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Review

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Systemic Acquired Resistance (50 Years after Discovery): Moving from the Lab to the Field

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ABSTRACT: Induction of plant defense(s) against pathogen challenge(s) has been the object of progressively more intense research in the past two decades. Insights on mechanisms of systemic acquired resistance (SAR) and similar, alternative processes, as well as on problems encountered on moving to their practical application in open field, have been carefully pursued and, as far as possible, defined. In reviewing the number of research works published in metabolomic, genetic, biochemical, and crop protection correlated disciplines, the following outline has been adopted: 1, introduction to the processes currently considered as models of the innate immunity; 2, primary signals, such as salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA), involved with different roles in the above-mentioned processes; 3, long-distance signals, identified from petiole exudates as mobile signaling metabolites during expressed resistance; 4, exogenous inducers, including the most significant chemicals known to stimulate the plant resistance induction and originated from both synthetic and natural sources; 5, fungicides shown to act as stimulators of SAR in addition to their biocidal action; 6, elusive mechanism of priming, reporting on the most recent working hypotheses on the pretranscriptional ways through which treated plants may express resistance upon pathogen attack and how this resistance can be transmitted to the next generation; 7, fitness costs and benefits of SAR so far reported from field application of induced resistance; 8, factors affecting efficacy of induced resistance in the open field, indicating that forces, unrevealed under controlled conditions, may be operative in the field; 9, concluding remarks address the efforts required to apply the strategy of crop resistance induction according to the rules of integrated pest management.

KEYWORDS: *induced resistance, SAR, ISR, BABA-IR, SA, JA, ABA, NPR1, long-distance signals, BTH, chitosan, laminarin, saccharin, silicon, fungicides, probenazole, phosphites, priming, integrated pest management*

INTRODUCTION

After the original observation by Ross that plants may acquire a sort of unspecific, systemic immunity following a preliminary localized infection, the related process, known as systemic acquired resistance (SAR), has been thoroughly investigated.[1] A number of studies, covering phytopathological, chemical and biochemical, genetic and genomic, molecular, and agronomic sciences, have contributively assembled and continue to reveal an impressive volume of facts and information, including a successfully potential way of exploiting the phenomenological process in crop protection. Models aimed to suggest and understand how the process of SAR may develop, in either its innate or evolved expression, as well as in its chemically induced potency, have been frequently published.[2−5] Recent reviews, in addition to the modes of action, also deal with the effectiveness of induced resistance under field conditions.[6−8]

Despite all of these thorough investigations, a molecular basis of SAR is still far from being clearly formulated. Although being the most investigated model of plant-induced resistance, it represents just one of the multifunctional defense mechanisms of plants. The number of facts emerging in recent decades allows, in fact, the consideration of SAR as one of the players in a multifaceted defense system inducible in plants according to distinct pathways, characterized by different signals, metabolites, and genes. SAR is then defined as the process depending on salicylic acid and involving the transduction protein NPR1 to develop a defense response. A second process, called induced systemic resistance (ISR), is induced by symbionts and orchestrates a pathway depending on other hormones, such as jasmonate and ethylene. A third mechanism of defense, the so-called β-aminobutyric acid-induced resistance (BABA-IR), has emerged in the past decade through the discovery that BABA exogenous application can activate multiple immune responses by potentiating SA-inducible defenses and priming for pathogen-induced callose deposition, independent of salicylic acid (SA) and jasmonic acid (JA). However, priming for callose deposition requires intact biosynthesis and perception of abscisic acid (ABA).[9] These mechanisms are interconnected and contribute to create a network of defenses controlled by the plant's innate immune system that may, more properly, be included in the generic term of induced resistance, of which SAR represents the best known, but not the only, process involved in response to pathogen challenges.

To account for a general view of innate immunity, several models assume that early (or basal) plant defenses are triggered by suitable pattern recognition receptors (PRRs) that recognize pathogen/microbe-associated molecular patterns (PAMPs/MAMPs).[10] Pathogen evolution would have overcome this line of defense by means of secreted effectors that suppress the so-called pathogen-triggered immunity (PTI). As a counter-attack, plants would have developed R proteins that recognize

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specific effectors originating the effector-triggered immunity (ETI, formerly called R-gene-based or vertical immunity). The resulting effects would recall the gene-for-gene concept and conform with the hypersensitive response (HR) and the entire development of SAR.[11] The responses to pathogen attack may be preactivated (alerted) or regulated by a number of signals and triggered by endogenous or synthetic inducers.

SIGNALS AND ENDOGENOUS INDUCERS

Primary Signals. Associated with a burst of reactive oxygen species (ROS), the early responses of plants to pathogens orchestrate a number of reactions, metabolites, and genes, strictly involved in defenses. The low molecular weight compounds that typically accumulate in infected tissues, sometimes named hormones, are generically classified as signals. The growing number of signals rising in the current literature makes their correct placement in a developing network uncertain and changeable. To avoid an arbitrary arrangement of sequential events, they will be reported in the order they have been discovered (Figure 1).

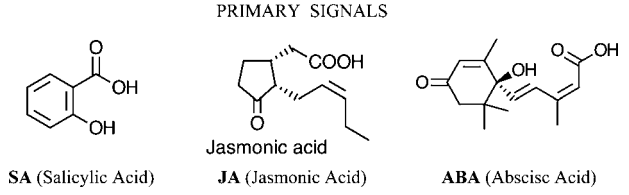

Figure 1. Induced resistance primary signals. SA is strictly required for systemic acquired resistance (SAR) activation; JA, besides being a signal in plant responses to wounds and damages caused by herbivores, is mainly involved in induced resistance (ISR) by rhizobacteria and other symbionts, often in tandem with ethylene; ABA intact pathway is required for BABA-IR.

Salicylic Acid (SA). SA is the best-known signal, proven to be essential to express SAR in most plants either in the pathogen-inoculated leaves or in all distal ones, even if it may require being translocated in the form of methyl ester.[12] As soon as activated in response to the stress produced by the infection, the synthesis of SA, normally occurring by hydroxylation of benzoic acid, is undertaken by the chorismate route via an isochorismate synthase (ICS). The chemical properties of SA are suitable to render it a player in a number of reactions and interactions with enzymes and molecular species playing important roles in SAR.[13] However, its major function has been generally established to be the induction of PR-1 genes expression, involving the essential mediation of the NPR1 protein. Recent investigations showed that the oligomeric form of NPR1, present in the cytoplasm, is disassembled and translocated to the nucleus, where the monomer NPR1 promotes efficient expression of defense genes (*PR1*) following a pathogen challenge or under SA induction. However, in the absence of infection or in uninduced state, no spurious activation of responsive genes occurs because NPR1 and TGA transcription factors do not interact with each other and *PR1* is not expressed.[14] According to a recent study on its mediation, NPR1 has been circumstantially shown to bind SA through a coordination with the transition metal Cu: this is assumed to make a bridge involving two oxygen atoms from hydroxyl and carboxyl groups of SA and two sulfur atoms of Cys 521 and Cys 529 of the protein (Figure 2). The complex was taking place with an estimated low apparent dissociation

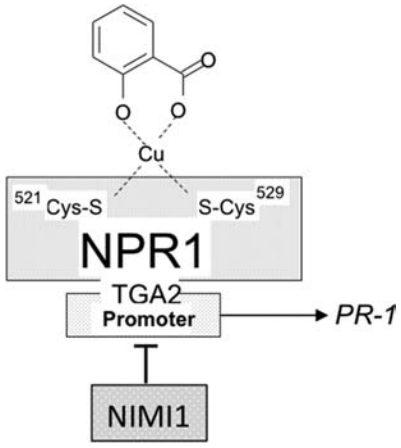

Figure 2. Possible mechanism through which NPR1 promotes the expression of *PR-1* genes. Using truncated parts of the protein, NPR1 has been shown to bind SA through a coordination with the transition metal Cu involving two oxygen atoms from hydroxyl and carboxyl groups of SA and two sulfur atoms of Cys 521 and Cys 529 of the protein. This binding would enhable NPR1 to interact with the transcriptional factor TGA2 leading to the expression of defense genes (PR-1). The cofactor NIMI1 (noninducible immunity 1) is a negative regulator of defense inhibiting gene expression through association with the NPR1-TGA complex. Adapted from Wu et al.[15]

constant $K_d = 1.4 \times 10^{-7}$ M (140 nM). This binding would cause a conformational change in the dimer form of NPR1, releasing the C-terminal transactivational domain from the autoinhibitory N-terminal one and so enabling the NPR1 transcriptional domain to activate the expression of defense genes (*PR-1*).[15]

However, the binding between SA and NPR1 is still a matter of debate and requires further structural analyses, as very recently suggested in a review by Fu and Dong.[8]

As said above, NPR1 is stored in the cytoplasm as an oligomer to prevent unwanted SAR activation in the absence of pathogen challenge. However, another function of oligomers is to maintain NPR1 homeostasis when the monomers are transported to the nucleus during SAR induction and there continuously degraded through the 26S proteasome pathway.[16] NPR1 degradation occurs both in absence of SA and/or in the presence of high levels of this hormone, that is, during pathogen attack, and is mediated by two NPR1 paralogues, NPR3 and NPR4, that are SA receptors with different binding affinities.[17,18] SA promotes the interaction of NPR1−NPR3 and disrupts that between NPR4 and NPR1, thus controlling NPR1 levels (Figure 3). In particular, in a very early stage of infection by a pathogen triggering ETI, high SA levels facilitate NPR3-mediated degradation of NPR1 in the challenged cell, leading to HR. This degradation necessarily occurs before NPR1 can regulate its target genes. Instead, in neighboring cells SA concentration is not sufficient to maintain NPR3−NPR1 interaction but enough to separate NPR4 from NPR1. This leads to NPR1 accumulation that prevents cell death and allows SAR establishment.[8,18]

To further complicate things, NPR1, besides interacting with the transcription factors of TGA family, also binds other cofactors (noninducible immunity 1 (NIM1)) that are negative regulators of defense.[19] Among the three *Arabidopsis* NIMINs, NIMIN1 and NIMIN2 interact with the C-terminal region of NPR1, whereas NIMIN3 binds to the N-terminus of NPR1. It has been suggested that NIMIN1 inhibits gene expression not

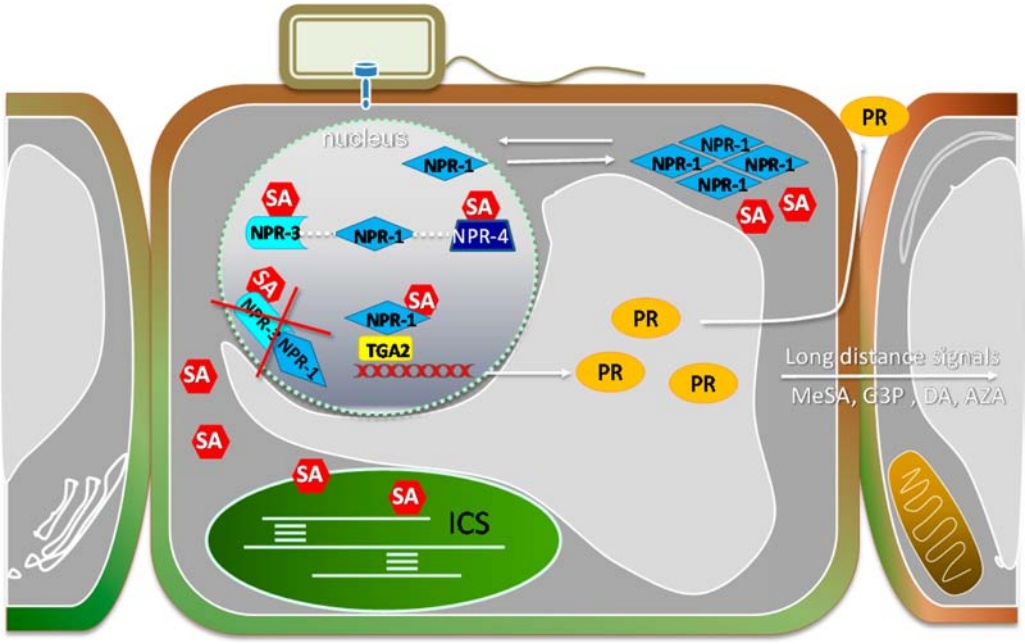

Figure 3. In response to the infection, the synthesis of salicylic acid (SA), normally occurring by hydroxylation of benzoic acid, is undertaken by the chorismate route through the isochorismate cleavage (ICS) in chloroplasts. Accumulation of SA in the cell changes the redox state, thus allowing oligomeric NPR-1 to disassemble and to migrate into the nucleus where its concentration is controlled by SA levels and the SA receptor proteins NPR-3 and NPR-4. When SA levels are high (i.e., in the cell directly contacted by pathogen effector) NPR-3 remains linked to NPR-1 and the complex is then degraded by the 26S proteasome, leading to PCD. On the contrary, intermediate SA levels (i.e., in the neighboring cells) do not allow interactions between NPR-1 and NPR-4; thus, free NPR-1 can interact with the transcription factor TGA2, promoting the transcription of *PR* genes and the synthesis of PR proteins. These are secreted in the attempt to impair pathogen survival. Long-distance mobile signals are also produced, including methyl salicylate (MeSA), glycerol-3-phosphate (G3P), abietane diterpenoid dehydroabietinal (DA), and azelaic acid (AZA). Adapted in a simplified form from Fu and Dong.[8]

through direct promoter binding but rather through association with the NPR1−TGA complex.[20]

SA and the related SAR have been generally associated with defenses against biotrophic or hemibiotrophic pathogens. By contrast, resistance against necrotrophs has been more frequently credited as being mediated by JA, often in tandem with ethylene (ET).[21]

Jasmonic Acid. JA and SA are known to orchestrate distinct pathways/networks with cross-talk between one another that may convey antagonistic or synergistic or additive messages. JA is also a signal involved in plant responses to wounds and damage caused by herbivores, in some way reminiscent of those triggered by necrotizing pathogens.

Again, JA and ET are induced by beneficial microbial species such as root-colonizing bacteria, mycorrhizal fungi, and *Trichoderma* spp. to prime plants to enhance their levels of defenses upon pathogen attack. This type of symbiotic benefit, called induced systemic resistance (ISR), is apparently elicited by microbial components, such as lipopolysaccharides (LPS) and exopolysaccharides (EPS), but a number of other determinants, including siderophores, antibiotics, biosurfactants, and various metabolites, have been found to trigger immune responses. Consistent with their beneficial role, these plant−microbe interactions produce resistance against pathogens mostly by priming for enhanced defenses.[22] An interesting feature of many of these interactions is the dependence of their ISR not only on JA and ET but also on the transcriptional regulator NPR1. This in fact has often been found to be essential in inducing the priming for expression of JA/ET genes and increased deposition of callose at the site of pathogen entry. In these cases NPR1 is assumed to play a totally different function from that previously mentioned as coactivator of SA-responsive PR genes. A regulatory role for NPR1 has been suggested to be played in cytosol to determine the fate of the cross-talk between SA and JA.[23,24] Certainly, if the cross talk would take place systemically, it could be very dangerous for the plant as the infection of a biotrofic pathogen could facilitate the subsequent attack by a necrotrophic one. However, it has been demonstrated that in distal tissues from an ETI triggered cell death, cross-talk is inhibited to avoid necrotrophic pathogens taking advantage of the repressed JA pathway.[24] A large body of facts about the reciprocal antagonism between SA and JA across plant species and phylogenesis has been the object of a recent review.[25]

However, interactions are not restricted to the cross-talk between primary signals. They also extend to involve even effectors of pathogens that may take advantage to enhance their virulence. Coronatine (COR) is a molecule effector produced by some pathogen strains of *Pseudomonas syringae*. It acts as a potent virulence factor in various bacterial infections, although its most significant action is mimicking the JA−isoleucine conjugate (JA−Ile), which suppresses SA-mediated plant responses (Figure 4). By virtue of this resemblance, COR may act as a potent agonist of JA−Ile and has been found to strongly induce jasmonate-responsive genes. By functioning as a jasmonate analogue, it has been shown to overcome the SA-dependent defenses during bacterial infection of *Arabidopsis thaliana*.[26,27] Interestingly, COR is also able to induce stomatal reopening through a signaling cascade facilitating bacterial penetration into the plant.[28]

Abscisic Acid (ABA). ABA is another hormone that may be heavily involved in many disease resistance processes but in a

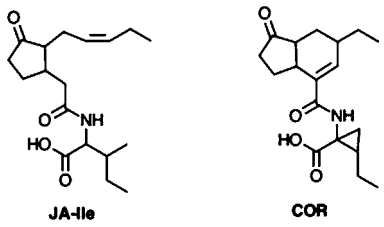

Figure 4. Coronatine (COR), a toxin produced by *Pseudomonas syringae*, mimics jasmonic acid–isoleucine conjugate (JA–Ile), which suppresses SA-mediated plant responses. Because of this resemblance, COR may act as a potent agonist of JA–Ile and has been found to strongly induce jasmonate-responsive genes, thus overcoming SA-dependent defenses during bacterial infection of *Arabidopsis thaliana*.

complex, intricate, and contradictory way. The name of ABA is bound to its role of accelerating leaf abscission, a process that occurs with deposition of callose as a barrier between living and dead cells. A number of studies brought evidence that ABA induces callose deposition as a barrier to infection by fungal and bacterial pathogens.[29] Resistance to tobacco necrosis virus, induced by chitosan, was also shown to imply callose deposition mediated by increased level of ABA[30] (Figure 5). At the same time, treatment with this hormone was found to suppress SAR induction by inhibiting its development both upstream and downstream of SA.[31] Again, using chitosan and Flg22, Ton's group recently found that variations in growth conditions greatly influence a plant's overall capacity to deposit callose after ABA pretreatment, and this variability correlates with the amount of H_2O_2 accumulation.[32] These findings further demonstrated that callose is a defense response controlled by distinct signaling pathways, depending on the environmental conditions and the challenging PAMP.

ABA is known to play multiple roles as an abiotic stress mediator. One of these roles is exerted during drought, when it induces closure of stomata. This process is apparently reproduced as a defense response to halt bacteria penetration as soon as plants perceive the related PAMPs.[33] However, ABA was also shown to suppress bacteria-induced callose in *Arabidopsis*.[34]

A negative role of ABA has been described in a study on tomato mutants with reduced levels of this hormone (*sitiens* plants). These plants were more resistant to *Botrytis cinerea* than the wild-type (wt) ones. Exogenous application of ABA restored susceptibility to *Botrytis cinerea* in *sitiens* plants and increased susceptibility in wt plants. In this study, ABA was found to partly repress phenylalanine ammonia-lyase (PAL) activity in wt plants and negatively modulate the SA-dependent pathway in tomato,[35] with high levels of H_2O_2 at the initial stages of tissue penetration by *Botrytis cinerea*.[36]

In the context of associated redox regulating system, biosynthesis of ABA has been tentatively assumed to support a role of oxidized state of redox-buffering capacity in enabling reactive oxygen species accumulation, whereas violaxanthin de-epoxidase (VDE) antagonizes ABA production, which is driven by 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Figure 5).[29,37,38]

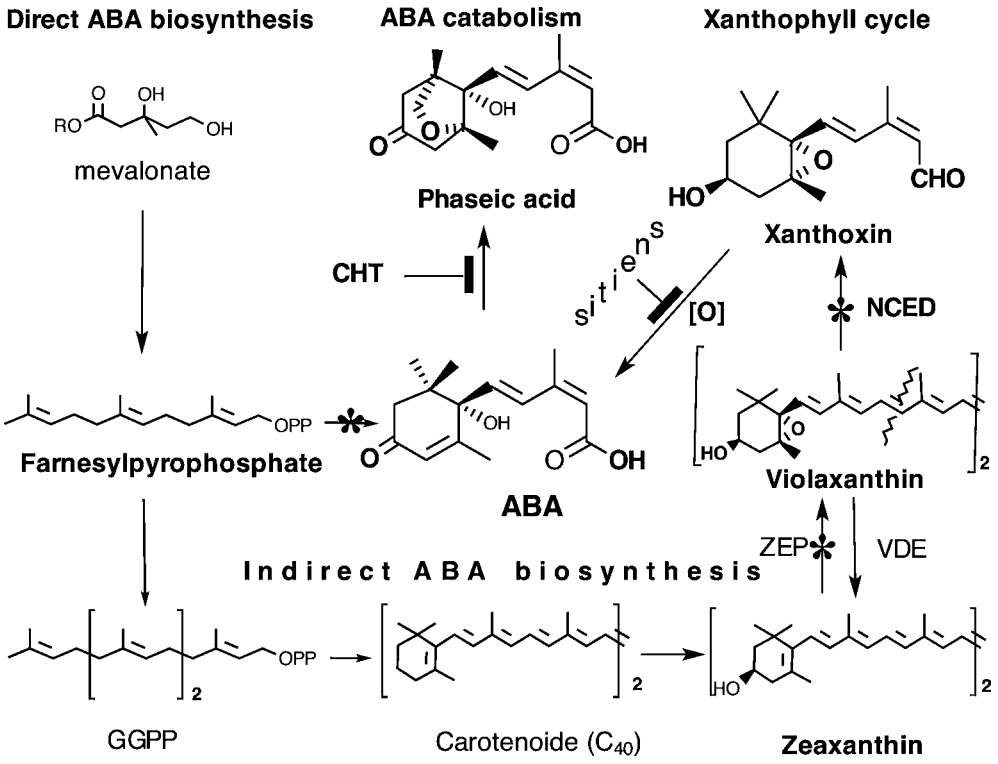

Figure 5. The physiological level of ABA depends on the balance between the rate of its synthesis and that of its catabolism to the biologically inactive phaseic acid. The increased abscisic acid (ABA) content in leaves of chitosan (CHT)-treated bean plants could be due to the activation of either the direct ABA biosynthetic route, via farnesyl pyrophosphate (star), or the indirect carotenoid pathway, through the xanthophyll cycle. In the second instance, CHT could positively regulate the key enzymes zeaxanthin epoxidase (ZEP, star) and 9-*cis*-epoxycarotenoid dioxygenase (NCED, star). Violaxanthin de-epoxidase (VDE) antagonizes ABA production, which is driven by NCED. Alternatively, CHT could reduce the rate of ABA catabolism. ABA production is also impaired in tomato *sitiens* plants by blocking the last oxidative step of its biosynthesis.

The multifaceted role of ABA tends to be rationalized by more authors in a context of overlapping of the ABA−SA cross-talks with the ABA mediation in abiotic and biotic stresses. According to this view, the effects change from the preinvasive to the postinvasive pathogen stages, when the ABA−SA cross-talk is conditioned by the combined effects of PAMPs and pathogen secreted effectors to become predominantly antagonistic.[29,39]

Long-Distance Signals. Analysis of a series of metabolites isolated from petiole exudates (PEXs) of pathogen-inoculated leaves identified several diverse signals that may be involved in long-distance spreading of acquired resistance[40] (Figure 6).

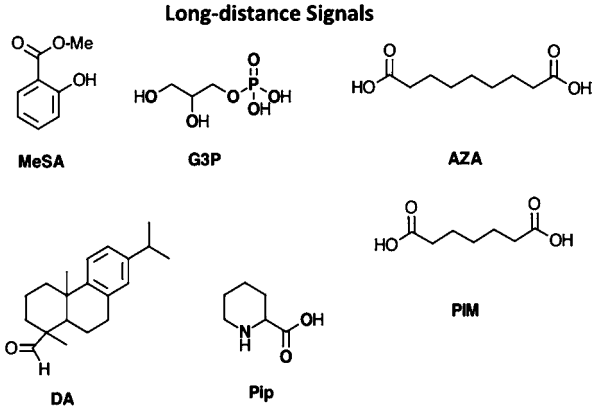

Figure 6. Main signals that may be involved in long-distance spreading of acquired resistance.

Methyl Salicylate (MeSA). MeSA is one of these metabolites, proposed to be the phloem-mobile signal prompt to release SA in distal leaves by virtue of a SA-inhibitable methyl esterase. Once in the distal leaves, MeSA is in fact hydrolyzed by suitable esterases that have been characterized and shown to be required for spreading SAR. In tobacco and *Arabidopsis* the esterase has been identified in SA-binding protein 2 (SABP2). In potato the same role has been found to be played by the orthologue of SABP2, called StMeS1. The esterase activity is feedback inhibited by high levels of SA. An exogenous inhibitor of this esterase has been identified in 2,2,2,2′-tetrafluoroacetophenone, a compound capable of occupying the same active site of SA, as shown by the X-ray crystal structure of the protein.[41] Another SA methyltransferase from rice (OsBSMT1), when overexpressed in transgenic *Arabidopsis*, induced PR1 transcript production in adjacent wild-type plants in an ICS1-independent and NPR1-dependent manner upon *Psm* infection.[42] This indicates that MeSA may be involved in the induction of defenses not only in the systemic tissue of the same plants but also in the nearby plants through its volatilization. An indirect proof comes from the fact that exogenous application of MeSA induced systemic immunity in wild-type tobacco plants.[43] Interestingly, during infection by *Psm*, it has been shown that pathogen toxin COR is indirectly responsible for MeSA volatilization outside the leaf tissue, possibly to lower the SA level and facilitate the infection.[44] However, the data of this work, in contrast to what was found in tobacco, appeared to render the role of MeSA dispensable for SAR in *Arabidopsis thaliana*, suggesting that the presence and role of the SAR mobile signal may depend on the plant species and/or the pathosystem.[45]

Glycerol-3-phosphate (G3P). G3P is the alcoholic matrix of glycerolipids, which are essential in growth and defenses. G3P, or a derivative, is a mobile signal and is also required for the translocation of the lipid transfer protein defective in induced resistance 1 (DIR1) to distal tissues. Therefore, the cooperation of both G3P and DIR1 is essential for induction of SAR.[46]

Dehydroabietinal (DA). DA, an interesting long-distance signaling metabolite, has recently been isolated and characterized as an abietane diterpenoid (Figure 6). This aldehyde may also act as a potent inducer of SAR, being active at picomolar concentrations when applied to leaves of *Arabidopsis*, tobacco, and tomato. Locally applied deuterated DA was reported to be rapidly translocated to distal leaves and induce SA systemic accumulation together with the expression of *PR-1* and *ICS1*, the gene encoding the *isochorismate synthase* responsible for SA synthesis during SAR. Other genes that are needed for biologically induced SAR were also required for the development of the DA-induced expression. The dependence of DA-induced resistance on *NPR1* and *ICS1* was considered as a good indication that DA acts upstream of SA in the developing pathway of SAR.[47]

Azelaic Acid (AZA). AZA is a nonandioic acid, another mobile metabolite endowed with priming properties of systemic immunity. This compound was first isolated from petiole exudates of bacterially infected leaves of *Arabidopsis*, where it has been found to accumulate. Unlike DA, AZA does not induce directly SA, but was stated to prime plants to produce high levels of SA and the SA-associated signaling marker *PR1* as soon as challenged by the pathogen *Pseudomonas syringae*.[48] In the same work, AZA was also found to transiently induce a gene, called *azelaic acid induced 1* (*AZI1*), encoding a protein, AZI1, credited with being involved in the generation of the vascular sap that confers disease resistance. Mutation of the *AZI1* gene resulted in plants incapable of displaying systemic immunity triggered by AZA. Therefore, azelaic acid and AZI1 were stated to be components of SAR involved in priming defenses.

However, a thorough investigation on the biogenesis of AZA and its homologous pimelic acid (heptandioic acid) PIM (Figure 6), carried out by the group of M. J. Mueller at Wuerzburg University, brought to light different facts and interpretations.[49] In their experiments, AZA pretreatment of *Arabidopsis* leaves did not induce resistance against virulent *Pst*, whereas infection with avirulent *Pst* was shown to produce in a few hours enhanced levels of AZA and PIM together with the markers of nonenzymatic lipid oxidation and dramatic up-regulation of enzymatic lipid peroxidation giving out JA. The authors were able to demonstrate that the accumulation of both AZA and PIM as esters of oxidized glycerolipids may occur upon pathogen infection by a free radical fragmentation of the polyunsaturated acyl chains initiated by a postulated singlet oxygen, independent of catalysis by lipoxygenases. In fact, they obtained these esters in infected mutants deficient in genes responsible for the 9-lipoxygenase and LOX2, the main 13-lipoxygenase. Therefore, they proposed a mechanism for the glycerolipid fragmentation that, in a simplified form, is shown in Figure 7.[50] According to the conclusions of the authors, AZA should be considered as just a marker for free radical-induced lipid fragmentation associated with oxidative membrane damage and cell death.[49]

Pipecolic Acid (Pip). A thorough analysis of the pool of amino acids extracted from petiole exudates of leaves

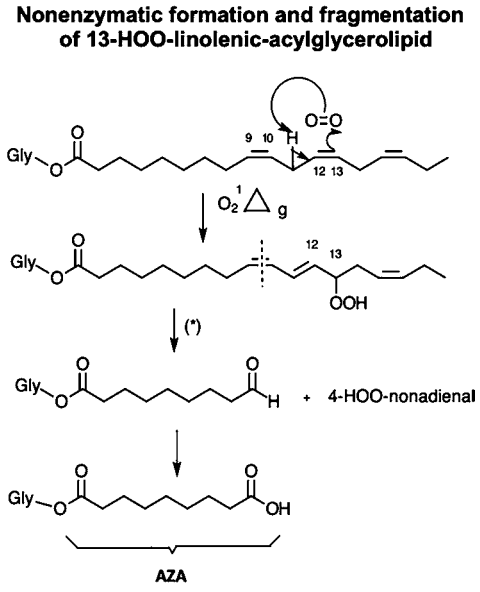

Figure 7. Nonenzymatic formation and fragmentation of 13-HOO-linolenic-acylglycerolipid to account for the enhanced accumulation of AZA in *avrPst*-infected leaves of *Arabidopsis*. (*) for reactions implicit in the formal cleavage between carbons 9 and 10, see Schneider et al., *J. Biol. Chem.* **2008**, *283* (23), 15539−15543.

inoculated with SAR-inducing *Pseudomonas syringae* pv *maculicola* (*Psm*) led to identification of the lysine catabolite pipecolic acid (piperidine-2-carboxylic acid) as the component massively increased with respect to leaves treated with a $MgCl_2$ solution.[51] In the inoculated leaves the accumulation of Pip was shown to be independent of SA as well as of the SAR regulator flavin-dependent monooxygenase 1 (*FMO1*), suggesting that it occurs upstream of both SA and *FMO1*. The induction was also positively influenced by *NPR-1* and phytoalexin-deficient 4 (*PAD4*). In distal, noninoculated leaves, systemic accumulation of Pip was just preceding that of SA at the onset of SAR and was markedly reduced in the SAR-defective *fmo1* and *ics1* mutants, but a localized bacterial treatment was sufficient to induce significant levels of Pip even in these mutants. By contrast, the level of Pip in distal leaves was not enhanced in *npr1* and *pad4* mutants.

The pathogen-induced Lys catabolism was also associated with high expression of AGD2-like defense response protein 1 (*ALD1*), which encodes an aminotransferase assumed to be involved in Pip biosynthesis. In fact, mutant plants *ald1* failed to produce Pip and SAR in *Psm*-infected leaves, whereas exogenous application of Pip complemented the defects in *ALD1*. Interestingly, BABA application led to Pip accumulation and induced resistance to *Psm* in an *ALD1*-dependent manner, indicating that Pip also modulates BABA-IR resistance against bacterial pathogen infection. All together, these results led to the conclusion that Pip orchestrates an amplification of resistance, with positive regulation of SA biosynthesis and priming for SAR induction.[51]

Exogenous Inducers. The idea that the activation of natural plant defenses, such as SAR, could be reproduced even by replacing the preliminary necrotic lesions by a chemical treatment was realized by White with the exogenous application of salicylates to defend the tobacco plant against different pathogens.[52] White was therefore a pioneer of what is known nowadays as chemical-induced resistance.

Successively, agrochemical research by Novartis brought to light various chemicals that may be considered as functional analogues of SA and may be even more potent than SA as exogenous inducers. Besides being a more practical way of triggering the plant inducible defenses, this technology extends further inquiries on the molecular base of what occurs in the armamentarium of the plant in the portion of time between the inducer application and the pathogen attack. The implicit process, known as priming, will be considered in a later section after a preliminary survey of the performances of the best known exogenous inducers (Figure 8) and the defense pathway

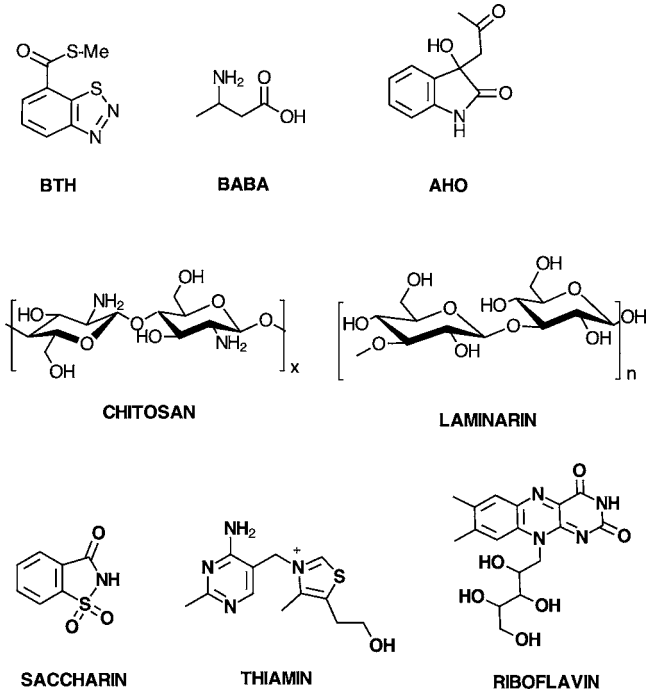

Figure 8. Some of the best-known exogenous inducers.

they activate (Figure 9). This survey does not include inducers represented by plant extracts such as Milsana (ethanolic extract of *Poligonum japonicum* L.) and Stifenia (extract of *Trigonella foenum graecum* L.) because, among their unspecified multifunctional compounds, the role of those involved in SAR elicitation is unclear.

BTH. Identified as the safest and most efficient from a series of analogues, the methyl ester of benzo(1,2,3)-thiadiazole-7-carbothiolic acid (BTH) was brought to the market with the common name of acibenzolar-*S*-methyl and has been tested on a number of pathosystems.[53] It has been shown to interact with NPR1 through a binding of affinity similar or even better than that displayed by SA with NPR1 as receptor (Figure 3).[15] A common effect of BTH as an inducer is the direct activation of *PR-1* and, in general, of the PR proteins. Other analogies with SA are the inhibition of catalase and ascorbate peroxidases besides structural elements that are compatible with this type of effects (see ref 13 and refs cited therein). A study of its fate in tomato plants showed that BTH rapidly translocates to apical leaves and is converted to the free carboxylic acid 3 days after application.[54]

Early work brought evidence that, in tobacco plants, BTH was able to induce resistance against biotrophic fungal pathogens as well as against *Pseudomonas syringae* and tobacco mosaic virus. Instead, protection against necrotrophic fungi

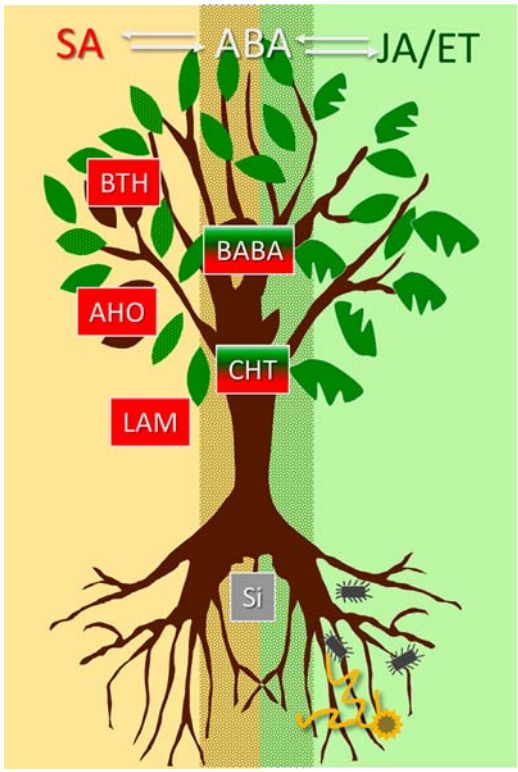

Figure 9. Defense pathways activated by exogenous inducers (BTH, benzothiadiazole; AHO, 3-(2-oxopropyl)-3-hydroxyoxindole; LAM, laminarin; Si, silicon), rhizobacteria, mycorrhizae, and phytophages. *β*-Aminobutyric acid (BABA) and chitosan (CHT), besides depending on abscisic acid (ABA) mediated callose synthesis, involve salicylic acid (SA) or jasmonic acid/ethylene (JA/ET) pathway depending on the treated plant. The three pathways interact with each other to different extents depending on the host plant and the pathosystem.

could not be observed. A number of genes encoding PR proteins were coordinately expressed after BTH application, with a maximum increase of *PR-1* transcript at a concentration of ca. 36 μM.[53]

In a further work, application of 0.3−1 mM BTH to *Arabidopsis* allowed the detection of the maximum expression of PR-1, PR-2, and PR-5 genes at 1 day from treatment. Resistance was then induced to protect the plant against *Peronospora parasitica* and *Pseudomonas syringae*. The protection was also reproduced in transgenic (NahC) plants unable to accumulate SA, indicating that BTH acts independently on SA.[55] When treatment with 0.3 mM BTH was applied to *Phaseolus vulgaris*, an oxidative burst took place early, with high levels of H_2O_2 and peroxidase activity. This was associated with a high and persisting expression of *PAL*, steadily increasing without phenolic deposition up to 7 days from application, when plants were inoculated with *Uromyces appendiculatus*. The first impairment to fungal colonization occurred between 24 and 72 h later and resulted in a thicker extrahaustorial matrix and deposition of phenolics around the haustorial neck. These responses were found to culminate 7 days after inoculum, with hyphae heavily stained with osmium and heavy phenolic deposits encapsulating the haustoria.[56]

A study of induced resistance against the powdery mildew *Blumeria graminis* in barley gave a quantitative measure of the efficacy of BTH at different times elapsed from treatment to fungal inoculation, called induction times (i.t.): after only 3 days of i.t., the infection on the primary leaf was reduced by 68.9%, whereas, when the induction was protracted to 5 days, the infected areas were reduced by 77.2%. A similar reduction was also measured in secondary untreated leaves when the induction was extended to 10 days. The most evident biochemical effects were observed after the pathogen challenge, when the oxidative burst caused diffusion of H_2O_2 to the whole cells that were involved in HR-like symptoms and phenols deposition in nonpenetrated callose papillae.[57] Effects on resistance induced by BTH in soybean against *Phytophthora sojae* have been described by other authors using electron microscopy. These effects were dominated by phenolic deposition in host and fungal cell walls, in particular around haustoria, similar to those previously mentioned in bean resistance against *Uromyces appendiculatus*. In addition, expressions of *PR-1*, *PR-3a-b*, *PR-9*, and *PR-10* were activated at different times and levels.[58] The last-mentioned effects may deserve some comment on the role of PR-proteins: whereas their up-regulation may contribute to resistance in soybean hypocotyls, as suggested by the authors, the role played by *PR-3a* and *PR-3b*, which encode proteins displaying chitinase activity, remains unclear because *Phytophthora* species, as oomycetes, do not have chitin in their cell walls.

BTH was also used to protect soybean from hypocotyl rot caused by *Rhizoctonia solani*. Interestingly, the mycelium growth of this pathogen was found to be partially inhibited in vitro by BTH, its radial growth being reduced 40% at the BTH highest dose of 2.4 mM (mancozeb at 0.2 mM showed a reduction of 94%). However, this activity did not affect the virulence of *Rhizoctonia solani*, and the inoculum precultured on BTH produced the same disease severity as that maintained in the unamended medium. Therefore, the seed treatment with BTH significantly reduced the hypocotyl rot symptoms caused by the pathogen, and this protecting effect was strictly correlated with the chitinase activity already induced before inoculation 2 days after the treatment. The chitinase activity increased according to the dose of BTH, starting at 0.2 mM and reaching a plateau at 0.4 mM, the dose at which the maximum of severity reduction (50% with respect to control) was observed. A dose-dependent inhibition of seminal root growth was also detected at 2 days from the treatment and reached a maximum of 53% in biomass dry weight at a dose of 2.4 mM. The type of root growth reduction observed up to the dose of 0.4 mM was later rapidly recovered, but when caused at 2.4 mM could not be recuperated.[59]

Recently, some fluorine-containing esters of the BTH benzothiadiazole carboxylic acid were synthesized and evaluated as SAR inducers, showing excellent activity against cucumber *Erysiphe cichoracearum* and *Colletotrichum lagenarium* in assay screening. Field test results demonstrated that two of them were more potent than BTH toward these pathogens.[60]

Finally, BTH- and laminarin (see ahead)-treated plants seem to be more attractive to a wide variety of parasitoids after herbivore attack, another important feature that improves their defense armamentarium.[61] Interestingly, through the suppression of certain dominating plant volatiles, elicitor-treated plants may be less apparent to herbivores that use herbivore-induced plant volatiles (HIPVs) for host location. These findings show that inducers of pathogen resistance are compatible with the biological control of insect pests and may even help to improve it.[61]

BABA. *β*-Aminobutyric acid has been the object of several studies following the old discovery that its application by soil drench protected pea plants from the disease caused by the

oomycete *Aphanomyces euteiches*.[62] More recent work confirmed an extension of its spectrum of induced activity to cover protection of several crops from diseases caused by downy mildews and necrotrophic fungi, as well as by bacterial and viral pathogens. Induction of plant resistance as BABA mode of action was inferred by the absence of any direct toxicity on these pathogens. However, an investigation on the signaling pathway associated with its induced resistance brought to light remarkable changes according to plant and pathosystem. Whereas in noninoculated tomato plants it was able to induce rapid accumulation of PR-1 proteins, when applied to tobacco leaves, it was found to develop typical symptoms of a hypersensitive reaction (HR) before eliciting systemic accumulation of SA.[63] Dependence of BABA-induced resistance on SA was supported by its failure in transgenic NahG plants.[64] More recently, a robust study with suitable selected mutants elucidated the BABA-induced priming phenomenon in *Arabidopsis*. The authors obtained evidence that treatment with BABA results in priming for multiple defense mechanisms: one of these is the SA-dependent resistance, demonstrated by an increased expression of the marker gene *PR-1* upon infection by *Pseudomonas syringae* pv *tomato* or *Botrytis cinerea*; a second important induced mode of action is the priming for resistance by formation of callose-rich papillae against the oomycete *Hyaloperonospora parasitica* and the necrotrophic pathogens *Alternaria brassicola* and *Plectrosphaerella cucumerina*. A mutant affected in the gene encoding the abscisic acid biosynthetic enzyme zeaxanthin epoxidase (ZEP, see Figure 5) failed to prime for formation of callose. During infection callose is considered to act as a physical barrier or as a matrix that concentrates antimicrobial compounds at the attempted sites of fungal penetration.[65,66] BABA was also found to repress the JA response induced by the coronatine virulence factor and effective priming the up-regulation of genes responsive to SA and BTH during *Pst* DC 3000, in agreement with the above-mentioned reports.[67]

When BABA treatment was used to induce resistance in *Vitis vinifera* against *Plasmopara viticola*, the effects in reducing mycelial growth and sporulation were associated with the expression of marker genes for the SA (*PR-1*) and JA (*PR-4* and *LOX-9*) pathways. The JA pathway appeared to be particularly involved, leading to a primed deposition of callose and lignin around the infection sites. In fact, similar effects (even if with a lower resistance efficacy) were observed by exogenous application of JA, whereas both BTH and, curiously, ABA failed to induce significant resistance.[68] In a further work by the same research group, a close biochemical examination of the phenylpropanoid pathway induced by BABA treatment upon *Plasmopara viticola* infection showed the accumulation of specific stilbene phytoalexins, including resveratrol and viniferins.[69]

AHO. An interesting example of a natural inducer of SAR is represented by this isatin derivative (3-hydroxy-3-(2-oxopropyl)-1*H*-indol-2-one, Figure 8), which was isolated from extracts of the ornamental *Strobilanthes cusia* and found to induce resistance in plants to a broad range of diseases.

The mode of action of AHO conforms to the activation of genes typical of the responses mediated by SA, but, unlike most inducers that act downstream of SA, it sensitizes a point in the activation chain upstream of SA. In fact, it did not induce resistance in *nahG* transgenic plants unable to accumulate SA. In tobacco plants, AHO enhanced resistance against the viral pathogen TMV and the powdery mildew *Erysiphe cichoracearum*. Among the biochemical effects induced, the most evident ones have been reported to be the *PR-1* expression and a notable increase of PAL activity, anticipating the increase in SA level. The last-mentioned effect suggested the hypothesis that the initial activation of PAL could be responsible for triggering the biosynthetic pathway of SA via enhanced production of *trans*-cinnamic acid.[70]

Chitosan (CHT). Oligomers of chitosan are originated by deacetylation of chitin, the linear polymer of (1→4)-*β*-linked *N*-acetyl-D-glucosamine, a known component of the fungal cell wall, but more abundant in the hexoskeleton of crustaceans. The deacetylation may occur by enzymatic reaction or, more practically, by chemical hydrolysis giving out sequenes of D-glucosamine (see Figure 8) in various proportions and with a large range of molecular weights. These sequences may assume, under physiological conditions, polycationic nature.

Various works have described the antimicrobial properties of CHT, including bacterial and fungal pathogens. Different mechanisms have been suggested to rationalize this activity, based on electrostatic interactions between the protonated amino groups and negative essential ions, the binding to microbial DNA, and metal ion chelation. A review has been published on this subject and gives useful references on chemical modifications to improve the water solubility of CHT, a critical aspect that generally limits its applications.[71]

However, many pieces of evidence show that CTH can also elicit plant defenses upon pathogen challenge by inducing accumulation of callose and phenolics. For instance, in studies aimed to test the activity of chitosaccharides, partially acetylated chitosans, when injected into wheat leaves, have been found to elicit both PAL and peroxidase activities, with a higher content of lignin in the cell walls.[72] Other studies brought evidence that CHT-treated plants respond to viral infections with typical signals of induced resistance, the efficacy of which apparently depends more on the plant than on the type of virus. Thorough investigations by Faoro et al.[73] on CHT-treated bean leaves brought to light the appearance of small clusters of dead cells, resembling microscopic HR, randomly distributed in the mesophyll. These treated leaves, together with the untreated distal ones, resulted in ful or partial protection when challenged by tomato bushy stunt tombus virus.[73]

The effects of CHT treatments have been thoroughly compared with those of parallel BTH applications in induced resistance against powdery mildew in barley. Both were found to induce responses typical of SAR, such as oxidative burst and phenolics deposition, but with a greater efficacy shown by BTH, which, unlike CTH, was able to promote a significant increase in HR response after the pathogen challenge. This difference in the HR response was inverted with respect to that observed by the same authors in bean plants, indicating that the mechanism of elicitation may differ depending primarily on the host.[57] Recently, a new CHT formulation (Kendal Cops (Kc)) was assessed in treatments of grapevine against powdery mildew and compared with parallel applications of standard fungicides (penconazole and methyldinocap). Under high disease pressure producing an infection severity of 87.5% in the control grapes, the disease was reduced to 2.4% in grapes treated with the experimental formulation (CHT 0.1%) and to 0.92% in the fungicide-treated ones. Total polyphenol content and antioxidant power in both grapes and related wines were significantly higher from the Kc-treated plants than from fungicide-treated ones.[74]

CHT and BTH have also been compared in their potential for protecting grapes against *Botrytis cinerea*. Both inhibited the radial growth of this fungus in vitro, with CHT showing a higher fungitoxicity (EC_{50} = 1.77 mg mL^{-1}) than BTH (EC_{50} = 3.44 mg mL^{-1} = 16 mM). When applied on plant, the resistance was induced more effectively by BTH, with the greatest reduction in lesion size obtained in grapes pretreated at 3 mg mL^{-1}.[75]

Laminarin. This elicitor is a linear β-1,3 glucan from the marine brown alga *Laminaria digitata* that induces typical SAR features, including ion fluxes, ROS production, activation of MAPKs cascade, callose deposition, phytoalexin production, and the expression of *PR* genes.[76−78] Indeed, the elicitation of plant defenses by glucans was shown a long time ago by using a β-1,3-linked D-glucan isolated from *Phytophtora infestans*, which strongly inhibited the development of lesions in tobacco tissues inoculated with various viruses.[79,80] In the past decade many papers have reported the efficacy of laminarin in controlling fungal, bacterial, and viral diseases, including downy mildew and gray mold of grapevine,[76,81] *Erwinia carotovora*, and TMV infection in tobacco.[82,83] In the case of TMV, sulfated laminarin resulted in a better local protection in both *Arabidopsis* and tobacco than did laminarin, and the two forms acted synergistically when used in mixture.[83,84] Furthermore, sulfated laminarin, but not laminarin, induced SA accumulation and PR-1 expression in treated plants. With regard to perception mechanisms of β-1,3 glucans, glucan-binding proteins (GBP) have been identified, which appear typical of Fabaceae family and do not possess any of the functional domains found in other innate immunity receptors.[85−87] These proteins contain two domains, one with glucan binding activity, and the other showing similarity to fungal glucan endoglucosidase enzymes. The latter would allow the release of the true elicitor (hepta-β-glucoside) near the elicitor binding site to facilitate its detection. The glucan perception of Fabaceae represents an example of a very sophisticated receptor system in plants, where the ligand is processed by an intrinsic part of the receptor complex itself, resulting in the amplification and tailoring of the best-fitting ligand molecules.[85]

Saccharin. 1,2-Benzisothiazoline-3-one-1,1-dioxide (Figure 8), a well-known sweetener used in foods and drinks, is also a good SAR elicitor, being able to induce resistance against a broad spectrum of pathogens in both cereals and leguminous plants.[88−92] Saccharin is, of course, a major metabolite of probenazole, a fungicide also known to stimulate defense mechanisms in rice plants against blast caused by *Magnaporthe grisea* and bacterial blight caused by *Xanthomonas oryzae* (see next section). In some cases saccharin application with root-drench treatment was more effective than foliar application in protecting soybeans against rust, and protection lasted about 15 days.[92] In a very recent work by Delgado et al.[93] saccharin was tested against rust by *Uromyces appendiculatus* in artificially inoculated plants and against both rust and angular leaf spot by *Phaeoisariopsis griseola* (ALS) in naturally infected bean plants. In the greenhouse experiments, beans sprayed with saccharin (0.24 mg mL^{-1}) and inoculated with *Uromyces appendiculatus* 6 days later showed a reduced number, size, and sporulation of pustules. In three follow-up experiments upon natural infection conditions during 2009 and 2010 and summer/autumn 2010, saccharin, sprayed every 14 days, was able to protect bean plants against rust in all three trials, but significantly reduced ALS only during summer/autumn and spring 2010. Increase in seed weight was observed when saccharin-treated plants were infected after preflowering, but not when rust infection occurred earlier and the pathogen pressure was higher. This is in disagreement with previous observations by Walters et al.,[90] which reported that effects of saccharin on growth rate and grain yield of barley plants increased in both parameters only under high disease pressure, proof that the fitness cost of induced resistance is almost unpredictable, being dependent on many factors, as reported ahead.

Silicon (Si). The second most abundant element in the Earth's crust[94] is absorbed by plant roots (Figure 9) as water-soluble orthosilicic acid ($Si(OH)_4$) and converted to insoluble silica in cell walls, intercellular spaces, and a subcuticular layer of leaves.[95] Silicon is known to increase the tolerance against both abiotic and biotic stresses in many plant species, and it is the only nutrient that is not detrimental when in excess.[94,96,97] It increases the tolerance toward both (hemi)biotrophic and necrotrophic pathogens in many plant species besides being also effective against salinity, drought, and other abiotic stresses.[96,98] Interestingly, silicon protects plants apparently without fitness costs.[99,100] At first, the protection conferred by silicon was ascribed to deposition of silica in the leaves, which would form a physical barrier impenetrable by pathogens.[101] However, as the beneficial effects of silicon are lost within a short period of time after application, it appears that its role as a modulator of basal defense responses is prevalent over its function as a mechanical barrier.[96,102−106] In any case, there are now many pieces of evidence that silicon triggers in plants a wide spectrum of defense responses. For example, in cucumber it enhanced the activity of chitinases, peroxidases, polyphenol oxidases, and flavonoid phytoalexins, and these responses were induced only after infection with *Pythium* spp.,[107] a typical feature of priming agents (see next section). Other proof of the priming effect by silicon was brought by Fauteux et al.,[108] showing that the stimulating effect of this element on the biosynthesis of SA, JA, and ET appeared only after challenge with *Erysiphe cichoracearum*. Again, in rice silicon activated the ET pathway involved in the resistance to *Magnaporthe oryzae* after pathogen challenge.[109] The resistance was characterized by increased accumulation of defense-related enzymes, including glucanase, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, as well as glycosylated phenolics and diterpenoid phytoalexins.[110−112] Another example of priming by silicon was shown in tomato plants after infection with *Ralstonia solanacearum*, when both JA and ET signaling pathways were induced, leading to increased resistance to the fungus.[106] In several powdery mildew diseases, that is, by *Podosphaera pannosa* in rose and by *Blumeria graminis* f. sp. *tritici* in wheat, silicon provided resistance by increasing papillae formation, callose synthesis, and H_2O_2 deposits, besides the synthesis of fungitoxic phenolics and flavonoids.[113−115]

From the long list of papers reporting successful results in controlling plant diseases with silicon, this inducer appears to activate/modulate multiple pathways, such as SA, JA, and ET, depending on the pathosystem and many other physiological and environmental factors. A very recent and exhaustive review by Van Bochkaven et al.[116] addresses these topics, highlighting the regulatory mechanisms that might account for broad-spectrum plant disease resistance induced by silicon, including priming of plant immune responses and alterations in phytohormone homeostasis.

Vitamins. In the past decade a new role for vitamins as chemical inducers of SAR has received attention owing to their safety and cost-effectiveness.[117] Three vitamins, vitamin B1

(thiamin), vitamin B2 (riboflavin), and vitamin K3 (menadione), have been found to induce SAR against fungal, bacterial, and viral infections in *Arabidopsis*, rice, cucumber, tobacco, and tomato.[118,119] In particular, thiamin activates the expression of SAR-related genes in rice, tobacco, and vegetable crops, improving the resistance of the plants to several pathogens.[118] Riboflavin, too, was shown to display effects similar to those typical of synthetic inducers, and its use on tobacco leaves has proved to control several diseases.[120] In mixtures with methionine, it was found to reduce the symptoms of powdery mildew infection in strawberry plants.[121] Treatments with 0.5 mM riboflavin have been proved to control *Phytophthora parasitica* in *Arabidopsis* and *Alternaria alternata* in tobacco.[119]

As a photosensitizer, riboflavin may produce singlet oxygen or oxygen superoxide. Resistance of rice to rice blast, induced by its application, has been suggested to depend in part on mediation of ROS produced by riboflavin photoactivation.[122] However, no HR symptoms have been reported during the induction of resistance in tobacco and *Arabidopsis*. In this view it was assumed that riboflavin may activate a novel signal transduction pathway.[119]

More recently, *p*-aminobenzoic acid (PABA, also referred to as vitamin Bx) was found to induce SAR against *Xanthomonas axonopodis* pv *vesicatoria* in pepper plants in both greenhouse and field experiments.[123] Moreover, dipping pepper seedlings in 1 mM PABA made plants more resistant to naturally occurring cucumber mosaic virus (CMV) infection in the field as assessed by the lower CMV RNA at 40 and 105 days post treatment. Interestingly, expression of the *Capsicum annuum* pathogenesis-related *PR-4* gene was primed in response to pathogen infection as assessed by quantitative real-time PCR, and the induced resistance to both bacterial and viral pathogens was not associated with apparent fitness allocation costs.[123]

A more complete list of low-molecule inducers of disease resistance has been shown in the review by Schreiber and Desveaux,[5] which also outlines criteria and projects for designing high-throughput screenings (HTS) as a source of new inducers.

FUNGICIDES AND OTHER AGROCHEMICALS WITH RESISTANCE INDUCTION ACTIVITY

Systemic fungicides are known to display a very specific mechanism of action. Nevertheless, some of them have also been shown to elicit plant defenses as a secondary mode of action and, occasionally, this has been appraised to occur even with other pesticides. An early instance, among anti-oomycetes, has been discovered in the action of metalaxyl when the elicitation of glyceollin was associated with the control of *Phytophthora megasperma* in soybean.[124] A role of plant defense mechanism in the antifungal action of metalaxyl, fosetyl, and $Cu(OH)_2$ was demonstrated in the control of *Peronospora parasitica* in *Arabidopsis* plants.[125]

Other examples are reviewed here in more detail.

Probenazole (Oryzemate = 3-Allyloxy-1,2-benziso-thiazole-1,1-dioxide). This saccharin derivative has been largely used for more than 30 years against rice blast, caused by *Magnaporthe grisea*, and bacterial leaf blight, caused by *Xanthomonas oryzae* pv *oryzae*.[126] Contrary to many other fungicides, its extensive use for so a long time has not developed resistance in the target pathogens,[126] possibly because its action mechanisms include SAR induction, by stimulating a site upstream of the point of SA accumulation in the SAR-signaling pathway.[127] Although generally used in rice crops, probenazole has also been shown to control southern corn leaf blight on maize, caused by *Cochliobolus heterostrophus*, without detrimental effects on plant growth and with lower environmental impact than the fungicide maneb usually employed to fight this harmful disease of maize.[128] Signals detected during probenazole-induced resistance in rice are those typically associated with oxidative burst, but also include enhancement of unsaturated fatty acids that may impair conidia development.[129] One of the most evident features of defense primed by this fungicide was the rapid lignification to the inoculum of *Pyricularia oryzae*. When PAL or other enzymes of the phenylpropanoid pathway were inhibited, the plant became more susceptible to the disease.[130] Interestingly, when tested in a screening model of cultured parsley cells, probenazole failed to give a positive result, but saccharin was active and was then proposed to be the active metabolite responsible for the SAR induction (see ref 13 and references cited therein).

Pyraclostrobin (Methyl *N*-{2-[1-(4-Chlorophenyl)-1*H*-pyrazol-3-yloxymethyl]phenyl}-(*N*-methoxy)carbamate). This compound belongs to the strobilurin class of fungicides, which includes a variety of synthetic plant-protecting compounds with broad-spectrum antifungal activity. Conrath's group demonstrated for the first time that pyraclostrobin, besides being a fungicide, enhanced the resistance of tobacco to both TMV and *Pseudomonas syringae* pv *tabaci*.[131] Pyraclostrobin was also active at improving TMV resistance in *nahG* transgenic tobacco plants, suggesting that it enhances TMV resistance in tobacco either by acting downstream of SA in the SA signaling mechanism or by functioning independently of SA.[131] Intriguingly, SA can activate two different pathways to counteract virus infection: by stimulating the transcription of RNA-dependent RNA polymerase 1 (RdRp1), which mediates the induction of RNA silencing, and by inhibiting the respiratory electron transport chain in mitochondria.[132] This inhibition leads to ROS enhancement in mitochondria, which is detected by sensor proteins and the signal transduced to the nucleus, where it may induce both defense and alternative oxidase (AOX) genes. AOX negatively regulates the amplitude and duration of ROS generation. Inhibitors of the respiratory electron transport, such as antimycin A (AA), cyanide (CN^-), and pyraclostrobin, induce ROS accumulation in mitochondria as well, activating the expression of defense genes.[132] As pyraclostrobin does not induce accumulation of pathogenesis-related protein PR-1 in infiltrated plants until TMV challenging, it can be regarded as a priming agent for induced resistance[131] (see next section). Further evidence of this priming effect comes from a recent work of Udayashankar et al.[133] in which it has been shown that pyraclostrobin treatment of common bean seeds, besides ameliorating germination and seedling vigor, primed plants for enhanced resistance to bean common mosaic virus (BCMV) infection. In screenhouse experiments, pyraclostrobin seed treatment at 10 μg mL^{-1} resulted in 76% protection against BCMV, whereas under field conditions the protection ranged around 65%.

Phosphites (Phosphonates). Phosphites, also known as phosphonates, are inorganic salts (mainly K salts) of the phosphorous acid (H_3PO_3) or esters of this acid, such as fosetyl-Al (aluminum salt of the monoethyl ester of phosphorous acid). Besides their action as fungicides against different pathogens,[134,135] they have been known for a long time as SAR elicitors in many hosts.[136−140] Phosphites exhibit an acceptable efficacy also against apple scab (*Venturia inaequalis*), pear scab (*Venturia pirina*), and pecan scab

(*Fusicladium effusum*), although the control of these diseases is less successful than with conventional fungicides.[139,141] However, phosphites are particularly efficient against oomycetes and have been used for more than 30 years in the management of *Phytophthora* diseases in many crops.[142] Again, with regard to oomycetes, several studies have shown the effectiveness of potassium phosphite in controlling grapevine downy mildew caused by *Plasmopara viticola*.[143,144] Recently, Pinto et al.[145] demonstrated in a two-year field experiment in vineyards that potassium phosphite provided protection levels of 38.19% in the first season and 45.29% in the second season, performing better than fungicide treatments. Despite the above successful results, the use of phosphite in European viticulture is partially hampered by European Union regulation 149/2008 that limits the amount of residual phosphorous acid on the grape to 74.5 mg/kg. To avoid the risk of exceeding this threshold, an integrated management of downy mildew with phosphites up to preveraison, followed by copper, is an effective solution with the beneficial side effect of improving polyphenol and melatonin contents in wine.[146]

The phosphite mechanisms of action are very complex and still poorly known. Certainly, they include direct inhibition of pathogen growth and stimulation of host defenses.[147] Recently, Eshraghi et al.[148] showed that phosphite-treated *A. thaliana* leaves responded to *Phytophthora cinnamomi* zoospore inoculation with a rapid increase in callose deposition and hydrogen peroxide production. Furthermore, callose papillae appeared 6 h earlier than in nontreated inoculated seedlings, and the production of H_2O_2 in the leaves at the site of hyphal penetration and in the distal leaves of the treated plant was greatly enhanced with respect to untreated controls. These results indicate that phosphite primes the plant for a rapid and intense response to infection involving heightened activation of a range of defense responses. Defense genes from both the SA and JA/ET pathways were activated, suggesting a multiple recruitment of host defense mechanisms.[148]

Lactofen (Ethyl *O*-[5-(2-Chloro-*α*,*α*,*α*-trifluoro-*p*-tolyloxy)-2-nitrobenzoyl]-DL-lactate). Some herbicides may also show activity as fungicides and as defense activators. The diphenyl ether herbicide lactofen, an inhibitor of protoporphyrinogen oxidase, induces cell death and expression of PR1, PR5, and PR10 proteins in soybean plants. The potential for disease protection of this herbicide has been proved, showing that it activates glyceollin accumulation when soybean tissues are challenged with the wall glucan elicitor from *Phytophthora sojae*.[149]

■ ELUSIVE MECHANISM OF PRIMING

As above-reported, according to the present state of knowledge, plant resistance has been assumed to result from two successive lines of defense against pathogen attacks. The first line would be triggered by recognition of PAMPs, exemplified by amino acid sequences of flagellin (flg 22) and lipopolysaccharides (LPS), typically present in bacteria, or by chitin and ergosterol, associated with fungal pathogens, or, again, by the cell-wall *β*-glucan that characterizes the oomycetes. Perception of these elicitors by receptors pertaining to proteins at the cell surface of infected plants triggers a cascade of gene activation through responses collectively named PAMP-triggered immunity (PTI), controlled by a multitude of genes (formerly known as horizontal resistance).

Inhibition or avoidance of PTI by evolutionary progress of pathogens, resulting in pathogen-delivered effectors, is assumed to be, or have been, followed by the counterattack of disease resistance (R) proteins. Recognition of pathogen effectors by R proteins would have originated the second stronger line of resistance, termed effector-triggered immunity (ETI), formerly known as gene-for-gene resistance or vertical resistance.

Despite their chronological connotation, the two lines of defense may equally take place and show significant overlap even in their transcriptomes, emphasizing the fact that ETI may include amplified aspects of PTI.[150] In a study in which *Arabidopsis thaliana* was elicited by two typical bacterial PAMPs, flg 22 and LPS, the systemic resistance responses were shown to be identical to those typical of SAR, indicating that this can be induced without producing tissue HR-associated necrosis.[151] More generally, perception of plants sense microbe-associated molecular patterns (MAMPs) has been shown to induce activation of several defense responses, already described as typical effects of SAR, such as production of ROS, cell wall reinforcement, and callose deposition. Accordingly, intimate interactions between MAMP-triggered and SA-mediated signaling have been clearly demonstrated.[152]

In light of the above considerations, the following question may arise: How can the effects typical of SAR, triggered by PTI and ETI, be promptly reproduced upon pathogen attack in plants merely pretreated by an exogenous inducer? In other words, what are the mechanisms through which the exogenous inducer effectively alerts (primes) the plant to promptly react with all of its available defenses when challenged by a pathogen?

The first perception of an exogenous inducer, assumed to occur by an array of unknown sensory elements capable of affecting suitable gene expression, is highly conjectural. The first signals of this perception merely allow the determination of whether the inducer acts down- or upstream of SA. Then, the early events concretely detectable are typical metabolites, proteins, and enzyme activities that imply a previous activation of related genes. Whereas some of the genes clearly involved are already accessible for the normal physiological metabolism and need only to be up-regulated, as for the important PAL contribution, others must be de novo activated, as for the PR proteins.[153] Here we enter in a complex labyrinth of intersecting pathways where the real function of involved genes often rests on the loss of function associated with their defective mutants. In other words, the proof of a function is from an absence rather than for a real effect.

The most important contribution to discern the complexity of the entire phenomenon of chemically induced resistance came from the dissection of its development in two phases: priming for defenses (lapse of time from chemical treatment to inoculation) and their expression upon pathogen challenge. This distinction, originated from the studies carried out mainly by Conrath and co-workers[154] in the past two decades, stimulated the research aimed to understand the mechanism that the chemical inducer must activate to alert the plant for exploiting its natural response to the pathogen attack. In fact, the priming phase of chemical induction is highly elusive. The mere expression of *PR-1*, even though representing the most reliable marker of SAR, does not appear to be, per se, responsible for the entire barrier of defenses capable of halting the infection. However, the physiological state of the tissues promptly changes as soon as the pathogen attack starts to invade their cells. After this moment, an arsenal of defenses is rapidly mobilized, from PAL to phytoalexins, phenolics, callose, lignin, and so on. These imply the expression of several genes

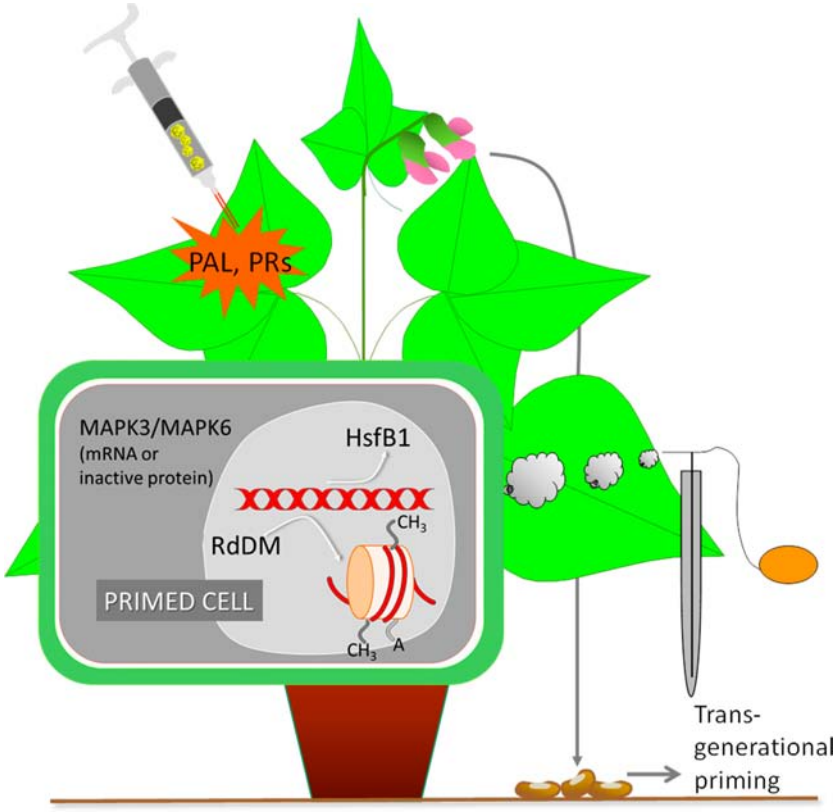

Figure 10. Low doses of exogenous inducers do not directly activate defense mechanisms but prime plants to respond more efficiently and rapidly under pathogen inoculation, that is, synthesizing phenylalanine ammonia-lyase (PAL) and pathogenesis-related proteins (PRs). In primed plant cells, mitogen-associated kinases (MAPK3 and MAPK6) are in a dormant form, whereas changes in DNA organization include RNA-directed DNA methylation (RdDM) that lead to DNA hypomethylation, driven by small interfering RNAs (siRNAs), and histone acetylation. Priming state also requires (at least in *Arabidopsis* primed by benzothiadiazole) the expression of the transcription coactivator heat-shock factor HsfB1 gene.

that in the priming phase must have been kept in a pretranscriptional state.[155,156] During the past decade two approaches have been pursued to identify a possible model for the pretranscriptional state:

(1) At first, the role of mitogen-activated protein kinases (MPKs) has been credited with crucial importance to mediate priming by an increased accumulation of these dormant defense regulatory proteins, which would require a secondary post-translational modification to become active upon a subsequent pathogen challenge.[154] Later, it was suggested that transcription factors of defense gene induction could accumulate to higher levels in primed plants. Both hypotheses have been validated in the past few years. In particular, Beckers et al. showed that when *Arabidopsis* plants are subjected to a priming treatment with BTH, they accumulate inactive MAPK3 and MPK6 that account for an enhanced kinase activity following secondary stress treatment and a faster and stronger induction of the PAL gene.[157] Emerging methods to identify MPK substrates by affinity chromatography may help to reveal the nature of other elusive signals.[158,159] However, these proteins have limited turnover, thus not explaining how a single priming stimulus can lead to a long-lasting effect typical of induced resistance. An explanation for the long-lasting changes in defense gene responsiveness comes from the different approach described below.

(2) This second more recent approach applies to principles of epigenetics, addressing its focus on chromatin as a possible substrate of memory for the plant stress response in SAR.[160] Covalent modifications of histones are known to occur by acetylation and methylation of their basic groups (mainly lysine and arginine) and have been assumed to serve as docking sites for transcriptional coactivator proteins or somehow have a role in gene activation.[161,162] A hypothesis on the molecular mechanism of priming was formulated to depend on chromatin chemical modifications conditioning defense genes for more robust activation.[163] Following this idea, Conrath found that priming the promoter of the transcription coactivator gene *WRKY 29* by BTH was associated with histone chemical changes. The expression of *WRKY 29* was not activated until the plants were challenged with an additional stress stimulus, such as an infection with *Psm* or water infiltration. These data support the hypothesis that histone modifications may serve as a sort of memory for the priming in systemic plant immunity.[164] An important mechanism by which plants can achieve targeted DNA methylation is through RNA-directed DNA methylation (RdDM),[165] which is a form of gene silencing directed by small interfering RNAs (siRNAs).[166]

Recent studies have provided support for the occurrence of epigenetic inheritance of disease resistance by transgenerational SAR transmitted through DNA hypomethylation[167] (Figure 10).

Interestingly, the RdDM pathway seems to control trans-generational priming also in the case of JA-dependent defenses, as shown by Rasmann et al. using *Arabidopsis* mutants of RdDM pathway to assess the contribution of siRNAs in transgenerational priming of induced defense against the specialist herbivore *Pieris rapae*.[168] Finally, in a study in which *Arabidopsis* plants had been treated with the chemical

inducer BABA or with a necrotizing inoculation of avirulent bacteria, the priming induction was found to be transmitted to their first progeny, where it was associated with enhanced resistance to both virulent bacteria and virulent strains of the biotrophic oomycete *Hyaloperonospora arabidopsidis*.[169] The enhanced resistance correlated with increased levels of the SA-dependent *PR* gene transcripts. Unlike other studies, the transgenerational priming was not transferred over the first progeny.[169]

The notion these discoveries imply is that the priming is a fundamental means that nature has devised to memorize a stress to face subsequent stresses. The chemical activation of the priming should be considered as a way of amplifying a natural process planned to resist both biotic and abiotic stressors, as indirectly proved by a very recent finding showing that the expression of the transcription coactivator heat-shock factor HsfB1 gene in *Arabidopsis* is required for BTH primed expression of defense genes and induced resistance in this plant species.[170]

FITNESS COSTS AND BENEFITS OF SAR

Induced resistance as SAR requires significant consumption of resources by the plant to activate its defenses in terms of new expressed genes, proteins, and metabolic pathways. The selective benefit of defense induction over constitutive resistance may be appreciated by considering the failure of the latter to adequately compensate for the costs it inflicts despite reducing pathogen attack. A number of mutants constitutively expressing elevated levels of SA and typical traits or signals of SAR have been shown to produce stunted growth or dramatic fitness costs.[171] However, the complex and unpredictable effects of naturally induced SAR on fitness may also be appreciated by considering that wild-type *A. thaliana* showed a higher fitness than the *npr1* mutant (impaired to express SAR) when the plants were challenged by *Hyaloperonospora parasitica* under low-nutrient conditions, but the beneficial effect disappeared under high-nutrient conditions. Instead, all of the *cpr* mutants, constitutively expressing SAR, failed to show a fitness benefit in comparison to wild type under *Hyaloperonospora parasitica* infection, suggesting that in nature SAR is only inducible to prevent excessive fitness costs.[172] Generally accepted is the notion that the induction of resistance at the right time of a pathogen attack avoids the endless costs that the plant should sustain under enemy-free conditions and so has been favored by natural and man-made selection.[173]

Nevertheless, adequate forms and levels of energy must be spent when SAR is involved, from the priming to the challenging phase. A recent study by authoritative experts has outlined the array of genes, signals, and pathways that have been so far identified to be involved in SAR. In view of the significant fitness costs that all of these activities require, the authors provocatively wonder if they are all really needed.[174] The question arising when we consider the costs the plant incurs when treated with a chemical inducer is whether these costs are strictly needed to impair the infection or also include a price the plant must play at the detriment of yield and quality of the produce.

The first evidence that wheat plants grown under limited nitrogen supply incur severe fitness costs when treated with BTH in the absence of pathogens was the finding that their growth was reduced and the seed yield impaired.[175] This type of fitness cost was called allocation cost, being due to the need for the plant to allocate a limited resource to defenses activation to the detriment to those required by the growth and reproduction. Since then, several papers have been published on costs, benefits, and trade-offs of induced resistance.

A review by Vallad and Goodman discussed the results of field trials on SAR and ISR according to various taxonomic groups of crops up to 2003.[176] The cited results of most field experiments give an idea about the physiological costs, due to resistance induction, resulting in some reduction of crop yield, although these reductions are generally at the limit of significance. Among monocots, BTH was found to control important diseases of wheat even better than standard fungicides, but the latter were shown to improve the wheat yield much better. BTH was also reported to control several diseases of dicots as effectively as most standard fungicides, whereas some minor efficacy was shown in its control of white rust with respect to strobilurins and against the combined effects of *Alternaria solani* and *Phytophthora* spp. The mentioned review carefully evaluated a range of BTH application rates and reported a trade-off between effective disease control and either phytotoxic effects or reduced plant productivity, not ignoring the statistical significance of the results. Considering the potential for future directions in conventional agriculture, it remarks that the efficacy of SAR induced by BTH depends on a great number of variables.[176]

A more recent review by Cipollini and Heil reports on costs and benefits of resistance separately induced to herbivores and pathogens.[177] About allocation costs of SAR, it remarks that studies using artificial pathogen challenge hardly represent natural conditions, which are often characterized by low pathogen pressure and low resources available. Implicit is the notion that benefits of SAR are mainly evident only under high pathogen pressure, but the conclusive synthesis of all studies leads to the statement that, despite inherent costs, the benefits of induced resistance outweigh them.[177]

A large body of evidence shows that a substantial part of the defenses triggered by chemical inducers are expressed only after the challenge by the pathogen. An interesting proof of dramatically different effects on fitness produced by priming and direct induction has been demonstrated by van Hulten et al.[178] These authors used low doses of BABA for priming and high doses of BABA or BTH for a direct induction of *Arabidopsis* defenses against attack by *Pseudomonas syringae* or *Hyaloperonospora parasitica*. They found that the direct induction (*PR-1* expressed directly after treatment in non-inoculated plants) seriously affected growth and seed set, whereas the priming (*PR-1* expressed only after inoculation) produced only marginal effects on the fitness and yet provided substantial protection.[178]

All results so far reported show that fitness costs may depend on several, not always evident, factors, including the nature of the chemical inducer, the dose applied, the species and variety of plant, the pathosystem, crop conditions, and so on. In contrast with more or less severe costs reported by other authors in BTH treatments, Iriti and Faoro did not observe appreciable fitness costs when they studied all parameters on which fitness depends during the SAR activated in bean by BTH under pathogen-free conditions.[179] This is clearly consistent with the absence of adverse effects to be expected during the priming phase of induction. In addition, the same authors produced evidence that BTH enhances significantly the content of beneficial metabolites when used to control *Botrytis cinerea* in grapevine.[180,181]

FACTORS AFFECTING EFFICACY OF INDUCED RESISTANCE IN THE OPEN FIELD

Fitness costs are not the only problem to consider when dealing with the practical use of the chemical activation of induced resistance under field conditions. The limited number of field trials carried out under totally natural conditions shows a tendentially better performance on dicots than on monocots. The latter, however, appear to preserve longer the induction without requiring repeated applications as often needed by dicots (see later for a tomato disease). As already mentioned, no improvement in the yield has been detected in the treatment of wheat by BTH to control some fungal diseases such as powdery mildew and *Septoria tritici*.[182] The most recent literature shows that protection offered by the limited number of inducers available on the market (mainly BTH, BABA, CHT) is generally never complete and often inferior to that provided by systemic fungicides on sensitive pathogens.[6,7]

One of the factors that may seriously limit the efficacy of the chemical induction is the host genotypic dependence of the expression of inducible defenses. In a study aimed to assess the genotype effect on the expression of induced resistance in spring barley, several cultivars with different genetic ratings of basal resistance were tested for the efficacy of their treatments with a mixture of BTH, BABA, and *cis*-jasmone in the control of *Rhinchosporium secalis* and *Blumeria graminis* f. sp. *hordei*. The study was carried out either under controlled-environment conditions or in open-field experiments.[183] The results showed a clear dependence of disease control on cultivar, with very different levels of reduced infection detected, without any relationship to the basal resistance ratings. A second remarkable outcome was the inconsistency regarding the years of field experiments: control of *Rhinchosporium secalis* was lacking on two cultivars in 2007 when levels of both pathogens were low, but was significant in 2009 when levels of *Rhinchosporium secalis* were moderate. Similarly, control of powdery mildew appeared to be proportional to this pathogen pressure, being weak in 2007 and excellent in 2008, when the performance of the inducers combination was even better than that provided by fungicide treatment. Whereas this behavior may suggest a link between efficacy of induced resistance and pathogen pressure, it is apparently in contrast with results of other authors on control efficacy shown by BTH in response to a tomato disease.[183]

Unexpected results have also been described as needing caution for applications in horticultural crops. The use of BTH to induce resistance to *Colletotrichum orbiculare* in cucumber was shown to encounter genotypic variability. Moreover, when challenged with the necrotroph *Didymella bryoniae*, some cultivars expressed resistance, but others were found to become even more susceptible to this pathogen.[184]

The review of Vallad and Goodman[176] reports the successful control of several diseases of apple and Japanese pear with BTH in field trials. Control of white rust of spinach showed the use of BTH to be as effective as the fungicide mixture of mefenoxam and copper hydroxide and less effective with respect to strobilurins. Comparable and sometimes better efficacy than standard fungicides was shown by BTH field treatments of solanaceous crops of tobacco, tomato, and pepper in the control of diseases caused by bacterial and fungal pathogens, but a poor effect resulted against infections caused by *Alternaria solani*, *Phytophthora infestans*, and *Xanthomonas axonopodis*.[176]

A recent review by Walters et al.[7] lists a number of most recent field trials and highlights instances where the application of induced resistance resulted in effectiveness significantly lower than expected. In addition to variation in host genotypes and crop nutrition, a number of factors, generically associated with the environment, have in fact emerged as responsible for a poor expression of induced resistance. Environment may reserve many unexpected and uncontrollable challenges, from potential trade-offs between antagonistic induction systems to interaction of induced resistance with abiotic stresses. An additional matter of concern is the question of whether, in the field, plants are already in an induced state. Cases of defense genes already expressed in untreated plants have been reported. Walters and Fountaine detected activities of peroxidase, cinnamyl dehydrogenase, chitinase, and glucanase already induced in untreated plants of spring barley.[6] Although, in the few cases when prior induction was detected, the plant's ability to successively respond to a new induction was not compromised, the question of its quantitative and qualitative expression remains open to doubt.[7]

In trials aimed to control bacterial spot on tomato, biweekly applications of BTH were found to not significantly reduce the epidemics compared with standard bactericide treatment. The effectiveness of induced resistance was, in fact, decreased after 9−12 days from treatment and could be repristinated only with weekly applications at 75−200 μM. Apart from the rate of BTH applied, the foliar adsorption appeared to be influenced by other factors, such as plant age and health, application methods, and environmental conditions.[185]

CONCLUDING REMARKS

The chemical approach to induce and activate SAR represents a sustainable way to control plant diseases by exploiting a natural phenomenon. As such, it may be considered as an alternative, or complementary, strategy to the use of fungicides. With respect to the latter, it appears to be less liable to select resistant strains of pathogens and offers an environmentally safe and friendly technology.

Despite these positive aspects, so far it has not yet been met with enthusiastic favor by the farmers. This has objective causes that must be removed or overcome. In a few words, farmers will not adopt new, more innovative pest management strategies unless they are convinced that they will be successful.

Some difficulties that may help to understand the tepid interest are here summarized:

(1) Phytotoxicity may sometimes affect treatments at relatively high doses (e.g., registration in Japan for use of BTH in the control of rice disease was recently lost, presumably for this reason).[186]

(2) Fitness costs are controversial, and the results indicate that it must be considered in a correct context, closely depending on the conditions of pathogen pressure forecasted.

(3) The reliability of the effectiveness of the inducer must be experimentally compared with that of systemic fungicides, but requires the adoption of appropriate time of priming induction

(4) Efficacy depends on a number of variables: dose, plant species, and cultivar, growth stage of plant, pathogen pressure, resource availability, and climatic conditions.

The last point requires a great challenge to be faced promptly with the aim of reproducing in the open field the effects of induced resistance that have been shown to rely on strong scientific evidence under controlled conditions. Several academic and agronomic centers are working to apply suitable

methodologies for reducing the gap in moving this technology from the laboratory to the field, as emerged during a recent meeting on stimulators of plant defenses at Avignon.[187,188] The urgency of reducing this gap is imposed by European Directive 2009/128/EC,[189] which requires that, by January 2014, all Member States must implement their crop protection activity according to the principles of integrated pest management. In this view, the adoption of alternative treatment methods, aimed to reduce use and risk of pesticides, is highly encouraged and demands adequate efforts to develop plant resistance inducers that might provide a significant level of crop protection. Field applications of inducers in mixtures with reduced doses of fungicides may represent a reasonable chance to be assessed. According to the view of Walters,[7] the main factors that can limit the effectiveness of inducers in the open field are genotype dependence, nutrient soil properties, environment, and prior induced state of plant. The first two of these factors may be a matter controllable to a great extent by farmers or, for them, by crop protectionists. The last two factors appear, at the moment, to be largely outside human control and require more knowledge and information on potential adverse factors that may be implicit in the environment. More field trials of new and existing inducers should eventually answer many questions and solve critical problems in the adoption of this technology.

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Notes

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REFERENCES

(1) Ross, A. F. Systemic acquired resistance induced by localized virus infections in plants. *Virology* **1961**, *14*, 340−358.

(2) Sticher, L.; Mauch-Mani, B.; Métraux, J. P. Systemic acquired resistance. *Annu. Rev. Phytopathol.* **1997**, *35*, 235−270.

(3) Hammerschmidt, R. Induced disease resistance: how do induced plants stop pathogens? *Physiol. Mol. Plant Pathol.* **1999**, *55*, 77−84.

(4) Conrath, U. Priming of induced plant defense responses. *Adv. Bot. Res.* **2009**, *51*, 361−395.

(5) Schreiber, K.; Desveaux, D. Message in a bottle: chemical biology of iduced disease resistance in plants. *Plant Pathol.* **2008**, *24* (3), 245−268.

(6) Walters, D. R.; Fountaine, J. M. Practical application of induced resistance to plant diseases: an appraisal of effectiveness under field conditions. *J. Agric. Sci.* **2009**, *147*, 523−535.

(7) Walters, D. R.; Ratsep, J.; Havis, N. D. Controlling crop diseases using induced resistance: challenges for the future. *J. Exp. Bot.* **2013**, *64* (5), 1263−1280.

(8) Fu, Z. Q.; Dong, X. Systemic acquired resistance: turning local infection into global defense. *Annu. Rev. Plant Biol.* **2013**, *64*, 839−863.

(9) Ton, J.; Mauch-Mani, B. Amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* **2004**, *38*, 119−130.

(10) Jones, J. D. G.; Dangl, J. L. The plant immune system. *Nature* **2006**, *444*, 323−329.

(11) Thomma, B. P. H. J.; Nùrnberger, T.; Joosten, M. H. A. J. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* **2011**, *23* (1), 4−15.

(12) Pieterse, C. M.; Leon-Reyes, A.; Van der Ent, S.; Van Wees, S. C. Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol.* **2009**, *5* (5), 308−316.

(13) Gozzo, F. Systemic acquired resistance in crop protection; from nature to a chemical approach. *J. Agric. Food Chem.* **2003**, *51*, 4487−4503.

(14) Mukhtar, S.; Nishimura, M. T.; Dangl, J. NPR1 in plant defense: it's not over 'till it's turned over. *Cell* **2009**, *137*, 804−806.

(15) Wu, Y.; Zhang, D.; Chu, J. Y.; Boyle, P.; Wang, Y.; Brindle, I. D.; De Luca, V.; Desprès, C. The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Rep.* **2012**, *1*, 639−647.

(16) Spoel, S. H.; Mou, Z.; Tada, Y.; Spivey, N. W.; Genschik, P.; Dong, X. Proteasome-mediated turnover of the transcription co-activator NPR1 plays dual roles in regulating plant immunity. *Cell* **2009**, *137* (5), 860−872.

(17) Zhang, Y.; Cheng, Y. T.; Qu, N.; Zhao, Q.; Bi, D.; Li, X. Negative regulation of defense responses in Arabidopsis by two NPR1 paralogs. *Plant J.* **2006**, *48*, 647−656.

(18) Fu, Z. Q.; Yan, S.; Saleh, A.; Wang, W.; Ruble, J.; et al. NPR3 and NPR4 are receptors f or the immune signal salicylic acid in plants. *Nature* **2012**, *486*, 228−232.

(19) Weigel, R. R.; Bauscher, C.; Pfitzner, A. J. P.; Pfitzner, U. M. NIMIN-1, NIMIN-2 and NIMIN-3, members of a novel family of proteins from Arabidopsis that interact with NPR1/NIM1, a key regulator of systemic acquired resistance in plants. *Plant Mol. Biol.* **2001**, *46*, 143−160.

(20) Weigel, R. R.; Pfitzner, U. M.; Gatz, C. Interaction of NIMIN1 with NPR1 modulates PR gene expression in Arabidopsis. *Plant Cell* **2005**, *17*, 1279−1291.

(21) Glazebrook, J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* **2005**, *43*, 205−227.

(22) Van Wess, S. C. M.; Van der Ent, S.; Pieterse, C. M. J. Plant immune responses triggered by beneficial microbes. *Curr. Opin. Plant Biol.* **2008**, *11*, 443−448.

(23) Koornneef, A.; Pieterse, C. M. J. Cross talk in defense signaling. *Plant Physiol.* **2008**, *146*, 839−844.

(24) Spoel, S. H.; Johnson, J. S.; Dong, X. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 18842−18847.

(25) Thaler, J. S.; Humphrey, P. T.; Whitteman, N. K. Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* **2012**, *17* (5), 260−270.

(26) Brooks, D. M.; Bender, C. L.; Kunkel, B. N. The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in *Arabidopsis thaliana*. *Mol. Plant Pathol.* **2005**, *6* (6), 629−639.

(27) Katsir, L.; Schilmiller, A. L.; Staswick, P. E.; Sheng, Y. H.; Gregg, A. H. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (19), 7100−7105.

(28) Zheng, X. Y.; Spivey, N. W.; Zeng, W.; Liu, P. P.; Fu, Z. Q.; et al. Coronatine promotes *Pseudomonas syringae* virulence in p lants by activating a signaling cascade that inhibits salicylic acid accumulation. *Cell Host Microbe* **2012**, *11*, 587−596.

(29) Ton, J.; Flors, V.; Mauch-Mani, B. The multifaceted role of ABA indisease resistance. *Trends Plant Sci.* **2009**, *14*, 310−317.

(30) Iriti, M.; Faoro, F. Abscissic acid is involved in chitosan-induced resistance to tobacco necrosis virus. *Plant Physiol. Biochem* **2008**, *46*, 1106−1111.

(31) Yasuda, M.; Ishikawa, A.; Jikumanu, Y.; Seki, M.; et al. Antagonistic interaction between SAR and the abscissic acid-mediated abiotic stress response in *Arabidopsis*. *Plant Cell* **2008**, *20* (6), 1678−1692.

(32) Luna, E.; Pastor, V.; Robert, J.; Flors, V.; Mauch-Mani, B.; Ton, J. Callose deposition: a multifaceted plant defense response. *Mol. Plant-Microbe Interact.* **2011**, *24* (2), 183−193.

(33) Melotto, M.; Underwood, W.; Koczan, J.; Nomura, K.; He, S. Y. Plant stomata function in innate immunity against bacterial invasion. *Cell* **2006**, *126*, 969−980.

(34) Clay, N. K.; Adio, A. M.; Denoux, C.; Jander, G.; Ausubel, F. M. Glucosinolate metabolites required for an Arabidopsis innate immune response. *Science* **2009**, *323*, 95−101.

(35) Audenaert, K.; De Meyer, G. B.; Hòfte, M. M. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses SA-dependent signaling mechanisms. *Plant Physiol.* **2002**, *128*, 491−501.
(36) Asselbergh, B.; Curvers, K.; Franca, S. C.; Audenaert, K.; et al. Resistance to *Botrytis cinerea* in *sitiens*, an ABA-deficient tomato mutant, involves timely production of H_2O_2 and cell wall modification in the epidermis. *Plant Physiol.* **2007**, *144*, 1863−1877.
(37) Pastori, G. M.; Kiddle, G.; Antoniw, J.; et al. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signalling. *Plant Cell* **2003**, *15* (4), 939−951.
(38) Nambara, E.; Marion-Poll, A. Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* **2005**, *56*, 165−185.
(39) Cao, F. Y.; Yoshioka, K.; Desveaux, D. The roles of ABA in plant-pathogen interactions. *J. Plant Res.* **2011**, *124*, 489−499.
(40) Dempsey, D. A.; Klessig, D. F. SOS − too many signals for SAR? *Trends Plant Sci.* **2012**, *17* (9), 538−545.
(41) Manosalva, P. M.; Park, S.-W.; Forouhar, F.; Tong, L.; Fry, W. E.; Klessig, D. F. *Methyl Esterase 1 (StMES1)* is required for SAR in Potato. *Mol. Plant−Microbe Interact.* **2010**, *23* (9), 1151−1163.
(42) Koo, Y. J.; Kim, M. A.; Kim, E. H.; Song, J. T.; et al. Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in *Arabidopsis thaliana*. *Plant Mol. Biol.* **2007**, *64*, 1−15.
(43) Park, S. W.; Kaimoyo, E.; Kumar, D.; Mosher, F.; Klessig, D. F. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* **2007**, *318*, 113−116.
(44) Attaran, E.; Zeier, T. E.; Griebel, T.; Zeier, J. Methyl salicylate production and jasmonate signalling are not essential for systemic acquired resistance in Arabidopsis. *Plant Cell* **2009**, *21*, 954−971.
(45) Spoel, S. H.; Dong, X. How do plants achieve immunity? Defence without specialized immune cells. *Nat. Rev. Immunol.* **2012**, *12*, 89−100.
(46) Chanda, B.; Xia, Y.; Mandal, M.; et al. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nat. Genet.* **2011**, *43*, 421−427.
(47) Chaturvedi, R.; Venables, B.; Petros, R. A.; Nalam, V.; et al. An abietane diterpenoid is a potent activator of SAR. *Plant J.* **2012**, *71*, 161−172.
(48) Jung, H. W.; Tschaplinski, T. J.; Wang, L.; Glazebrook, J.; Greenberg, J. T. Priming in systemic plant immunity. *Science* **2009**, *324*, 89−91.
(49) Zoeller, M.; Stingl, N.; Kriscke, M.; Fekete, A.; Waller, F.; Berger, S.; Mueller, M. J. Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. *Plant Physiol.* **2012**, *160*, 365−378.
(50) Schneider, C.; Porter, N. A.; Brash, A. R. Routes to 4-hydroxynonenal: fundamental issues in the mechanisms of lipid peroxidation. *J. Biol. Chem.* **2008**, *283* (23), 15539−15543.
(51) Nàvarovà, H.; Bernsdorff, F.; Döring, A.-C.; Zeier, J. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell* **2012**, *24*, 5123−5141.
(52) White, R. F. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology* **1979**, *99*, 410−417.
(53) Friedrich, L.; Lawton, K.; Ruess, W.; et al. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant J.* **1996**, *10*, 61−70.
(54) Scarponi, L.; Buonaurio, R.; Martinetti, L. Persistence and translocation of a benzothiadiazole derivative in tomato plants in relation to systemic acquired resistance against *Pseudomonas syringae* pv *tomato*. *Pest Manag. Sci.* **2001**, *57*, 262−268.
(55) Lawton, K. A.; Friedrich, L.; Hunt, M.; et al. Benzothiadiazole induces resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* **1996**, *10*, 71−82.
(56) Maffi, D.; Iriti, M.; Pigni, M.; Vannini, C.; Faoro, F. *Uromyces appendiculatus* infection in BTH-treated bean plants: ultrastructural details of a lost fight. *Mycopathologia* **2011**, *171*, 209−221.
(57) Faoro, F.; Maffi, D.; Cantu, D.; Iriti, M. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. *BioControl* **2008**, *53*, 387−401.
(58) Han, Q.; Feng, H.; Zhao, H.; Huang, L.; et al. Effect of benzothiadiazole on inducing resistance of soybean to *P. sojae*. *Protoplasma* **2013**, *250*, 471−481.
(59) Faessel, L.; Nassr, N.; Lebeau, T.; Walter, B. Effects of the plant defence inducer, acibenzolar-S-methyl, on hypocotyl rot of soybean caused by *Rhizoctonia solani* AG-4. *Phytopathology* **2008**, *156*, 236−242.
(60) Du, Q.; Zhu, W.; Zhao, Z.; Qian, X.; Xu, Y. Novel benzo-1,2,3-thiadiazole-7-carboxylate derivatives as plant activators and the development of their agricultural applications. *J. Agric. Food Chem.* **2012**, *60*, 346−353.
(61) Sobhy, I. S.; Erb, M.; Sarhan, A. A.; El-Husseini, M. M.; et al. Less is more: treatment with BTH and laminarin reduces herbivore-induced volatile emissions in maize but increases parasitoid attraction. *J. Chem. Ecol.* **2012**, *38*, 348−360.
(62) Papavizas, G. C. Greenhouse control of *Aphanomyces* root rot of peas with aminobutyric acid and methyl aspartic acid. *Plant Dis. Rep.* **1964**, *48*, 537−541.
(63) Cohen, Y.; Niderman, T.; Mosinger, E.; Fluhr, R. β-Aminobutyric acid induces accumulation of pathogenesis-related proteins in tomato and resistance to late bright infections caused by *Phytophthora infestans*. *Plant Physiol.* **1994**, *107*, 59−66.
(64) Siegrist, J.; Orober, M.; Buchenauer, H. β-Aminobutyric acid-induced enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiol. Mol. Plant Pathol.* **2000**, *56*, 95−106.
(65) Ton, J.; Jakab, G.; Toquin, V.; Mauch-Mani, B. Dissecting the β-aminobutyric acid-induced priming phenomenon in Arabidopsis. *Plant Cell* **2005**, *17*, 987−999.
(66) Flors, V.; Ton, J.; van Doorn, R.; Jakab, G.; Garcia-Augustin, P.; Mauch-Mani, B. Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant J.* **2008**, *54*, 81−92 and references cited therein..
(67) Tsai, C.-H.; Singh, P.; Chen, C.; Thomas, J.; Weber, J.; Mauch-Mani, B.; Zimmerli, L. Priming for enhanced defense responses by specific inhibition of the Arabidopsis responses to coronatine. *Plant J.* **2011**, *65* (3), 469−479.
(68) Hamiduzzaman, M. M.; Jakab, G.; Barnavon, L.; Neuhaus, J.; Mauch-Mani, B. β-Aminobutyric acid-induced resistance against downy mildewin grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Mol. Plant−Microbe Interact.* **2005**, *18* (8), 819−829.
(69) Slaughter, A. R.; Hamiduzzaman, M. M.; Gindro, K.; Neuhaus, J.-M.; Mauch-Mani, B. β-Aminobutyric acid-induced resistance in grapevine against downy mildew: involvement of pterostilbene. *Eur. J. Plant Pathol.* **2008**, *122* (1), 185−195.
(70) Li, Y. M.; Zhang, Z.; Jia, Y.; Shan, Y.; et al. 3-Acetonyl-3-hydroxyoxindole: a new inducer of SAR in plants. *Plant Biotechnol. J.* **2008**, *6*, 301−308.
(71) Goy, R. C.; de Britto, D.; Assis, O. B. G. A review of the antimicrobial activity of chitosan. *Polimeros: Cienc. Tecnol.* **2009**, *19* (3), 241−247.
(72) Vander, P.; Vàrum Kjell, M.; Domard, A.; El Gueddari, N. E.; Moerschbacher, B. M. Comparison of the ability of partially N-acetylated chitosans and chitooligosaccharides to elicit resistance reactions in wheat leaves. *Plant Physiol.* **1998**, *118*, 1353−1359.
(73) Faoro, F.; Sant, S.; Iriti, M.; Maffi, D.; Appiano, A. Chitosan-elicited resistance to plant viruses: a histochemical and cytochemical study. In *Chitin Enzymology 2001*; Muzzarelli, R. A. A., Ed.; AtecEdizioni: Grottammare, Italy, 2001; pp 57−62.
(74) Iriti, M.; Vitalini, S.; Di Tommaso, G.; D'Amico, S.; Borgo, M.; Faoro, F. New chitosan formulation prevents grapevine powdery

mildew infection and improves polyphenol content and free radical scavenging activity of grape and wine. *Aust. J. Grape Wine* **2011**, *17*, 263−269.
(75) Munoz, Z.; Moret, A. Sensitivity of *Botrytis cinerea* to chitosan and acibenzolar-S-methyl. *Pest Manag. Sci* **2010**, *66*, 974−979.
(76) Aziz, A.; Poinssot, B.; Daire, X.; Adrian, M.; Bezier, A.; et al. Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Mol. Plant−Microbe Interact.* **2003**, *16*, 1118−1128.
(77) Daxberger, A.; Nemak, A.; Mithofer, A.; Fliegmann, J.; Ligterink, W.; et al. Activation of members of a MAPK module in β-glucan elicitor-mediated non-host resistance of soybean. *Planta* **2007**, *225*, 1559−1571.
(78) Trouvelot, S.; Varnier, A. L.; Allegre, M.; Baillieul, F.; et al. A β-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including Hrlike cell death. *Mol. Plant−Microbe Interact.* **2008**, *21*, 232−243.
(79) Hodgson, W. A.; Munro, J.; Singh, R. P.; Wood, F. A. Isolation from *Phytophthora infestans* of a polysaccharide that inhibits potato virus X. *Phytopathology* **1969**, *59*, 1334−1335.
(80) Singh, R. P.; Wood, F. A.; Hodgson, W. A. Nature of virus inhibition by a olysaccharide from *Phytophthora infestans*. *Phytopathology* **1970**, *60*, 1566−1569.
(81) Steimetz, E.; Trouvelot, S.; Bordier, A.; Heloir, M.-C.; Poinssot, B.; Adrian, M.; Daire, X. Does leaf position influence induced resitance to grape downy mildew? *IOBC−WPRS Bull.* **2013**, *89*, 171−175.
(82) Klarzynski, O.; Plesse, B.; Joubert, J. M.; Yvin, J. C.; et al. Linear β-1,3 glucans are elicitors of defense responses in tobacco. *Plant Physiol.* **2000**, *124*, 1027−1037.
(83) Menard, R.; Alban, S.; de Ruffray, P.; Jamois, F.; Franz, G.; et al. β-1,3 Glucan sulfate, but not β-1,3 glucan, induces the salicylic acid signaling pathway in tobacco and Arabidopsis. *Plant Cell* **2004**, *16*, 3020−3032.
(84) Menard, R.; de Ruffray, P.; Fritig, B.; Yvin, J. C.; Kauffmann, S. Defense and resistance-inducing activities in tobacco of the sulfated β-1,3 glucan PS3 and its synergistic activities with the unsulfated molecule. *Plant Cell Physiol.* **2005**, *46*, 1964−1972.
(85) Fliegmann, J.; Mithofer, A.; Wanner, G.; Ebel, J. An ancient enzyme domain hidden in the putative β-glucan elicitor receptor of soybean may play an active part in the perception of pathogen-associated molecular patterns during broad host resistance. *J. Biol. Chem.* **2004**, *279*, 1132−1140.
(86) Mithofer, A.; Lottspeich, F.; Ebel, J. One-step purification of the β-glucan elicitor-binding protein from soybean (*Glycine max* L) roots and characterization of an anti-peptide antiserum. *FEBS Lett.* **1996**, *381*, 203−207.
(87) Umemoto, N.; Kakitani, M.; Iwamatsu, A.; Yoshikawa, M.; Yamaoka, N.; Ishida, I. The structure and function of a soybean β-glucan elicitor-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1029−1034.
(88) Siegrist, J.; Glenewinkel, D.; Schmidke, M. Chemically induced resistance in green bean against bacterial and fungal pathogens. *J. Plant Dis. Prot.* **1997**, *104*, 599−610.
(89) Oostendorp, M.; Kunz, W.; Dietrich, B.; Staub, T. Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol.* **2001**, *107*, 19−28.
(90) Walters, D. R.; Paterson, L.; Walsh, D. J.; Havis, N. D. Priming for plant defense in barley provides benefits only under high disease pressure. *Physiol. Mol. Plant Pathol.* **2009**, *73*, 95−100.
(91) Boyle, C.; Walters, D. R. Saccharin-induced protection against powdery mildew in barley: effects on growth and phenylpropanoid metabolism. *Plant Pathol.* **2006**, *55*, 82−91.
(92) Srivastava, P.; George, S.; Marois, J.; Wright, D. L.; Walker, D. R. Saccharin induced systemic acquired resistance against rust (*Phakopsora pachyrhizi*) infection in soybean: effects on growth and development. *Crop Prot.* **2011**, *30*, 726−732.
(93) Delgado, D. Z.; de Freitas, M. B.; Stadnik, M. J. Effectiveness of saccharin and ulvan as resistance inducers against rust and angular leaf spot in bean plants (*Phaseolus vulgaris*). *Crop Prot.* **2013**, *47*, 67−73.
(94) Epstein, E. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11−17.
(95) Ma, J. F.; Yamaji, N.; Mitani-Ueno, N. Transport of silicon from roots to panicles in plants. *Proc. Jpn. Acad. Ser. B−Phys. Biol. Sci.* **2011**, *87*, 377−385.
(96) Fauteux, F.; Rémus-Borel, W.; Menzies, J. G.; Bélanger, R. R. Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiol. Lett.* **2005**, *249*, 1−6.
(97) Ma, J. F.; Tamai, K.; Yamaji, N.; Mitani, N.; Konishi, S.; et al. A silicon transporter in rice. *Nature* **2006**, *440*, 688−691.
(98) Cooke, J.; Leishman, M. R. Is plant ecology more siliceous than we realise? *Trends Plant Sci.* **2011**, *16*, 61−68.
(99) Epstein, E. Silicon: its manifold roles in plants. *Ann. Appl. Biol.* **2009**, *155*, 155−160.
(100) Ma, J. F.; Yamaji, N. Silicon uptake and accumulation in higher plants. *Trends Plant Sci.* **2006**, *11*, 392−397.
(101) Jones, L. H. P.; Handreck, K. A. Silica in soils, plants and animals. *Adv. Agron.* **1967**, *19*, 107−149.
(102) Samuels, A. L.; Glass, A. D. M.; Ehret, D. L.; Menzies, J. G. Mobility and deposition of silicon in cucumber plants. *Plant Cell Environ.* **1991**, *14*, 485−492.
(103) Fawe, A.; Abou-Zaid, M.; Menzies, J. G.; Bélanger, R. R. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathology* **1998**, *88*, 396−401.
(104) Chain, F.; Cote-Beaulieu, C.; Belzile, F.; Menzies, J. G.; Bélanger, R. R. A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. *Mol. Plant−Microbe Interact.* **2009**, *22*, 1323−1330.
(105) Zargar, S. M.; Nazir, M.; Agrawal, G. K.; Kim, D. W.; Rakwal, R. Silicon in plant tolerance against environmental stressors: towards crop improvement using omics approaches. *Curr. Proteomics* **2010**, *7*, 135−143.
(106) Ghareeb, H.; Bozso, Z.; Ott, P. G.; Repenning, C.; Stahl, F.; Wydra, K. Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. *Physiol. Mol. Plant Pathol.* **2011**, *75*, 83−89.
(107) Chérif, M.; Asselin, A.; Bélanger, R. R. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* **1994**, *84*, 236−242.
(108) Fauteux, F.; Chain, F.; Belzile, F.; Menzies, J. G.; Bélanger, R. R. The protective role of silicon in the Arabidopsis-powdery mildew pathosystem. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17554−17559.
(109) Iwai, T.; Miyasaka, A.; Seo, S.; Ohashi, Y. Contribution of ethylene biosynthesis for resistance to blast fungus infection in young rice plants. *Plant Physiol.* **2006**, *142*, 1202−1215.
(110) Rodrigues, F. A.; McNally, D. J.; Datnoff, L. E.; Jones, J. B.; Labbe, C.; et al. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. *Phytopathology* **2004**, *94*, 177−183.
(111) Rodrigues, F. A.; Jurick, W. M.; Datnoff, L. E.; Jones, J. B.; Rollins, J. A. Silicon influences cytological and molecular events in compatible and incompatible rice-*Magnaporthe grisea* interactions. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 144−159.
(112) Cai, K. Z.; Gao, D.; Luo, S. M.; Zeng, R. S.; Yang, J. Y.; Zhu, X. Y. Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. *Physiol. Plant.* **2008**, *134*, 324−333.
(113) Bélanger, R. R.; Benhamou, N.; Menzies, J. G. Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*). *Phytopathology* **2003**, *93*, 402−412.
(114) Rémus-Borel, W.; Menzies, J. G.; Bélanger, R. R. Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 108−115.
(115) Shetty, R.; Jensen, B.; Shetty, N. P.; Hansen, M.; Hansen, C. W.; et al. Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. *Plant Pathol.* **2012**, *61*, 120−131.

(116) Van Bockhaven, J.; De Vleesschauwer, D.; Höfte, M. Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *J. Exp. Bot.* **2013**, *64*, 1281−1293.
(117) Lyon, G. Agents that can elicit induced resistance. In *Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection*; Walters, D. N. A., Lyon, G., Eds.; Blackwell Publishing: Oxford, UK, 2007; pp 9−29.
(118) Ahn, I. P.; Kim, S.; Lee, Y. H. Vitamin B1 functions as an activator of plant disease resistance. *Plant Physiol.* **2005**, *138*, 1505−1515.
(119) Dong, H.; Beer, S. Riboflavin induces disease resistance in plants by activating a novel signal transduction pathway. *Phytopathology* **2000**, *90*, 801−811.
(120) Dong, H.; Liu, A.; Wang, Y.; Liu, B.; Fan, H. et al.Control of brown spot by induced resistance in tobacco: preparation SRS2, its functions to control the disease and to improve qualitative and economic properties of the cured leaves. In *Induced Resistance Against Diseases in Plants*; Dong, H., Ed.; Science Press: Beijing, China, 1995; pp 422−427.
(121) Wang, S.; Tzeng, D. D. Methionine-riboflavin mixtures with surfactants and metal ions reduce powdery mildew infection in strawberry plants. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 987−991.
(122) Aver'yanov, A. A.; Lapikova, V. P.; Nikolaev, O. N.; Stepanov, A. I. Active oxygen-associated control of rice blast disease by riboflavin and roseoflavin. *Biochemistry (Moscow)* **2000**, *65*, 1292−1298.
(123) Song, G. C.; Choi, H. K.; Ryu, C.-M. Vitamin Bx-mediated induced resistance against cucumber mosaic virus and *Xanthomonas axonopodis* in the pepper field. *IOBC−WPRS Bull.* **2013**, *89*, 195−198.
(124) Ward, E. W. A. Suppression of metalaxyl activity by glyphosphate: evidence that host defense mechanisms contribute to metalaxyl inhibition of *Phytophthora megasperma* f.sp. *glycines* in soybeans. *Physiol. Plant Pathol.* **1984**, *25*, 381−386.
(125) Molina, A.; Hunt, M. D.; Ryals, J. A. Impaired fungicide activity in plants blocked in disease resistance signal transduction. *Plant Cell* **1998**, *10*, 1903−1914.
(126) Iwata, M. Probenazole − a plant defence activator. *Pesticide Outlook* **2001**, *12*, 28−31.
(127) Yoshioka, K.; Nakashita, H.; Klessig, D. F.; Yamaguchi, I. Probenazole induces systemic acquired resistance in Arabidopsis with a novel type of action. *Plant J.* **2001**, *25*, 149−157.
(128) Yang, K.-H.; Huang, C.-J.; Liu, Y.-H.; Chen, C.-Y. Efficacy of probenazole for control of southern orn leaf blight. *J. Pestic. Sci.* **2011**, *36*, 235−239.
(129) Kessmann, H.; Staub, T.; Hofmann, C.; Maetzke, T.; Herzog, J.; Ward, E.; Uknes, S.; Ryals, J. Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol.* **1994**, *32*, 439−459 and references cited therein..
(130) Thieron, M.; Reisener, H. J.; Scheinpflug, H. Systemic acquired resistance in rice: regulation of host defence reactions. In *Modern Fungicides and Antifungal Compounds*; Lyr, H., Russel, P. E., Sisler, H. D., Eds.; Intercept: Andover, UK, 1996; pp 493−502.
(131) Herms, S.; Seehaus, K.; Koehle, H.; Conrath, U. A strobilurin fungicide enhances the resistance of tobacco against tobacco mosaic virus and *Pseudomonas syringae* pv *tabaci*. *Plant Physiol.* **2002**, *130*, 120−127.
(132) Singh, D. P.; Moore, C. A.; Gilliland, A.; Carr, J. P. Activation of multiple anti-viral defense mechanisms by salicylic acid. *Mol. Plant Pathol.* **2004**, *5*, 57−63.
(133) Udayashankar, A. C.; Nayaka, C. S.; Archana, B.; Nayak, U.; Niranjana, S. R.; Prakash, H. S. Strobilurins seed treatment enhances resistance of common bean against bean common mosaic virus. *J. Phytopathol.* **2012**, *160*, 710−716.
(134) Fenn, M.; Coffey, M. D. Studies on the *in-vitro* and *in-vivo* antifungal activity of fosetyl-Al and phosphorous acid. *Phytopathology* **1984**, *74*, 606−611.
(135) Wilkinson, C. J.; Shearer, B. L.; Jackson, T. J.; Hardy, G. E. St. J. Variation in sensitivity of Western Australian isolates of *Phytophthora cinnamomi* to phosphite in vitro. *Plant Pathol.* **2001**, *50*, 83−89.
(136) Guest, D. I.; Grant, B. R. The complex action of phosphonates as antifungal agents. *Biol. Rev.* **1991**, *66*, 159−187.
(137) Becot, S.; Pajot, E.; Le Corre, D.; Monot, C.; Silue, D. Phytogard (K_2HPO_3) induces localized resistance in cauliflower to downy mildew of crucifers. *Crop Prot.* **2000**, *19*, 417−425.
(138) Jackson, T. J.; Burgess, T.; Colquhoun, I.; Hardy, G. E. St. J. Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathol.* **2000**, *49*, 147−154.
(139) Percival, G. C.; Noviss, K.; Haynes, I. Field evaluation of systemic inducing resistance chemicals at different growth stages for the control of apple (*Venturia inaequalis*) and pear (*Venturia pirina*) scab. *Crop Prot.* **2009**, *28*, 629−633.
(140) Miller, J. S.; Olson, N.; Woodell, L.; Porter, L. D.; Clayson, S. Post-harvest applications of zoxamide and phosphite for control of potato tuber rots caused by oomycetes at harvest. *Am. J. Potato Res.* **2006**, *83*, 269−278.
(141) Bock, C. H.; Brenneman, T. B.; Hotchkiss, M. V.; Wood, B. W. Evaluation of a phosphite fungicide to control pecan scab in the southeastern USA. *Crop Prot.* **2012**, *36*, 58−64.
(142) Hardy, G. E. St. J.; Barrett, S.; Shearer, B. L. The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathol.* **2001**, *30*, 133−139.
(143) Reuveni, M.; Reuveni, R. Efficacy of foliar sprays of phosphates in controlling powdery mildews in field-grown nectarine, mango trees and grapevines. *Crop Prot.* **1995**, *14*, 311−314.
(144) Dalbó, M. A.; Schuck, E. Avaliação do uso de fosfitos para o controle do míldio da videira. *Agropecuaria Catarinense* **2003**, *16*, 33−35.
(145) Pinto, K. M. S.; do Nascimento, L. C.; de Souza Gomes, E. C.; da Silva, H. F. C.; dos Reis Miranda, J. Efficiency of resistance elicitors in the management of grapevine downy mildew *Plasmopara viticola*: epidemiological, biochemical and economic aspects. *Eur. J. Plant Pathol.* **2012**, *134*, 745−754.
(146) Vitalini, S.; Gardana, C.; Zanzotto, A.; Fico, G.; Faoro, F.; Simonetti, P.; Iriti, M. From vineyard to glass: agrochemicals enhance the melatonin and total polyphenol contents and antiradical activity of red wines. *J. Pineal Res.* **2011**, *51*, 278−285.
(147) Daniel, R.; Guest, D. Defence responses induced by potassium phosphonate in *Phytophthora palmivora*-challenged *Arabidopsis thaliana*. *Physiol. Mol. Plant Pathol.* **2006**, *67*, 194−201.
(148) Eshraghi, L.; Anderson, J.; Aryamanesh, N.; Shearer, B.; McComb, J.; Hardy, G. E. St. J.; O'Brien, P. A. Phosphite primed defence responses and enhanced expression of defence genes in *Arabidopsis thaliana* infected with *Phytophthora cinnamomi*. *Plant Pathol.* **2011**, *60*, 1086−1095.
(149) Graham, M. Y. The diphenylether lactofen induces cell death and expression of defense- related genes in soybean. *Plant Physiol.* **2005**, *139*, 1784−1794.
(150) Craig, A.; Ewan, R.; Mesmar, J.; Gudipati, V.; Sadanandom, A. E3 ubiquitin ligases and plant innate immunity. *J. Exp. Bot.* **2009**, *60* (4), 1123−1132.
(151) Mishina, T. E.; Zeier, J. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. *Plant J.* **2007**, *50*, 500−513.
(152) Tsuda, K.; Sato, M.; Glazebrook, J.; Cohen, J. D.; Katagiri, F. Interplay between MAMP-triggered and SA-mediated defense responses. *Plant J.* **2008**, *53*, 763−775.
(153) Bolton, M. D. Primary metabolism and plant defense − fuel for the fire. *Mol. Plant−Microbe Interact.* **2009**, *22* (5), 487−497.
(154) Conrath, U.; Beckers, G. J.; Flors, V.; García-Agustín, P.; Jakab, G.; Mauch, F.; Newman, M. A.; Pieterse, C. M.; Poinssot, B.; Pozo, M. J.; Pugin, A.; Schaffrath, U.; Ton, J.; Wendehenne, D.; Zimmerli, L.; Mauch-Mani, B.; Prime-A-Plant Grp, B. Priming: getting ready for battle RID D-3308-2011 RID A-9326-2011. *Mol. Plant−Microbe Interact.* **2006**, *19*, 1062−1071.
(155) Conrath, U. Molecular aspects of defence priming. *Trends Plant Sci* **2011**, *16* (10), 524−531.

(156) van Verk, M. C.; Bol, J. F.; Linthorst, H. J. Prospecting for Genes involved in transcriptional regulation of plant defenses, a bioinformatics apprach. *BMC Plant Biol.* **2011**, *11* (88), 1−12.
(157) Beckers, G. J. M.; Jaskiewicz, M.; Liu, Y.; Underwood, W. R.; He, S. Y.; Zhang, S.; Conrath, U. Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* **2009**, *21*, 944−953.
(158) O'Shaughnessy, E. C.; Palani, S.; Collins, J. J.; Sarkar, C. A. Tunable signal processing in synthetic MAP kinase cascades. *Cell* **2011**, *144*, 119−131.
(159) Hoehenwarter, W.; Thomas, M.; Nukarinen, E.; Egelhofer, V.; Rohrig, H.; Weckwerth, W.; Conrath, U.; Beckers, G. J. M. Identification of novel *in vivo* MAP kinase substrates in *Arabidopsis thaliana* through use of tandem metal oxide affinity chromatography. *Mol. Cell. Proteomics* **2013**, *12.2*, 369−380.
(160) Pastor, V.; Luna, E.; Mauch-Mani, B.; Ton, J.; Flors, V. Primed plants do not forget. *Environ. Exp. Bot.* **2012**, DOI: 10.1016/j.envexpbot.2012.02.013.
(161) Kanno, T.; Kanno, Y.; Siegel, R. M.; Jang, M. K.; Lenardo, M. J.; Ozato, K. Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. *Mol. Cell* **2004**, *13*, 33−43.
(162) Ruthenburg, A. J.; Allis, C. D.; Wysocka, J. Methylation of lysine 4 and histone H3: intricacy of writing and reading a single epigenetic mark. *Mol. Cell* **2007**, *25*, 15−30.
(163) Bruce, T. J. A.; Matthes, M. C.; Napier, J. A.; Pickett, J. A. Stressful "memories" of plants: evidence and possible mechanisms. *Plant Sci.* **2007**, *173*, 603−608.
(164) Jaskiewicz, M.; Conrath, U.; Peterhänsel, C. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* **2011**, *12* (1), 50−55.
(165) Wassenegger, M.; Heimes, S.; Riedel, L.; Sanger, H. L. RNA-directed de-novo methylation of genomic sequences in plants. *Cell* **1994**, *76*, 567−576.
(166) Naumann, U.; Daxinger, L.; Kanno, T.; Eun, C.; Long, Q.; Lorkovic, Z. J.; Matzke, M.; Matzke, A. J. M. Genetic evidence that DNA methyltransferase DRM2 has a direct catalytic role in RNA-directed DNA methylation in *Arabidopsis thaliana*. *Genetics* **2011**, *187*, 977−979.
(167) Luna, E.; Bruce, T. J. A.; Roberts, M. R.; Flors, V.; Ton, J. next-generation systemic acquired resistance. *Plant Physiol.* **2012**, *158*, 844−853.
(168) Rasmann, S.; De Vos, M.; Casteel, C. L.; Tian, D.; Hatlischke, R.; Sun, J. Y.; et al. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* **2012**, *158*, 854−863.
(169) Slaughter, A.; Daniel, X.; Flors, V.; Luna, E.; Hohn, B.; Mauch-Many, B. Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiol.* **2012**, *158*, 835−843.
(170) Pick, T.; Jaskiewicz, M.; Peterhänsel, C.; Conrath, U. Heat shock factor HsfB1 primes gene transcription and systemic acquired resistance in Arabidopsis. *Plant Physiol.* **2012**, *159*, 52−55.
(171) Heil, M.; Baldwin, I. T. Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends Plant Sci.* **2002**, *7* (2), 61−67.
(172) Heidel, A. J.; Dong, X. N. Fitness benefits of systemic acquired resistance during *Hyalopperonospora parasitica* infection in *Arabidopsis thaliana*. *Genetics* **2006**, *173*, 1621−1628.
(173) Walters, D.; Heil, M. Costs and trade-offs associated with induced resistance. *Physiol. Mol. Plant Pathol.* **2007**, *71*, 3−17.
(174) Dempsey, D. M. A.; Klessig, D. F. SOS − too many signals for SAR ? *Trends Plant Sci.* **2012**, *17* (9), 538−545.
(175) Heil, M.; Hilpert, A.; Kaiser, W.; Linsenmair, E. Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance incur allocation costs? *J. Ecol.* **2000**, *88*, 645−654.
(176) Vallad, G. E.; Goodman, R. M. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci.* **2004**, *44*, 1920−1034.
(177) Cipollini, D.; Heil, M. Costs and benefits of induced resistance to herbivores and pathogens in plants. *CAB Rev.: Perspect. Agric., Vet. Sci., Nutr. Nat. Resour.* **2010**, *5* (005), 1−25.
(178) van Hulten, M.; Pelser, M.; van Loon, L. C.; Pieterse, C. M. J.; Ton, J. Costs and benefits of priming for defense in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (14), 5602−5607.
(179) Iriti, M.; Faoro, F. Does benzothiadiazole-induced resistance increase fitness cost in bean ? *J. Plant Pathol.* **2003**, *85* (4, Special Issue), 265−270.
(180) Iriti, M.; Faoro, F. Fitness costs of chemically-induced resistance: double edged sword or (un)stable equilibrium? *J. Plant Patol.* **2006**, *88* (1), 5−6.
(181) Iriti, M.; Rossoni, M.; Borgo, M.; Ferrara, L.; Faoro, F. Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: primary versus secondary metabolism. *J. Agric. Food Chem.* **2005**, *53*, 9133−9139.
(182) Stadnik, M. J.; Buchenauer, H. Effects of benzothiadiazole, kinetin and urea on the severity of powdery mildew and yield of winter wheat. *Z. Pflanzenkrankh. Pflanzenschutz* **1999**, *106*, 476−489.
(183) Walters, D. R.; Havis, N. D.; Paterson, L.; Taylor, J.; Walsh, D. J. Cultivar effects on the expression of induced resistance in spring barley. *Plant Dis.* **2011**, *95*, 595−600 and references cited therein..
(184) da Rocha, A. B.; Hammerschmid, R. History and perspectives on the use of disease resistance inducers in horticultural crops. *HortTechnology* **2005**, *15* (3), 518−529.
(185) Huang, C.-H.; Vallad, G. E.; Zhang, S.; Wen, A.; et al. Effect of application frequency and reduction rates of acibenzolar-*S*-methyl on the field efficacy of induced resistance against bacterial spot on tomato. *Plant Dis.* **2012**, *96* (2), 221−227.
(186) Ishii, H. Fungicide research in Japon − an overview. In *Modern Fungicides and Antifungal Compounds V*; Dehne, H. W., Deising, H. B., Gisi, U., Kuck, K. H., Russell, P. E., Lyr, H., Eds.; Deutsche Phytomedizinische Gesellschaft: Braunschweig, Germany, 2008.
(187) Corio-Costet, M.-F.; Dufour, M.-C. Comment évaluer l'efficacité d'un stimulateur et son niveau de protection? Du Geneu au Champ. *Colloque Elicitra-Avignon*, June 13−14, 2013; http://www.elicitra.org/index.php?rub=programme_et_presentations.
(188) Bardin, M.; Mauch-Mani, B.; Mazzotta, S.; Nicot, P.; Pieterse, C.; Poesse, J.-L.; Ponchet, M.; Schmitt, A. Leading from success in the lab to success in the field. Proceedings of IOBC-WPRS Working group Induced resistance in plants against insects and diseases, Avignon, France, June. *IOBC−WPRS Bull.* **2013**, *89*, 1−462.
(189) Directive 2009/28/CE; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:en:PDF.