

D6.4 Publications

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Beneficial Microbes to Optimize pest control in Sustainable Tomato production

BeMOST

HFRI-FM17-50

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Scientific area:	Agricultural Sciences – Food Science &
	Technology
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	1. University of Thessaly
Cooperative Organizations:	2. University Hohenheim
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Summary

D6.4 is a deliverable of WP6 which objectives are:

6.1. To develop and implement the plans for effective dissemination of the project, its activities and results employing a range of communication and dissemination tools;

6.2. To raise public awareness in the project aims and results;

6.3. To coordinate communication activities aiming at the scientific community and stakeholders.

In the context of the WP6 objectives, D6.4 reports on the publication of the project's scientific results in peer-reviewed journals, abstracts to conferences and technical journals. Publications to journals and abstracts are attached to the Annex.





Publications

During the project, BeMOST members contributed to the following publications.

Publication #1	
Title	Pappas M.L., P. Baptista, G.D. Broufas, A. Dalakouras, W.
	Djobbi, V. Flors, M. Msaad Guerfali, S. Khayi, R. Mentag, V.
	Pastor, J.A. Pereira, P. Sánchez-Bel, K. Papadopoulou
	(2020). Biological and Molecular Control Tools in Plant
	Defense. In "Plant Defence: Biological Control", Eds Jean-
	Michel Mérillon and Kishan Gopal Ramawat. Springer, 3-43.
Dates	21/10/2020
Туре	Book chapter
Dublication #2	
	Comence K. Mauriadau C. Arematria T. Kakasianni M.
IITIE	Samaras K., Mourtiadou S., Arampatzis T., Kakagianni M.,
	Feka M., Wackers F., Papadopoulou K.K., Broutas G.D.,
	Pappas M.L. (2023). Plant-Mediated Effects of Beneficial
	Microbes and a Plant Strengthener against Spider Mites in
	Tomato. Plants, 12 (4), art. no. 938, doi:
	10.3390/plants12040938
Dates	18/02/2023
Туре	Journal article
Publication #3	
Title	Pappas M (2022) Microbes enhance tomato defences
	AGRO.TECH Magazine, 48-49 (in Greek).
Dates	July-August 2022
Туре	Journal article
.	
Publication #4	
Title	Pappas M.L., Samaras K., Ntalia P., Broufas G.D. (2023).
	Spider mites perform worse on soil microbe-inoculated
	plants: from the lab to the greenhouse. In Proceedings of the
	8th Meeting of the IOBC-WPRS Working Group "Integrated
	Control of Plant-Feeding Mites", Broufas G., Le Hesran S.,
	Marčić D., Palevsky E., Simoni S., Vangansbeke D., Walzer
	A., Zemek R. (eds.).
Dates	09/2023
Туре	IOBC Bulletin article



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Publication #5	
Title	Pappas M.L. (2023). Beneficial soil microbes and zoophytophagous predators as plant 'vaccination' agents against arthropod pests. In: Proceedings of the joint Meeting of the Working Groups Integrated Control of Protected Crops, Temperate and Mediterranean Climate, Rapisarda C., Dreux L., Gobin B. and Messelink G. (eds).
Dates	08/2023
Туре	IOBC Bulletin article

Publication #6	
Title	Samaras K., Ntalia P., Bechtsoudis A., Broufas G.D., Pappas
	M.L. (2023). Plant-mediated effects of beneficial soil microbes on natural enemies. In: Proceedings of the joint Meeting of the Working Groups Integrated Control of Protected Crops, Temperate and Mediterranean Climate, Rapisarda C., Dreux L., Gobin B. and Messelink G. (eds).
Dates	08/2023
Туре	IOBC Bulletin article

and worked on the following manuscripts which are planned to be submitted for publication:

Publication #	
Title	Arampatzis Th., Mourtiadou S., Zamioudis C., Papadopoulou K., Broufas G.D. & Pappas M.L. Plant-mediated effects of soil bacteria against thrips, whiteflies, aphids and <i>Tuta absoluta</i> in tomato (e.g. <i>Pest Management Science</i>)
Dates	To be submitted
Туре	Journal article
Dates Type	To be submitted Journal article

Publication #	
Title	Mourtiadou S., Arampatzis Th., Kakagianni M., Feka M., Broufas G.D., Papadopoulou K. & Pappas M.L. Plant- mediated effects of soil fungi against thrips, whiteflies, aphids and <i>Tuta absoluta</i> in tomato (e.g. <i>Frontiers in Plant Science</i>)
Dates	To be submitted
Туре	Journal article



The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant" (Project Number: 50).



Publication #	
Title	Samaras K., Weinhold A., van Dam N., Broufas G.D. &
	Pappas M.L. Plant-mediated effects of soil microbes on natural enemies of pests (e.g. <i>Frontiers in Plant Science</i>)
Dates	To be submitted
Туре	Journal article

Conferences & Meetings

BeMOST participated in the following scientific conferences and meetings.

Conference #1	Entomology 2020
Title	Mourtiadou S., Arampatzis T., Kakagianni M., Feka M.,
	Papadopoulou K., Broufas G. & Pappas M. L. (2020). Plant-
	mediated effects of beneficial soil microbes against arthropod
	pests. Entomology 2020 Virtual Annual Meeting,
	Entomological Society of America (ESA).
Dates	11-25/11/2020
Туре	Oral presentation
Place	Online

Conference #2	BES Annual Meeting 2021
Title	Samaras K., Ntalia P., Mourtiadou S., Arampatzis T., Broufas
	G. & M.L. Pappas (2021). The hidden role of beneficial soil
	microbes against spider mites, whiteflies and thrips in tomato.
Dates	12-15/12/2021
Туре	Poster
Place	Liverpool, UK & Online

Conference #3	IHC 2022
Title	Samaras K., Ntalia P., Broufas G. & M.L. Pappas (2022). Beneficial soil microbes to enhance tomato resistance against arthropod pests.
Dates	14-20/08/2022
Туре	Oral
Place	Angers, France



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Conference #4	19th Panhellenic Entomological Congress
Title	Samaras K., Ntalia P., Bechtsoudis A., G.D. Broufas & M.L.
	Pappas (2022). Effects of beneficial soil microbes on the
	biology of natural enemies.
Dates	23/5-27/5/2022
Туре	Poster
Place	Agrinio, Greece

Conference #5	19th Panhellenic Entomological Congress
Title	Ntalia P., Samaras K., Bechtsoudis A., G.D. Broufas & M.L.
	Pappas (2022). Effects of beneficial soil microbes on the
	behavior of natural enemies.
Dates	23/5-27/5/2022
Туре	Poster
Place	Agrinio, Greece

Conference #6	19th Panhellenic Entomological Congress
Title	Pappas ML., Samaras K., Ntalia P., Feka M., Papadopoulou
	K. & G.D. Broufas (2022). Effects of beneficial soil microbes
	against herbivorous pests.
Dates	23/5-27/5/2022
Туре	Oral
Place	Agrinio, Greece

Conference #7	British Ecological Society Annual Meeting 2022
Title	Pappas ML, Avramidou M., Samaras K., Ntalia P., Kakagianni M., Papadopoulou K. & G.D. Broufas (2022). Beneficial soil microbe-mediated tomato responses against spider mites.
Dates	23-27/5/2022
Туре	Oral
Place	Online

Conference #8	European Congress of Entomology 2023 (ECE2023)
Title	Pappas M.L., Samaras K., Ntalia P., Mourtiadou S., Arampatzis T., Avramidou M., Feka M., Kakagianni M., Weinhold A., Steppuhn A., van Dam N.M., Papadopoulou K., & G.D. Broufas (2023). Beneficial microbes to optimize pest control in sustainable tomato production
Dates	16-20/10/2023
Туре	Oral



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Place	Heraklion, Greece
Conference #9	8th Meeting of the IOBC-WPRS Working Group "Integrated
	Control of Plant-Feeding Mites"
Title	Pappas M.L., Samaras K., Ntalia P., Broufas G.D. (2023).
	Spider mites perform worse on soil microbe-inoculated
	plants: from the lab to the greenhouse.
Dates	4-7/09/2023
Туре	Oral
Place	Belgrade, Serbia
Conference	Joint Meeting of the Working Groups Integrated Control of
#10	Protected Crops, Temperate and Mediterranean Climate
Title	Pappas M.L. (2023). Beneficial soil microbes and

Title	Pappas M.L. (2023). Beneficial soil microbes and
	zoophytophagous predators as plant 'vaccination' agents
	against arthropod pests.
Dates	27-31/08/2023
Туре	Oral
Place	Brest, France

Conference	Joint Meeting of the Working Groups Integrated Control of
#11	Protected Crops, Temperate and Mediterranean Climate
Title	Samaras K., Ntalia P., Bechtsoudis A., Broufas G.D., Pappas M.L. (2023). Plant-mediated effects of beneficial soil microbes on natural enemies.
Dates	27-31/08/2023
Туре	Poster
Place	Brest, France

Conference #12	10th International Conference Mikrobiokosmos
Title	Pappas M.L., Samaras K., Ntalia P., Broufas G.D. (2023). Plant-mediated effects of beneficial soil microbes on herbivore populations in the greenhouse.
Dates	30/11-02/12/2023
Туре	Poster
Place	Larissa, Greece





BeMOST results were presented to the following scientific meetings.

Meeting #1	Soildatiry Horizon EU project meeting (invitation: Prof.
	Cristina Cruz)
Title	Presentation of the BeMOST project, objectives & results
Dates	25/11/2022
Туре	Oral
Place	Online
-	
Meeting #2	Acarological Society of America (invitation: Dr. Emilie
	Demard)
Title	Presentation of the BeMOST project, objectives & results
Dates	15/11/2023
Туре	Oral
Place	Online

Meeting #3	Koppert NL meeting (invitation: Dr. Susanne Lommen)
Title	Presentation of the BeMOST project, objectives & results
Dates	28/11/2023
Туре	Oral
Place	Online

Delays & Difficulties

Main delays and difficulties faced during the project are related to the covid-19 pandemic because of the travel restrictions and the cancellation or postponement of all physical conferences.



The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant" (Project Number: 50). 10



Annex

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Chapter 1 Biological and Molecular Control Tools in Plant Defense



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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 signal-ling [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 nonadapted 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, plantderived 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 plantpathogen 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 Ca2+ 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 defenserelated 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 time-frame 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 plantstress 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 methvlation 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 RNA imechanism 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 highpressure 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 spraving 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 esteraseweresprayed 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. lycopersi*cum* 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 plantmicrobe 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 soilborne 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 twospotted 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. occidentalisand 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. pyg-maeus* 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.

1.6 Endophytic Fungi in Plant Defense

1.6.1 Endophytic Fungi Mediating ISR

Endophytic microorganisms occur ubiquitously in plants, where they spend part of (facultative endophytes) or all (obligate endophytes) their life-cycle, without causing any signs of disease [204]. Endophytes colonize all plant organs, generally the inter- and intracellular spaces of their inner tissues [204]. They may derive from the surrounding environment, such as the rhizosphere and phyllosphere, but also from vegetative planting material or from seeds [205]. Endophytic communities are very diverse and their composition is influenced by a broad spectrum of factors, such as host genetics [206], geographic location [207], local environmental conditions [208], pathogen infections [209] and anthropogenic influence [210]. The capacity of endophytes to confer resistance or tolerance to the host plant is largely attributed to endophytic production of bioactive metabolites in colonized plants. These compounds may suppress biotic stressors either directly, by antibiosis, parasitism and competition, or indirectly via the induction of plant defenses [211]. These mechanisms frequently operate simultaneously. Some of the compounds that inhibit biotic stressors directly include defense metabolites (e.g., terpenoids, alkaloids and polypeptides), volatile organic compounds (e.g., acids, alcohols, alkyl pyrones, ammonia, esters, hydrogen cyanide, and ketones), iron-chelating compounds (e.g., siderophore), quorum sensing inhibitors and hydrolytic enzymes [212-215].

Elicitation of ISR by endophytes has been reported to be important to fight an array of pathogens, including fungi [216–219], bacteria [220, 221], oomycetes [222] and recently, arthropods [149–151]. Some of the most important endophytes found to induce ISR in crop plants include fungi belonging to the genera *Trichoderma*, *Penicillium*, *Fusarium* and *Phoma*, and bacteria of the genera *Pseudomonas* and *Bacillus* [223–225].

Knowledge on cellular, molecular, and biochemical defense responses activated by endophytes against pathogens or pests is so far limited. The few studies performed suggest that endophytes activate ISR response via their contact with the plant receptor (*i.e.*, PRRs) in the same manner as pathogens, but differ in the induction of defense responses [226, 227]. Elicitors involved in ISR triggered by endophytes are not so well characterized as compared to pathogens. Endophytic elicitors identified so far are common among all microbes and include endophytic-cell components, such as chitin or chitin derivative (*e.g.*, chitosan), β -glucans, ergosterol and flagellin, as well as proteins (*e.g.*, cerato-platanins), peptides (*e.g.*, peptaibols, elicitins), lipopolysaccharides and enzymes (*e.g.*, xylanases, proteinases and cellulases) secreted by endophytes [224, 228–230].

Increasing evidence suggests that endophytes defend themselves from plant defense mechanisms. Endophytes can, for instance, prevent themselves from being recognized by plant receptors [231] or succeed in being perceived in a different way as compared to pathogens [232], and can also protect themselves from ROS generated by the plant as a defense response [233]. Upon recognition of the endophyte by

the plant, a set of signal molecules are generated to induce and amplify out the defense response at long distance. JA and ET are known to be the major signal molecules involved in systemic defense responses of plants mediated by endophytes [162, 234, 235]. Despite the common association of SA with SAR, this plant hormone was also shown to induce systemic responses, activated by endophytes [66, 236]. However, in a pathosystem involving F. oxysporum Fo47, against Fusarium wilt disease in tomato, induced resistance triggered upon endophytic colonization was demonstrated to be independent of the SA/JA/ET pathways [237]. These contradictory results open several questions related with the necessity of phytohormones to induce endophyte-mediated resistance and the classification of induced resistance response as ISR or SAR. Defense responses can include strengthening of structural barriers by callose accumulation, generation of ROS, synthesis of pathogenesis-related (PR) proteins (which have a recognition role in defense and stress as well as antimicrobial activity), production of defense-related enzymes (e.g., peroxidases, polyphenol oxidases, phenylalanine ammonia-lyase), anti-microbial metabolites (e.g. phenolic and flavonoid compounds) and proteins that inhibit pathogen growth, along with the increased anti-oxidant capacity of the host [217-220, 236].

1.6.2 Endophytic Entomopathogenic Fungi as Biocontrol Agents

Endophytic entomopathogenic fungi (EEPF) are naturally occurring soil microbes [238] which show similar characteristics to the non-clavicipitaceous (class III) endophytic fungi [239]. Among these traits are their occurrence primarily or exclusively on foliar tissues, their horizontal transmission (via airborne spores) and high diversity of host range [239–241]. EEPF are classified in two groups, the generalist facultative insect pathogens (mainly Hypocreales species) that inspire a broad research interest, and the host-specific obligate pathogens (Entomophthorales and a small number of Hypocreales species) with a narrow host spectrum [242].

The dual ability of EEPF to establish themselves as both endophytes and entomopathogens [243] provide a successful crop protection method in a sustainable agriculture context. Studies on EEPF carried out some decades ago report *Beauveria bassiana* [244], *Metarhizium anisopliae* [245], *Verticillium* (=*Lecanicillium*) *lecanii* [246], *Paecilomyces farinosus* (Holmsk.) (=*Isaria farinosa*) [247], *Paecilomyces* sp. [248], *Paecilomyces varioti* [249], *Cladosporium* [250], and *Purpureocillium lilacinum* (formerly*Paecilomyces lilacinus*) [251] as pathogenic agents against thrips, aphids, whiteflies, mosquitoes, fruit flies, mites and other arthropods and plant parasitic nematodes [252]. Because of their peculiar life-style (i.e. symptomless endophytes of plants and infectious to herbivorous insects), EEPF have received much attention recently as promising biological control agents [253–255]. However, the mechanisms underlying their interactions with plants and pests remain poorly understood while their insect-killing capabilities have prompted many studies on the basis of their biotechnological potential [240]. Notably, latest research has focused on the role of EEPF in secondary metabolites production as well as their ability to promote plant growth and enhance resistance [256].

ISR has been demonstrated for diverse EEPF. Perhaps, the most striking example is the genus *Beauveria* (particularly the species *bassiana*), which accounts for the 67% of EEPF studied [240]. Inoculation of cultivated cotton seeds with *B. bassiana* conidia resulted in lower survival and development of the corn earworm Helicoverpa zea [257]. Likewise, Rachiplusia nu larvae consumption on colonized corn plants with B. bassiana was reduced [258]. Against Aphis gossypii, inoculation of cotton seeds had a negative effect on reproduction with an increased mortality after successful establishment of B. bassiana [259]. Beauveria bassiana was also used as an endophyte against the leaf miner Liriomyza huidobrensis resulting in reduced oviposition, mortality, longevity and adult emergence [260]. These authors further confirmed that colonization is species-specific, depending on the host plant, the fungal isolate and plant part. Assessing the effect of B. bassiana on the growth of Arabidopsis thaliana and its resistance against two herbivorous species (Myzus persicae and P. xylostella) and a facultative parasitic Ascomycete fungus (Sclerotinia sclerotiorum), Raad et al. [261] found a decreased leaf lesion area caused by the pathogen. Nonetheless, population growth of *M. persicae* or *P. xylostella* was not affected. A deeper transcriptomic, phytohormone and glucosinolate analysis showed that the expression of genes involved in plant defense varied; conversely, JA and SA levels as well as those of leaf glucosinolates remained unchanged. This was again a confirmation for the species-specificity of the induced defense mechanism. Beauveria bassiana can also act against bacteria by lowering the severity of Xanthomonas bacterial blight [262], and also confer resistance against the Zucchini Yellow Mosaic Virus (ZYMV) in colonized squash plants [263]. The latter was the first report on *B. bassiana* being involved in plant defense against viruses followed by other reports such as ISR against melon viruses [264]. Several other studies were carried out with other EEPF such as Metarhizium species (acridum, robertsii, anisopliae, brunneum, pingshaense), Purpureocilium lilacinum, Isaria fumosorosea, Clonostachys rosea and Lecanicillium lecanil that showed successful endophytism in different host plants and plant parts, with induction of systemic resistance [240].

Direct effects of EEPF on plant biotic stressors are attributed to mycoparasitism, competition with other endophytes or the production of secondary metabolites. Mycoparasitism is defined as an antagonistic interaction between two fungal organisms by the production of extracellular enzymes such as chitinases, cellulases and glucanases by the parasite to digest the host cell wall [265]. It has been described in depth for *Trichoderma* spp. [266, 267] and *Lecanicillium* spp. [213, 268] under laboratory conditions. Likewise, Griffin [269] showed an ability of *B. bassiana* to parasite the fungus *Pythium myriotylum*, a serious pathogen of many crops. Competition for space and resources can occur between EEPF and pathogens thus conferring protection and reducing the probability of colonization by pathogens [243]. In the case of initial colonization by EEPF, resources are expected to be exhausted, limiting nutrient availability for the pathogen. Consequently, the disease is expected to be suppressed [270] as for example, with *B. bassiana* inoculated to

grapevine that was shown to control the infection by the pathogen *Plasmopara viti-cola*via an antagonistic effect.

Antibiosis and feeding deterrence are non-entomopathogenic mechanisms of EEPF [240, 260, 271, 272]. They are well known to be sources of secondary metabolites that exert inhibitory effects on pests and pathogens. Beauvericine [273, 274], oosporein [275, 276] and bassianolide [277] are toxic secondary metabolites produced by *Beauveria* spp. These metabolites pose insecticidal, antibacterial, antifungal and antiviral activities [278]. Most of the work carried out has demonstrated the secretion of these metabolites *in vitro*; however, their production *in planta* is not evidenced since production may be temporary or degrading rapidly [272]. Our knowledge of the mechanisms of action of EEPF as entomopathogens and as plant growth promoters is well advanced during this last decade. However, there is still a lot to investigate in particular the relationship between EEPF and their plant hosts with the community of symbionts they harbour.

1.7 Aspects of Commercial Application

Compared to conventional agrochemicals, RNA molecules (dsRNAs, sRNAs) seem to win the race in terms of environmental considerations and risk assessment. RNAi molecules are not toxic to humans even when present in their diet [107]. Moreover, their mode of action is extremely specific, since it is based on a nucleotidic complementarity of 20-25 bases with their target. Thus, off-target effects are practically minimized. Concerning cost issues, a rough estimation has suggested that for fieldscale application of RNA molecules against pests and pathogens 10 g of dsRNA per hectare is required [279]. For laboratory experiments limited amounts of dsRNA may be generated by the commercially available *in vitro* transcription kits (average cost 100 USD per 1 g of dsRNA). Yet, for field-scale applications alternative dsRNA production systems need to be sought for, such as the one provided by RNAagri (https://www.rnagri.com/) and AgroRNA (www.agrorna.com) wherein bacteria engineered to produce the desired dsRNA multiply in large fermentators and huge quantities of encapsidated dsRNA are isolated with low-cost methods (average cost 2 USD per 1 g of dsRNA). Yet, the degradation rate of the applied RNA in field conditions due to nucleases and/or hydrolysis is an issue that needs to be taken into consideration. To this end, lipid double hydroxide clay nanosheets ('BioClay') have been developed, wherein the dsRNA is bound to clay nanosheets and is significantly resistant from degradation [117]. Similarly, for enhanced biopesticide efficacy, Nanosur (www.nanosur.com) offers formulated RNAs for improved translocation across cellular membranes and reduced degradation. Moreover, chemical enhancers such as Sortin1 and Isoxazolone have recently been developed whose mere application in plants seems to boost host RNAi machinery [280]. Cumulatively, the above discussed advances have facilitated the development of commercial RNA-based plant defense products that are soon to emerge in the market, such as 'BioDirect' (Bayer/Monsanto), which is designed for pest (Colorado potato beetle, brassicas flea beetle, varroa mites), virus (Tospovirus) and weed (glyphosate resistance) control.

Current applied and fundamental research has offered a plethora of potential chemical stimulants of the plant immune system that have the potential to protect crops in a more sustainable way. Most chemicals discovered by means of induced resistance and priming are natural compounds that contribute to signal subsequent plant defenses. Hence, their potential use as active matters in the future design of agrochemicals offers an opportunity of a new generation of sustainable products. However, despite the enormous interest for these compounds to fit in a new generation agriculture, the legislation needs to be redefined in parallel to adapt new discoveries to the applied field. Indeed, although a low environmental impact of these naturally occurring metabolites is expected, knowledge of their impact on non-target crops and organisms is important. Furthermore, an adapted legislation, out of the phytosanitary frame but supervised, is needed for their wide acceptance and use in the field.

Considered as a relevant tool to unfold and sustain agriculture, EEPF have already been used as potent tools in empowering sustainable agriculture. Nevertheless, more research is required to invest in technical challenges. As stated above, EEPF can be exploited in crop protection for both their entomopathogenic and non-entomopathogenic roles. Their endophytism offers an advantage compared to entomopathogenic fungi used as contact biocontrol agents that are limited by their susceptibility to biotic and abiotic factors. EEPF action as entomopathogens on the other hand, has been extensively studied for plant-surface inhabiting pests. However, their effects against endophytic insects, whose larvae feed internally in stems, flowers, seeds, parenchyma leaves and fruits are not proven yet. Moreover, colonization of plant parts by EEPF differs among plants and fungal species and its persistence is not completely prevailed [270]. A recent transcriptomic analysis of Beauveria-colonised plants showed a reprogramming of plant defense pathways [261]. Hence, further studies are required for a better understanding of mechanisms regulating plant responses to EEPF and those governing EEPF-mediated tritrophic interactions. Finally, current EEPF formulations (e.g. BotaniGard ES/WP, Mycotrol, Naturalis L, BioCeres WP, Velifer, balance, XPulse, PFR-97 WDG, Semaspore Bai and MeloCon WG) rely on a single microorganism and are commercialised to serve exclusively as insecticides [252]. Endophyte consortium formulations would be of interest, possibly offering synergistic efficiency. Nonetheless, the validation and commercialisation of economically viable EEPF constitutes laborious challenges and potential risks i.e., introducing organisms into new ecosystems, toxicosis risks [281] and field efficiency. EEPF were proved harmless for beneficial insects (e.g. bees) [260, 282, 283]. Yet, they may interfere with nest-mate recognition and also promote inter-colony transmission of diseases in honey bees [284]. Future studies are crucial to cast light on EEPF side-effects on beneficial organisms.

The same holds for all pest control strategies presented in this chapter. Biological pest control with the use of natural enemies (predators, parasitoids) is an important alternative to chemical control, commonly employed in sustainable crop production to suppress pest populations, especially in greenhouse crops. Despite the vast

fundamental knowledge on mechanisms of plant defense and RNAi and their effects on plant pathogens and pests, our understanding of their impact on beneficial organisms (natural enemies, pollinators) is largely poor. Plant defense and RNAi tools may negatively affect natural enemies directly, by causing mortality or slowing down their development, or via interfering with their herbivorous prey.

1.8 Conclusions & Future Considerations

Increasing population on Earth makes uncertain the future regulation of food security and supply. The United Nations have gathered these needs up and push for reaching certain goals of sustainability until 2030 [285], and fulfilling by the end of the millennium other objectives, like the end of hunger and poverty. All these new policies, strongly supported by higher education and research institutions, prompt us to revisit current agricultural practices [286], also by considering environmental sustainability. Future agro-technological considerations may include the concept of defense priming as well as RNAi, as new strategies in crop protection by key pests and pathogens [110, 287]. Moreover, the long lasting and transgenerational aspects of priming should be considered. They can add value to this sustainable concept, by providing defense to plants without requiring additional treatments [80–82]. The mechanisms described in this chapter, tackle the cross-kingdom (including bacteria, plants and pests) and -scale (from molecular to applied in fields) relevance of this type of adaptive immunity, highlighting ecological implications in plant defense [288].

Furthermore, soil-borne beneficial microorganisms are of particular interest as vaccination agents of crops, capable of enhancing plant resistance to biotic stressors. An important prerequisite for the development and application of effective beneficial inocula is a solid knowledge of the mode of action of these organisms, the mechanisms and regulatory pathways involved in microbe-induced resistance and, how context dependency influences beneficial interactions among crops and pests/ pathogens. The ability of beneficial microorganisms to activate phytohormone-mediated plant defense responses is well-established. However, most studies on the molecular mechanisms that govern the complex multi-partite interactions of plants are limited to a few model plants and also refer to certain pathosystems. Hence, little is known of the universality of these mechanisms in crop plants and their pathosystems. An untargeted approach to identify microbe-induced defense mechanisms is needed and the molecular tools are now applicable to economically important plants, too.

The advancements in Next Generation Sequencing (NGS) technologies provide an unprecedented insight into the genetic patrimony of different living organisms. These technologies revolutionized the methods of deciphering DNA sequences as well as the exchange, storage and analysis of enormous quantities of resulting sequence data. The generation of sequencers belonging to the third generation (e.g. PacBio, Oxford Nanopore Technologies), that enable long length sequences read
and accuracy [289, 290], allow for genome assemblies of organisms identified as suitable for biological control, such as the beneficial microbes dealt with in this chapter. In the last decades, several genomes of biocontrol agents have been sequenced and assembled leading the way for understanding their biology and functional characteristics that are beneficial to plant defense and biocontrol activities against plant pathogens and pests. The development of NGS has also facilitated comparative and functional genomics in these organisms that allow for a better description of genes responsible for the main beneficial properties of biocontrol agents in different ecosystems. In addition, important reservoirs of genes that could play key roles in ecosystem functioning may be accessed. For example, it was recently shown that two bacterial genes in biocontrol Pseudomonas strains are necessary for the acidification of the rhizosphere, which in turn modulates plant immunity to facilitate normal plant growth [291]. In yet another case, genome reconstruction at strain-level derived from a metagenomics analysis of the endophytic community in sugar-beet, a novel gene cluster encoding nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) was identified as essential for disease suppression by the endophytic community [68].

In conclusion, it becomes more and more obvious that disease/pest suppression is the final outcome of complex and multipartite plant-microbe interactions leading to either coevolution or physiological adaptation in a context-specific manner. The challenge is to take a holistic perspective in future studies to assess the suppressive function of microbial assemblages at a community level and apply molecular tools not only on harmful organisms but also on the ecosystem. Such a community-level approach is crucial to determine the feasibility of novel biocontrol molecular tools in sustainable crop production.

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Article Plant-Mediated Effects of Beneficial Microbes and a Plant Strengthener against Spider Mites in Tomato

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Abstract: The two-spotted spider mite *Tetranychus urticae* is a polyphagous herbivore with a worldwide distribution, and is a serious pest in tomato and other crops. As an alternative to chemical pesticides, biological control with the release of natural enemies such as predatory mites represent an efficient method to control *T. urticae* in many crops, but not in tomato. Other biological control agents, such as beneficial microbes, as well as chemical compounds, which can act as plant defense elicitors that confer plant resistance against pests and pathogens, may prove promising biological solutions for the suppression of spider mite populations in tomato. Here, we assessed this hypothesis by recording the effects of a series of fungal and bacterial strains and the plant strengthener acibenzolar-s-methyl for their plant-mediated effects on *T. urticae* performance in two tomato cultivars. We found significant negative effects on the survival, egg production and spider mite feeding damage on plants inoculated with microbes or treated with the plant strengthener as compared to the control plants. Our results highlight the potential of beneficial microbes and plant strengtheners in spider mite suppression in addition to plant disease control.

Keywords: defense elicitor; pest control; soil microbes; spider mites; tomato

1. Introduction

Feeding the increasing human population in a sustainable manner represents a major challenge. If left uncontrolled, herbivorous arthropod pests can be highly destructive to crops, causing significant yield losses, often above 30% [1]. Pesticide application remains the most common method of controlling such pests, despite policies that promote the use of non-chemical methods in crop production. This global trend is in part driven by a strong demand for agricultural products with reduced load of chemicals [2–4]. Novel strategies, complementary or alternative to the existing ones, are required to control arthropod pests of crops in the most efficient and environmentally friendly manner.

Biological control, i.e., the use of beneficial agents against harmful organisms, together with breeding for resistance, are the most promising alternatives to chemical control in crop production [5]. Nevertheless, breeding for resistance in modern crops is often hindered by the complex genetic nature of the traits involved, the narrow range of effectiveness (limited to only a few pest species) and the demonstrated ability of pests to overcome resistance mechanisms [6–9]. Hence, biological control is currently the most widely applied alternative method to control various arthropod pests in organic farming and IPM programs.

Among biocontrol agents, selected root-colonizing microbes (bacteria and fungi) have long been recognized for their ability to antagonize soil-borne pathogens, improve plant



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). growth and nutrition, and also stimulate (prime) the plant immune system against future attackers [10]. Defense priming triggered by soil-borne microbes is generally referred to as Induced Systemic Resistance (ISR). Microbe-mediated ISR is associated with enhanced expression of defense-related genes that only becomes evident upon attack [11,12]. Thus, ISR may provide plants with a cost-effective mechanism of protection against aboveground herbivores [13,14]. For example, an endophytic fungal strain (*Fusarium solani* strain K) was shown to enhance tomato resistance against spider mites [15], indicating that selected microbes can also contribute to the control of important agricultural pests such as insects and mites. Hence, soil-borne beneficial microbes are of particular interest as 'plant vaccination' agents, capable of enhancing plant resistance to biotic stressors [16]. Yet, to date, we only have limited and scattered data on the effects of soil-borne beneficial microbes in providing protection to economically important crops against herbivores [17] and soil microbes currently marketed by the biocontrol industry are only provided as plant growth regulators and/or biofungicides.

Plant defenses can also be induced by chemical compounds besides beneficial soil microbes [18–20]. Plant strengtheners, for example, include synthetic compounds which are commercially available to improve plant vigor and protect plants against pathogens. Considering that plant defenses against pathogens and herbivores can be mediated by the same signaling pathways, plant strengtheners can be elicitors that also induce resistance against herbivores [21–23]. Hence, beneficial soil microbes and plant strengtheners can be efficient alternatives to chemical pesticides in integrated pest management.

Mechanisms involved in plant defense induction by microbes or chemical elicitors may mediate both direct and indirect responses against herbivores [14,24,25]. Direct effects in particular can be directly effective against arthropods; for instance, when they exhibit an increased sensitivity to jasmonic acid (JA) [26,27]. In the present study, we assessed the plant-mediated effects of a series of commercial and laboratory fungal and bacterial strains, as well as the plant strengthener acibenzolar-S-methyl (Table 1), against the two-spotted spider mite *Tetranychus urticae* in tomato. Spider mites are mesophyll cell-content feeders and *T. urticae* is a polyphagous pest that infests a high number of crops of different plant families. Since tomato defenses against spider mites are mediated by the phytohormones JA, salicylic acid and ethylene [28,29], we hypothesized that spider mites could be affected by plant responses elicited by the beneficial microbes and the plant strengthener. To the best of our knowledge, the plant-mediated effects of beneficial soil microbes or plant strengtheners on herbivorous mites have been scarcely addressed so far [30–33].

Tomato cy: ACE			
Tomato cv: ACE			
Fungi			
Trichoderma harzianum T-22TRIANUM-P [®] KOPPERT35 $1 \times 10^9 \text{ cfu/g}$			
$ \begin{array}{c} \mbox{Asperello}^{\mbox{\ensuremath{\mathbb{S}}}} T34 \mbox{ Biocontrol}^{\mbox{\ensuremath{\mathbb{S}}}}, \\ Trichoderma \mbox{ asperellum T34} & \mbox{Biobest Group NV} & 35 \\ 1 \times 10^9 \mbox{ cfu/g} \end{array} $			
$\begin{tabular}{lllllllllllllllllllllllllllllllllll$			
Plant strengthener			
Acibenzolar-S-methylBION 50 WG5Syngenta Hellas5			

Table 1. Strains of beneficial microbes and a plant strengthener tested for their plant-mediated effects against spider mites in tomato.

Strain	Origin (Product/Lab)	Dosage (mg/pot)	
Tomato cv: Moneymaker			
Fungi			
Isaria fumosorosea Apopka 97	PreFeRal [®] , Biobest Group NV 2×10^9 cfu/g	0.64	
Trichoderma atroviride SC1	Vintec [®] , Bi-PA NV/SA 1×10^{10} cfu/g	0.09	
Trichoderma asperellum TV1	Xedavir, Intrachem Hellas 1 × 10 ⁷ cfu/g	350	
Trichoderma asperellum T34	Asperello [®] T34 Biocontrol TM , Biobest Group NV 1×10^9 cfu/g	3.50	
Rhizoglomus irregulare QS69	Advantage, INOQ GmbH 3.6 $ imes$ 10 ⁴ propagules/g	10	
Funneliformis mossae	Lab [34] 2×10^5 cfu/g	10	
Rhizophagus irregularis	Lab [34] 2×10^5 cfu/g	10	
Rhizophagus irregularis (DAOM) 197198	DAOM Agronutrition 5×10^4 cfu/mL	10 µL	
	Bacteria		
Pseudomonas sp. DSMZ 13134	$\begin{array}{c} \text{Proradix}^{\textcircled{\text{\tiny B}}},\\ \text{Anthesis}\\ 6.6\times10^{10}~\text{cfu}/\text{g} \end{array}$	0.08	
Bacillus amyloliquefaciens MBI600	Serifel [®] , BASF Hellas $5.5 imes 10^{10}$ cfu/g	0.32	
Bacillus amyloliquefaciens FZB24	Taegro [®] , Syngenta 1 × 10 ¹⁰ cfu/g	0.24	
Bacillus pumilus QST 2808	Sonata [®] , Bayer 1×10^9 cfu/gr	6.4	
Bacillus amyloliquefaciens subsp. plantarum D747	$\begin{array}{c} \text{Amylo-X}^{\mathbb{B}},\\ \text{K}^{\mathbb{B}}\text{N Efthymiadis}\\ 2\times10^{11} \text{ cfu/g} \end{array}$	1.60	

Table 1. Cont.

2. Results

2.1. Plant-Mediated Effects on Spider Mite Performance

2.1.1. Spider Mite Performance on Tomato Plants cv. Ace 55

The number of mites found alive was significantly lower for the plant strengthener treatment, whereas the plants of all other treatments hosted a similar number of spider mites, which was also significantly lower compared to control plants (F = 42.75; df = 4, 80; p < 0.05, Figure 1A).

Furthermore, all fungal strains tested resulted in a significant reduction in spider mite oviposition (F = 42.75; df = 4, 80; p < 0.05, Figure 1B) on tomato plants of the cultivar Ace 55, with females laying approx. 31–50% fewer eggs on treated compared to control plants (F = 18.66; df = 4, 80; p < 0.05, Figure 1B).



Figure 1. Effects of soil application of beneficial fungi and a plant strengthener on spider mite (*Tetranychus urticae*) performance on tomato cv. Ace 55. Box plots of (**A**) the live adult females and (**B**) spider mite eggs per plant recorded on treated and control plants (n = 18). In each panel, significant differences between treatments are indicated by different letters (Tukey's HSD, p < 0.001).

Compared to the microbial products, the application of the plant strengthener resulted in an even more pronounced reduction in spider mite oviposition, at approx. 50% of the level observed in the control plants (Figure 1B).

Tomato treatment with the different microbial products also resulted in a significant reduction in the damage inflicted by spider mites over the four days of feeding compared to the control plants (Figure 2). Notably, the application of the plant strengthener resulted in the greatest reduction in the feeding damage, both compared to the microbial-treated and the control plants (F = 125.02; df = 4, 40; p < 0.001, Figure 2).

2.1.2. Spider Mite Performance on Tomato Plants cv. Moneymaker

Bacterial strains tested significantly reduced the number of live spider mites (F = 14.27; df = 5, 72; p < 0.001, Figure 3A), as well as the number of spider mite eggs (F = 10.12; df = 5, 72; p < 0.001, Figure 3B) per plant.

Similarly, all tested fungal strains significantly reduced the number of live spider mites (Figure 4(A1): F = 29.76; df = 5, 72; p < 0.001; Figure 4(B1): F = 80.432; df = 3, 56; p < 0.001), as well as the number of spider mite eggs per plant (Figure 4(A2): F = 42.68; df = 5, 72; p < 0.001; Figure 4(B2): F = 38.05; df = 3, 56; p < 0.001), with *R*. *irregularis* QS69 and 197,198 strains resulting in the lowest number of live spider mites and eggs.

Although a direct comparison among the two microbe groups (fungi vs. bacteria) cannot be made, microbes of both groups were shown to result in a similar reduction in spider mites compared with the control (Figures 3 and 4).



Figure 2. Effects of soil application of beneficial fungi and a plant strengthener on the feeding damage inflicted by spider mites (*Tetranychus urticae*) on tomato cv. Ace 55. Box plots of plant damaged area recorded on treated and control plants (n = 10). In each panel, significant differences between treatments are indicated by different letters (Tukey's HSD, p < 0.001).



Figure 3. Effects of soil application of beneficial bacteria on spider mite (*Tetranychus urticae*) performance on tomato cv. Moneymaker. Box plots of (**A**) the live adult females and (**B**) spider mite eggs per plant recorded on treated and control plants (n = 15). In each panel, significant differences between treatments are indicated by different letters (Tukey's HSD, p < 0.001).



Figure 4. Effects of soil application of beneficial fungi on spider mite (*Tetranychus urticae*) performance on tomato cv. Moneymaker. Box plots of (**A1**,**B1**) the live adult females and (**A2**,**B2**) spider mite eggs per plant recorded on treated and control plants (n = 15). In each panel, significant differences between treatments are indicated by different letters (Tukey's HSD, p < 0.001).

2.2. Plant Growth Parameters

Overall, no significant effects were recorded in the stem and root weight of tomato plants which were inoculated with the different microbes and infested with spider mites. Stem weight was found to be similar between the different experiments regardless of the bacterial (means ranging from 0.58 to 0.74 g; Microbe (M): F = 1.647; df = 5, 180; p = 0.150; Infestation (I): F = 0.255; df = 1, 180; p = 0.614; M × I: F = 0.633; df = 5, 180; p = 0.675) or the fungal species (means ranging from 0,49 to 0,55 g in group A plants: Microbe (M): F = 0.948; df = 5, 180; p = 0.452; Infestation (I): F = 1.089; df = 1, 180; p = 0.298; M × I: F = 0.073; df = 5, 180; p = 0.452; Infestation (I): F = 1.089; df = 1, 180; p = 0.298; M × I: F = 0.073; df = 5, 180; p = 0.421; Infestation (I): F = 0.06; df = 1, 120; p = 0.937; M × I: F = 0.289; df = 3, 120; p = 0.833).

The same was seen in terms of the weight of the tomato roots, with no significant effects found related to the inoculation of the plants with the different bacterial (means ranging from 0.079 to 0.099 g; Microbe (M): F = 1.954; df = 5, 180; p = 0.88; Infestation (I): F = 0.025; df = 1, 180; p = 0.874; M × I: F = 0.176; df = 5, 180; p = 0.971) or fungal species (means ranging from 0.059 to 0.071 g in group A plants: Microbe (M): F = 1.22; df = 5, 180; p = 0.302; Infestation (I): F = 0.551; df = 1, 180; p = 0.459; M × I: F = 0.484; df = 5, 180; p = 0.788, and from 0.137 to 0.122 g in group B plants: Microbe (M): F = 0.601; df = 3, 120; p = 0.616; Infestation (I): F = 0.053; df = 1, 120; p = 0.819; M × I: F = 0.478; df = 3, 120; p = 0.698)).

3. Materials and Methods

3.1. Plants

Tomato (*Solanum lycopersicum* L.) plants cv. Ace 55 (Vf) and Moneymaker were used in experiments, as well as in herbivore rearing. Plants were grown from seeds sown in pots (\emptyset 12 cm) that were filled with sterilized peat (Klasmann-TS2). All plants were maintained in climate chambers (25 ± 2 °C, 16:8 LD, 60–70% RH) and watered every other day. When used in the experiments, plants were 4–5 weeks old.

3.2. Herbivores

Spider mites (*Tetranychus urticae*) from laboratory rearing, established with individuals collected from greenhouse tomatoes, were used in the experiments. The mites were reared on detached tomato leaves placed on wet cotton wool in plastic trays at 25 ± 2 °C, 16:8 LD, 60–70% RH. Fresh tomato leaves were added every three days on the trays, which were regularly filled with water as required to maintain leaf vigor. Young female mites (2–4 days old) were used in the experiments. These were obtained by infesting tomato plants with a high number (approx. 300) of female mites that were allowed to lay eggs for 48 h at 25 ± 2 °C, 16:8 LD. The next day, the mites were removed and the plants were maintained at the same conditions until adult mites emerged (after approx. 16 days).

3.3. Plant Treatments

3.3.1. Experiments with Tomato Plants cv. Ace 55

We assessed the effects of three commercial fungal products in tomato plants against spider mites and the plant strengthener acibenzolar-S-methyl, a synthetic analogue of salicylic acid (SA) (Table 1). The products were dissolved in water and drenched in sterilized peat in pots where young tomato plants cv. Ace 55 were transplanted 2 days before (10 days from seed sowing). After 3 weeks, the plants were infested on 3 leaflets with spider mites which were reared on 'Ace 55' tomato leaflets (15 females per leaflet). Leaflets were selected as described in [28]. Oviposition and survival were recorded 4 days afterwards by removing the infested leaflets and checking them under a stereoscope. During the experiments, the plants were maintained at 25 ± 1 °C, 16:8 LD, 60–70% RH and watered every other day. Two time replicates with nine plants per treatment were used. We used a separate cohort of plants to assess the impact of the treatments on spider mite feeding damage (five plants per treatment, repeated in two independent experiments). In these experiments, plants were infested with 45 spider mite females per plant as described above. Feeding damage was recorded on spider-mite-infested leaflets which were collected and scanned digitally, and damaged leaf area was assessed manually calculated in Photoshop following the steps under 'Plant Damage Quantification' as described in [35].

3.3.2. Experiments with Tomato Plants cv. Moneymaker

We assessed the effects of five commercial strains of bacteria and eight strains of fungi, two strains from laboratory and six commercial strains, against spider mites in tomato plants cv. Moneymaker (Table 1). The products (commercial strains) were dissolved in water and drenched in sterilized peat in pots where young tomato plants cv. Moneymaker had been transplanted 2 days before (10 days from seed sowing). Lab fungal strains were routinely cultured on potato dextrose broth (PDB) at 25 °C for 5 days in the dark. Conidial suspensions were prepared and applied as water drench one week after seed sowing as described in Pappas et al. [15]. After 3 weeks, plants were infested with spider mites reared on cv. Moneymaker tomato leaflets as described above. Oviposition and survival were recorded 4 days after inoculation. During the experiments, the plants were maintained at 25 ± 1 °C, 16:8 LD, 60–70% RH and watered every other day. The experiment was conducted three times independently. In each experiment, five plants were used per treatment.

3.4. Plant Growth Parameters

Another set of experimental plants cv. Moneymaker was inoculated with beneficial microbes and infested with a standard number of spider mites as described above. Each beneficial microbe was applied in pots with sterilized peat in which tomato plants had been growing. The plants were inoculated with the microbe under study two days after having been transplanted. Ten days after inoculation, the plants were infested with 45 *T. urticae* females per plant. Four days after spider mite introduction, the performance of tomato plants was assessed by recording the dry weight of the above- and belowground plant parts of microbe-inoculated control and herbivore-infested plants.

3.5. Statistics

To evaluate the effect of the microbials and the plant strengthener (fixed factor) on the number of spider mite eggs, mite survival and mite damage, a mixed-model ANOVA with replication in time as the random factor was used. In case of significant differences, means were further separated by Tukey's HSD post hoc test. Similarly, to evaluate the effect of microbial application and infestation by the spider mites (fixed factors) on plant growth parameters (shoot and root dry weight), a mixed-model ANOVA was used with repetition in time as the random factor. Prior to statistical analysis, normality and homogeneity of variances were checked with the Shapiro–Wilk and Levene's tests, respectively. Significance levels were $\alpha = 0.05$ for all tests and statistics were performed using SPSS [36].

4. Discussion

In the present study, we tested to what extent treating tomato plants with different beneficial microbes or a plant strengthener affects tomato resistance to spider mites. We found that the number of live spider mites was lower on treated compared to control plants, irrespective of the microbial group (bacteria or fungi) or the application of the plant strengthener or the tomato cultivar. In accordance, we recorded a significantly lower egg production and also observed that feeding damage inflicted by spider mites was lower on treated compared to control plants. Finally, plant biomass was not affected by the application of the microbes in herbivore-infested plants compared to the control plants. We argue that these results indicate plant defense induction capabilities in both the tested microbes as well as the plant strengthener, with some variation was recorded between and within the two microbe groups (fungi and bacteria) and between the microbes and the plant strengthener.

Activating the plant's inherent defense system with the application of beneficial soil microbes or plant strengtheners represents a novel strategy to biologically fend off plant herbivorous pests. Currently, beneficial microbes used against arthropod pests are mainly entomopathogens that typically act on the pest directly. They are known as 'biopesticides' in the sense that they are naturally occurring or derived from natural products, and can be formulated and applied on crops in ways similar to conventional pesticides. Among biopesticides, *Bacillus thuringiensis* (*Bt*) is the most widely applied entomopathogenic bacterium against arthropod pests, whereas *Metarhizium*, *Beauveria* and *Isaria* are examples of entomopathogenic fungi. *Pseudomonas*, *Trichoderma* and *Bacillus* (other than *Bt*) are used as biofungicides [24,25,37]. Microbes as biopesticides offer the advantage of lower or no toxicity compared to synthetic pesticides. Nevertheless, their target range can be narrow

and even strain-specific. This selectivity of many of the currently available biopesticides means that there is an urgent need for the diversification of the biocontrol toolbox with biocontrol agents that have a wider target-pest range. Beneficial soil microbes and plant strengtheners may offer such an opportunity to impact a broad range of biotic stressors by activating plant defense responses. Among the broad number of currently identified soil bacteria and fungi, a relatively low number of species have been tested for their plant-mediated effects against arthropods, and none of these have reached the biocontrol market in that capacity. The same holds for the plant-mediated effects of plant strengtheners such as acibenzolar-S-methyl, which is commercially available as a fungicide and acts by mimicking the natural systemic acquired resistance of plants against pathogens [38–40].

The plant-mediated effects of microbes against spider mites have mainly been studied for entomopathogenic fungi when applied as soil drench or after treating seeds or roots in tomato, bean and strawberry [41–45], and several promising strains of *Metarhizium*, *Beauveria* and *Cordyceps* entomopathogens with plant protection capabilities have been identified. Nevertheless, the plant-mediated effects of other beneficial microbes such as plant-growth-promoting fungi (PGPF) or rhizobacteria (PGPR) on spider mites have been rarely addressed so far. An exception is the study of the beneficial soil endophytic fungus Fusarium solani strain K which was shown to negatively affect spider mite performance in tomato via the elicitation of plant defense responses [15], and different PGPR in strawberry [46], as well as the work of Pappas et al. [47], who identified a series of effective beneficial fungi and bacteria against spider mites in pepper. With regard to the effects of arbuscular mycorrhizal fungi (AMF), previous studies have shown variable effects on spider mites. Spider mite performance was shown to be enhanced by the AMF *Glomus mosseae* on bean plants [30–32], whereas spider mite performance in Lotus japonicus was differentially affected by four different AMF species belonging to different genera depending on the AMF species [48], and negatively affected in citrus plants [49]. It is evident that this important group of plant-interacting organisms need a more thorough evaluation as putative biocontrol agents.

Notably, in our study all fungal strains studied were shown to negatively affect spider mite performance when applied as water drench, while the AMF *Rhizoglomus irregularis* strains were the most promising of all. In addition, the bacteria tested were also shown to negatively affect spider mite performance in tomato. Putative mechanisms involved in the recorded effects could be the production of secondary metabolites, antibiotic effects, feeding deterrents and plant defense induction [16,24,25,41,50–53], or even the entomopathogenic activity of the microbes colonizing the plant, as has been reported for *C. fumosorosea* [16,54]. In our study, using two different plant cultivars, plant nutritional benefits translated to plant growth were not recorded, while spider mites were negatively affected on microbetreated plants. In addition, we recorded a difference in the number of live spider mites on plants and non-inoculated control plants, suggesting that recorded differences cannot be attributed to plant responses affecting spider mite reproduction only. Further studies are needed at the molecular and chemical levels to elucidate which of the above mechanisms underlies the reported findings.

Compared to the plant-mediated effects of beneficial microbes, the application of the plant strengthener acibenzolar-S-methyl resulted in more pronounced negative effects on spider mite performance. These effects were reflected in the number of live spider mites and their eggs, as well as at the resulting feeding damage on the acibenzolar-S-methyl-treated plants compared to control plants. Other studies have shown acibenzolar-S-methyl and SA to be involved in induced defense responses against phloem feeders such as aphids in tomato [55,56]. Furthermore, acibenzolar-S-methyl was shown to be effective against mesophyll cell-content feeders such as spider mites when sprayed on tomato and apple trees [38,39,57] or applied in the soil of lima bean plants [58]. Several mechanisms related to the application of acibenzolar-S-methyl have been proposed/demonstrated, ranging from the activation of defense-related enzymes to the expression of pathogenesis-related (PR) genes, as well as the alteration of volatile blend emissions [19,20,39,40]. Studying the

molecular and chemical mechanisms involved in tomato–spider mite interactions after acibenzolar-S-methyl application, coupled with behavioral and life-history experiments, will enable us to explain the recorded effects on mite performance.

The plant growth parameters studied in this work were shown not to be affected by the application of the microbes tested. Specifically, dry root and shoot weights of plants were not affected by the application of the microorganisms, irrespective of the spider mite infection. One possible explanation of the absence of effects may be the short duration of the experiments. Studying the effects on plant growth parameters at later stages, i.e., when plants will be inoculated with the microbes under study for longer time periods after transplantation, spanning several weeks or after repeated applications of the microbes, could reveal possible negative or positive effects. On the other hand, the absence of significant effects may be indicative of a trade-off in the plant's investment in defense responses elicited by soil microbes at the expense of its growth. Specifically with regard to herbivory, the net benefit of microbial application would depend on the trade-off between induced plant defenses versus plant nutritional quality or quantity alteration [14,24,59–61]. In the present study, spider mites were adversely impacted on plants treated with the microbes, suggesting the absence of nutritional benefits or that defense induction outcompetes the putative benefits of improved nutrition for the herbivore. Long-term experiments are needed to clarify the plant-growth-promotion effects of the tested microbes versus plant defense induction against aboveground herbivores.

5. Conclusions

Collectively, our data support the hypothesis that beneficial soil microbes, as well as the plant strengthener acibenzolar-S-methyl, alter tomato responses to the detriment of the mite population. The putative mechanisms involved should be further explored to assess the extent to which these mechanisms may involve defense induction, priming or plant growth promotion. Our experiments were conducted with tomato plants in pots in sterilized peat under controlled conditions; hence, further experiments in the greenhouse/field could provide additional evidence for the effectiveness of the tested microbes and the plant strengthener in shaping plant–herbivore interactions. The number of spider mite individuals (45 females/plant) used in our experiments to infest plants may resemble the early infestation events when spider mites begin to colonize plants. Accordingly, a previous study suggests an action threshold level of eight mites per leaflet on a second or third recently expanded tomato leaf to avoid yield losses by *T. urticae* [62]. Ultimately, the net benefit of the tested elicitors for the plant and their potential as novel tools in pest control should be confirmed by studying their effects on plant fitness and reproductive output.

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Σέρκος Χαρουτουνιάν

Πρόεδρος του ΕΛΓΟ - ΔΗΜΗΤΡΑ

«Η μετάβαση στην ψηφιακή εποχή πρέπει να πετύχει»



ΑΦΙΕΡΩΜΑΤΑ

Γεωργία ακριβείας: Μηχανήματα - εφαρμογές και drone (Σ.ΜΗ.Ε.Α.)



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ΓΕΩΡΓΙΑ

Μικροοργανισμοί βελτιώνουν την άμυνα της τομάτας

Το ερευνητικό έργο BeMOST αναπτύσσει μια καινοτόμα στρατηγική για την αντιμετώπιση φυτοφάγων εχθρών της τομάτας



Άρθρο της Μαρίας Λ. Παππά, αναπληρώτριας καθηγήτριας του Δημοκρίτειου Πανεπιστημίου Θράκης, επιστημονικής υπεύθυνης του έργου BeMOST

ία από τις μεγαλύτερες προκλήσεις στον τομέα της γεωργικής παραγωγής είναι η ανάπτυξη ενός αειφόρου μοντέλου παραγωγής, μειωμένων εισροών, που θα εξασφαλίζει την κάλυψη των επισιτιστικών αναγκών ενός ραγδαία αυξανόμενου ανθρώπινου πληθυσμού.

Στο πλαίσιο αυτό, σημαντική παράμετρο αποτελεί η προκαλούμενη απώλεια παραγωγής από φυτοφάγα είδη εχθρών. Για την αντιμετώπιση των εχθρών των καλλιεργειών, η χρήση χημικών παρασιτοκτόνων, παρά τις αρνητικές επιδράσεις τους στο περιβάλλον, συνεχίζει να αποτελεί το πλέον αποτελεσματικό μέσο φυτοπροστασίας. Ωστόσο, οι απαιτήσεις των καταναλωτών για τρόφιμα υψηλής ποιότητας, απαλλαγμένα από υπολείμματα γεωργικών φυτοπροστατευτικών προϊόντων, καθιστούν αναγκαία την ανάπτυξη συγχρόνων συστημάτων παραγωγής μειωμένων εισροών.

Το ερευνητικό έργο BeMOST, με τίτλο «Ωφέλιμοι μικροοργανισμοί της ριζόσφαιρας για την αντιμετώπιση σημαντικών εχθρών της τομάτας», στοχεύει σε μια καινοτόμο προσέγγιση βιολογικής φυτοπροστασίας, αξιοποιώντας ωφέλιμους μικροοργανισμούς για την προστασία των καλλιεργειών από φυτοφάγους εχθρούς μέσω της ενεργοποίησης της άμυνας των φυτών.

Φορέας υλοποίησης του έργου είναι το Δημοκρίτειο Πανεπιστήμιο Θράκης, με συνεργαζόμενους φορείς το Πανεπιστήμιο



Θεσσαλίας, το University of Hohenheim και το German Centre for Integrative Biodiversity Research (iDiv).

Άμυνα των φυτών

Τα φυτά αντιδρούν στην τροφική δραστηριότητα φυτοφάγων εχθρών τους μέσω της ενεργοποίησης άμεσων και έμμεσων μηχανισμών άμυνας. Πολλοί από τους παραπάνω μηχανισμούς ξεκινούν με την αναγνώριση του εχθρού και τη σηματοδότηση της άμυνας, οδηγώντας, για παράδειγμα, στην παραγωγή αμυντικών ενώσεων που επηρεάζουν αρνητικά τον φυτοφάγο εχθρό.

Ένας σημαντικός αριθμός μικροοργανισμών της ριζόσφαιρας είναι γνωστό ότι έχουν την ικανότητα να ανταγωνίζονται παθογόνους μικροοργανισμούς του εδάφους, να βελτιώνουν την ανάπτυξη των φυτών και να ενισχύουν το ανοσοποιητικό σύστημα των φυτών έναντι μελλοντικών προσβολών. Ενδιαφέρον παρουσιάζει το γεγονός ότι ορισμένοι ωφέλιμοι μικροοργανισμοί της ριζόσφαιρας –όπως είναι μυκόρριζες, ενδοφυτικοί μύκητες και ριζοβακτήρια που προάγουν την ανάπτυξη των φυτών– προκαλούν στα φυτά την επαγωγή αποκρίσεων άμυνας παρόμοιων με αυτές που αναπτύσσουν έναντι των εχθρών τους.

Ωστόσο, έως σήμερα, έχουμε μόνο περιορισμένα και αποσπασματικά δεδομένα σχετικά με τις επιδράσεις ωφέλιμων μικροοργανισμών στην επαγόμενη άμυνα έναντι φυτοφάγων εχθρών σε καλλιεργούμενα φυτικά είδη. Επιπλέον, οι γνώσεις μας σχετικά με τους ρυθμιστικούς μηχανισμούς, στους οποίους βασίζονται αυτές οι αλληλεπιδράσεις, είναι περιορισμένες.

Αντικείμενο του έργου

Το ερευνητικό έργο BeMOST προωθεί την κατανόηση των πολύπλοκων άμεσων και έμμεσων βιοτικών αλληλεπιδράσεων που σχετίζονται με την επαγωγή αποκρίσεων άμυνας των φυτών σε μία από τις σημαντικότερες καλλιέργειες λαχανικών (τόσο σε ευρωπαϊκό όσο και σε παγκόσμιο επίπεδο), την τομάτα.

Στο έργο περιλαμβάνονται επιζήμια είδη εχθρών της τομάτας, όπως είναι ο κοινός τε-

Απεικόνιση του ερευνητικού έργου BeMOST

τράνυχος (Tetranychus urticae), το λεπιδόπτερο (Tuta absoluta), ο αλευρώδης (Trialeurodes vaporariorum), ο θρίπας (Frankliniella occidentalis) και η αφίδα (Myzus persicae). Επιπλέον, μελετώνται οι επιδράσεις υποσχόμενων μικροοργανισμών στους φυσικούς τους εχθρούς, όπως σε αρπακτικά και παρασιτοειδή.

Οι κύριοι σκοποί του έργου είναι η διερεύνηση του βαθμού στον οποίο η ενεργοποίηση των μηχανισμών άμυνας των φυτών από ωφέλιμους μικροοργανισμούς της ριζόσφαιρας μπορεί να επηρεάσει φυτοφάγα είδη αρθροπόδων, τους φυσικούς τους εχθρούς, καθώς και την απόδοση των φυτών με πειράματα εργαστηρίου και θερμοκηπίου, για τη βελτίωση της βιολογικής αντιμετώπισης εχθρών των καλλιεργειών.

Ειδικότεροι στόχοι του έργου είναι: a) ο εντοπισμός ωφέλιμων μικροοργανισμών της ριζόσφαιρας που επάγουν την αντοχή της τομάτας σε επιζήμιους φυτοφάγους εχθρούς, β) η κατανόηση σε μοριακό και βιοχημικό επίπεδο των μηχανισμών που εμπλέκονται στις αλληλεπιδράσεις φυτών, μικροοργανισμών και φυτοφάγων εχθρών, και γ) η ανάπτυξη νέων στρατηγικών βιολογικής αντιμετώπισης εχθρών των καλλιεργειών με την αξιοποίηση κατάλληλων στελεχών μικροοργανισμών της ριζόσφαιρας.

Οφέλη και καινοτομία

Η ενεργοποίηση του αμυντικού συστήματος των φυτών από ωφέλιμους μικροοργανισμούς της ριζόσφαιρας αποτελεί καινοτόμο στρατηγική στη βιολογική αντιμετώπιση φυτοφάγων εχθρών. Ορισμένοι μόνο μικροοργανισμοί διατίθενται σήμερα σε εμπορική κλίμακα, κυρίως ως ρυθμιστές της ανάπτυξης των φυτών ή βιομυκητοκτόνα, ενώ οι γνώσεις μας σχετικά με τις επιδράσεις τους σε φυτοφάγους εχθρούς είναι περιορισμένες.

Το BeMOST αξιολογεί την αποτελεσματικότητα επιλεγμένων μικροοργανισμών στον έλεγχο σημαντικών εχθρών, θέτοντας τις βάσεις για την ανάπτυξη νέων προϊόντων και την ανάδειξη σημαντικών άγνωστων δράσεων εμπορικών μικροβιακών σκευασμάτων στην αύξηση της αντοχής των φυτών έναντι εχθρών, όπως και ενώσεων και γονιδίων που σχετίζονται με την επαγόμενη από μικροοργανισμούς αυξημένη αντοχή των φυτών σε φυτοφάγους εχθρούς.

Η χρήση ωφέλιμων μικροοργανισμών που βελτιώνουν την ανάπτυξη και ενισχύουν τους μηχανισμούς άμυνας των φυτών θα συμβάλει στη βελτίωση της βιολογικής κα-



ΑΡΙΣΤΕΡΑ: Πειραματικά φυτά τομάτας. ΔΕΞΙΑ: Φυτά τομάτας με έντονη προσβολή από τετράνυχο.

Το έργο BeMOST αναμένεται να αναδείξει μικροοργανισμούς που ενισχύουν την άμυνα της τομάτας έναντι φυτοφάγων εχθρών

ταπολέμησης εχθρών μεγάλης οικονομικής σημασίας, μειώνοντας αφενός την εξάρτηση των παραγωγών από τη χρήση συνθετικών φυτοπροστατευτικών προϊόντων και αφετέρου το αντίστοιχο κόστος στην τομάτα και σε άλλες καλλιέργειες.

Πρόοδος του έργου

Το έργο BeMOST διανύει το τρίτο έτος της συνολικής του διάρκειας. Στο χρονικό αυτό διάστημα έχουν πραγματοποιηθεί πειράματα εργαστηρίου στα οποία δοκιμάστηκε σημαντικός αριθμός μυκήτων και βακτηρίων με την ανάδειξη αρκετών πολλά υποσχόμενων μικροοργανισμών έναντι φυτοφάγων εχθρών της τομάτας. Μεταξύ αυτών αναδείχθηκαν δύο μύκητες και ένα βακτήριο, για τους οποίους συνεχίζεται η μελέτη των εμπλεκόμενων μηχανισμών. Συγκεκριμένα γίνονται αναλύσεις έκφρασης των γονιδίων και συσσώρευσης φυτοορμονών, ενζύμων και μεταβολιτών.

Επιπλέον, οι πολλά υποσχόμενοι μικροοργανισμοί αξιολογήθηκαν ως προς τις επιδράσεις τους σε φυσικούς εχθρούς των φυτοφάγων, όπως είναι αρπακτικά έντομα και ακάρεα, καθώς και παρασιτοειδή έντομα. Σε εξέλιξη βρίσκονται πειράματα θερμοκηπίου στα οποία μελετάται η επίδραση μικροοργανισμών με ριζοπότισμα στη δυναμική των πληθυσμών σημαντικών φυτοφάγων εχθρών της τομάτας. Με την ολοκλήρωση του έργου, πέραν του εντοπισμού μικροοργανισμών που επηρεάζουν αρνητικά τους φυτοφάγους εχθρούς στην τομάτα, θα έχουν αναδειχθεί και ενώσεις φυτικής προέλευσης με αποτελεσματικότητα στην ενίσχυση της άμεσης και έμμεσης άμυνας της τομάτας έναντι των εχθρών της.

Υποστήριξη από το ΕΛ.ΙΔ.Ε.Κ.

Το ερευνητικό έργο BeMOST υποστηρίζεται από το Ελληνικό Ίδρυμα Έρευνας και Καινοτομίας (ΕΛ.ΙΔ.Ε.Κ.), στο πλαίσιο της Δράσης «1η Προκήρυξη ερευνητικών έργων ΕΛ.ΙΔ.Ε.Κ. για την ενίσχυση των μελών ΔΕΠ και Ερευνητών/τριών και την προμήθεια ερευνητικού εξοπλισμού μεγάλης αξίας» (αριθμός έργου: 50).



Spider mites perform worse on soil microbe-inoculated plants: from the lab to the greenhouse

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Abstract: Spider mites are key pests in several crops causing significant yield losses. Specifically, the two spotted spider mite *Tetranychus urticae*, is a polyphagous pest which is usually controlled in Integrated Pest Management (IPM) schemes with biological control i.e. the release of predators and application of chemicals. In this context, beneficial soil microbes are applied as biofungicides or plant growth promoting agents. Herein, we explored the plant-mediated effects of a series of beneficial soil fungi and bacteria on spider mites infesting tomato plants aboveground. Experiments were conducted in the lab to assess short-term effects on spider mite performance. Subsequently, promising microbes were assessed for their effects on the population dynamics of the mites with greenhouse experiments. Our results show that inoculating tomato plants with microbes can result in decreased spider mite performance both in the short- and the long-term. Furthermore, biological control with the release of the zoophytophagous predator *Macrolophus pygmaeus* resulted in a stronger reduction in the number of spider mites and eggs, in inoculated plants. Overall, our study highlights the role of beneficial soil microbes in shaping plant-mite interactions to the benefit of the plant.

Key words: microbes, performance, population dynamics, *Tetranychus* sp., tomato.

Summary: Tomato is one of the most important vegetable crops worldwide. It is attacked by several arthropod pests which are distributed worldwide on several vegetable and horticultural crops with spider mites alone, causing more than 1 billion euro of damage in horti- and agriculture yearly (Bolland et al. 1998). Although biological control against spider mites is well-developed in other vegetable crops, these are still a severe threat in tomato and pesticides need to be frequently applied eventually, leading to resistance development, environmental pollution, and health and societal negative impacts (Van Leeuwen et al. 2010). Aiming at increasing the biocontrol toolbox to sustainably suppress tomato pests, assessing the role of beneficial soil microbes against aboveground pests is in line with European environmental and economic policies that request for environmentally friendly strategies to combat pests and reduce chemical inputs to agricultural cropping systems (EU 128/2009/EC 2009). Particularly, because beneficial soil microbes such as plant growth promoting fungi, mycorrhizae and rhizobacteria are known to antagonize soil pathogens, to improve plant growth, and to prime the plant immune system against future attackers (Pineda et al., 2013; Pieterse et al., 2014; Finkel et al., 2017).

Towards this direction, we assessed a series of fungal and bacterial strains for their plantmediated effects on spider mite performance with lab experiments. Plants were inoculated with the beneficial microbe and then infested with a standard number of pest individuals. Differences in oviposition, and survival among microbe-inoculated and control plants were recorded. Each beneficial microbe was applied in sterilized peat where tomato (cv. Moneymaker) plants in pots had been growing. The plants were transplanted and after 2 days were inoculated with the microbe under study. After another 21 days, the plants were infested with 45 T. urticae females per plant for 4 days. Afterwards, survival as well as number of eggs laid were recorded. According to the analyses of the results, we concluded on the microbes Bacillus amyloliquefaciens QST763 and Trichoderma harzianum T-22 to proceed with the greenhouse experiments on the population dynamics of spider mites since both were shown to perform very well with regard to their plant protection capabilities. Each beneficial microbe was applied in peat where tomato plants in pots had been growing. In these experiments, an additional treatment of combined application of beneficial microbes and a natural enemy (M. pygmaeus) was included. The plants were transplanted in the greenhouse and after 2 days were inoculated with the microbes. After another 21 days, the plants were infested with spider mites (10 females/plant). Two weeks later, we released four *M. pygmaeus* females per plant and after one week we sampled plants to record the population dynamics of both the herbivore and the predator for another six weeks. At each sampling point, the number of mites and eggs on each plant were recorded, as well as the number of live predators (adults and nymphs). The results confirmed the significant effects of tomato inoculation with T. harzianum T-22 and B. amyloliquefaciens QST713, as depicted by the gradual decrease in spider mite populations. In addition, we recorded an increase in the number of the predators that was also shown to result in lower number of spider mite eggs and individuals when plants were inoculated with the microbes.



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Beneficial soil microbes and zoophytophagous predators as plant 'vaccination' agents against arthropod pests

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Abstract: Unable to run away from their enemies, plants are continuously challenged by biotic stressors. Besides herbivorous arthropods and pathogenic microorganisms, plant interactors also include beneficial organisms such as predators and microbes that can be promising biocontrol agents. Beneficial soil microbes in particular are known to elicit plant responses and therefore may be capable of protecting plants against herbivores. Similarly, beneficial arthropods such as zoophytophagous predators have been shown to elicit defense-related responses in plants impacting herbivores indirectly, via their phytophagy. On the other hand, beneficial soil microbes showing intimate relationships with plants may not only affect herbivores but also their natural enemies through the induction of plant defenses. Here, I explore promising opportunities for controlling pests on the basis of our current knowledge on parameters that determine plant defense. I specifically refer to soil microbes and zoophytophagous predators and address their use as plant 'vaccination' agents to prime plants against future attackers. Research on plant-arthropod-microbe interactions is relatively poor but necessary to identify beneficial interactions and further develop biocontrol strategies for sustainable crop production.

Key words: biological control, microbes, tomato, plant defense, predators

Extended Summary: Plants employ sophisticated mechanisms to defend themselves against herbivorous arthropods. These involve the expression of direct defenses such as toxins and antidigestive proteins as well as indirect defenses via the emission of plant volatiles to attract the natural enemies of pests (Karban and Baldwin, 1997; Dicke and Baldwin, 2010). Defense induction can occur after exposure of plants to herbivores but also, when plants are exposed to beneficial non-pathogenic organisms such as root-colonizing microbes for example, plant growth fungi, mycorrhizae and rhizobacteria which are known to antagonize soil pathogens, to improve plant growth, and to prime the plant immune system against future attackers (Pineda et al., 2013; Pieterse et al., 2014; Finkel et al., 2017). Importantly, other beneficial organisms such as mirid zoophytophagous predators have been shown to induce plant defenses in ways similar to pure herbivores (Pérez-Hedo et al., 2022). Studying such beneficial plant-arthropod-microbe interactions could result in the development of sustainable plant protection tools and strategies for the suppression of key arthropod pests of plants.

In this context, a number of case studies are presented focusing on the application of beneficial soil microbes and their plant-mediated effects on aboveground herbivores, as well as their effects on natural enemies of pests. Particularly, interactions between mirid predators, plants and microbes are studied to identify promising ones for biological pest control. For example, spider mites are shown to perform worse on tomato plants inoculated with the beneficial endophytic fungus, *Fusarium solani* strain K (FsK) via the alteration of tomato

defense responses against spider mites (Pappas et al., 2018). In addition, the attraction of FsKinoculated tomato plants to the mirid predator *Macrolophus pygmaeus* was enhanced compared to non-inoculated plants, while the mirid predator *Nesidiocoris tenuis* induced less necrotic rings via feeding on FsK-inoculated tomato plants (Garantonakis et al., 2018; Pappas et al., 2018). Finally, research gaps are explored to identify opportunities for the development of sustainable pest control tools via for example, the application of plant defense elicitors, metabolites or peptides to directly affect pest populations or the behavior of their natural enemies. Understanding the chemical and molecular mechanisms involved in successful plantmicrobe-arthropod interactions is crucial for the development of environmentally friendly alternatives to chemical control.



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Plant-mediated effects of beneficial soil microbes on natural enemies

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Abstract: Plants have evolved sophisticated mechanisms to defend themselves against herbivory. One such mechanism is the release of volatiles by infested plants which inform natural enemies about the presence of prey, eventually resulting in pest suppression. Plantpredator interactions can be affected by beneficial microbes living belowground as they are known to enhance the resistance of plants against pests. Nevertheless, the plant-mediated effects of soil microbes on the performance of natural enemies have not been widely studied. Here we studied whether beneficial soil microbes affect the biology and behavior of aboveground predators and parasitoids. We recorded the survival, offspring production, prey consumption/parasitism, as well as the behavioral responses of two mirids, a phytoseiid predator and a parasitoid as affected by the inoculation of tomato plants with beneficial microbes. Our results highlight the variable effects of microbes on natural enemies depending on the microbe, the herbivore and the natural enemy species studied.

Key words: biological control, microbes, natural enemies, plant defense, tomato

Extended Summary: Plants interact with various pathogenic and beneficial organisms that are found above- or belowground. To defend themselves against harmful organisms, such as herbivorous arthropods, plants employ a series of constitutive and inducible defences which target directly the pest or indirectly by attracting natural enemies (Karban and Baldwin, 1997; Schaller, 2008). The latter can be facilitated by the release of volatiles that inform predators about prey availability on infested plants (Kessler and Baldwin, 2001; Dicke et al., 2010), therefore increasing the numbers of natural enemies landing on plants to feed on pests and suppress their populations. On the other hand, beneficial interactions between plants and predators may be facilitated by organisms living belowground such as beneficial microbes. These are of particular interest as they are known to enhance plant responses against pathogens and herbivorous pests (Pineda et al., 2013; Pieterse et al., 2014; Finkel et al., 2017). Nevertheless, their impact on the ability of plants to attract predators has not been widely studied.

In the present study, we assessed the plant-mediated effects of two beneficial microbes, the fungus *Trichoderma harzianum* T22 and the bacterium *Bacillus amyloliquefaciens* QST713 on the performance and behavior of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis*, the predatory mite *Amblyseius swirskii* and the parasitoid *Encarsia formosa* in tomato. We hypothesized that the tested microbes would alter tomato plant-mediated responses and thus affect the performance of the natural enemies aboveground. Three to four weeks old experimental plants were grown from seeds that were surface-sterilized and sown in pots, each containing sterilized peat. Microbe inoculation was performed at the recommended

dose for field application two days after transplantation. After 2 weeks, plants were infested with herbivore individuals and after one week, individuals each predator (M. pygmaeus, N. tenuis and A. swirskii) were transferred on the plants for a period of 5 and 14 days, respectively and their survival and nymph production were recorded on inoculated plants as compared to non-inoculated plants. In addition, the effects of plant inoculation with microbes on the predation of M. pygmaeus, N. tenuis, and A. swirskii on different species of herbivorous pests, as well as the parasitism efficiency of E. formosa were studied. Finally, we performed olfactometer experiments giving a choice to the natural enemies between all different combinations of plants infested with different herbivores and inoculated with soil microbes or not.

Our results show that tomato inoculation with soil microbes can affect its indirect defenses provided by natural enemies, with survival, offspring production, and prey consumption or parasitism being significantly affected, depending on the microbe and species of prey. Furthermore, tomato inoculation with microbes can enhance the attractiveness of the plants to natural enemies as compared to non-inoculated plants. In this context, we are currently analyzing the volatile blend emitted from the headspace of inoculated plants and testing specific volatile compounds for their effects on the behavior of natural enemies. Furthermore, we are assessing the effects of microbes on the performance of natural enemies under greenhouse conditions.



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Plant-mediated effects of beneficial soil microbes against arthropod pests

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Rooted and unable to flee, plants often interact with both arthropods and microbes. Besides pathogens that cause diseases in crops, plant interactors also include beneficial microbes. Certain soil microbes in particular are known for their ability to improve plant growth, antagonize pathogens and prime plants against future attacks via the elicitation of plant defense responses. Hence, they might also be capable of protecting plants against herbivores and serve as promising biological control agents in Integrated Pest Management programs. The aim of the present study was to assess the plant-mediated effects of several commercial and lab-owned microbial strains against tomato pests such as spider mites, whiteflies and aphids. We found that herbivore performance can be substantially hampered on microbe-inoculated plants as compared to uncolonized plants. Furthermore, we assessed the impact of microbe inoculation on plant performance. Our results will help in understanding tomato-arthropod-microbe interactions, also in developing biocontrol strategies for sustainable pest control.

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The hidden role of beneficial soil microbes against spider mites, whiteflies and thrips in tomato

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Plant-growth promoting fungi and rhizobacteria have shown potential in protecting plants against pathogens. Nevertheless, our knowledge on their effects against herbivorous pests remains largely unknown. We assessed the plant-mediated effects of soil microbes against tomato pests, specifically the mite *Tetranychus urticae*, the whitefly *Trialeurodes vaporariorum* and the thrips *Frankliniella occidentalis*. Context-dependency was addressed by recording the effects of microbes in different tomato cultivars. We show that herbivore performance can be affected by soil microbes and that variation can be recorded among cultivars. Overall, we highlight the role of soil microbes as biocontrol agents in suppressing herbivore populations in tomato, possibly via the induction of plant defenses.

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Beneficial soil microbes to enhance tomato resistance against arthropod pests

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Soil-borne beneficial microbes have been recognized for their ability to improve plant growth, antagonize pathogens and prime plant immunity against future attackers. Among root-colonizing microbes, plant-growth promoting fungi and rhizobacteria have shown potential in protecting plants not only against pathogens but also aboveground herbivores via the elicitation of systemic defense responses. Nevertheless, current knowledge on the effects of beneficial soil microbes against certain herbivorous pests such as key pests of tomato remains limited. In this study, we present a synthesis of our findings on the impact of a series of soil fungi and bacteria on enhancing direct resistance against key tomato pests, namely the two-spotted spider mite, greenhouse whitefly, green peach aphid, western flowers thrips and tomato stem borer. Biological control with the use of predatory insects and mites represents an effective alternative to chemicals. Here, we also explore the effects of soil microbes on indirect tomato resistance to herbivorous pests. Tomato inoculation with microbes is shown to differentially affect the studied herbivores as well as their natural enemies. Our results highlight the potential of beneficial soil microbes in pest control and the necessity to understand the molecular and chemical mechanisms underlying their plant-mediated effects.

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Επιδράσεις ωφέλιμων μικροοργανισμών της ριζόσφαιρας στη συμπεριφορά φυσικών εχθρών

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Οι οργανισμοί με τους οποίους τα φυτά αλληλεπιδρούν περιλαμβάνουν μακρο- και μικροοργανισμούς που βρίσκονται στο υπέργειο ή το υπόγειο τμήμα τους. Για να αμυνθούν έναντι των επιβλαβών για αυτά οργανισμών, όπως τα φυτοφάγα αρθρόποδα, τα φυτά έχουν αναπτύξει μηχανισμούς που στοχεύουν αφενός στον να αποκρούσουν τους εχθρούς τους, αφετέρου να προσελκύσουν θηρευτές. Το τελευταίο επιτυγχάνεται με την έκλυση πτητικών ενώσεων που δηλώνουν την παρουσία ατόμων της λείας στα προσβεβλημένα φυτά, αυξάνοντας τον αριθμό των φυσικών εχθρών που προσελκύονται σε αυτά προκειμένου να τραφούν με τους φυτοφάγους εχθρούς και να καταστείλουν τους πληθυσμούς τους. Από την άλλη πλευρά, οι ωφέλιμες αλληλεπιδράσεις μεταξύ φυτών και αρπακτικών μπορεί να επηρεαστούν από μικροοργανισμούς της ριζόσφαιρας. Οι οργανισμοί αυτοί παρουσιάζουν ιδιαίτερο ενδιαφέρον καθώς είναι γνωστό ότι ενισχύουν τις αποκρίσεις των φυτών έναντι παθογόνων μικροοργανισμών και φυτοφάγων εχθρών. Ωστόσο, οι επιδράσεις τους στην ικανότητα των φυτών να προσελκύουν αρπακτικά δεν έχει διερευνηθεί σε μεγάλο βαθμό. Στην εργασία αυτή, παρουσιάζουμε τα αποτελέσματα πειραμάτων ολφακτομέτρου που πραγματοποιήθηκαν με φυσικούς εχθρούς, συγκεκριμένα ενήλικα άτομα των αρπακτικών εντόμων Macrolophus pygmaeus Rambur (Hemiptera: Miridae) και Nesidiocoris tenuis Reuter (Hemiptera: Miridae), του ακάρεως Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) και του παρασιτοειδούς Encarsia formosa Gahan (Hymenoptera: Aphelinidae) στα οποία δόθηκε η επιλογή ανάμεσα σε όλους τους διαφορετικούς συνδυασμούς φυτών που είχαν προσβληθεί με φυτοφάγους εχθρούς και είχαν εμβολιαστεί ή όχι με επιλεγμένους ωφέλιμους μικροοργανισμούς της ριζόσφαιρας. Από τα αποτελέσματα φαίνεται ότι, η ελκυστικότητα εμβολιασμένων με μικροοργανισμούς φυτών τομάτας παραλλάσει ανάλογα με το είδος τόσο του φυτοφάγου όσο και του φυσικού εχθρού. Συνολικά, τα αποτελέσματά μας μπορεί να είναι χρήσιμα στην ανάπτυξη νεών αειφόρων εργαλείων αντιμετώπισης εχθρών των καλλιεργειών και αναδεικνύουν την ανάγκη η μελέτη των αλληλεπιδράσεων εντόμων-φυτών να τοποθετούνται στο ευρύτερο πλαίσιο της βιοκοινότητας.

Λέξεις-κλειδιά: βακτήρια, βιολογική καταπολέμηση, μύκητες, φυσικοί εχθροί, φυτική άμυνα



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Επιδράσεις μικροοργανισμών της ριζόσφαιρας στη βιολογία φυσικών εχθρών

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Για να αμυνθούν έναντι των φυτοφάγων εχθρών τους, τα φυτά έχουν αναπτύξει πολύπλοκους μηχανισμούς που περιλαμβάνουν μια σειρά από προϋπάρχουσες και επαγώμενες αμυντικές αποκρίσεις. Η έκλυση πτητικών ενώσεων είναι μία τέτοια απόκριση που στοχεύει στην προσέλκυση φυσικών εχθρών σε προσβεβλημένα φυτά για την ανεύρεση λείας. Ωστόσο, άλλες αποκρίσεις των φυτών, όπως αυτές που επάγωνται από ωφέλιμους μικροοργανισμούς της ριζόσφαιρας μπορεί να είναι επιβλαβείς για τους φυσικούς εχθρούς, εάν για παράδειγμα τα φυτά δεν είναι σε θέση να «αναγνωρίσουν» τα ωφέλιμα αρθρόποδα από τους φυτοφάγους εχθρούς ή εάν τα αρπακτικά καταναλώνουν άτομα λείας που αναπτύσσονται σε φυτά στα οποία έχουν επαχθεί οι μηχανισμοί άμυνας. Στην εργασία αυτή, εξετάσαμε κατά πόσον ωφέλιμοι μικροοργανισμοί της ριζόσφαιρας θα μπορούσαν να επηρεάσουν χαρακτηριστικά της βιολογίας αρπακτικών και παρασιτοειδών. Για τον σκοπό αυτό, καταγράψαμε την επιβίωση και την παραγωγή απογόνων των αρπακτικών Macrolophus pygmaeus Rambur (Hemiptera: Miridae), Nesidiocoris tenuis Reuter (Hemiptera: Miridae) ka Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) σε φυτά που είχαν εμβολιαστεί ή όχι με μικροργανισμούς της ριζόσφαιρας και στη συνέχεια προσβληθεί ή όχι με διαφορετικούς φυτοφάγους εχθρούς. Επιπλέον, κατανάλωση αρπακτικά, αξιολογήσαμε тпу λείας από тα καθώς και τηv αποτελεσματικότητα παρασιτισμού Encarsia formosa Gahan (Hymenoptera: TOU Aphelinidae). Τα αποτελέσματά μας δείχνουν ότι, οι επιδράσεις του εμβολιασμού φυτών τομάτας με μικροργανισμούς της ριζόσφαιρας στους φυσικούς εχθρούς παραλλάσουν μεταξύ των διαφορετικών ειδών, ανάλογα με το είδος τόσο του μικροοργανισμού όσο και του φυτοφάγου. Συνολικά, υπογραμμίζουμε τη σημασία της μελέτης των επιδράσεων μικροοργανισμών της ριζόσφαιρας σε φυσικούς εχθρούς για τον εντοπισμό αειφόρων εργαλείων αντιμετώπισης των εχθρών των καλλιεργειών.

Λέξεις-κλειδιά: αρθρόποδα, αρπακτικά, βιολογική καταπολέμηση, μικροοργανισμοί, φυτική άμυνα, τομάτα



Το ερευνητικό έργο υποστηρίχτηκε από το Ελληνικό Ίδρυμα Έρευνας και Καινοτομίας (ΕΛ.ΙΔ.Ε.Κ.) στο πλαίσιο της Δράσης «1η Προκήρυξη ερευνητικών έργων ΕΛ.ΙΔ.Ε.Κ. για την ενίσχυση των μελών ΔΕΠ και Ερευνητών/τριών και την προμήθεια ερευνητικού εξοπλισμού μεγάλης αξίας» (Αριθμός Έργου: 50). Επιδράσεις ωφέλιμων μικροοργανισμών της ριζόσφαιρας σε φυτοφάγους εχθρούς

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Τα φυτά συχνά καλούνται να αντιμετωπίσουν βιοτικούς στρεσογόνους παράγοντες, μεταξύ αυτών φυτοφάγα αρθρόποδα και παθογόνους μικροοργανισμούς. Ωστόσο, αλληλεπιδρούν και με ωφέλιμους οργανισμούς όπως αρπακτικά έντομα και μικροοργανισμούς. Συγκεκριμένα, ορισμένα μικροοργανισμοί της ριζόσφαιρας είναι γνωστοί για την ικανότητά τους να βελτιώνουν την ανάπτυξη των φυτών, να ανταγωνίζονται παθογόνα και να προετοιμάζουν τα φυτά έναντι μελλοντικών προκλήσεων μέσω της επαγωγής αποκρίσεων φυτικής άμυνας. Επομένως, παρουσιάζουν ενδιαφέρον για χρήση στη φυτοπροστασία ως παράγοντες βιολογικής καταπολέμησης σε προγράμματα ολοκληρωμένης διαχείρισης εχθρών. Σε αυτήν την εργασία, αξιολογήσαμε τις επιδράσεις μέσω του φυτού μιας σειράς ωφέλιμων μυκήτων και βακτηρίων της ριζόσφαιρας έναντι κύριων εχθρών της τομάτας που ανήκουν σε διαφορετικές συντεχνίες και τύπους διατροφής, όπως τα είδη Tetranychus urticae Koch (Acari: Tetranychidae), Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae), Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) και Myzus persicae (Sulzer) (Hemiptera: Aphididae). Συγκεκριμένα, έγινε καταγραφή χαρακτηριστικών της βιολογίας, όπως η επιβίωση, η ταχύτητα ανάπτυξης και η ωοπαραγωγή αναλόγως του είδος του εχθρού σε εμβολιασμένα φυτά σε σύγκριση με τα φυτά του μάρτυρα. Επιπλέον, έγινε καταγραφή των επιδράσεων επιλεγμένων μικροοργανισμών σε διαφορετικές ποικιλίες τομάτας. Τα αποτελέσματά μας δείχνουν ότι, ο εμβολιασμός φυτών με διαφορετικά βακτηριακά και μυκητιακά στελέχη επηρεάζει τους πληθυσμούς των φυτοφάγων εχθρών μέσω του φυτού. Ωστόσο, καταγράψαμε σημαντική παραλλακτικότητα σε αυτές τις επιδράσεις ανάλογα με τους μικροοργανισμούς καθώς και τα είδη φυτοφάγων που μελετήθηκαν. Συνολικά, η εργασία μας αναδεικνύει τον ρόλο των μικροοργανισμών της ριζόσφαιρας ως παραγόντων βιολογικής καταπολέμησης στην καταστολή των πληθυσμών φυτοφάγων εχθρών, πιθανά μέσω της επαγωγής αποκρίσεων άμυνας των φυτών.

Λέξεις-κλειδιά: αρθρόποδα, βακτήρια, βιολογική καταπολέμηση, μύκητες, φυτική άμυνα, τομάτα



Το ερευνητικό έργο υποστηρίχτηκε από το Ελληνικό Ίδρυμα Έρευνας και Καινοτομίας (ΕΛ.ΙΔ.Ε.Κ.) στο πλαίσιο της Δράσης «1η Προκήρυξη ερευνητικών έργων ΕΛ.ΙΔ.Ε.Κ. για την ενίσχυση των μελών ΔΕΠ και Ερευνητών/τριών και την προμήθεια ερευνητικού εξοπλισμού μεγάλης αξίας» (Αριθμός Έργου: 50).

Beneficial soil microbe-mediated tomato responses against spider mites

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Soil microbes are known to be capable of protecting plants against herbivores via the elicitation of plant defense responses. However, little is known on their effects in shaping plant-mite interactions. Herein, we assessed the effects of several fungal and bacterial strains on the performance of the spider mite *Tetranychus urticae* (TSSM). TSSM performance was shown to be negatively affected on inoculated plants. We also evaluated the role of the most promising microbes in altering gene expression in response to TSSM and found evidence for the induction of defenses in inoculated plants. Our results highlight the potential of soil microbes against mites in sustainable crop production.

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Keywords: Solanum, plant defense, Tetranychidae, mite pest, Phytoseiidae, biological control

OC131. Induced resistance by arbuscular mycorrhizal fungi in tomato: a new tool for integrated pest management programs

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Beneficial soil microorganisms can boost plant defences increasing their resistance to herbivores. Our research has revealed the contribution of arbuscular mycorrhizal fungi to improve plant direct and indirect defences against chewing herbivores in tomato. Mycorrhizal colonization in tomato reduced the performance of the generalist chewer *Spodoptera exigua* and the specialist leafminer *Tuta absoluta*. The reduction was associated to a primed accumulation of antiherbivore metabolites in challenged leaves including alkaloids and polyamine conjugates. Moreover, the volatile blends in mycorrhizal and non mycorrhizal plants differ, and enhanced attraction of natural enemies of the pests-commonly used in biocontrol programs- have been observed in challenged mycorrhizal plants. Comparisons across different experimental scales from controlled lab set-ups to commercial production conditions evidenced the robustness of the effects, the compatibility with other biocontrol methods, and accordingly, the potential of mycorrhiza induced resistance to be incorporated in current Integrated Pest Management Programs.

Keywords: *Tuta absoluta*, tomato, primed defenses, Nesidiocoris tenuis, HIPVs, multitrophic interactions

OC132. Beneficial microbes to optimize pest control in sustainable tomato production

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Plants are continuously challenged by biotic stressors such as herbivorous arthropods and pathogenic microbes. Nevertheless, plants also interact with beneficial organisms such as certain soil microbes which are known for their ability to improve plant growth, antagonize pathogens and prime plants against future attacks via plant defense elicitation. In this work, we hypothesized that beneficial soil microbes can protect plants also against herbivores hence, serve as biological control agents in Integrated Pest Management programs. We assessed the plant-mediated effects of a series of beneficial soil fungi and bacteria against key pests of tomato namely, *Tetranychus urticae, Trialeurodes vaporariorum, Frankliniella occidentalis, Tuta absoluta* and *Myzus persicae*, and their natural enemies, and studied the molecular and chemical mechanisms underlying beneficial microbe-tomato interactions which enhance tomato resistance against key pests. Our results identified promising bacterial and fungal strains with efficacy against tomato pests via the plant, as well as specific molecular and chemical components of tomato direct and indirect defense that were differentially affected by tomato inoculation with these microbes.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant" (Project Number: 50).

Keywords: arthropod pests, beneficial fungi, plant defense, predators, tomato

OC133. The multifaceted nature of the modulation of plant responses to insect herbivory by beneficial microbes

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Inoculation of plants with beneficial microbes is increasingly recognized as a powerful tool to enhance plant defense against insect pests. Arbuscular Mycorrhizal (AM) fungi can sensitize the plant's immune system, resulting in priming of defences against leaf-chewing insects. However, meta-analyses show that effects of AM fungi on plant defense range from increased resistance to increased susceptibility. We present two studies in ribwort plantain (*Plantago lanceolata*) showing that such contrasting effects may reflect ontogenetic changes during plant life, and unexpected ways in which AMF interfere with plant responses to herbivory.First, we show that the direction of effects of AMF on the leaf chewing insect *Mamestra brassicae* shifts during plant ontogeny. In young plants, AMF enhances leaf nutritional quality, increasing the insect's efficiency of conversion of ingested food (ECI). However, as plants age, effects of induction of



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Plant-mediated effects of beneficial soil microbes on herbivore populations in the greenhouse

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Plants have evolved sophisticated mechanisms to defend themselves against their enemies. Besides the latter, they also interact with beneficial organisms such as soil microbes and zoophytophagous predators which are known to prime plants against future attacks via plant defense elicitation. In this work, we assessed the plant-mediated effects of two beneficial soil microbes, namely *Trichoderma harzianum* T22 and *Bacillus amyloliquefaciens* QST713, shown previously to negatively affect herbivore performance in the lab, on the population dynamics of the two spotted spider mite *Tetranychus urticae* and the whitefly *Trialeurodes vaporariorum* with greenhouse experiments. Our results show that inoculating tomato plants with microbes can result in decreased herbivore performance in the greenhouse. Furthermore, biological control with the release of mirid predators was not affected by microbial inoculation of the plants. Overall, our study highlights the added value of beneficial soil microbes in pest control as well as their compatibility with natural enemies.



The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-and the procurement of high-cost research equipment grant" (Project Number: 50).