liquid chromatography (HPLC) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) have made possible the quantitative identification of sugars, amino acids, and secondary metabolites (Zhang and Turgeon, 2018). Nuclear magnetic resonance (NMR) spectroscopy can also identify many compounds quickly, non-targeted, and through a largely non-invasive approach without derivatization (Krishnan *et al*., 2005). Metabolite analyses are using several joined omics strategies including LC-MS, gas chromatography-MS, and NMR to map out the changing metabolome during aphid feeding (Will *et al*., 201). Proteomic approaches using 2D electrophoresis, MALDI-TOF, and shotgun LC-MS/MS are used to identify stress proteins, enzymes, and defense peptides that are dynamic in phloem sap during aphid infestation (Carella *et al*., 2016; Kehr, 2013). The technology described here has given us unprecedented insight into how sap can be treated as a dynamic biochemical interface describing plant–aphid interaction.

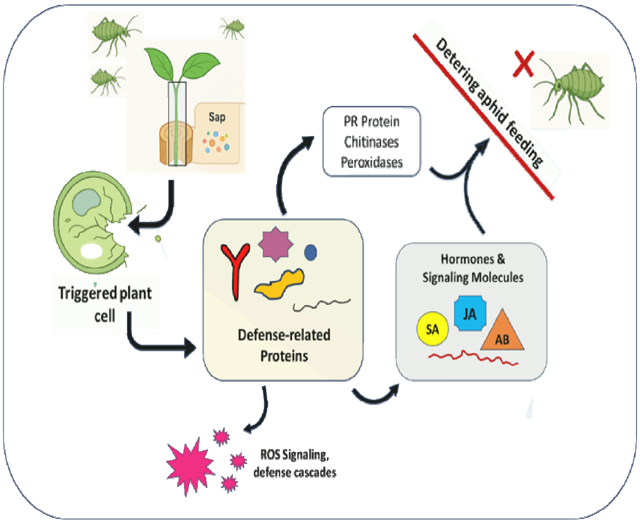

Fig. 2 : Activated plant defense responses to aphid feeding.

Advantages and Limitations of Sap Analysis

Sap analysis's primary advantage is that it offers a direct biochemical representation of the resource and signals sequestered for aphids when feeding, linking sap composition change to insect performance and plant resistance characteristics. Sap analysis is useful due to its delivery of systemic defenses signals, but is an ideal matrix to study long-distance signaling by plants under stress (Dinant *et al*., 2010). On the other hand, methodological limitations, including the small sample volume (extremely small), potential for contamination, and difficulty isolating cell type specific contribution, compromise interpretation. Additionally, the sap composition could reflect dynamic alterations due to biochemical processes, thus temporal parameters need to be designed carefully, such that a meaningful biological variation is examined (Hijaz & Killiny, 2014). Irrespective of these shortcomings, sap analysis is still a vital tool for dissecting plant-aphid interactions, especially when combined simultaneously with transcriptomic data and/or physiological parameters.

Metabolite-Level Changes in Vascular Sap after Aphid Feeding

Sugars and Osmotic Balance

Sugars are the main energy source for aphids and changes in their levels affect feeding behavior and performance. Sucrose is the main transport sugar in phloem sap and aphid infestation will change levels of sucrose either by hydrolysis into hexoses or with the involvement of the plant via metabolic regulation (Douglas, 2006). Increased levels of glucose and fructose were found in susceptible plants where the aphids are provided assimilable carbon, whereas resistant genotypes appeared to prevent sugar hydrolysis or control phloem loading to limit accessibility of aphids (Ryan *et al*., 2015). These changes will impact not only aphid nutrition, but also change the osmotic balance of phloem sap, impacting the turgor-driven translocation of phloem sap (Dinant *et al*., 2010).

Amino Acid Shifts in Abundance

Amino acid availability governed aphid's growth, because phloem-feeding insects depend on amino acids as their source of nitrogen. Aphid feeding is often associated with increased levels of the amino acid's aspartate, glutamate, proline, and serine that correlate to increased aphid fecundity (Sandström *et al*., 2000). In addition to changing levels of free amino acids in the phloem, resistant host plants can also restrict levels of essential amino acids (e.g., methionine and lysine), and the ratios of different amino acids, potentially mediating nutritional quality of phloem sap as a whole (Karley *et al*., 2002). Levels of proline, which are typically elevated in stressed plants, further demonstrate how complex these interactions can be since proline acts as both an osmoprotectant for stressed plants and an enhancer of aphid performance (Ghosh *et al*., 2022). These findings collectively, suggest that the dynamics of amino acids in phloem sap represent a sensitive metabolic response where aphid success is influenced by host genotype, range and combinations of amino acids present in sap, and other stressors affecting plant metabolism.

Organic Acids and Secondary Metabolites

In addition to sugars and amino acids, phloem sap contains organic acids (e.g. malate, citrate) which can help regulate pH and fulfill a variety of metabolic signaling functions during aphid infestation (Hijaz & Killiny, 2014). Secondary metabolites can also be modified to dramatic degrees. In the case of members of the Brassicaceae family of plants, glucosinolates represent an important class of defense and they can saturate phloem sap as a result of aphid feeding, albeit many aphid species have subsequently developed detoxification related strategies (Kim & Jander, 2007). Phenolics and alkaloids can similarly obtain levels in phloem sap as a response to feeding. Phenolics are thought to be deterrents exudates and alkaloids have been categorized as anti-nutritive (Will *et al*., 2013). These chemical defenses provide resistance by modifying aphid feeding rates, stylet penetration success, or survivability. The metabolite composition of vascular sap can thus be thought of as both nutritional pallet and chemical battleground, in which plant and aphid are actively interacting with one another.

Protein and Enzyme Dynamics in Sap

Vascular sap is not merely a transport system for nutrients and metabolites, but a flow of active materials containing various proteins and enzymes that coordinate a plant's defense against aphids. The plant defense proteins, in combination with aphid secreted effectors, dictate the outcomes of the relationship. For example, Pathogenesis-related (PR) proteins such as chitinases, β-1,3-glucanases, or thaumatin-like proteins are often loaded into the phloem sap by aphids. PR proteins can degrade pathogen- or insect-derived cell wall structures directly, or by acting as a signal for systemic acquired resistance (SAR) (Carella *et al*., 2016; Kehr, 2006). Oxidative enzymes like peroxidases and polyphenol oxidases can induce oxidative damage to the plant cell wall, and promote accumulation of antimicrobial secondary metabolites to conceptualise biochemical barriers that decrease the fluency of aphid stylet penetration and feeding (Gaupels & Ghirardo, 2012).

ROS signaling and defense cascades: Reactive oxygen species (ROS) play an essential role in early defense signaling. Enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) are generally found in vascular sap and assist in attaining ROS homeostasis. A rapid increase in ROS in plants can provide a systemic alert signal to activate downstream defenses. However, uncontrolled accumulation can lead to oxidative damage during feeding that can be used by aphids to weaken the plant tissue (Foyer & Shigeoka, 2011; Dafoe *et al.*, 2009).

Aphid-induced effectors: Some of these defense proteins are countered by aphids using salivary effectors secreted into the phloem sap. Some well characterized effectors, including Mp10 and Mp55 from *Myzus persicae*, target relevant ROS regulating enzymes or restrict accumulation of specific PR proteins to inhibit the signaling by plant defense processes (Bos *et al.*, 2010; Rodriguez & Bos, 2013). This molecular suppression facilitates prolonged phloem sap ingestion and successful colonization. The arms race that is occurring between defense proteins and effectors illustrates the dynamic nature of sap proteomes while herbivores feed.

Hormonal Signals in Vascular Sap During Aphid Infestation

Phytohormones moving in vascular sap are critical to how plants respond to aphid feeding. The phloem functions to not only transport assimilates but to also serve as a long-distance signaling system of hormones responsible for coordinating local and systemic defenses.

SA-JA-ethylene cross-talk: Salicylic acid (SA), jasmonic acid (JA), and ethylene work in a tightly regulated, interplaying signaling triad to coordinate plant responses to aphid infestation. Typically, aphids induce SA-dependent pathways for Induced Defenses that are associated with biotrophic organisms, and affected JA signaling pathways that are characterized as Induced Defenses of necrotrophic pathogens and chewing herbivores (Morkunas *et al.*, 2011; Walling, 2008). Moreover, ethylene will often act in synergy and antagonistically with SA and JA, redirecting outputs dependent on the plant and aphid species (Thompson & Goggin, 2006). This hormonal cross-talk enables the plant to fulfill the energy demands of growth vs. defense, while avoiding excess damage.

Role of ABA and auxins: Abscisic acid (ABA), a well-established signaling molecule for drought and other abiotic stresses, is thought to newly serve as an inhibitor of aphid-plant interactions. High concentrations of ABA have been noted to diminish the JA dependent defenses and allow for aphid colonization (Hillwig *et al.*, 2016). While ABA accumulates in the vascular sap and may persist in xylem and phloem sap, it also helps to promote callose deposition in sieve plates and inhibit phloem, which may limit aphid feeding but phloem feeding, as JA promotes, will still have to take place. Auxins also can be targeted by aphids to alter sink strength in order to redirect resources to facilitate feeding (Giron *et al.*, 2007). Thus, both auxins and ABA manipulate the capability of plants to respond and leverage defense responses, while the aphids demonstrate the full capability to alter the plant's defenses and degree of susceptibility to herbivore attack.

Transport of phytohormones as systemic signals: Phytohormones are systemic in their distribution, moving through phloem sap and providing rapid transport of messages between local feeding sites and distant tissues. There is evidence of the presence of hormonal signals, such as SA and JA, in phloem exudates (Carella *et al.* 2016) and it has been established that SA and JA act as signals to enhance resistance via systemic acquired resistance (SAR) and induced systemic resistance (ISR). The systemic transport of phytohormones further allows the plant to coordinate a whole plant defense against aphids based on the local recognition events that link systemic phytohormone transport with reprogramming of metabolism and the expression of defense related genes. In conclusion, hormonal signaling in vascular sap is a central layer of regulation during the course of the plant-aphid interaction in which the balance between induction and suppression of defense signals ultimately dictates whether a plant resists or succumbs to infestation.

Molecular Signatures in Sap

Vascular sap is composed of a range of molecular messengers, such as RNAs, peptides, and small metabolites that act as the systemic regulators of plant defense and physiology. These mobile signatures are dynamically changed upon aphid infestation, acting as defense signals for the plant and potential targets to aphids and microbes.

RNAs, peptides, and small molecules as systemic defense messengers, it’s an assortment of messenger RNAs (mRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs) and phloem-mobile peptides represent a wide range of possibilities for RNA and peptide trafficking to function in long-distance signaling (Kehr & Kragler, 2018). Signaling peptides (CLE and CEP families) and secondary metabolites (such as phenolics and glucosinolates) act as systemic cues by signaling distal tissues about the local aphid attack to successfully initiate and activate systemic defense (Turnbull & Lopez-Cobollo, 2013). These molecules provide signals but are also a part of systemic reprogramming of metabolism, stiffening cell walls, and activation of transcriptional defense networks. Aphid induced changes in phloem-mobile transcripts, such as the transcription profile of phloem sap is altered by aphid feeding, with significant enrichment or depletion of transcripts associated with defense, hormone signaling, and secondary metabolism (Louis *et al.*, 2014). For instance, miRNAs influenced by auxin and jasmonic acid pathways showed a change in abundance during infestation, effectively modifying hormonal balance towards either resistance or susceptibility (Kettles & Kaloshian, 2016). These changes reflect an ongoing molecular conversation, where the plant is attempting to activate resistance mechanisms, and aphids are counteracting it by inhibiting or redirecting systemic signaling.

Plant, microbe & aphid interaction at the sap level: Vascular sap also serves as an ecological interface where plant-microbe-aphid (three part) interactions merge. Endophytic microbes play a role in altering sap chemistry; sometimes contributing to the sap's resistance against aphids through induced systemic resistance (ISR) (Zamioudis & Pieterse, 2012). Aphids are also vectors of viruses, which take advantage of their phloem mobility to move through the vascular system and spread throughout the plant, and they can thus alter sap and plant composition while lowering plant defenses to promote viral replication, and aphid performance (Mauck *et al.*, 2012). This three-part interaction illustrates the complexity of identifying and interpreting sap mediated molecular signatures that occur in exchange and modulation of the systemic cues provided by the plant, aphids, and microbes.

Long-Term Perspectives up to date

Over the past thirty years, the study of vascular sap has evolved from simple biochemical methods into integrative omics-based approaches, providing new insights into plant aphid interactions. Early studies focused on measuring sugars, amino acids, and proteins in phloem sap to define food availability and aphid feeding preferences (Kock *et al.*, 2004). Biochemical descriptions laid the groundwork for the discovery of sap as a signal, not simply a food source. More recently, using approaches including high throughput proteomics, metabolomics, and transcriptomics, studies are now able to describe the molecular diversity of sap under stress and track the systemic defense pathways it perturbs (Kehr, 2013; Carella *et al.*, 2016). Comparative studies with resistant vs. susceptible genotypes will helps to catch the exact scenario, one major trend that has come to light has been the stark contrast in the composition of sap from resistant plants and sap from the susceptible plants. Resistant genetic lines frequently indicate higher amounts of PR proteins and other defense proteins (i.e. peroxidases), secondary metabolites (i.e. glucosinolates in Brassicaceae; phenolics in legumes), and stress-related RNA in sap than their susceptible counterparts (Louis & Shah, 2013). While susceptible, investigated lines exhibit phloem profiles enriched with nutrients and suppression of defense signaling, favoring the proliferation of aphids. These comparisons illustrate the value of sap analysis in predictive abilities in screening and breeding programs for insect resistance. Studies among diverse crops**,** sap profiling research in *Arabidopsis thaliana* demonstrated that salicylic acid and phloem-mobile RNAs are involved in systemic acquired resistance to *Myzus persicae* (Thompson & Goggin, 2006). Metabolomic and proteomic studies of Brassica crops such as mustard and canola reported changes to glucosinolates and antioxident enzymes in response to aphid feeding, related to the level of resistance specific to the genotype (Hao *et al.*, 2020). For wheat, sap studies indicated changes in amino acid composition following attacking by the Russian wheat aphid (*Diuraphis noxia*), and revealed how resistant cultivars can restrict aphid performance by altering the phloem nutritional status (Rojas *et al.*, 2020). In legumes, comparative sap work on pea and soybean, revealed that plant hormones and defense proteins are contributing to limiting establishment of pea aphid (*Acyrthosiphon pisum*) populations (Drzewiecka *et al.*, 2014).

Overall past studies of vascular sap research have shown a big paradigm shift that we have moved from a purely nutrient-based view of sap to appreciating it as an active systemic integration point of defense proteins, metabolites, RNAs, and hormonal signals. As omics technologies continue to be developed, single-cell phloem and combined ecological studies become commonplace, the potential to improve our understanding of how plants engage aphids and speed up the breeding of resistance into diverse crop systems will be valuable for sustainable systems.

Implications for Crop Protection and Breeding

The examination of vascular sap composition as a result of aphid infestation reveals some potential concepts for more sustainable pest management methods, and could help lead to breeding for resistance strategies. Because sap is the main feeding site for phloem-feeding aphids, it's biochemical and molecular properties has potential as useful indicators of a plant's defense status and resistance potential. In sap markers for resistance screening**,** there is a fairly consistent pattern across comparative studies between resistant and susceptible genotypes that suggest that resistant plants exhibit higher levels of sap profile measures such as: 1) pathogenesis-related (PR) proteins; 2) peroxidases; 3) glucosinolates; 4) phenolics; and 5) altered amino acid ratios (Hao *et al.*, 2020; Louis & Shah, 2013). Importantly, it has also been suggested that other phloem mobile RNAs and defense-related phytohormones including salicylic acid present in plant sap may be exploited as biomarkers for early detection of aphid resistance (Kehr, 2013). These sap markers can be used in high-throughput phenotyping platforms to tap into a large number of resistant germplasm (genetic material) for breeding programs.

Integrating sap analysis into breeding programs: In contemporary breeding methods, molecular and biochemical markers are frequently used as selection aids to accelerate selection. Sap-based assays (e.g., proteomics, metabolomics and phloem hormones) could be incorporated into breeding frameworks across breeding pipelines as an addition to phenotypic evaluations screen for aphid resistance. Resistant genotypes identified by using sap markers may be advanced in measures of marker-assisted selection (MAS) and genomic selection and reduces the identification of resistant genotypes based only on field screening. (Rojas *et al.*, 2020).

Biotechnological prospects: Biotechnology offers new avenues for phloem engineering, where the composition of the phloem can be engineered to deter feeding by aphids. Transgenic strategies that increase the expression of PR proteins, alter phloem amino acid profiles, and increase glucosinolates have demonstrated some success at reducing aphid performance (Zhu-Salzman & Zeng, 2015). In addition, RNA interference (RNAi) tools can silence aphid effectors delivered into the phloem, and restore host protein activity of defense proteins (Pitino *et al.*, 2011). As RNAi and other gene silencing systems match with CRISPR-based gene editing and sap omics, designing crop types with modified phloem profiles for aphid resistance could become a reality. In conclusion, vascular sap offers a promising venue for breeding disease-resistant aphids. The identification of validated sap resistance markers and the development of bio tools lead to crop varieties for aphid resistance with durable and broad-spectrum resistance which will lessen the need for chemical pesticides while promoting sustainable agriculture.

Future Directions

While there is considerable understanding of the vascular sap responses to aphid feeding, a wide range of possibilities exist to further understand these responses, as well as utilize this knowledge to protect crops. Future studies should implement integrated multi-omics approaches that can encompass sap metabolomics, transcriptomics, proteomics, and small RNA profiling. This systems level approach would create a broader scope of how defense pathways, nutrient and signaling molecules are all impacted during the unfurling of aphid infestation (Carella *et al.*, 2016; Kehr & Kragler, 2018). This integration may additionally develop stronger and more consistent biomarkers of resistance across crops and environments. Present methods predominantly rely only on endpoint sampling of exudates and do not produce sufficient temporal resolution. The development of microfluidic devices, in vivo biosensors, and non-invasive imaging may help provide better, real-time observation of sap's composition during aphid feeding (Vanbel *et al.*, 2013). These technologies will allow for a better understanding of the temporal shifts of hormones, metabolites, and RNAs and this timing will elucidate the regulation of defense when activated. The influence of climate change - regarding increased CO_2, higher temperatures and drought stress - on sap composition and aphid-plant interactions remains poorly understood. Changes in the phloem sugar-to-amino acid ratios, changes in hormone signaling and changes in plant secondary metabolism due to climate change may all have an impact on aphid performance and the durability of resistance (Sun *et al.*, 2018). Understanding these aspects is essential for breeding crops with climate-resilient pest resistance.

Knowledge gaps

There are definite gaps remaining in relating sap molecular changes to whole-plant and ecological outcomes. For instance, how do specific sap-mobile RNAs or peptides induce systemic defense gene expression? While beneficial endophytes or microbiomes may alter the chemistry of sap in ways that would enhance resistance, to what degree is this possible? There are also opportunities for more comparative studies of resistance and susceptibility across genotypes of crops in order to later define/generate universal vs. species-specific defense strategies. We will need an interdisciplinary approach with more breakthroughs in using sap as a pathway for better understanding plant–aphid interactions, and emerging as an exciting platform for sustainable crop protection strategies.

Conclusion

Vascular sap describes a constantly evolving battlefield where the opposing strategies of plants and aphids play out. Phloem and xylem sap are both the avenues of most nutrients, hormones, proteins, and mobile RNAs and act as the site of communication and action against aphid colonization. While sap transports a plethora of defense proteins, signaling molecules, and hormones, aphids are able to circumvent this by injecting their own salivary effectors to change the chemistry of the sap and ultimately suppress host immunity. The molecular exchange and interaction of vascular sap during aphid feeding represents more than a mere transit mechanism, but an active environment of constant exchanges of molecules where resistance or susceptibility is determined. The increasing integration of a wide variety of new concepts and approaches, including proteomics, metabolomics, and transcriptomics, has characterized sap as a source rich in diagnostic markers of plant defensive status. These concepts are important for the agriculture sector because sap analysis adds new options into pest management practices that are sustainable and may ultimately foster sustainable pest strategies. Plant breeders gain the opportunity to evaluate molecular markers for tolerance, more efficiently screen additional germplasm, move sap measurements into their selection pipelines, and even modify phloem components of the plant to improve tolerance against aphids. Sap-based strategies (in addition to supporting breeding) may support biotechnology applications, ecological insect pest management, and climate resilience pest management strategies to protect crops. Ultimately, using vascular sap as an avenue to understanding plant–aphid interactions and as a breeding trait in applied agricultural research provides numerous opportunities to decrease pesticide use, increase resilience of crops, and develop sustainable agricultural practices to address declining climate resilience and increasingly worse pest pressures.

Author Contributions

Ranjithkumar V, first author and corresponding author, contributed to collection of reviews and manuscript writing, conceptual idea, writing part of the manuscript and manuscript correction; Ajithkumar V contributed to writing part of the manuscript; Gayathri G contributed to manuscript correction and English language use

Declarations (Conflict of interest): The authors declare that they have no competing interests or other interests that might be perceived to influence the results and conclusions reported in this paper. Also, there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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