Plant Defense Signaling from the Underground Primes Aboveground Defenses to Confer Enhanced Resistance in a Cost-Efficient Manner

Marieke Van Hulten, Jurriaan Ton, Corné M.J. Pieterse, and Saskia C.M. Van Wees

Abstract Plants can be induced to develop below and aboveground enhanced resistance to pathogens and herbivorous insects by root-colonizing beneficial micro-organisms. The resistance induced is broad-spectrum and can be long lasting. The enhanced resistance is based at least partially on priming of defense responses, leading to a more rapid or more intense mobilization of defense responses upon encounter with harmful organisms. Several molecular players in local and systemic tissues of plants treated with resistance-inducing microbes have been identified and are reviewed in this chapter. We also discuss the ecological consequences of expression of induced resistance through a primed defense response.

1 Introduction

Below the soil surface, interactions between plants and microbes take place. Plant roots are quickly colonized by members of the indigenous microflora. Colonization by pathogens could have deleterious effects on the plant, but interactions between plants and microbes can also be advantageous for both the plant and the microbe. A well-known example of symbiosis between plants and soil-borne micro-organisms is that between plants and arbuscular mycorrhizal fungi, where the fungus aids the plant in the uptake of water and mineral nutrients such as phosphate by enhancing its absorbance surface through the fungal mycelium, while the plant provides carbohydrates to the fungus (Harrison 2005). Another classical example of symbiosis is the interaction between legume plants and *Rhizobium* spp. bacteria, in which the bacteria induce the formation of root nodules where they fix atmospheric nitrogen to convert it into organic nitrogenous compounds that become available

M. Van Hulten, J. Ton, C.M.J. Pieterse, and S.C.M. Van Wees (✉)
Faculty of Science, Plant–Microbe Interactions, Department of Biology, Utrecht University, Utrecht 3508 TB, The Netherlands
e-mail: s.vanwees@uu.nl

for the plant, while the bacteria obtain various organic photosynthetic compounds from the plant (Spaink 2000). Plant growth-promoting rhizobacteria (PGPR) form yet another class of beneficial soil-borne micro-organisms. PGPR such as *Pseudomonas* spp. and *Baccilus* spp. colonize the rhizosphere, where they live off nutrients exuded by plant roots. They can improve plant growth either directly by augmenting photosynthesis (Zhang et al. 2008) or indirectly by suppressing plant diseases (Van Loon et al. 1998; Bloemberg and Lugtenberg 2001). Disease suppression can be established through direct effects on soil-borne pathogens, such as competition for nutrients or secretion of toxic compounds (Van Loon et al. 1998; Weller et al. 2002; De Bruijn et al. 2007). However, root colonization by PGPR was also documented to suppress diseases caused by foliar pathogens in aboveground tissue (Kloepper et al. 2004; Van Loon and Bakker 2006; Van Wees et al. 2008). This plant-mediated effect of PGPR on pathogens is dependent on activation of the host's immune response and is commonly referred to as induced systemic resistance (ISR; Van Loon 2000). In addition to PGPR, soil-borne beneficial fungi can also trigger an ISR response in plants – for instance, plant growth-promoting fungi (PGPF) that include members of *Trichoderma* spp. (De Meyer et al. 1998; Harman et al. 2004; Shoresh et al. 2005; Vinale et al. 2008; Segarra et al. 2009) and *Piriformospora* spp. (Waller et al. 2005). Moreover, mycorrhizal association has also been reported to protect systemic plant tissues (Pozo and Azcón-Aguilar 2007).

Here, the molecular mechanisms of ISR triggered by PGPR are reviewed in light of the ecological perspective of the costs and benefits that are associated with plant defense.

## 2 Perception of PGPR by the Plant

Typically, ISR is effective against a broad range of taxonomically different pathogens (Van Loon et al. 1998; Van Wees et al. 2008) and also against herbivorous insects (Zehnder et al. 2001; Van Oosten et al. 2008). ISR induction is dependent on the combination of the plant and the beneficial micro-organism. PGPR strains that induce ISR in one species may not do so in another species and vice versa, suggesting host specificity in PGPR detection. For example, *Pseudomonas putida* WCS358 induces ISR in Arabidopsis (*Arabidopsis thaliana*), but not in its closely related crop relative radish (Van Peer et al. 1991; Van Peer and Schippers 1992; Leeman et al. 1995; Van Wees et al. 1997). Conversely, *Pseudomonas fluorescens* WCS374 is capable of inducing ISR in radish but not in Arabidopsis (Leeman et al. 1995; Van Wees et al. 1997).

The establishment of a symbiotic interaction requires a complex dialog between the plant and the micro-organism. The plant can detect microbe-associated molecular patterns (MAMPs) of beneficial micro-organisms, such as flagellin and lipopolysaccharides (LPS), which is in analogy to the detection of pathogen-associated molecular patterns (PAMPs) of pathogenic microbes (Nürnberger et al. 2004). In support of this, purified flagellin and LPS of beneficial rhizobacteria are reported

to elicit ISR. That bacterial mutants lacking one of these determinants are still capable of protecting plants suggests that multiple MAMPs are involved in the induction of ISR (Bakker et al. 2007). However, while PAMP detection triggers a primary defense response in plants, called PAMP-triggered immunity (PTI), which keeps nonadapted pathogens at bay (Jones and Dangl 2006; Schwessinger and Zipfel 2008), perception of beneficial microbes does not trigger such a substantial defense response (Verhagen et al. 2004; Van Wees et al. 2008), or to a much lesser extent (Liu et al. 2007), and the benefactor remains accommodated by the plant.

3 ISR Signal Transduction

ISR induced by several beneficial *Pseudomonas* strains was shown to function independent of the plant defense hormone salicylic acid (SA; Pieterse et al. 1996). This is in contrast to another well-studied form of systemically induced resistance, namely systemic acquired resistance (SAR), which is triggered upon infection by pathogens (Durrant and Dong 2004). However, the use of signaling mutants indicated that components of the signaling pathways controlled by the hormones jasmonic acid (JA) and ethylene (ET) response are required for ISR (Pieterse et al. 1998; Van Wees et al. 2008). This indicates that distinct signaling cascades underlie each form of systemically induced resistance. Concordantly, SAR is predominantly effective against biotrophic pathogens that are resisted through SA-dependent defenses, while ISR is most efficient against necrotrophic pathogens and insects (Fig. 1), which are susceptible to JA-dependent defenses. For instance, SAR was shown to protect *Arabidopsis* plants against turnip crinckle virus in *Arabidopsis*, while ISR did not (Ton et al. 2002). Conversely, ISR induced by *P. fluorescens* WCS417 was shown to be effective in *Arabidopsis* against the necrotrophic pathogens *Alternaria brassicicola* (Ton et al. 2002), *Botrytis cinerea* (Van der Ent et al. 2008), and *Plectosphaerella cucumerina* (Segarra et al. 2009), while pathogen-induced SAR was not. ISR was reported to be effective against *Spodoptora exigua*, a generalist herbivore on *Arabidopsis*; SAR could also induce resistance against this insect (Van Oosten et al. 2008). Both ISR and SAR led to protection against the biotrophic oomycete *Hyaloperonospora arabidopsidis* and the (hemi-)biotroph *Pseudomonas syringae* (Ton et al. 2002). However, this resistance was shown to be accomplished through activation of distinct signaling pathways in SAR and ISR (Cao et al. 1994; Lawton et al. 1995; Pieterse et al. 1998; Van Wees et al. 1999, 2000; Van der Ent et al. 2009b). In agreement with this, SAR and ISR have been shown to have an additive effect on the level of induced resistance against *P. syringae* (Van Wees et al. 2000). For multiple plant–benefical microbe interactions, the involvement of JA and/or ET signaling components has been reported, indicating that ISR signaling is the common route to induce systemic resistance (Van Wees et al. 2008). However, several examples of PGPR and PGPF that trigger SA-dependent SAR signaling leading to enhanced systemic resistance have been documented as well (Van Loon and Bakker 2005; Van Wees et al. 2008).
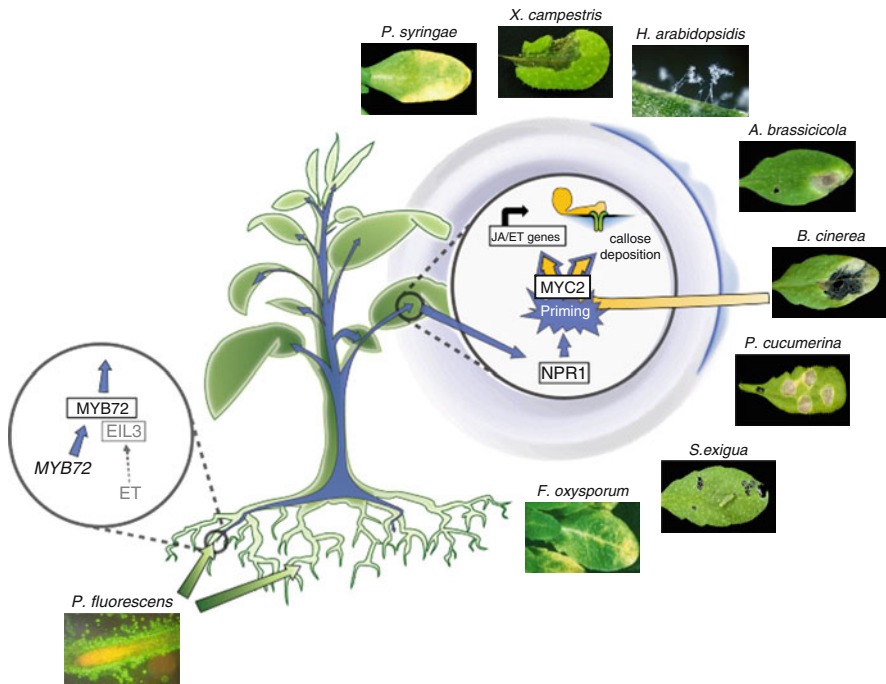
Fig. 1 Spectrum of effectiveness of *Pseudomonas fluorescens* WCS417-mediated ISR in Arabidopsis. ISR induced by the beneficial rhizobacterium *P. fluorescens* WCS417 is effective against the hemibiotrophic bacterial pathogens *Pseudomonas syringae* (Pieterse et al. 1996) and *Xanthomonas campestris* (Ton et al. 2002), the biotrophic oomycete *Hyaloperonospora arabidopsidis* (Ton et al. 2002), the necrotrophic fungal pathogens *Alternaria brassicicola* (Ton et al. 2002), *Botrytis cinerea* (Van der Ent et al. 2008) and *Plectosphaerella cucumerina* (Segarra et al. 2009), the fungal root pathogen *Fusarium oxysporum* (Van Wees et al. 1997), and the insect herbivore *Spodoptora exigua* (Van Oosten et al. 2008). The TF MYB72 is involved in ISR locally in the roots (formation or translocation of the ISR signal; Van der Ent et al. 2008) and the TF MYC2 is required for the priming response in systemic ISR-expressing tissue (Pozo et al. 2008). Adapted from Van Wees et al. (2008)

Microarray analysis of Arabidopsis root tissue identified a differential expression of 94 genes during rhizosphere colonization by *P. fluorescens* WCS417 (Verhagen et al. 2004). One of these genes was the R2R3-MYB-like transcription factor (TF) gene *MYB72* (Verhagen et al. 2004). Interestingly, *myb72* knockout mutants were incapable of mounting ISR, indicating that local *MYB72* induction is required for ISR (Van der Ent et al. 2008; Fig. 1). Interestingly, in *Arabidopsis*, MYB72 is also essential for the induction of ISR by the beneficial fungus *Trichoderma asperellum* T34 (Segarra et al. 2009), suggesting a regulating role for MYB72 in the induction of ISR by taxonomically different organisms. MYB72 may be an early point of convergence in ISR signaling elicited by different MAMPs (Segarra et al. 2009). *MYB72* overexpressors do not show enhanced levels of disease resistance (Van der Ent et al. 2008), indicating that although *MYB72* induction is required, it is

not sufficient for ISR induction, suggesting the involvement of an additional signal. It was demonstrated that MYB72 binds to the ETHYLENE INSENSITIVE3-LIKE (EIL3) TF *in vitro*, linking MYB72 function to the ET response pathway. In this respect it is noteworthy that the Arabidopsis *ethylene insensitive root 1* (*eir1*) mutant, which is insensitive to ET in the roots but not in the shoot (Roman et al. 1995), is incapable of mounting an ISR response after root colonization by WCS417r, while leaf infiltration with WCS417r still triggered ISR in this mutant (Knoester et al. 1999). These results indicate that ISR requires an intact ET responsiveness at the site of elicitation (Knoester et al. 1999).

While most SA signaling mutants of *Arabidopsis* are still able to express beneficial microbe-induced ISR, the SA-nonresponsive mutant *npr1 (nonexpressor of PR genes 1*; Cao et al. 1994; Delaney et al. 1995; Shah et al. 1997) of Arabidopsis is disturbed in WCS417-ISR (Pieterse et al. 1998). SA triggers the reduction of inactive NPR1 oligomers into active monomers, which subsequently translocate to the nucleus (Mou et al. 2003). In the nucleus NPR1 can interact with different TFs to regulate the expression of downstream genes, like *PR-1* (Fan and Dong 2002; Wang et al. 2006). NPR1 functions in the ISR signal transduction pathway likely downstream of the JA- and ET-dependency and does not activate *PR* gene expression (Pieterse et al. 1998). Evidence is accumulating that the role of NPR1 in ISR is connected to a cytosolic function of NPR1 (Stein et al. 2008), which is in line with a role of NPR1 in the cytosol in cross-talk between SA and JA signaling (Spoel et al. 2003; Leon-Reyes et al. 2009). Several examples of NPR1-dependency of ISR triggered by PGPR and PGPF in different plant species have been documented (Van der Ent et al. 2009a).

4 Priming for Enhanced Defense

Large-scale transcriptome analysis of ISR-expressing leaves in plants of which the roots were treated with WCS417 or other beneficial microbes revealed that there is no or only a weak direct induction of gene expression in systemic tissue (Verhagen et al. 2004; Liu et al. 2007; Van Wees et al. 2008). However, subsequent infection with a pathogen led to an augmented expression of a large number of genes in ISR expressing plants compared to control plants (Van Wees et al. 1999; Verhagen et al. 2004; Ahn et al. 2007; Cartieaux et al. 2008). In analogy to a similar phenomenon in animals, the enhanced defensive capacity without direct induction of defense responses in the absence of pathogens is called priming (Conrath et al. 2002, 2006). The set of genes that showed WCS417-primed induction after *P. syringae* infection of Arabidopsis was particularly enriched in JA/ET-regulated genes (Verhagen et al. 2004) that are responsive to JA/ET-inducing pathogens and insects, like *P. syringae, A. brassicicola, Pieris rapae,* and *Frankliniella occidentalis* (De Vos et al. 2005; Van der Ent et al. 2009a). Indeed, WCS417-ISR against the bacterial pathogen *P. syringae* and the insect *Spodoptera exigua* is associated with primed expression of the JA/ET-dependent genes *VSP2* and

PDF1.2, respectively (Van Wees et al. 1999; Hase et al. 2003; Pozo et al. 2008; Van Oosten et al. 2008). The specific priming of JA/ET-dependent defense responses during WCS417-mediated ISR fits with the dependency of ISR on JA/ET-dependent signaling pathways (Pieterse et al. 1998). Recently, Pozo et al. (2008) found an overrepresentation of MYC2 TF binding sites in the promoters of priming-responsive genes in ISR-expressing plants. MYC2 is a well-known player in JA-regulated signaling (Lorenzo et al. 2004). Mutant *jin1* which is impaired in MYC2 was incapable of mounting WCS417-ISR against *P. syringae* and *H. arabidopsidis*, indicating a central role for the MYC2 TF in WCS417-ISR (Pozo et al. 2008; Fig. 1). Also ISR induced by the beneficial fungus *Piriformospora indica* against *Golovinomyces orontii* in *Arabidopsis* was demonstrated to depend on MYC2 (Stein et al. 2008), supporting a role for MYC2 as an important regulator of priming during ISR induced by different microbes.

Besides priming of certain JA/ET-dependent responses, WCS417 also primes the plant to reinforce the cell wall at the site of pathogen attack. An enhanced deposition of callose-rich papillae is observed upon infection by the oomycete *H. arabidopsidis* in WCS417-pretreated plants (Van der Ent et al. 2008). In addition to forming a physical barrier for pathogen penetration, callose depositions are also considered to be a matrix for the accumulation of defense compounds such as H_2O_2, phenolics, and various proteins and glycoproteins with hydrolytic and antifungal properties (Zeyen et al. 2002). Priming for enhanced deposition of callose-containing papillae during WCS417-ISR in Arabidopsis is dependent on the ISR regulators MYB72, NPR1, and MYC2 (Pozo et al. 2008; Van der Ent et al. 2008, 2009b). Moreover, this ISR response is dependent on the phosphoinositide (PtdIns)- and abscisic acid (ABA)-dependent signaling components IBS2 and IBS3 (Van der Ent et al. 2009b), which were previously identified to be required for primed callose deposition induced by the priming agent *β*-aminobutyric acid (BABA; Zimmerli et al. 2001; Van der Ent et al. 2009b).

Priming of defense responses is also characteristic of other induced resistance phenomena. For instance, mycorrhizal fungi can prime plants for enhanced JA-regulated defense activation (Pozo and Azcón-Aguilar 2007). Insect herbivory can prime plants for a faster and stronger defense response to subsequent stresses in both systemic tissue (De Vos et al. 2006) and in neighboring plants by means of the production of volatile organic compounds (Engelberth et al. 2004; Ton et al. 2007). Although pathogen-induced SAR is accompanied by a large-scale transcriptional reprogramming of the cell (Maleck et al. 2000) and direct accumulation of PR proteins (Van Loon 1997), priming of certain defense responses also occurs during SAR (Cameron et al. 1999; Van Wees et al. 1999). While some PR proteins are thought to contribute to resistance because several of them possess antimicrobial activity (Van Loon and Van Strien 1999; Van Loon et al. 2006), direct activation of these *PR* genes alone seems not sufficient to explain the broad range of protection (Van Loon 1997). The fact that priming can be induced in various ways and has been observed in different plant species ranging from monocots to dicots, conferring protection against a wide variety of pathogens, insects, and abiotic stresses (Conrath et al. 2006) suggests that priming appears to be a common feature of the plant's immune system.

5 Mechanisms of Priming of Defense Responses

Although plants do not possess an adaptive immune system, priming may be considered as a form of immunological memory in plants. The molecular mechanisms underlying priming are the subject of research by us and other scientists. Since defense genes are not activated directly in primed plants, it was hypothesized that the primed state is based on accumulation or posttranslational modification of signal molecules that remain inactive until a subsequent stress stimulus is perceived (Conrath et al. 2006). Due to the enhanced level of signaling components, subsequent pathogen attack would lead to an increased activation of the appropriate defense pathway and thus to a potentiated activation of defense-related genes. TFs are plausible candidates to contribute to the onset of priming. Using reverse transcription quantitative polymerase chain reaction (RTq-PCR) technology, Van der Ent et al. (2009b) recently analyzed the expression of all putative *Arabidopsis* TF genes during WCS417-ISR. The expression of 121 different types of TF genes was induced or repressed, including induction of the *MYC2* gene, which had previously been identified as a player in priming during ISR (Pozo et al. 2008). Interestingly, the AP2/ERF (APETALA2/ETHYLENE-RESPONSIVE FACTORS) TF family was notably overrepresented among the upregulated TFs in ISR-expressing plants. MYC2 and several AP2/ERF TFs are implicated in the regulation of JA/ET-dependent defenses (Lorenzo and Solano 2005; Pré et al. 2008).

Also priming induced by the nonprotein amino acid BABA is accompanied by direct upregulation of TF genes (Van der Ent et al. 2009b). However, while several TFs that had previously been implied in regulation of JA/ET-dependent signal transduction pathways were upregulated during ISR, pretreatment with BABA induced the expression of 22 out of the 72 known WRKY genes in the *Arabidopsis* genome (Van der Ent et al. 2009b). WRKY TF genes have previously been implicated in the regulation of several SA-dependent defense-related genes (Dong et al. 2003). The BABA-responsiveness of a subset of the SA-regulated WRKYs is in agreement with BABA-induced priming of SA-dependent defenses (Zimmerli et al. 2000). WCS417- and BABA-induced priming is associated with induced expression of divergent sets of TF genes, which are in accordance with the defense responses that are primed by these inducers. However, the exact role of the priming-related TFs in the regulation of priming remains to be elucidated. Transcriptome analyses of pathogen-induced SAR expressing tissue demonstrated that TF genes are induced (Maleck et al. 2000; Wang et al. 2006), but it is unknown whether this is related to priming or to direct induction of defense responses.

There is no significant activation of defense-related genes during priming induced by treatment with WCS417 or BABA, which suggests that the accumulating TFs remain inactive until the perception of a subsequent stress signal. One way to activate TFs posttranslationally is via phosphorylation. For instance, phosphorylation of a bZIP TF is crucial for abscicic acid (ABA)-induced transcriptional activity (Kagaya et al. 2002). Interestingly, mitogen activated protein kinases (MPKs), which can phosphorylate proteins, were also recently shown to be primed

by low concentrations of the SA analog benzothiadiazole (BTH; Beckers et al. 2009). Inactive MPK3 and MPK6 proteins accumulated in response to BTH and only subsequent exposure to pathogens led to activation of these primed MPKs (Beckers et al. 2009). Epigenetic regulation forms another possible mechanism for the priming phenomenon (Bruce et al. 2007). An altered methylation status or modification of nucleosomal histones could ensure a more accessible chromatin structure for activation of TF genes or defense-related genes, which could facilitate a quicker or more potent transcriptional response to subsequent pathogen attack. The SA-dependent SAR response is documented to be associated with epigenetic regulation as well (Mosher et al. 2006). Recently, Jung et al. (2009) discovered that the metabolite azeleic acid seems an important signal molecule in the establishment of pathogen-induced SAR. Azeleic acid is translocated in the vascular sap from local pathogen-infected tissue to systemic tissue. Application of azeleic acid confers enhanced disease resistance in Arabidopsis which is associated with priming for enhanced accumulation of SA upon pathogen challenge (Jung et al. 2009).

6   Costs of Induced Defenses

While some defense compounds are constitutively present such as toxic compounds that form a pre-existing chemical barrier against pathogens (Osbourn 1996), others are expressed only upon attack by a pathogen or herbivore. The inducible responses can be subjected to priming. Two prominent hypotheses have been proposed to explain the spatial and temporal variation in plant defense. These are the optimal defense theory (ODT), which predicts that plant parts with high fitness value will be highly defended, and the growth-differentiation balance hypothesis (GDBH), which assumes that a balance must be maintained between resources used for growth and defense (Barto and Cipollini 2005). It is assumed that inducible defenses are too costly to be expressed constitutively under enemy free conditions. In agreement with this, the constitutive SA-dependent defense expressing *Arabidopsis* mutant *cpr1* (*constitutive expressor of PR genes 1*) is severely compromised in growth and seed production compared to wild-type plants (Bowling et al. 1994; Heidel et al. 2004; Van Hulten et al. 2006). This indicates a severe fitness penalty for the constitutive expression of SA-inducible defenses, which may explain why SA-dependent defenses are not expressed constitutively (Bowling et al. 1994; Heidel et al. 2004). In agreement with this, Heidel et al. (2004) observed that *cpr1* also displays a decreased fitness under field conditions, in spite of its enhanced resistance. Interestingly, in the same field experiment, SA-insensitive *npr1* mutants that are defected in plant defense exhibited a decreased fitness as well, suggesting that there is a delicate balance between the costs and the benefits of inducible plant defense. Mutants that constitutively express JA- and ET-dependent defenses, such as *cev1* (*constitutive expression of VSP1*), also exhibit undersized measures (Ellis and Turner 2001). Moreover, several studies have demonstrated a fitness reduction upon direct induction of defenses by exogenous application of SA or (Me)JA

(Baldwin 1998; Agrawal et al. 1999; Heil et al. 2000; Van Dam and Baldwin 2001; Cipollini 2002; Heidel et al. 2004).

Fitness costs can arise from various processes (Heil 2002; Heil and Baldwin 2002; Walters and Heil 2007). Allocation costs occur when limited resources are allocated to resistance traits and not to growth and reproduction. In agreement with this, several studies have reported that photosynthesis is repressed during pathogen infection, presumably to free resources needed for defense (Berger et al. 2007). One can predict that plants experience more costs of defense-related traits under low-nutrient conditions. Conversely, resistance levels may be impaired due to limiting resources. Both predictions have been empirically confirmed (Cipollini 2002; Dietrich et al. 2004, 2005).

Ecological costs occur when defense expression affects other organisms besides the challenging pathogen. Activation of certain defenses may for instance have a negative effect on interactions with plant-beneficial organisms, such as mycorrhizal fungi (Glandorf et al. 1997). Moreover, resistance against one pathogen may result in enhanced susceptibility to another pathogen or insect. There is ample evidence of cross communication between the SA and JA/ET defense pathways, which can act both synergistically or antagonistically (Reymond and Farmer 1998; Rojo et al. 2003; Bostock 2005; Beckers and Spoel 2006; Koornneef and Pieterse 2008; Pieterse et al. 2009). For instance, Spoel et al. (2007) recently showed that SA-mediated defenses triggered in *Arabidopsis* upon infection with *P. syringae* rendered the infected tissue more susceptible to *A. brassicicola*. Pathway crosstalk is thought to be a mechanism for fine-tuning defense responses by prioritizing which defensive strategy to employ to cope with the different organisms that (simultaneously) interact with the plant. Koornneef et al. (2008) demonstrated that timing of elicitation of SA and JA signaling pathways is crucial for determining which defense pathway to prioritize, suggesting that there is a window of opportunity during which JA- and SA-regulated defense responses can have cross-effects on organisms other than the attacker. Therefore, laboratory studies that concentrate on single plant-attacker combinations may not take all ecological costs into account. The challenge lies in unraveling the costs of defense mechanisms in a multitrophic environment, such as appears in nature.

7 Fitness Benefits of Priming Under Disease Pressure

While the inducibility of defenses may save resources under enemy-free conditions compared to constitutive activation of defenses, it also causes a time slot between attack and the expression of inducible defenses in which the plant is vulnerable to the attacker. Priming may be a mechanism to ease the trade-off dilemma between costly defense activation and effective protection against harmful organisms (Goellner and Conrath 2008), since primed plants do not activate defenses directly upon induction treatment but activate their defenses faster and stronger when subsequently attacked by pathogens or insects (Conrath et al. 2002, 2006).

Most studies on the costs and benefits of plant defense have concentrated on direct activation of defenses, rather than on priming. Moreover, putative benefits of defenses under disease pressure were often not taken into account (Walters and Boyle 2005). We recently demonstrated in Arabidopsis that priming induced by BABA has clear benefits under conditions of disease pressure (Van Hulten et al. 2006). In the absence of pathogens, priming had no or only marginal effect on the relative growth rate (RGR) and seed production of the plant, whereas there were large effects when defenses were directly activated. In the presence of pathogens, a clear fitness advantage was observed for primed plants over non-primed plants and also over plants in which defenses were activated already before pathogen challenge (Van Hulten et al. 2006). These results clearly indicate that under conditions of disease pressure, the benefits of priming outweigh the costs. In agreement with our study, Walters et al. (2009) recently demonstrated in barley (*Hordeum vulgare*) that priming induced by saccharin, a metabolite of the synthetic SAR-inducer probenazole, had no significant effect on plant growth rate and grain yield in the absence of pathogen infection. However, priming significantly increased plant fitness under high inoculum pressure by the hemibiotrophic fungus *Rhynchosporium secalis*. To take unforeseen ecological costs into account, putative fitness costs of saccharin treatment under natural conditions were also evaluated in a field study. Saccharin treatment increased grain yield slightly but not significantly compared to untreated controls, indicating that priming for enhanced defense responses induced by saccharin did not incur allocation costs in barley under field conditions (Walters et al. 2009).

The studies of Van Hulten et al. (2006) and Walters et al. (2009) are the only ones to date that describe the fitness costs and fitness benefits associated with priming of defense. In these studies priming had been induced either chemically or genetically by the *edr1* mutation, which affects a MPKKK (MPK kinase kinase) that is a negative regulator of SA-inducible defense responses in Arabidopsis (Frye and Innes 1998; Frye et al. 2001). No study has yet been designed to elucidate fitness effects of ecologically more relevant induction of priming, like that induced by beneficial micro-organisms. However, Raupach and Kloepper (1998) reported that in two field trials, conducted in separate years, seed treatment of cucumber with PGPR *Baccilus* spp. increased plant growth and reduced disease severity against different pathogens. In another field study, *Bacillus* pretreatment protected cucumber plants from cucumber beetles and the beetle-transmittable bacterial wilt disease (Zehnder et al. 2001). This was accompanied by significant yield increases. Also field-grown tomato plants were protected by *Baccilus* spp. against the cucumber mosaic virus and tomato mottle virus, which was associated with increased plant yield compared to untreated plants (Zehnder et al. 2001). An increase in yield was not observed during a similar field trial conducted in the consecutive cropping season, but viral titers were also not affected by the bacteria in this second year (Zehnder et al. 2001). These results demonstrate fitness benefits for plants that interact with PGPR. These interactions do not seem to incur costs on the plant. Although the priming phenomenon was not the emphasis of these studies, priming is often found to be the underlying mechanism of systemic plant protection by PGPR (Van Wees et al. 2008; Van der Ent et al. 2009a).

8 To What Extent are Plants in the Field Already Primed?

Priming has predominantly been studied under tightly controlled growth conditions with optimum temperature, light intensity, humidity, and nutrient availability for plant growth. In natural environments the above mentioned parameters could be less favorable for the plant. Furthermore, in nature plants interact with many organisms, such as pathogens, herbivores, other plants, and beneficial micro-organisms (Pieterse and Dicke 2007). All these parameters may exert an effect on the plant and consequently on the priming response. Conversely, under natural conditions the priming response may have different effects on plant fitness or the plant's ability to respond to stresses than under controlled conditions, because of the different environment of the plant. This way, effects of priming may become apparent that would not be detected under controlled greenhouse conditions. Studies on the fitness consequences of plants that exhibit a primed defense state in the field will be instrumental to better understand the ecological impact of priming of defense responses. In earlier studies priming-inducing agents have shown to protect plants in the field (Beckers and Conrath 2007; Goellner and Conrath 2008). However, in these studies, the primed state was not verified, which is crucial to impute any protective effect of the agents to a primed state because most chemical priming agents can also activate defenses directly when applied at higher doses (Kohler et al. 2002; Van Hulten et al. 2006). Therefore, from these field studies, it cannot be concluded that the induced protection is caused by priming because contribution of direct activation of defenses can not be excluded. Also in field studies with PGPR and PGPF that can induce resistance through priming of defenses, additional protective mechanisms can not be excluded because many of these beneficials are capable of exerting direct effects on pathogenic organisms through competition for nutrients or production of antibiotics.

Since priming can be induced by a plethora of organisms, whether beneficial or harmful, it is possible that plants in the field are already primed to some extent through their continual interaction with the biotic (and abiotic) environment. Walters (2009) recently suggested that in several published field studies, defenses had already been induced in plants to some extent prior to induction treatment. For example, treatment of field-grown wheat with BTH, a functional analog of SA, did not induce SAR-related genes, which may be due to the already high expression levels of these genes before treatment (Pasquer et al. 2005). However, Herman et al. (2007) reported that tomato plants in the field responded to treatment with ASM, a functional analog of SA, with significant induction of defense-related gene expression, despite their enhanced basal level of expression in the field compared to in the greenhouse. Moreover, a second treatment with ASM resulted in a significantly stronger expression response relative to non-pretreated plants. This latter finding implies that although plants may be already primed, this does not compromise their ability to express even higher levels of induced resistance upon subsequent induction.

Priming of defense responses is an inducible phenomenon, indicating that by default the plant is in a noninduced or nonprimed physiological state. It would be

interesting to investigate whether plants that grow in their natural habitat, in which they coevolved with indigenous micro-organisms and are well adapted to the local environment, have naturally acquired the primed state. For this purpose, proper controls need to be included (e.g., mutant plants that are affected in priming only) and markers strictly correlated with priming should be assessed. These tools are not known at the moment.

9 Outlook

In recent years, knowledge on resistance induced by beneficial root-colonizing microbes has greatly expanded. There is ever-growing information available on microbes with resistance-inducing activity, plant species that are perceptive to resistance-inducing microbes, and pathogens/insects to which the induced resistance is effective. The picture is emerging that the plant defense signaling pathway that is triggered by ISR-inducing beneficials depends on responsiveness to the plant hormones JA and ET. ISR is not accompanied by massive changes in gene expression. Instead, ISR is established through priming the plant for enhanced defense responses upon encountering a pathogen or insect. Priming seems to be a cost-efficient defense mechanism. Hence, beneficial microbes-induced priming may be an ecologically relevant feature of plants.

Priming by beneficial microbes may be a valuable tool for sustainable crop protection. For effective use of priming agents in agriculture, it is critical to investigate whether and to what extent the specific crops in the field may be already primed by their interacting environment and whether this could be further enhanced by application of priming agents. However, the fact that JA-dependent ISR and SA-dependent SAR can have additive effects (Van Wees et al. 2000) implies that different defense pathways can be primed simultaneously, leaving room for enhancement of naturally primed defenses. PGPR can present an attractive alternative to chemical pesticides for protection against pathogens and insects. A major advantage of PGPR is that once systemic resistance is induced, the natural defense mechanisms of the plant are operative for prolonged periods (Van Loon et al. 1998). However, complete disease control is rarely provided by resistance-inducing agents. Research aimed at determining the factors that influence the success rate of the PGPR is necessary for broad implementation of biocontrol agents that consistently provide acceptable levels of disease control in crop protection programs. Besides inducing of resistance PGPR can also directly control soil-borne pathogens, through competition of nutrients or production of antibiotics. Therefore, (a combination of) PGPR strains that can protect plants through different mechanisms are ideally suited to confer consistent, long-lasting protection of crops against various diseases and pests. Mixtures of PGPR strains have indeed been shown to enhance biological control in cucumber and radish against different pathogens compared to the effect of single treatments (Raupach and Kloepper 1998; De Boer et al. 2003).

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