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Plant Defence: Biological Control

Second Edition

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Biological control of pests, weeds, and plant and animal diseases utilising their natural antagonists is a well-established but rapidly evolving field of applied ecology. Despite its documented applications and systematic development efforts for longer than a century, biological control still remains a grossly underexploited method of pest management. Its untapped potential represents the best hope to providing lasting, environmentally sound, and socially acceptable control of most problem pests in agriculture, and of invasive alien organisms threatening global biodiversity. Based on the overwhelmingly positive features of biological control, it is the prime candidate in the search for reducing dependency on chemical pesticides. Public demand for finding solutions based on biological control is the main driving force in the rapid developments in the various strategies of utilising natural enemies for controlling noxious organisms. This book series is intended to accelerate these developments through exploring the progress made within the various aspects of biological control, and via documenting these advances to the benefit of fellow scientists, students, public officials, and the public at large. Each of the books in this series is expected to provide a comprehensive, authoritative synthesis of the topic, likely to stand the test of time.

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Plant Defence: Biological Control

Second Edition

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Preface to Second Edition

We are pleased to present the second revised edition of *Plant Defence: Biological Control*. Production of a second edition in itself is evidence that the first one was a great success. The work on biological control agents is preferred due to their environmental friendly role. Firstly, the use of biodegradable and eco-friendly control agents is a necessity as continuous long-term use of chemical pesticides has detrimental effect on soil health and consequently residue present in plants has long-term effect on human health. Secondly, search for safe alternative agents like biological control of diseases still continues. This endeavour this is a timely compilation of state-of-the-art information dealing with various aspects of biological control agents. The present book is organised into four parts: I. Biology of Plant Defence, II. Use of Natural Compounds, III. Use of Biological Agents, and IV. Market and Commercialization. Most of the chapters are new and a few are updated.

The book will be useful to upper students studying crop protection, agricultural sciences, applied entomology, plant pathology and plant sciences. Biological and agricultural research scientists in biotechnology, forestry, plant pathology and post-harvest technology, crop management and environmental sciences, agrochemical and crop protection industries, and in academia will find this book very useful. The editors wish to thank all contributors as well as the staff at Springer for their cooperation in completion of this book.

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Preface to First Edition

Approximately 6.6 billion humans inhabit the Earth. Notably, the human population has grown nearly tenfold over the past three centuries and has increased by a factor of four in the last. Therefore, demand for food, feed and fodder is ever increasing.

Plant diseases worldwide are responsible for billions of dollars' worth of crop losses every year. Productivity of crops is at risk due to the incidence of pests, pathogens and animal pests. Crop losses to pests can be substantial and may be reduced by various control activities. Estimates of crop loss are available for major food and cash crops at the world level. Among crops, the total loss potential of pests worldwide varies from 25 to 40%. Globally, enormous crop losses are caused by plant diseases, which can occur from the time of seed sowing to harvesting and storage. Important historical evidences of plant disease epidemics are Irish Famine due to late blight of potato (Ireland, 1845), Bengal famine due to brown spot of rice (India, 1942) and Coffee rust (Sri Lanka, 1967). Such epidemics have left their effect on the economy of the affected countries and deep scars in the memory of human civilization.

Plant diseases, caused primarily by fungal and bacterial pathogens, cause losses to agricultural and horticultural crops every year. These losses can result in reduced food supplies, poorer quality of agricultural products, economic hardship for growers and processors, and ultimately higher prices for consumers. For many diseases, traditional chemical control methods are not always economical nor are they effective, and fumigation as well as other chemical control methods may have unwanted health, safety and environmental risks.

Biological control involves use of beneficial microorganisms, such as specialised fungi and bacteria, to attack and control plant pathogens and diseases they cause. Biological control offers an environmental friendly approach to the management of plant diseases and can be incorporated into cultural and physical controls and limited chemical uses for an effective integrated pest management system. Due to the high cost of synthetic pesticides and concerns over environmental pollution associated with the continuous use of these chemicals, there is a renewed interest in the use of botanicals and biological control agents for crop protection. Benefits and risks are always associated with new technologies and their utilization. These types

of considerations have encouraged microbiologists and plant pathologists to gain a better knowledge of biocontrol agents to understand their mechanism of control and to explore new biotechnological approaches to induce natural resistance. This book provides a comprehensive account of interaction of host and its abiotic stress factors and biotic pathogens and development of biological control agents for practical applications in crops and tree species from temperate to subtropical regions. The contents are organised into the following parts:

• General Biology of Parasitism
• Applications of Biological and Natural Agents for Disease Resistance
• Host Parasite Interaction
• Mechanism of Defence

The chapters have been written by well-known researchers in their field.

The book is primarily designed for use by upper undergraduates and postgraduates studying crop protection, agricultural sciences, applied entomology, plant pathology and plant sciences. Biological and agricultural research scientists in biotechnology, forestry, plant pathology and post-harvest technology, crop management and environmental sciences, agrochemical and crop protection industries, and in academia will find this book very useful. Libraries in all universities and research establishments where agricultural and biological sciences are taught should have multiple copies of this very valuable book on their shelves. The editors wish to thank all contributors as well as the staff at Springer for their cooperation in completion of this book.

Bordeaux, France   Jean-Michel Mérillon

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1.1 Introduction

A major challenge of humankind is to feed the increasing human population in a sustainable manner. If left uncontrolled, herbivorous pests and pathogens can be highly destructive to crops causing significant yield losses, often above 30% [1, 2]. Pesticide application, an important component of the so-called Green Revolution, remains currently the most common method to control key pests and pathogens of

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crops, despite being incompatible with current regulations(e.g. Directive 2009/128/EC) that promote the reduced input of pesticides and the use of non-chemical methods in crop production, a global trend driven by a strong demand for agricultural products with reduced load of chemicals that also contribute to the increasing levels of pesticide resistance in populations of crop pests.

To limit environmental impacts of harmful pesticides and improve agricultural sustainability, a conversion to a new green movement is required [3] taking into account the complexity of the ecological nature of the problem. Novel strategies, complementary and/or alternative to the existing ones are required to control pests and pathogens in the most efficient and environmental-friendly manner. A growing emphasis on biological control tools such as the use of beneficial organisms and/or environmentally friendly (non-GMO) molecular tools is necessary to overcome technical challenges that are crucial in food production and pest/disease control. This has to be achieved with an approach to minimize environmental risks.

To this end, we herein focus on biological control and the theoretical framework underlying plant defense responses against biotic stressors such as herbivorous arthropods and pathogenic microorganisms with the aim to identify biological and relevant molecular tools that could be used to combat harmful key pests and diseases of crops. We further focus on beneficial soil microbes and zoophytophagous predators and present solid evidence about their potential in plant defense induction and in sustainable crop protection. Molecular tools that could be exploited in agriculture are addressed in light of the mechanisms involved in positive interactions among beneficial organisms and plants, resulting in the production/activation of chemicals such as peptides, toxins, anti-digestive compounds and secondary metabolites (e.g. volatiles). In addition, we refer to the development of molecular biopesticides based on RNA molecules designed to selectively downregulate genes involved in pathogenicity of pests and pathogens through RNA interference (RNAi). This chapter ends with a special section on endophytic fungi as a case study of beneficial microbes that display both plant growth promoting and plant protection capabilities.

1.2   Basal Plant Defenses Against Arthropods and Pathogens

To cope with pathogens and herbivorous pests, plants have evolved sophisticated defense mechanisms broadly classified as passive or constitutive and active or inducible (Fig. 1.1). Passive or constitutive defense mechanisms are constitutively expressed and provide protection from initial invasion or attack [4–6]. Against pathogens, these may include physical barriers, such as wax layers [7], cuticle [8] and cell wall [9], as well as preformed chemical compounds with antimicrobial (generically called phytoanticipins) and lytic effects [4, 10]. If these preformed barriers are overcome, pathogens can still be confronted by inducible host plant defense mechanisms, which prevent further colonization or pathogen spread [4]. Similarly, arthropods are confronted with an array of constitutive and/or inducible plant
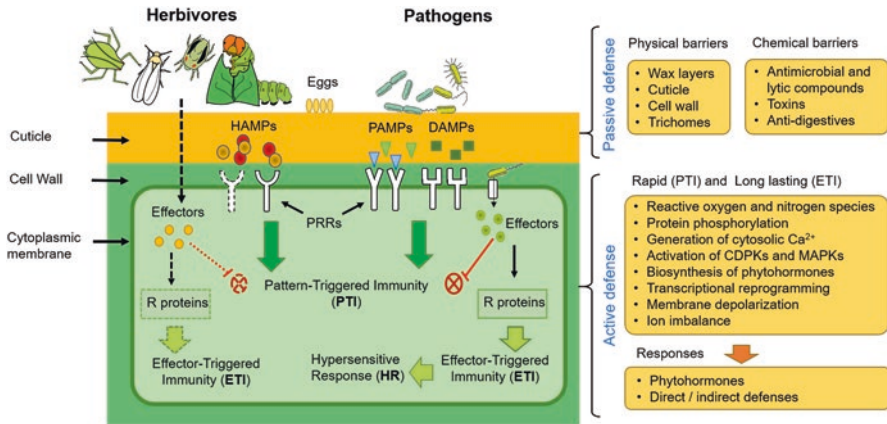
Fig. 1.1 Global overview of plant defense responses against herbivores and pathogens. Herbivore-, pathogen- or damage-associated molecular patterns (HAMPs, PAMPs and DAMPs, respectively) are recognized by pattern recognition receptors (PRRs) and lead to pattern-triggered immunity (PTI). Effector-like molecules from herbivores and pathogens can suppress PTI and result to effector-triggered susceptibility (ETS). The recognition of these molecules by plant resistance proteins (R proteins) lead to effector-triggered immunity (ETI) that, in the case of pathogens, often culminates in hypersensitive response (HR). Uncharacterized elements are indicated by dashed lines. Defense mechanisms (passive and active defense) operating during herbivore attack and pathogen infestation are indicated on the right

defenses such as physical traits (trichomes, wax layers, etc.) and chemicals (toxins, anti-digestive compounds, secondary metabolites) that aim at killing, deterring or retarding the population growth of pests [5]. Plants can also defend themselves indirectly by emitting volatile compounds that attract the natural enemies of herbivores [11, 12]. As with pathogens, inducible plant defenses against herbivores are initiated upon recognition of the attacker and downstream activation of defense signalling [13, 14]. Compared to constitutive defenses, induced plant responses are considered to be cost-saving, preventing auto-intoxication and more advantageous as they can be tailored to the attacker after specific cues recognition by the plant [15–17].

1.2.1 Pathogen Perception by Plants and Defense Induction

The first defensive line of plant immunity relies on the perception of pathogen- or damage-associated molecular patterns (PAMPs and DAMPs, respectively) by receptors called pattern recognition receptors (PRRs) localized on the plant plasma membrane [18] (Fig. 1.1). All plant PRRs identified to date belong to receptor-like kinases (RLKs) or receptor-like proteins (RLPs) [19]. RLKs are proteins with an extracellular domain involved in the perception of signal molecules (*i.e.*, PAMPs/DAMPs), and additionally of a transmembrane domain and an intracellular kinase

domain, which amplify or transduce these signals into the cell, respectively [20]. RLPs have a similar structural organization but lack the intracellular kinase domain [20]. Recent studies suggest that sensing of PAMPs/DAMPs could be also happening through membrane lipids [21]. PAMPs comprise a diverse array of structural components of the pathogen, such as bacterial flagellin, fungal cell wall-derived chitins and glucans, as well as pathogen-specific lipopolysaccharides, proteins, peptidoglycan, elongation factors (*e.g.*, EF-Tu) or microbial nucleic acids [19, 20, 22]. DAMPs are molecules of plant origin released upon pathogen-induced cell damage, and include mainly cell wall or cytosolic proteins, peptides, nucleotides, and amino acids [23].

The recognition of PAMPs/DAMPs by PPRs can activate the immune plant response, a process collectively termed 'pattern-triggered immunity' (PTI) [24]. In this process, a complex network of signalling events is activated, leading to a series of cellular and physiological responses. Such signalling events include, for instance, the rapid generation of cytosolic Ca^{2+} and reactive oxygen species (ROS) or reactive nitrogen species, ion efflux, protein phosphorylation, activation of Ca^{2+}-dependent protein kinases (CDPKs) and mitogen-activated protein kinases (MAPKs), increased biosynthesis of phytohormones, and transcriptional reprogramming [20, 25]. This complex signaling network leads to the establishment of a number of plant defense responses, such as plasmodesmata closure to inhibit molecular exchanges among cells, stomatal closure to limit pathogen entry, production of antimicrobial compounds (*e.g.*, phytoalexins) and generation of ROS either to signal downstream defenses or inhibit growth of pathogens, callose deposition to provide a physical barrier for pathogen attacks, and accumulation of pathogenesis-related proteins such as lytic enzymes (chitinases, glucanases, and proteases) [20].

In general, PTI is sufficient to fight off most pathogens, in particular host non-adapted pathogens [18]. However, some pathogens have developed strategies to evade PTI and for these, plant initiates a second layer of inducible defense, termed as Effector-Triggered Immunity (ETI), resulting in an incompatible reaction [26] (Fig. 1.1). In general, ETI activation results from the intracellular recognition of pathogen effector molecules by plant resistance proteins (R proteins) [26]. These effectors, synthetized by the pathogen and injected into the host cell cytosol, have an important function in pathogenesis [27]; some enhance pathogen virulence and suppress PTI, while others aid pathogens to propagate on their host by reprogramming host cell metabolism and physiology, causing effector-triggered susceptibility (ETS) [27]. Plants, in turn, recognize these effectors by receptor R proteins in a specific manner [28]. Recognition by R proteins can be mediated either through direct physical interaction with the effector (ligand-receptor model) or indirectly by detecting modifications on other host proteins caused by effector activity (guard model) [29]. Most of the R proteins identified so far belong to the nucleotide binding leucine-rich repeat (NB-LRR) type [26]. In comparison with PTI, ETI is a stronger and more efficient response, and often culminates in hypersensitive response (HR), a type of programmed cell death that limits the spread of the pathogen from infection sites [24]. Several studies suggest that ETI utilizes the same defense signalling network as PTI, but in distinct ways, emitting stronger and longer-lasting

responses [29]. In general, ETI restores and amplifies PTI basal transcriptional programs and antimicrobial defences [24]. Both PTI and ETI can induce immune responses against pathogens on uninfected distal tissues [30]. Among the diverse chemical signals identified so far, the plant hormone salicylic acid (SA), has been found to play an important role in systemic resistance that provides broad spectrum and long-lasting protection to future infections [30]. Establishment of systemic resistance involves the generation of signals in the damaged tissue, and their further transport via vascular system to sites further from the injury location.

1.2.2 Herbivore Perception by Plants and Defense Induction

Herbivore-associated molecular patterns (HAMPs) include all herbivore-derived signalling molecules that, when in contact with the host plant, are capable of eliciting defense responses [31, 32]. HAMPs can be elicitors deriving from the herbivores found in their saliva, regurgitant or other secretions such as honeydew and those used for eggs attachment to the plant surface [33–35]. Furthermore, plant-derived DAMPs such as cell wall fragments, or endogenous compounds released upon the disruption of plant tissue during herbivory can be responsible for the elicitation of non-specific plant defense responses [14].

Plants can detect herbivorous arthropods based on their HAMPs. These are presumed to be recognized by receptors leading to PTI [14, 36] (Fig. 1.1). Despite our vast knowledge on different types of PRRs involved in pathogen recognition by plants, to date only a few examples exist for PRRs involved in plant-herbivore interactions [32, 37]. As with the R-gene mediated recognition of effectors in plant-pathogen interactions, indications exist about the evolvement of similar recognition mechanisms underlying plant-herbivore interactions that may lead to ETI (Fig. 1.1); however, much less is known about such effectors and respective plant receptors [36, 37]. Polyphosphoinositides generated at the plasma membrane are believed to act as second messengers just as they do during pathogenesis [38]. Changes in the plasma membrane potential follow ion fluxes across the plasma membrane and afterwards, protein kinase cascades can activate ROS production such as hydrogen peroxide that can have direct effects on herbivores or change cell's redox status. The increase in cytosolic Ca^{2+} can also activate nitric oxide-mediated processes that precede phytohormone (JA) upregulation [39]. These responses occur not only locally but also in distal undamaged tissues. As with pathogens, a complex signalling network modulates the expression of defense-related genes and the production of defensive compounds that are active against herbivores [13]. The phytohormones jasmonic acid (JA) and SA, ethylene (ET) and abscisic acid (ABA) are key regulators in plant defense against herbivores, modulating the expression of defense-related genes and the production of defensive compounds [14, 40]. Cross-talk among the phytohormonal pathways (e.g. JA and SA antagonism) is considered to be fine-tuning plant defenses against specific attackers [41–43].

As with pathogens efficiently evading PTI, many arthropods have evolved a variety of strategies to cope with plant defenses including behavioural adaptations and mechanisms to decrease exposure (e.g. via detoxification or sequestration) or sensitivity (e.g. via target-site sensitivity) to defense compounds [34]. Furthermore, certain herbivores are known to be able to manipulate sink source flows or to suppress plant defenses [14, 34–36, 44]. Similar to pathogen effectors, effector-like molecules from herbivores, specifically those secreted via their saliva into the host plant are presumed to also interfere with PTI and lead to ETS [36, 37]. However, as with HAMPs and PRRs, our knowledge on herbivore effectors is still limited.

1.3   Plant Defense Priming

Plants are surrounded by multiple threats that they must face by responding effectively to survive. After specific attacker's recognition, plants need to re-organize all immune machinery to counteract the attack. The speed and intensity of the response will determine the final output. As described above, at first, plants may use constitutive defense barriers, and if those are not efficient enough, inducible defenses are activated to defeat pathogens and pests. To mount an efficient response, plants need to sense "the non-self". Different stimuli can prepare plants to gain these inducible defenses and set plants' immunity in a manner that they can respond in a shorter time and more efficiently to pathogen/pest attack [45, 46] (Fig. 1.2). Upon perception of appropriate stimuli ('sense of danger') different physiological and molecular changes, timely and quantitatively, prepare defenses for future attacks, resulting in incompatible interactions. Those changes taking place between the sensing of the stimuli and the presence of the challenge are known as the 'priming state' [46] (Fig. 1.2). During this phase, the plant adapts its immune responses by learning from experience.

Distinct stimuli may trigger the priming state, like beneficial organisms, arthropods, pathogens, and avirulent bacteria, as well as chemical compounds or even abiotic cues that may stimulate the production of active compounds. A silent timeframe comes until the challenge shows up (the 'priming phase'). Hence, when the plant is exposed to a subsequent stress, it is sensitized to respond faster and with higher intensity, and this is the so-called 'post-challenge primed state'. In this phase, there is an enhancement in the response following perception of danger and signal transduction. For example, sour orange citrus displays constitutive priming against the two-spotted spider mite *Tetranychus urticae* due to a high level of flavonoids and a faster activation of the oxylipin pathway [47].

Among the different stimuli, there are genes that confer constitutive priming. For instance, a mutation in the gene *NRT2.1* that functions as a transceptor in *Arabidopsis* confers constitutive priming against the pathogen *Pseudomonas syringae* pv tomato DC3000 [48]. The knockdown of *NRT2.1* allows a lower sensitivity to the toxin coronatine, preventing the plant from the effector manipulation. Another example of constitutive priming in *Arabidopsis* is generated by the mutant *edr1* (*ENHANCE*
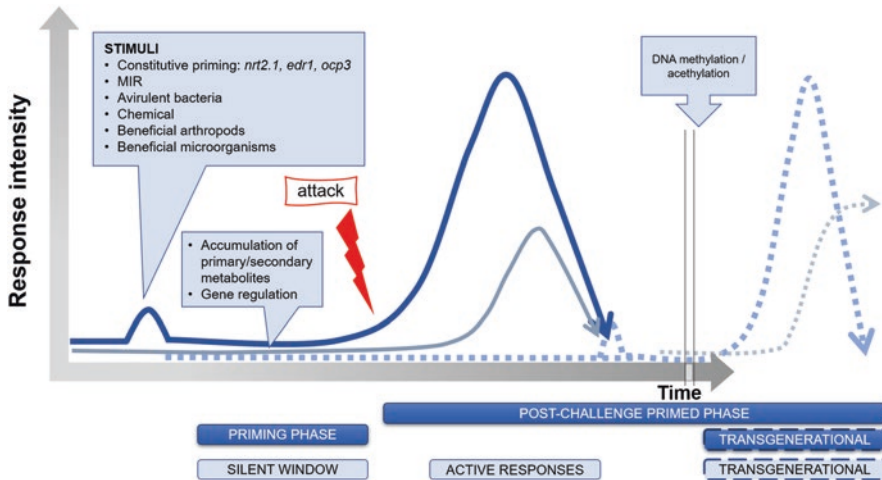
Fig. 1.2 Intervals of action in priming defenses. Different stimuli in plants can produce a transient and small response that tend to equilibrate afterwards. Priming inducers may range from biological (MIR, beneficial microorganisms and arthropods, avirulent bacteria) to chemical (BABA, I3CA) or genetic inputs (for example, downregulation of NRT2.1, OPC3 or EDR1). When plant defenses go to basal levels, a memory window lasts until the threat appears. This period is the so-called 'priming phase'. Along this phase, different players have been described, such as changes in primary and secondary metabolism, although this is dependent on the interaction between the priming inducer and the plant species. Then, after the attack of a pathogen/pest, the post-challenge primed phase starts. At this stage, primed plants (dark continuous blue line) respond faster and stronger to the challenge than non-primed plants (grey continuous line). Different mechanisms may orchestrate and coordinate a horizontal response to overcome the infection/attack. The intensity of the response in the long term depends on the interaction between plant -pathogen/pest- priming inducer, and may be associated with changes in the chromatin and histone modifications. Stressful memories can be transmitted to the offspring (transgenerational phase) through epigenetic modifications if the presence of the stress persists along time (blue dashed line corresponds to response intensity of plants that are still primed and grey dashed line, to the ones that have not been primed before). The dark blue squares show the names of the priming periods of priming and light blue squares show the type of defense responses ("silent", active responses or transgenerational)

DISEASE RESISTANCE1), also displaying priming of ROS and callose accumulation in response to PAMPs [45], and thus being more resistant to *P. syringae* and *Hyaloperonospora arabidopsidis* [49]. Additionally, the mutant *edr1* can also express constitutively two MAPK kinases MPK3–MPK6 that have been associated to priming [50].

Lack of activity of other genes may also confer constitutive priming. This is the case of the *OVEREXPRESSOR OF CATIONIC PEROXIDASE 3 (OCP3),* which mediates the response to necrotrophic pathogens and tolerance to abiotic stress [51, 52]. Mechanisms behind *OCP3* constitutive priming are the accumulation of ROS and the activation of the kinase cascade in a controlled manner, in which a positive interplay between ABA-JA and callose are key elements to mount defense priming. Interestingly, the *Arabidopsis* mutant *vtc1*, which is impaired in the production of

ascorbic acid, also shows constitutive priming of *PR1* and SA [53]. Thus, these genes may function as nodes that balance plant decisions relative to growth, abiotic stress tolerance or resistance to biotic insults. Loss of function mutants of these genes may be constitutively prepared for hyperactivation of defense responses without costs in plant fitness.

1.3.1 Mechanisms Regulating the Priming Phase

Despite the pre-challenge phase has been described in the past as uneventful and without fitness cost, now it is known to be associated with several molecular changes. Subtle changes during that phase may be translated into fitness cost, that it can be compensated by the final result when a threat appears [54]. A plant strategy during this "silent" phase (Fig. 1.2) is the accumulation of hormone and metabolite conjugates that will be hydrolysed to their active form upon a challenge. Following certain priming stimuli such as β-aminobutyric acid (BABA) and avirulent bacteria, the two main glycosylated forms of SA (SAG and SGE) are accumulated [55]. Other glucose conjugates of phytoanticipins also accumulate at this stage, such as the aliphatic and indolic glucosinolates [56] or benzoxazinoids [45], which are sequestered in the vacuole allowing their faster release upon pathogen/herbivore attack.

An open debate is whether changes and induced resistance by beneficial organisms may be mediated by defense priming [54] (TIPS). Among them, Arbuscular Mycorrhizal Fungi (AMF) were shown to protect a wide range spectrum of plant species against pathogen insults [57]. Reasonably, since AMF symbiosis and interactions with beneficial microorganisms take place before the challenge, there are obvious metabolic changes in the symbiont. Mycorrhiza-Induced Resistance (MIR) is a particular defense priming since in the priming phase, there is a whole molecular and metabolic dialogue between the plant and AMF leading to the symbiosis. In fact, priming during MIR is under consideration since it may be tissue dependent. MIR is effective against several root and foliar pathogens and current studies aim to elucidate the changes in the priming phase related to MIR.

Since carbon–rich compounds, amino acids and lipids are the main metabolites exchanged between AMF and the host plant, AM symbiosis is expected to impact primary metabolism. Several metabolites related to carbon metabolism were accumulated in AM-*Lotus japonicus* plants before challenge [58]. Tomato plants colonized by *Rhizoglomus irregularis* (formerly *Glomus intraradices)* showed enhanced OPDA content and up-regulation of *LOX-D* gene expression level in the priming phase [59]. Changes in the pre-challenge priming state usually targets the primary metabolism, such as sugar and amino acid pathways, not only in AM priming but also with other priming stimuli. Using qPCR and mutant approaches, an ABA-dependent regulation of starch degradation after BABA and I3CA priming was shown [60], and the sugar-derivative glycerol-3-phosphate has been reported as a key signal in the azelaic acid-induced systemic immunity and priming [61].

Amino acids are the precursors of many secondary metabolites that can participate in the subsequent defense responses. Pastor et al. [62] reported changes in *Arabidopsis* primary metabolism, mainly in tricarboxylic acid (TCA) metabolites such as citrate, fumarate, malate and 2-oxoglutarate as well as an enhanced biosynthesis of phenylpropanoid pathway following BABA priming before challenge. In the same study, authors compared changes occurring after BABA and *P. syringae* pv tomato (*PstAVRpt2*) priming treatment and found that pathways that were up-regulated after BABA priming were repressed after *PstAVRpt2* treatment. BABA is a water-soluble chemical compound that is rapidly distributed throughout the plant while the bacteria use the plant sensing mechanisms to coordinate the interaction between themselves and the plant. The different responses to these two priming stimuli recorded by the authors, highlighted that not only plant species but also the nature of the stimulus is important for the priming response. Hence, priming is a horizontal phenomenon that triggers multiple metabolic pathways shortly after infection/attack, resulting in enhanced defensive responses.

1.3.2 Mechanisms Regulating Post-Challenge Primed State: Internal and External Strategies

The spatiotemporal input of priming has been recently revisited as the 'internal' and 'external' strategies of plant defense [63]. As part of the internal plant defense responses, priming is a mechanism regulating the boosted defense reaction upon challenge along with systemic acquired resistance [46]. This internal response in primed plants, the so called 'post-challenge primed state', ranges from hours after challenge to longer period, which may also be extended to the progeny [46, 64, 65] (Fig.1.2). This transgenerational, epigenetically regulated defense priming may be fixed along evolution terms by genetic adaptations, leading to ETI. Conversely, defense priming regulates boosted responses during the external strategies that are based, on the one hand, on interactions with microbes at the root or shoot level that trigger the well-known induced systemic resistance [46, 66] (ISR) and, on the other hand, on recruitment of natural enemies, the so-called 'induced indirect defense'. During herbivory, VOCs are released within the first few hours after attack and attraction of natural enemies takes place at shorter term [67]. In a longer term, priming by beneficial microbes leads to the formation of disease-suppressive microbiomes [68, 69] that may protect plants through antibiosis, competition and induced resistance [70–72].

As regards internal strategies, several mechanisms were shown to be involved during the post-challenge priming state (Fig. 1.2). One of the first responses of primed plants after PAMPs perception is stronger production of H_2O_2, preceding an earlier and stronger callose accumulation [45]. Surprisingly, primed plants that are effectively protected by this battery of early responses do not trigger, or even down regulate, subsequent immune responses [73]. When the activation of subsequent

defensive layers is required, in addition to the biosynthesis of phytohormones that is costly and takes longer time, primed plants were also shown to target signaling cascades in a non-costly manner as a fast and strong immune response. For example, priming activates a subset of glycosyl hydrolases releasing active forms from inactive glycosylated hormones [48, 55, 74] while, Beckers et al. [75] defined an enhanced accumulation of non-active MPK3 and MPK6 in primed plants that were rapidly phosphorylated once the challenge was present triggering much faster *PR1*, *PAL* gene transcription and other SA-dependent responses. The accumulation of a specific set of secondary metabolites defined as the 'priming fingerprint' is described as one of the latest short-term responses of primed plants [76]. Primed defenses are defined as a horizontal plant response that is dependent on the plant-stress interaction. The range of mechanisms implicated in the long-lasting defense response entails an effort from the scientific community, and different laboratories are tackling the basis of mechanisms behind epigenetic changes and transmission of priming defenses to the offspring, against biotic and abiotic stress. Nevertheless, still further research is needed to gain knowledge in this area from the molecular level to higher scale for practical use in agriculture.

1.3.3   Transgenerational Priming State

As time following the 'post-challenge priming state' progresses, the direct, hormonal-regulated immune responses decay in intensity and epigenetic mechanisms start being more relevant [45, 46] (Fig. 1.2). One of the first reports describing chromatin remodeling as a long-term priming and SAR was proposed by Jaskiewicz et al. [77]. SAR-related priming was associated with relaxed density of the chromatin that increased methylation and acetylation of histones packing WRKY promoters. This histone modification leads to a faster gene transcription following a pathogen or herbivore attack and a subsequent faster and more efficient defense response. Following this pioneer publication, shortly after, increasing evidence of DNA methylation associated with heterochromatin [78] was shown to be involved in long-term priming [79]. In this latter work, the primed expression of WRKY and SA-dependent genes was regulated via the RNA-directed DNA methylation pathway. Later, transgenerational priming and SAR-associated priming were shown to be regulated in the progeny of primed plants by epigenetic changes [80, 81]. Noteworthy, transgenerational priming is not only functional in SA-dependent immune responses but also in JA-dependent defenses against insect attacks [82].

1.3.4   Induced Indirect Resistance

The so-called 'external strategies' of plants are long been known. The study of beneficial insects that are attracted by plants following herbivory can be useful in Integrated Pest Management (IPM) programs. Plants in response to HAMPs release HIPVs that improve the recruitment of beneficial arthropods [67, 83]. Importantly, external strategies of plants can be enhanced via priming, for example, when plants are exposed to appropriate stimuli. In fact, several interesting studies in phylogenetically distant plant species such as maize and citrus show similar outputs when susceptible plants are exposed to VOCs [84, 85]. Maize plants exposed to VOCs released by plants treated by caterpillar regurgitant were more efficient to mount effective defenses against *Spodoptera littoralis* [84]. In addition, maize plants primed with VOCs were more attractive to the parasitic wasp *Cotesia marginiventris* while control plants and plants only treated with VOCs did not result in a significant attraction. Similarly, mite-susceptible citrus genotypes can express resistance after priming by VOCs released by resistant citrus attacked by the spider mite *T. urticae*. VOCs-mediated priming results in enhanced resistance against spider mites and priming of JA-dependent responses [85]. Thus, priming against herbivores, either by stimulating direct (internal) or indirect (external) defenses, is another example of adaptive immune responses of plants [86, 87]. Notably, plants are not only able to be attractive to aboveground beneficial arthropods but also to beneficial microbes present in the rhizosphere [88]. It is well-known that plants exposed to phosphorous deficiency are more attractive to mycorrhizal fungi by the release of strigolactones at the very early stages of the mycorrhizal symbiosis, which at a later stage ends up in MIR that is also mediated via priming [57, 59].

1.4   RNA Interference in Plant Defense

In addition to the aforementioned strategies, plants have developed a powerful nucleotide sequence-specific defense mechanism based on RNA interference (RNAi). RNAi is triggered by double stranded RNA (dsRNA) molecules that are cleaved by DICER-LIKE (DCL) endonucleases into by 20–25-nt small RNA (sRNA) duplexes [89, 90]. One of the two strands of the occurring sRNA duplex associate with ARGONAUTE (AGO) effectors proteins and recognize (1) complementary mRNA for degradation or translational inhibition and (2) cognate DNA for methylation and heterochromatinization [91, 92]. In plants, a plethora of sRNAs regulate development, control genome stability, fine-tune epigenome plasticity, tame transposon activity and mediate pathogen defense [93–96]. Concerning the latter aspect, plant viruses having RNA or DNA genome generate through replication or transcription dsRNA intermediates which are processed by plant DCLs into sRNAs that target the viral RNA genome for degradation and viral DNA genome for methylation [97, 98]. Indeed, it has been proposed that RNAi mechanism in plants

has evolved as a major antiviral defense mechanism [93, 99]. Recently, it has been suggested that RNAi is also involved in antifungal defense, since plants send sRNAs into fungal pathogens in order to target essential fungal genes, as cotton does against *Verticillium dahliae*, Arabidopsis against *Botrytis cinerea* and wheat against *Fusarium graminearum* [100–102].

The tremendous gene silencing potential of RNAi has not skipped the attention of plant biotechnologists. During the last two decades, plant scientists have transformed a plethora of plants expressing dsRNAs against various viruses, fungi, oomycetes, insects, mites and nematodes, all resulting in very high levels of plant defense against each corresponding target [98, 103–108]. Common denominator in all these approaches was the use of a transgene consisting of an invertedly repeated cDNA that, upon transcription, would generate dsRNA molecules that would trigger RNAi against the selected target. However, since the use of transgenes, transgenic plants and genetically modified organisms (GMOs) in general have been met with considerable public and scientific concern, plant biologists have lately resorted to GMO-free RNAi approaches by simply exogenously applying dsRNAs and sRNAs inplants against various pests and pathogens using methods such as high-pressure spraying and trunk injection [109–111] (Fig. 1.3). RNAi-based biopesticides, consisting solely of dsRNA and/or sRNA molecules, could exhibit an extremely specific mode of action since they require only 21 nt homology with their target, thus aiming specific regions of specific genes in specific species, practically eliminating undesired off-target effects. Importantly, according to the 40th annual meeting of the Toxicology Forum, the exogenous application of RNA molecules pose no threat to human health even when present in diet [107]. Not surprisingly, the non-GMO, non-toxic and highly specific character of RNA-based tools has rendered them a vital importance in modern crop protection platforms [112, 113].

1.5 Exploiting Biological and Molecular Tools in Plant Defense

1.5.1 RNA-based Strategies Against Viruses, Viroids, Fungi and Insects

Viruses cause epidemics on almost all agronomical important crops, posing a serious threat to global food security and being responsible for yield losses roughly estimated to cost worldwide more than 30 billion USD annually [114]. Most plant viruses exhibit a single stranded RNA genome and replicate in plant cell cytoplasm through dsRNA intermediates, thus serving as targets for host RNAi machinery. Hence, a well-established strategy involves pre-treating of plants with dsRNAs/sRNAs designed to target specific viral regions (e.g. coat or movement protein) in order to resist imminent viral infection (Fig. 1.3). Indeed, leaf spraying and/or mechanical inoculation of RNAi molecules targeting viral sequences resulted in
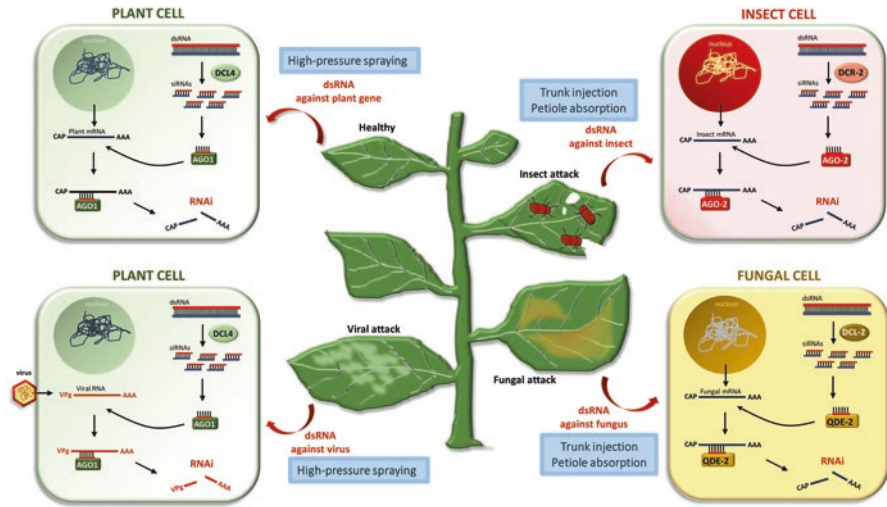
Fig. 1.3 Transgene-free RNA-based molecular control tools in plant defense involve the exogenous application of in vitro and/or in vivo transcribed dsRNA molecules in plants with the objective to trigger RNAi against (1) plant/weed genes, (2) viruses/viroids, (3) fungi/oomycetes and (4) insects/mites. In cases (1) and (2), the exogenously applied dsRNA needs to be efficiently taken up by the plant cell in order to be processed by plant DCLs into siRNAs that will target for degradation the corresponding transcripts in the cytoplasm. To achieve efficient delivery inside the plant cell, the dsRNA needs to be applied by high-pressure spraying which allows the mechanical disruption of the plant cell wall. In cases (3) and (4), the exogenously applied dsRNA is supposed to trigger RNAi not inside the plant cell but inside the fungal and/or insect cell. To increase RNAi efficiency inside the fungal and insect cells, the applied dsRNA needs to avoid processing by plant DCLs and, instead, be processed solely by the fungal or insect Dicers into siRNAs which will target the corresponding fungal or insect mRNAs for degradation. To achieve this, the exogenous dsRNA needs to be applied by trunk injection and/or petiole absorption, since by these two methods the dsRNA is transported exclusively through the plant xylem and apoplast (where no plant DCLs are present) to distant tissues and are thus accessible to be taken up by the plant tissue-penetrating fungi and by the chewing and/or xylem sap-feeding insects. However, trunk injection and petiole uptake are not suitable in the case of phloem-sap feeding insects (e.g. aphids) since in that case the xylem-residing dsRNA would be inaccessible to them. In the latter case, high pressure spraying of dsRNA would be more advisable, since it allows the symplastic delivery of RNA molecules to systemic tissues. Image adopted by permission from Dalakouras et al. [110]. Copyright American Society of Plant Biologists

significant viral resistance (1) in *N. benthamiana* (against Pepper Mild Mottle Virus, Tobacco Etch Virus, Alfalfa Mosaic Virus, Tobacco Mosaic Virus), (2) in *N. tabacum* (against Tobacco Mosaic Virus, Potato Virus Y, Cucumber Mosaic Virus), (3) in *Cucumis sativus* (against Zucchini Yellow Mosaic Virus), (4) in *Vigna unguiculate* (against Bean Common Mosaic Virus), (5) in *Zea mays* (against *Sugarcane Mosaic Virus*), (6) in *Carica papaya* (against Papaya Ringspot Virus) and (7) in *Pisum sativum* (against Pea Seed-borne Mosaic Virus) [115–124]. Closely related to viruses are viroids which are non-encapsidated, non-coding, circular, single stranded RNA pathogens [125]. Similar to antiviral applications, mechanical inoculation in

Solanum lycopersicum, *Gynura aurantiaca* and *Dendranthema grandiflora* leaves of dsRNAs targeting regions of potato spindle tuber viroid, citrus exocortis viroid and chrysanthemum chlorotic mottle viroid, respectively, resulted in considerable resistance of these plants to the corresponding viroids [126].

Fungal pathogens are responsible for devastating crop diseases worldwide. According to a *Molecular Plant Pathology* survey, the 'top 10' fungal plant pathogens list includes, in rank order, *Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp., *Fusarium graminearum*, *Fusarium oxysporum*, *Blumeria graminis*, *Mycosphaerella graminicola*, *Colletotrichum* spp., *Ustilago maydis* and *Melampsora lini* [127]. It is thus of utmost importance that novel, sustainable-but-effective tools are developed against these pathogens. RNA-based approaches could play here a foremost role as well (Fig.1.3). However, as precondition, it needs to be ascertained that the target-fungus under consideration contains an active RNAi machinery; notably, *Saccharomyces cerevisiae* and *Ustilago maydis* lack RNAi components and thus cannot serve as targets for RNA-based approaches [128]. Nevertheless, most fungi do encode DCLs and AGOs and even RNA-dependent RNA polymerases and are thus susceptible to RNAi. Indeed, exogenous application of RNAi molecules in (1) *Hordeum vulgare* (against *Fusarium graminearum*), (2) *Triticum aestivum* (against *Fusarium asiaticum*), (3) *S. lycopersicum* (against *B. cinerea*) and (4) *Brassica napus* (against *Sclerotinia sclerotum*) compromised fungal infection in these plants [129–132].

But perhaps the most important implications of exogenous RNAi reside in insect management (Fig. 1.3). Similar to antifungal approaches, the applied RNA needs to be delivered inside the insect cell. Yet, this is not as straightforward as it may seem. The uptaken (by the insect) RNA needs to survive the salivary nucleases in the midgut and haemolymph, absorbed by epithelial cells and systemically spread in order to trigger homogeneous RNAi of an essential gene throughout the insect body. Yet, despite these negative prospects, such a task is indeed feasible. Thus, (1) when dsRNA designed to target arginine kinase of *Diaphorina citri*, *Bactericera cockerelli* and *Homalodisca vitripennis* was injected in the trunk of *Citrus aurantifolia* and *Vitis vinifera*, it suppressed the corresponding pest populations [133]. Similarly, pest mortality was observed when (2) sRNAs targeting the *Plutella xylostella* acetylcholine esterase were sprayed in *Brassica oleracea*; (3) dsRNA targeting the *Diabrotica virgifera* vacuolar ATPase was applied *in S. lycopersicum*; (4) dsRNA targeting *Nilaparyata lugens P450* was root-absorbed by *Oryza sativa* roots; and (5) dsRNA targeting the *Tuta absoluta vacuolar ATPase* was absorbed by *S. lycopersicum* petioles [126, 134–137]. The prevailing assumption is that coleopterans are the most susceptible to exogenously applied RNAi, while lepidopterans and hemipterans are significantly resistant to it, seemingly because lepidopterans restrict the absorbed dsRNA to endocytic compartments, and hemipterans inject nucleases into the plant tissue before feeding [138]. However, the use of liposomes, chitosan nanoparticles, cationic core-shell nanoparticles, and guanylated polymers promise to significantly increase dsRNA stability in such applications [139, 140]. Overall, RNA-based plant defense approaches are highly promising pest and pathogen

control methods, complementary to plant resistance strategies, such as induced defense and priming.

1.5.2 *Priming-based Biological Control and Induced Resistance: Applied Aspects*

Knowledge on priming during the last 5–6 years has grown exponentially and many published studies have paid attention to the mechanisms underlying this adaptive immune response [46, 63, 141]. Most studies focus on model plant species covering fundamental aspects of priming and, research in applied aspects of priming in common crops has received much less attention. Reasonably, since the availability of molecular tools in common crops is less abundant, most research data of priming in crops such as potato, wheat, barley, cowpea or citrus refer to yield improvement, disease phenotypes or pest resistance and sometimes, hormonal or metabolic imbalances during post-challenge primed state [46, 87, 142, 143].

Accordingly, our knowledge on the mechanisms underlying biocontrol priming in crops is scarce. In many cases, the application of the triggering priming agent, either a chemical or a beneficial organism, is reported not to display a benefit on crop growth, until a disease infestation or insect attack. In barley, it was shown that saccharin treatments did not increase plant growth, although primed plants increased grain yield in the presence of the fungus *Rhynchosporium secalis* [144]. Seemingly, plant colonization by AMF has rather variable outputs in terms of growth [145]. Despite these limitations, the low or non-existent benefits of priming sensing during the priming phase counterweights the benefits following disease or insect attack.

In semi-field experiments, priming triggered by mycorrhizal symbiosis was shown to be functional in potato against the herbivore *Trichoplusia ni* [142]. Although mycorrhization had no effects on potato growth, it effectively reduced larval weight that may be explained by enhanced JA-dependent responses. In studies on priming in citrus trees, sour orange rootstock was found to display constitutive priming against spider mites [47, 85]. Interestingly, rootstock resistance is transmitted to the scion, therefore these findings can be applied to commercial varieties to stimulate plant immunity in the field. Another unexplored field aspect is the improvement of IPM strategies by using citrus plants that are more attractive to natural enemies. Recently, it was shown that sour orange recruits more efficiently the generalist predatory mite *Euseius stipulatus* that may improve the efficiency of pest control in agriculture [146]. Priming has also been shown in a context of treatments with natural extracts such as mint volatiles that were proven to confer enhanced defenses in field trials on soybean against both the herbivore *Spodoptera litura* and the fungus *Phakopsora pachyrhizi* [147]. Therefore, defense priming known as 'green vaccination' has been proposed as the perfect match to IPM strategies which, following appropriate field experimentation, could be transferred to applied science [148].

1.5.3   Priming Induced by Beneficial Organisms

Beneficial microbes belonging to the rhizosphere are known to induce resistance against a broad spectrum of pathogens and pests. Root-associated microorganisms that colonize root surfaces, or those that may enter the host tissue, can also sensitize plants against aboveground pathogens or pests systemically, via ISR [66, 149–151]. The rhizosphere contains the major part of the microbiota of plants, and part of the microbial community is involved in plant growth stimulation via plant growth-promoting microorganisms (PGPM) and in boosting the plant immune system thus, impacting plant health [152–154]. Best known beneficial microorganisms include different phyla of the bacteria *Actinobacteria, Proteobacteria* and *Firmicutes* [152, 155] and fungi, such as Ascomycota (*Trichoderma* sp.) and Glomeromycota (AMF) [156–158].

The interaction of microorganisms in the rhizosphere with plant roots is plant-microbe dependent [152]. The establishment of mutualistic symbiosis with mycorrhizal fungi is fine-tuned by the plant, which controls the recruitment and the entrance of the fungi [159]. On the contrary, *Trichoderma* fungi exert nutrient competition, or mycoparasitism in the rhizosphere [160]. Also, *Trichoderma* induce ISR through volatile compounds in the shoots against pathogenic fungi, priming JA responses [161]. The mechanisms behind this sort of induced resistance are SA-independent. Instead, they use the JA/ET dependent signaling to combat aerial attacks, with the overaccumulation of the AP2/ERF family of transcription factors (TF), which has been demonstrated to participate in the regulation of ET/JA-dependent defences [162]. The TF *MYC2* also plays an important role in ISR, since it was discovered to bind in a common site found in ISR-primed genes in *Arabidopsis* [163]. Experiments with *myc2* mutants showed that *Pseudomonas fluorescens* WCS417r and *Piriformospora indica*, two beneficial root-associated microorganisms inducing ISR, were unable to induce resistance against *P. syringae* and *H. parasitica*, pointing to this TF as an essential element in ISR.

Additionally, certain *Fusarium* fungi may be useful for the biocontrol of soil-borne microorganisms and herbivorous pests. For example, *Fusarium solani* strain K (FsK) is a root-restricted endophytic fungal isolate that colonizes tomato roots [164]. In tomato, FsK can confer ethylene-dependent resistance against fungal root and foliar pathogens [164]. FsK-colonized plants were recently shown to be more resistant to plant damage caused by the zoophytophagous predator *Nesidiocoris tenuis*, possibly via the JA and/or ethylene signaling pathways [165] and to the two-spotted spider mite, *T. urticae* [166]. FsK-colonization of tomato plants was shown to result in differential expression of defense-related genes as well as volatile emission in response to spider mite feeding. Notably, FsK colonized plants were more attractive to *Macrolophus pygmaeus*, a natural enemy of spider mites [166]. In addition, certain strains of the soil-borne *F. oxysporum* were shown to be efficient in controlling *V. dahliae* in eggplant through SA-dependent responses increasing the expression of *PR1* [167]. The efficacy in protecting plants by this fungus has been also shown in olive and pepper plants against *V. dahliae* and *Phytophthora capsici,*

by the induction of *PR1* gene among others [168, 169]. Interestingly, the strain *F. oxysporum* 47 (Fo47) could not protect these plants from foliar infection by *B. cinerea*. Perhaps the induction of SA in plants colonized by Fo47 blocks other defenses that influence other diseases. This fungus may act at several levels like the production of VOCs, plant growth promotion, antibiosis and mycoparasitism *in vitro*, induced resistance, also by competition at the root site [170].

Other beneficial microorganisms that are emerging as potential biocontrol agents, are strains belonging to the *Rhizobia* genus. Traditionally, this genus has been considered an essential player in nitrogen fixation and uptake by the plant. Nevertheless, evidence suggests additional roles in plant defense regarding root diseases. *Rhizobium* bacteria can produce and release proteolytic enzymes and parasite fungi in the rhizosphere such as pathogenic strains of *F. oxysporum* [171]. Also, *Rhizobium leguminosarum* strain Rl was able to protect chickpea against the pathogen *F. oxysporum* f. sp. *ciceris* (Foc) [172]. This protection is also present against other microorganisms (bacteria, viruses) and nematodes, via ISR [173]. Additional responses like emissions of antimicrobial VOCs, siderophore production, competition and changes in volatile plant compounds are also contributing to plant defense by *Rhizobium* [173].

Besides beneficial soil microbes, zoophytophagous predators such as the mirids *M. pygmaeus, N. tenuis* and *Orius laevigatus* have been shown to induce plant defenses against herbivorous pests via their phytophagy [83, 174–178]. Exposing plants to *M. pygmaeus* negatively affected the performance of the two spotted spider mite *T. urticae* in tomato and the western flower thrips *Frankliniella occidentalis* in pepper [174, 175, 178]. These negative effects against pests were attributed to the increased accumulation of transcripts and the activity of proteinase inhibitors (PI) in the mirid-exposed tomato plants [175], and to the activation of the JA-related responses in pepper plants [178]. Furthermore, tomato and pepper plants exposed to *N. tenuis*werefound to be more attractive to predator conspecifics [179] and to the parasitoid *Encarsia formosa,* a biological control agent of whiteflies [176]. This indirect plant defense response was related to changes in the volatile blend released by the mirid-exposed plants, via the activation of ABA and JA signaling pathways [176]. Notably, mirid-induced plants were shown to be less attractive to key pests such as the tomato leaf miner *T. absoluta*, the whitefly *Bemisia tabaci*, the western flower thrips *F. occidentalis*and the two-spotted spider mite *T. urticae* [83, 176, 177, 180, 181]. Overall, the above studies suggest that zoophytophagous predators may serve as 'plant vaccination agents' at the early stages of the establishment of a crop directly affecting herbivores via predation and indirectly, via the induction of direct and indirect plant defense responses, eventually enhancing their overall biocontrol efficiency [174, 182].

Interestingly, zoophytophagous predators have been recently shown to positively interact with beneficial soil microbes to the benefit of their host plant. The colonization of tomato plants with a root restricted endophyte, the non-pathogenic stain FsK was shown to result in reduced feeding symptoms (necrotic rings on leaves and stems) by the zoophytophagous predator *N. tenuis* possibly via the upregulation of the ethylene and JA pathways [165], and to alter volatile blend emission by tomato

plants and enhance their attractiveness to *M. pygmaeus* [166]. Similarly, *M. pygmaeus* population growth was enhanced on tomato plants colonized by *Trichoderma longibrachiatum* that were also more attractive to conspecifics [183], and similar results were obtained for the AMF *Rhizophagus irregularis* [184]. Finally, inoculation of tomato plants with *Fusarium oxysporum* Fo162 was shown to enhance the efficiency of *M. pygmaeus* to control *T. vaporariorum*, possibly due to a shift in the feeding preference of the predator from plant- towards prey consumption [185]. Taken together, zoophytophagous predators engage in complex interactions with plants also involving beneficial soil microbes and the manipulation of innate plant defense responses. The outcomes of such interactions are currently shown to be positive in terms of plant protection. Further studies are required to understand underlying mechanisms and estimate field efficiency to be able to propose biocontrol strategies and management schemes involving zoophytophagous predators and microbe-inoculation in agricultural settings.

1.5.4   Chemical Priming

Most of chemical priming inducers are natural compounds isolated from challenged plants, or compounds mimicking the structures of natural immune inducers. They do not have *in vitro* antimicrobial activity, and target the main defense-related phytohormone pathways. The first chemical inducers of priming studied were SA and synthetic SA analogues such as 2,6-dichloroisonicotinic acid (INA) and thiadiazole-7-carbothioic acid (BTH). Both were shown to prime parsley cells to resist *Phytophtora sojae* [186]. Accumulation of SA is a common trait in SAR and mediates the activation of a set of pathogenesis-related (PR) genes. Mono- and dichloro substituted SA and fluoro-SA derivatives were found to induce PR proteins in tobacco against TMV infection [187, 188]. While SA regulates defense against biotrophic pathogens, JA and MeJA control mainly the immune responses against necrotrophic pathogens and herbivores. JA and several synthetic JA mimics have been shown to induce priming by activating JA signaling and defense responses in different plant species (reviewed by Zhou and Wang [189]). In most cases, when phytohormone analogues are used as priming agents, it is concentration that determines whether priming or direct defenses are displayed by the plant [49].

Besides the main phytohormones and their analogues, several chemical compounds such as BABA and Indol-3-carboxylic acid (I3CA) are known to prime the plants to cope with environmental and biotic stresses [190, 191]. Among these chemical inducers, BABA-IR has the widest protection spectrum; it has been shown to protect about forty plant species including mono- and dicotyledonous against several pathogens and pests, including viruses, Protista, bacteria, oomycetes, fungi and arthropods being effective in a wide range of applications (foliar spray, soil

drench etc.) [192]. Importantly, there are indications that BABA-mediated priming can reduce herbivores (aphids) growth without displaying direct negative effects on their parasitoids [193]. BABA-IR acts by potentiating defense mechanisms depending on the pathosystem [194]. Defense against *Plectosphaerella cucumerina* is mounted through an ABA-dependent signaling that contributes to callose accumulation, whilst defense priming against *P. syringae* pv *tomato* (*Pst*) is mediated by SA-dependent responses. Despite BABA-IR is known for almost 60 years now, it was only a few years ago when the receptor and the perception mechanism for BABA-IR was identified, being the *Impaired in BABA-induced Immunity 1* (*IBI1*) gene which encodes for an aspartyl-tRNA synthetase [195, 196]. A recent study has also identified BABA as an endogenous metabolite present in several plant species [197]. Studying the BABA-IR in *Arabidopsis* against *P. cucumerina,* Gamir *et al.* [191] described for the first time a common fingerprint of various priming stimulus within specific plant-pathogen interactions. In this study, I3CA was identified as one of the metabolites mediating BABA-IR. Further studies showed that I3CA was also capable to act as priming stimulus in *Arabidopsis* upon *P. cucumerina* by increasing ABA levels in the pre-challenge stage and enhancing callose deposition upon infection [60]. In addition, a series of secondary metabolites that were shown to mediate priming, can trigger defense priming on themselves, as is the case for pipecolic acid, dehydroabietal, imprimatins, azelaic acid and glycerol-3-phosphate among others [46].

Another class of chemical inducers are those that prime cells without targeting metabolism or a specific signaling pathway; this is the case of silicon as priming agent. Silicon does not react within the cell and its action is mainly based on its deposition within or between the cells, in the cell wall or as phytoliths [198]. Plants obtain Si as silicic acid ($Si(OH)_4$) from the soil and deposit it as silica which helps to construct mechanical barriers (phytoliths) and abrasive structures (Si-fortified leaf trichomes) to prevent insect feeding [199]. When *Spodoptera exempta* was fed on Si-treated grass they showed reduced insect growth rates and irreversible wear down of their mouthparts [200]; however, the exact mechanisms for Si-IR remain controversial. In addition to the physical benefits of silicon, systemic defense responses were recently shown to be stimulated following Si treatments (reviewed by Coskun et al. [201]). Perennial ryegrass grown in Si-amended soil showed increased papillae deposition and lignin-associated phenolic compounds against *M. oryzae* leading to a reduction of disease incidence and severity [202]. In addition, certain defense-related enzymes such as peroxidases (POX), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (POD) were increased in Si-treated rice (*O. sativa*) upon *Cnaphalocrocis medinalis* attack [203]. Hence, the current understanding of fundamental and mechanistic aspects of priming generate enough knowledge to design new sustainable technological tools that may be complementary to IPM improving the efficiency of crop protection.