20 Electrophysiology and Plant Responses to Biotic Stress

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Plants respond actively to biotic stress by sensing and triggering cascades of signals that lead to the production of toxic compounds, spreading from secondary metabolites to reactive oxygen species. Here, we show that the evaluation of plasma transmembrane potential (V_m) is a powerful tool for the deciphering of earlier events following biotic attacks. After a short introduction and definition of abiotic and biotic stress, we describe how plants react to herbivore attack by changing V_m and how this can be measured using electrophysiology.

20.1 Abiotic and biotic stress

20.1.1 What is an abiotic stress?

One important feature distinguishing plants from other complex multicellular organisms is that plants are static organisms and thus cannot escape environmental challenges. Abiotic stresses are caused by physical Earth's forces such as salt, water, light, heat and cold stresses. Although clearly different from each other in their physical nature, each of them elicit specific plant responses as well as activate some common reactions in plants (Zhu 2001). Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment (Wang et al. 2003). Abiotic stress is the primary cause of crop loss worldwide (more than 50% yield reduction for most major crop plants; Boyer 1982; Bray et al. 2000). Abiotic stress often leads to morphological, physiological, biochemical and molecular changes affecting plant growth and productivity (Wang et al. 2001). Abiotic stresses may activate cell signaling pathways (Knight and Knight 2001; Zhu 2001, 2002) and cellular responses (Wang et al. 2003) that can lead to alteration of the transmembrane potential (V_m). In general, V_m variations depend on unbalanced ion distribution across the plasma membrane and depolarization occurs

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when cations (such as K^+ and Ca^{2+}) are allowed to enter the cell or upon anion efflux. On the other hand, hyperpolarization mainly depends on the activity of the plasma membrane H^+-ATPase or when inward anion channels (or outward cation channels) are opened. The primary candidate for intercellular signaling in higher plants is the stimulus-induced change in V_m and excitation waves transmit information from one part of the plant to another with a speed of propagation of the action potential that in soybean can reach 40 m s^{-1} (Shevstova et al. 2001). Since ion fluxes through channels directly influence V_m, it seems reasonable to assume that molecules able to act on channel activity might be considered as important factors inducing electrical signals (Maffei et al. 2004). Under abiotic stress, the up-regulation of free radical scavenging systems is a common component of the response (Pasternak et al. 2005), as are heat stress (Dat et al. 1998; Larkindale and Knight 2002), UV-radiation stress (Brosche and Strid 2003), photoinhibition (Muller-Moule et al. 2003), heavy metal stress (Pinto et al. 2003) and anoxia (Blokhina et al. 2001). All of them may have consistent repercussions on the balance of ions across the plasma membrane, and hence on V_m. Emerging evidence suggests a broader role for common signals (such as reactive oxygen species) that mediate responses to abiotic environment, developmental cues, infection and the programmed cell death in different cell types (Torres and Dangl 2005) making tools to detect abiotic stress responses useful to quantify other plant responses. While trying to balance water deficits and carbon assimilation, plants must integrate additional information on light quality, nutrient status and temperature to make "informed decisions" to add to the pressure posed by the presence of biotic stress.

20.1.2 What is a biotic stress?

As primary producers in the food chain, plants are the source of carbon, protein, vitamins and minerals for all heterotrophic organisms, from bacteria to humans. Thus we can define biotic stress as the pressure posed on plants by living organisms. In recent years, the molecular basis of biotic stress responses in plants (Maleck et al. 2000) has been identified (reviewed by Karpinski et al. 2003). Among biotic stress, the most studied are microbial infections and herbivore attack. Based on their effects on the plant, microbes interacting with plants can be classified as pathogenic, saprophytic and beneficial. Pathogens can attack leaves, stems or roots. Current models of the mechanisms of plant defense against pathogen infection are based on animal models, and have been recently linked to the light-sensing network and to the oxygen-evolving complex in photosystem II (PSII) (Abbink et al. 2002). Much progress has been made in understanding the mechanisms by which plants detect and defend themselves against pathogens (Kunkel and Brooks 2002). Progress has been done in cloning and characterization of plant disease resistance genes that govern the recognition of specific pathogen strains

(Staskawicz et al. 2001; Dangl and Jones 2001), the deciphering of signal transduction pathways for the activation of defense responses (Feys and Parker 2000; Glazebrook 2001), and the characterization of endogenous plant signaling molecules involved in plant defense [salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Dong 1998; Thomma et al. 2001)]. The current advances of the roles of the SA, JA and ET signaling pathways in pathogen defense has been summarized in several recent reviews (Kunkel and Brooks 2002 and references therein). There is also a growing body of literature that reports that the JA, SA and ET defense signaling pathways do not function independently, but show an active crosstalk (Kunkel and Brooks 2002). Recent studies indicate that defense signaling may be even more complex than expected, and that additional plant signaling pathways are likely to be involved in regulating pathogen defense, most of them involving ion fluxes and then variations in V_m.

Recognition is considered to be the initial key event in the response of plants to microbes. Recognition can occur through physical interaction, such as through adhesins, fimbriae, flagella, and type III and type IV secretion systems, or through signaling by small molecules (Lugtenberg et al. 2002). Early events during pathogen attack, before gene expression, involve the release of cell wall oligosaccharides (so-called elicitors) which can be recognized by specific receptors able to trigger signaling cascades involving ion fluxes and activation of reactive oxygen species (ROS) forming enzymes (Kombrink and Somssich 1995). One of the two of the earliest occurrences following recognition are a calcium flux across the plasmalemma and the generation of O_2^- and H_2O_2, the so-called "oxidative burst" (Mur et al. 2005) with these two events appearing to be mutually regulated (Grant et al. 2000). The generation of the plant oxidative burst has been linked to the initiation of electron flow across the plasmalemma via a NADPH oxidase complex, analogous to that found in mammalian neutrophils (Mur et al. 2005).

The evolution of plant secondary compounds is often considered to be tightly associated with defense against biotic stress, and it has recently been proposed that plant chemical defense could also be involved in abiotic stress responses, such as photodamage (Holopainen 2004). Thus, plants possess biochemical defense mechanisms which prevent or reduce further damage from both abiotic and biotic stress. The defense includes the induction of both de novo biosynthesis and rapid accumulation of secondary metabolites, referred to as phytoalexins (Mithöfer et al. 2004). These compounds are low molecular weight organic molecules not present in all plants that may also exhibit antibiotic activities (Mithöfer et al. 2004). Regardless of the plant species, major classes of secondary metabolites are phenylpropanoids, terpenoids, and nitrogen-containing organic compounds. Secondary plant compounds are present both as constitutive as well as inducible plant defenses. Volatile organic compounds (VOCs) emitted by plants can form as by-products of plant processes and can be emitted to the atmosphere owing to their volatility (Holopainen 2004). Some volatile compounds appear to behave like

signals for plant protection and communication. Herbivore induced plant volatiles (HIPV) are VOCs emitted from aerial and underground plant organs after herbivore damage (Kessler and Baldwin 2001; Holopainen 2004). HIPV may act as an indirect plant defense by repelling non-specific herbivores or by attracting predators and parasitoids of herbivores (Heil 2004). Evidence for trade-offs between resistance to pathogens and herbivores were reported (Felton and Korth 2000).

20.2 Plant responses to herbivore attack

Plant responses to herbivore attack are complex and involve an array of signals, leading to activation of multiple defenses. Feeding herbivores cause extensive and irreversible wounding along with an introduction of salivary secretions. Both, wounding and components from the insects' secretions have an obvious, but clearly different impact on the plants response (Schittko et al. 2001 and references cited therein). In the model system *Nicotiana attenuata* and its specialist herbivore *Manduca sexta*, feeding elicits a JA burst, a large transcriptional reorganization of the plant host and, after hours, a systemic release of VOCs (Halitschke et al. 2003). Principally the same sequence is passed through in the interaction between Lima bean and spider mites (Arimura et al. 2000), and in the interaction of corn plants (*Zea mays*) with the beet armyworm (*Spodoptera exigua*) (see also Gatehouse 2002).

Recently, Maffei, Bossi and co-workers of the Max Planck Institute of Jena (Maffei et al. 2004) presented novel facets to the previously known sequence and demonstrated that herbivore attack onto a Lima bean leaf is associated with: a) a strong V_m depolarization at the bite zone causing a wave of V_m depolarization spreading throughout the entire attacked leaf and; b) a consistent influx of Ca^{2+}, at the very edge of the bite, which is halved by application of the Ca^{2+} channel blocker verapamil. Regurgitants (R) and *N*-acyl-amino acid conjugates interact with the plasma membrane and alter V_m. R from Lima bean reared larvae altered V_m in a concentration-independent fashion and its effect is clearly different from that observed in V_m studies with the individual compounds (Maffei et al. 2004). A non-linear response of V_m to the concentration of R and R-factors was observed. Possibly the effects are related to different modes of membrane V_m depolarization by either micellar transport of ions or pore formations by the conjugates and other components of R (Abramson and Shamoo 1979). Volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine), which was isolated from the oral secretions of beet armyworm (*Spodoptera exigua*) larvae and increases the emission of VOCs when applied to maize, was the first reported herbivore-specific elicitor. Unfortunately, volicitin was completely inactive on lima bean V_m (Maffei et al. 2004), moreover, neither enantiomer of volicitin was active in the induction of VOCs (Felton and Korth 2000). The

time-course and distance-dependence spreading of the V_m depolarization upon herbivore attack in intact leaves is probably associated with a molecule able to disperse within tissues at a relatively high speed. Recent results from perfusing leaves with H_2O_2 (Maffei et al. 2006) and Ethephon (the ethylene releasing agent) (unpublished data) indicate a V_m depolarizing effect of these molecules. Another interesting target is the analysis of the early events in the interaction of volatiles (including VOCs, ethylene, hydrogen peroxide and NO) emitted from wounded plants and/or perceived by neighboring healthy plants. Preliminary results already indicate compound-specific variations in V_m (Maffei et al., unpublished data). Using spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*) (Takabayashi and Dicke 1996), it has been shown that not only the attacked plant but also neighboring plants are affected, becoming more attractive to predatory mites and less susceptible to spider mites (Bruin et al. 1992). The mechanism involved in such interactions, however, remains elusive. Arimura et al. (2000) showed that uninfested lima bean leaves activate five separate defense genes when exposed to volatiles from conspecific leaves infested with *T. urticae*, but not when exposed to volatiles from artificially wounded leaves. These data indicate that gene activation is preceded by perception of VOCs and signal transduction; all involving the plant cell plasma membrane. Both wounding and the introduction of herbivore-specific elicitors appear to be essential for the full induction of defense responses. Recent studies applying a continuous rather than a single instance of mechanical damage (pattern wheel) to Lima bean leaves clearly resulted in the emission of volatile blends resembling those that occur after herbivore damage (Mithöfer et al. 2005). In accordance with Arimura and co-workers (2005), we can conclude that early and secondary cell signaling for herbivore-induced plant responses comprise: (1) the reception of an extracellular signal(s) such as high- or low-molecular weight factors from the herbivore (e.g. fatty acid–amino acid conjugates), (2) V_m depolarization and an intracellular calcium influx, (3) the activation of protein kinase/phosphatase cascades, and (4) the release of linolenic acid from the cell membrane and subsequent activation of the octadecanoid pathway which leads finally to the synthesis of JA and other oxylipins.

Until recently, herbivore-induced indirect defenses have largely been a laboratory phenomenon, but a recent study of *N. attenuata* plants growing in natural populations demonstrated, by manipulating the release of single compounds in the herbivore-induced VOC bouquet, that VOC emission resulted in increased predation rate of *Manduca* eggs by a generalist predator and decreased oviposition rate by the adult moths (Baldwin et al. 2001).

Recent physiological studies have linked the plant signal transduction pathways that result in induction of direct defenses in leaves to indirect defences that act through the production of volatiles that attract natural enemies of herbivores (Agrawal 2000).

20.3 Plant responses to plant attack

VOCs are also emitted by plants to cope with other plants for nutrition in what is called allelopathy. Allelopathy is the negative effect of chemicals released by one plant species on the growth or reproduction of another (Inderjit and Callaway 2003). Plants synthesize a great variety of terpenoid natural products, which can be involved in allelopathic interactions. The ability of allelochemicals to alter membrane permeability and affect V_m (thus inhibiting mineral absorption) has been investigated since the 1970s (Balke 1985).

Many phenolic compounds induce efflux of anions and cations, and inhibition of uptake may depend on alterations and perturbations induced in the inner membrane by specific binding or by prevention of the development of an electrochemical V_m (Moreland and Novitzky 1987). Isosakutranetin (ISK; 5,7-dihydroxy 4′-methoxy flavanone) is a plant exudate with known cytotoxic and fungicide properties. When tested on wheat roots it inhibited K^+-dependent H^+ extrusionand net K^+ uptake. ISK acts on wheat roots as an inhibitor of K^+ permeation suggesting a major role of ISK as an allelopathic molecule (Sacco and Maffei 1997).

Monoterpenoids are the major components of some essential oils: they have toxic effects on seed germination (Robinson 1983; Rice 1984), growth of some bacterial strains (Knobloch et al. 1989; Economou and Nahrstedt 1991), development and growth of some insects (Lee et al. 1999), growth of pathogenic fungi (Adam et al. 1998). Increasing the concentration of peppermint essential oil from 100 up to 900 ppm caused an increasing depolarization of cucumber root V_m (from 5 to 110 mV) (Maffei et al. 2001). A plot of log of octanol–water partition coefficient (K_{ow}) against their depolarizing effect showed a significant negative correlation, suggesting that among all monoterpenoids increased membrane depolarization depends on lower K_{ow} (Maffei et al. 2001). Recent findings have shown that monoterpenes affect biological membranes by damaging their structure and changing their lipid packing density which increases ion permeability and perturbs membrane-bound enzyme function (Griffin et al. 2000). Maffei et al. (2001) found that decreasing water solubility of monoterpenes increases the possibility for terpenoids to interact with and disrupt membrane integrity, thus causing a rapid and reversible membrane V_m depolarization.

Another important allelopathic molecule is juglone. Significant inhibition of transpiration and stomatal conductance reported by Jose and Gillispie (1998) in hydroponically grown corn and soybeans exposed to juglone suggests that this phytotoxin may interfere with normal water transport. Furthermore, a decrease in H^+-ATPase activity was positively correlated with increasing juglone concentration in corn and soybean root microsomal membranes (Hejl and Koster 2004). These data support the hypothesis that juglone-mediated reductions in growth arise from the decreased ability of the roots to translocate water secondary to inhibition of plasma membrane H^+-ATPase activity. These data also were corroborated by observations that seedlings appeared wilted, like drought-affected plants, and the roots

appeared flaccid, even though submerged in nutrient solution. Since the plant cell plasma-membrane H^+-ATPase and associated membrane proteins play an essential role in the maintenance of cell turgor and uptake of components essential for growth (Babakov et al. 2000), significant reduction in mineral and water uptake by roots subsequent to H^+-ATPase inhibition in root cells would lead to closing of stomata and have a strong indirect effect on numerous essential plant functions, such as respiration, photosynthesis, and protein synthesis, resulting in decreased growth (Hejl and Koster 2004).

20.4 Methods in plant electrophysiology following herbivore attack

20.4.1 Our model system for electrophysiology

The system developed to measure V_m in leaves is the result of many technical tests which gave at the end a useful set of both electrical, electronic and hydraulic instrumentations, with the aim of on-line (or real-time) recording of electrical variations through the plant plasma membrane.

This system was initially developed to measure membrane potential variations of the aquatic plant *Elodea densa* (Bellando et al. 1995). It consists mainly of a home-made Plexiglas block, a polymethyl methacrylate (PMMA) polymer, or even Teflon, a more inert polymer of polytetrafluoro ethylene (PTFE), unfortunately not transparent; some wells and sockets which were made and dug, as shown in Fig. 20.1. The main use of this block is to perfuse

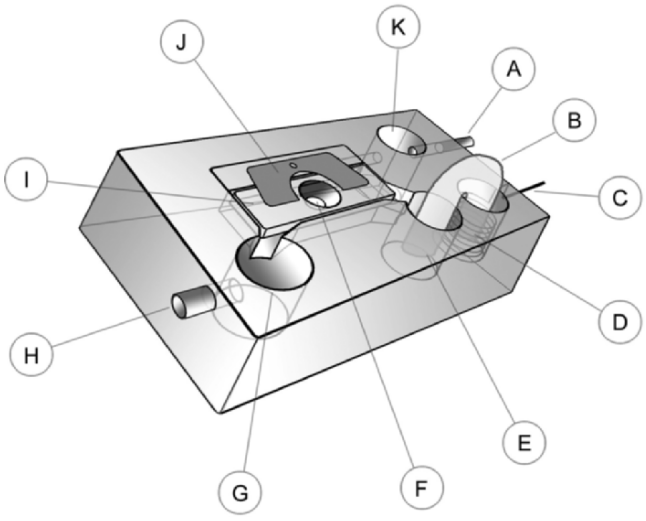

Fig. 20.1. Schematic representation of the system used for the evaluation of V_m. See description in the text

buffers or specific chemical through a leaf segment, allowing electrophysiological measurements. A small squared part of a leaf, following incubation in a fresh buffer, is placed in the central socket (Fig. 20.1F) of the block and then fixed on top (Fig. 20.1J) with a plastic holed lid (Fig. 20.1F). The hole in the lid allows the operator to reach the leaf fragment and directly measure V_m. As previously reported (Maffei et al. 2004, 2006) an external tubing system managed by a eight barrel multi channel peristaltic pump allows perfusion of buffer. Pump speed is normally 1 ml/min, flowing through the port shown as Fig. 20.1A; different other molecules could also flow through the same port (Fig. 20.1A), in this case a special setting of two-three way valves between the pump and the aperture (Fig. 20.1A) allows a convenient switching from the normal buffer to a new chemical, without stopping the V_m evaluation. The buffer driven through [A] by the peristaltic pump, reaches the first well (Fig. 20.1 K) where bubbles, if present in the perfusion liquid or in tubes, can easily emerge and dissolve. Then the perfusion medium runs directly in the central socket (Fig. 20.1F) where the leaf piece is posed and fixed and where molecules, if present in the liquid, can act on plant tissues. This central socket is in communication with two other wells (Fig. 20.1G, E); both wells are deeper than the central socket, thus allowing buffers or other liquids to flow in continuously from the central part. The well marked with [E] serves to contain one of the two ends of the salt bridge (Fig. 20.1B) and from the well [G] liquids flow out through the exhaust tube (Fig. 20.1H), to be collected or sent to waste. The well [G] is quite useful in those cases where an overflow from [A] occurs: in this situation liquids can be removed directly from the well [G] with a pipette quickly and safely. There is a last well marked with [D] in Fig. 20.1: it serves to contain the other end of the salt bridge, and, more importantly, contains a silver wire solenoid which is directly connected to the outside of the well with a male plug (Fig. 20.1C), allowing electrical connections to the circuit (see below for the electrical settings).

The core of the entire system is the electrical circuit which allows V_m measurement. In order to measure V_m we used very thin tip (2–3 μm) borosilicate glass capillaries (WPI Inc., model 1B150F-4) which are obtained with a capillary puller (Narishige model PE-21) and filled with a 3 M KCl solution prepared in ultra-pure water (Millipore). Due to the very thin tip, the 3 M KCl solution in the inner part of the glass electrode permits an efficient electrical conductance with a very low (fM) loss on ions from the electrode to the cellular matrix. Fig. 20.2 shows the glass electrode on its way to the plant cell to be impaled.

Glass microelectrodes are directly connected to a probe (WPI inc.) by means of an electrode holder (WPI Inc.); this probe does the first step of the signal cleaning up and stabilization, and is connected to a signal amplifier (WPI inc. model Electro 705). The amplifier takes the electrical signal coming from the cell and brings it amplified, cleaned and stabilized to an oscilloscope (Tektronics model TDS 210), for further digital elaboration and data storage. The signal is measured and recorded in mV. The oscilloscope also allows

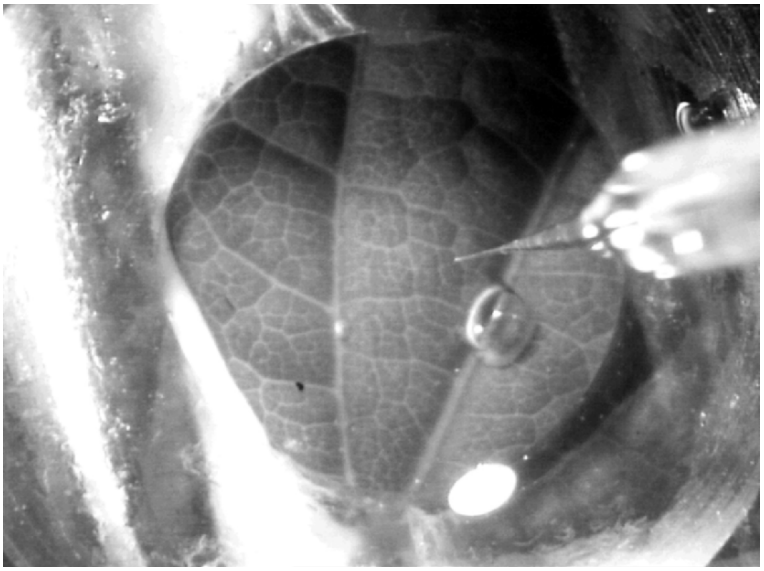

Fig. 20.2. Microcapillary made of borosilicate is used as an electrode in order to impale plant cells and detect V_m

seeing the wave of the electrical signal, the shape of which gives important information about cell condition, electrode integrity and, in general, the electrical conditions of the entire system. The oscilloscope is also plugged to the ground, to complete the electrical circuit; ground is represented by the silver wire solenoid in the well (Fig. 20.1D). The ground is a special silver–silver chloride electrode which acts as signal transducer by converting ionic currents in solution to an electric current within a wire, the same operation done by the probe attached to the glass electrode. The silver wire is plated with chlorine by electroplating, which ensures stability and good conductibility to the silver wire. The well where the ground is present is filled with a 3 M KCl^+ saturated $AgCl_3$ solution which allows electrical communication with the salt bridge (Fig. 20.1B). The salt bridge is the electric link between the ground and the buffer solution in which the plant material is immersed; it is a curled glass pipe filled with agarose jellified solution containing 3 M KCl. The entire system, set up as explained, gives a signal with a wave shape quite near to a square wave; in fact parts of system acts as an electrical capacitor. To solve this problem, a special device, a variable resistance connected in parallel, is mounted between the silver–silver chloride electrode and the oscilloscope, resulting in straight line electrical signal more convenient for measuring and recording. Figure 20.3 depicts the scheme of how this variable resistance is working. “IN” represents the wire coming from the ground, “OUT” is the wire connected to the oscilloscope, 1 is a switch, the general ground is marked with 2 and the variable resistance is marked with 3.

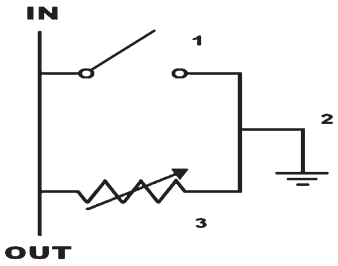

Fig. 20.3. Scheme of the circuit used to trigger the signal

Finally, Fig. 20.4 shows a simplified scheme of the electrical circuit.

All instruments and electrical devices are very sensitive to both physical vibration and environmental electrical noises, thus all the equipment is mounted on a stable work table, electrically grounded and kept under a Faraday cage; all cables and wires, when not shielded properly, are wrapped with aluminum foil in order to reduce noise. Obtaining a good V_m is a very delicate operation and the use of a micromanipulator, which can move in three directions, is fundamental. A stereo microscope or a special video camera is also needed in order to see exactly where to position the electrode onto the leaf tissue. Data recording is performed mainly with two different systems: data or electrical signals from the V_m are simply directed to a normal paper recorder which plots an immediate graphical image of what is going on during an experiment. The second is represented by the oscilloscope's signal that can go directly to a computer: the oscilloscope takes the analogical electrical signals and transform them into digital data, transferred by a serial

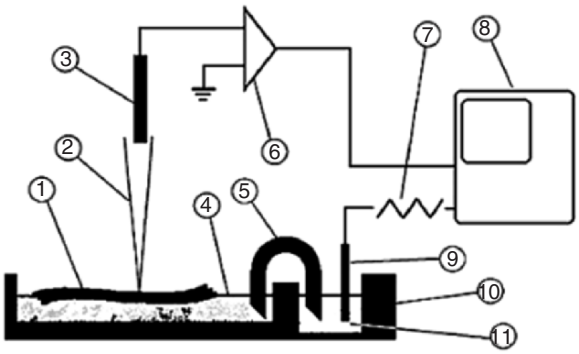

Fig. 20.4. General simplified representation of the system used to detect V_m. *1*, leaf; *2*, borosilicate capillary electrode; *3*, probe; *4*, perfusion liquid; *5*, 3 M agarose/KCl salt bridge; *6*, amplifier; *7*, variable resistance; *8*, oscilloscope; *9*, $Ag/AgCl_3$ electrode; *10*, plexiglas block; *11*, 3 M $KCl/AgCl_3$ saturated solution

cable to the computer; a special software (WaveWork v 4.2a Scope K&S Elecktronik) can draw these data in graphics and can transform them in text tables.

All the system described above is useful for experiments in which V_m variations, if present, are depending on the kind of solution perfused to the plant and on the molecules of interest present in the medium. For this reason, experiments can only be carried out with the treatment with molecules easily miscible in aqueous media. Many compounds are dissolved in organic solvents, like methanol, and then dissolved in the aqueous buffer, but some of them may have a very low polarity. These molecules are also volatile, making difficult to keep their concentration at a constant value during the experiment. To overcome problems linked to molecule solubility, we developed a new system, mainly based on the above described system. Figure 20.5 shows how a plant cutting can be analyzed. A plastic block (Fig. 20.5G) in which an entire aerial part of a plant (Fig. 20.5A) is positioned and immersed in buffer (Fig. 20.5H). The membrane potential is captured with a glass electrode (Fig. 20.5B) directly over an entire leaf fixed on a stative (Fig. 20.5C); the other parts of the electric devices are similar to those described above, silver–silver chloride ground and salt bridge (Fig. 20.5F) operate in the same way as above. The novelty of the system is mainly represented by the plastic chamber (Fig. 20.5D) which hosts both the plant leaf and the electrode, allowing evaluation of gaseous treatment over the plant; the plant and the electrode are gently sealed with rubber in order to allow small movements of the electrode and to maintain the plant into a closed environment. Special ports (Fig. 20.5E, I) are made in order to drive in and out a volatile compound allowing a real-time recording of a V_m induced by a controlled gaseous treatment of a particular molecule.

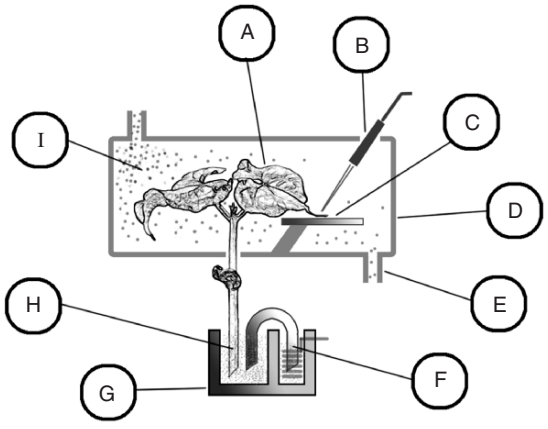

Fig. 20.5. Scheme of the new system used for the detection of V_m in intact leaves. See text for description

20.4.2 Bite and wounds: is there any difference?

The results of the measurement of membrane potential after mechanical wounding and herbivore attack indicate a specific response of the leaf tissue. Lima bean leaf V_m varies according to the cell type. Preliminary tests on intact leaves allowed evaluating the average V_m of epidermal, guard cell, palisade and spongy parenchyma cells. Epidermal cells have an average V_m of −50 mV (± 5.7 mV), guard cells have an average V_m of −200 mV (± 12.2 mV), palisade cells have an average V_m of −140 mV (± 9.8 mV), and spongy parenchyma cells have an average V_m of −100 mV (± 10.5 mV). Different trials demonstrated that Lima bean palisade cells are the most responsive cells, when leaf tissues are attacked by larvae of *S. littoralis* (Maffei et al. 2004).

To study the early effects at the bite zone and subsequent signal spreading, V_m was evaluated at increasing distances from the site of damage. The response was a strong V_m depolarization in the bite zone, followed by a transient V_m hyperpolarization and, finally, a constant V_m depolarization throughout the rest of the attacked leaf. Figure 20.6 shows the V_m variations superimposed on the wounded Lima bean leaf tissue. The ordinates represent V_m expressed in mV, while in the abscissa the bands (and the corresponding histogram bars) represent different distances (and the corresponding V_m values) from the bite zone. The V_m of the mechanically wounded leaf (control) is represented by the dashed line. Exponential interpolation shows the trend of V_m variation. A strong V_m depolarization was found up to about 1.5 mm from the bite zone, whereas a V_m hyperpolarization was found at ~2.5–3 mm from the bite zone, immediately followed by a second strong V_m depolarization. V_m differences from control in the zone from 3.5 to ~6 mm from the bite zone were not significant, but V_m displayed depolarized values from 6 mm throughout all the attacked leaf (Fig. 20.6).

The trend of the V_m variation prompted a series of experiments aimed to better understand the nature and the reasons for this effect. The first attempt was to probe whether the feeding activity of the herbivore was perceived as a V_m variation even at considerable distances from the bite zone in the same leaf. An intact leaf from a potted plant was fixed to the V_m apparatus and the V_m determined. When V_m reached a constant value *S. littoralis* was allowed to start its feeding activity. Figure 20.7 depicts V_m variations as a function of time and distance from mechanically wounded (MW) Lima bean leaf tissue, starting with a potential of about −137 mV, and V_m from a leaf under attack by *S. littoralis*. It is evident that feeding activity starts a series of V_m variations eventually leading to V_m depolarization within the first 15 min after the onset of the feeding activity. In particular when V_m was taken from palisade cells at an average distance of 5 mm a strong and transient hyperpolarization occurred within 5 min after the herbivore bite, followed by a constant depolarization. The same pattern was observed when V_m palisade cell was measured at a distance of 30 mm from the bite zone, but depolarization was higher than in cells at 5 mm distance. Finally in palisade cells which were 60 mm

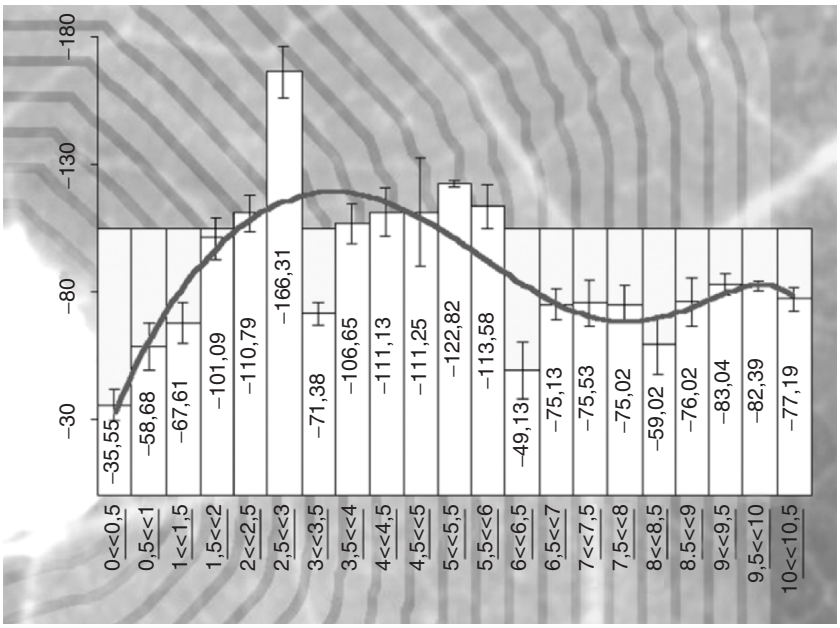

Fig. 20.6. Lima bean (*Phaseolus lunatus*) leaf V_m values as a function of distance from the bite zone 15 min after herbivore damage. The histogram superimposed on Lima bean leaf wounded by a larva of *Spodoptera littoralis* represents V_m values (and standard deviations) measured at increasing distances from the bite zone. *Upper bars* represents the average V_m value from a mechanically wounded Lima bean leaf. In the close vicinity of the bite zone (up to 1.5 mm) there is a strong drop in the V_m (depolarization), whereas at about 2.5–3 mm from the bite zone an increase of V_m is observed (hyperpolarization). About 6 mm from the bite zone throughout all leaf there is a constant V_m depolarization

distant from the bite zone V_m depolarization occurred within 2–3 min from the bite event and no hyperpolarization was observed (Fig. 20.7). From Fig. 20.7, it is evident that the recognition of the bite activity of *S. littoralis* is quickly perceived in the same leaf at increasing distances from the bite area. However, the attempt to find variations in neighboring leaves (OL) resulted in no obvious variations as did mechanical wounding (MW) on the same leaf (Fig. 20.7).

20.4.3 Action potentials and membrane potentials: continuous recording

In general, cells are electrically coupled by plasmodesmata to provide signal conduction, thus, application of mechanical or chemical damage to intact plants (e.g. connected to the V_m detector in rooted plants in pot) results in fast action potentials which can propagate up to several cm per second (Fromm et al. 1995; Volkov et al. 2001). Usually, after signal transmission the resting

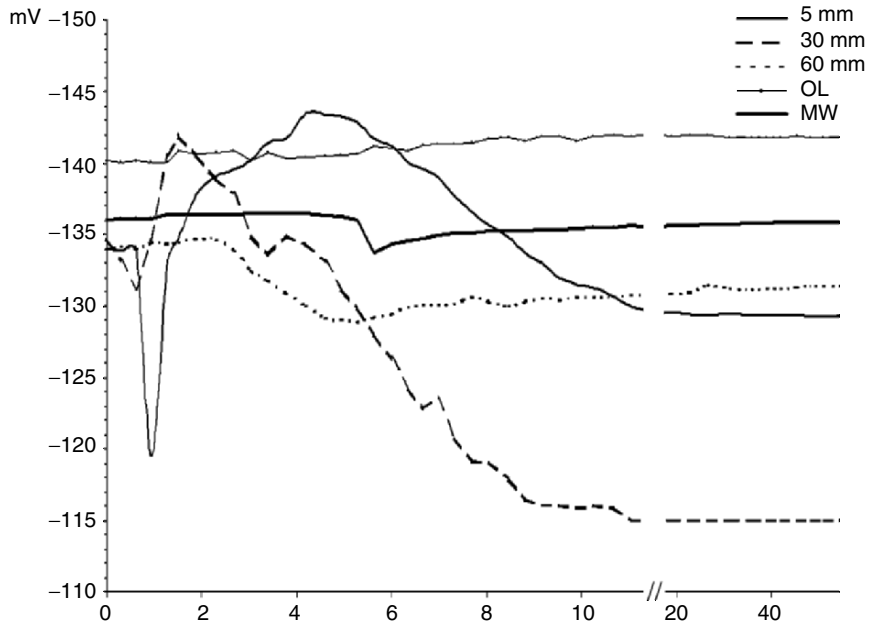

Fig. 20.7. Time-course of the V_m variations in palisade cells distant 5, 30 and 60 mm from the bite activity of *S. littoralis*. The feeding larva induces a series of V_m variations leading to V_m depolarization after about 15 min from the onset of the feeding activity. In particular, when V_m was taken at an average distance of 5 mm, a strong and transient hyperpolarization occurred within 5 min after the herbivore bite, followed by a constant depolarization. The same pattern was observed when V_m was measured at a distance of 30 mm from the bite zone, but depolarization was higher than in cells at 5 mm distance. In cells 60 mm distant from the bite zone, V_m depolarization occurred within 2–3 min after the bite, and no hyperpolarization was observed. MW=V_m value after mechanical wounding; OL=V_m value of the opposite leaf

potential remains stable. In order to evaluate the speed of depolarization in an intact leaf we grounded the basal part of the Lima bean stem and impaled a palisade cell with a micropipette. When the V_m was stable (around –140 mV), a mechanical damage was done with a small forceps at different distances from the impaled cell on the same leaf. An immediate action potential was recorded and when V_m was again stable a drop of a strong oxidant was applied at the wounded zone. After the application of the oxidant a small action potential was also recorded. After a few seconds a significant V_m depolarization was recorded and the original resting potential was not re-established, indicating that the voltage change was not an action potential. The V_m was then allowed to reach a stable value. The new value was a V_m hyperpolarized value with respect the beginning of the experiment. A second damage was then performed in another part of the leaf and an action potential was recorded, once

again a drop of a strong oxidant was applied and a V_m depolarization was observed after a longer period (Fig. 20.8).

In Lima bean the speed of the fastest V_m depolarization after a strong oxidant application was found to be about 1 mm s^{-1}, and the speed of the slowest V_m depolarization was 4 mm s^{-1}, that is 0.1 cm s^{-1} and 0.4 cm s^{-1}, respectively. In a putative isotropic and constant system, given these transmission rates, the diffusion coefficient *(D)* of a putative chemical signal would be (according to the Einstein random walk equation):

$$D_{fast} = d^2/2t = (0.1)^2/2\,(1) = 5\times 10^{-3}\,\mathrm{cm^2/s} = 5\times 10^{-7}\,\mathrm{m^2\,s^{-1}}$$

$$D_{slow} = d^2/2t = (0.4)^2/2\,(1) = 8\times 10^{-2}\,\mathrm{cm^2/s} = 8\times 10^{-6}\,\mathrm{m^2\,s^{-1}}$$

where *t* is time, and where fast and slow represent the speed of propagation after the first and the second strong oxidant application. Using the Stokes–Einstein equation, the radius *(r)* of a spherical molecule that has such a diffusion coefficient is:

$$\mathrm{D} = kT/6\pi r\eta \text{ or } \mathrm{r} = k\mathrm{T/D}6\pi\eta$$

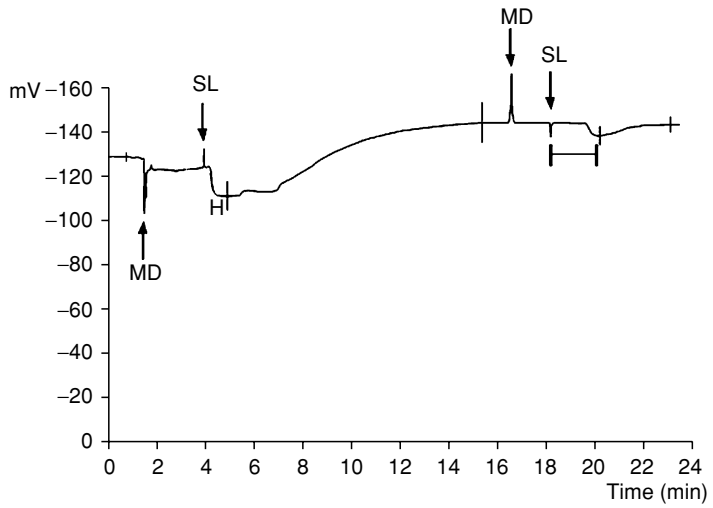

Fig. 20.8. Action potentials and membrane potentials (V_m) in Lima bean intact leaves in response to mechanical wounding and application of a strong oxidant (*SL*). V_m was measured in a palisade cell and mechanical damage (MD) was performed at different distances from the impaled cell. Actions potentials are evidenced by a fast potential change that returns to the same value. After the application of a strong oxidant a small action potential was recorded. After a few seconds a significant V_m depolarization was recorded and the original resting potential was not re-established, indicating that the voltage change was not an action potential. After some minutes the V_m is stable, but at higher (hyperpolarized) values. A second MD was then applied in another part of the leaf and an action potential was recorded, once again a drop of a strong oxidant was applied and a V_m depolarization was observed after a longer period. Metric bars indicate standard deviation

where k is Boltzmann's constant, T is absolute temperature, and η is viscosity (Pa s). If T=298 K, η=0.001 Pa s, and k=1.38×10^{-23} J/K, then r_{fast}=4.37×10^{-13} m, and r_{slow}=2.73×10^{-14} m.

Since this is about or less than the radius of a single carbon ion, it is unlikely that in this hypothetical system any molecule can diffuse so quickly. If we consider that the leaf mesophyll is not a constant environment and considering the various resistances to the spread of a signal it is reasonable to argue that the measured message might travel from the wound site by electrical signals.

20.4.4 Much more than a bite: the effect of larvae regurgitates

In order to evaluate which molecule may be responsible of V_m variations a series of experiments was carried out using regurgitate (R) collected from larvae previously feeding on Lima bean leaves for 24 h (Maffei et al. 2004).

Perfusion with R caused a V_m depolarization, however the effect was found not to be linearly linked to concentration. In fact, perfusion with 100 µg ml^{-1} R depolarized V_m more than perfusion with 250 µg ml^{-1}, but less than perfusion with 500 µg ml^{-1}. Interestingly, when R was washed out with fresh buffer, palisade V_m experienced a hyperpolarization for all concentrations, with an opposite trend as observed during depolarization (Fig. 20.9).

Since previous studies have demonstrated that R of *S. littoralis* contains several surface active, amphiphilic compounds, especially *N*-acyl glutamine conjugates (Spiteller and Boland 2003a,b) these compounds were used to study V_m variations.

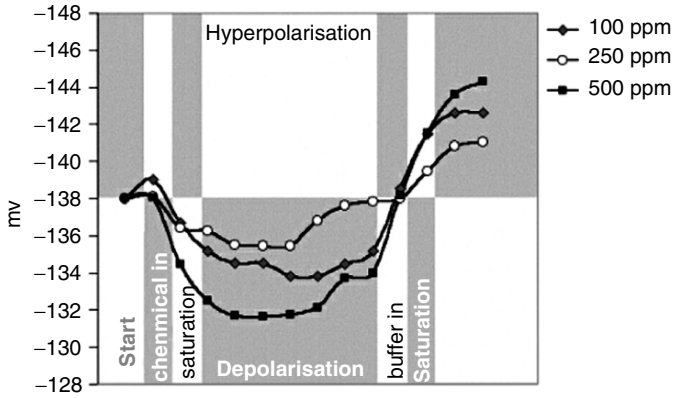

Fig. 20.9. Effect of *S. littoralis* oral secretions and regurgitants (*R*) on the V_m of Lima bean palisade cells. At the lowest concentration (100 µg ml^{-1}) R caused an intermediate V_m depolarization when compared to concentrations of 250 and 300 µg ml^{-1}. After washing the tissues with fresh buffer V_m was hyperpolarized at all concentrations, and once again R at 100 µg ml^{-1} had an intermediate value (modified from Maffei et al. 2004)

The effect of racemic volicitin (Alborn et al. 1997, 2000) and that of the naturally occurring (17*S*)-*N*-(17-hydroxylinoleoyl)-L-glutamine (Spiteller et al. 2001) on V_m was low (Maffei et al. 2004). In order to assess whether fatty acid chain length and degree of saturation have an impact on V_m, leaves were perfused with *N*-palmitoleoyl-L-glutamine and *N*-linolenoyl-L-glutamine. Perfusing cells with *N*-palmitoleoyl-L-glutamine caused a V_m depolarization at the lowest concentration used (25 μg ml^{-1}), but a V_m hyperpolarization when higher concentrations (100–300 μg ml^{-1}) were applied (Maffei et al. 2004). Removal of conjugates with fresh buffer had no effect on 25 and 300 μg ml^{-1} concentrations. A V_m depolarization was, however, observed in perfusion with 100 μg ml^{-1} (Maffei et al. 2004). Perfusion with *N*-linolenoyl-L-glutamine caused no V_m variation when used at 50 and 500 μg ml^{-1}, whereas the strongest V_m depolarization was observed at 100 μg ml^{-1} (Maffei et al. 2004).

To study the impact of the fatty acid and amino acid building blocks of the conjugates, Lima bean leaves were individually treated with linolenic acid and glutamine. Linolenic acid caused no obvious effect on V_m at low concentrations (10 and 50 μg ml^{-1}), while a weak V_m depolarization was observed when leaf tissues were perfused with L-glutamine (Maffei et al. 2004).

Because of their molecular architecture, *N*-acyl glutamines are amphiphilic compounds with a pronounced ability to form micelles, similar to known detergents such as sodium dodecyl sulfate (SDS). In order to test whether a detergent has an effect on V_m, increasing concentrations of SDS were applied to Lima bean leaves. Low concentrations had no effect, whereas at high concentration (500 mM) a clear V_m depolarization was observed, even after washing with fresh buffer (Maffei et al. 2004).

Application of the fatty acid or amino acid components of the conjugates shows virtually no effect for linolenic acid but a clear V_m depolarization for glutamine. The latter effect could play a role during larval feeding after enzymatic cleavage of the conjugates and may rely on transport processes (e.g. symport) of the amino acid (Delrot et al. 2001) and/or interaction of free glutamine with receptors. However, as yet, nothing is known about the stability of *N*-acyl amino acids in the plant cells.

20.5 Conclusions

Millions of years of continuous interaction between plants and herbivores/pathogens allowed the evolution of defense mechanisms from both sides, granting an equal and co-evoluted fitness to stress conditions. On the one side, plants have evolved the ability to respond to herbivores/pathogens by producing toxic weapons (such as many secondary metabolites) and refining the capability to detect and respond quickly to tissue damage by activating cascade signals and gene activation or to attract predators of the attacking biota. On the other hand, herbivores and pathogens evolved the ability to detoxify

poisons and to reduce plant responses by inhibiting signal transduction and/or gene activation. In all of this, the first barrier between a plant and its invader is the plasma membrane. Alteration of the balanced flux of different ions and organic acids/molecules generates a quick response that can be defined as one of the early events following a biotic attack. Depolarization of the V_m is one of the first responses of the plasma membrane and is mainly depending on anion efflux followed by calcium release from internal stores or influx from the apoplast (Hammond-Kosack and Jones 2000; Maffei et al. 2006). Thus electrophysiology is indeed a valuable tool to study and understand what is going on at the very beginning of plant interaction with other organisms (including other plants) and V_m evaluation, more that the single patch analysis, gives a tissue image of cooperative interplay among wounded and unwounded cells. Much more has to be done in this field, but the promising results obtained in intact rooted plants following biotic and abiotic stress may lead to interesting new discoveries.

References

Abbink TEM, Peart JR, Mos TNM, Baulcombe DC, Bol JF, Linthorst HJM (2002) Silencing of a gene encoding a protein component of the oxygen-evolving complex of photosystem II enhances virus replication in plants. Virology 295:307–319

Abramson JJ, Shamoo AE (1979) Anionic detergents as divalent-cation ionophores across black lipid-membranes. J Membr Biol 50:241–255

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998) Antifungal activities of *Origanum vulgare* subsp. Hirtum, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. J Agric Food Chem 46:1739–1745

Agrawal AA (2000) Mechanisms, ecological consequences and agricultural implications of tritrophic interactions. Curr Opin Plant Biol 3:329–335

Alborn HT, Jones TH, Stenhagen GS, Tumlinson JH (2000) Identification and synthesis of volicitin and related components from beet armyworm oral secretions. J Chem Ecol 26:203–220

Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. Science 276:873–949

Arimura GI, Ozawa R, Shimoda T, Nishioka T, Boland W, Takabayashi J (2000) Herbivory-induced volatiles elicit defence genes in lima bean leaves. Nature 406:512–515

Arimura GI, Kost C, Boland W (2005) Herbivore-induced, indirect plant defences. Biochim Biophys Acta 1734:91–111

Babakov AV, Chelysheva VV, Klychnikov OI, Zorinyanz SE, Trofimova MS, De Boer AH (2000) Involvement of 14-3-3 Proteins in Osmotic Regulation of H^+-ATPase in Plant Plasma Membrane. Planta, 211:446–448

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant-insect interactions. Curr Opin Plant Biol 4:351–358

Balke NE (1985) Effects of allelochemicals on mineral uptake and associated physiological processes. In: Thompson AC (ed) The chemistry of allelopathy. Biochemical interactions among plants. America Chemical Society, Washington, D.C., pp 161–178

Bellando M, Marrè MT, Sacco S, Talarico A, Venegoni A, Marrè E. (1995) Transmembrane potential-mediated coupling between H^+ pump operation and K^+ fluxes in *Elodea densa* leaves hyperpolarized by fusicoccin, light or acid load. Plant Cell Environ 18:963–976

Blokhina OB, Chirkova TV, Fagerstedt KV (2001) Anoxic stress leads to hydrogen peroxide formation in plant cells. J Exp Bot 52:1179–1190
Boyer JS (1982) Plant productivity and environment. Science 218:443–448
Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. In: Gruissem W, Buchannan B, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249
Brosche N, Strid A (2003) Molecular events following perception of ultraviolet-B radiation by plants. Physiol Plant 17:1–10
Bruin J, Dicke M, Sabelis MW (1992) Plants are better protected against spider-mites after exposure to volatiles from infested conspecifics. Experientia 48:525–529
Dangl JL, Jones JD (2001) Plant pathogens and integrated defense responses to infection. Nature 411:826–833
Dat JF, Foyer CH, Scott IM (1998) Changes in salicylic acid and antioxidants during induction of thermotolerance in mustard seedlings. Plant Physiol 118:1455–1461
Delrot S, Atanassova R, Gomès E, Coutos-Thévenot P (2001) Plasma membrane transporters: a machinery for uptake of organic solutes and stress resistance. Plant Sci 161:391–404
Dong X (1998) SA, JA, ethylene, and disease resistance in plants. Curr Opin Plant Biol 1:316–323
Economou D, Nahrstedt A (1991) Chemical, physiological, and toxicological aspects of the essential oil of some species of the genus *Bystropogon*. Planta Med 57:347–351
Felton GW, Korth KL (2000) Trade-offs between pathogen and erbivore resistance. Curr Opin Plant Biol 3:309–314
Feys BJ, Parker JE (2000) Interplay of signaling pathways in plant disease resistance. Trends Genet 16:449–455
Fromm J, Hajirezaei M, Wilke L (1995) The biochemical response of electrical signaling in the reproductive system of *Hibiscus* plants. Plant Physiol 109:375–384
Gatehouse JA (2002) Plant resistance towards insects herbivores: a dynamic interaction. New Phytol 156:145–169
Glazebrook J (2001) Genes controlling expression of defense responses in *Arabidopsis*—2001 status. Curr Opin Plant Biol 4:301–308
Grant JJ, Yun BW, Loake GJ (2000) Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. Plant J 24:569–582
Griffin S, Markham JL, Dennis G, Grant Wyllie S (2000) Using atomic force microscopy to view the effects of terpenoids on the stability and packing of phosphatidylcholine supported lipid bilayers. Proceedings of the 31st international symposium on essential oils, Hamburg, 10–13 September 2000
Halitschke R, Gase K, Hui D, Schmidt DD, Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. Plant Physiol 131:1894–1902
Hammond-Kosack K, Jones JDG (2000) Responses to plant pathogens. In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD, pp 1102–1156
Hejl AM, Koster KL (2004) Juglone disrupts root plasma membrane H^+-ATPase activity and impairs water uptake, root respiration, and growth in soybean (*Glycine max*) and corn (*Zea mays*). J Chem Ecol 30:453–471
Heil M (2004) Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by wild Lima bean (*Phaseolus lunatus*). J Chem Ecol 30:1289–1295
Holopainen JK (2004) Multiple functions of inducible plant volatiles. Trends Plant Sci 9:529–533
Inderjit, Callaway RM (2003) Experimental designs for the study of allelopathy. Plant Soil 256:1–11

Jose S, Gillispie AR (1998) Allelopathy in black walnut (*Juglans nigra* L.) alley cropping. II. Effects of juglone on hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) growth and physiology. Plant Soil 203:199–205

Karpinski S, Gabrys H, Karpinska B, Mullineaux, P (2003) Light perception in plant disease defence mechanisms. Curr Op Plant Biol 6:390–396

Kessler A, Baldwin IT (2001) Defensive function of herbivoreinduced plant volatile emissions in nature. Science 291:2141–2144

Knight H, Knight MR (2001) Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci 6:262–267

Knobloch K, Pauli A, Iberl B, Weigand H, Weis N (1989) Antibacterial and antifungal properties of essential oil components. J Essent Oil Res 1:119–128

Kombrink E, Somssich IE (1995) Defense responses of plants to pathogens. Adv Bot Res 21:1–34

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene and salicylic acid. Plant Physiol 128:682–695

Lee S, Tsao R, Coats JR (1999) Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrisomelidae), twospotted spider mite (Acari: Tetranychidae) and house fly (Diptera: Muscidae). J Econ Entomol 92:56–67

Lugtenberg BJJ, Chin-A-Woeng TFC, Bloemberg GV (2002) Microbe-plant interactions: principles and mechanisms. Antonie van Leeuwenhoek 81:373–383

Maffei M, Camusso W, Sacco S (2001) Effects of *Mentha* x *piperita* essential oil and monoterpenes on cucumber root membrane potential. Phytochemistry 58:703–707

Maffei M, Bossi S, Spiteller D, Mithoefer 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, Mithöfer A, Arimura G-I, Uchtenhagen H, Bossi S, Bertea CM, Starvaggi Cucuzza L, Novero M, Volpe V, Quadro S, Boland W (2006) Effects of feeding *Spodoptera littoralis* on Lima bean leaves. III. Membrane Depolarization and Involvement of Hydrogen Peroxide. Plant Physiol 140:1022–1035

Maleck K, Levine A, Eulgem T, Morgan A, Schmidt J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat Genet 26:403–409

Mithöfer A, Schulze B, Boland W (2004) Biotic and heavy metal stress response in plants: evidence for common signals. FEBS Lett 566:1–5

Mithöfer A, Wanner G., Boland W (2005) Effects of feeding *Spodoptera littoralis* on Lima Bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. Plant Physiol 137:1160–1168

Moreland DE, Novitzky WP (1987) Effects of phenolic acids, coumarins, and flavonoids on isolated chloroplasts and mitochondria. In: Walzer GR (ed) Allelochemicals: role in agricolture and forestry. American Chemical Society, Washington D.C., pp 247–261

Muller-Moule P, Havaux M, Niyogi KK (2003) Zeaxanthin deficiency enhances the high light sensitivity of an ascorbate-deficient mutant of *Arabidopsis*. Plant Physiol 133:748–760

Mur L, Kenton P, Draper J (2005) *In planta* measurements of oxidative bursts elicited by avirulent and virulent bacterial pathogens suggests that H_2O_2 is insufficient to elicit cell death in tobacco. Plant Cell Environ 28:548–561

Pasternak T, Rudas V, Potters G, Jansen MAK (2005) Morphogenic effects of abiotic stress: reorientation of growth in *Arabidopsis thaliana* seedlings. Environ Exp Bot 53:299–314

Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P (2003) Heavy metal-induced oxidative stress in algae. J Phycol 39:1008–1018

Rice EL (1984) Allelopathy, 2nd edn. Academic Press, Orlando

Robinson T (1983) The organic constituents of higher plants, 5th edn. Cordus Press, North Amherst, Mass.

Sacco S, Maffei M (1997) The effect of isosakuranetin (5,7-dihydroxy 4′-methoxy flavanone) on potassium uptake in wheat root segments. Phytochemistry 46:245–248
Schittko U, Hermsmeier D, Baldwin IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. II. Accumulation of plant mRNAs in response to insect-derived cues. Plant Physiol 125:701–710
Shvetsova T, Mwesigwa J, Volkov AG (2001) Plant electrophysiology: FCCP induces action potential and excitation waves in soybean. Plant Sci 161:901–909
Spiteller D, Boland W (2003a) *N*-(15,16-dihydroxylinoleoyl)-glutamine and *N*-(15,16-epoxylinoleoyl)-glutamine isolated from oral secretions of lepidopteran larvae. Tetrahedron 59:135–139
Spiteller D, Boland W (2003b) Identification and synthesis of *N*-(17-acyloxyacyl)-glutamine conjugates from oral secretion of lepidopteran larvae. J Org Chem 68:8743–8749
Spiteller D, Pohnert G, Boland W (2001) Absolute configuration of volicitin; an elicitor of plant volatile biosynthesis from Lepidopteran larvae. Tetrahedron Lett 42:1483–1485
Staskawicz BJ, Mudgett MB, Dangl JL, Galan JE (2001) Common and contrasting themes of plant and animal diseases. Science 292:2285–2289
Takabayashi J, Dicke M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. Trends Plant Sci 1:109–113
Thomma BP, Penninckx IA, Broekaert WF, Cammue BP (2001) The complexity of disease signaling in *Arabidopsis*. Curr Opin Immunol 13:63–68
Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8:397–403
Volkov AG, Dunkley TC, Morgan SA, Ruff D II, Boyce YL, Labady AJ (2001) Bioelectrochemical signalling in green plants induced by photosensory systems. Bioelectrochemistry 63:91–94
Wang W, Vinocur B, Shoseyov O, Altman A (2001) Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. Acta Hort 560:285–292
Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14
Zhu JK (2001) Cell signaling under salt, water and cold stresses. Curr Opin Plant Biol 4:401–406
Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–73