Defense Priming: An Adaptive Part of Induced Resistance

Brigitte Mauch-Mani,[1] Ivan Baccelli,[1] Estrella Luna,[2] and Victor Flors[3]

[1]Institute of Biology, Faculty of Science, University of Neuchâtel, 2000 Neuchâtel, Switzerland; email: brigitte.mauch@unine.ch, ivan.baccelli@unine.ch

[2]Plant Production and Protection (P^3) Institute for Translational Plant and Soil Biology, Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, United Kingdom; email: e.luna-diez@sheffield.ac.uk

[3]Metabolic Integration and Cell Signaling Group, Departamento de Ciencias Agrarias y del Medio Natural, Universitat Jaume I, 12071 Castellón, Spain; email: flors@uji.es

Annu. Rev. Plant Biol. 2017. 68:485–512

First published online as a Review in Advance on February 2, 2017

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-042916-041132

Keywords

priming, induced resistance, adaptive immunity, stimuli, transgenerational resistance, response to stress

Abstract

Priming is an adaptive strategy that improves the defensive capacity of plants. This phenomenon is marked by an enhanced activation of induced defense mechanisms. Stimuli from pathogens, beneficial microbes, or arthropods, as well as chemicals and abiotic cues, can trigger the establishment of priming by acting as warning signals. Upon stimulus perception, changes may occur in the plant at the physiological, transcriptional, metabolic, and epigenetic levels. This phase is called the priming phase. Upon subsequent challenge, the plant effectively mounts a faster and/or stronger defense response that defines the postchallenge primed state and results in increased resistance and/or stress tolerance. Priming can be durable and maintained throughout the plant's life cycle and can even be transmitted to subsequent generations, therefore representing a type of plant immunological memory.

Contents

INTRODUCTION 486
PRIMING-INDUCING STIMULI 488
Pathogen-Derived Stimuli 488
Beneficial-Microbe-Derived Stimuli 488
Arthropod-Derived Stimuli 494
Chemical Stimuli 495
Abiotic Stimuli 496
THE PRIMING PHASE: CHANGES FOLLOWING STIMULATION 496
Physiological and Transcriptional Changes 496
Metabolic Changes 498
Epigenetic Changes 499
THE POSTCHALLENGE PRIMED STATE: ENHANCED RESPONSIVENESS UPON CHALLENGE 501
Enhanced Perception of the Attacker 501
Enhanced Signal Transduction and Defense Responses 502
DURATION OF THE PRIMED STATE 503
Long-Term Responses Within the Same Generation 503
Transgenerational Immune Resistance 503
Costs 504
Plants Can Also Forget 504
Transgenerational Resistance in Crops 505
CONCLUSIONS 505

INTRODUCTION

Plants are exceptional organisms in that they are sessile and therefore cannot escape potential threats by pathogens, arthropods, or adverse environmental conditions. Constitutive physical and chemical defense mechanisms such as waxy cuticles, cell walls, and phytoanticipins contribute to their survival (2). Against pathogens, plants can also defend themselves in a more specific manner by using extracellular or intracellular protein receptors, termed pattern recognition receptors and resistance proteins, to recognize pathogen-derived molecules such as pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and effectors, ultimately leading to PAMP-triggered immunity and effector-triggered immunity (37). Immunity relies on inducible defense mechanisms that plants deploy in response to an attack, and pathogens in turn try to suppress these mechanisms.

Systemic acquired resistance: increased defense capacity against a broad range of pathogens following local induction by a pathogen or its molecules

Induced systemic resistance: increased defense capacity of an entire plant against a broad range of pathogens following local induction by beneficial microorganisms

However, exposure to PAMPs, DAMPs, effectors, certain physical or chemical stimuli, and/or root-colonizing nonpathogenic microorganisms can result in plant expression of a suite of defense responses in both a local and systemic manner (**Figure 1**). These responses are typically associated with systemic acquired resistance, induced systemic resistance, and mycorrhiza-induced resistance. These different types of resistance help the plant to contain the attacker and are characterized, for instance, by the direct induction of antimicrobial proteins. But this is not always the case. The number of examples of a potentiated defensive capacity without a concomitant induction of specific defense genes has been steadily increasing over the last 15–20 years, and this phenomenon has been termed priming. Priming was, however, overlooked in studies of induced resistance

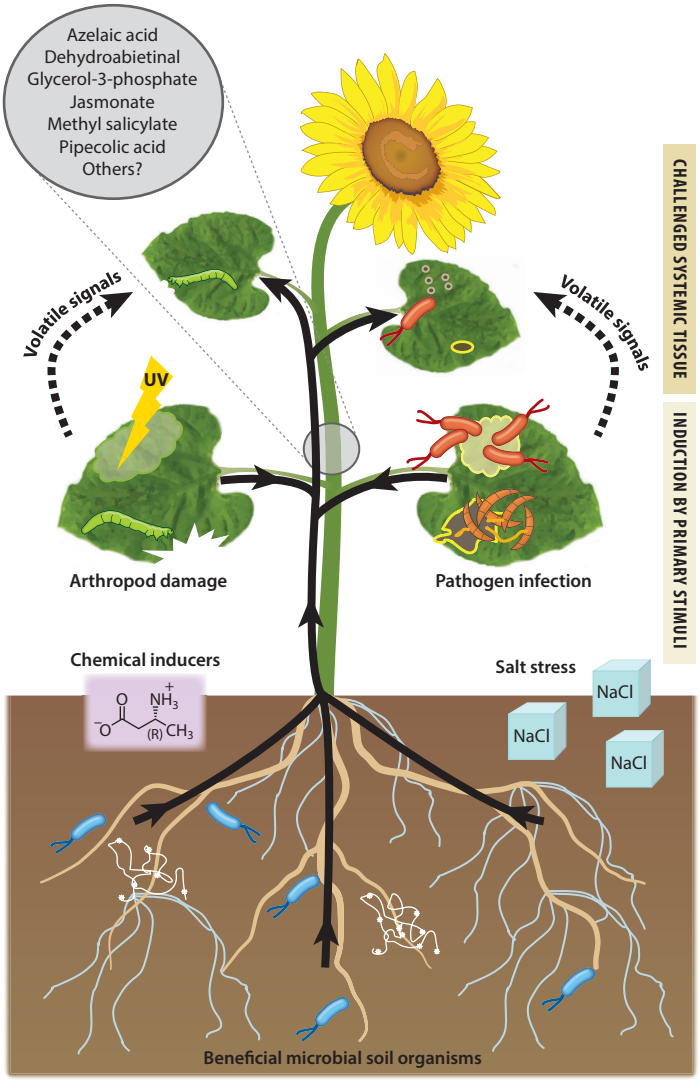

Figure 1

Induced resistance triggered by various biotic and abiotic stressors. Beneficial interactions with rhizobacteria, plant-growth-promoting fungi, and mycorrhiza, as well as chemical inducers and abiotic stress at the root level, can lead to an increased defensive capacity of aboveground parts. Pathogen attack on the leaves, exposure to abiotic stress, and damage inflicted by arthropods are also able to induce resistance in systemic parts of the plant. The signals warning the unattacked parts either are transported inside the plant (*solid black arrows*) or reach the distal part in the form of volatiles (*dashed black arrows*).

until the first comprehensive approaches were published in 2002 and 2006 (30, 32). These early reviews focused on the plant's conditioning for boosted responses against pathogens. It is now generally accepted that priming is an intrinsic part of induced resistance: The plant takes defensive measures against the potential attacker while also preparing its defensive system for a faster and/or stronger reaction in the future. Interestingly, priming is effective not only against pathogens, but also against insect arthropods (40, 46) and abiotic stresses (12, 126).

Initially, it was presumed that plants exposed to a priming stimulus did not change their metabolism, and no gene expression changes were detected until the plant was exposed to a challenge infection (70, 149, 150). Because of the rapid increase in analytical power and the recent advances in -omics techniques, holistic approaches to the study of defense priming (10) have now demonstrated that priming stimuli trigger direct changes in the plant that are crucial for the enhanced defensive behavior. In contrast to the expression of directly induced defenses, however, no or only minimal fitness costs in terms of growth and seed or fruit production are associated with defense priming. Thus, the memory of the stimuli, low fitness costs, a more robust defense, and better performance in the presence of the challenge are essential checkpoints to experimentally ascertain the presence of defense priming (89).

In the following sections, we review the priming phenomenon from the initial stimuli to the changes that take place in the plant to create a more robust and efficient defense. We also discuss both long-term and transgenerational aspects of priming.

Priming: enhanced sensitivity and responsiveness to stress that results from a prior experience and leads to increased resistance and/or abiotic stress tolerance

Priming stimulus: a signal that prepares a plant for enhanced responsiveness by causing subtle physiological, molecular, and epigenetic changes

Plant-growth-promoting rhizobacteria (PGPRs): beneficial rhizosphere bacteria that live on roots and their surroundings and promote plant growth

Plant-growth-promoting fungi (PGPFs): beneficial soil fungi that live on roots and their surroundings and promote plant growth

PRIMING-INDUCING STIMULI

Plants possess a remarkable capacity to perceive numerous environmental signals that allow them to respond to their surroundings. Stimuli from pathogens, beneficial microbes, or arthropods, as well as chemicals and abiotic cues, can trigger the establishment of priming by acting as warning signals (**Figure 2**, **Table 1**).

Pathogen-Derived Stimuli

Pathogens themselves, or pathogen-derived molecules, can act as priming stimuli in plants. Broadly, molecules produced by pathogens are referred to as PAMPs and effectors, whereas molecules released by the host plant following an attack are referred to as DAMPs (16, 37, 127). Pathogen-derived molecules can be of different biochemical natures (peptides, polysaccharides, or lipids) and are perceived by plants through appropriate protein receptors (pattern recognition receptors or resistance proteins) (16, 37, 127). Unlike effectors, PAMPs, by definition, are not strain or species specific and do not contribute to pathogen virulence; they are thus present in both pathogenic and nonpathogenic microorganisms (127). Structural molecules such as lipopolysaccharides and flagellin (or its derived 22-amino-acid peptide, flg22) from bacteria, chitin from fungi, and β-glucans from fungi and oomycetes (16, 127) are clear examples of PAMPs. Lipopolysaccharides and flg22 are probably the best-known priming PAMPs (45, 91, 96). These molecules are also present in beneficial microbes, where the term microbe-associated molecular pattern (MAMP) (16, 105) is used instead of PAMP.

Beneficial-Microbe-Derived Stimuli

Beneficial microbes include plant-growth-promoting rhizobacteria and fungi that exert positive effects in the interplay between microbes and hosts.

Plant-growth-promoting rhizobacteria and fungi. Plant-growth-promoting rhizobacteria (PGPRs) and plant-growth-promoting fungi (PGPFs), both of which induce systemic resistance, can also trigger defense priming (105). The subtle costs associated with these interactions are negligible under pathogenic pressure, and many studies have provided evidence that the induced resistance they trigger is based on priming (3, 19, 73, 76, 105). The most studied PGPRs belong to the genus *Pseudomonas*, followed by those belonging to the genera *Serratia* and *Bacillus*. The

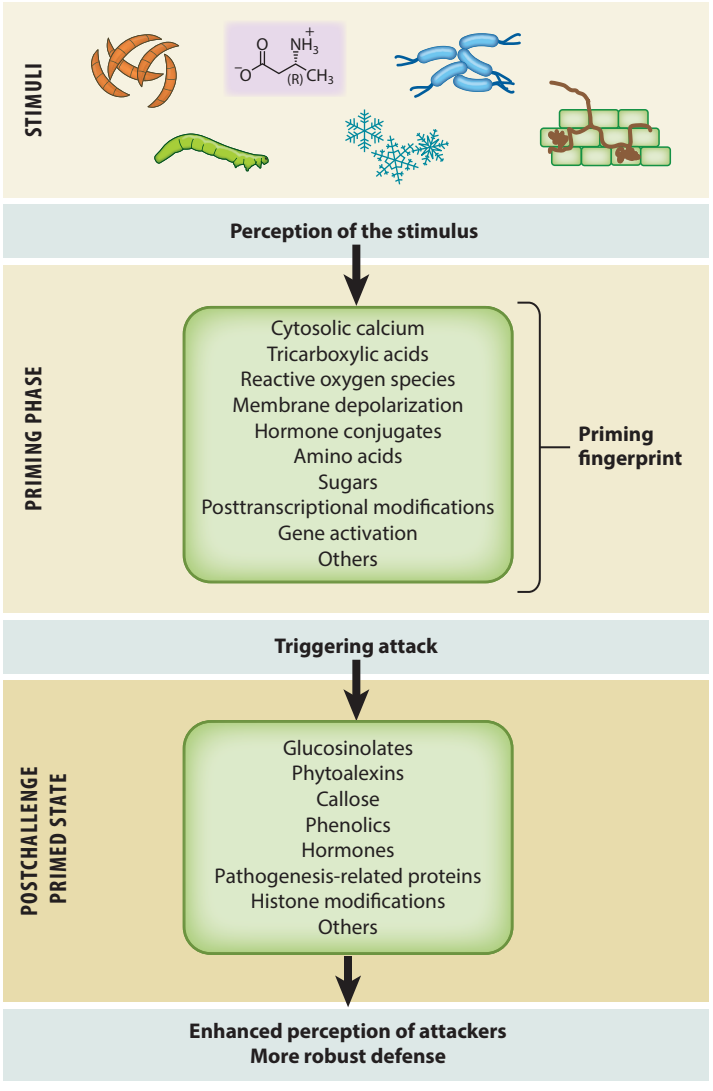

Figure 2

The sequential steps of defense priming. Stimuli stemming from pathogenic or beneficial fungi, bacteria, rhizobacteria, arthropods, and abiotic stresses are perceived by the plant, leading to a slight induction of various compounds and activities in the so-called priming phase. These compounds are referred to as the priming fingerprint, and a subset of these compounds may be common to several stimuli. Upon challenge with an attacker, these primed plants display an enhanced perception of the attackers and therefore are able to mount a more robust defense against it in the postchallenge primed state.

most studied PGPFs are *Trichoderma* spp., nonpathogenic strains of *Fusarium* spp., *Piriformospora indica*, and arbuscular mycorrhizal fungi (AMFs) from the genus *Glomeromycota* (73, 105).

The goal of the initial chemical interplay between microbe and plant is the establishment of symbiosis. However, the involved signals can also serve as stimuli for defense priming. For instance, the first chemical stimuli exchanged to start the symbiosis are hormones and flavonoids from the host and nodulation (Nod) factors from rhizobacteria (97). Beneficial microbes also need

Table 1 Priming stimuli and changes induced in plants during the priming phase, the postchallenge primed state, and the long-term/transgenerational primed state

Priming phase		Postchallenge primed state		Long-term/ transgenerational primed state		
Inducing stimuli	**Induced changes**	**Challenge**	**Enhanced responses**	**Challenge**	**Long-lasting responses**	**Reference(s)**
Pathogen/microbe-derived stimuli						
Lipopolysaccharides	Transient gene induction of *THT*; transient increase of TyDC enzymatic activity in pepper leaves	*Xanthomonas campestris* pv. *vesicatoria* or *X. campestris* pv. *campestris*	Enhanced transcription of *PR1*, *PR2*, and *THT* genes; enhanced TyDC enzymatic activity; increased synthesis of antibacterial hydroxycinnamoyl-tyramine conjugates	ND	ND	96
N-acyl-homoserine lactones	Induction of genes encoding signaling kinases, receptor kinases, and calcium signaling proteins; downregulation of genes encoding glucanases; no effect on hormones in *Arabidopsis*	*Pst* DC3000 or flg22	Enhanced transcription of receptor kinases, calmodulin-binding proteins, G proteins, and genes related to the cell wall, secondary metabolism, and flavonoids; enhanced callose deposition; enhanced accumulation of soluble and cell wall–bound phenolic compounds; enhanced accumulation of SA and *cis*-OPDA; accelerated stomatal closure	ND	ND	112
Other inducing stimuli: flg22, lipochitooligosaccharides, chitin, chitosan, ergosterol, β-glucans, plant elicitor peptides, avirulent pathogens, trichothecenes, pyocyanin, 2,4-diacetylphloroglucinol, iron-dependent siderophores (pyoverdin), Nod factors						
Plant-growth-promoting rhizobacteria						
Pseudomonas putida LSW17S	No local or systemic transcript induction of *PR1* and *PDF1.2* genes in *Arabidopsis*	*Pst* DC3000	Enhanced transcription of *PR1*, *PR2*, *PR5*, and *PDF1.2* genes; enhanced H_2O_2 and callose accumulation	*Pst* DC3000	Enhanced transcription of *PR1* and *PDF1.2* upon challenge up to 10 days after LSW17S inoculation	3

Burkholderia phytofirmans PsJN	No major transcriptomic or metabolomic changes in grape	Cold	Enhanced expression of *STS*, *PAL*, *LOX*, and *CBF4* genes as well as genes encoding chitinases and glucanases; increased proline content; enhanced increase in malondialdehyde and aldehyde content; enhanced H_2O_2 accumulation	ND	ND	126
Other inducing stimuli: *Serratia* spp., *Bacillus* spp.						
Plant-growth-promoting fungi						
Trichoderma asperelloides T203	No major transcriptomic changes; changes in amino acids, polyamines, sugars, and citric acid cycle intermediates in *Arabidopsis* leaves	*Pst* DC3000	Enhanced transcription of *PR1*, *PR2*, *PR14*, and ethylene- or JA-responsive genes (*EIR1*, *ETO3*, *LOX2*, and *ERF13*); decreased transcription of *WRKY40*	ND	ND	19
Rhizophagus irregularis	Systemic induction of genes functioning in protection against oxidative stress, transcriptional control, signaling (*OsMPK6*), Ca^{2+} signaling, SA-dependent responses (*OsNPR1*), and JA-dependent responses (*OsJAMyb*, *OsMPK7*, and *OsAOC*); downregulation of the defense genes *OsPR1a*, *OsPR5*, *OsPBZ1*, and *OsPR10* in rice leaves	*Magnaporthe oryzae*	Enhanced transcription of the defense genes *OsPR1a*, *OsPR5*, *OsPR10*, and *OsPBZ1*	ND	ND	24
Other inducing stimuli: *Piriformospora indica*, *Glomeromycota* spp., *Fusarium* spp.						

(*Continued*)

Table 1 (*Continued*)

Priming phase		Postchallenge primed state		Long-term/ transgenerational primed state		
Inducing stimuli	**Induced changes**	**Challenge**	**Enhanced responses**	**Challenge**	**Long-lasting responses**	**Reference(s)**
Arthropod-derived stimuli						
Oviposition by *Spodoptera exigua*	No major changes in defense metabolites in *Nicotiana attenuata* leaves	*Spodoptera exigua* larvae or wounding plus oral secretions	Enhanced production of caffeoyl putrescine and trypsin protease inhibitors; no increase in JA or JA-Ile levels; enhanced transcription of *NaMyb8*	ND	ND	11
Herbivore-induced plant volatiles (indole)	No induction of volatiles; no induction of ABA, JA, or JA-Ile in maize	Wounding plus *Spodoptera littoralis* regurgitant	Enhanced production of green leaf volatiles, ABA, JA, JA-Ile, and mono-, homo-, and sesquiterpenes	ND	ND	41
Other inducing stimuli: herbivory, oral secretions, trichome sensing, other herbivore-induced plant volatiles						
Natural or synthetic chemicals						
BABA	In *Arabidopsis*: accumulation of aspartic acid; induction of tricarboxylic acids such as citrate, fumarate, (*S*)-malate, and 2-oxoglutarate; induction of free SA and SA glucosides, JA conjugates, indolic derivatives, xanthosine, and amino acid imbalance; induction of phenylpropanoids and octadecanoids; induction of *RBOHD* and *GSH1* transcription; transient induction of *PR1*	*Plectosphaerella cucumerina*	Subcellular translocation of IBI1; enhanced production of indole-3-carboxylic acid; enhanced H_2O_2 and callose accumulation; enhanced transcription of *PR1*; decreased transcription of *APX1* and *GSH1*	*Pst* DC3000 or BABA	Resistance and enhanced transcription of *PR1* in the following generation	48, 83,100, 102, 116, 130
Other inducing stimuli: pipecolic acid, azelaic acid, dehydroabietinal, glycerol-3-phosphate, sulfonamides, vitamin B_1 (thiamine), vitamin B_2 (riboflavin), probenazole, PS3, SA, methyl salicylate, BTH, JA, ABA, menadione, silicon, indole-3-carboxylic acid, hexanoic acid						

Abiotic stimuli						
Mild repetitive heat, cold, or salt treatment	No direct induction of PAMP-triggered-immunity-responsive gene transcription, but enrichment of H3K9K14ac, H3K4me2, and H3K4me3 for *FRK1*, *WRKY53*, *WRKY70*, *NHL10*, and *PR1*; enrichment of RNA polymerase II to PAMP-triggered-immunity genes in *Arabidopsis* leaves	*Pst* DC3000 *hrcC* or flg22	Enhanced transcription of *PR1*, *WRKY70*, and the PAMP-triggered-immunity-responsive genes *WRKY53*, *FRK1*, and *NHL10*; enhanced callose accumulation	*Pst* DC3000	Resistance and enrichment of H3K9K14ac up to 5 days after treatment	115
Wounding	Cytosolic Ca^{2+} increase and ROS burst; transient induction of *GST1* in *Arabidopsis* leaves	*Botrytis cinerea*	Enhanced camalexin production; enhanced transcription of camalexin biosynthetic genes and *GST1*; enhanced MPK3 and MPK6 activation	ND	ND	14, 28
Other inducing stimuli: heavy metal stress (copper), ultraviolet light, mechanical stimulation						

References are representative examples from the literature. Abbreviations: ABA, abscisic acid; *APX1*, *ASCORBATE PEROXIDASE 1*; BABA, β-aminobutyric acid; BTH, benzothiadiazole; *CBF4*, *C-REPEAT-BINDING FACTOR 4*; *EIR1*, *ETHYLENE INSENSITIVE ROOT 1*; *ERF13*, *ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 13*; *ETO3*, *ETHYLENE OVERPRODUCING 3*; flg22, flagellin-derived 22-amino-acid peptide; *FRK1*, *FLG22-INDUCED RECEPTOR KINASE 1*; *GSH1*, *GAMMA-GLUTAMYL CYSTEINE SYNTHETASE 1*; *GST1*, *GLUTATHIONE-S-TRANSFERASE 1*; H3K4me2, dimethylation of histone H3 at lysine 4; H3K4me3, trimethylation of histone H3 at lysine 4; H3K9K14ac, acetylation of histone H3 at lysines 9 and 14; IBI1, IMPAIRED IN BABA-INDUCED IMMUNITY 1; Ile, isoleucine; JA, jasmonate; *LOX*, *LIPOXYGENASE*; MPK, MITOGEN-ACTIVATED PROTEIN KINASE; *NaMyb8*, *Nicotiana attenuata Myb 8*; ND, not determined; *NHL10*, *NDR1/HIN1-LIKE 10*; Nod, nodulation; OPDA, 12-oxo-phytodienoic acid; *OsAOC*, *Oryza sativa allene oxide cyclase*; *OsJAMyb*, *Oryza sativa jasmonate-inducible Myb*; *OsMPK*, *Oryza sativa mitogen-activated protein kinase*; *OsNPR1*, *Oryza sativa nonexpressor of PR genes 1*; *OsPBZ1*, *Oryza sativa probenazole-induced protein 1*; *OsPR*, *Oryza sativa pathogenesis-related*; *PAL*, *PHENYLALANINE AMMONIA-LYASE*; PAMP, pathogen-associated molecular pattern; *PDF1.2*, *PLANT DEFENSIN 1.2*; *PR*, *PATHOGENESIS-RELATED*; PS3, sulfated laminarin; *Pst*, *Pseudomonas syringae* pv. *tomato*; *RBOHD*, *RESPIRATORY BURST OXIDASE HOMOLOG D*; ROS, reactive oxygen species; SA, salicylic acid; *STS*, *STILBENE SYNTHASE*; *THT*, *TYRAMINE HYDROXYCINNAMOYL TRANSFERASE*; TyDC, tyrosine decarboxylase.

to suppress local immune responses in the host; two well-known effectors from mycorrhizal fungi are secreted protein 7 (SP7) and mycorrhiza-induced small secreted protein 7 (MiSSP7) (144). In addition, the interaction between beneficial microbes and host plants is mediated by compounds with eliciting activity. From PGPRs, for instance, we can mention 2,4-diacetylphloroglucinol, pyocyanin, *N*-acyl-homoserine lactones, pyoverdin and other iron-dependent siderophores, and biosurfactants (34, 105).

Trichoderma. Beneficial fungi from the genus *Trichoderma* have been defined as opportunistic avirulent plant symbionts (55). Many *Trichoderma* symbiotic strains are used as biofertilizers to protect crops against fungal diseases, and several recent publications have pointed to plant sensitization as the main mechanism behind their beneficial effect on defenses (19, 88, 105). For example, ergosterol contained in the cell membranes of *Trichoderma* fungi is recognized by plants as a MAMP (16) and may promote a primed defense.

Mycorrhiza. AMFs protect plants against different pathogens (68). In 2005, Lee et al. (74) demonstrated that *Rhizophagus irregularis* (formerly *Glomus intraradices*) could protect against anthracnose disease. Despite the evidence demonstrating the effectiveness of mycorrhiza-induced resistance, mycorrhizal stimuli that trigger responses in plants are still mostly elusive (68).

A stimulus that marks the symbiotic interaction is the accumulation of H_2O_2 (43). Although this accumulation seems to lead to arbuscule degradation during the asynchronous process, the increase of H_2O_2 could indirectly serve as a priming signal in AMF-colonized roots against soil pathogens. As a further confirmation, these peroxides accumulate in the cytoplasm rather than in the apoplast, thus pointing to a signaling role in mycorrhizal roots (43). Lipochitooligosaccharides are likely stimuli from AMFs. *R. irregularis*, for example, secretes sulfated and nonsulfated lipochitooligosaccharides (85). Although no studies have reported that lipochitooligosaccharides are the ultimate signals that trigger mycorrhiza-induced resistance, this important discovery opens a new field of study. Delaux et al. (35) proposed a tool kit of plant-symbiotic microbes that includes proteins, small molecules, and microRNAs as important players in the establishment of symbiosis and subsequent signaling. Importantly, two genes, *NOD FACTOR PERCEPTION* (*NFP*) and *DOES NOT MAKE INFECTIONS 2* (*DMI2*), both of which code for receptor-like kinases, can perceive lipochitooligosaccharide signals and activate downstream phosphorylation events (35), thus leading to priming.

The overall scenario becomes even more complicated when arbuscular mycorrhizal plants develop in natural environments where a complex microbiome is present in the soil and where interactions between these plants and other beneficial microbes occur (23, 90). Obviously, when the entire mycorrhizosphere is participating, stimuli responsible for AMF-triggered priming are not univocal (23).

Arthropod-Derived Stimuli

Herbivore-associated stimuli can be of biological or physical origin. Biological stimuli include oral secretions, insect-associated microbes, insect-associated molecular patterns (IAMPs), and oviposition signals (56, 58, 61); physical signals consist of spatiotemporal repeated patterns and trichome sensing of insects walking on leaf surfaces (58, 61, 103). Moreover, herbivore-induced plant volatiles have been described as elicitors of priming because they act as stimuli to neighboring plants (46). All of these stimuli are produced during challenge with an arthropod, which obviously triggers direct defenses in the plant, but when these physical or biological stimuli are

used experimentally, they can induce a faster and/or stronger defensive behavior in the attacked plants.

Oral secretions. Oral secretions are important elements that help plants distinguish between mechanical wounding and insect herbivory. Among the many components found in caterpillar secretions, fatty acid–amino acid conjugates are responsible for triggering specific responses in attacked plants (61). Volicitin was one of the first fatty acid–amino acid conjugates reported in lepidopteran larvae (5). In addition, insect oral peptides and sulfated fatty acids act as primary stimuli in insect-plant interactions (4, 58).

Volatile organic compounds (VOCs): chemicals with high water pressure emitted by plants that can be stimuli to prime distal parts or neighboring plants

Oviposition signals. Stimuli provided by oviposition may play a dual role depending on the challenge that follows the stimulus. In fact, they can induce priming or the suppression of host defenses according to the subsequent challenge (21, 57).

Physical stimuli. In addition to the stimuli described above, trichomes can perceive insect contact and prepare the plant to defend against herbivore attack (103). Moreover, certain entomophytophagous beneficial insects can act as stimulants by injecting stylets into the plant's stem and activating indirect defense mechanisms and antixenosis (104).

Volatile organic compounds. Arthropods can trigger the release of plant volatile organic compounds (VOCs) that can prime distal plant parts and neighboring plants (46). A relevant subset of priming stimuli within VOCs induced by insects is the herbivore-induced plant volatiles (46, 71). Engelberth et al. (40) described how *Arabidopsis* plants exposed to several green leaf volatiles (small aliphatic alcohols and aldehydes) displayed primed jasmonate (JA)–dependent signals that were enhanced only following infestation with a caterpillar. Among the relevant set of VOCs that induce priming, terpenoids are the main priming stimuli against *Spodoptera littoralis* in maize (128). Recently, a more detailed study of herbivore-induced plant volatiles has revealed that indole is present in the blend of volatiles released by infested leaves and that it triggers priming by enhancing the terpene levels in systemic leaves and neighboring plants (41).

Chemical Stimuli

Numerous chemical compounds, often of natural origin (see sidebar titled Priming Chemicals or Natural Compounds?), have been shown to act as priming stimuli. These chemicals normally induce a much more reproducible response, and for this reason investigators frequently prefer to use them to carry out molecular and genetic studies on priming. Because of the large number of chemicals known, in this review we report on a few whose mechanisms of action are best understood.

PRIMING CHEMICALS OR NATURAL COMPOUNDS?

Priming stimuli cover a wide range of physical, biological, or chemical environmental inputs to which a plant responds by acquiring a memory. These inputs induce low-cost changes in the plant that include the accumulation of numerous metabolites. Many of these natural molecules, when applied exogenously, can themselves act as priming stimuli, generating a plant memory that boosts induced defenses and improves the plant's performance upon challenge. This is the case, for example, for some hormones and, as shown recently, for BABA as well (126a).

Among these chemicals are β-aminobutyric acid (BABA), probenazole, benzothiadiazole (BTH), and salicylic acid (SA), all of which can induce resistance in plants by protecting against a broad range of pathogens (98). SA is a hormone that triggers several direct responses in plants, but at low doses it has been reported to enhance flg22-induced MITOGEN-ACTIVATED PROTEIN KINASE 3 (MPK3) and MPK6 activation (145). BTH and BABA have been thoroughly studied as priming agents against pathogens and insects (8, 10, 101). Similarly to SA, both of these chemicals may directly induce defenses when applied at high doses (135). Dempsey & Klessig (36) reviewed natural secondary metabolites that had been found to mediate systemic acquired resistance, including JA, azelaic acid, dehydroabietinal, glycerol-3-phosphate, methyl salicylate, and pipecolic acid. These compounds, however, are likely to trigger priming, as has been confirmed, for example, for azelaic acid and pipecolic acid (67, 95). Because the molecular mechanisms behind the induced resistance by chemicals are not fully understood, it is not always easy to classify them as priming stimuli. **Table 1** lists chemicals with priming activity, and recently published reviews have summarized the available information (10, 31, 49).

β-Aminobutyric acid (BABA): a nonprotein amino acid effective as a priming stimulus against a wide range of biotic and abiotic stresses

Priming phase: the biological process of acquisition of priming, which occurs from the time of stimulation through the exposure to a challenging stress

Abiotic Stimuli

A study carried out on *Arabidopsis* demonstrated that repetitive exposure of a plant to mild abiotic cues, such as heat, cold, or salt, can enhance resistance against virulent *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 by acting at the epigenetic level (115) (**Table 1**). Importantly, when plants were subjected to long-term exposure or high salt concentrations, priming did not occur (115).

Different forms of abiotic stimulation can also induce resistance in plants. Interesting examples include mechanical stimulation by repetitive leaf rubbing or bending (15) and wounding (28). In addition, submergence (62) and exposure to ultraviolet light or ozone (143) can induce protection against pathogens, although the role of defense priming is not clear. Finally, heavy metal stress caused by copper primes for enhanced VOC and JA production upon caterpillar feeding in maize plants (140).

THE PRIMING PHASE: CHANGES FOLLOWING STIMULATION

The priming phase refers to the biological process of acquiring priming, which takes place from the initial stimulation through the exposure to a challenging stress. It includes all changes that occur in the plant after the perception of a stimulus and prepare the plant for enhanced responsiveness when a challenge occurs. These changes can take place at the physiological, molecular, and epigenetic levels; can occur within seconds or hours after stimulation; can be transient or maintained throughout the lifetime of a plant; and can even be inherited by subsequent generations. Different priming stimuli may cause similar changes as well as specific ones. Stimulus specificity may reside, for example, in the activation of only some of the responses described below (**Table 1**).

Physiological and Transcriptional Changes

Transient changes in the level of intracellular calcium occur within a few seconds or minutes and are among the best-known early responses to stimulation. Cytosolic calcium rapidly increases, for instance, in cells neighboring a wound site or after leaf rubbing, and the calcium increase is crucial for local priming by wounding (14, 28). PAMPs (16, 65) and insect feeding (57) but not BABA (39) have been reported to transiently impact calcium levels during the priming phase. Calcium fluxes could also play a role during AMF root colonization: For example, *Oryza sativa* calcium-dependent

protein kinase 18 (OsCPK18) is strongly upregulated at the gene level in cortical cells, suggesting that an increase in cytoplasmic calcium is triggered by AMFs (25).

The increase in cytosolic calcium triggers ion fluxes across the membranes that lead to membrane depolarization (65)—another transient physiological event that has been reported, for instance, after PAMP perception (65). Interestingly, membrane depolarization triggers electrical signaling that can transmit the local perception of wounding to undamaged leaves and activate JA signaling in those leaves (94).

The increase in intracellular calcium can precede the generation of reactive oxygen species (ROS) (14, 65), the so-called ROS burst. In 1998, Alvarez et al. (6) demonstrated that, after inoculation with avirulent *P. syringae*, both the localized oxidative burst and the subsequent secondary microbursts in distal leaves were necessary to establish systemic acquired resistance. A fine-tuning of ROS homeostasis seems to also be crucial for priming, as reported in *Arabidopsis* after treatment with BABA (102). Balmer et al. (10) have published a review that provides more information on the involvement of ROS during the priming phase.

Stimulus perception and downstream cellular immune responses are rapidly linked by sequential phosphorylation events (31). For instance, PAMPs trigger the activation of the protein kinase BRI1-ASSOCIATED RECEPTOR KINASE (BAK1), which mediates the activation of MPKs (29). Importantly, treatments with BTH induce the accumulation of inactive unphosphorylated MPK3 and MPK6 (13), which plays a pivotal role in the rapid activation of phosphorylation-dependent defense mechanisms (31).

It is widely accepted that local and systemic transcriptional reprogramming may occur during the priming phase (51, 112, 134). For example, quantitative polymerase chain reaction analysis revealed that application of BABA or inoculation with *Pseudomonas fluorescens* WCS417r in *Arabidopsis* induced the expression of transcription factors associated with defense response mechanisms (134). Importantly, transcriptional changes induced by different priming stimuli are partially specific: For example, 30% of the induced genes were different after treatment of grapevine leaves with laminarin or its sulfated derivative (PS3) (51), and almost 90% of the induced genes did not overlap after treatment with BABA or inoculation with *P. fluorescens* WCS417r in *Arabidopsis* (134).

Massive transcriptomic changes have been also reported following mycorrhizal colonization of maize and tomato plants by the AMF *R. irregularis* (27, 53). In maize, one group of the induced genes was related to anthocyanin and lipid metabolism, most likely dependent on the improved phosphorus status of mycorrhizal plants (53). Interestingly, leaf analysis also revealed a systemic induction of defense-related genes and a concomitant induction of secondary metabolites in addition to changes in genes involved in primary metabolism, such as the metabolism of carbohydrates, organic acids, and amino acids (53). In a parallel study performed in tomato plants, *R. irregularis* inoculation caused changes in systemic leaves for 742 out of 21,113 genes analyzed by RNA sequencing (RNA-seq) and induced resistance against *Xanthomonas campestris* pv. *vesicatoria* (27). Changes in gene expression affected hormone metabolism, biotic and abiotic stress responses, signaling, and transport, suggesting that this transcriptional reprogramming may facilitate defense responses to subsequent infection with *X. campestris* (27).

Some studies have investigated changes at the protein level induced by priming-inducing chemicals during the priming phase (10). BABA, for example, may in some cases directly induce pathogenesis-related (PR) proteins (8), and lipopolysaccharides can transiently increase the enzymatic activity of a tyrosine decarboxylase (96). Importantly, protein levels corresponding to pattern recognition receptors and coreceptors increase after treatment with BTH (124), suggesting that following stimulation, plants prepare their defensive system for an enhanced sensitivity against potential attackers (31).

Finally, several proteins have been reported to act as negative regulators of defense priming. For instance, genetic mutations in the genes encoding proteins involved in the regulation of nitrogen uptake (22), chloroplastic transcription factors (50), DNA-dependent RNA polymerases (77), and MPKs (47) result in mutants that are constitutively primed.

Metabolic Changes

The accumulation of inactive forms of defense-related hormones seems to be implied in the sensitization of defenses (22). For instance, the constitutively primed *Arabidopsis* mutant *nitrate transporter 2.1* (*nrt2.1*) has low basal levels of free SA that rapidly increase after challenge with *Pst* (22). Similar mechanisms seem to occur with hormone conjugates, phytoanticipins, and indolic glucosinolates (10, 49). In addition, priming activators can increase the levels of compounds involved in primary metabolism, such as amino acids, tricarboxylic acids, glycerol-3-phosphate, *myo*-inositol, and xylitol (49, 100), as well as the levels of methyl salicylate and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, as found in tomato after seed treatment with JA (117). Importantly, treatment with BABA also induces the accumulation of aspartic acid as a direct consequence of the blockage of the enzymatic activity of IMPAIRED IN BABA-INDUCED IMMUNITY 1 (IBI1), an aspartyl-tRNA synthetase that functions as the BABA receptor (83). In addition, treatment with BABA or infection with avirulent *Pst AvrRpt2* causes similar metabolomic changes in *Arabidopsis* (100). On the basis of studies that have analyzed different priming stimuli (49, 100, 101), a common subset of shared compounds can be identified that are then referred to as the priming fingerprint (49). These compounds undergo a slight induction after stimulation, but their accumulation following challenge is faster and/or stronger in challenged plants than it is in unstimulated controls (49).

Beneficial microorganisms can induce metabolic changes in colonized plants that can be helpful for the plant to enhance responsiveness upon subsequent challenge (see sidebar titled Evaluation of Priming Induced by Beneficial Organisms). For instance, maize roots colonized by PGPRs of the genus *Azospirillum* significantly affect the benzoxazinoid profile in a strain- and cultivar-dependent manner (136). The metabolic fraction analyzed by liquid chromatography–mass spectrometry showed no overlap in a principal component analysis among different *Azospirillum* strains, either in the root extracts or in the shoot (136). Some benzoxazinoids, such as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one or its glucoside, were detectable only with specific strains. This hints at specific mechanisms of interaction and recognition between the host plant and PGPRs (136).

EVALUATION OF PRIMING INDUCED BY BENEFICIAL ORGANISMS

Root colonization by beneficial organisms induces considerable changes in the host (10, 109). Arbuscular mycorrhizal fungi, for example, cause significant changes in the host plant (109), which apparently rules out the possibility that mycorrhiza-induced resistance takes place through priming. In this situation, the absence of fitness costs in the plant is a desirable trait to determine whether mycorrhiza-induced resistance can still be considered defense priming. Although most research reports point to clear growth promotion and other beneficial effects of arbuscular mycorrhizal fungi, some studies have demonstrated that a massive colonization or certain environmental conditions influencing symbiont growth may incur additional costs, thereby reducing plant growth (119). The consideration of defense priming as a mechanism behind mycorrhiza-induced resistance therefore requires a careful study of the individual cases.

Importantly, some bacterial strains are well known for their ability to release VOCs that can mediate induced systemic resistance, such as 2,3-butanediol (111). Interestingly, Farag et al. (42) reported that volatiles released by PGPRs were strain dependent. Further study is needed to decipher the metabolomic changes occurring in the host plant after PGPR colonization that might share a common priming fingerprint.

More information is available concerning the interaction between plants and beneficial fungi. In this kind of interaction, PGPFs must first be perceived and then establish in the root, after which metabolic changes related to priming take place in the shoot. The initial fungal stimuli are linked to the perception of fungal chitin oligomers (54). Upon colonization, the root switches from a permissive penetration stage to increasing amounts of SA, presumably to control colonization and prevent excessive carbohydrate waste. During fungal spread and arbuscule formation in mycorrhized plants, other plant hormones are affected. JA and abscisic acid, for instance, are used by the host plant to keep AMFs under a certain level of colonization, and they mediate the activation of defense mechanisms that confer priming (107). Because AMFs use plant carbohydrates for their own metabolism, they are important sinks for carbon and affect the carbon balance of the leaf primary metabolism (113). Additionally, AMFs also influence nitrogen uptake efficiency (118). Both AMF and nutritional starvation alter the carbon/nitrogen balance in plants, which can play a role in signal delivery (84). Thus, alteration of carbon/nitrogen status in the symbiont may be another important priming stimulus.

Metabolic changes associated with the release of VOCs triggered by mycorrhiza have also been studied. For instance, *Medicago truncatula* plants colonized by *R. irregularis* did not show differences in the blend of basal VOCs emitted by mycorrhizal and nonmycorrhizal plants but did show a different volatile profile upon herbivore damage driven by mycorrhization (75). Another study reported the role of alkanes, alcohols, and phenyl compounds as an important pool of secondary metabolites released by *Sorghum bicolor* roots colonized by *R. irregularis* (123).

Finally, common mycelial networks of AMFs have recently received considerable attention (121). Unstressed plants that are connected by these networks with neighboring stressed plants show enhanced expression of defense-related genes, accumulation of defensive enzymes, and induction of the JA pathway, culminating in the release of aphid-repelling VOCs (121), suggesting that these networks can transmit certain stimuli. The signals mediating this transmission could be amino acids, lipids, and phosphate transporters that can be delivered into the hyphal cytoplasmic stream (9). Another candidate could be electrical signals mediated by variations in the level of Ca^{2+} and propagated by glutamate receptors (94), which could elicit responses or signal to neighboring plants connected by common mycelial networks.

Epigenetic Changes

Genetic imprinting, paramutations, transposon activity, and gene silencing are epigenetic phenomena known to take place in all living organisms. The mechanisms behind these phenomena involve changes in chromatin structure, which can ultimately alter genomic processes such as gene transcription, replication, and recombination (see sidebar titled Epigenetic Phenomena).

Priming smells of epigenetics. Priming is based on the assumption that certain alterations serve as pivotal changes that affect a future response. Therefore, several research groups have hypothesized that changes at the epigenetic level could influence the way plants respond to biotic and abiotic stresses (20, 133). The rationale behind the response would be that the initial stimulus alters the chromatin structure in a way that leaves gene promoters more accessible and therefore easy to activate. The idea that "priming smells of epigenetics" was built up after the discovery

EPIGENETIC PHENOMENA

Epigenetics, first defined by Conrad Waddington in 1942, refers to inheritable changes in phenotype or gene expression that do not affect the DNA sequence. The mechanisms behind these phenomena involve changes in chromatin structure and compaction, which can ultimately alter genomic processes such as gene transcription, replication, and recombination. Changes in chromatin compaction consist of posttranslational modifications of histones, such as methylation and acetylation, the creation of histone variants, and changes in DNA methylation. Well-characterized examples of posttranslational modifications that change compaction are acetylation of histone H3 at lysine 9 (H3K9ac) and trimethylation of histone H3 at lysine 27 (H3K27me3), marks associated with positive and negative gene transcriptional activity, respectively (99, 147). DNA methylation in plants has evolved from a resistance capacity to foreign DNA and involves modulation of genomic imprinting, gene expression, and transposon activity (52, 148). Posttranscriptional gene-silencing processes in living cells can also be mediated by RNA interference through microRNAs and small interfering RNAs. Small interfering RNAs are targeted by RNA-silencing complexes that, in association with RNA-dependent DNA polymerases, result in epigenetic marks such as changes in DNA methylation or chromatin modifications.

that the SUPPRESSOR OF NPR1 INDUCIBLE (SNI1) protein suppresses SA-dependent *PR* gene expression through posttranslational modifications of histone H3 (93). The following years bloomed with studies that ultimately supported these hypotheses.

Chromatin modifications. Studies have investigated the role of epigenetic mechanisms in the activation of defense-dependent genes. Alvarez-Venegas et al. (7) reported that the expression of the *WRKY70* gene, which encodes an SA-dependent transcription factor involved in crosstalk with JA, was dependent on trimethylation of histone H3 at lysine 4 (H3K4me3). Moreover, chromatin changes made by the ATP-dependent chromatin remodeler SPLAYED (SYD) were implicated in the activation of the JA-dependent *PLANT DEFENSIN 1.2* (*PDF1.2*) gene after infection with *Botrytis cinerea* (137). The role of histone replacement by variants also affects the activation and expression of SA-dependent defense genes. *Arabidopsis* mutants disrupted in the enzymes needed to incorporate histone variant H2A.Z into the nucleosome showed an upregulation of SA-dependent genes that resulted in enhanced resistance to *Pst* (86).

However, the ultimate proof that certain stimuli can change the epigenetic status came from a study by Jaskiewicz et al. (64), who showed that changes in posttranslational modifications are unlinked from direct gene expression. After BTH treatments, the chromatin structure surrounding the promoter of *WRKY29*, *WRKY6*, and *WRKY53* (which encode transcription factors associated with changes controlled by SA) was associated with changes in the acetylation and methylation levels of histones H3 and H4 at different residues (64). BTH did not activate gene expression, but these changes facilitated a faster and stronger expression after subsequent infection by *P. syringae* pv. *maculicola* (64).

In addition, several studies have investigated changes in chromatin after BABA treatment. For instance, Po-Wen et al. (106) showed that the enhanced responsiveness of PAMP-triggered-immunity-responsive genes after BABA treatment is mediated by acetylation of histone H3 at lysines 9 and 14 (H3K9K14ac) and dimethylation of histone H3 at lysine 4 (H3K4me2). A recent study performed in common bean has also demonstrated that priming by treatments with BABA or 2,6-dichloroisonicotinic acid results in changes in H3K4me3 levels in defense-related genes (87). Finally, environmental stress by heat, cold, or salt triggers posttranslational modifications and

facilitates the expression of PAMP-triggered-immunity-responsive genes, which are responsible for enhanced resistance to *Pst* (115).

The role of DNA methylation and demethylation in bacterial disease has been documented. For instance, infections with *Pst* DC3000 failed to develop in mutants impeded in DNA methylation (38). In addition, bacterial infection leads to a reduction of the levels of DNA methylation during defense response, and DNA hypomethylation during pathogen infection promotes the expression of defense-related genes (146). In agreement with this, a recent publication has demonstrated the link between methylation status and priming (78). In this study, mutants displaying contrasting DNA methylomes showed an opposite resistance phenotype to *Hyaloperonospora arabidopsidis* and *Plectosphaerella cucumerina* that was dependent on the capacity of plants to prime both the activation of defense-dependent genes and callose deposition (78).

Postchallenge primed state: the state in which plants express a more robust defensive behavior upon challenge by activating faster and/or stronger defenses

Regulation by RNA-directed DNA methylation was first linked to the activation of defense responses by Agorio & Vera (1), who demonstrated that the RNA-binding ARGONAUTE 4 (AGO4) protein plays a role in defense and that an *ago4* mutant is more susceptible to *Pst*. Since then, studies have demonstrated that other mutants blocked in RNA-directed DNA methylation processes are more resistant to pathogens, including *H. arabidopsidis* and *Pst* (66, 82), and more susceptible to necrotrophic pathogens (78). Moreover, López et al. (77) demonstrated the link between posttranslational modifications and DNA methylation using RNA polymerase V mutants that showed enrichment in H3K4me3 at the promoter of defense genes, leading to priming of SA-dependent genes and resistance to *Pst*.

In conclusion, the changes described above alter the chromatin structure at the promoter regions and can ultimately destabilize the chromatin structure of neighboring regions, thus facilitating the access of transcription components (31). Therefore, it is clear that modifications of histones or DNA methylation levels can serve as pivotal mechanisms to facilitate transcription of defense-dependent genes upon subsequent challenge.

THE POSTCHALLENGE PRIMED STATE: ENHANCED RESPONSIVENESS UPON CHALLENGE

Enhanced Perception of the Attacker

A key trait for resistance in plants is fast and efficient perception of their surroundings, and recent discoveries point to enhanced perception of attackers as an important aspect of defense priming. This postchallenge primed state is suggested by the evidence that *Arabidopsis* plants treated with the SA analog BTH increased their levels of pattern recognition receptors and coreceptors, such as BAK1, FLAGELLIN-SENSITIVE 2 (FLS2), and CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1), and showed enhanced responsiveness to flagellin and chitin (124). The aminotransferase AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1) plays a role in regulating levels of pattern recognition receptors, coreceptors, and PAMP responsiveness and also mediates pipecolic acid accumulation (26). Another player acting at this level is the plasma membrane–localized protein lectin receptor kinase VI.2 (LecRK-VI.2), which is associated with FLS2 and is required for BABA-induced resistance and priming of PAMP-triggered immunity (63). Importantly, priming by BABA relies on a subcellular translocation of its receptor, IBI1, from the endoplasmic reticulum to the cytoplasm after challenge (83).

Boosted perception is due not only to cell receptors, but also to physical structures that help plants to monitor their surroundings, as is the case for trichomes. Chemical treatment with methyl jasmonate induces enhanced production of trichomes in tomato leaves, preparing the plant for increased sensitivity to the presence of herbivores (17, 103).

Enhanced Signal Transduction and Defense Responses

Primed plants can show potentiated ROS generation in response to a challenge. Priming treatments with SA, BTH, BABA, and PS3, for example, boost ROS generation upon challenge with pathogens, PAMPs, or DAMPs (8, 39, 51, 102, 124, 142). In turn, PAMPs potentiate ROS generation when challenged by endogenous danger signals, such as plant elicitor peptides (45). Boosted ROS generation also occurred in PGPR-bacterized grapevine plants upon exposure to cold stress (126).

Wang et al. (139) recently suggested that interplay between ROS signaling and chloroplastic Ca^{2+} generation would facilitate the primed state for stomatal closure when plants sense a stressful environment. Indeed, primed plants have exhibited accelerated stomatal closure, a defensive measure aimed at hampering bacterial ingress (8, 105). *N*-acyl-homoserine lactones, which are bacterial quorum-sensing molecules, are among the priming stimuli that have been shown to boost this response (112). Interestingly, primed plants are also able to block pathogen-mediated (coronatine-dependent) reopening of stomata during *Pst* DC3000 infection (132), a result further supported by the discovery of the *Arabidopsis* mutant *nrt2*, which displays constitutive priming and reduced sensitivity to coronatine (22). An improved stomatal response can also lead to abiotic stress tolerance. As reported in BABA-treated plants, a decrease in stomatal conductance may help improve water use efficiency and lead to drought stress tolerance (8).

Primed callose deposition is a widely used marker of successful enhanced responsiveness to fungi, oomycetes, bacteria, or PAMP application at the sites of attempted colonization or application (8, 102, 106, 115, 124). Enhanced callose accumulation is not exclusively a hallmark of priming by pathogen-derived or chemical stimuli. In 2005, Lee et al. (74) reported for the first time a primed callose response in plants colonized by AMFs: Cucumber plants colonized by the AMF *R. irregularis* showed enhanced callose formation after 5 days of infection with *Colletotrichum orbiculare* compared with nonmycorrhized plants (74). Interestingly, the growth conditions of the plant can influence the callose response: High light intensity, for example, can boost flagellin-induced callose (81).

As one of the main signaling components during defense, MPKs also exhibit enhanced activation in primed plants upon pathogen challenge or abiotic stress (13, 145). For example, their primed status can be observed in terms of longer and more intense phosphorylation, as reported, for example, for MPK3 in *Arabidopsis* upon challenge with *P. syringae* pv. *maculicola* and for leaf infiltration (13). Conrath et al. (31) recently reviewed the role of MPKs in defense priming.

Earlier and/or stronger gene expression upon challenge is one of the most common responses detected in primed plants (28, 115) (**Table 1**). Tomato plants colonized by the AMF *Funneliformis mosseae* (syn. *Glomus mosseae*) showed, for example, enhanced expression of the *LIPOXYGENASE D* (*LOXD*), *ALLENE OXIDE CYCLASE* (*AOC*), and *SERINE PROTEASE INHIBITOR I* (*PI-I*) and *PI-II* genes upon chewing of the leaves by the caterpillar *Helicoverpa armigera* (122), indicative of priming of the oxylipin pathway and JA-related defenses. By contrast, tomato plants colonized by the same AMF showed enhanced expression of *PR1*, *PR2*, and *PR3*, in addition to enhanced expression of *LOX*, *AOC* and *PHENYLALANINE AMMONIA-LYASE* (*PAL*) genes, in leaves upon *Alternaria solani* attack (120), indicative of priming not restricted to JA signaling. In tomato plants, the increased resistance induced by *Trichoderma harzianum* against *B. cinerea* was associated with enhanced expression of the JA-responsive gene encoding prosystemin (88). Interestingly, oviposition by *Helicoverpa zea* on tomato leaves led to increased transcription of the *PI-II* gene and enhanced production of JA upon subsequent wounding and application of oral secretions, a challenging treatment to mimic herbivory by newly hatched neonate larvae (72). Studies performed with the priming inducer BABA have provided a comprehensive picture of the hormonal interplay in defense priming (44, 129, 150; for a review, see 8). Studies have also shown that pathways

dependent on JA, SA, abscisic acid, ethylene, phosphatidylinositol, and phenylpropanoids are involved in BABA priming, depending on the plant and the challenging stress (8, 129).

Primed plants also show increased protein accumulation and enzymatic activity upon challenge, as extensively documented, for instance, for PR proteins after BABA priming (8). However, an increased enzymatic activity was also reported after AMF preinoculation of tomato plants: *F. mosseae* colonization led to increased enzymatic activity of β-1,3-glucanase (PR2), chitinase (PR3), PAL, and LOX upon foliar challenge with *A. solani* (120).

In addition to the responses mentioned above, boosted synthesis of metabolites, amino acids, and phytoalexins has been frequently detected in primed plants upon subsequent challenge (8, 10, 28, 96). PGPR colonization of grapevine, for example, boosted the proline increase during cold stress (126).

The hypersensitive response is the best-known defense reaction mounted by plants with a certain degree of resistance at the sites of attempted infection. Primed plants can show a potentiated hypersensitive response upon challenge, as has been found, for example, after priming by BABA or PS3 (8, 131).

Finally, it is worth mentioning that priming can also influence tritrophic interactions, because primed plants can display enhanced attractiveness to predators. Bean plants colonized by the AMF *F. mosseae* showed enhanced population growth of the predatory mite *Phytoseiulus persimilis*, the natural enemy of the herbivorous spider mite *Tetranychus urticae* (59).

DURATION OF THE PRIMED STATE

Unlike mammals, plants have a nonadaptive immune system that relies on biochemical changes. Nevertheless, priming of induced resistance influences responses after an initial stimulus, and it therefore represents a type of immunological memory that allows plants to remember stressful situations. Epigenetic modifications are one of the mechanisms that enable plants to acquire memory and can cause long-term alterations to gene responsiveness.

Long-Term Responses Within the Same Generation

Initial epigenetic changes in chromatin structure via DNA methylation and posttranslational modifications provide long-term memory within a generation that allow the plant to keep defense mechanisms primed for future attacks (101). Luna et al. (80) showed that the histone methyltransferase KRYPTONITE (KYP) is required for long-lasting priming by BABA against *H. arabidopsidis* in *Arabidopsis*. However, they found no direct changes in posttranslational modification at the promoter level of BABA-primed defense regulatory genes, such as the *PR1* or *WRKY* transcription factor genes, pointing to an epigenetic *trans*-regulation role of defense responses (80). Changes in the DNA methylation status of the plant can also affect long-term responses to biotic and abiotic stresses. Interestingly, a recent study reported that DNA methylation and demethylation do not play a role in systemic acquired resistance four weeks after the initial stimulus (78).

Other studies have reported long-lasting induced resistance to pests and pathogens. For instance, it is possible to achieve durable induced resistance in tomato after seed treatment with BABA or JA (141). This long-lasting resistance was based on priming of gene expression and did not cause any reduction in growth (141).

Transgenerational Immune Resistance

Plants can rapidly acquire stress tolerance through physiological changes that are often associated with developmental costs. Consequently, plants need to be able to revert the acquired tolerance

once the stress has ceased. Epigenetic modifications provide an excellent evolutionary strategy for plant adaptation to environmental challenges because they are heritable, occur rapidly, and can be reversed.

Transgenerational priming state: a priming state that is visible in the progeny of stimulated plants and mediated by epigenetic mechanisms

The discovery that changes in DNA methylation are inheritable prompted hypotheses about epigenetic traits being passed on to subsequent generations. The first study showing higher resistance in the progeny of plants infected with tobacco mosaic virus was published in 1983 (110), with similar findings later described (60). Interestingly, Molinier et al. (92) first observed that exposure to UV or flg22 resulted in greater homologous recombination frequency in subsequent generations, pointing to an epigenetic regulation of environmentally induced changes. Changes in homologous recombination frequency were also reported in the progeny of plants infected with tobacco mosaic virus (18), along with enhanced resistance to tobacco mosaic virus and bacterial and fungal pathogens (69).

In 2012, three independent studies described a transgenerational priming state that manifested as enhanced resistance in the progeny of plants exposed to bacterial infection (79), BABA treatment (116), and herbivory (108). Infections with a pathogenic strain of *P. syringae* enhanced resistance to both the same pathogen and *H. arabidopsidis*, a phenomenon that was based on priming of SA-dependent defenses (79). Similarly, *Arabidopsis* plants treated with either BABA or an avirulent strain of *P. syringae* produced progeny that were more resistant to *H. arabidopsidis* (116). Finally, caterpillar attack resulted in progeny that were more resistant to herbivory infestation in both *Arabidopsis* and tomato, a transgenerational induced resistance based on priming of JA-dependent defenses (108). Studies demonstrating transgenerational resistance after bacterial and herbivory infestation further endeavored to elucidate the epigenetic changes mediating this phenomenon. After directly comparing the transgenerational resistance of mutants in RNA-directed DNA methylation, CpNpG methylation, and de novo CpNpN methylation with that of their wild-type relatives, Luna and colleagues (79, 82) suggested that non-CG methylation plays a pivotal role in transgenerational inheritance of defense priming.

Costs

Transgenerational resistance can incur associated costs. For instance, crosstalk between SA- and JA-dependent resistance has been demonstrated in the progeny of virulent *Pst*-infected plants (79). SA-primed progeny downregulated JA-dependent defenses, resulting in plants that were more susceptible to the necrotrophic fungus *Alternaria brassicicola* (79). López et al. (77) also demonstrated epigenetic regulation of SA-JA crosstalk using mutants impaired in enzymes that mediate RNA-directed DNA methylation, which were more resistant to biotrophic pathogens but more susceptible to necrotrophs. Thus, different immune responses can achieve transgenerational priming with a certain level of specificity to the parental stimulus, resulting in costs associated with the inheritance of defense-hormone crosstalk.

Plants Can Also Forget

Priming generally results in low fitness costs for the plant (89). However, it could lead to the downregulation of some resistance pathways or could sensitize plants such that they respond to false alarm signals. For these reasons, Crisp et al. (33) recently hypothesized that plants might be better at forgetting previous stresses in order to avoid compromising development, yield, and ultimately survival. This is in accordance with the hypothesis that the durability of transgenerational defense priming over stress-free generations may be linked to the level of the stress originally encountered (79). For example, infections with virulent *Pst* and herbivory attack were able to induce a

long-lasting resistance that was maintained over at least one stress-free generation, whereas resistance after infection with avirulent *Pst* was lost at this stage (79, 108, 116). Transgenerational resistance can be sustained through more generations when the initial stress is repeatedly applied, thus warning the progeny of a persistent stress (114). These differences hint at a dependent relationship between the intensity of the stimulation and the durability of the transgenerational resistance. From an ecological perspective, this outcome makes perfect sense: If the stress pressure is high, then the progeny will likely encounter the same stress suffered by the parental plants. Accordingly, owing to the fast and reversible nature of epigenetic modifications, it is likely that transgenerational immune priming is erased after certain stress-free generations, thus removing the plausible costs.

Transgenerational Resistance in Crops

Since the discovery that defense priming can be transmitted to subsequent generations, several publications have described similar findings in crops (138). Rasmann et al. (108) demonstrated that transgenerational resistance to herbivory attack can be achieved in *Solanum lycopersicum*. However, transgenerational resistance can also be obtained in legumes (87, 125). Therefore, transgenerational immune priming does not seem to be limited to short-life model species, such as *Arabidopsis*, and is achievable in economically relevant crops with longer life spans. Studying the mechanisms behind this phenomenon will open opportunities to optimize resistance in cultivars via epigenetic exploitation.

CONCLUSIONS

Priming is an effective strategy to combat biotic and abiotic stresses, and it therefore represents a potential approach to enhance plant protection in agricultural systems (138). As there is an urgent need for new strategies that do not rely on pesticides or single resistance genes, the exploitation of the capacity of the plant immune system in combination with other strategies may hold the potential to achieve better protection of crops. The attractiveness of priming for agricultural protection is also associated with the fact that this phenomenon, unlike the direct activation of defenses, does not incur major developmental costs (135). There has already been a considerable translation of knowledge from the laboratory to the field (31, 138).

Importantly, certain side effects need to be considered before priming can be fully integrated into an agricultural setting. For instance, a stimulation level that is too high might lead to direct resistance induction, thus compromising fitness. Moreover, plants can be more prone to responding to false alarm signals that do not represent a threat, causing them to unnecessarily reallocate energy resources. The most prominent challenge lies in the fact that, in the field, many biotic and abiotic stresses happen concurrently. Little is known about how plants then set their defensive priorities, which makes it difficult to predict their reaction and obtain a robust and broad-spectrum resistance response. In the future, this might even become more difficult in view of the unpredictable effects of climate change.

SUMMARY POINTS

1. Priming is an intrinsic part of all induced resistance mechanisms in plants.
2. Priming can be induced by a wide variety of biotic and abiotic stimuli.

3. Stimuli induce physiological, molecular, and epigenetic changes that prepare the plant for enhanced responsiveness.
4. The primed state can last for the lifetime of a plant and can even be transmitted to its descendants.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the Swiss National Science Foundation for supporting this work through grant 31003A-160162 to B.M.-M. The work by E.L. was supported by a research fellowship at the Plant Production and Protection (P^3) center of the University of Sheffield. We also acknowledge project grant AGL2015-64990-C2-2-R, funded by the Spanish MINECO (FEDER), to V.F. We apologize to researchers whose studies were not included in this review owing to word and reference limits.

LITERATURE CITED

1. Agorio A, Vera P. 2007. ARGONAUTE4 is required for resistance to *Pseudomonas syringae* in *Arabidopsis*. *Plant Cell* 19:3778–90
2. Agrios GN. 2005. How plants defend themselves against pathogens. In *Plant Pathology*, pp. 207–48. San Diego, CA: Academic. 5th ed.
3. Ahn IP, Lee SW, Suh SC. 2007. Rhizobacteria-induced priming in *Arabidopsis* is dependent on ethylene, jasmonic acid, and *NPR1*. *Mol. Plant-Microbe Interact.* 20:759–68
4. Alborn HT, Hansen TV, Jones TH, Bennett DC, Tumlinson JH, et al. 2007. Disulfooxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. *PNAS* 104:12976–81
5. Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276:945–49
6. Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. *Cell* 92:773–84
7. Alvarez-Venegas R, Al Abdallat A, Guo M, Alfano JR, Avramova Z. 2007. Epigenetic control of a transcription factor at the cross section of two antagonistic pathways. *Epigenetics* 2:106–13
8. Baccelli I, Mauch-Mani B. 2016. Beta-aminobutyric acid priming of plant defense: the role of ABA and other hormones. *Plant Mol. Biol.* 91:703–11
9. Bago B, Zipfel W, Williams RM, Jun J, Arreola R, et al. 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiol.* 128:108–24
10. **Balmer A, Pastor V, Gamir J, Flors V, Mauch-Mani B. 2015. The 'prime-ome': towards a holistic approach to priming. *Trends Plant Sci.* 20:443–52**
11. Bandoly M, Hilker M, Steppuhn A. 2015. Oviposition by *Spodoptera exigua* on *Nicotiana attenuata* primes induced plant defence against larval herbivory. *Plant J.* 83:661–72
12. Beckers GJ, Conrath U. 2007. Priming for stress resistance: from the lab to the field. *Curr. Opin. Plant Biol.* 10:425–31
13. Beckers GJ, Jaskiewicz M, Liu Y, Underwood WR, He SY, et al. 2009. Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* 21:944–53
14. Beneloujaephajri E, Costa A, L'Haridon F, Metraux JP, Binda M. 2013. Production of reactive oxygen species and wound-induced resistance in *Arabidopsis thaliana* against *Botrytis cinerea* are preceded and depend on a burst of calcium. *BMC Plant Biol.* 13:160

10. Presents the latest results in the field of priming based on -omics research.

15. Benikhlef L, L'Haridon F, Abou-Mansour E, Serrano M, Binda M, et al. 2013. Perception of soft mechanical stress in Arabidopsis leaves activates disease resistance. *BMC Plant Biol.* 13:133
16. Boller T, Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379–406
17. Boughton AJ, Hoover K, Felton GW. 2005. Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *J. Chem. Ecol.* 31:2211–16
18. Boyko A, Kathiria P, Zemp FJ, Yao Y, Pogribny I, Kovalchuk I. 2007. Transgenerational changes in the genome stability and methylation in pathogen-infected plants. *Nucleic Acids Res.* 35:1714–25
19. Brotman Y, Lisec J, Meret M, Chet I, Willmitzer L, Viterbo A. 2012. Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. *Microbiology* 158:139–46
20. Bruce TJA, Matthes MC, Napier JA, Pickett JA. 2007. Stressful "memories" of plants: evidence and possible mechanisms. *Plant Sci.* 173:603–8
21. Bruessow F, Gouhier-Darimont C, Buchala A, Metraux JP, Reymond P. 2010. Insect eggs suppress plant defence against chewing herbivores. *Plant J.* 62:876–85
22. Camañes G, Pastor V, Cerezo M, García-Andrade J, Vicedo B, et al. 2012. A deletion in *NRT2.1* attenuates *Pseudomonas syringae*-induced hormonal perturbation, resulting in primed plant defenses. *Plant Physiol.* 158:1054–66
23. Cameron DD, Neal AL, van Wees SC, Ton J. 2013. Mycorrhiza-induced resistance: more than the sum of its parts? *Trends Plant Sci.* 18:539–45
24. Campos-Soriano L, Garcia-Martinez J, San Segundo B. 2012. The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. *Mol. Plant Pathol.* 13:579–92
25. Campos-Soriano L, Gomez-Ariza J, Bonfante P, San Segundo B. 2011. A rice calcium-dependent protein kinase is expressed in cortical root cells during the presymbiotic phase of the arbuscular mycorrhizal symbiosis. *BMC Plant Biol.* 11:90
26. Cecchini NM, Jung HW, Engle NL, Tschaplinski TJ, Greenberg JT. 2015. ALD1 regulates basal immune components and early inducible defense responses in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 28:455–66
27. Cervantes-Gámez RG, Bueno-Ibarra MA, Cruz-Mendívil A, Calderón-Vázquez CL, Ramírez-Douriet CM, et al. 2016. Arbuscular mycorrhizal symbiosis-induced expression changes in *Solanum lycopersicum* leaves revealed by RNA-seq analysis. *Plant Mol. Biol. Rep.* 34:89–102
28. Chassot C, Buchala A, Schoonbeek HJ, Metraux JP, Lamotte O. 2008. Wounding of Arabidopsis leaves causes a powerful but transient protection against *Botrytis* infection. *Plant J.* 55:555–67
29. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nurnberger T, et al. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* 448:497–500
30. Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab J, et al. (Prime-A-Plant Group). 2006. Priming: getting ready for battle. *Mol. Plant-Microbe Interact.* 19:1062–71
31. Conrath U, Beckers GJM, Langenbach CJG, Jaskiewicz MR. 2015. Priming for enhanced defense. *Annu. Rev. Phytopathol.* 53:97–119
32. Conrath U, Pieterse CM, Mauch-Mani B. 2002. Priming in plant-pathogen interactions. *Trends Plant Sci.* 7:210–16
33. Crisp PA, Ganguly D, Eichten SR, Borevitz JO, Pogson BJ. 2016. Reconsidering plant memory: intersections between stress recovery, RNA turnover, and epigenetics. *Sci. Adv.* 2:e1501340
34. De Vleesschauwer D, Höfte M. 2009. Rhizobacteria-induced systemic resistance. In *Advances in Botanical Research*, Vol. 51: *Plant Innate Immunity*, ed. LC Van Loon, pp. 223–81. San Diego, CA: Academic
35. Delaux PM, Sejalon-Delmas N, Becard G, Ane JM. 2013. Evolution of the plant-microbe symbiotic 'toolkit.' *Trends Plant Sci.* 18:298–304
36. Dempsey DA, Klessig DF. 2012. SOS—too many signals for systemic acquired resistance? *Trends Plant Sci.* 17:538–45
37. Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat. Rev. Genet.* 11:539–48

38. Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, et al. 2012. Widespread dynamic DNA methylation in response to biotic stress. *PNAS* 109:E2183–91
39. Dubreuil-Maurizi C, Trouvelot S, Frettinger P, Pugin A, Wendehenne D, Poinssot B. 2010. β-Aminobutyric acid primes an NADPH oxidase–dependent reactive oxygen species production during grapevine-triggered immunity. *Mol. Plant-Microbe Interact.* 23:1012–21
40. Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH. 2004. Airborne signals prime plants against insect herbivore attack. *PNAS* 101:1781–85
41. Erb M, Veyrat N, Robert CA, Xu H, Frey M, et al. 2015. Indole is an essential herbivore-induced volatile priming signal in maize. *Nat. Commun.* 6:6273
42. Farag MA, Ryu CM, Sumner LW, Pare PW. 2006. GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. *Phytochemistry* 67:2262–68
43. Fester T, Hause G. 2005. Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. *Mycorrhiza* 15:373–79
44. Flors V, Ton J, van Doorn R, Jakab G, Garcia-Agustin P, Mauch-Mani B. 2008. Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant J.* 54:81–92
45. Flury P, Klauser D, Schulze B, Boller T, Bartels S. 2013. The anticipation of danger: Microbe-associated molecular pattern perception enhances AtPep-triggered oxidative burst. *Plant Physiol.* 161:2023–35
46. Frost CJ, Mescher MC, Carlson JE, De Moraes CM. 2008. Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146:818–24
47. Frye CA, Tang D, Innes RW. 2001. Negative regulation of defense responses in plants by a conserved MAPKK kinase. *PNAS* 98:373–78
48. Gamir J, Pastor V, Cerezo M, Flors V. 2012. Identification of indole-3-carboxylic acid as mediator of priming against *Plectosphaerella cucumerina*. *Plant Physiol. Biochem.* 61:169–79
49. Gamir J, Sanchez-Bel P, Flors V. 2014. Molecular and physiological stages of priming: how plants prepare for environmental challenges. *Plant Cell Rep.* 33:1935–49
50. Garcia-Andrade J, Ramirez V, Flors V, Vera P. 2011. Arabidopsis *ocp3* mutant reveals a mechanism linking ABA and JA to pathogen-induced callose deposition. *Plant J.* 67:783–94
51. Gauthier A, Trouvelot S, Kelloniemi J, Frettinger P, Wendehenne D, et al. 2014. The sulfated laminarin triggers a stress transcriptome before priming the SA- and ROS-dependent defenses during grapevine's induced resistance against *Plasmopara viticola*. *PLOS ONE* 9:e88145
52. Gehring M, Henikoff S. 2007. DNA methylation dynamics in plant genomes. *Biochim. Biophys. Acta* 1769:276–86
53. Gerlach N, Schmitz J, Polatajko A, Schluter U, Fahnenstich H, et al. 2015. An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. *Plant Cell Environ.* 38:1591–612
54. Gutjahr C. 2014. Phytohormone signaling in arbuscular mycorhiza development. *Curr. Opin. Plant Biol.* 20:26–34
55. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2:43–56
56. Hilker M, Fatouros NE. 2015. Plant responses to insect egg deposition. *Annu. Rev. Entomol.* 60:493–515
57. Hilker M, Meiners T. 2006. Early herbivore alert: insect eggs induce plant defense. *J. Chem. Ecol.* 32:1379–97
58. Hilker M, Meiners T. 2010. How do plants "notice" attack by herbivorous arthropods? *Biol. Rev. Camb. Philos. Soc.* 85:267–80
59. Hoffmann D, Vierheilig H, Schausberger P. 2011. Mycorrhiza-induced trophic cascade enhances fitness and population growth of an acarine predator. *Oecologia* 166:141–49
60. Holeski LM, Jander G, Agrawal AA. 2012. Transgenerational defense induction and epigenetic inheritance in plants. *Trends Ecol. Evol.* 27:618–26
61. Howe GA, Jander G. 2008. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* 59:41–66
62. Hsu FC, Chou MY, Chou SJ, Li YR, Peng HP, Shih MC. 2013. Submergence confers immunity mediated by the WRKY22 transcription factor in *Arabidopsis*. *Plant Cell* 25:2699–713

63. Huang PY, Yeh YH, Liu AC, Cheng CP, Zimmerli L. 2014. The Arabidopsis LecRK-VI.2 associates with the pattern-recognition receptor FLS2 and primes *Nicotiana benthamiana* pattern-triggered immunity. *Plant J.* 79:243–55

64. Jaskiewicz M, Conrath U, Peterhansel C. 2011. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep.* 12:50–55

65. Jeworutzki E, Roelfsema MR, Anschutz U, Krol E, Elzenga JT, et al. 2010. Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca-associated opening of plasma membrane anion channels. *Plant J.* 62:367–78

66. Jin H. 2008. Endogenous small RNAs and antibacterial immunity in plants. *FEBS Lett.* 582:2679–84

67. Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. 2009. Priming in systemic plant immunity. *Science* 324:89–91

68. Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ. 2012. Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.* 38:651–64

69. Kathiria P, Sidler C, Golubov A, Kalischuk M, Kawchuk LM, Kovalchuk I. 2010. Tobacco mosaic virus infection results in an increase in recombination frequency and resistance to viral, bacterial, and fungal pathogens in the progeny of infected tobacco plants. *Plant Physiol.* 153:1859–70

70. Katz VA, Thulke OU, Conrath U. 1998. A benzothiadiazole primes parsley cells for augmented elicitation of defense responses. *Plant Physiol.* 117:1333–39

71. Kim J, Felton GW. 2013. Priming of antiherbivore defensive responses in plants. *Insect Sci.* 20:273–85

72. Kim J, Tooker JF, Luthe DS, De Moraes CM, Felton GW. 2012. Insect eggs can enhance wound response in plants: a study system of tomato *Solanum lycopersicum* L. and *Helicoverpa zea* Boddie. *PLOS ONE* 7:e37420

73. Kloepper JW, Ryu CM, Zhang S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94:1259–66

74. Lee CS, Lee YJ, Jeun YC. 2005. Observations of infection structures on the leaves of cucumber plants pre-treated with arbuscular mycorrhiza *Glomus intraradices* after challenge inoculation with *Colletotrichum orbiculare*. *Plant Pathol. J.* 21:237–43

75. Leitner M, Kaiser R, Hause B, Boland W, Mithofer A. 2010. Does mycorrhization influence herbivore-induced volatile emission in *Medicago truncatula*? *Mycorrhiza* 20:89–101

76. Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ. 2007. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *Plant J.* 50:529–44

77. López A, Ramírez V, García-Andrade J, Flors V, Vera P. 2011. The RNA silencing enzyme RNA polymerase V is required for plant immunity. *PLOS Genet.* 7:e1002434

78. López Sánchez A, Stassen JHM, Furci L, Smith LM, Ton J. 2016. The role of DNA (de)methylation in immune responsiveness of Arabidopsis. *Plant J.* 88:361–74

79. Luna E, Bruce TJ, Roberts MR, Flors V, Ton J. 2012. Next-generation systemic acquired resistance. *Plant Physiol.* 158:844–53

80. Luna E, Lopez A, Kooiman J, Ton J. 2014. Role of NPR1 and KYP in long-lasting induced resistance by β-aminobutyric acid. *Front. Plant Sci.* 5:184

81. Luna E, Pastor V, Robert J, Flors V, Mauch-Mani B, Ton J. 2011. Callose deposition: a multifaceted plant defense response. *Mol. Plant-Microbe Interact.* 24:183–93

82. Luna E, Ton J. 2012. The epigenetic machinery controlling transgenerational systemic acquired resistance. *Plant Signaling Behav.* 7:615–18

83. Luna E, van Hulten M, Zhang Y, Berkowitz O, Lopez A, et al. 2014. Plant perception of β-aminobutyric acid is mediated by an aspartyl-tRNA synthetase. *Nat. Chem. Biol.* 10:450–56

84. Maekawa S, Sato T, Asada Y, Yasuda S, Yoshida M, et al. 2012. The Arabidopsis ubiquitin ligases ATL31 and ATL6 control the defense response as well as the carbon/nitrogen response. *Plant Mol. Biol.* 79:217–27

85. Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, et al. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* 469:58–63

64. Shows that certain stimuli can change the epigenetic status of a plant.

68. Demonstrates that mycorrhization can induce a priming state in a plant.

86. March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, Reyes JC. 2008. Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. *Plant J.* 53:475–87
87. Martinez-Aguilar K, Ramirez-Carrasco G, Hernandez-Chavez JL, Barraza A, Alvarez-Venegas R. 2016. Use of BABA and INA as activators of a primed state in the common bean (*Phaseolus vulgaris* L.). *Front. Plant Sci.* 7:653
88. Martinez-Medina A, Fernandez I, Sanchez-Guzman MJ, Jung SC, Pascual JA, Pozo MJ. 2013. Deciphering the hormonal signalling network behind the systemic resistance induced by *Trichoderma harzianum* in tomato. *Front. Plant Sci.* 4:206
89. Martinez-Medina A, Flors V, Heil M, Mauch-Mani B, Pieterse CMJ, et al. 2016. Recognizing plant defense priming. *Trends Plant Sci.* 21:818–22
90. Martinez-Medina A, Pascual JA, Perez-Alfocea F, Albacete A, Roldan A. 2010. *Trichoderma harzianum* and *Glomus intraradices* modify the hormone disruption induced by *Fusarium oxysporum* infection in melon plants. *Phytopathology* 100:682–88
91. Mishina TE, Zeier J. 2007. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. *Plant J.* 50:500–13
92. **Molinier J, Ries G, Zipfel C, Hohn B. 2006. Transgeneration memory of stress in plants. *Nature* 442:1046–49**
93. Mosher RA, Durrant WE, Wang D, Song J, Dong X. 2006. A comprehensive structure–function analysis of *Arabidopsis* SNI1 defines essential regions and transcriptional repressor activity. *Plant Cell* 18:1750–65
94. Mousavi SA, Chauvin A, Pascaud F, Kellenberger S, Farmer EE. 2013. *GLUTAMATE RECEPTOR-LIKE* genes mediate leaf-to-leaf wound signalling. *Nature* 500:422–26
95. Navarova H, Bernsdorff F, Doring AC, Zeier J. 2012. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell* 24:5123–41
96. Newman MA, von Roepenack-Lahaye E, Parr A, Daniels MJ, Dow JM. 2002. Prior exposure to lipopolysaccharide potentiates expression of plant defenses in response to bacteria. *Plant J.* 29:487–95
97. Oldroyd GE, Harrison MJ, Paszkowski U. 2009. Reprogramming plant cells for endosymbiosis. *Science* 324:753–54
98. Oostendorp M, Kunz W, Dietrich B, Staub T. 2001. Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol.* 107:19–28
99. Pasini D, Hansen KH, Christensen J, Agger K, Cloos PA, Helin K. 2008. Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes Dev.* 22:1345–55
100. Pastor V, Balmer A, Gamir J, Flors V, Mauch-Mani B. 2014. Preparing to fight back: generation and storage of priming compounds. *Front. Plant Sci.* 5:295
101. Pastor V, Luna E, Mauch-Mani B, Ton J, Flors V. 2012. Primed plants do not forget. *Environ. Exp. Bot.* 94:46–56
102. Pastor V, Luna E, Ton J, Cerezo M, García-Agustín P, Flors V. 2013. Fine tuning of reactive oxygen species homeostasis regulates primed immune responses in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 26:1334–44
103. Peiffer M, Tooker JF, Luthe DS, Felton GW. 2009. Plants on early alert: glandular trichomes as sensors for insect herbivores. *New Phytol.* 184:644–56
104. Pérez-Hedo M, Bouagga S, Jaques JA, Flors V, Urbaneja A. 2015. Tomato plant responses to feeding behavior of three zoophytophagous predators (Hemiptera: Miridae). *Biol. Control* 86:46–51
105. Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52:347–75
106. Po-Wen C, Singh P, Zimmerli L. 2013. Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic *Pectobacterium carotovorum* bacteria. *Mol. Plant Pathol.* 14:58–70
107. Pozo MJ, Lopez-Raez JA, Azcon-Aguilar C, Garcia-Garrido JM. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytol.* 205:1431–36

92. Shows that exposure to UV or flg22 results in greater homologous recombination frequency in subsequent generations.

108. Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, et al. 2012. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* 158:854–63

109. Rivero J, Gamir J, Aroca R, Pozo MJ, Flors V. 2015. Metabolic transition in mycorrhizal tomato roots. *Front. Microbiol.* 6:598

110. Roberts DA. 1983. Acquired resistance to tobacco mosaic virus transmitted to the progeny of hypersensitive tobacco. *Virology* 124:161–63

111. Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW. 2004. Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiol.* 134:1017–26

112. Schenk ST, Hernandez-Reyes C, Samans B, Stein E, Neumann C, et al. 2014. *N*-acyl-homoserine lactone primes plants for cell wall reinforcement and induces resistance to bacterial pathogens via the salicylic acid/oxylipin pathway. *Plant Cell* 26:2708–23

113. Schweiger R, Muller C. 2015. Leaf metabolome in arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 26:120–26

114. Singh P, Roberts MR. 2015. Keeping it in the family: transgenerational memories of plant defence. *CAB Rev.* 10:26

115. Singh P, Yekondi S, Chen PW, Tsai CH, Yu CW, et al. 2014. Environmental history modulates *Arabidopsis* pattern-triggered immunity in a HISTONE ACETYLTRANSFERASE1-dependent manner. *Plant Cell* 26:2676–88

116. Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B. 2012. Descendants of primed Arabidopsis plants exhibit resistance to biotic stress. *Plant Physiol.* 158:835–43

117. Smart LE, Martin JL, Limpalaer M, Bruce TJ, Pickett JA. 2013. Responses of herbivore and predatory mites to tomato plants exposed to jasmonic acid seed treatment. *J. Chem. Ecol.* 39:1297–300

118. Smith FA, Grace EJ, Smith SE. 2009. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytol.* 182:347–58

119. Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu. Rev. Plant Biol.* 62:227–50

120. Song YY, Chen D, Lu K, Sun Z, Zeng R. 2015. Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. *Front. Plant Sci.* 6:786

121. Song YY, Ye M, Li CY, He X, Zhu-Salzman K, et al. 2014. Hijacking common mycorrhizal networks for herbivore-induced defence signal transfer between tomato plants. *Sci. Rep.* 4:3915

122. Song YY, Ye M, Li CY, Wang RL, Wei XC, et al. 2013. Priming of anti-herbivore defense in tomato by arbuscular mycorrhizal fungus and involvement of the jasmonate pathway. *J. Chem. Ecol.* 39:1036–44

123. Sun X-G, Tang M. 2013. Effect of arbuscular mycorrhizal fungi inoculation on root traits and root volatile organic compound emissions of *Sorghum bicolor*. *S. Afr. J. Bot.* 88:373–79

124. Tateda C, Zhang Z, Shrestha J, Jelenska J, Chinchilla D, Greenberg JT. 2014. Salicylic acid regulates *Arabidopsis* microbial pattern receptor kinase levels and signaling. *Plant Cell* 26:4171–87

125. terHorst CP, Lau JA. 2012. Direct and indirect transgenerational effects alter plant-herbivore interactions. *Evol. Ecol.* 26:1469–80

126. Theocharis A, Bordiec S, Fernandez O, Paquis S, Dhondt-Cordelier S, et al. 2012. *Burkholderia phytofirmans* PsJN primes *Vitis vinifera* L. and confers a better tolerance to low nonfreezing temperatures. *Mol. Plant-Microbe Interact.* 25:241–49

126a. Thevenet D, Pastor V, Baccelli I, Balmer A, Vallat A, et al. 2017. The priming molecule β-aminobutyric acid is naturally present in plants and is induced by stress. *New Phytol.* 213:552–59

127. Thomma BP, Nurnberger T, Joosten MH. 2011. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* 23:4–15

128. Ton J, D'Alessandro M, Jourdie V, Jakab G, Karlen D, et al. 2007. Priming by airborne signals boosts direct and indirect resistance in maize. *Plant J.* 49:16–26

129. Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, et al. 2005. Dissecting the β-aminobutyric acid-induced priming phenomenon in Arabidopsis. *Plant Cell* 17:987–99

130. Ton J, Mauch-Mani B. 2004. β-Amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* 38:119–30

108. Demonstrates that transgenerational resistance to herbivory attack can be achieved in model and crop plants.

124. Reports that, in *Arabidopsis*, microbial pattern receptor kinase levels and signaling are regulated by SA.

128. Shows that, in maize, priming is mediated by airborne signals.

131. Trouvelot S, Varnier AL, Allegre M, Mercier L, Baillieul F, et al. 2008. A β-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. *Mol. Plant-Microbe Interact.* 21:232–43
132. Tsai CH, Singh P, Chen CW, Thomas J, Weber J, et al. 2011. Priming for enhanced defence responses by specific inhibition of the Arabidopsis response to coronatine. *Plant J.* 65:469–79
133. van den Burg HA, Takken FLW. 2009. Does chromatin remodeling mark systemic acquired resistance? *Trends Plant Sci.* 14:286–94
134. Van der Ent S, Van Hulten M, Pozo MJ, Czechowski T, Udvardi MK, et al. 2009. Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation. *New Phytol.* 183:419–31
135. van Hulten M, Pelser M, van Loon LC, Pieterse CM, Ton J. 2006. Costs and benefits of priming for defense in *Arabidopsis*. *PNAS* 103:5602–7
136. Walker V, Bertrand C, Bellvert F, Moenne-Loccoz Y, Bally R, Comte G. 2011. Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytol.* 189:494–506
137. Walley JW, Rowe HC, Xiao Y, Chehab EW, Kliebenstein DJ, et al. 2008. The chromatin remodeler SPLAYED regulates specific stress signaling pathways. *PLOS Pathog.* 4:e1000237
138. Walters DR, Ratsep J, Havis ND. 2013. Controlling crop diseases using induced resistance: challenges for the future. *J. Exp. Bot.* 64:1263–80
139. Wang W-H, He E-M, Guo Y, Tong Q-X, Zheng H-L. 2016. Chloroplast calcium and ROS signaling networks potentially facilitate the primed state for stomatal closure under multiple stresses. *Environ. Exp. Bot.* 122:85–93
140. Winter TR, Borkowski L, Zeier J, Rostas M. 2012. Heavy metal stress can prime for herbivore-induced plant volatile emission. *Plant Cell Environ.* 35:1287–98
141. Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, et al. 2012. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytol.* 193:770–78
142. Xu J, Xie J, Yan C, Zou X, Ren D, Zhang S. 2014. A chemical genetic approach demonstrates that MPK3/MPK6 activation and NADPH oxidase-mediated oxidative burst are two independent signaling events in plant immunity. *Plant J.* 77:222–34
143. Yalpani N, Enyedi AJ, León J, Raskin I. 1994. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. *Planta* 193:372–76
144. Yamazaki A, Hayashi M. 2015. Building the interaction interfaces: host responses upon infection with microorganisms. *Curr. Opin. Plant Biol.* 23:132–39
145. Yi SY, Min SR, Kwon SY. 2015. NPR1 is instrumental in priming for the enhanced flg22-induced MPK3 and MPK6 activation. *Plant Pathol. J.* 31:192–94
146. Yu A, Lepere G, Jay F, Wang J, Bapaume L, et al. 2013. Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense. *PNAS* 110:2389–94
147. Zhou J, Wang X, He K, Charron JB, Elling AA, Deng XW. 2010. Genome-wide profiling of histone H3 lysine 9 acetylation and dimethylation in *Arabidopsis* reveals correlation between multiple histone marks and gene expression. *Plant Mol. Biol.* 72:585–95
148. Zilberman D. 2008. The evolving functions of DNA methylation. *Curr. Opin. Plant Biol.* 11:554–59
149. Zimmerli L, Jakab G, Metraux JP, Mauch-Mani B. 2000. Potentiation of pathogen-specific defense mechanisms in *Arabidopsis* by β-aminobutyric acid. *PNAS* 97:12920–25
150. Zimmerli L, Metraux JP, Mauch-Mani B. 2001. β-Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus *Botrytis cinerea*. *Plant Physiol.* 126:517–23

135. Reports that priming is ecologically less costly for a plant than direct defense.