The role of early signaling events in plant-insect interactions

Running title: early events in plant-insect interactions

Simon A. Zebelo[a] and Massimo E. Maffei*[b]

[a] Department of Entomology and Plant Pathology, Auburn University, 301 Funchess Hall, Auburn 36849, Alabama, USA.

[b] Department of Life Sciences and Systems Biology, Innovation Centre, University of Turin, Via Quarello 15/A, Turin 10135, Italy.

Simon A. Zebelo (saz0002@auburn.edu)

Massimo E. Maffei (massimo.maffei@unito.it)

*Corresponding author: Massimo E. Maffei, Tel: +390116705967; Fax: +390112365967

Date of submission: 30/09/2014

Number of Figures: 3

Number of Tables: 0

Total world count: 7803

Abstract.

The response of plants to the stress caused by herbivores involves several different defense mechanisms. These responses begin at the plant cell plasma membrane, where insect herbivores interact physically by causing mechanical damage and chemically by introducing elicitors or triggering plant-derived signaling molecules. The earliest plant responses to herbivore contact are represented by ion flux unbalances generated in the plant cell plasma membrane at the damaged site. Differences in the charge distribution generate plasma transmembrane potential (Vm) variation, the first event that eventually leads to the initiation of signal transduction pathways and gene expression. Calcium signaling and the generation of reactive oxygen and nitrogen species are early events closely related to Vm variations. This review provides an update on recent developments and advances in plant early signaling in response to herbivory, with particular emphasis on the electrophysiological variations of the plasma membrane potential, calcium signaling, cation channel activity, the production of reactive oxygen and nitrogen species, and the formation of a systemically moving signal from wounded tissues. The roles of calcium dependent protein kinases and calcineurin signaling are also discussed.

Key words Biotic stress, Calcium signaling, Plant electrophysiology, Reactive oxygen and nitrogen species, Signal transduction, Transmembrane potential (Vm).

Introduction

Over the course of millions of years of evolution, plants have developed defensive strategies against herbivorous insects that have led to myriad molecular interactions at both the genomic and metabolic levels. The ability for an organism to survive depends on its ability to respond quickly and efficiently to external stimuli and to develop effective and sustainable defenses.

These defenses are intended to directly protect the plant with toxic compounds (or using mechanical defense structures such as thorns and glandular trichomes) or to indirectly protect it through molecular interactions that may attract predators or parasitoids of the herbivorous attacker (Wu and Baldwin, 2010; Baldwin, 2010). Plants have evolved means to recognize and respond quickly to herbivory. These means include the perception of molecular patterns and defense effectors (Bos *et al.*, 2010; Bonaventure *et al.*, 2011; Maffei *et al.*, 2012), the elevation of cytosolic calcium ($[Ca^{2+}]_{cyt}$) (Reddy *et al.*, 2011), the depolarization of the plasma transmembrane potential (Vm) (Bricchi *et al.*, 2010), ion efflux/influx (Bricchi *et al.*, 2013), mitogen-activated protein kinase (MAPK) activation and protein phosphorylation (Arimura and Maffei, 2010; Arimura *et al.*, 2011), the activation of NADPH oxidase, and the production of reactive oxygen (ROS) and nitrogen (RNS) species (Miller and Mittler, 2006; Bricchi *et al.*, 2010; Arimura *et al.*, 2011; Marino *et al.*, 2012). These cascades lead to a rise in the production of phytohormones jasmonic acid (JA) and salicylic acid (SA) (Zipfel, 2009; Consales *et al.*, 2012; Erb *et al.*, 2012; Bricchi *et al.*, 2013), an increase in the production of ethylene (Arimura *et al.*, 2009; Onkokesung *et al.*, 2010; Diaz, 2011; Scala *et al.*, 2013), the expression of late defense response genes involved in the emission of volatile organic compounds (VOCs) (Wu and Baldwin, 2010; Baldwin, 2010; Maffei *et al.*, 2011; Karban *et al.*, 2011) and the production of toxic compounds (Karban, 2010). These events start locally at the feeding site but can spread systemically throughout the plant (Wu and Baldwin, 2009; Maffei, 2010; Bricchi *et al.*, 2012). Although the individual responses that comprise these pathways have been widely catalogued, the connections between them and their interdependence have received little research attention to date.

Plant responses to herbivory have been previously reviewed both prior to gene expression changes (Ebel and Mithöfer, 1998; Garcia-Brugger *et al.*, 2006; Maffei *et al.*, 2007; Zipfel, 2009; Arimura *et al.*, 2011) and after gene expression changes (Howe and Jander, 2008; Mithöfer *et al.*, 2009a; Wu and Baldwin, 2010; Baldwin, 2010; Bonaventure *et al.*, 2011; Bonaventure, 2012; Pearse and Karban, 2013). The aim of this review is to provide an update on recent developments and advances in our understanding of early plant signaling in response to herbivory, with particular emphasis on the electrophysiological variations of the plasma membrane potential, calcium signaling, cation channel activity, reactive oxygen and nitrogen species production and the presence of a systemically moving signal from the wounded tissues.

Defense in preparation for attack: the sensitivity of the plasma membrane and the role of symplastic signaling

The use of electrophysiology in the study of plant cells has witnessed a slow and steady increase for a number of purposes in recent years (Ochatt, 2013). Electrical signals are known to travel along the plant at different rates, carrying different messages (Gurovich and Hermosilla, 2009; Volkov *et al.*, 2010; Oyarce and Gurovich, 2011; Volkov, 2012; Volkov *et al.*, 2013). Vm depolarization is correlated to increases in the cytosolic calcium ion levels, ion channel activity and ROS and RNS bursts. All these events occur seconds to minutes after herbivory and are among the earliest plant defense responses.

The plasma membrane recognizes changes in the environment surrounding the cell, starting a cascade of electric signaling that eventually results in specific responses. Leaf damage by insect herbivores implies the direct delivery of elicitors or the indirect generation of plant cell wall-derived elicitors that may bind specific receptors at the plant plasma membrane. Emerging

evidence indicates that many high-affinity receptors for insect herbivores are located in the plant cell plasma membrane (Maffei *et al.,* 2012). The elicitor-receptor reaction produces variations in the Vm, which is defined as the difference in the electrochemical gradient between the interior and exterior of the plant cell. These variations can lead to either more positive (depolarization) or more negative (hyperpolarization) Vm values, and such events eventually lead to the generation of signaling cascades (Zebelo and Maffei, 2012a). In the *Spodoptera littoralis-Phaseolus lunatus* interaction, both direct herbivory and the insect's oral secretions (OS) have been demonstrated to induce a rapid Vm depolarization (Maffei *et al.,* 2007; Bricchi *et al.,* 2010; Bricchi *et al.,* 2012). The same response has been shown in higher plant species like *Arabidopsis thaliana* (Zebelo and Maffei, 2012b; Bricchi *et al.,* 2013) and *Ginkgo biloba* (Mohanta *et al.,* 2012), as well as in lower plant species like the fern *Pteris vittata* (Imbiscuso *et al.,* 2009). Interestingly, a significant Vm depolarization was observed in response to almost every stylet puncture during *Myzus persicae* phloem feeding (Bricchi *et al.,* 2012).

It is known that systemic signaling induced by biotic stressors is transduced by either chemical or electrical signals (Masi *et al.,* 2009; Zimmermann *et al.,* 2009; Zebelo *et al.,* 2012; Zebelo and Maffei, 2012b; Baluska and Mancuso, 2013; Salvador-Recatala *et al.,* 2014; Mousavi *et al.,* 2014).

OS from some insect herbivores contain effectors that overcome antiherbivore defenses. Herbivores possess diverse microbes in their digestive systems and salivary glands that can modify plant-insect interactions. For example, Colorado potato beetle (*Leptinotarsa decemlineata*) larvae exploit bacteria in their OS to suppress the anti-herbivore defenses of tomato plants (*Solanum lycopersicum*) (Chung *et al.,* 2013). Furthermore, applying bacteria isolated from larval OS to wounded plants confirmed that microbial symbionts are responsible

for this defense suppression. A further demonstration that salivary components are necessary to trigger plant responses to herbivore larvae has been recently provided. The ablation of the ventral eversible gland (VEG) of *S. littoralis* prompted a significant reduction in the Vm depolarization and significantly reduced both the cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) and the H_2O_2 burst (Zebelo and Maffei, 2012b). Moreover, VEG-ablated larvae induced a reduced defense-related enzyme expression and a reduced emission of plant volatiles (Zebelo *et al.*, 2014).

In general, three mechanisms are recognized for the transmission of electrical signals following herbivory: action potentials (APs), variation potentials (VPs) and system potentials (SPs). The OS of insect herbivores are known to cause both APs and VPs, but it is still unclear whether insect herbivory can cause SPs. SPs have been described as novel electrical long distance signals in plants that are induced by wounding, acting as the forerunners of slower chemical signals (Zimmermann *et al.*, 2009). SPs serve as back up APs and VPs and can remain overlapped with APs and VPs in some instances. Although SPs have been demonstrated only in mechanically damaged tissues, it is difficult to exclude the occurrences of SPs following herbivore feeding (Zebelo and Maffei, 2012a).

APs comprise a generic long distance signaling system that may act to potentiate a host response to subsequent signals delivered through alternative long distance information packages. An AP is a momentary change in the electrical potential of plant cells that sense stimuli from environmental stressors, eventually leading to intercellular and intracellular communication. A number of substances strongly depolarize the plasma membrane and thus presumably activate voltage-gated ion channels. Although in principle it is possible that (anion) channels are directly activated by depolarization, the temporal sequence of the ion flux kinetics of barley leaves shows that Ca^{2+} is lost from the apoplast well before the apoplastic anion concentration (measured as

Cl^-) starts to increase (Felle and Zimmermann, 2007). Therefore, channel activity is involved in APs. The more the channels are activated, the more rapid the depolarization will be, eventually leading to an accelerated depolarization that is measured as a membrane potential 'break-through' typical of an AP. APs generated by herbivory propagate as a fast electrical signals that travel through the entire plant from the point of origin of the perceived input at a speed up to 40 cm sec^{-1} (Volkov, 2012). Zebelo and co-workers demonstrated that herbivore-induced plant volatiles (HIPVs) trigger APs and VPs on nearby receiver plants (Zebelo *et al*., 2012).

It is generally accepted that wounded leaves communicate their damaged status to other leaves through a long-distance process. Using non-invasive electrodes, the surface potential changes in *A. thaliana* were mapped after leaf wounding, and it was found that membrane depolarization is correlated with JA signaling domains in undamaged leaves. Furthermore, of the 34 screened membrane protein mutant lines, the mutations in several clade *3 GLUTAMATE RECEPTOR-LIKE* genes (GLRs 3.2, 3.3 and 3.6) attenuated wound-induced surface potential changes, showing a reduced JA-response gene expression in leaves distal to wounds in a *glr3.3 glr3.6* double mutant (Mousavi *et al*., 2013). These results open new avenues for research in organ-to-organ wound signaling, demonstrating the existence of plant genes with functions related to those important for synaptic activity in animals. An open question remains: how are electrical signals propagated through the plant body? While animals have a nervous system that is specialized in the conduction of electrical signals, nothing similar is present in plants. Central to the success of these defenses is the need for local and systemic communication between cells. For plant cells, which are surrounded by cell walls, symplastic continuity is achieved through the presence of plasmodesmata (PD). These plasma-membrane-lined channels, bridging the cell wall, provide symplastic continuity and provide soluble and membrane environments for the

passage of small and large molecules and the potential for electrical conduction (Maule *et al.*, 2011). PD-located proteins (PDLPs) are type-I membrane proteins with receptor-like properties, although the nature of their potential ligands is not known (Amari *et al.*, 2010). Using *Arabidopsis* plants mutated for *pdlp* genes, Bricchi and co-workers (2013) not only implicated PDs directly for their role in the defense against herbivory but also showed that some molecular responses to herbivory can be genetically distinguished from each other and from the overall defense response. However, although PDs have been correlated with gap junctions, no synaptic mechanisms of molecules are present to justify what has been demonstrated in the animal nervous system. Little is known about the electrophysiological responses of phloem sieve elements in wounding, and whether natural damaging stimuli induce propagating electrical signals in these tissues. Very recently, the use of living aphids and the direct current (DC) version of the electrical penetration graph (EPG) were used to detect changes in the membrane potential of *Arabidopsis* sieve elements (SEs) during caterpillar wounding. Feeding wounds in the lamina induced rapid depolarization waves in the affected leaf, increasing to the maximum amplitude (c. 60 mV) within 2 s. Major damage to the midvein induced fast and slow depolarization waves in unwounded neighbor leaves but only slow depolarization waves in non-neighboring leaves. The slow depolarization waves rose to a maximum amplitude (c. 30 mV) within 14 s. The distal electrical signals elicited by caterpillar cutting are indistinguishable from those elicited by a purely mechanical stimulus, demonstrating that the mechanical aspect of insect chewing is sufficient to induce the full electrophysiological response recorded in the phloem sieve elements of unwounded leaves (Salvador-Recatala *et al.*, 2014). Moreover, no remote electrical signals were recorded from the sieve elements of *glr3.3a glr3.6a* plants, indicating that GLR3.3 and/or GLR3.6 are necessary for the production of both the slow and fast

(action potential-like) remote depolarization waves. Therefore, it is conceivable that the depolarization waves produced by the sieve elements in response to remote wounding contributed to the global wound-activated surface potentials (Salvador-Recatala *et al.*, 2014).

Genetically encoded voltage-sensitive fluorescent proteins (VSFPs) are being used in neurobiology as non-invasive tools to study synchronous electrical activities in specific groups of nerve cells. This "light-based electrophysiology" has been recently adapted for use in plant systems. The production of transgenic plants engineered to express different versions of VSFPs that are targeted to the plasma membrane and internal membranes of root cells has allowed the recording of concurrent changes in the plasma membrane potential in populations of cells and at multiple membrane systems within single cells in response to various stimuli in living plants. Such coordinated electrical changes may globally orchestrate cell behavior to elicit successful reactions of the entire root to varying and unpredictable environments (Matzke and Matzke, 2013).

Another interesting connection to electrical signaling could be the recent discovery that plants are able to discriminate between the vibrations caused by chewing and those caused by wind or insect song. A vibration-signaling pathway would complement the known signaling pathways that rely on volatile, electrical, or phloem-borne signals. It has been suggested that vibrations may represent a new long distance signaling mechanism in plant-insect interactions that might contribute to the systemic induction of chemical defenses (Appel and Cocroft, 2014). The mechanisms used by plants to detect and respond to mechanical vibration have received experimental attention from several groups (Gagliano *et al.*, 2012; Monshausen and Haswell, 2013). Mechanoreception is thought to begin with the triggering of mechanosensors in the cell wall and/or plasma membrane, causing fluxes of calcium, ROS, and H^+. These trigger

downstream responses involving many plant hormones and the rapid expression of genes that respond early to many plant stresses (Appel and Cocroft, 2014). Vm changes that occur following herbivory have also been associated with the same signaling events.

In the next section, we describe recent findings in early calcium signaling and ion concentration variations upon herbivory following Vm depolarization.

Calcium and other ions act as second messengers in plant-insect interactions

The candidate ion species responsible for Vm variations in plant cells following herbivory are calcium (Ca^{+2}), protons (H^{+}), potassium (K^{+}) and chlorine (Cl^{-}). Herbivore feeding causes a dramatic Ca^{2+}cytosolic ion influx limited to a few cell layers lining the wounded zone (Maffei *et al.*, 2004; Howe and Jander, 2008). This response is limited to herbivory or biotrophic activity; neither single nor repeated mechanical wounding induces such significant changes in the cytosolic Ca^{2+} ion influx (Bricchi *et al.*, 2010). The fact that single or repeated mechanical wounding alone is not sufficient to elicit significant $[Ca^{2+}]_{cyt}$ variations points to oral factors [or herbivore-associated elicitors (Bonaventure *et al.*, 2011)] as triggers for a $[Ca^{2+}]_{cyt}$ burst. Insect feeding and isolated insect-derived elicitors are known to cause changes in the Ca^{2+} homeostasis resulting from the tight regulation of protein channels and transporters located in the plasma membrane and organelle membranes (Jammes *et al.*, 2011) and Ca^{2+} sensors (Arimura and Maffei, 2010; Kudla *et al.*, 2010; Batistic and Kudla, 2012). These events have been associated with Vm depolarization (Bricchi *et al.*, 2013). In plants, $[Ca^{2+}]_{cyt}$ is maintained in the nM range (100–200 nM), whereas in many organelles and in the apoplast, $[Ca^{2+}]$ reaches the mM range (Dodd *et al.*, 2010). The dynamics of spatial and temporal Ca^{2+} changes in the cytosol and/or in other compartments of the plant cell are now accepted to generate “calcium signatures”, which

might be responsible for the initiation of specific downstream events that could eventually lead to the appropriate responses (Mithöfer *et al.*, 2009b; Batistic and Kudla, 2012; Short *et al.*, 2012).

Several techniques have been used and developed to localize, measure and monitor $[Ca^{2+}]_{cyt}$ variations. A large number of fluorescent Ca^{2+} indicators are available for studying Ca^{2+} in plant cells (Mithöfer *et al.*, 2009b). The loading of Ca^{2+}-sensitive fluorescent probes into plant cells is an essential step in measuring the activities of cytoplasmic free Ca^{2+} ions with a fluorescent imaging technique. However, barriers to the loading of the test compounds or the Ca^{2+}-sensitive fluorescent dyes could be represented by the low permeability of the cell wall and by a massive cuticle. This would allow the penetration of only a limited number of cell layers, most likely near the infection zone. In addition to bio-luminescent techniques using aequorin, two fluorescent Ca^{2+} indicators have been recently used to successfully demonstrate the induction of Ca^{2+} signatures upon herbivory: Fluo-3 AM (Kanchiswamy *et al.*, 2010) and Calcium Orange™ (Bricchi *et al.*, 2010; Mohanta *et al.*, 2012; Bricchi *et al.*, 2013). Despite their proven efficacy, these two indicators do not allow for a precise quantification of $[Ca^{2+}]_{cyt}$ variations. Another way to fine-tune the Ca^{2+} variations is by using the Yellow Cameleon (YC) Ca^{2+}-sensor (Russell, 2011). Recently, Maffei and co-workers (2014) used a Cameleon YC3.6 reporter protein expressed in *Arabidopsis thaliana* to quantify $[Ca^{2+}]_{cyt}$ variations upon mechanical damage (MD) to leaves, herbivory by 3rd and 5th instar larvae of *Spodoptera littoralis* and *S. littoralis* OS applied to MD. YC3.6 allowed for a clear distinction between MD and herbivory and quantitatively discriminated calcium responses between the two larvae instars (Verrillo *et al.*, 2014). The GFP probes in particular can be targeted to well-defined subcellular locales, enabling high-resolution mapping of these signals within the cell (Swanson *et al.*, 2011).

The development of various Ca^{2+} probes from the past six decades, the improvements that have been developed in this field, the limitations of each probe and important points to consider while planning ideal Ca^{2+} imaging experiments in plant science are all topics that have been recently reviewed (Kanchiswamy *et al.*, 2014).

Calcium sensors

Calcium sensors play a major role in calcium signaling upon herbivory. In the standard model, Ca^{2+}-sensor proteins, such as CaM (calmodulin), detect Ca^{2+} signals and subsequently regulate downstream targets to advance the signal transduction cascade (Du *et al.*, 2011). In *Arabidopsis*, 7 genes encode the 4 CaM isoforms (*CaM1/4*;*CaM2/3/5*;*CaM6*;*CaM7*), which differ only in one to five amino acid residues (Batistic and Kudla, 2012). *Arabidopsis SIGNAL RESPONSIVE1* (*AtSR1* or *CAMTA3*) encodes a calmodulin (CaM)-binding transcription factor involved in the mediation of biotic stress responses (Galon *et al.*, 2008). AtSR1 is an important component of plant resistance to insect herbivory as well as one of only three described proteins involved in Ca^{2+}/CaM-dependent signaling to function in the regulation of glucosinolate metabolism, providing a novel avenue for future investigations into plant–insect interactions (Laluk *et al.*, 2012). Ca^{2+}/CaM-binding is also critical for the AtSR1-mediated herbivore-induced wound response. Interestingly, *atsr1* mutant plants are more susceptible to herbivore attack than wild-type plants. The complementation of *atsr1* mutant plants by overexpressing the wild-type AtSR1 protein can effectively restore plant resistance to herbivore attack. However, when mutants of AtSR1 with impaired CaM-binding ability were overexpressed in *atsr1* mutant plants, plant resistance to herbivore attack was not restored, suggesting a key role for Ca^{2+}/CaM-binding in wound signaling (Qiu *et al.*, 2012).

In addition to CaM, plants possess many CaM-like (CML) proteins (50 in *Arabidopsis*) that are predicted to function as Ca^{2+} sensors, but which remain largely uncharacterized (Vadassery *et al.*, 2012b). Nevertheless, it is known that most CMLs are cytoplasmic proteins and that some CMLs undergo lipid modifications resulting in membrane binding (Batistic and Kudla, 2012). Among the CMLs, two are particularly involved in the plant immune and biotic response: CML43 and CML42.

CML43 displays characteristics typical of Ca^{2+} sensors, and its GUS reporter activity strongly increased when *Arabidopsis*-transformed plants were exposed to the defense compound SA (salicylic acid). Therefore, CML43 functions as a Ca^{2+} sensor in the plant immune response as well (Bender *et al.*, 2014). The perception of microbe-associated molecular patterns (MAMPs) is closely connected to plant responses to insect herbivory. MAMPs typically induce a transient Ca^{2+} burst, resulting in a rapid (within seconds) increase in the free cytosolic Ca^{2+}, which subsequently (within minutes) declines to steady-state Ca^{2+} levels (Frei dit Frey *et al.*, 2012).

Plant gene expression induced by OS revealed the up-regulation of a gene encoding the calmodulin-like protein CML42, which negatively regulates plant defense. CML42 is localized to the cytosol and nucleus. Its up-regulation is negatively regulated by the JA receptor Coronatine Insensitive1 (COI1), as a loss of functional COI1 results in prolonged CML42 activation. CML42 thus acts as a negative regulator of plant defenses by decreasing COI1-mediated JA sensitivity and the expression of JA-responsive genes, independent of herbivory-induced JA biosynthesis. Furthermore, the results indicate that CML42 acts as a crucial signaling component connecting Ca^{2+} and JA signaling (Vadassery *et al.*, 2012a). CML42 is also involved in abiotic stress responses, as kaempferol glycosides were down regulated in *cml42* and impaired in ultraviolet B resistance. Under drought stress conditions, the level of abscisic acid

accumulation was higher in *cml42* plants. Thus, CML42 might serve as a Ca^{2+} sensor, with multiple functions in insect herbivory defense and abiotic stress responses. A porin-like protein (PLP), most likely of bacterial origin, was identified in the collected OS of *S. littoralis* larvae. PLP exhibits channel-forming activity and up-regulates CML42 in *Arabidopsis*; however, it is not sufficient to elevate in vivo $[Ca^{2+}]_{cyt}$. Because membrane channel formation is a widespread phenomenon in plant-insect interactions, this PLP might represent an example for microbial compounds from the insect gut, which are initially involved in plant-insect interactions (Guo *et al.*, 2013).

In addition to calmodulin, plants have two other main families of Ca^{2+} sensors: calcineurin B-like (CBLs) and calcium dependent protein kinases (CPKs). After CBL proteins sense Ca^{2+} signatures, these proteins interact selectively with CBL-interacting protein kinases (CIPKs), thereby forming the CBL/CIPK complexes involved in decoding calcium signals (Zhu *et al.*, 2013; Kim, 2013). The CBL-CIPK system shows variety, specificity, and complexity in response to different stresses, and the CBL-CIPK signaling pathway is regulated by complex mechanisms in plant cells involving crosstalk with other signaling pathways (Yu *et al.*, 2014). In vivo and in vitro data have indicated that the kinase CIPK26, upon interaction with the calcium sensors CBL1 or CBL9, enhances the activity of the NADPH oxidase RBOHF via phosphorylation (Drerup *et al.*, 2013; Kimura *et al.*, 2013). Moreover, CBL/CIPK may control the activity of a K^+ channel from the Shaker family, VvK1.2 in grapevine (Cuellar *et al.*, 2013), whereas protein kinase CIPK9 interacts with the calcium sensor CBL3 and plays crucial roles in K^+ homeostasis in *Arabidopsis* (Liu *et al.*, 2013). Both ROS and K^+ are involved in plant responses to herbivory (see below). In tea (*Camelia sinensis*) leaves exposed to a mild infestation of green leafhopper (*Empoasca vitis*), a subtractive cDNA library was constructed using the suppression subtractive

hybridization strategy. Genes involved in the CBL–CIPK pathway were up-regulated by herbivory. The expression of a CBL-interacting protein kinase 19, *Cs-Ev9*, significantly increased in the Fuzao2 tea cultivar infested by the tea leafhopper (by approximately 18 times with respect to the controls), providing the first evidence for the involvement of the CBL–CIPK pathway in response to herbivory (Yang *et al.*, 2011).

CPKs are multifunctional proteins with Ca^{2+} binding and signaling capabilities in single proteins to directly translate Ca^{2+} signals into phosphorylation events (Tena *et al.*, 2011; Boudsocq and Sheen, 2013; Valmonte *et al.*, 2014). Recent studies have shown that CPKs play a significant role in herbivore-elicited signaling cascades. Upon herbivory, the plant responds by activating Ca^{2+} signatures and corresponding CPKs such as CPK3 and CPK13 in *Arabidopsis*. These CPKs transcriptionally regulate the plant defensin gene (*PDF1.2*) independent of phytohormone signaling cascades in response to *S. littoralis* insect damage (Arimura and Maffei, 2010; Kanchiswamy *et al.*, 2010). This regulation occurs through the phosphorylation of the HSFB2A transcription factor, which positively regulates the expression of *PDF1.2* independent of ethylene, JA and abscisic acid. CPK3 also induces the negative feedback regulation of herbivore-induced Ca^{2+} signals, indicating that CPKs can play redundant and specific roles in plant defense (Kanchiswamy *et al.*, 2010).

Ca^{2+}-ATPases

Herbivory induces extracellular stimuli that trigger increases in the cytosolic calcium levels, which are detrimental to plants. To cope with such stresses, plants need to develop efficient efflux mechanisms to maintain ionic homeostasis. The Ca^{2+}-ATPases are members of the P-type ATPase superfamily, which performs many fundamental processes in organisms by actively

transporting ions across cellular membranes (Bose *et al.*, 2011). Moreover, ATP-dependent P-type calcium ATPases can modulate the biotic stress response by activating the components of signaling pathways. The plasma membrane and endomembrane-bound Ca^{2+} channels regulate cytoplasmic [Ca^{2+}] levels, promoting Ca^{2+} homeostasis by mediating the influx and efflux of Ca^{2+}. Furthermore, the type IIB Ca^{2+}-ATPases have a CAM-binding domain, which, upon binding with Ca^{2+}, forms a complex (Ca^{2+}/CaM) and initiates many signal networks (Huda *et al.*, 2013). Upon herbivory, the initial Ca^{2+} burst is followed by a consistent decrease in the cytosolic [Ca^{2+}], which implies the involvement of a Ca^{2+} efflux-mediated increased Ca^{2+}-ATPase activity (Maffei *et al.*, 2007).

Potassium channels

Recently, the question on whether Vm depolarization was caused by a calcium-signaling pathway was addressed. An *Arabidopsis* line (*pdko3*) with a mutation in the genes encoding plasmodesmal proteins was found to be defective in some of the typical plant responses to herbivory. Following herbivory and the release of the *S. littoralis* OS containing a putative β-galactofuranose polysaccharide, the mutant line, unlike WT plants, shows an almost complete loss of Vm depolarization. However, both the *pdko3* and wild type (WT) plants show increased accumulation of cytosolic Ca^{2+}. Thus, mutations in genes for plasmodesmal proteins have provided valuable genetic tools for the dissection of the complex spectrum of responses to herbivory and have shown us that the responses to herbivory imply a Ca^{2+}-independent Vm depolarization (Bricchi *et al.*, 2013). To understand the cause of Vm depolarization, the K^+ channel activity was measured by confocal imaging when *Arabidopsis* plants were damaged by *S. littorals* feeding or OS introduced to mechanically damaged leaves. Potassium represents the major osmotically active cation in plant cells and is known to be fundamental for plant functions

such as the control of membrane potential (Geiger *et al.*, 2009). Upon herbivory, the increased concentration of cytosolic Ca^{2+} triggers the opening of inward rectifier K^+ channels, which are mainly responsible for the herbivore-induced Vm depolarization. In the *pdko3 Arabidopsis* mutant, the connection between increased calcium and K^+ channel activation is broken, leading to significantly decreased Vm depolarization. However, the lower absolute levels of cytosolic calcium in the *pdko3* leaves open the possibility of a threshold effect in K^+ channel activation (Bricchi *et al.*, 2013).

Elicitor-dependent accumulation of second messengers such as Ca^{2+} and reactive oxygen/nitrogen species are central to many signaling and regulation processes in plants. However, the mechanisms that govern the reciprocal interrelation of Ca^{2+}, ROS and RNS signaling are only beginning to emerge. NADPH oxidases of the respiratory burst oxidase homolog (RBOH) family are critical components contributing to the generation of ROS whereas Calcineurin B-like (CBL) Ca^{2+} sensor proteins together with their interacting kinases (CIPKs) have been shown to function in many Ca^{2+} signaling processes, including in the control of K^+ channels. Figure 1 shows the role of Ca^{2+} signaling pathways following herbivory.

The second line of defense uses oxidative weapons: ROS and RNS

The unraveling of the mechanisms connected to changes in the Vm, $[Ca^{2+}]_{cyt}$, ROS and RNS production upon insect perception will undoubtedly shed critical light on the mechanisms used by plants to sense insect attacks (Bonaventure, 2012). ROS and RNS constitute key features underpinning the dynamic nature of cell signaling systems in plants. Despite their importance in many aspects of cell biology, our understanding of oxidative and especially of nitrosative signaling and their regulation remains poor (Molassiotis and Fotopoulos, 2011).

The role of ROS

Emerging evidence has begun to implicate ROS as pivotal redox-based signaling molecules in the plant defense response (Skelly and Loake, 2013). The modification of cysteines in key regulatory proteins by changes in redox homeostasis is appearing as a central responsive molecular switch that controls the function of a variety of regulators of plant defense responses (Yun *et al.*, 2012). ROS such as H_2O_2 can also activate lipoxygenases to initiate the biosynthesis of oxylipins such as JA (Porta and Rocha-Sosa, 2002); indeed, JA treatment alone produces a H_2O_2 burst (Ozawa *et al.*, 2009). Mechanically damaged Lima bean leaves react rapidly and dramatically to H_2O_2 by inducing a strong Vm depolarization. However, leaves wounded by *S. littoralis* already show a reduced starting Vm, with the consequence of dramatically lower or even no responsiveness to H_2O_2 application (Mithöfer *et al.*, 2009a). Thus, the calcium-induced potassium-dependent depolarization of the Vm following herbivory is linked to a reduction in the downstream responses of the attacked leaves to signaling molecules such as H_2O_2 (Bricchi *et al.*, 2010).

The production of ROS such as H_2O_2 depends on the activity of oxidases. Plant NADPH oxidases, also known as respiratory burst oxidase homologues (RBOHs), are the most thoroughly studied enzymatic ROS-generating systems. Our understanding of their involvement in various plant processes has increased considerably in recent years (Marino *et al.*, 2012). The ability of RBOHs to integrate calcium signaling and protein phosphorylation with ROS production, coupled with genetic studies demonstrating their involvement in many different biological processes in cells, places RBOHs at the center of the ROS network of cells and demonstrates their important function in plants (Suzuki *et al.*, 2011). In *Arabidopsis*, a Ca^{2+}-signaling network regulates the formation of ROS. After interaction with the calcium sensors CBL1 or CBL9, the

kinase CIPK26 enhances the activity of the NADPH oxidase RBOH via phosphorylation. The CBL-interacting protein kinase CIPK26 specifically interacts with the N-terminal domain of RBOH in yeast two-hybrid analyses and with the full-length RBOH protein in plant cells. In addition, CIPK26 phosphorylates RBOH in vitro, and the co-expression of either CBL1 or CBL9 with CIPK26 strongly enhances ROS production by RBOHF in HEK293T cells. Together, these findings identify a direct connection between CBL-CIPK-mediated Ca^{2+} signaling and ROS signaling in plants and provide evidence for the synergistic activation of the NADPH oxidase RBOH by direct Ca^{2+}-binding to its EF-hands and Ca^{2+}-induced phosphorylation by CBL1/9CIPK26 complexes (Drerup *et al.,* 2013). Moreover, the direct binding of CIPK26 to RBOH negatively modulates ROS production and plays a role in the regulation of ROS signaling in plants (Kimura *et al.,* 2013). In *Arabidopsis*, extracellular ATP causes the production of ROS in intact roots, with the plasma membrane NADPH oxidase AtRBOHC being the major contributor. This resulted in the stimulation of plasma membrane Ca^{2+}-permeable channels, which contribute to the elevation of cytosolic free Ca^{2+}. The disruption of this pathway in the *AtrbohC* mutant impaired the extracellular ATP-induced increase in ROS, the activation of Ca^{2+} channels, and the transcription of the *MAP kinase3* gene that is known to be involved in stress responses, showing that higher plants evolved a mechanism to transduce the ATP signal at the plasma membrane (Demidchik *et al.,* 2009). In the model plant *Nicotiana attenuata*, transcripts of the respiratory burst NADPH oxidase homolog NaRBOHD are rapidly and transiently induced by wounding and are amplified when *Manduca sexta* OS are added to the wounds. The *M. sexta* OS contain fatty-acid-amino-acid-conjugates (FACs), which are responsible for the increase in NaRBOHD transcripts. Silencing NaRBOHD significantly reduced the ROS levels after OS elicitation, without influencing the expression of the pivotal hormones that regulate plant

resistance to herbivores. In addition, NaRBOHD-silenced plants were more vulnerable to insect herbivores, especially to the larvae of the generalist *S. littoralis*. These results indicate that NaRBOHD-based defenses play an important role in late herbivore-elicited responses (Wu *et al.*, 2013).

The evidence of ROS involvement in plant-insect interactions is growing. The accumulation of reactive oxygen species seems to play a major role in determining the extent of tissue alterations during gall morphogenesis (Carneiro *et al.*, 2014). The detection of enzymes related to ROS production has been described for galls induced by sucking insects (de Oliveira and dos Santos Isaias, 2010). The potential role of ROS in the defense systems of wheat (*Triticum aestivum*) and rice (*Oryza sativa*) against Hessian fly (*Mayetiola destructor*) larvae reveals a rapid and prolonged accumulation of H_2O_2 in wheat plants at the attack site during incompatible interactions. Increased accumulation of both H_2O_2 and $O_2^{-}\bullet$ was detected in rice plants during non-host interactions with the larvae. Class III peroxidases might play a role in ROS generation in resistant wheat and non-host rice plants during the response to Hessian fly attacks (Liu *et al.*, 2010). Moreover, insect infestation impacts the stress signaling network through the effects on the ROS and cellular redox metabolism, with particular emphasis on the roles of ROS in the plant responses to phloem-feeding insects (Kerchev *et al.*, 2012). Pea aphid (*Acyrthosiphon pisum*) causes oxidative stress conditions in pea leaves through the enhanced production of H_2O_2 and $O_2^{-}\bullet$. The early, strong generation of H_2O_2 was observed at 24 h in aphid-infested leaves, whereas the strong generation and continuous increase of $O_2^{-}\bullet$ production in aphid-infested leaves from 0 to 96 h enhanced the defense potential to protect against aphid herbivory. The relative release of H_2O_2 and $O_2^{-}\bullet$ was estimated with confocal microscopy by staining leaves with specific fluorochromes [dichlorodihydro-fluorescein diacetate (DCFH-DA) and

dihydroethidium (DHE)] to reveal the fine-tuned regulatory mechanisms involved in plant-aphid interactions (Van *et al.*, 2013).

The concentration of H_2O_2 and malondialdehyde (MDA) and the activities of several ROS scavenging enzymes and related gene expression [e.g., superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)] were measured in different species damaged by mechanical and herbivore wounding. After infestation with chewing insects, the POD activity in the sap and total soluble protein of tomato, cowpea and cotton were enhanced. Similar results were observed with the feeding of sap sucking insects (Singh *et al.*, 2013). In poplar, the concentration of H_2O_2 and MDA and the activities of SOD, CAT and POD were enhanced by herbivore wounding treatment, but not as much as by mechanical wounding, suggesting that H_2O_2, SOD, CAT and POD are associated with insect resistance (An *et al.*, 2010). In the "living fossil" plant *Ginkgo biloba*, a significantly higher H_2O_2 production is observed 30 min after herbivory than in mechanically damaged leaves; however, by 4 h after feeding, the values dropped to the control levels. The use of DPI (diphenyleneiodonium, a suicide inhibitor of the phagocytic NADPH oxidase and an inhibitor of NADH-dependent H_2O_2 production by peroxidase) inhibited herbivory-dependent H_2O_2 production to the control levels, whereas verapamil (a voltage-gated Ca^{2+} channel antagonist with a significant effect on herbivore-induced Ca^{+2} release) significantly increases the level of H_2O_2 upon herbivory. These data confirm the existence of an interplay between H_2O_2 and calcium homeostasis (Mohanta *et al.*, 2012).

Another interesting question is how much insects can tolerate the increased ROS production in the attacked leaves. In the emerald ash borer (*Agrilus planipennis*), antioxidant genes quench ROS from both dietary and endogenous sources (Rajarapu *et al.*, 2011), whereas *Lymantria dispar* caterpillar larvae can tolerate elevated levels of ROS in their midguts without nutritionally

significant changes in the compositions of susceptible essential amino acids in their food (Barbehenn *et al.*, 2012). The abundance of vitamin C (VitC) in plants influences their susceptibility to insect feeding as VitC is an essential dietary nutrient that serves as an antioxidant in the insect midgut and is also a substrate for plant-derived ascorbate oxidase, which can lead to the generation of toxic reactive oxygen species. Herbivores appear to influence both the de novo synthesis and redox cycling of VitC in their host plants, thereby potentially altering the nutritional value of crops and their susceptibility to pests. The recent development of genetically modified crops with enhanced VitC content provides both an impetus and a tool for further studies on the role of VitC in plant-insect interactions (Goggin *et al.*, 2010).

Involvement of NRS

The available descriptions of NO-mediated physiological processes are continuously increasing. Although it was initially associated with plant defense responses against pathogens, reports indicate that NO is also involved in plant responses to insects. *S*-nitrosoglutathione reductase (GSNOR) reduces the NO adduct *S*-nitrosoglutathione (GSNO), an essential reservoir for NO bioactivity involved in plant-herbivore interactions. Using a virus-induced gene silencing (VIGS) system, the activity of GSNOR was knocked down in *N. attenuata*. The silencing of GSNOR decreased the herbivory-induced accumulation of JA and ethylene without compromising the activity of two mitogen-activated protein kinases (MAPKs), salicylic acid-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK) (Wuensche *et al.*, 2011a). *M. sexta* feeding decreased the activity of trypsin proteinase inhibitors (TPIs), which were detected in GSNOR-silenced plants. Therefore, GSNOR plays an important role in plant resistance to herbivory and JA signaling and suggests the potential involvement of NO in plant-herbivore interactions (Wuensche *et al.*, 2011a).

Nitric oxide-associated protein 1 (NOA1) is required for plant resistance in plant-herbivore interactions. NOA1-silenced *N. attenuata* plants treated with mechanical wounding and *M. sexta* OS showed elevated levels of herbivory-induced JA but reduced concentrations of most carbon-based defensive compounds. These data suggest the involvement of NOA1 in *N. attenuata*'s defense against *M. sexta* attack, and highlight its role in photosynthesis and in the biosynthesis of jasmonates and secondary metabolites (Wuensche *et al.*, 2011b).

Nilaparvata lugens, the brown plant hopper (BPH), is one of the most destructive phloem-feeding insects to attack rice throughout Asia. BPH feeding increases the level of endogenous NO in the leaf and sheath tissue of both resistant and susceptible rice cultivars within 1 h of infestation. The production of NO in response to BPH feeding appears to depend primarily on the activity of nitric oxide synthase (NOS). The application of exogenous NO stimulated the expression of certain drought stress-related genes, reduced the plant height, delayed leaf senescence and reduced the seedling mortality caused by BPH feeding. This suggests that NO signaling plays a role in the rice tolerance response to BPH feeding (Liu *et al.*, 2011). In the interaction between *S. littoralis* and Lima bean, both herbivory and robotic wounding induced marked accumulation of NO but with distinct temporal patterns. Herbivory caused a rapid and transient increase of NO levels, whereas the response to robotic wounding was of equal intensity but delayed (Bricchi *et al.*, 2010). Interestingly, the time course of NO production parallels the induction of the volatile compound emission observed in Lima beans (Arimura *et al.*, 2011), suggesting a possible involvement of NO in the signaling pathway leading to volatile emission. Figure 2 summarizes the changes in the ROS and RNS signaling pathways in response to herbivory.

A rapid chemical signal provides a systemic warning

Systemic responses are used by plants to prevent attacking herbivores from moving from wounded zones, where plant defenses are built up in response to feeding, to reach unattacked leaves. The existence of such a systemic signaling molecule was first reported in tomato plants in the pioneer studies of Ryan and co-workers (Green and Ryan, 1972). Since then, regulation of defense genes in systemic tissues upon herbivory has been increasingly reported (Champigny and Cameron, 2009), and considerable research effort has focused on the identification of systemic wound signals and the mechanisms by which they are generated, transported and perceived in distal leaves. The signaling species implicated in wound-induced systemic responses include oligosaccharides, reactive oxygen species, systemin, green leaf volatiles, hydraulic signals, electrical signals and various plant hormones (Koo *et al.*, 2009 and references cited therein). The nature of the systemic wound signal remains unknown. In the current model, a rapidly transmitted wound signal triggers the systemic synthesis of JA, which, upon conversion to JA-Ile, activates the expression of early response genes by the SCF^{COI1}/JAZ pathway (Koo *et al.*, 2009). In addition to the responses in the attacked leaf, the enhanced levels of defense gene transcripts are found in unelicited leaves on the same plant. This between-leaf systemic signaling of defense responses occurs without MAPK activation in systemic leaves and suggests the existence of a mobile signal other than the one activating responses within the attacked leaves (Wu *et al.*, 2007). However, the nature of the signal and its means of propagation are still debated. Given how rapidly MAPKs are activated in undamaged regions, it may be that the signal is carried by electricity or rapidly propagated hydraulic signals. Another hypothesis is that relatively small molecules, such as FACs or FAC-elicited signal compounds, enter the xylem vessels through wounds and are carried to specific regions of the leaf (Wu *et al.*, 2007). Whether electric signaling or xylem transport is involved in activating MAPKs in undamaged regions

needs further investigation. Increasing evidence excludes the possibility that plant phytohormones (e.g., JA or its derivatives such as the JA isoleucine conjugate or its methyl ester) might be the travelling molecules because they are synthesized in the systemic leaves rather than transported (Wang *et al.*, 2008); moreover, insect elicitors induce a higher JA accumulation in distant tissues (VanDoorn *et al.*, 2010; Bricchi *et al.*, 2013).

Quite recently, evidence on the role of the symplastic connection for the transmission of the moving signal was provided. Hydroponically cultured *Arabidopsis* provides an ideal tool to study shoot-to-root signaling, allowing for changes in Vm in the root to be measured in real time following biotic or chemical stress on the shoots. When the root-Vm was measured in WT *Arabidopsis*, it was found to be significantly depolarized 7 - 8 min after either herbivory or the application of OS or FRα, a β-galactofuranose isolated from *S. littoralis* OS. On the other hand, the *pdko3* mutant showed no root-Vm depolarization after herbivory or the application of either OS or FRα. The rate of signal transduction was ~110 cm h^{-1}, which matches the rate of phloem transport (from 30 to 150 cm h^{-1}). To test the phloem transport hypothesis, petioles of the treated leaves were subjected to either cold (4°C) or heat (60°). Both treatments completely abolished the WT herbivore-, OS- and FRα-induced root Vm depolarization, indicating the requirement of functional sieve elements. Therefore, because the *pdko3* mutant does not transmit the signal, it appears that that disruption of plasmodesmata function reduces the systemic signaling (Bricchi *et al.*, 2013). From all these studies, it is clear that elucidating the nature of the mobile signals in different species will contribute to an understanding of the rates of signal transmission and the speed of systemic plant responses to herbivory. In our laboratory, we tested different species and analyzed the time of root Vm depolarization upon leaf herbivory (*S. littoralis*). The rate of transmission was found to correspond to the phloem rate (Maffei *et al.*, unpublished results).

However, different biotic attacks may trigger different rates of plant responses. For instance, in *Arabidopsis*, the plasma membrane responds with a Vm depolarization depending on the nature of biotic attack. The Vm response is much more rapid upon *S. littoralis* (30 min -2 h) herbivory than following an attack by the aphid *Myzus persicae* (4-6 h). Furthermore, pathogenic infection with *Pseudomonas syringae* induces a very late Vm depolarization (16 h). Regardless of the nature of the biotroph and the rate of response, the Vm depolarization occurs at the same intensity, which in *Arabidopsis* corresponds to a Vm depolarization of approximately 40 mV. This suggests the occurrence of a Vm threshold, which is reached for successful herbivory/infection (Bricchi *et al.*, 2012). The question of why the Vm polarization occurs at different times appears to be associated with the mode of the biotic damage. The fast clipping and consistent plant tissue removal by chewing herbivores induces a "quantitative" response that is proportional to the level of tissue damage; on the other hand, the stylet probing and the phloem feeding of aphids induces a reduced level of damage that requires more time for a plant response. Finally, bacterial growth and tissue damage takes time, which appears to be proportional to the Vm depolarization. Therefore, besides the nature of the moving molecule, it will be important to evaluate the nature of the elicitor that induces its synthesis.

Because the use of plasmodesmata mutants in *Arabidopsis* suggests the involvement of phloem elements as the possible carriers of the moving signal(s), this hypothesis restricts the molecular size of the moving signal to plasmodesmata constraints, including the flux rate [3.3-8.5 µm s^{-1}, (Rutschow *et al.*, 2011)], their specificity and directionality (Christensen *et al.*, 2009), their variable flux properties between species and tissue types (Maule *et al.*, 2011), the role of ROS as potent molecules that have an impact on the efficiency of PD trafficking (itez-Alfonso *et al.*, 2011) and their size exclusion limits (Burch-Smith and Zambryski, 2012; Lee, 2014). Figure 3

illustrates a symplastic model for the transmission of the moving signal as well as for the effects of disrupting the symplastic continuity of the systemic response.

Concluding remarks

Depolarization of the Vm is one of the first responses of the plasma membrane. It depends mainly on the ion fluxes, including the opening of K^+ channels, calcium release from internal stores or influx from the apoplast. The cytosolic calcium increase triggers protein calcium sensors including calmodulin, calcium-dependent protein kinases and calcineurin signaling, which activate a cascade of signals and eventually leads to transcription factor activation and transcriptional regulation. Calcium also triggers hydrogen peroxide production through the activation of CBL1/CBL9-CIPK26 interaction, which eventually phosphorylates RBOH and thus activates the production of ROS. Calcium homeostasis changes and cell redox status alterations also trigger NO production, which can either directly or indirectly affect the plant cell responses to herbivory. A growing body of evidence indicates the presence of mobile signaling molecules that travel from the wounded tissues towards systemic organs. Although the nature of this molecule remains unknown, its presence depends on the activity of insect's oral secretions, where specific elicitors (FACs or oligosaccharides) are necessary and sufficient to start the response process. Both the xylem and phloem are good candidates, given the path of the molecule toward the systemic tissues. In *Arabidopsis*, the elicitation with *S. littoralis* β-galactofuranose requires functioning plasmodesmata in order for the signaling molecule to reach the systemic tissues, justifying a model where the symplastic route is used.

Understanding fast responses of plants to the surrounding environment is of interest not only from an ecological and evolutionary perspective but also for the development of novel crop

protection strategies. Owing to the massive damage that herbivores cause to valuable crops, the deciphering of early signals from plants represents one of the most exciting areas research in the first line of defense.

Three areas where future efforts might result in major breakthroughs are related to the identification of herbivore-specific signal molecules, their recognition, and further signal transduction. The challenge for further research is to determine their mode of action, whether these signals are transduced by receptor-mediated processes or whether they simply interact with the plant membranes to initiate signal transduction pathways. One approach to achieve this goal is the use of plant mutants that are not responsive to a particular herbivory-related signal, as the *Arabidopsis pdko3* mutant or the several *N. attenuata* silenced plants (Yang *et al.,* 2012; Bricchi *et al.,* 2013; Woldemariam *et al.,* 2013; Wu *et al.,* 2013). The characterization and use of such mutants could result in the identification of genes encoding proteins involved in signal perception. Such studies can not only uncover individual signaling pathways but can also establish links in a network of alternative routes regulating the multitude of inducible plant defenses.

References

Amari K, Boutant E, Hofmann C, Schmitt-Keichinger C, Fernandez-Calvino L, Didier P, Lerich A, Mutterer J, Thomas CL, Heinlein M, Mely Y, Maule AJ, Ritzenthaler C. 2010. A family of plasmodesmal proteins with receptor-like properties for plant viral movement proteins. PLoS Pathog. **6,** e1001119.

An Y, Shen Y, Zhang Z. 2010. Effects of Damage Stress on Membrane Lipid Peroxidation and Antioxidant Enzymes in Poplar Leaves. Res.Soil Water Cons. **17,** 241-244.

Appel H, Cocroft R. 2014. Plants respond to leaf vibrations caused by insect herbivore chewing. Oecologia **175,** 1257-1266.

Arimura G, Maffei ME. 2010. Calcium and secondary CPK signaling in plants in response to herbivore attack. Biochem.Biophys.Res.Comm. **400,** 455-460.

Arimura G, Matsui K, Takabayashi J. 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: Proximate factors and their ultimate functions. Plant Cell Physiol. **50,** 911-923.

Arimura G, Ozawa R, Maffei ME. 2011. Recent Advances in Plant Early Signaling in Response to Herbivory. Int.J.Mol.Sci. **12,** 3723-3739.

Baldwin IT. 2010. Plant volatiles. Curr.Biol. **20,** R392-R397.

Baluska F, Mancuso S. 2013. Root apex transition zone as oscillatory zone. Front.Plant Sci. **4**.

Barbehenn RV, Niewiadomski J, Kochmanski J, Constabel C. 2012. Limited Effect of Reactive Oxygen Species on the Composition of Susceptible Essential Amino Acids in the Midguts of Lymantria Dispar Caterpillars. Arch.Insect Biochem.Physiol. **81,** 160-177.

Batistic O, Kudla J. 2012. Analysis of calcium signaling pathways in plants. Biochim.Biophys.Acta **1820,** 1283-1293.

Bender KW, Dobney S, Ogunrinde A, Chiasson D, Mullen RT, Teresinski HJ, Singh P, Munro K, Smith SP, Snedden WA. 2014. The calmodulin-like protein CML43 functions as a salicylic-acid-inducible root-specific Ca2+ sensor in Arabidopsis. Biochem.J. **457,** 127-136.

Bonaventure G. 2012. Perception of insect feeding by plants. Plant Biol. **14,** 872-880.

Bonaventure G, VanDoorn A, Baldwin IT. 2011. Herbivore-associated elicitors: FAC signaling and metabolism. Trends Plant Sci. **16,** 294-299.

Bos JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA. 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PloS Genet. **6,** e1001216.

Bose J, Pottosin II, Shabala SS, Palmgren MG, Shabala S. 2011. Calcium efflux systems in stress signaling and adaptation in plants. Front.Plant Sci. **2**.

Boudsocq M, Sheen J. 2013. CDPKs in immune and stress signaling. Trends Plant Sci. **18,** 30-40.

Bricchi I, Leitner M, Foti M, Mithöfer A, Boland W, Maffei ME. 2010. Robotic mechanical wounding (MecWorm) versus herbivore-induced responses: early signaling and volatile emission in Lima bean (Phaseolus lunatus L.). Planta **232,** 719-729.

Bricchi I, Bertea CM, Occhipinti A, Paponov IA, Maffei ME. 2012. Dynamics of Membrane Potential Variation and Gene Expression Induced by Spodoptera littoralis, Myzus persicae, and Pseudomonas syringae in Arabidopsis. PLoS One **7**.

Bricchi I, Occhipinti A, Bertea CM, Zebelo SA, Brillada C, Verrillo F, De Castro C, Molinaro A, Faulkner C, Maule AJ, Maffei ME. 2013. Separation of early and late responses to herbivory in Arabidopsis by changing plasmodesmal function. Plant J. **73,** 14-25.

Burch-Smith TM, Zambryski PC. 2012. Plasmodesmata Paradigm Shift: Regulation from Without Versus Within. Annu.Rev.Plant Biol. **63,** 239-260.

Carneiro R, Castro A, Isaias R. 2014. Unique histochemical gradients in a photosynthesis-deficient plant gall. South Afr.J.Bot. **92,** 97-104.

Champigny MJ, Cameron RK. 2009. Action at a Distance: Long-Distance Signals in Induced Resistance. Adv.Bot.Res. **51,** 123-171.

Christensen NM, Faulkner C, Oparka K. 2009. Evidence for Unidirectional Flow through Plasmodesmata. Plant Physiol. **150,** 96-104.

Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc.Natl.Acad.Sci.USA **110,** 15728-15733.

Consales F, Schweizer F, Erb M, Gouhier-Darimont C, Bodenhausen N, Bruessow F, Sobhy I, Reymond P. 2012. Insect oral secretions suppress wound-induced responses in Arabidopsis. J.Exp.Bot. **63,** 727-737.

Cuellar T, Azeem F, Andrianteranagna M, Pascaud F, Verdeil JL, Sentenac H, Zimmermann S, Gaillard I. 2013. Potassium transport in developing fleshy fruits: the grapevine inward K+ channel VvK1.2 is activated by CIPK-CBL complexes and induced in ripening berry flesh cells. Plant J. **73,** 1006-1018.

de Oliveira DC, dos Santos Isaias RM. 2010. Cytological and histochemical gradients induced by a sucking insect in galls of *Aspidosperma australe* Arg. Muell (Apocynaceae). Plant Sci. **178,** 350-358.

Demidchik V, Shang ZL, Shin R, Thompson E, Rubio L, Laohavisit A, Mortimer JC, Chivasa S, Slabas AR, Glover BJ, Schachtman DP, Shabala SN, Davies JM. 2009. Plant extracellular ATP signalling by plasma membrane NADPH oxidase and Ca^{2+} channels. Plant J. **58,** 903-913.

Diaz J. 2011. Information flow in plant signaling pathways. Plant Sign.Behav. **6,** 339-343.

Dodd AN, Kudla J, Sanders D. 2010. The Language of Calcium Signaling. Annu.Rev.Plant Biol. 593-620.

Drerup MM, Schlucking K, Hashimoto K, Manishankar P, Steinhorst L, Kuchitsu K, Kudla J. 2013. The Calcineurin B-Like Calcium Sensors CBL1 and CBL9 Together with Their Interacting Protein Kinase CIPK26 Regulate the Arabidopsis NADPH Oxidase RBOHF. Mol.Plant **6,** 559-569.

Du LQ, Yang TB, Puthanveettil SV, Poovaiah BW. 2011. Calmodulin-Binding Proteins in Plants: Decoding of Calcium Signal Through Calmodulin. In: Luan S, ed. *Coding and decoding of calcium signals in plants.* Berlin: Springer-Verlag, 177-233.

Ebel J, Mithöfer A. 1998. Early events in the elicitation of plant defence. Planta **206,** 335-348.

Erb M, Meldau S, Howe GA. 2012. Role of phytohormones in insect-specific plant reactions. Trends Plant Sci. **17,** 250-259.

Felle HH, Zimmermann MR. 2007. Systemic signalling in barley through action potentials. Planta **226,** 203-214.

Frei dit Frey N, Mbengue M, Kwaaitaal M, Nitsch L, Altenbach D, Haweker H, Lozano-Duran R, Njo MF, Beeckman T, Huettel B, Borst JW, Panstruga R, Robatzek S. 2012. Plasma membrane calcium ATPases are important components of receptor-mediated signaling in plant immune responses and development. Plant Physiol. **159,** 798-809.

Gagliano M, Renton M, Duvdevani N, Timmins M, Mancuso S. 2012. Out of Sight but Not out of Mind: Alternative Means of Communication in Plants. PLoS One **7**.

Galon Y, Nave R, Boyce JM, Nachmias D, Knight MR, Fromm H. 2008. Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in Arabidopsis. Febs Lett. **582,** 943-948.

Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A. 2006. Early signaling events induced by elicitors of plant Defenses. Mol.Plant-Microbe Interact. **19,** 711-724.

Geiger D, Becker D, Vosloh D, Gambale F, Palme K, Rehers M, Anschuetz U, Dreyer I, Kudla J, Hedrich R. 2009. Heteromeric AtKC1.AKT1 Channels in Arabidopsis Roots Facilitate Growth under K(+)-limiting Conditions. J.Biol.Chem. **284,** 21288-21295.

Goggin FL, Avila CA, Lorence A. 2010. Vitamin C content in plants is modified by insects and influences susceptibility to herbivory. Bioessays **32,** 777-790.

Green TR, Ryan CA. 1972. Wound-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects. Science **175,** 776-777.

Guo H, Wielsch N, Hafke JB, Svatos A, Mithoefer A, Boland W. 2013. A porin-like protein from oral secretions of Spodoptera littoralis larvae induces defense-related early events in plant leaves. Insect Biochemistry and Molecular Biology **43,** 849-858.

Gurovich LA, Hermosilla P. 2009. Electric signalling in fruit trees in response to water applications and light-darkness conditions. J.Plant Physiol. **166,** 290-300.

Howe GA, Jander G. 2008. Plant immunity to insect herbivores. Annu.Rev.Plant Biol. **59,** 41-66.

Huda KMK, Banu MSA, Tuteja R, Tuteja N. 2013. Global calcium transducer P-type Ca-2-ATPases open new avenues for agriculture by regulating stress signalling. J.Exper.Bot. **64,** 3099-3109.

Imbiscuso G, Trotta A, Maffei M, Bossi S. 2009. Herbivory induces a ROS burst and the release of volatile organic compounds in the fern *Pteris vittata* L. J.Plant Interact. **4,** 15-22.

itez-Alfonso Y, Jackson D, Maule A. 2011. Redox regulation of intercellular transport. Protoplasma **248,** 131-140.

Jammes F, Hu HC, Villiers F, Bouten R, Kwak JM. 2011. Calcium-permeable channels in plant cells. Febs J. **278,** 4262-4276.

Kanchiswamy CN, Malnoy M, Occhipinti A, Maffei ME. 2014. Calcium imaging perspective in plants. Int.J.Mol.Sci. **15,** 3842-3859.

Kanchiswamy CN, Takahashi H, Quadro S, Maffei ME, Bossi S, Bertea C, Zebelo SA, Muroi A, Ishihama N, Yoshioka H, Boland W, Takabayashi J, Endo Y, Sawasaki T, Arimura G. 2010. Regulation of Arabidopsis defense responses against *Spodoptera littoralis* by CPK-mediated calcium signaling. Bmc Plant Biol. **10,** 97.

Karban R, Shiojiri K, Ishizaki S. 2011. Plant communication - why should plants emit volatile cues? J.Plant Interact. **6,** 81-84.

Karban R. 2010. Neighbors affect resistance to herbivory--a new mechanism. New Phytol **186,** 564-566.

Kerchev PI, Fenton B, Foyer CH, Hancock RD. 2012. Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant Cell Environ. **35,** 441-453.

Kim KN. 2013. Stress responses mediated by the CBL calcium sensors in plants. Plant Biotechnol.Rep. **7,** 1-8.

Kimura S, Kawarazaki T, Nibori H, Michikawa M, Imai A, Kaya H, Kuchitsu K. 2013. The CBL-interacting protein kinase CIPK26 is a novel interactor of Arabidopsis NADPH oxidase AtRbohF that negatively modulates its ROS-producing activity in a heterologous expression system. J.Biochem. **153,** 191-195.

Koo AJ, Gao X, Jones A, Howe GA. 2009. A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. Plant J. **59,** 974-986.

Kudla J, Batistic O, Hashimoto K. 2010. Calcium Signals: The Lead Currency of Plant Information Processing. Plant Cell **22,** 541-563.

Laluk K, Prasad K, V, Savchenko T, Celesnik H, Dehesh K, Levy M, Mitchell-Olds T, Reddy A. 2012. The Calmodulin-Binding Transcription Factor SIGNAL RESPONSIVE1 is a Novel Regulator of Glucosinolate Metabolism and Herbivory Tolerance in Arabidopsis. Plant Cell Physiol. **53,** 2008-2015.

Lee JY. 2014. New and old roles of plasmodesmata in immunity and parallels to tunneling nanotubes. Plant Sci. **221,** 13-20.

Liu LL, Ren HM, Chen LQ, Wang Y, Wu WH. 2013. A Protein Kinase, Calcineurin B-Like Protein-Interacting Protein Kinase9, Interacts with Calcium Sensor Calcineurin B-Like Protein3 and Regulates Potassium Homeostasis under Low-Potassium Stress in Arabidopsis. Plant Physiol. **161,** 266-277.

Liu X, Williams CE, Nemacheck JA, Wang H, Subramanyam S, Zheng C, Chen MS. 2010. Reactive Oxygen Species Are Involved in Plant Defense against a Gall Midge. Plant Physiol. **152,** 985-999.

Liu Y, He J, Jiang L, Wu H, Xiao Y, Liu Y, Li G, Du Y, Liu C, Wan J. 2011. Nitric oxide production is associated with response to brown planthopper infestation in rice. Journal of Plant Physiology **168,** 739-745.

Maffei M. 2010. Sites of synthesis, biochemistry and functional role of plant volatiles. S.Afr.J.Bot. **76,** 612-631.

Maffei M, Bossi S, Spiteller D, Mithöfer A, Boland W. 2004. Effects of feeding *Spodoptera littoralis* on lima bean leaves. I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. Plant Physiol. **134,** 1752-1762.

Maffei ME, Gertsch J, Appendino G. 2011. Plant volatiles: Production, function and pharmacology. Nat.Prod.Rep. **28,** 1359-1380.

Maffei ME, Mithöfer A, Boland W. 2007. Before gene expression: early events in plant-insect interaction. Trends Plant Sci. **12,** 310-316.

Maffei ME, Arimura GI, Mithoefer A. 2012. Natural elicitors, effectors and modulators of plant responses. Nat.Prod.Rep. **29,** 1288-1303.

Marino D, Dunand C, Puppo A, Pauly N. 2012. A burst of plant NADPH oxidases. Trends Plant Sci. **17,** 9-15.

Masi E, Ciszak M, Stefano G, Renna L, Azzarello E, Pandolfi C, Mugnai S, Baluska F, Arecchi FT, Mancuso S. 2009. Spatiotemporal dynamics of the electrical network activity in the root apex. Proc.Natl.Acad.Sci.U.S.A. **106,** 4048-4053.

Matzke AJM, Matzke M. 2013. Membrane "potential-omics": toward voltage imaging at the cell population level in roots of living plants. Front.Plant Sci. **4**.

Maule AJ, Benitez-Alfonso Y, Faulkner C. 2011. Plasmodesmata – membrane tunnels with attitude. Curr.Opin.Plant Biol. **14,** 683-690.

Miller G, Mittler R. 2006. Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann.Bot. **98,** 279-288.

Mithöfer A, Boland W, Maffei ME. 2009a. Chemical ecology of plant insect interactions. In: Parker J, ed. *Molecular Aspects of Plant Disease Resistance.* Chirchester: Wiley-Blackwell, 261-291.

Mithöfer A, Mazars C, Maffei ME. 2009b. Probing spatio-temporal intracellular calcium variations in plants. Meth.Mol.Biol. 79-92.

Mohanta TK, Occhipinti A, Zebelo AS, Foti M, Fliegmann J, Bossi S, Maffei ME, Bertea CM. 2012. *Ginkgo biloba* responds to herbivory by activating early signaling and direct defenses. PLoS One **7,** e32822.

Molassiotis A, Fotopoulos V. 2011. Oxidative and nitrosative signaling in plants: two branches in the same tree? Plant Sign.Behav. **6,** 210-214.

Monshausen GB, Haswell ES. 2013. A force of nature: molecular mechanisms of mechanoperception in plants. J.Exper.Bot. **64,** 4663-4680.

Mousavi SA, Chauvin A, Pascaud F, Kellenberger S, Farmer EE. 2013. GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. Nature **500,** 422-+.

Mousavi SA, Nguyen CT, Farmer EE, Kellenberger S. 2014. Measuring surface potential changes on leaves. Nat.Protocols **9,** 1997-2004.

Ochatt S. 2013. Plant cell electrophysiology: Applications in growth enhancement, somatic hybridisation and gene transfer. Biotechnol.Adv. **31,** 1237-1246.

Onkokesung N, Galis I, von Dahl CC, Matsuoka K, Saluz HP, Baldwin IT. 2010. Jasmonic Acid and Ethylene Modulate Local Responses to Wounding and Simulated Herbivory in *Nicotiana attenuata* Leaves. Plant Physiol. **153,** 785-798.

Oyarce P, Gurovich L. 2011. Evidence for the transmission of information through electric potentials in injured avocado trees. J.Plant Physiol. **168,** 103-108.

Ozawa R, Bertea CM, Foti M, Narayana R, Arimura GI, Muroi A, Horiuchi JI, Nishioka T, Maffei ME, Takabayashi J. 2009. Exogenous polyamines elicit herbivore-induced volatiles in Lima bean leaves: Involvement of calcium, H_2O_2 and jasmonic acid. Plant Cell Physiol. **50,** 2183-2199.

Pearse IS, Karban R. 2013. Do plant-plant signals mediate herbivory consistently in multiple taxa and ecological contexts? J.Plant Interact. **8,** 203-206.

Porta H, Rocha-Sosa M. 2002. Plant lipoxygenases. Physiological and molecular features. Plant Physiol. **130,** 15-21.

Qiu Y, Xi J, Du L, Suttle JC, Poovaiah B. 2012. Coupling calcium/calmodulin-mediated signaling and herbivore-induced plant response through calmodulin-binding transcription factor AtSR1/CAMTA3. Plant Mol.Biol. **79,** 89-99.

Rajarapu SP, Mamidala P, Herms DA, Bonello P, Mittapalli O. 2011. Antioxidant genes of the emerald ash borer (Agrilus planipennis): Gene characterization and expression profiles. J.Insect Physiol. **57,** 819-824.

Reddy ASN, Ali GS, Celesnik H, Day IS. 2011. Coping with stresses: Roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell **23,** 2010-2032.

Russell JT. 2011. Imaging calcium signals in vivo: a powerful tool in physiology and pharmacology. Brit.J.Pharmacol. **163,** 1605-1625.

Rutschow HL, Baskin TI, Kramer EM. 2011. Regulation of Solute Flux through Plasmodesmata in the Root Meristem. Plant Physiol. **155,** 1817-1826.

Salvador-Recatala V, Tjallingii W, Farmer EE. 2014. Real-time, invivo intracellular recordings of caterpillar-induced depolarization waves in sieve elements using aphid electrodes. New Phytol. **203,** 674-684.

Scala A, Allmann S, Mirabella R, Haring MA, Schuurink RC. 2013. Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. Int.J.Mol.Sci. **14,** 17781-17811.

Short EF, North KA, Roberts MR, Hetherington AM, Shirras AD, McAinsh MR. 2012. A stress-specific calcium signature regulating an ozone-responsive gene expression network in Arabidopsis. Plant J. **71,** 948-961.

Singh H, Dixit S, Singh P, Verma PC. 2013. Differential peroxidase activities in three different crops upon insect feeding. Plant Sign.Behav. **8**.

Skelly MJ, Loake GJ. 2013. Synthesis of Redox-Active Molecules and Their Signaling Functions During the Expression of Plant Disease Resistance. Antiox.Redox Signal. **19,** 990-997.

Suzuki N, Miller G, Morales J, Shulaev V, ngel Torres M, Mittler R. 2011. Respiratory burst oxidases: the engines of ROS signaling. Curr.Opin.Plant Biol. **14,** 691-699.

Swanson SJ, Choi WG, Chanoca A, Gilroy S. 2011. In vivo imaging of Ca^{2+}, pH, and eeactive oxygen species using fluorescent probes in plants. Annu.Rev.Plant Biol. **62,** 273-297.

Tena G, Boudsocq M, Sheen J. 2011. Protein kinase signaling networks in plant innate immunity. Curr.Opin.Plant Biol. **14,** 519-529.

Vadassery J, Reichelt M, Hause B, Gershenzon J, Boland W, Mithoefer A. 2012a. CML42-Mediated Calcium Signaling Coordinates Responses to Spodoptera Herbivory and Abiotic Stresses in Arabidopsis. Plant Physiol. **159,** 1159-1175.

Vadassery J, Scholz SS, Mithofer A. 2012b. Multiple calmodulin-like proteins in Arabidopsis are induced by insect-derived (Spodoptera littoralis) oral secretion. Plant Sign.Behav. **7,** 1277-1280.

Valmonte GR, Arthur K, Higgins CM, MacDiarmid RM. 2014. Calcium-Dependent Protein Kinases in Plants: Evolution, Expression and Function. Plant Cell Physiol. **55,** 551-569.

Van CM, Bednarski W, Borowiak-Sobkowiak B, Wilkaniec B, Samardakiewicz S, Morkunas I. 2013. Oxidative stress in pea seedling leaves in response to Acyrthosiphon pisum infestation. Phytochemistry **93,** 49-62.

VanDoorn A, Kallenbach M, Borquez AA, Baldwin IT, Bonaventure G. 2010. Rapid modification of the insect elicitor N-linolenoyl-glutamate via a lipoxygenase-mediated mechanism on *Nicotiana attenuata* leaves. Bmc Plant Biol. **10**.

Verrillo F, Occhipinti A, Kanchiswamy CN, Maffei ME. 2014. Quantitative analysis of herbivore-induced cytosolic calcium by using a Cameleon (YC 3.6) calcium sensor in *Arabidopsis thaliana* . J.Plant Physiol. **171,** 136-139.

Volkov A. 2012 Plant Electrophysiology: Methods and Cell Electrophysiology. Heildelberg: Springer.

Volkov AG, Vilfranc CL, Murphy VA, Mitchell CM, Volkova MI, O'Neal L, Markin VS. 2013. Electrotonic and action potentials in the Venus flytrap. J.Plant Physiol. **170,** 838-846.

Volkov AG, Foster JC, Markin VS. 2010. Signal transduction in *Mimosa pudica*: biologically closed electrical circuits. Plant Cell Environ. **33,** 816-827.

Wang L, Allmann S, Wu J, Baldwin IT. 2008. Comparisons of Lipoxygenase3- and Jasmonate-Resistant4/6-Silenced Plants Reveal That Jasmonic Acid and Jasmonic Acid-Amino Acid Conjugates Play Different Roles in Herbivore Resistance of Nicotiana Attenuata. Plant Physiol. **146,** 904-915.

Woldemariam MG, Son TD, Oh Y, Gaquerel E, Baldwin IT, Galis I. 2013. NaMYC2 transcription factor regulates a subset of plant defense responses in Nicotiana attenuata. Bmc Plant Biol. **13**.

Wu JQ, Baldwin IT. 2009. Herbivory-induced signalling in plants: Perception and action. Plant Cell Environ. **32,** 1161-1174.

Wu J, Baldwin IT. 2010. New Insights into Plant Responses to the Attack from Insect Herbivores. Annu.Rev.Genet. **44,** 1-24.

Wu J, Hettenhausen C, Meldau S, Baldwin IT. 2007. Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. Plant Cell **19,** 1096-1122.

Wu J, Wang L, Wuensche H, Baldwin IT. 2013. Narboh D, a Respiratory Burst Oxidase Homolog in Nicotiana attenuata, is Required for Late Defense Responses After Herbivore Attack. J.Integr.Plant Biol. **55,** 187-198.

Wuensche H, Baldwin IT, Wu J. 2011a. S-Nitrosoglutathione reductase (GSNOR) mediates the biosynthesis of jasmonic acid and ethylene induced by feeding of the insect herbivore *Manduca sexta* and is important for jasmonate-elicited responses in *Nicotiana attenuata*. J.Exper.Bot. **62,** 4605-4616.

Wuensche H, Baldwin IT, Wu J. 2011b. Silencing NOA1 Elevates Herbivory-Induced Jasmonic Acid Accumulation and Compromises Most of the Carbon-Based Defense Metabolites in *Nicotiana attenuata*. J.Integr.Plant Biol. **53,** 619-631.

Yang DH, Hettenhausen C, Baldwin IT, Wu J. 2012. Silencing Nicotiana attenuata Calcium-Dependent Protein Kinases, CDPK4 and CDPK5, Strongly Up-Regulates Wound- and Herbivory-Induced Jasmonic Acid Accumulations. Plant Physiol. **159,** 1591-1607.

Yang HM, Xie SX, Wang L, Jing SL, Zhu XP, Li XW, Zeng W, Yuan HY. 2011. Identification of up-regulated genes in tea leaves under mild infestation of green leafhopper. Sci.Hortic. **130,** 476-481.

Yu QY, An LJ, Li WL. 2014. The CBL-CIPK network mediates different signaling pathways in plants. Plant Cell Rep. **33,** 203-214.

Yun BW, Spoel SH, Loake GJ. 2012. Synthesis of and signalling by small, redox active molecules in the plant immune response. Biochim.Biophys.Acta **1820,** 770-776.

Zebelo AS, Piorkowski J, Disi J, Fadamiro H. 2014. Secretions from the ventral eversible gland of *Spodoptera exigua* caterpillars activate defense-related genes and induce emission of volatile organic compounds in tomato, *Solanum lycopersicum*. Bmc Plant Biol. **14,** 140.

Zebelo SA, Maffei ME. 2012a. Signal transduction in plant–insect interactions: From membrane potential variations to metabolomics. In: Volkov A, ed. *Plant Electrophysiology*. Berlin Heidelberg: Springer-Verlag, 1-30.

Zebelo SA, Maffei ME. 2012b. The Ventral Eversible Gland (VEG) of *Spodoptera littoralis* triggers early responses to herbivory in *Arabidopsis thaliana*. Arthr.Plant Interact. **6,** 543-551.

Zebelo SA, Matsui K, Ozawa R, Maffei ME. 2012. Plasma membrane potential depolarization and cytosolic calcium flux are early events involved in tomato (*Solanum lycopersicum*) plant-to-plant communication. Plant Sci. **196,** 93-100.

Zhu S, Zhou XP, Wu XM, Jiang ZH. 2013. Structure and function of the CBL-CIPK Ca2+-decoding system in plant calcium signaling. Plant Mol.Biol.Rep. **31,** 1193-1202.

Zimmermann MR, Maischak H, Mithöfer A, Boland W, Felle HH. 2009. System Potentials, a Novel Electrical Long-Distance Apoplastic Signal in Plants, Induced by Wounding. Plant Physiol. **149,** 1593-1600.

Zipfel C. 2009. Early molecular events in PAMP-triggered immunity. Curr.Opin.Plant Biol. **12,** 414-420.

Figure 1. Calcium signaling pathways after herbivory. Following herbivory, insect-originated elicitors bind on putative polysaccharide (β-galactofuranose) or FACs receptors, leading to cytosolic Ca^{2+} homeostasis. This homeostasis is regulated by influx and efflux channels as well as by ATP-dependent Ca^{2+} pumps. The cytosolic Ca^{2+} increase induces the release of Ca^{2+} from cellular stores (mitochondria, vacuoles and the endoplasmic reticulum) via Ca^{2+} channels. The cytosolic Ca^{2+} increase triggers two cascades of signaling events: 1) the activation of inward K^+ channels, which cause Vm depolarization, and 2) the activation of CBL-interacting protein kinases (CBL-CIPK), calmodulin-like protein (CML42 & CML43), and calcium dependent protein kinases (CPK3 & CPK13) signaling pathways, which are involved in the activation of transcription factors (e.g., HSFB2A) that lead to the expression of defense genes in the nucleus. The broken arrows indicate the calcium pathways contributing to the increased cytosolic calcium concentrations.

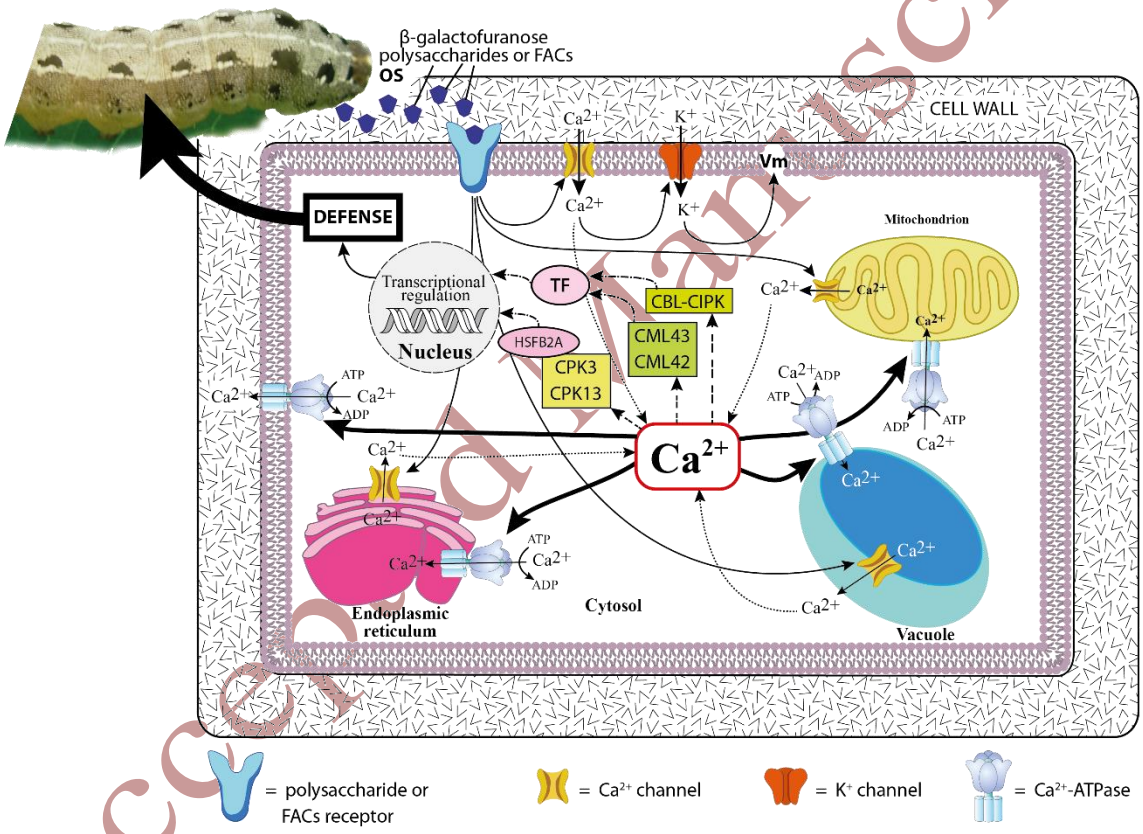

Figure 2. **The role of ROS and NRS in plant-insect interactions**. Upon herbivory, insect originated elicitors bind putative polysaccharide (β-galactofuranose) or FACs receptors, leading to the cytosolic Ca^{2+} homeostasis (see Figure 1). The cytosolic Ca^{2+} increase triggers the CBL-CIPK signaling pathways, which activate the plasma membrane NADP oxidase (ROBH). This in turn generates the superoxide radical anion, which is eventually dismutated to hydrogen peroxide (H_2O_2) by the catalytic activity of superoxide dismutase (SOD). The activity of ROBH is also regulated by external ATP. H_2O_2 acts directly on herbivores or enters the cytosol through hydroperoxiporins, increasing the cytosolic H_2O_2 concentration. By the action of mitochondrial Fenton reaction and the activity of catalase (CAT) peroxidase (POD) and ascorbate peroxidase (APX), the levels of H_2O_2 are reduced. In contrast, the activity of peroxisomal xanthine oxidase, mitochondrial CIPI/CIPIII, Ubiquinone, and chloroplastic PSI catalyze the conversion of O_2 to superoxide radical anion (O_2^{-}•), which contributes to the elevation of the H_2O_2 cytosolic concentration by the action of SOD. Cytosolic Ca^{2+} increase may lead to nitric oxide synthase (NOS) activity, thus increasing the cytosolic NO level. The NO adduct *S*-nitrosoglutathione (GSNO) level is reduced by the activity of *S*-nitrosoglutathione reductase (GSNOR), producing the oxidized glutathione disulphide (GSSG) and NH_3. Nitric oxide-associated protein 1 (NOA1) and N-nitroso species, as well as the activity of GSNOR have been associated with the phytohormone JA, triggering the transcriptional regulation of defense genes.

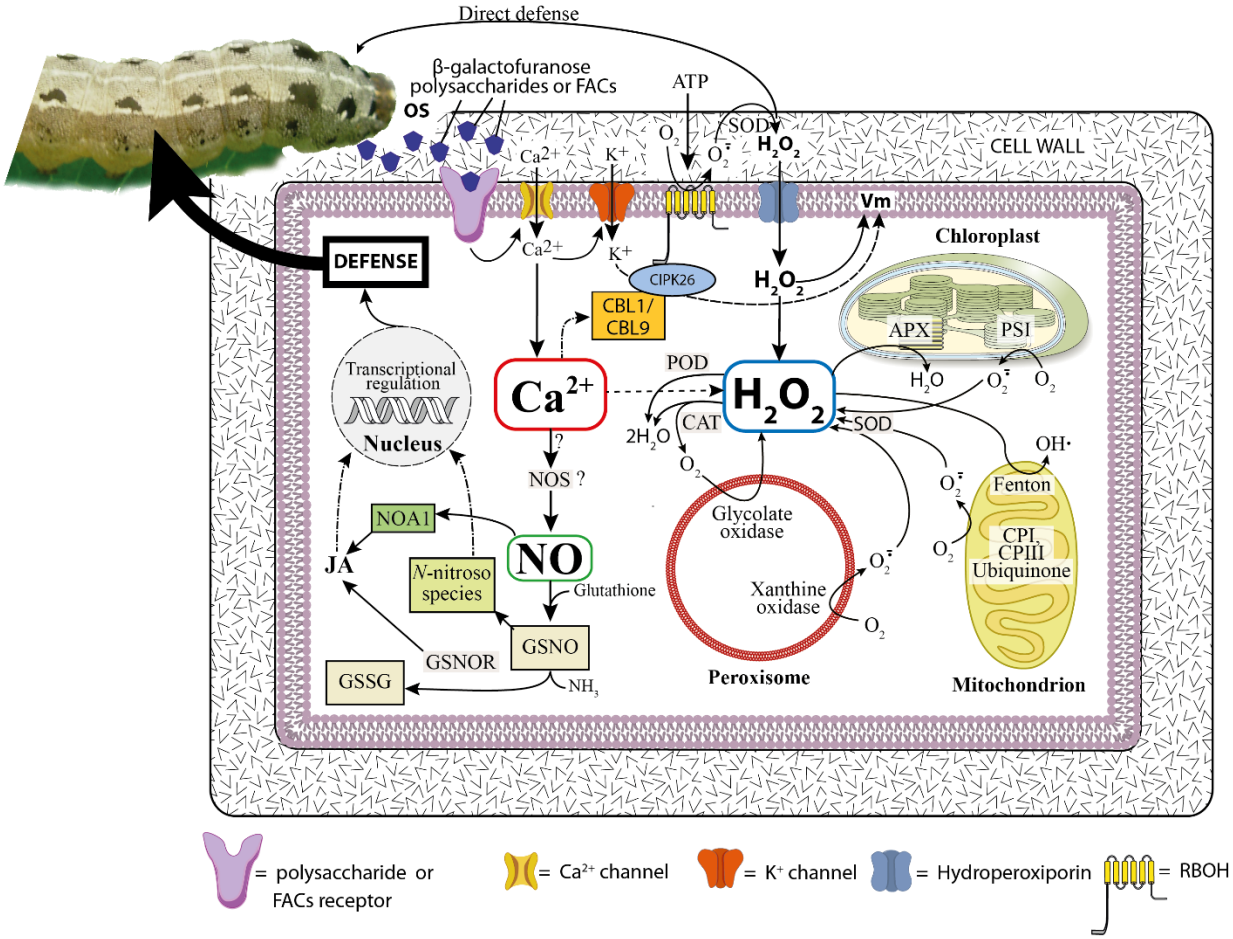

Figure 3. *Arabidopsis* model for the transmission of a moving signal from wounded to systemic tissues. Upon herbivory and the release of factors present in insect oral secretions (including a putative β-galactofuranose polysaccharide and/or FAC), both the *pdko3* and wild type (WT) plants show an increased accumulation of $[Ca^{2+}]_{cyt}$, NO and H_2O_2. In contrast, unlike WT plants, the mutant shows an almost complete loss of voltage-gated K^+ channel activity and Vm depolarization, a loss of shoot-induced root-Vm depolarization, a loss of gene expression activation and regulation of the JA defense pathway and a much diminished release and altered profile of the VOCs. These events implicate the symplastic route as probable candidate for the means by which moving molecules reach systemic tissues.