DEFENSE REMODELLING BY ECTOMYCORRHIZAL FUNGI IN NON-HOSTS

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AFFIDAVIT

Hereby, I confirm that this dissertation entitled “Defense remodelling by ectomycorrhizal fungi in non-hosts” has been written independently and no other sources and aids were used than quoted.

Kishore Vishwanathan

Goettingen, July 31, 2019

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ABSTRACT

Root colonizing beneficial microorganisms promote growth of host plants and protect them against biotic threats. These microbes are considered as sustainable replacements for the hazardous chemical treatments in agriculture. Ectomycorrhizal fungi, a class of beneficial fungi colonizing trees of a certain genera, protects the host's systemic tissue from herbivory by priming defense genes and metabolites. Whether host-symbiotic interaction is a requisite for induced systemic resistance (ISR) has not been determined so far. This thesis aimed to investigate host specificity in ISR by studying the altercations in a non-mycorrhizal host, *Arabidopsis thaliana* (Col-0), against cabbage looper (*Trichoplusia ni*) after root treatment with an ectomycorrhizal fungus (*L. bicolor*).

To find out if mycorrhization is essential for ISR, Col-0 roots were treated with *L. bicolor* and the foliar tissue was challenged with *T. ni* larvae. Though there was no visible penetration of *L. bicolor* in Col-0 roots, ISR was triggered by negatively affecting *T. ni* weight gain. The expression of defense genes (*VSP*, *PDF1.2* or *PR1*) or phytohormone accumulation (JA, JA-Ile, ABA, SA) in Col-0 leaves was not significantly influenced by *L. bicolor*. However, *L. bicolor*-triggered ISR was attributed by increasing the accumulation of the secondary metabolite, camalexin, in systemic leaves. Further characterization of the ISR signalling pathway using *Arabidopsis* mutants, showed that *L. bicolor*-induced ISR is dependent on the *CYP79B2/B3* and *CYP81F2* of the tryptophan pathway, jasmonic acid signalling *via COI1* and salicylic acid signalling *via SID2* and *NPR3/4* and the iron homeostasis regulator, *MYB72*.

Unlike other beneficial microbes that suppress basal plant defenses, *L. bicolor* induced oxidative burst and activated the mitogen-activated protein kinase signalling cascade in Col-0. Also, Col-0 root treatment with heat-killed *L. bicolor* induced ISR against the larvae. These observations indicated that cell wall component(s) of *L. bicolor* can trigger ISR. The fungal cell wall component, chitin, when treated on Col-0 roots, reduced *T. ni* growth by 38% and *L. bicolor*-triggered ISR was dependent on the chitin receptor, *CERK1*. Moreover, at the expense of triggering ISR against herbivory, chitin induced susceptibility in Col-0 against the hemibiotrophic pathogen, *Pseudomonas syringae*. Data from this thesis highlights that host adaptability or symbiotic association is not necessary for altering systemic defense responses. Screening other microbial patterns with hosts and non-hosts can provide us answers whether ISR is a broadly conserved mechanism in systemic plant immunity.

ABBREVIATIONS

ABA	Abscisic acid
ABA-GE	(+)-abscisic acid D-glucopyranosyl ester
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
AOS	Allene oxide synthase
AP	Alkaline phosphatase
APS	Ammonium persulphate
Arabidopsis	*Arabidopsis thaliana*
BAK	*BRI1* associated receptor kinase
BGLU42	β-glucosidase
bp	Base pairs
BR	Brassinosteroid
BRI	BR insensitive
BSA	Bovine serum albumin
BTB-POZ	Protein-protein interaction motif
C	Celsius
CBP60g	60g Calmodulin-binding protein
cDNA	Complementary deoxy ribonucleic acid
CERK	Chitin elicitor receptor kinase
CFU	Colony forming units
cm	Centimetre
COI	Coronatine insensitive
Col-0	*Arabidopsis thaliana* wildtype Columbia-0
CSP	Common symbiotic signalling pathway
CT	Cycle threshold
CYP	Cytochrome P450
Da	Dalton
DAMP	Damage associated molecular pattern
ddH_2O	Double deionised water
DEG	Differentially expressed gene
dinor-OPDA	Dinor-12-oxo-phytodienoic acid

Abbreviations

DIR1	Defective in induced resistance 1
DNA	Deoxy ribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DP	Declustering potential
DTT	Dithiothreitol
D5-IAA	Indole-2,4,5,6,7-D5-3-acetic acid
EDTA	Ethylene-diamine-tetra-acetic acid
EFR	LRR-RLK receptor for Ef-Tu
Ef-Tu	Elongation factor
EIN	Ethylene insensitive
EMF	Ectomycorrhizal fungi
ERF	Ethylene response factor
ESI	Electrospray ionization
Et	Ethylene
et al.	And others
ETI	Effector triggered immunity
ETS	Effector triggered susceptibility
FIT	Fe-deficiency induced transcription factor
flg22	Flagellin peptide with 22 amino acids
FLS	Flagellin sensitive
FMO1	Flavin-dependent monooxygenase 1
FRO2	Ferric reduction oxidase 2
F6'H1	Feruloyl-CoA 6'-Hydroxylase1
g	Gram
GA	Gibberellic acid
GC	Gas chromatography
GST	Glutathione-S-Transferase
GUS	β-glucuronidase
HSD	Honest significant difference
IAA	Indole-3-acetic acid
IAR	IAA conjugate resistant
IC	Isochorismate
ICA	Indole-3-carboxylic acid

Abbreviations

ICS	Isochorismate synthase
ILL	IAA-Leucine resistant like gene
IRT1	Iron regulated transporter 1
ISR	Induced systemic resistance
ISS	Induced systemic susceptibility
JA	Jasmonic acid
JA-Ile	Jasmonic acid isoleucine
JA-Val	Jasmonic acid L-valine
JAZ	Jasmonate ZIM-domain protein
JIN	Jasmonate insensitive
L	Litre
LB	Luria-Bertani
L. bicolor	*Laccaria bicolor*
LC	Liquid chromatography
LCO	Lipochitooligosaccharide
log_2	Logarithmic base 2
log_{10}	Logarithmic base 10
LRR	Leucin rich repeat
LSD	Least significant difference
LTB	Lactophenol trypan blue
LYM	Lysin motif
m	Metre
mA	Milli-Ampere
MAMP	Microbe associated molecular pattern
MAPK	Mitogen activated protein kinases
MAPKK	MAPK kinase
MAPKKK	MAPKK kinase
MeJA	Methyl jasmonate
MES	2-(N-Morpholino)ethanesulfonic acid
mg	Milligram
$MgSO_4$	Magnesium sulphate
milliQ	Type 1 ultrapure
min	Minutes

Abbreviations

MiSSP7	Mycorrhiza induced small secreted protein 7
MiSSP8	Mycorrhiza induced small secreted protein 8
mm	Millimetre
mM	Millimolar
MMN	Modified Melin Norkrans
mmol	Millimole
MP	Milk powder
MS	Mass spectrometer
½MS (or) 1MS	Murshige and Skoog medium
MTBE	Methyl-tert-butyl ether
MYB	MYB family transcription factor
MYC	MYC transcription factor
Myc	Mycorrhization signal
n	Number
NB	Nucleotide binding
ng	Nanogram
NIM	Non-inducible immunity
NIMIN	NIM1 interacting
NINJA	Novel interactor of JAZ
nm	Nanometre
nM	Nanomolar
NPR1	Non-expressor of PR genes 1
NPR3	Non-expressor of PR genes 3
NPR4	Non-expressor of PR genes 4
Noco2	*Hyaloperonospora arabidopsis* Noco2 strain
Nod	Nodulation
OD_{600}	Optical density at 600 nm
OPC4	*S*-[2-[3-[[(2*R*)-4-[[[(2*R*,3*R*,5*R*)-5-(6-aminopurin-9-yl)-4-hydroxy-3-phosphonooxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]oxy-2-hydroxy-3,3-dimethylbutanoyl]amino]propanoylamino]ethyl]4-[(2*S*)-3-oxo-2-[(*Z*)-pent-2-enyl]cyclopentyl]butanethioate
OPDA	12-Oxo-phytodienoic acid

Abbreviations

ORA59	Octadecanoid responsive Arabidopsis 59
p	Probability value
p-	Promoter
PAGE	Polyacrylamide gel electrophoresis
PAL	Phenylalanine ammonia-lyase
PAMP	Pathogen associated molecular pattern
P. involutus	*Paxillus involutus*
PDF1.2	Plant defensin 1.2
PEN2	Penetration 2
PGPR	Plant growth promoting rhizobacteria
pH	Potential of Hydrogen
PR	Pathogenesis related
***Pst* DC3000**	*Pseudomonas syringae* pathovar Tomato DC3000
PTI	Pattern triggered immunity
pv	Pathovar
PVDF	Polyvinylidine difluoride
qRT-PCR	Real time quantitative reverse transcription polymerase chain reaction
R	Resistance
RA	Raphanusamic acid
RIN	RNA integrity number
RLK	Receptor like kinase
RLU	Relative luminescence units
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
s	Second
SA	Salicylic acid
SAG	Salicylic acid glucoside
SAR	Systemic acquired resistance
SARD	SAR deficient
SCF E3	SKP-Cullin-F box E3 ubiquitin ligase complex

Abbreviations

SD	Standard deviation
SDS	Sodium dodecyl sulphate
SE	Standard error
SF2	Nuclear phosphoprotein
SID	Salicylic acid induction deficient
SOD	Superoxide dismutase
SP	Secreted protein
TBST	Tris buffered saline with 0.1% Tween 20
TEMED	Tetramethylethylenediamine
TGA	TGA family transcription factor
TIR	Toll/Interleukin
TPL	*TOPLESS*
TPR	TPL related proteins
T. ni	*Trichoplusia ni*
Tris	Tris(hydroxymethyl)aminomethane
U	Enzyme unit
UPLC	Ultra-performance liquid chromatography
V	Volume
V	Volt
VSP1	Vegetative storage protein 1
VSP2	Vegetative storage protein 2
W	Weight
WRKY	WRKY transcription factor
X	Times/fold
xg	G force or relative centrifugal force
x-gluc	5-Bromo-4-chloro-3-indolyl-beta-D-glucuronic acid
&	And
~	Approximately
°	Degree
/	Per
%	Percentage
µE	Micro Einstein
µg	Microgram

Abbreviations

µL	Microlitre
µmol	Micromole
1D-SOMs	One dimensional self-organizing maps
12-COOH-JA	12-Carboxy-jasmonic acid
12-COOH-JA-Ile	12-Carboxy-jasmonoyl-isoleucine
12-HSO4-JA	12-Hydroxy-jasmonic acid sulphate
12-*O*-Glc-JA	12-*O*-Glucosyl-jasmonic acid
12-*O*-Glc-JA-Ile	12-*O*-Glucosyl-jasmonoyl isoleucine
12-OH-JA	12-Hydroxy-jasmonic acid
12-OH-JA-Ile	12-Hydroxy-jasmonoyl-isoleucine
2,3-DHBA	2,3-Dihydroxybenzoic acid
3'	3 prime end
5'	5 prime end

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1. INTRODUCTION

1.1. Plant innate immunity

Plants are constantly exposed to a diverse range of micro-organisms present in the natural environment. The invading microbes can either be pathogenic or beneficial to the plants. On a general basis, these microbes can be classified as biotrophic, hemi-biotrophic or necrotrophic organisms depending on their lifestyles (Laluk, 2011). The biotrophs depend on a living and intact host for their survival and reproduction. The necrotrophs kill the host cells and rely on dead matter to complete their life cycle. The hemi-biotrophic microbes engage in a biotrophic interaction with the host and then switch over to necrotrophy during their life cycle (Glazebrook, 2005). To counter all these kinds of microbes, plants have developed an innate immune system to detect these threats and activate appropriate defense mechanisms. Plants sense microbial presence by recognizing the conserved signatures termed microbe-associated molecular patterns (MAMPs) (Choi & Klessig, 2016) or the conserved patterns for disease causing pathogens called pathogen-associated molecular patterns (PAMPs) (Jones & Dangl, 2006). The MAMPs/PAMPs are specific to microbes and are not present in plants (Zipfel et al., 2006). The most common MAMPs/PAMPs include flagellin (flg22), the highly conserved peptide of the bacterial motility structure flagellum; chitin and peptidoglycans, the cell wall components of fungi and bacteria respectively; elongation factor (Ef-Tu), the proteins involved in prokaryotic cell cycle and elongation (Mazzotta & Kemmerling, 2011). Plants utilize trans-membrane proteins called receptor like kinases like LRR-RLK, to detect these MAMPs/PAMPs and activate various local defense responses (Swiderski et al., 2009). Some of the identified receptor domains for MAMPs/PAMPs include FLS2 which recognizes flg22 (Chinchilla et al., 2005), EFR for Ef-Tu (Zipfel et al., 2006), CERK1 for chitin (Miya et al., 2007) and LYM1 LYM3 CERK1 for peptidoglycan (Lajunen et al., 2011). Perception of these patterns by the receptors lead to the activation of downstream defense responses called pattern-triggered immunity (PTI) (Nürnberger et al., 2004). PTI signalling response is the plant's first line of defense. Phosphorylation of the mitogen-associated protein kinase (MAPK) cascades, reactive oxygen species (ROS) production, calcium spiking, callose deposition, alkalization of the extracellular space are the basal PTI responses that are triggered after perception of mi-crobes or pests (Mazzotta & Kemmerling, 2011; Wu et al., 2014; Zipfel, 2009).

Introduction

To surpass PTI responses and successfully invade plants, microbes secrete effector proteins (Figure 1). These effectors target and weaken the plant defense components, thereby leading to effector-triggered susceptibility (ETS) (Jones & Dangl, 2006). Effectors usually target hormone signalling or cell developmental processes to evade the basal defense responses (Boller & He, 2009). For example, pathogens like *Xanthomonas campestris* or *Pseudomonas syringae* secrete effector proteins to modulate plant immunity (Ossier et al., 1999; Schechter et al., 2004). Not just pathogens, even beneficial microbes like *Laccaria bicolor* and *Rhizophagus irregularis* secrete effectors MiSSP7, MiSSP8 or SP7 respectively, to target jasmonic acid and ethylene signalling pathways and plant developmental mechanisms for root colonization (Kloppholz et al., 2011; Pellegrin et al., 2019; Plett et al., 2014, 2011). In return, plants have evolved resistance (R) genes and proteins which directly or indirectly bind to these effectors to induce effector-triggered immunity by activating defense responses (ETI) (Dangl & Jones, 2001; van der Hoorn & Kamoun, 2008). Most of the R proteins belong to the NB-LRR proteins with nearly 100 different TIR-NB-LRR class of R proteins found in *Arabidopsis thaliana* (Swiderski et al., 2009). ETI defense responses are much stronger than PTI responses and result in hypersensitive cell death (Jones & Dangl, 2006) (Figure 1). In this way, plants engage locally in either compatible or incompatible interactions with innumerable micro-organisms.

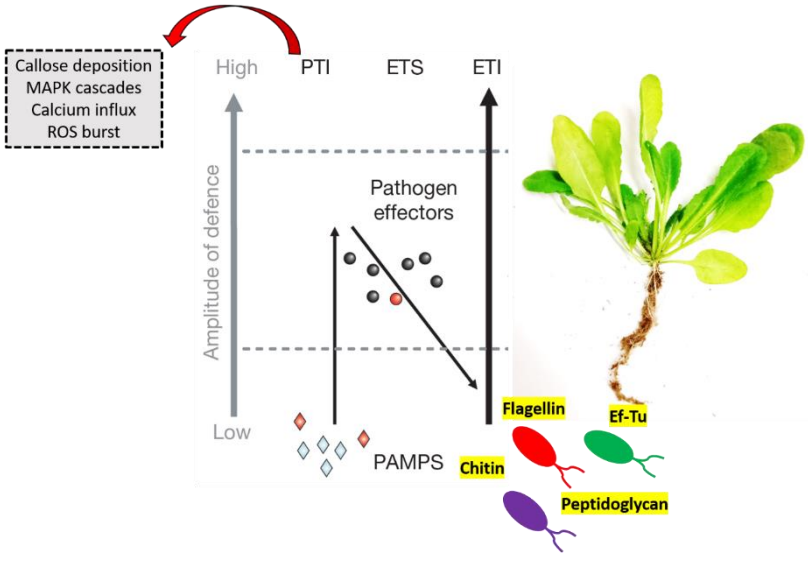


Figure 1. Zig zag model of plant immunity.

Plants recognize MAMPs/PAMPs (flagellin, chitin, Ef-Tu, peptidoglycan (and other diamond shaped PAMPs)) from invading microbes to mount PTI responses (marked in red arrow) like ROS burst, MAPK phosphorylation, calcium influx and callose deposition. Microbes suppress PTI defenses by secreting effectors (black and red circles) which can be perceived directly or indirectly by plant R proteins to induce ETI. Image modified from Jones & Dangl (2006).

1.2. Phytohormones

Both PTI and ETI responses depend on hormone signalling pathways to regulate the defense gene expression (Pieterse et al., 2012). Phytohormones are mobile signals which influence plant development and immunity (Verhage et al., 2010). Some of the major phytohormones involved in plant growth and development include auxin, cytokinin, gibberellin, abscisic acid (ABA) and brassinosteroid (BR) (Pieterse et al., 2012). Auxin is responsive to light, gravity and plays an important role in vascular and root development (Woodward & Bartel, 2005). Cytokinins promote generation of shoot meristems while having a negative influence on auxin-induced root branching and elongation (Riefler et al., 2006). Leaf unrolling, stem and pollen tube growth are influenced by BRs (Nakashita et al., 2003). While seed germination and flowering are regulated by gibberellic acid (GA) (Willige et al., 2007), ABA controls seed maturation and stomatal conductance (Leung & Giraudat, 1998). In addition to contributing to plant growth, ABA is also involved in plant response to abiotic stress (Ton et al., 2009). The major phytohormones which act against biotic stress include salicylic acid (SA), jasmonic acid (JA) and ethylene (Et) (Van Loon et al., 2006; Vlot et al., 2009). Wounding and leaf-chewing insects trigger JA responses, necrotrophic pathogens induce JA/Et signalling and SA is effective against biotrophic pathogens (Glazebrook, 2005; Howe et al., 2007; McConn et al., 1997; Vlot et al., 2009).

Plants experience growth/defense trade-off, where investment of resources in plant immunity compromises growth and vice versa (Conrath, 2011; Martinez-Medina et al., 2016). To mitigate the trade-off, plants must cautiously modulate or balance the developmental and defense cues. Therefore, all the above mentioned phytohormones interact with each other in a synergistic or antagonistic manner to optimize plant fitness (Martinez-Medina et al., 2016). Hence, the growth and development-related hormones like ABA, auxin, GA, cytokinin, etc., have also been reported to be involved in plant defense (Bari & Jones, 2009). The most common examples for signal crosstalk are the SA-JA antagonism in biotic stress response and auxin-cytokinin interactions in root development (Leon-Reyes et al., 2010; J. Liu et al., 2017; Van der Does et al., 2013). Exogenous application of SA or infection by biotrophic pathogens suppresses JA signalling responses in the plant (Leon-reyes et al., 2009; Spoel et al., 2003). The SA receptor and positive regulator *NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1* (*NPR1*), regulates SA and JA antagonism under high SA concentration (Leon-Reyes et al., 2010; Pieterse et al., 2012). While SF2 is involved in the negative feedback loop of

cytokinin biosynthesis, it is also present in the auxin pathway as a signalling repressor (J. Liu et al., 2017).

1.2.1. Jasmonate signalling pathway

The phytohormone JA is synthesized by the octadecanoid pathway (Santino et al., 2013). JA is an oxylipin, derived from the pre-cursor α-linolenic acid (Kazan & Manners, 2008). α-linolenic acid undergoes multiple conversions in chloroplast and peroxisomes to produce JA from oxo-phytodienoic acid (OPDA). JA undergoes further conversion in the cytosol to functional derivatives like methyl jasmonate (MeJA) or jasmonoyl-isoleucine (JA-Ile) (Kazan & Manners, 2008). JA and its by-products are commonly called as jasmonates and JA-Ile has been recognized as the mobile signal for activating the signalling processes in the jasmonate pathway (Thines et al., 2007). Genetic studies on the model plant species, *Arabidopsis* have identified several genes that perceive JA-Ile and are involved in regulating the jasmonate signalling pathway (Figure 2). CORONATINE INSENSITIVE 1 (COI1) is the jasmonate receptor of the SCF E3 ubiquitin complex consisting of JASMONATE INSENSITIVE 1 (JIN1) which regulates transcription of other activators and repressors of the signalling cascade (Chini et al., 2007; Thines et al., 2007; Yan et al., 2009). Upon perception of JA-Ile, SCF^{COI1} binds to the JASMONATE ZIM DOMAIN (JAZ) proteins which are negative regulators of jasmonate signalling and ubiquitinates them for degradation (Chini et al., 2007; Pauwels & Goossens, 2011; Thines et al., 2007). All the twelve members of the JAZ protein family in *Arabidopsis*, play pivotal roles in repressing jasmonates, and thereby, modulate the plant growth-defence trade off. They maintain the equilibrium by recruiting the co-repressor, its related proteins and the adaptor proteins namely TOPLESS (TPL), TPL Related Proteins (TPRs) and NOVEL INTERACTOR of JAZ (NINJA), respectively (Pauwels et al., 2010). The degradation of JAZ proteins pave way for the transcription of MYC, WRKY, MYB and other transcription factors which independently result in the expression of defense related genes (Kazan & Manners, 2008). Transcription factor WRKY57 in *A. thaliana* and GbWRKY1 in cotton activate *JAZ1* to promote *Botrytis cinerea* and *Verticillium dahliae* susceptibility (Jiang & Yu, 2016; Li et al., 2014). Jasmonate response to herbivore attack is regulated by the ABA-mediated MYC2 branch to express the *VEGETATIVE STORAGE PROTEIN 2* (*VSP2*) gene (Figure 2). On the other hand, pathogen attack triggers the ETHYLENE RESPONSE FACTOR (ERF) which mediates jasmonate and ethylene signalling. The ERF activates OCTADECANOID-

RESPONSIVE ARABIDOPSIS 59 (ORA59) transcription factor, which in turn leads to the expression of *PLANT DEFENSIN 1.2* (*PDF 1.2*) to defend against necrotrophic pathogens (Verhage et al., 2010, 2011) (Figure 2).

In addition to their role in biotic stress responses, JA signalling also plays an important role in plant developmental and physiological processes. For instance, MYB108 transcription factor in the JA pathway which induces wounding-mediated cell death, also regulates stamen and pollen maturation in *Arabidopsis* (Mandaokar & Browse, 2009). It is also interesting to note that during wounding response, JA-Ile mediated JA signalling activation is accompanied by an inactivation pathway in parallel. The auxin-amidohydrolases *IAR3* and *ILL6* are triggered to deconjugate the JA-Ile to 12-hydroxyjasmonic acid (Widemann et al., 2013). Therefore, jasmonate derivatives are crucial plant signals for wounding response (Koo & Howe, 2009), defense against leaf chewing insects and nectrophic pathogens (Nickstadt et al., 2004).

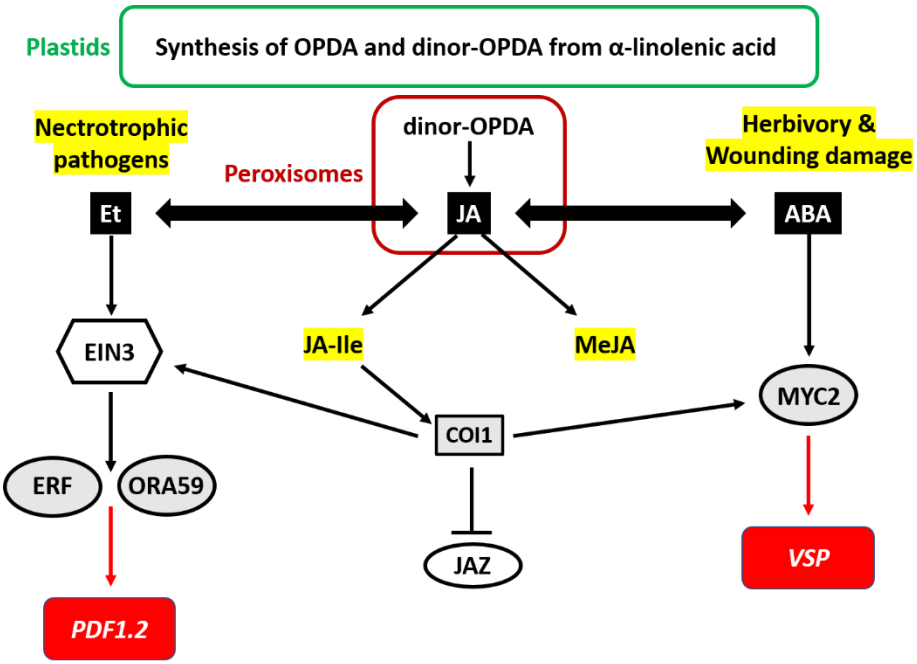


Figure 2. JA signalling pathway.

dinor-OPDA synthesized in the plastids are converted to JA in the persoxisomes. The functional derivative of JA, JA-Ile, binds with SCF^{COI1} receptor complex to degrade JAZ proteins. Herbivory or wounding triggers the ABA branch of JA signalling leading to the expression of *VSP*. Necrotrophic pathogens trigger the Et branch leading to the transcriptional activation of *PDF1.2*. Phytohormones are mentioned in black boxes, JA derivatives are highlighted in yellow, transcription factors are indicated in grey circles, and defense marker genes are mentioned in red boxes. *EIN3* is a positive regulator of JA/Et signalling which is also involved in repression of SA synthesis. Image modified from Santino et al., (2013).

1.2.2. Salicylic acid signalling

The phenolic compound, SA is synthesized by two pathways in plants. In one pathway, isochorismate synthase (ICS) catalyzes chorismate from the shikimate pathway to the SA precursor, isochorismate, terming this the isochorismate (IC) pathway (Wildermuth et al., 2001). Phenylalanine ammonia-lyase (PAL) is the enzyme involved in SA biosynthesis from chorismate in the second pathway (Fu & Dong, 2013). During PTI response, SA biosynthesis is highly dependent on the ICS pathway in which chorismate is converted to IC and subsequently to SA in the chloroplasts (Garcion et al., 2008; Wildermuth et al., 2001). SA-mediated responses lead to transcriptional activation of *PR* gene expression. The transcriptional regulation of ICS is however controlled by the homologs CBP60g (CALMODULIN BINDING PROTEIN 60g) and SARD1 (SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1) (Wang et al., 2011; Zhang et al., 2010). SA regulation of defense genes expression *via* ICS is dependent on the receptor and activator, *NPR1* (Wu et al., 2012). *NPR1* oligomers are localized in the cytosol when SA is absent (Figure 3). Higher SA in the cytosol alters the redox status, which reduces *NPR1* to monomers and translocate the monomeric *NPR1* to the nucleus (Tada et al., 2013). The clade II TGA transcription factors (TGA2/TGA5/TGA6) bind to *NPR1* in the presence of SA to induce the SA-responsive genes like *PATHOGENESIS-RELATED GENE 1* (*PR1*) (Gatz, 2013; Zhang et al., 2003). In the absence of SA, TGA2 may form a complex with NIMIN1 (NPR1/NIM1 INTERACTING PROTEIN 1) and TPL to repress the transcription of *PR1* (Hermann et al., 2013; Seyfferth & Tsuda, 2014). SA antagonizes JA signalling *via NPR1*, acting downstream of *ISOCHORISMATE SYNTHASE 1/SALICYLIC ACID INDUCTION DEFICIENT 2 (ICS1/SID2)* (Spoel et al., 2003). *NPR3* and *NPR4* are further NPR proteins whose functions are not completely clear.

Wu et al., (2012) reported that SA binds to *NPR1* and relieves the C-terminal transactivation domain from the suppression of the N-Terminal BTB/POZ domain to regulate the transcription of defense genes. Contrary to that, Fu et al., (2012) reported that *NPR1* does not bind to SA whereas, *NPR3* and *NPR4* are adaptors for Cullin E3 ubiquitin ligase degrading *NPR1* under high and low concentrations of SA respectively. However, it was shown recently that *NPR3* and *NPR4* are also SA receptors which act independent of *NPR1* (Ding et al., 2018). Ding et al (2018) also showed that unlike *NPR1*, both *NPR3* and *NPR4* are co-repressors of SA mediated transcription of defense genes.

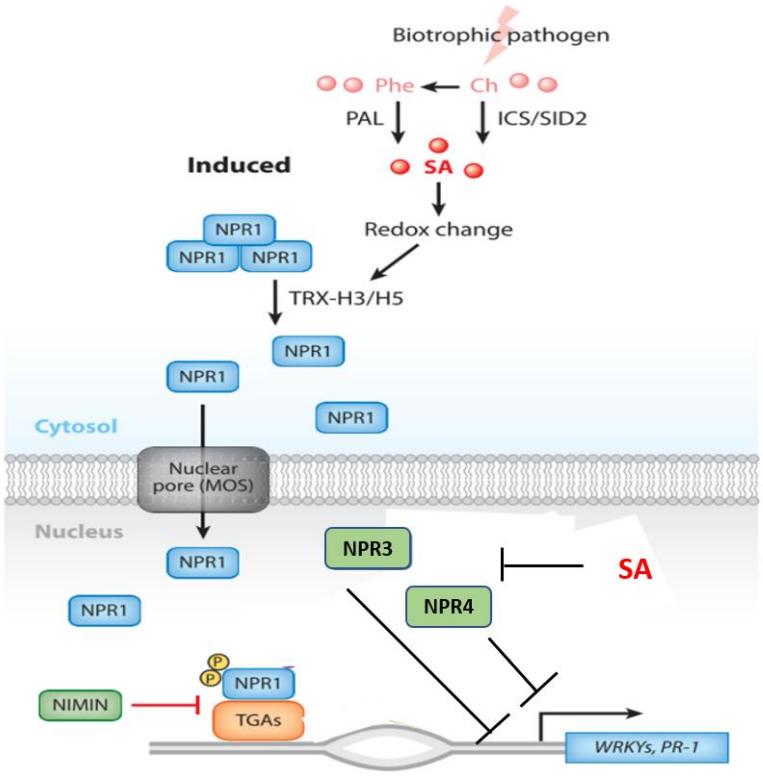


Figure 3. SA signalling pathway.

Upon infection by biotropic pathogen, SA (red circles) is accumulated in the tissue from the precursors chorismate (Ch) and phenylalanine (Phe) (both in pink circles). The redox potential reduces *NPR1* and the oligomers are transported to the nucleus. *NPR1* binds to the TGA transcription factors to activate transcription of defense genes. SA binds with *NPR3* and *NPR4* independent of *NPR1*, to repress the transcriptional activation of *PR* defense genes. Red box: transcription factors, blue box: positive regulator, green box: negative regulator of SA signalling. Image modified from Pieterse et al., (2012).

1.3. Secondary metabolites

Secondary metabolites include low molecular weight antimicrobial compounds which are either synthesized constitutively (phytoanticipins) or upon sensing stimuli (phytoalexins) (Piasecka & Jedrzejczak-Rey, 2015; Van Etten et al., 2007). The functions of these defense compounds range from cell wall reinforcement to toxicity against invading pathogens or deterrence of pests (Bennett & Wallsgrove, 1994; Clay et al., 2009). The diversity of secondary metabolites involved in biotic stress responses among different species of even the same clade is huge (Ver et al., 2011). The biosynthesis of some major defense metabolites has been uncovered for *Arabidopsis thaliana* (Piasecka & Jedrzejczak-Rey, 2015). These include cyanogenic glucosides, aliphatic and indolic glucosinolates, saponins and benzoxazine glucosides (Piasecka & Jedrzejczak-Rey, 2015). Of which, sulphur containing compounds like indole-

type phytoalexins and glucosinolates are the most well characterized metabolites in *Arabidopsis* for plant defense (Bednarek, 2012). The sulphur-containing phytochemicals are synthesized by the tryptophan pathway. Cytochrome P450 monooxygenase enzymes like *CYP79B2* and *CYP79B3* metabolize tryptophan to indole-3-acetaldoxime. Indole-3-acetaldoxime branches to synthesize camalexin *via CYP71A13* and indolic glucosinolates by *CYP81F2/PEN2* (Bednarek, 2012; Piasecka & Jedrzejczak-Rey, 2015). These tryptophan derived metabolites are activated in plants against infection by fungal pathogens and oomycetes (Bednarek, 2012; Bennett & Wallsgrove, 1994; Iven et al., 2012; Lipka et al., 2005).

In addition to pre-/post-invasion responses, secondary metabolites also help restructuring the plant microbiome in the rhizosphere (Voges et al., 2019). Phenolic compounds called coumarins are synthesized in a *MYB72* dependent manner and excreted in the rhizosphere by β-glucosidase, *BGLU42* (Zamioudis et al., 2014). Scopoletin, a coumarin secreted from the roots upon sensing beneficial microbes, specifically inhibits the growth of pathogens and not the beneficial microbes (Stringlis et al., 2018).

1.4. Systemic defense in plants

Along with local defense responses, plants mount immunity in distal tissues after encountering microbial interaction (Durrant & Dong, 2004; Pieterse et al., 2014). The systemic defense mechanisms ensure that the entire plant is protected against invading pathogens and pests by a process called priming. The plant defense responses exhibit a significant increase upon perception of the priming stimulus. Upon sensing a challenge, the plant responds with a faster, stronger and sustained defense without compromising on the growth at a low fitness cost (Martinez-Medina et al., 2016). The two plant systemic defense mechanisms are systemic acquired resistance (SAR) induced by pathogenic microbes and induced systemic resistance (ISR) triggered by beneficial microbes. Both these mechanisms involve sensitizing the phytohormone signalling and defense genes expression to ward off pathogens and pests (Pieterse et al., 2014).

1.4.1. Systemic acquired resistance

Pathogen infection at the local tissue triggers a systemic defense in the distal issue, by depending on SA signalling (Conrath, 2006; Durrant & Dong, 2004). This process is called systemic acquired resistance (SAR) and leads to protection of plant's distal tissue from subsequent attacks (Vlot et al., 2009). The local infection induces mobile signals which prime the distal tissues by the accumulation of more SA, thus, activating *PR* genes, specifically *PR1*, *via* the master regulator *NPR1* (Durrant & Dong, 2004). The systemic defense can also be induced by exogenous application of SA leading to accumulation of PR proteins in the distal leaves (Fu & Dong, 2013). Hence, SA was assumed to be the mobile signal for triggering SAR, but grafting experiments with tobacco did not support this assumption (Vernooij et al., 1994). Transgenic rootstock expressing bacterial SA-degrading enzyme translocated a signal to the non-transgenic scions for resistance against a pathogen (Vernooij et al., 1994). Similarly, a lipid-transfer protein DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) was proposed to be involved in long distance signalling (Maldonado et al., 2002). DIR1 was expressed in sieve elements and companion cells (Champigny et al., 2013). However, *dir1-1* mutants were occasionally SAR-competent, thereby, ruling out the possibility of DIR1 forming a chaperone complex with the mobile signal in phloem (Champigny et al., 2013). FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1) converts pipecolic acid to N-hydroxypipecolic acid, which accumulates in the systemic tissues after pathogen infection (Hartmann et al., 2018). Various other metabolites like methyl salicylic acid, jasmonic acid, azelaic acid and glycerol-3-phosphate were also proposed to be the mobile signals for SAR (Chanda et al., 2011; Jung et al., 2009; Park et al., 2007; Truman et al., 2007). However, the actual mobile signal transported in SAR is still to be unraveled.

1.4.2. Induced systemic resistance

Induced systemic resistance (ISR) caused by beneficial microbe interaction with plants provides a broad defense spectrum against diverse threats (Pangesti et al., 2016; Shoresh, Yedidia, & Chet, 2004). ISR was first reported by van Peer, Niemann, & Schippers (1991) in carnation treated with plant-growth promoting beneficial rhizobacteria (PGPR) *Pseudomonas fluorescens* (now called *Pseudomonas simiae*) WCS417r. Similar reports emerged about other beneficial microbes like other Pseudomonads, PGPR *Bacillus* species, *Trichoderma* species and mycorrhizal fungi inducing resistance against pathogens (Alström, 1991; Fernández et al.,

2019; Haney et al., 2017; Martínez-Medina et al., 2017; Wei et al., 1996). In contrast to SAR, ISR by beneficial microbes was not dependent on accumulation of SA or activation of *PR* genes and proteins (Hoffland et al., 1995; Pieterse et al., 1996). It was observed that beneficial microbes trigger defense responses independent of SA but dependent on JA/Et signalling in systemic tissues (Pieterse et al., 1996, 1998). However, some beneficial microbes like *Pseudomonas aeruginosa* 7NSK2, *Pseudomonas fluorescens* SS101 or *Trichoderma* sp. have been observed to depend on SA mediated SAR pathway for systemic protection (Audenaert et al., 2002; Contreras-Cornejo et al., 2011; Mortel et al., 2012). Hence, there are no specific pathways which define ISR triggered by mutualists. Unlike SAR, the association with mutualists has not been reported to significantly enhance defense gene expression or hormone concentrations in the systemic tissues (Pieterse et al., 2014). However, both SAR and ISR (in the case of *P. simiae* WCS417r) are dependent on the SA transcriptional regulator *NPR1* for triggering the responses (Pieterse et al., 1998). It was reported by Spoel et al. (2003) that cytosolic NPR1 but not nuclear NPR1 is involved in modulation of JA signalling (Spoel et al., 2003).

1.5. Beneficial microbes

Plant-beneficial microbe interactions are predominantly confined to the rhizosphere. Plants exude their photosynthetically derived sugars in the rhizosphere to attract beneficial microbes (Bais et al., 2010). Soil inhabiting pathogenic microbes also sense the nutrient sink and tend to colonize the roots. On one hand, beneficial microbes inhibit pathogens by producing toxic compounds for pathogens to exert their dominance in root colonization (Lambert et al., 1987; Pieterse et al., 2014). On the other hand, the mutualists trigger plants to secrete metabolites which selectively inhibit pathogenic growth (Stringlis, Yu, et al., 2018). In this way, beneficial microbes directly or indirectly outcompete the pathogens to colonize the plant roots, and thereby establishing symbiosis. In addition to protection of the local tissue, they also engage in mutual nutrient transfer and systemic defense activation by ISR (Heijden et al., 2015). Hence, plant species were classified as hosts or non-hosts depending on their ability to engage in the above-mentioned associations with distinct symbionts.

1.5.1. Mycorrhizal fungi

Mycorrhizal fungi are plant growth promoting fungi which are commonly found in the rhizosphere along with PGPR rhizobacteria and endophytes (Bais et al., 2010). These beneficial fungal species engage in carbon-nutrient exchange with hosts by colonizing the roots and forming specialized structures called mycorrhiza (Heijden et al., 2015). The most common classes of mycorrhizhal fungi include arbuscular mycorrhiza, ectomycorrhiza, orchid mycorrhiza and ericoid mycorrhiza. Arbuscular mycorrhizal fungi (AMF), the most prominent mycorrhizal fungi, colonize around 72% of land-use plants (Brundrett & Tedersoo, 2018). They provide water and nutrients like nitrogen and phosphorus to the hosts, in exchange for carbon (Kiers et al., 2011). However, there are contradictory views on mutual exchange of carbon-nutrients with respect to other mycorrhizal fungi (Heijden et al., 2015).

Mycorrhizal associations are established as a result of molecular crosstalk between the host plant and the beneficial fungi. Primary and secondary metabolites exuded by the plant roots promote proliferation of mycorrhizal fungi in the rhizosphere (Garcia et al., 2015). The volatile organic compounds from the fungi also initiate early signalling events in the plant roots including lateral root formation (Ditengou et al., 2015). The host plants possess a common symbiotic signalling pathway (CSP) which is activated upon perception of Nod or Myc or lipochitooligosaccharides (LCOs) or chitin oligomers from these beneficial fungi (Ehrhardt et al., 1996; Haney et al., 2011). The CSP consists of a leucine-rich repeat receptor kinase, calcium- and calmodulin-dependent kinases (CASTOR and POLLUX) (Garcia et al., 2015). Unlike vast majority of vascular plants, parasitic plants, stonecrops and plants belonging to Brassicaceae and Proteaceae have lost the common symbiotic signalling pathway. Hence they cannot undergo mycorrhization and are considered as non-host species for the mycorrhizal fungi (Lambers & Teste, 2013).

1.5.2. Ectomycorrhizal fungi

Ectomycorrhizal fungi (EMF), evolved from wood and litter decayers, predominantly colonize certain tree species which constitute 2% of the vascular plants (Brundrett & Tedersoo, 2018; Heijden et al., 2015). There are evidences that the ectomycorrhizal fungi have evolved independently 200 times from ancestral brown saprotrophic fungi (Heijden et al., 2015). During this evolution, EMF lost their saprotrophic ability of lignocellulose decay mechanism but

have evolved pathogenic effector secretion system for colonizing host roots (Martin et al., 2016). Sesquiterpenes from the model EMF, *L. bicolor*, induces lateral root formation in both hosts and non-hosts, thereby initiating the mycorrhization event (Ditengou et al., 2015). LCOs produced by *L. bicolor* activate CSP in host plants for initiating root colonization (Cope et al., 2019). Then the EMF colonize host root species by forming a fungal sheath around the roots (Smith & Read, 2008). Unlike AMF which penetrates the root cells, the hyphae of EMF grow between the root cells (Smith & Read, 2008). The hyphae of EMF grows in the root apo-plastic space to establish the Hartig net which is a characteristic feature of all EMF (Smith & Read, 2008). Since the non-hosts are devoid of CSP, their roots cannot be mycorrhized by EMF.

Plant hormones play a role in influencing ectomycorrhizal association. Auxin responsible for root growth is involved in this signalling event (Felten et al., 2009). While root treatment with salicylic acid did not affect colonization, jasmonic acid and ethylene prevented mycorrhiza establishment (Plett et al., 2014). MiSSP7, a small protein secreted by the model EMF, *L. bicolor*, localizes in the nucleus of the poplar root cells to suppress JA-mediated degradation of *PtJAZ6* and is necessary for Hartig net formation (Plett et al., 2014, 2011). Another effector, MiSSP8 secreted by *L. bicolor*, was observed to be involved in hyphal aggregation during ectomycorrhizal symbiosis and could possibly be inherited from its ancestral saprotrophic lifestyle (Pellegrin et al., 2019).

The systemic altercations triggered by EMF in host plants have been studied by using plant growth and nutrient status as readouts. EMF colonization and fertilization had similar effects on the nitrogen levels in the foliar tissue of chestnut (Rieske et al., 2003). In most cases, EMF have not been reported to enhance the growth of the host plants like AMF (Gange et al., 2005; Garcia et al., 2015; Kaling et al., 2018). Phenolic compound synthesis was downregulated in poplar leaves after root mycorrhization by EMF (Kaling et al., 2018). In addition, the anti-herbivore defense systems were also activated in the foliar leaves leading to reduced egg deposition by the poplar leaf beetle, *Chrysomela populi* (Kaling et al., 2018). However, EMF effect on insect performance varies and depends on the infesting herbivore (Hartley & Gange, 2009). Though there are evidences about host protection against herbivory by EMF, the common host components involved in this ISR response have to be identified and validated. Insects trigger distinct defense responses in the host including volatile patterns and this variance is further enhanced by EMF root colonization (Clavijo Mccormick et al., 2014; Clavijo McCormick et al., 2012). Investigating EMF effects using alternative model plant species with abundant tools and resources can unravel the conserved ISR pathways induced by EMF.

1.6. Objectives

While the outcome of EMF impact on host defense has been reported, the interaction between EMF and non-host species has not been studied so far. This research was aimed to investigate the influence of EMF treatment on a non-host species and correlate this effect on systemic protection against biotic stress in a tripartite interaction. The model EMF, *L. bicolor* and the model plant species, *Arabidopsis thaliana* (will be mentioned as Col-0) were used as our working models. In addition to not being mycorrhized, Col-0 offers great advantage for this study because of its shorter growth phase and the flexibility in trying many established pathogen assays. The genome sequence information available for both *A. thaliana* and *L. bicolor* makes them a feasible model for genetic analyses. The system was challenged with Brassicaceae generalist herbivore, *Trichoplusia ni* and the hemi-biotrophic pathogen, *Pseudomonas syringae* pv. Tomato DC3000 (*Pst* DC3000) as readouts for systemic resistance by EMF. In the context of the main goal of this thesis, the experiments were designed and performed to address the following questions:

- Can *L. bicolor* induce systemic defense responses in Col-0?
- Which components are altered in Col-0 systemic tissue by *L. bicolor* for ISR?
- How does *L. bicolor* trigger the systemic defense responses in Col-0?

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Unless specified, all the chemicals were obtained from Carl Roth GmbH + Co. KG, Karlsruhe, Germany, with analytical grade specifications. The composition of media and buffers are reported in this section alongside the description of the specific method.

2.2. Preparation of *Arabidopsis* plants

2.2.1. Plant materials

Most of the *Arabidopsis thaliana* seeds were provided by Dr. Cara Haney, University of British Columbia, Canada. The *Arabidopsis* mutants analysed in this project include the JA biosynthesis mutant *aos* (Park et al., 2002) (provided by Prof. Dr. Ivo Feussner, University of Göttingen, Germany), the JA receptor mutant *coi1-16* (Ellis & Turner, 2002), the SA biosynthesis mutant *sid2-2* (Dewdney et al., 2000), the SA positive regulator mutant *npr1-1* (Cao et al., 1994), the SA negative regulator double mutant *npr3-2 npr4-2* (Zhang et al., 2006), the tryptophan metabolism double mutant *cyp79b2/b3* (Glawischnig et al., 2004), the camalexin biosynthesis mutant *cyp71a13-1* (provided by Dr. Pawel Bednarek, Polish Academy of Sciences, Poland), the indolic glucosinolates biosynthesis mutant *cyp81f2* (provided by Prof. Dr. Volker Lipka, University of Göttingen, Germany), the iron deficiency response regulator double mutant *myb72/10* (Palmer et al., 2013) and the chitin receptor mutant *cerk1-2* (Miya et al., 2007). *CYP7A12p*-, *MYB51p*- and *WRKY11p*-GUS (Millet et al., 2010) were the reporter lines used for GUS analysis.

2.2.2. Seed sterilization

Seeds were surface sterilized by treating with 70% ethanol for 1 min. The tubes containing seeds were mixed and centrifuged (5418R, Eppendorf AG, Hamburg, Germany) briefly before discarding ethanol. Seeds were then bleached with 10% DanKlorix (CP GABA GmbH,

Hamburg, Germany) for 2 min, followed by brief centrifugation. The bleach solution was discarded, and the seeds were washed thrice with sterile ddH_2O. Sterilized seeds were suspended in 500 µL of sterile ddH_2O and cold stratified for two days in darkness at 4°C.

2.2.3. Plant growth condition

2.2.3.1. *In vitro* experiments

Ten to twelve sterile Col-0 seeds were placed on square petri-dishes (Sarstedt AG & Co. KG, Nümbrecht, Germany) filled with ½ MS medium (concentration in g/L: MS medium with vitamins (Duchefa Farma B. V., Haarlem, Netherlands) 2.215, MES hydrate (Sigma-aldrich, Steinheim, Germany) 1, phyto-agar (Duchefa Farma B. V., Haarlem, Netherlands) 10; pH = 5.7 to 5.8). Plates were sealed with micropore tape (3M™ Micropore™ surgical tape, Minnesota, United States of America) and placed in acclimatized chamber (Percival Scientific, CLF Plant Climatics, Emersacker, Germany) in an upright position with 100 $\mu Em^{-2}s^{-1}$ light and 80% relative air humidity for 16/8 hours at 22/20 °C day/night phase.

2.2.3.2. Soil experiments

Jiffy-7 (Jiffy Products International AS, Norway, Article number 789005) peat pellets with diameter 4.2 cm were used as substrate for growing plants. The culturable beneficial microbes for inducing ISR are less abundant in the jiffy-7 pellets (Haney et al., 2017). Hence, unsterile pellets were used for the entire set of experiments. Pellets were soaked in holder trays (1020 Greenhouse growing tray with no drain holes, Tennessee, United States of America) containing tap water for 15 to 20 min. The pellets sucked up the water and expanded to a height of 42 mm. The moist pellets were filled in the sockets of 50 pellet piece trays (Landmark Plastic Corporation, Akron, United States of America) mounted over a sieve tray (1020 Greenhouse nursery carry tray, Hendersonville, United States of America) and then placed above the flat holder tray (Figure 4). Surface sterilized seeds were suspended in sterile 0.1 % (w/v) Phyto-agar solution to separate the seeds. Three seeds were placed/pellet using a glass pasteur pipette and the tray was covered with a Jiffy-7 dome (Jiffy Products International AS, Norway) to maintain humidity. The seeds germinated in the climate chamber (Kees Klima- und Kältetechnik GmbH, Ronnenberg, Germany) under short day conditions (8 hours) with 130 $\mu Em^{-2}s^{-1}$ light and 80% relative air humidity at 22 °C (day) and 20 °C (night).

On day 7, excessively germinated seedlings in every pellet were reduced to one seedling and the humidity domes were removed. Plants were watered twice every week, by filling the holder trays containing pellets with tap water. After 15 min, the water from the holder trays was drained and the plant trays were swapped inside the climate chamber in a random manner.

For *Hyaloperonospora arabidopsidis* experiments, 36 seeds were placed in every square disposable pots (Hermann Meyer KG, Rellingen, Germany) filled with peat (Typ N Erde, Hermann Meyer KG, Rellingen, Germany). On day 7, excess seedlings were thinned down to a total of 30 seedlings/pot. For the entire duration of the experiment, plants were grown under short day conditions (8 hours) with 130 $\mu Em^{-2}s^{-1}$ light and 80% relative air humidity at 22 °C (day) and 20 °C (night).

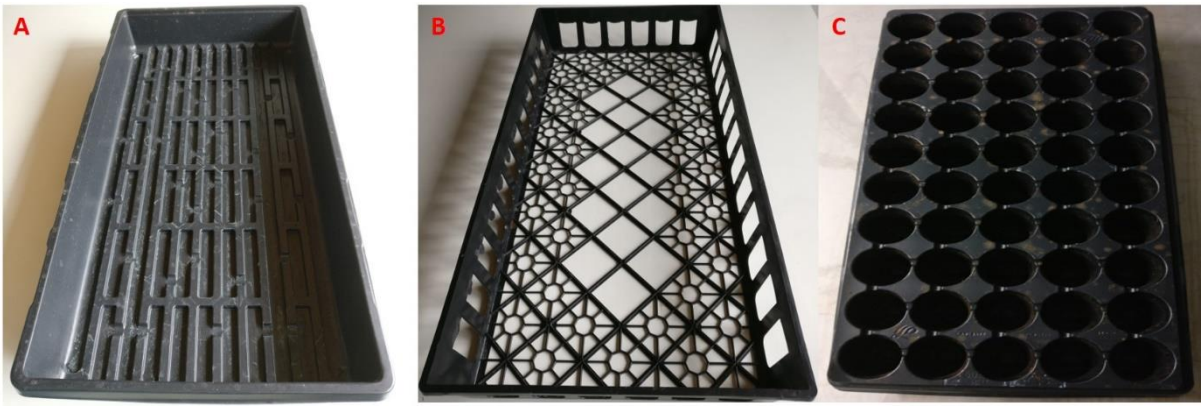


Figure 4. **Tray set-up for growing *Arabidopsis* in Jiffy-7 pellets.**
(A) Holder tray at the bottom (B) Sieve tray in the middle (C) Pellet trays on top.

2.3. Preparation of microbes and elicitors

2.3.1. Fungal strains

2.3.1.1. *Laccaria bicolor*

The ectomycorrhizal fungus, *Laccaria bicolor* (monokaryotic strain CII-H-82, S238N) was propagated by subculturing from the stock culture. 0.3 cm^2 fungal agar plugs from the stock culture were cut using a sterile scalpel in the biosafety cabinet (Thermo SCIENTIFIC SAFE 2020, Thermo Electron LED GmbH, Langenselbold, Germany). Three fungal plugs

were transferred and cultured on solid MMN medium (concentrations in g/L: glucose 10.0, ammonium tartrate 2.5, ammonium sulphate 0.25, potassium dihydrogen phosphate 0.5, magnesium sulphate heptahydrate 0.15, calcium chloride dihydrate 0.05, sodium chloride 0.025, 0.1% thiamine-hydrochlorate 0.1 mL and 1% ferric chloride hexahydrate 1mL; pH 5.2 to 5.4) in dark at 23 to 26°C for three weeks. Around 25 fungal agar plugs from a single colony were inoculated in conical flasks containing 200 mL of MMN medium and incubated on a shaker (GIGYROTORY® Shaker, New Brunswick Scientific Co., Inc, Edison, United States of America) at 120 rpm in dark at 23 to 26°C. When the fungi were three weeks old, the culture was transferred to 50 mL sterile FALCON® tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany) and homogenized using an ULTRA-TURRAX T25 tissue disruptor (Janke & Kunkel GmbH & Co. KG, IKA®-Labortechnick, Staufen, Germany). The FALCON tubes with slightly unscrewed caps were transferred back to the shaker and incubated at 23 to 26°C in dark for a week.

Two days before root inoculation, 4 to 5 FALCON tubes containing the fungal mycelium were homogenized again and incubated as mentioned above. Homogenization was performed to mildly wound *L. bicolor* because wounding increases the aggressiveness of the fungi (Kemppainen et al., 2005). On the day of inoculation, these FALCON tubes were centrifuged at 4500 rpm for 5 min at room temperature. The supernatant was discarded, and the pellet was re-suspended in 10 mM $MgSO_4$. OD of the inoculum in a 10 x 4 x 45 mm polystyrene cuvette, was measured at 600 nm (Sarstedt AG & Co. KG, Nümbrecht, Germany) using a Bio-Photometer (Eppendorf AG, Hamburg, Germany). 10 mM $MgSO_4$ served as the blank for OD measurement. Based on the OD value, *L. bicolor* suspension of OD_{600} = 1 was prepared using 10 mM $MgSO_4$. This solution was diluted with 10 mM $MgSO_4$ to prepare the inoculum with OD_{600} = 0.1. Inoculum of dead *L. bicolor* was prepared by heat killing *L. bicolor* suspension of OD_{600} = 1, in a water bath for 20 min at 65-80°C. Higher concentration of dead *L. bicolor* was used to nullify the variance resulting from the growth of live *L. bicolor* in the pellets at the end of the experiment (as performed by Millet et al., 2010 with *Pst* and *P. fluorescens* WCS417r). The heat-killed fungal solution was also plated on MMN medium to check for growth recovery. *Paxillus involutus*, another EMF was also propagated and cultured like *L. bicolor*.

2.3.1.2. *Hyaloperonospora arabidopsidis*

H. arabidopsidis Noco2 (Noco2) strain was provided by Prof. Dr. Volker Lipka (University of Göttingen, Germany). Col-0 seedlings were grown in a dense manner on 50 to 100 mm pots (Hermann Meyer KG, Rellingen, Germany) under short day conditions with 10 hours of 130 μEm^2s^{-1} at 22/18 °C of day/night phase. Noco2 strains were propagated on 2-week-old Col-0 seedlings by spraying an inoculum of $5x10^4$ spores using a common hand sprayer. The inoculated plants were covered with a lid and transferred to climate chambers (Percival Scientific, CLF Plant Climatics, Emersacker, Germany) with same short-day conditions as the growth chamber. On the next day, the pots containing infected plants were uncovered for 18 hours to dry up the water droplets and covered again with the lid. After 6 days, the shoots of the infected seedlings were harvested, suspended in dH_2O and sporangiospores were counted using a hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany).

2.3.2. Bacterial strains

The bacterial strain *Pseudomonas simiae* WCS417r (WCS417r) was provided by Dr. Cara Haney (University of British Columbia, Canada) and *Pseudomonas syringae* pv. *Tomato* (*Pst*) was given as a gift by Prof. Xin Li (University of British Columbia, Canada). The strains were cultured on LB medium (concentrations in g/L: tryptone (Duchefa Farma B. V., Haarlem, Netherlands), yeast Extract, sodium chloride, bactoagar 15) at 28°C. A day before inoculation, an overnight culture was prepared by inoculating the strains in LB medium and the culture was incubated at 28°C on a shaker (Innova4330, New Brunswick Scientific Co., Inc, Edison, United States of America). The bacterial cells from the overnight culture were centrifuged, re-suspended in 10 mM $MgSO_4$ and OD_{600} value was measured using the Bio-Photometer (Eppendorf AG, Hamburg, Germany). *Pst* DC3000 solution with OD_{600} = 1 was prepared by diluting the suspension using 10 mM $MgSO_4$ and the solution was further diluted to OD_{600} = 0.1. The *Pst* DC3000 final inoculum was adjusted to OD_{600} = 0.0002 using 10 mM $MgSO_4$.

2.3.3. MAMP and PAMP stock solutions

A 0.1mM flagellin stock solution was prepared by suspending flg22 peptide (Sigma-aldrich, Steinheim, Germany) in sterile ddH_2O. Chitin stock solution was prepared in two different ways for ISR and PTI response experiments using chitin from shrimp shells (Sigma-aldrich, Steinheim, Germany). For ROS and MAPK experiments, 10 mg of chitin from shrimp shells was ground using IKA® RW20 digital mixer (IKA®-Werke GmbH & Co. KG, Staufen, Germany) with 1 mL distilled water for 12 min to prepare the stock. For ISR experiments, 10 mg of chitin from shrimp shells was suspended in 1 mL of ddH_20 and autoclaved for 20 min. After centrifugation at 4500 rpm for 5 min at room temperature, the supernatant was collected and used as stock for ISR experiments. Chitin and flg22 stock solutions were stored at 4°C and -20°C respectively until further use.

2.3.4. Elicitor and microbial inoculation

When Col-0 or other mutant seedlings were 9 days old, 2 mL of *L. bicolor* inoculum (OD_{600} = 0.1) was pipetted on the peat surface surrounding every seedling root. 2 mL of WCS417 (OD_{600} = 0.01) was inoculated/seedling for beneficial rhizobacteria treatment. Similarly, 2 mL of heat-killed *L. bicolor* solution (OD_{600} = 2) was inoculated around the seedlings for dead *L. bicolor* treatment. Millet et al., (2010) showed that 500 µg/mL of chitin can trigger callose deposition in *Arabidopsis*. Hence for chitin treatment, 2 mL of 500 µg/mL chitin solution (final concentration = 1 mg/mL) was pipetted/plant. The control plants were mock inoculated with 2 mL of 10 mM $MgSO_4$.

Since the PTI experiments were performed in controlled *in vitro* conditions with less volume, low elicitor concentrations were enough to induce a response. Flg22 and chitin were used at a final concentration 100 nM and 10 µg/mL respectively (Millet et al., 2010) for ROS and MAPK experiments.

2.4. Biotic stress experiments

2.4.1. Caterpillar feeding assay

Herbivore experiments on *Arabidopsis* were done as described by Haney et al., (2017). Eggs of the Brassicaceae generalist herbivore, *Trichoplusia ni*, were purchased from from natural sciences and engineering research council (NSERC) Canada (Roe et al., 2018). The providers were requested to ship the eggs collected over 24 hours without media for synchronous hatching (Ali & Agrawal, 2012). *T. ni* eggs were incubated in an airtight box with moist paper towels for 2 days under the plant growth conditions to synchronize the circadian cycle of *T. ni* with the plants. Diurnal variances between the plants and caterpillars can cause discrepancies in the plant anti-herbivore defenses resulting in altered feeding behavior (Jander, 2012). Hence, the circadian rhythm of the caterpillars was synchronized with that of the plants.

A wet paper towel was placed inside the box to maintain humidity. When the eggs hatch, each larva was placed on a 4-week old *Arabidopsis* plant grown in a pellet and covered with a breathable nylon mesh net (Tech Textiles, Birmingham, United Kingdom) using a rubber band. The plants with the larvae were maintained under 16/8 hours in light/dark phase as described under chapter 2.4.1. After 7 days of feeding, the caterpillars were weighed individually using a microbalance (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany), and the larval weight gain was recorded. Due to the negligible mass of the caterpillars after hatching, the initial weight was recorded as zero (Figure 5). After weighing, the caterpillars were killed by freezing them overnight at -20°C.

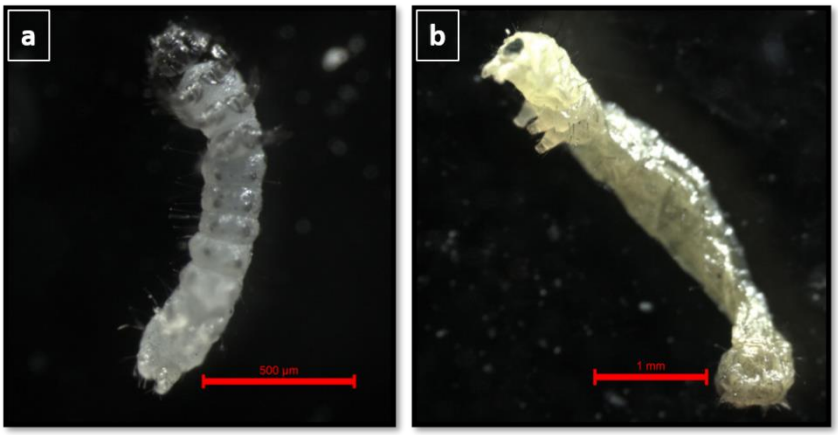


Figure 5. *Trichplusia ni* (or) the cabbage looper.

(a) *T. ni* when freshly hatched from the egg and (b) *T. ni* after 7 days of feeding on Col-0.

2.4.2. Biotrophic pathogen infection assays

2.4.2.1. *Pseudomonas syringae* DC3000

Pseudomonas syringae infection assays were performed as described by (Haney et al., 2017). The *Pst* DC3000 inoculum (OD_{600} = 0.0002) was prepared from an overnight culture as described in chapter 2.3.2. A total of six 5-week old plants from each treatment was used in this experiment and the plants were placed in the trays as shown in Figure 6. The trays were half filled with water and covered with a dome for at-least an hour in the plant growth room to induce stomatal opening. The water from the trays were drained not completely and 2 leaves/plant were marked with a permanent marker. *Pst* DC3000 inoculum (OD_{600} = 0.0002) was infiltrated into the marked leaves using a blunt (no needle) 1 mL syringe. The inoculum was forced into the entire leaf through the stomatal openings. Bacterial solution suspended on the leaf surfaces was dried using paper towels and the trays were covered again with lids. At 2 days post inoculation, leaf disks (9 mm diameter) from the infiltrated leaves were collected and transferred individually to sterile 2 mL tubes. The leaf disks were homogenized with a metal bead (4 mm diameter) and 100 µL sterile ddH_2O using a bead beater (Retsch GmbH, Haan, Germany) at 25 s^{-1} for 1.5 min. The homogenized suspension was serial diluted (1:10 increments) in a sterile 96 well round bottom plate (Sarstedt AG & Co. KG, Nümbrecht, Germany) filled with 90 µL of 10 mM $MgSO_4$ in every well. 8 µL of the serial diluted samples were plated on omnitrays (Sarstedt AG & Co. KG, Nümbrecht, Germany) containing LB medium

with rifampicin (50 µg/mL). The plates were incubated at 28 °C in dark and colony forming units (CFUs) were counted after 2 days.

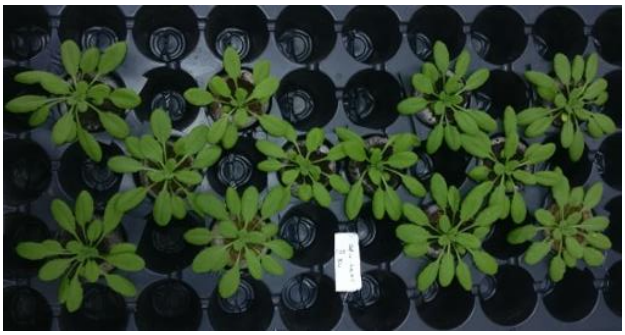


Figure 6. Plant set-up for *Pst* DC3000 infection.

Plants are arranged in this manner inside the trays for *Pst* DC3000 experiment to avoid damage or contact with other plants.

2.4.2.2. *Hyaloperonospora arabidopsidis*

Hyaloperonospora arabidopsis Noco2 (Noco2) strains were propagated on Col-0 seedlings as described in chapter 2.3.1.2. Two week old plants with or without *L. bicolor* were sprayed with freshly harvested Noco2 spores ($5x10^4$ spores/mL) and the pathogen assay was performed as described by (James, 2013). The trays were covered with lids and transferred to acclimatized chambers with 130 $\mu E.m^{-2}s^{-1}$ light and 80% relative air humidity for 8/16 hours at 22/18 °C in day/night phase for 5 days. Infected shoots from each pot were harvested and suspended together with ddH_20 in a 50 mL FALCON tube based on their weight. After vortexing for 30s, 10 µL of the suspension was pipetted into the counting chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany) and spores were counted using a light microscope (Carl Zeiss Microimaging GmbH, Göttingen, Germany). Average of 4 counts from at-least 4 technical replicates/treatment was normalized per gram of plant tissue to calculate the number of Noco2 spores infecting the seedlings.

2.5. Gene expression analyses

Eight 4-week-old Col-0 plants/treatment grown in jiffy pellets, were used for gene expression analyses. Two freshly hatched larvae were placed on every *T. ni* treatment plant and covered with mesh net. The control plants were mock covered with mesh nets without *T. ni* and all the plants were transferred to acclimatized chamber as explained in chapter 2.2.3.2. Visible *T. ni* feeding damages were observed in Col-0 leaves only after 24 hours. Hence, 24-hour after *T. ni* feeding was the time point chosen for gene expression, phytohormone and metabolite analysis. After 24 hours of feeding, the larvae were removed and one leaf/plant was harvested. Only leaves with prominent caterpillar feeding damages were harvested for *T. ni* treatment samples. Leaves collected from the plants with same treatment were pooled, flash frozen in liquid nitrogen and stored in the -80°C freezer (Ewald Innovationstechnik GmbH, Rodenberg, Germany) until further use.

2.5.1. qRT-PCR experiment

Frozen samples collected as mentioned above, were homogenized twice using the bead beater (Retsch MM400, Retsch GmbH, Haan, Germany) at a frequency of 25 s^{-1} for 1 min. RNA was extracted from 100 mg of the homogenized samples using the Qiagen RNAeasy extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The RNA concentration in the extract was measured using a NanoDrop™ Photometer (Thermo Fischer Scientific, Massachusetts, United States of America). DNA contaminants in the RNA samples were removed by treating 1 µg of extracted RNA with TURBO DNAse (AMBION GmbH, Kaufungen, Germany) in 1 30 µL reaction (34 ng/µL RNA) according to TURBO DNA-free™ kit protocol. 27 µL of DNA-free RNA was reverse transcribed in a 40 µL reaction (16 ng/µL RNA) containing cDNA synthesis mix (50 µM Oligo dT primers, 10 mM dNTP mix, 10x reverse transcription buffer, 24 mM magnesium chloride, 0.1 M DTT and 200 U/µL Superscript III reverse transcriptase (Invitrogen, Carlsbad, United States of America)) to synthesize single stranded cDNA by incubating at 65 °C for 5 min, 50 °C for 50 min, 85 °C for 5 min and then stored at -20 °C. 1.5 µL of synthesized cDNA along with 2.5 µM forward and reverse primers and 2x PowerUp™ SYBR™ Green master mix (Thermo Fischer Scientific, Massachusetts, United States of America) (Final concentration = 8 ng RNA) was used to quantify the gene expression using 7500 Fast Real-time PCR system (Applied Biosystems®, Foster City, United

States of America). Expression of all candidate genes were tested using primers mentioned in Supplementary Table 1. The cycle threshold (CT) values of each gene was normalized to the cycle threshold of the house-keeping gene, *EIF4A* (Millet et al., 2010).

2.5.2. Transcriptome analyses

The extracted RNA (as described above in chapter 2.5.1) was also used for transcriptome analyses. RNA quality of the samples was checked using a Bioanalyzer (Agilent 2100, Agilent, Santa Clara, United States of America) and the RNA integrity numbers (RIN) of the samples ranged between 6.8 and 8.2 (Table 2). Library construction and sequencing were conducted at Chronix Biomedical (Chronix Biomedical, Inc., Göttingen, Germany). RNA libraries were prepared using the TrueSeq RNA Library Prep Kit (Illumina, San Diego, United States of America). Single-end reads were sequenced with a length of 75 bp using an Illumina HighSeq 2000 (Illumina, San Diego, CA, USA).

The samples yielded 16 to 21 million reads (Table 1). Processing of the raw sequence data was performed by Dr. Dennis Janz (Department of Forest Botany and Tree Physiology, University of Göttingen, Germany) with the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Using FASTQ Trimmer, all nucleotides with a Phred quality score below 20 were removed from the ends of the reads, and sequences smaller than 38 bp or sequences with a Phred score below 20 for 10% of the nucleotides were discarded by the FASTQ Filter; adapter sequences and primers were removed with the FASTQ Clipper (http://hannonlab.cshl.edu/fastx_toolkit/). Raw sequence qualities were high, therefore read numbers/sample after processing remained between 16 and 21 million (Table 1). The processed sequences were mapped against the *Arabidopsis thaliana* transcriptome and differential gene expression analyses was performed as mentioned in Vishwanathan et al., 2020.

Table 1. Processing and mapping information of every samples' raw sequence data.

Sample ID	Treatment	Sample number	RIN Value	Raw	Trimmed	Filtered	Mapped	% Mapped
10A	Col-0 + Buffer	S20	7.50	18,323,659	18,318,236	18,087,530	17,175,563	94.96
10B	Col-0 + Buffer + *T. ni*	S19	6.80	20,203,128	20,194,911	19,905,113	18,891,328	94.91
13A	Col-0 + Buffer	S18	8.30	18,217,338	18,211,936	17,967,732	17,326,589	96.43
13B	Col-0 + Buffer + *T. ni*	S17	7.60	19,716,705	19,707,668	19,428,965	18,724,741	96.38
15A	Col-0 + Buffer	S16	7.50	19,706,504	19,697,626	19,414,133	18,621,357	95.92
15B	Col-0 + Buffer + *T. ni*	S15	7.20	17,364,845	17,357,509	17,100,108	16,447,675	96.18
17A	Col-0 + Buffer	S14	7.70	16,917,427	16,910,467	16,680,497	15,957,944	95.67
17B	Col-0 + Buffer + *T. ni*	S13	7.70	17,223,761	17,217,803	16,970,427	16,345,331	96.32
18A	Col-0 + Buffer	S12	7.90	19,157,737	19,149,695	18,878,821	18,195,574	96.38
18B	Col-0 + Buffer + *T. ni*	S11	7.60	20,488,629	20,480,817	20,206,424	19,341,346	95.72
20A	Col-0 + *L. bicolor*	S10	7.10	19,809,126	19,801,344	19,532,487	18,648,387	95.47
20B	Col-0 + *L. bicolor* + *T. ni*	S9	7.30	20,883,945	20,875,671	20,587,068	19,616,510	95.29
23A	Col-0 + *L. bicolor*	S8	8.20	17,267,981	17,262,882	17,029,735	16,397,067	96.28
23B	Col-0 + *L. bicolor* + *T. ni*	S7	7.00	18,245,284	18,236,417	17,966,636	17,202,430	95.75
25A	Col-0 + *L. bicolor*	S6	8.10	17,953,224	17,945,646	17,697,924	17,001,164	96.06
25B	Col-0 + *L. bicolor* + *T. ni*	S5	7.20	20,835,382	20,824,065	20,517,536	19,653,407	95.79
27A	Col-0 + *L. bicolor*	S4	7.90	19,920,333	19,910,035	19,606,826	18,731,911	95.54
27B	Col-0 + *L. bicolor* + *T. ni*	S3	7.60	18,454,061	18,447,710	18,197,177	17,531,189	96.34
28A	Col-0 + *L. bicolor*	S2	7.70	16,605,947	16,599,168	16,363,095	15,671,379	95.77
28B	Col-0 + *L. bicolor* + *T. ni*	S1	7.80	17,832,755	17,824,806	17,580,314	16,905,795	96.16

2.6. Metabolite measurements

2.6.1. Preparation of plant extract

Col-0 leaf samples were collected as described in chapter 2.5. Extraction was performed as described by Kusch et al., 2019. 100 mg of the leaf samples were homogenized using a bead beater (Retsch GmbH, Haan, Germany). Extraction was performed with 750 µL of methanol (LC-MS grade) containing internal standards and 2.5 mL of methyl-tert-butyl ether (MTBE) (Sigma-aldrich, Steinheim, Germany). The extracted solution was shaken for 1 hour at 200

rpm. 600 µL of water was added for phase separation and incubated for 10 min. After centrifugation at 450xg for 15 min, the upper phase (non-polar) was transferred to another tube. The lower phase (polar) was extracted with 700 µL of methanol:water (3:2.5, v/v) and 1.3 mL MTBE solution. The lower phase was shaken, phase separated and centrifuged as described above. Both the upper phases were pooled for targeted analysis of metabolites and phytohormones. The extracted lower and upper phases were pooled together for untargeted analysis of metabolites. The pooled phase solutions were evaporated using streaming nitrogen. The dried residue was suspended in solvent containing 100 µL acetonitrile:water (20:80, v/v) with 0.3 mmol L^{-1} ammonium formate (pH 3.5).

2.6.2. Targeted analysis of hormones

Phytohormone analysis was done by Dr. Krzysztof Zienkiewicz from the Goettingen Metabolomics and Lipidomics Laboratory, Göttingen Center for Molecular Biosciences (GZMB), and Department of Plant Biochemistry, University of Goettingen, according to the method described below.

The extraction of samples was performed as described in chapter 2.6.1. Reversed phase separation of constituents was performed as previously described (Kusch et al., 2019) using an ACQUITY UPLC® system (Waters Corp., Milford, MA, USA) equipped with an ACQUITY UPLC® HSS T3 column (100 mm x 1 mm, 1.8 µm; Waters Corp., Milford, MA, USA). Nanoelectrospray (nanoESI) analysis was carried out as described by Kusch et al., (2019) and phytohormones were ionized in a negative mode and determined in a scheduled multiple reaction monitoring mode with an AB Sciex 4000 QTRAP® tandem mass spectrometer (AB Sciex, Framingham, MA, USA). Mass transitions were as described by Iven et al., 2012, with some modifications specified in Supplementary Table 2.

2.6.3. Non-targeted metabolome analysis

Metabolite analysis was done by Dr. Kirstin Feussner from the Goettingen Metabolomics and Lipidomics Laboratory, Göttingen Center for Molecular Biosciences (GZMB), and Department of Plant Biochemistry, University of Goettingen, according to (Feussner & Feussner, 2019).

Two-phase extraction of samples was performed with MTBE, methanol and water as described in chapter 2.6.1. The metabolome analysis was done using LC-high resolution-MS (LC-HR-MS). The polar and non-polar extraction phase was ionized by positive and negative electrospray ionization (ESI). The metabolites in the samples were separated by using an Acquity UPLC system with UPLC eLambda 800 mm PDA detector equipped with Acquity UPLC BEH SHIELD RP18 column (1 x 100 mM, 1.7 µm particle size, Waters Corporation, Milford, USA). Sample raw data values were processed with Markerlynx 4.1 software resulting in two indices. The data was further processed by ANOVA with multiple corrections using Benjamin-Hochberg. The feature identities were confirmed by co-eluting with authentic standards.

2.7. Microscopic analysis

L. bicolor mycelium and dead root tissue were stained with Lactophenol trypan blue (LTB) (Koch & Slusarenko, 1990) and visualized using an inverted microscope (Carl Zeiss Microimaging GmbH, Göttingen, Germany). Col-0 seeds were sterilized and germinated on ½ MS media in petri dishes as described in chapter 2.2.3.1. When the seedlings were 5 days old, 10 µL of *L. bicolor* suspension in 10 mM $MgSO_4$ (OD_{600} = 0.1) was inoculated on every seedling root. Petri-plates were sealed with micropore tape and the root portion of the plate was covered with aluminium foil. The plates were placed back in the acclimatized chamber in an upright position as mentioned in chapter 2.2.3.1. When the seedlings were two weeks old, roots of 5 plants were harvested and immersed in a 2 mL reaction tube (Eppendorf AG, Hamburg, Germany) containing LTB solution (10 mL lactic acid, 10 mL glycerol, 10 mL ddH_20, 10 g solid phenol, 10 mg trypan blue) diluted in 1:1 ratio with 100% ethanol. The tubes were boiled in a water bath for 2 mins with their lids open. LTB solution was discarded, and the samples were de-stained with chloral hydrate solution (2.5 g chloral hydrate/mL of ddH_2O) for 2 to 3 hours at room temperature on an orbital shaker (Eppendorf AG, Hamburg, Germany). The solution was replaced with fresh chlorate hydrate solution and de-staining was performed overnight on the shaker at room temperature. On the next day, chloral hydrate solution was discarded, and the samples were treated overnight with 70% glycerol. Root samples were mounted on a glass slide using 70% glycerol, fixed and examined using the inverted microscope (Axio Observer Z1, Carl Zeiss Microimaging GmbH, Göttingen, Germany). The microscopic images were analysed using Zen Lite software.

2.8. MAPK activation experiment

Two sterilized and cold stratified Col-0 seeds were placed in every well of a 24-well plate (Sarstedt AG & Co. KG, Nümbrecht, Germany) containing 500 µL of 1MS solution with 0.5% (w/v) sucrose. The seeds were germinated under 100 µE light condition for 16/8 hours at 22/20°C in day/night phase. The medium was replaced by fresh 1MS medium containing 0.5% (w/v) sucrose on day 7 and day 14. On day 15, the entire seedlings were treated with 500 µL of 1MS medium containing 0.5% (w/v) sucrose, together with elicitors or dead or live *L. bicolor* (OD_{600} = 0.1). flg22 and chitin were used at a final concentration of 100 nM and 10 µg/mL respectively per well. After treatment time of 15 min, seedlings from 2 wells of the same treatment were removed, briefly dried in a paper towel and collected in a 1.5 mL reaction tube. The tubes with samples were flash frozen in liquid nitrogen and stored at -20 °C until further use.

2.8.1. Protein quantification

The frozen samples were ground with 200 µL of CERK1 extraction buffer (250 mM sucrose, 100 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid potassium salt (Sigma-Aldrich, St. Louis, United States of America) pH 7.5, 5% glycerol, 1 mM sodium molybdate dihydrate, 25 mM sodium fluoride, 50 mM sodium pyrophosphate decahydrate, 10 mM ethylenediaminetetraacetic acid, 1mM 1,4-dithiothreitol, 0.5% triton x-100) containing 500 µL protease inhibitor cocktail (4-2-aminoethyl benzene sulfonyl fluoride 1 g, bestatin hydrochloride (Serva Electrophoresis GmbH, Heidelberg, Germany) 5 mg, pepstatin A (Serva Electrophoresis GmbH, Heidelberg, Germany) 10 mg, leupeptin hemisulphate (Serva Electrophoresis GmbH, Heidelberg, Germany) 100 mg, E-64 (trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (Serva Electrophoresis GmbH, Heidelberg, Germany) 10 mg, 1-10-phenanthroline monohydrate (Sigma-aldrich, Steinheim, Germany) 10 g dissolved in 200 mL of dimethylsuphoxide) and sea sand with IKA® RW20 digital mixer for 30 s. After homogenization, 400 µL more CERK1 extraction buffer was added and the tubes were centrifuged at 13000 rpm, 4°C for 10 min. The supernatant was transferred to new tube and kept on ice. Protein concentration in the extract was quantified after mixing 3 µL of the extract with 1 mL BRADFORD solution ((Roti® - Quant, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) diluted to

1:5 ratio with milliQ water). Absorbance was measured after 5min using a photometer (Biochrom GmbH, Berlin, Germany, Biowave II) at 595 nm. BSA was used as the standard for protein quantification. The samples were normalised to 1 µg/mL protein concentration using CERK1 buffer and were later stored at -20°C. For SDS-PAGE and western blot, the normalised extracts were mixed with 4x SDS buffer (200 mM Tris pH 6.8, 400 mM 1,4-dithiothreitol, 8% sodium dodecyl sulphate, 40% glycerol, 0.1% (w/v) bromophenol blue (Sigma-aldrich, Steinheim, Germany)) and stored at -20°C.

2.8.2. SDS-PAGE

10% SDS gels were used for MAPK assay and the compositions of resolving and stacking gels are mentioned in Table 2. The gels were prepared in 1.5 mm Bio-Rad gel set-up (BIO-RAD Laboratories, Hercules, United States of America) with 10 well combs, covered with wet paper towels and stored at 4°C until use. Before loading the samples, the extracts with SDS loading buffer were heated at 98°C for 5 mins and briefly centrifuged at 13000 rpm. Combs were removed from the stacking gel and the gel was placed in the PAGE apparatus filled with 1x SDS running buffer (diluted from 10x SDS running buffer; concentration in g/L: Tris base – 30.4 g, glycine – 144.2 g, 1% (w/v) SDS dissolved in milliQ water). 6 µL of protein ladder (BIO-RAD Laboratories, Hercules, United States of America) and 15 µL of the samples were loaded in the respective wells. The gel was run at 30 mA in a PowerPac^{TM} HC (BIO-RAD Laboratories, Singapore) until bromophenol blue reaches the end of the gel (for 1.5 hours). After the run, the apparatus was disassembled, and the stacking gels were removed.

Table 2. Composition of separating and stacking gel for SDS-PAGE.

Chemicals and buffers	10% Resolving gel (mL)	Stacking gel (mL)
Tris buffer (1.5 M, pH 8.8)	33	-
Tris buffer (0.5 M, pH 6.8)	-	20.4
30% Acrylamide	16.5	4.15
10% APS	0.5	0.125
TEMED	0.02	0.0125
Total volume	50	25

2.8.3. Western blot

The PVDF membranes (Roti®-PVDF, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) which will be blotted with proteins were activated by placing the membranes in methanol for a few seconds. The western blot apparatus with the PVDF membrane, whatman papers (WhatmanTM, GE Healthcare UK Limited, Buckinghamshire, United Kingdom), sponge foam pads (Tran-Blot®, (BIO-RAD Laboratories, Hercules, United States of America) was assembled as shown in Figure 7. The set up was clamped and blotted at 80 V in BIO-RAD Trans-Blot® cell (BIO-RAD Laboratories, Hercules, United States of America) filled with transfer buffer (diluted from 20x transfer buffer stock: 1M Tris-base, 1 M boric acid, pH = 8.3). After 2 hours, the apparatus was disassembled, and the gels were discarded. The membrane was blocked with 4% MP (Milk powder) in 1xTBS-T buffer (diluted from 20x TBS-T buffer: 3M sodium chloride, 200 mM Tris pH 8.0, 1% (v/v) Tween-20) for 1 hour on a shaker at room temperature. The membrane was subsequently incubated overnight with the primary antibody, αpMPK (p44/42) (Bio-Rad Immunstar AP, Cell Signalling Technology, Massachusetts, United States of America) (diluted 1:5000 with 4% MP in 1xTBS-T) at 4°C on a shaker. The primary antibody solution was discarded on the next day, and the membrane was washed 5 times for 12 min with 4% MP in 1xTBS-T buffer. Then the membrane was incubated with anti-rabbit secondary antibody (Sigma A3687, Sigma-Aldrich, St. Louis, United States of America) (diluted 1:5000 with 4% MP in 1xTBS-T) for 2 hours on a shaker at room temperature. The antibody solution was discarded, and the membrane was washed 6 times for 5 min with 1x TBS-T buffer on a shaker at room temperature. Final washing of the membrane was done with 10 mL of AP buffer (100 mM Tris pH 9.5, 100 mM sodium chloride, 50 mM magnesium sulphate) for 5 mins, before transferring it to a plastic foil with 500 µL of the substrate (Phospho-MAPK/CDK substrate Rabbit mAb #2325, Cell Signalling Technology, Massachusetts, United States of America). The membrane was incubated briefly (2-5 min) in dark and then later developed using the ChemiDoc™ Touch imaging system (BIO-RAD Laboratories, Hercules, United States of America). After visualization, the proteins in the membrane were stained with Ponceau S staining solution (0.1 % (w/v) Ponceau S (Sigma-aldrich, Steinheim, Germany) (in 5% (v/v) acetic acid) for 15 min.

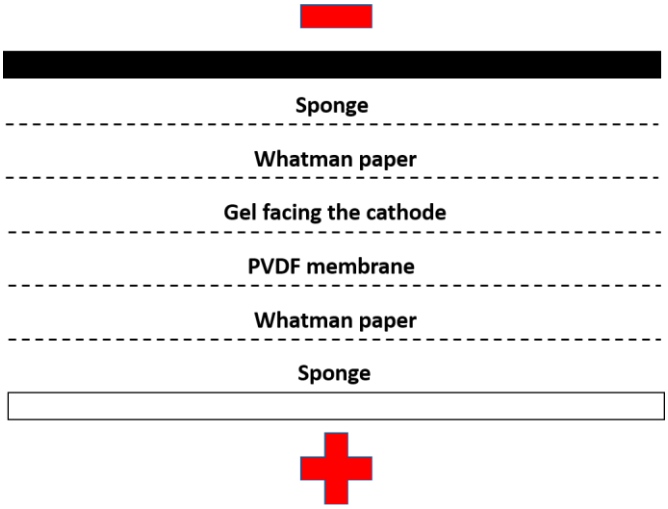


Figure 7. Western blot apparatus set-up.

Proteins separated by the SDS-PAGE was transferred to the PVDF membrane by performing western blot. The gel and membrane are arranged as shown in the figure.

2.9. Oxidative burst analysis

2.9.1. Local tissue response

ROS burst experiments with entire Col-0 seedlings was performed as described by (Cheng et al., 2015). A sterile Col-0 seed was placed in every well of a white 96-well plate (Sarstedt AG & Co. KG, Nümbrecht, Germany), filled with 200 µL of 1MS medium containing 0.5% (w/v) sucrose. The seeds were germinated under 100 µE light for 16/8 hours at 22/20°C in day/night phase. For suppression of ROS burst by *L. bicolor*, media in the wells were replaced with sterile ddH_20 on day 9 and the treatment wells were inoculated with live or dead *L. bicolor* (final OD_{600} = 0.1 in the plate). Oxidative burst reagent was prepared by adding luminol derivative L-012 (final concentration of 100 µM) and horseradish peroxidase (final concentration of 20 µg/mL) to 24 mL of milliQ water. On the next day, ddH_20 in the wells with or without dead or live *L. bicolor* were replaced with 200 µL of the oxidative burst reagent with or without elicitors in the corresponding wells. Luminescence was immediately measured in a TECAN infinite M200 plate reader (TECAN, Männedorf, Switzerland) for 60 cycles with kinetic interval of 1 min and integration time of 350 ms. For ROS induction by *L. bicolor*, seedlings were treated with freshly prepared live and dead *L. bicolor* (final OD_{600} = 0.1) in the respective wells before adding the oxidative burst reagent.

2.9.2. Systemic tissue response

Col-0 plants were grown and treated with *L. bicolor* as described in chapters 2.2.3.2 and 2.3.1.1. When the plants were 5 weeks old, a single leaf from 8 plants/treatment were harvested. Leaf disks were collected from the harvested leaves using a biopsy punch (0.4 mm diameter) and placed in a white 96-well plate filled with 100 µL of tap water. The plate was covered with a lid and placed on the lab bench overnight. On the next day, water was removed from the wells and replaced with oxidative burst reagent with or without elicitors. Luminescence was measured immediately in the TECAN infinite M200 plate reader as described in chapter 2.9.1.

2.10. GUS expression analyses

GUS expression analyses was performed as described by (Millet et al., 2010). The GUS-reporter lines with promoters for *CYP71A12*, *WRKY11* and *MYB51* were placed in 96 well flat-bottomed plates (Sarstedt AG & Co. KG, Nümbrecht, Germany) containing 100 µL of 1MS with 0.5% (w/v) sucrose. The seeds were germinated for 8 days in a growth room under 100 µE. $m^{-2}s^{-1}$ for 16/8 hours at 22/20 °C day/night phase. Media in the wells were replaced with sterile ddH_2O on day 9 and live or dead *L. bicolor* was inoculated (Final OD_{600} = 0.1 in the plate) into the corresponding treatment wells. On day 10, flg22 (final concentration = 100 nM) was added to the elicitor treatment wells. After 4 hours, the wells filled with ddH_2O with or without live or dead *L. bicolor* and flg22 were replaced with GUS staining solution (50 mM sodium phosphate buffer (pH = 7), 10 mM EDTA, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 0.5 mM x-gluc (Thermo Fischer Scientific, Massachusetts, United States of America), 0.01% triton X-100). The seeds were stained with GUS solution for 3 hours at 37°C and later washed thrice with water. The seedlings were de-stained with 95% ethanol overnight at 4°C. On the next day, ethanol was removed and GUS expression in the seedling roots (Figure 8) was observed under a light microscope (Carl Zeiss Microimaging GmbH, Göttingen, Germany).

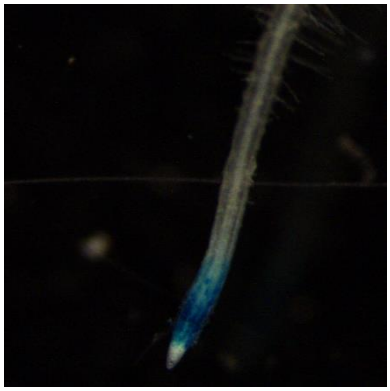


Figure 8. Flg22 induced GUS expression in the roots of *CYP71A12p*-GUS reporter line.

9-days old seedlings were treated with flg22 for 3 hours and later stained with GUS solution. flg22 treatment induces *CYP71A12* expression in the root elongation zone. Similarly, GUS expression analyses was recorded using *MYB51p*- and *WRKY11p*-GUS lines.

2.11. Statistical analyses

Statistical analyses for *T. ni* feeding, *Pst* DC3000, phytohormone, metabolite quantification and gene expression experiments was performed as described in (Vishwanathan et al., 2020). The relative luminescence units (RLU) data from all ROS experiments for different treatments was analyzed for statistical difference by two-way ANOVA. Tukey's HSD test was performed for multiple comparison analysis of the data. Significant effect of *L. bicolor* treatment for ISR against Noco2 was determined by performing Student's t-test.

3. RESULTS

3.1. *L. bicolor* induces systemic resistance in Col-0 against *T. ni*

3.1.1. *L. bicolor* affects larval weight gain

The systemic effect of *L. bicolor* treatment on a non-host species like Col-0 was determined by challenging this system with *T. ni*. The outcome was determined by weighing the larvae after feeding on buffer-treated and *L. bicolor*-treated Col-0 plants for 7 days. Caterpillars feeding on *L. bicolor* treated Col-0 plants had 22% less weight gain than those feeding on the control plants ($p < 10^{-7}$; Figure 9; Vishwanathan et al., 2020). Though Col-0 is a non-host, *L. bicolor* triggered ISR by negatively affected the weight gain of caterpillars.

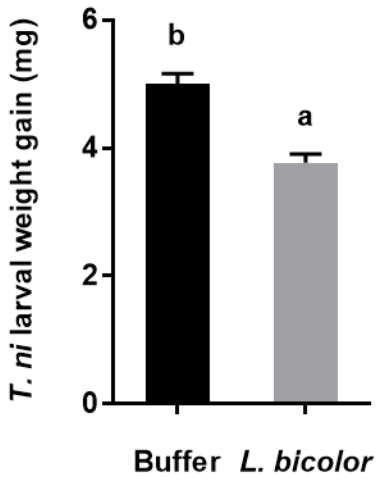


Figure 9. *L. bicolor* treatment induces systemic resistance in Col-0 against *T. ni*.

Col-0 plants were inoculated with *L. bicolor* and later fed by *T. ni* larvae. Caterpillar weight gain was measured after 7 days of feeding. Data show that *L. bicolor* treatment negatively affected the weight gain of the larvae. n = 20 experiments with at least 20 caterpillars/treatment (Vishwanathan et al., 2020). ANOVA was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.1.2. *L. bicolor* does not influence the transcriptome in systemic tissue

To test if *L. bicolor* inoculation activated systemic defenses to protect the plant against leaf chewing caterpillars, the expression of well-characterized defense marker genes as outlined in the introduction was determined. The expression of JA markers genes like *VSP1*, *VSP2* and *MYC2*; JA/Et marker genes like *PR4*, *ERF1* and *PDF1.2*; Et marker gene, *EIN3*; SA marker genes like *GST6*, *PR1*, *PR2*; Transcription factors like *WRKY70*, *MYB51* and the camalexin biosynthesis *CYP71A13* in Col-0 in response to *L. bicolor* inoculation and *T. ni* feeding, were analyzed by performing qRT-PCR. The CT values obtained for the candidate genes were normalized to that of the house keeping gene *EIF4A*. The data showed that *VSP1*, *VSP2*, *MYC2*, and *ERF1* gene expressions were significantly upregulated after *T. ni* feeding ($p < 0.05$; Figure 10; Vishwanathan et al., 2020). *VSP1*, *VSP2* and *PDF1.2* defense gene expressions were significantly increased in response to combined effect of *L. bicolor* and *T. ni* ($p < 0.05$; Figure 10; Vishwanathan et al., 2020). However, no significant effect was observed for *L. bicolor* treatment on Col-0 ($p < 0.4$; Figure 10; Vishwanathan et al., 2020).

Since *L. bicolor* treatment did not significantly influence the candidate marker gene expression as described above, transcriptomic analysis was performed. Leaf samples were collected from Col-0 plants treated with or without *L. bicolor* and *T. ni* (chapter 2.5) to extract RNA and identify significantly differentially expressed genes (DEGs) (chapter 2.5.2). Though genes were differentially regulated in response to *T. ni*, not even a single gene was significantly affected by *L. bicolor* treatment with or without *T. ni* (https://wwwdev.ebi.ac.uk/gxa/experiments/E-MTAB-8523/Results?accessKey=d8c24ec5-2a19-4372-9d11-bb2966a4b418). Both qRT-PCR and transcriptome analysis were performed using samples collected from 6 independent experiments. The single time point considered for the analyses and the variance associated between the samples collected from soil-grown plants could be reasons for not identifying candidate genes primed by *L. bicolor*. Gene expression analyses of leaf samples collected from plants grown and treated under axenic conditions can identify the genetic determinants of *L. bicolor*-triggered ISR.

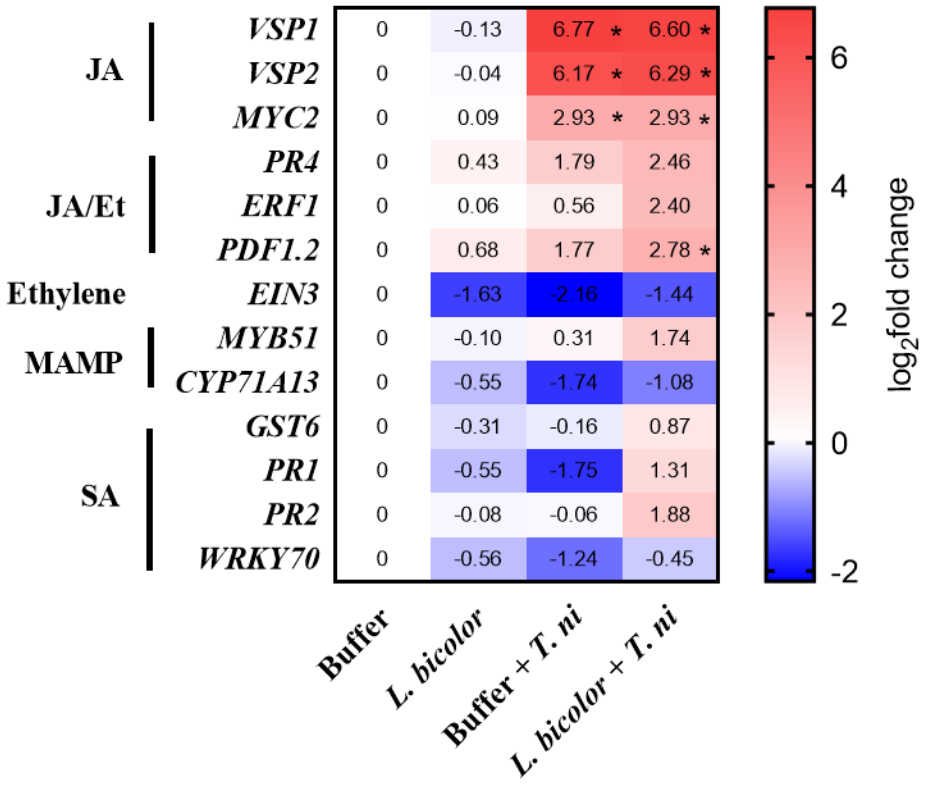


Figure 10. Defense gene expression in Col-0 leaves in response to *L. bicolor* and *T. ni*.

RNA was extracted from Col-0 leaves after root treatments with or without *L. bicolor* and later with or without *T. ni* feeding. Marker defense gene expression was analyzed by performing qRT-PCR. CT values of target genes were normalized to the house keeping gene *EIF4A*. Data show $\log_2$ relative fold change ($\log_2(2^{-\Delta\Delta CT})$) values in leaf samples collected from 6 independent experiments (n = 6) normalized to mock-inoculated control plants (Vishwanathan et al., 2020). Statistical significance was performed for $\log_2(2^{-\Delta\Delta CT})$ values using two-way ANOVA with Tukey's HSD test. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.1.3. Phytohormone levels were not primed by *L. bicolor*

To check if any of the major phytohormones accumulated for *L. bicolor* treatment, concentrations of JA-, SA- and ABA- derivatives were determined in the leaf samples of Col-0 plants as described in chapter 2.6. Like gene expression analyses, there were no significant changes in phytohormones' or their derivatives' concentration in the leaf samples in response to *L. bicolor* (Supplementary table 6.2.1). *L. bicolor* had no significant effect on the concentration of the active form of JA, JA-Ile ($p = 0.95$; Figure 11). Similarly, other phytohormones were also not responsive to *L. bicolor* but had increased concentrations upon *T. ni* feeding (Supplementary Figure 6.2.1). However, the concentration of the glycosylated jasmonate derivative, 12-OH-JA-Ile, increased 40-fold in Col-0 leaves when inoculated with *L. bicolor* and

fed by *T. ni* ($p < 0.01$; Figure 11). These results show that *L. bicolor* treatment in Col-0 roots did not significantly alter the phytohormone accumulation profile in the systemic leaves at 24 hours after *T. ni* feeding. However, priming of phytohormones cannot be excluded because analysis was performed from samples of only one time point. On the other hand, pooling leaves from plants grown in soil conditions can also contribute to the disparity between replicates. Hormone quantification of *in vitro* samples with considerable homogeneity and different time points could have determined whether the dynamics associated with metabolite synthesis and accumulation influences ISR by *L. bicolor* against caterpillar feeding.

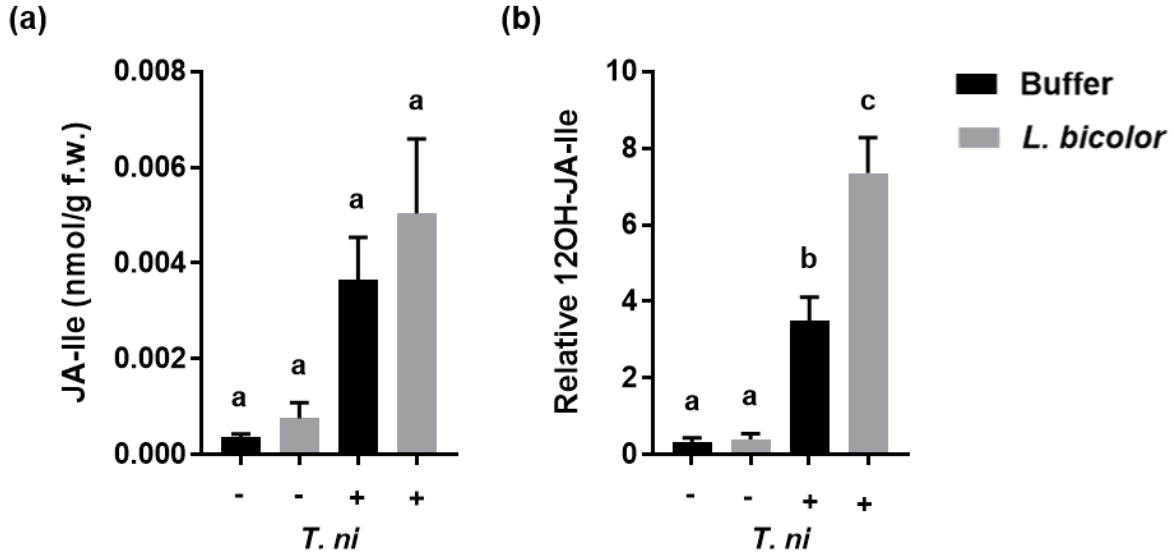


Figure 11. Influence of *L. bicolor* and *T. ni* treatment on (a) JA-Ile and (b) 12-OH-JA-Ile levels in Col-0 leaves.

Phytohormone concentrations were quantified in Col-0 leaves harvested from plants treated with or without *L. bicolor* and *T. ni*. Data show absolute mean of JA-Ile and concentration of 12OH-JA-Ile relative to the endogenous concentration in the buffer treated plants. n = 6 experiments with 8 plants/treatment. Two-way ANOVA and Fisher's LSD test was used for determining statistical significant with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.1.4. *L. bicolor* is dependent on JA and SA signalling pathways for regulating ISR

To test whether phytohormone signalling pathways were activated by *L. bicolor*, *T. ni* feeding experiments were performed (as described in chapter 2.4.1) with various hormone signalling mutants.

Results

The JA receptor mutant, *coi1-16* was treated with *L. bicolor* as described in chapter 2.3.1.1 and challenged with *T. ni* larvae. Although not significant, *coi1-16* which is insensitive to endogenous JA-Ile promoted weight gain of *T. ni* caterpillars ($p = 0.056$; Figure 12). Unlike Col-0, the *T. ni* larvae feeding on *L. bicolor*-treated *coi1-16* plants did not record less weight gain than the control ($p = 0.995$; Figure 12; Vishwanathan et al., 2020). Therefore, the ISR phenotype triggered by *L. bicolor* was abolished when *COI1* a member of the JA receptor complex was knocked out. This shows that *L. bicolor* triggered systemic defense in Col-0 is dependent on *COI1*.

To further characterize the JA signalling pathway, the JA biosynthesis mutant, *aos*, was treated with *L. bicolor* and fed by *T. ni*. Results based on a single individual experiment showed that *L. bicolor* treatment did not significantly affect *T. ni* larval weight gain in both Col-0 and *aos* ($p = 0.07$; Supplementary figure 6.2.2). Hence, independent experiments are required to determine if *L. bicolor* depends on jasmonate biosynthesis for triggering ISR. Ethylene which can be synergistic or antagonistic to JA could also play a role in this ISR signalling process. The ethylene signal transduction mutant *ein2-1* was checked for its role in leaf protection by *L. bicolor* against *T. ni*. Like the *aos* experiment, *L. bicolor* treated Col-0 did not have a significant negative influence on *T. ni* larval growth ($p = 0.54$; Supplementary figure 6.2.3) in the experiment performed. More replicates are necessary to test whether *L. bicolor*-triggered ISR depends on *EIN2*.

Another major phytohormone in induced systemic defenses and biotic stress responses is SA. SA signalling mutants were also tested for *L. bicolor* triggered ISR by performing caterpillar feeding experiments. SA had a strong antagonistic effect on JA. This observation was evident in Figure 12, where *T. ni* caterpillars after feeding on *sid2-2* and *npr1-1* mutants had significantly less weight than those feeding on Col-0 ($p < 0.001$). *L. bicolor* treatment did not significantly influence the *T. ni* larvae weight gain in the SA biosynthesis mutant, *sid2-2* ($p = 0.89$; Figure 12; Vishwanathan et al., 2020). Though not statistically significant, *L. bicolor* treatment reduced the larvae weight gain by approximately 30% in SA positive regulator mutant, *npr1-1* ($p = 0.1$; Figure 12; Vishwanathan et al., 2020). More independent caterpillar feeding experiments on *npr1-1* with and without *L. bicolor* can indicate whether the ISR pathway is also dependent on *NPR1*. These results show that *L. bicolor* triggered resistance is dependent on JA-and SA-signalling pathways.

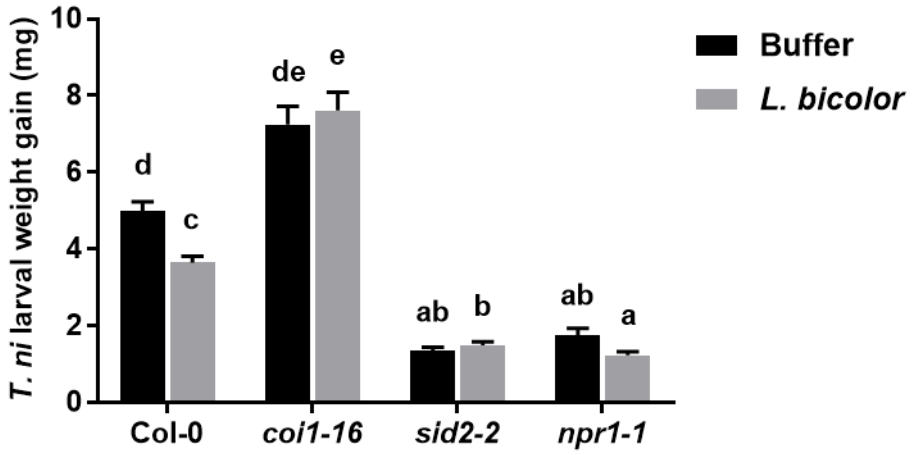


Figure 12. *L. bicolor*-triggered ISR is dependent on JA and signalling pathways.

JA receptor mutant, *coi1-16*, SA biosynthesis mutant, *sid2-2* and SA positive regulator mutant, *npr1-1* were inoculated with *L. bicolor* and fed by *T. ni*. Data show the ISR response against *T. ni* triggered by *L. bicolor* is dependent on JA signalling *via COI1* and SA signalling *via SID2* and maybe *NPR1*. n = 4 experiments for *coi1-16*, *npr1-1* and n = 3 experiments for *sid2-2* with at least 20 caterpillars/treatment (Vishwanathan et al., 2020). Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.1.5. Negative regulators of SA play a role in *L. bicolor* induced ISR

L. bicolor-triggered ISR seems to be partially dependent on or independent of *NPR1*, which regulates JA antagonism (Spoel et al., 2003). *NPR3* and *NPR4* are negative regulators of SA signalling and acting independent of *NPR1* (Ding et al., 2018). Hence, it was hypothesized that the negative regulators of SA might be involved in this ISR response. To check this, *L. bicolor* inoculated *npr3/4* double mutant was tested for its role in affecting *T. ni* feeding. Instead of promoting resistance, *L. bicolor* treatment significantly increased *T. ni* larval weight gain by 25% in *npr3/4* double mutants ($p < 0.01$; Figure 13). Therefore, *L. bicolor*-triggerd ISR was dependent on negative regulators of SA signaling for protecting the plants against *T. ni*.

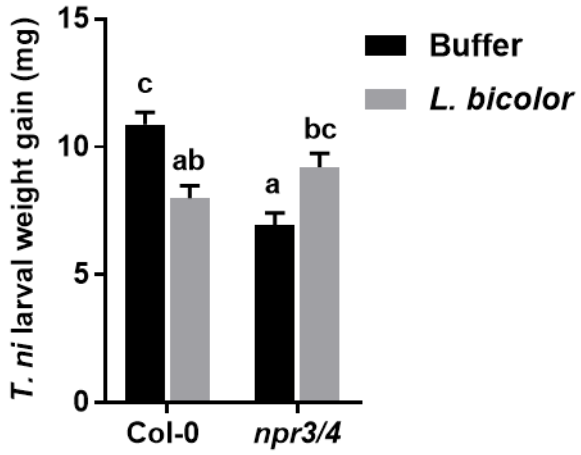


Figure 13. *NPR3* and *NPR4* are involved in *L. bicolor*-triggered ISR against herbivory.

npr3/4 mutants were inoculated with *L. bicolor* and fed by *T. ni*. Data show that unlike Col-0, *L. bicolor* treatment has a positive effect on *T. ni* caterpillars feeding on *npr3/4* mutants. n = 3 experiments with at least 20 caterpillars/treatment (Vishwanathan et al., 2020). Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.2. Other key players in ISR by *L. bicolor*

3.2.1. *MYB72* is a component of *L. bicolor*-triggered ISR

ISR in many cases, has been reported to be dependent on the iron homeostasis responses in plants (Romera et al., 2019). It is also shown that the iron deficiency response regulator, *MYB72*, is necessary for ISR (Martínez-Medina, Van Wees, et al., 2017; Van Der Ent et al., 2008). To check if this is the same for ISR by *L. bicolor*, caterpillar feeding experiments (chapter 2.4.1) were performed with *myb72-1*. The *T. ni* larvae feeding on *L. bicolor* treated *myb72-1* plants did not record significant difference in weight gain when compared with buffer treated plants ($p = 0.51$; Figure 14). *MYB72* and *MYB10* had been shown to have redundant roles in iron stress response (Palmer et al., 2013). Hence, the *myb72/10* double mutant was also tested to study the influence of *MYB10* in ISR. Like *myb72-1, L. bicolor* treatment did not negatively influence the weight gain of *T. ni* larvae, feeding on *myb72/10* plants ($p = 0.77$; Figure 14). Like in other reported beneficial microbial systems, ISR by *L. bicolor* also depended on *MYB72* for triggering systemic resistance. Since volatiles from *Trichoderma* spp. can also trigger ISR

by activating iron deficiency responses (Martínez-Medina, Van Wees, et al., 2017), *L. bicolor* volatiles could also be the stimuli for the systemic response in Col-0 against herbivore attacks.

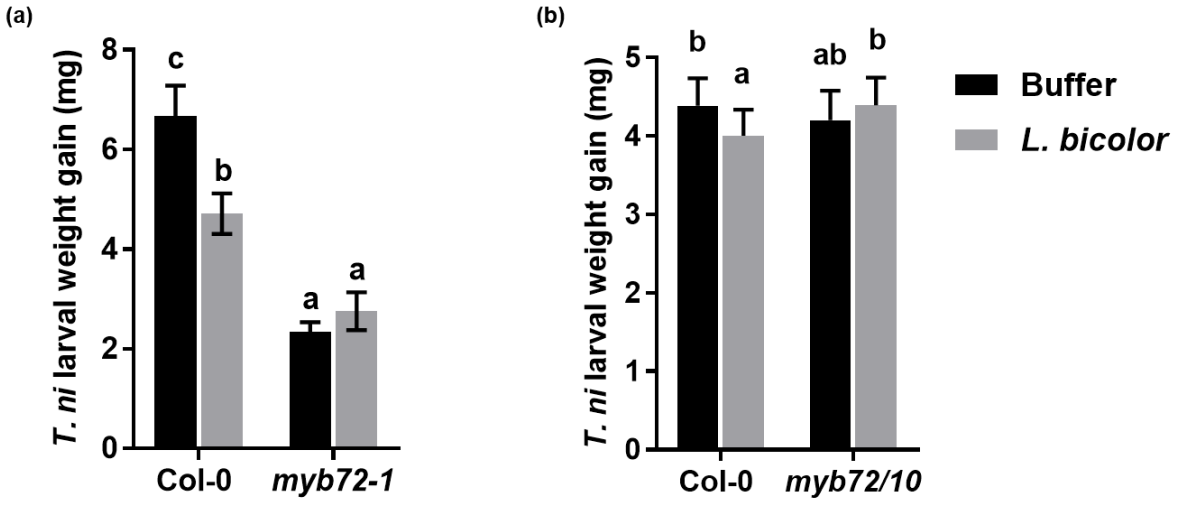


Figure 14. *L. bicolor* depends on *MYB72* for ISR against *T. ni*.

Iron stress response mutant roots, *myb72-1* (a) and *myb72/10* (b) were inoculated with *L. bicolor* and the leaves were fed by *T. ni*. Data show that *L. bicolor*-induced ISR response against *T. ni* larvae is dependent on iron homeostasis regulator, *MYB72*. n = 2 experiments for *myb72-1* and n = 5 experiments for *myb72/10* with at least 20 caterpillars/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.2.2. Camalexin accumulation in Col-0 leaves was increased by *L. bicolor*

Untargeted metabolite analysis was performed (chapter 2.6.1) to identify secondary metabolites which could be synthesized by the Col-0 leaves in response to *L. bicolor* and negatively affect caterpillar feeding. The data obtained from the analyses hint to a possible involvement of indolic glucosinolates in the defense regulation of *L. bicolor* with Col-0 (Supplementary figure 6.2.4). This observation was validated by performing *T. ni* feeding experiment with the indolic glucosinolate biosynthesis mutant, *cyp81f2*. The *cyp81f2* mutant cannot synthesize indolic glucosinolates and is devoid of callose deposition (Iven et al., 2012). Unlike Col-0, *T. ni* larvae feeding on *L. bicolor* inoculated *cyp81f2* plants had no significant growth reduction than control ($p = 0.92$; Figure 15). Indolic gluosinolates can thereby negatively influence the weight gain of *T. ni* caterpillars resulting from *L. bicolor* inoculation.

Results

Metabolite analysis was performed again with a targeted approach to check for accumulation of important metabolites, which have already been reported to function in plant defense. The targeted approach showed that camalexin was significantly primed by *L. bicolor* treatment ($p < 0.05$; Figure 15). It was observed that this phytoalexin accumulated in a significantly higher concentration when challenged with caterpillars ($p < 0.001$; Figure 15). Concentrations of other phytoalexins like glucobrassicin or raphanusamic acid were not affected by *L. bicolor* treatment (Supplementary figure 6.2.6). The mutant *cyp71a13-1* which cannot synthesize camalexin was treated with *L. bicolor* to confirm that camalexin is involved in this ISR. Preliminary results obtained from a single experiment showed that *L. bicolor* treatment did not have a significant influence on *T. ni* larval weight gain in *cyp71a13-1* mutants ($p < 0.98$; Supplementary figure 6.2.5).

Both camalexin and indolic glucosinolates are synthesized *via* the tryptophan pathway (Glawischnig et al., 2004; Ver et al., 2011). *cyp79b2/b3* double mutant cannot synthesize camalexin or indolic glucosinolates. The role of this secondary metabolite biosynthesis pathway in *L. bicolor* triggered ISR was determined by performing caterpillar feeding experiments with *cyp79b2/b3* double mutant. *L. bicolor* inoculation did not affect the larval feeding in *cyp79b2/b3* double mutant and had no significant impact on *T. ni* weight ($p = 0.07$, Figure 15; Vishwanathan et al., 2020). These results support that *L. bicolor* depends on the tryptophan pathway to prime camalexin to make the plants more robust against herbivory attacks.

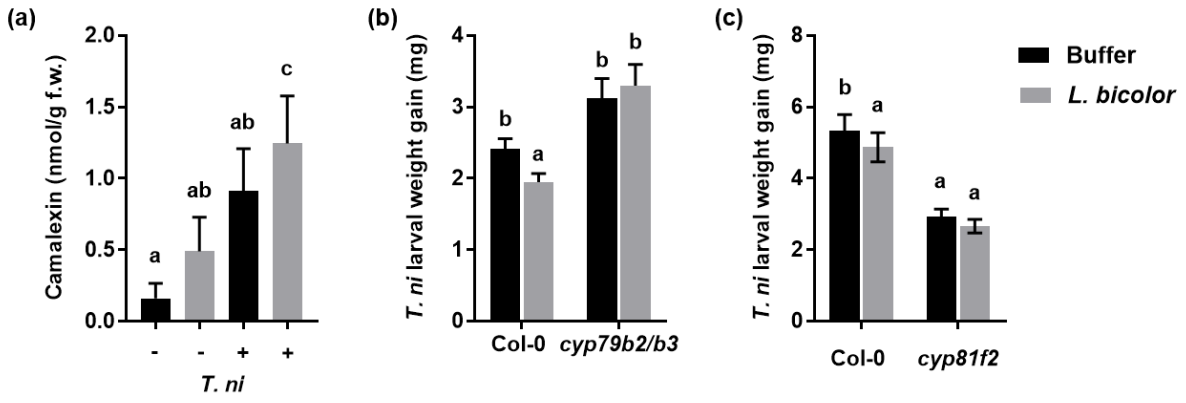


Figure 15. *L. bicolor* induced ISR against herbivory is dependent on the tryptophan pathway.

(a) Col-0 leaves from plants inoculated with buffer or *L. bicolor* and later challenged with or without *T. ni*. Data show camalexin levels in Col-0 systemic leaves collected from 4 independent experiments with 8 plants/treatment (n = 4). Two-way ANOVA with Fischer's LSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE. (b) *L. bicolor*-induced ISR against caterpillar feeding is lost in (b) *cyp79b2/b3* and (c) *cyp81f2* mutants. Data show caterpillar weight gain from n = 3 experiments for *cyp79b2/b3* and n = 4 experiments for *cyp81f2* with at least 20 caterpillars/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.3. Chitin perception triggers systemic resistance to herbivory

3.3.1. *L. bicolor* induces oxidative burst in Col-0

Like other plant invading microbes, the beneficial EMF, *L. bicolor* was expected to suppress the PTI responses to successfully colonize Col-0 roots. For determining the local tissue response, entire Col-0 seedlings were treated with *L. bicolor* as described in chapter 2.9.1. On the next day, the seedlings were treated with elicitors (chitin and flg22) and ROS production was measured. *L. bicolor* treatment significantly enhanced ROS generation in Col-0 seedlings compared to mock inoculated plants ($p < 0.001$; Figure 16). *L. bicolor* treatment did not suppress chitin or flg22 triggered ROS burst in the Col-0 seedlings (Figure 16). However, prior *L. bicolor* exposure significantly enhanced ROS production after chitin treatment ($p < 0.001$; Figure 16).

Suppression of elicitor triggered ROS burst was also analyzed in systemic tissues as explained in chapter 2.9.2. Leaf disks examined for ROS production showed that there was no

significant ROS generation in *L. bicolor* inoculated plants ($p = 0.99$; Figure 16). Elicitor treatment generated identical ROS burst in buffer- and *L. bicolor*-treated Col-0 plants ($p < 0.9$; Figure 16).

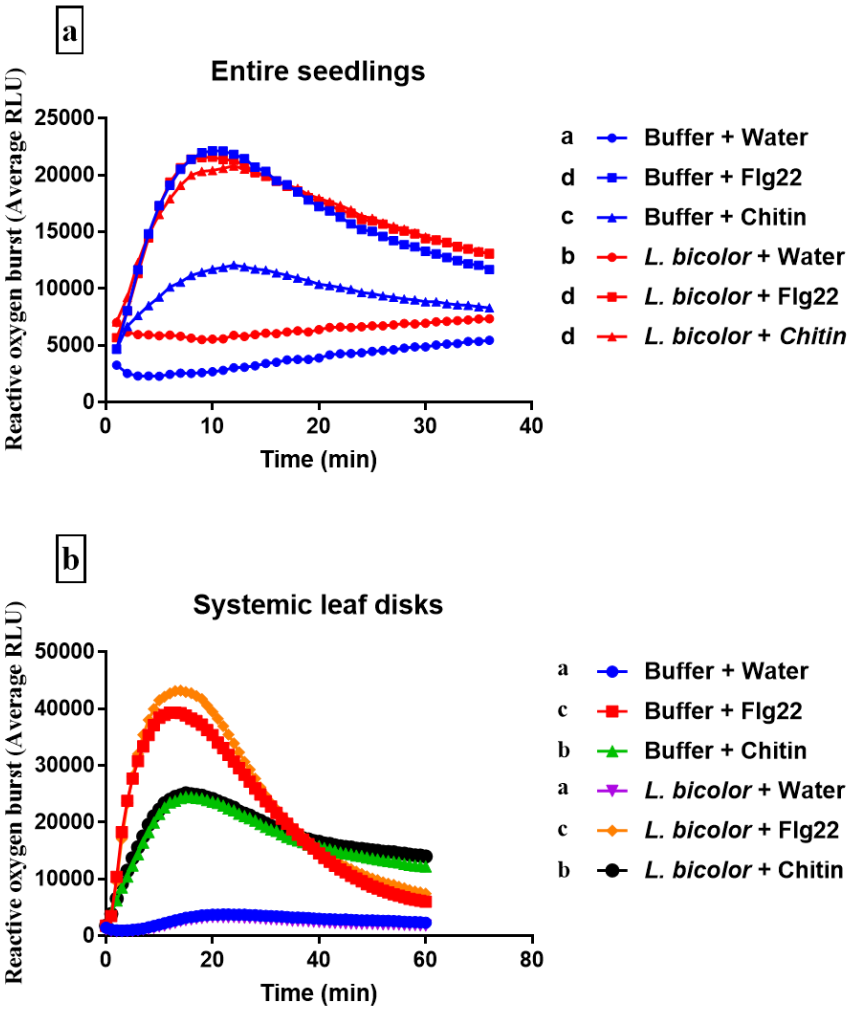


Figure 16. ROS burst responses in Col-0 seedlings to *L. bicolor* and elicitor treatments.

Experiment was performed using (a) entire seedlings (b) systemic leaf disks as described in chapter 2.9. Data show averages of RLU values of at least 2 independent experiments (n = 2) with 8 seedlings or leaf disks/treatment. Error bars are not included for easier visualization of the plots. Plots with error bars are shown in supplementary figure 6.2.7. Statistical analysis was performed as described in chapter 2.11. Different letters near the legends denote significant differences at $p < 0.05$.

3.3.2. *L. bicolor* activates MAPK signaling cascade in Col-0

Phosphorylation of MAPKKK activates the signalling cascade leading to phosphorylation of MAPKK and finally, MAPK6 and MAPK3, which are the common markers for PTI responses. The role of *L. bicolor* in suppressing or inducing the activation of the MAPK cascade was tested by performing MAPK experiments using entire seedlings as described in chapter 2.8. Fresh inoculation with live *L. bicolor* resulted in the phosphorylation of MAPK6 and MAPK3 (Figure 17; Vishwanathan et al., 2020). As expected, MAMPs present in dead *L. bicolor* also activated MAPK cascades leading to phosphorylation of MAPK6, MAPK3 and MAPK4 (Figure 17; Vishwanathan et al., 2020). Co-treatment of the seedlings with elicitors and live *L. bicolor*, did not result in suppression of MAPK6, MAPK3 and MAPK4 phosphorylation (Figure 17). Therefore, both live and dead *L. bicolor* activated MAPK cascades and also did not suppress elicitor-induced MAPK phosphorylation in Col-0.

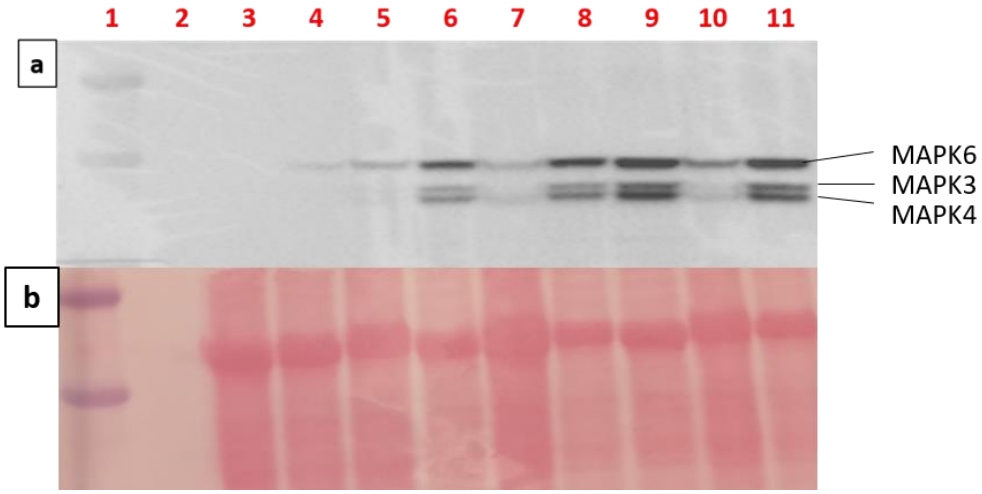


Figure 17. *L. bicolor* activates the MAPK signalling cascade in Col-0.

(a) Col-0 seedlings grown under axenic conditions, were treated with elicitors and *L. bicolor* for 15 min. Proteins were extracted and phosphorylation of MAPK6, MAPK3 and MAPK4 in the samples were identified using α-p44/42-ERK antibody. Experiment was repeated at least 3 times with 4 seedlings/treatment. Lanes: [1] marker, [2] empty, [3] buffer, [4] *L. bicolor*, [5] dead *L. bicolor*, [6] buffer + chitin, [7] *L. bicolor* + chitin, [8] dead *L. bicolor* + chitin, [9] buffer + flg22, [10] *L. bicolor* + flg22 and [11] dead *L. bicolor* + flg22 were observed with substrate exposure time of 240s. (b) Proteins separated and blotted in PVDF membrane were stained with Ponceau reagent.

3.3.3. Live and dead *L. bicolor* did not trigger GUS expression in Col-0 roots

Flg22 and chitin can induce the expression of MAMP responsive genes like *CYP71A12*, *WRKY11* and *MYB51* in the roots of Col-0 (Millet et al., 2010). The transgenic lines with GUS-responsive promoters for these candidate genes were analyzed for induction or suppression of PTI responses by *L. bicolor* as described in chapter 2.10. As shown in Figure 8, flg22 as the positive control induced GUS expression of these genes in the roots of *CYP71A12p*-GUS seedlings (Supplementary Table 6.1.3). Both live and dead *L. bicolor* treatment did not induce GUS expression (Supplementary Table 6.1.3). Ideally, dead *L. bicolor* solution which is supposed to contain a cocktail of fungal MAMPs, should induce GUS expression. Chitin (500 µg/mL) prepared by both autoclaving and grinding chitin oligomers from shrimp shells (chapter 2.3.3) was tested for the GUS expression in the reporter line. Contrary to Millet et al., 2010, even chitin treatment did not show GUS staining in reporter lines (Supplementary Table 6.1.3). The GUS expression analyses of MAMP responsive reporter lines did not respond to *L. bicolor* or dead *L. bicolor* or chitin.

3.3.4. *L. bicolor* does not penetrate Col-0 roots

To check *L. bicolor* can penetrate the non-host roots, Col-0 seedlings were grown and inoculated with *L. bicolor* as explained in chapter 2.7. When the seedlings were 2 weeks old, roots were harvested and stained with LTB solution (chapter 2.7). When visualized under microscope, there was no penetration of *L. bicolor* inside the Col-0 roots (Figure 18). The hyphae of *L. bicolor* were observed to be around but not infiltrating inside the plant roots. Therefore, *L. bicolor* is imparting systemic defense in Col-0 without penetrating the roots.

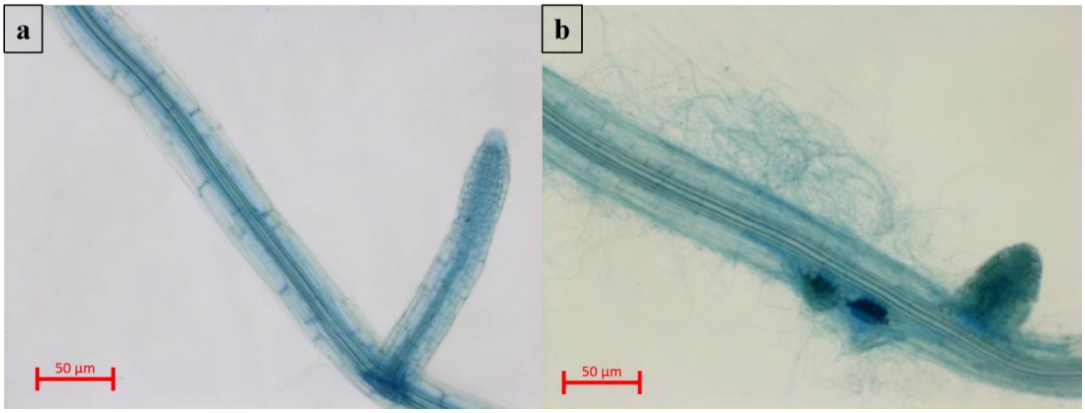


Figure 18. **Lactophenol trypan blue staining of Col-0 roots without (a) and with *L. bicolor* (b).**

L. bicolor colonies were inoculated on Col-0 roots. The roots were harvested and stained with Lactophenol trypan blue solution. The staining shows no visible penetration of *L. bicolor* into Col-0 roots. n = 2 experiments with 5 Col-0 roots/treatment.

3.3.5. Perception of chitin from *L. bicolor* triggers systemic resistance against herbivory

Since *L. bicolor* induces PTI responses instead of suppressing them, MAMPs from *L. bicolor* are probably triggering ISR. To test this hypothesis, heat-killed *L. bicolor* was inoculated on Col-0 roots as mentioned in chapter 2.3.1.1. The plants were later challenged with *T. ni* as described before (chapter 2.4.1). Like live *L. bicolor*, dead *L. bicolor* treatment significantly reduced caterpillar weight gain by 35%, compared to the control ($p < 0.0001$; Figure 19; Vishwanathan et al., 2020). This shows that MAMPs from *L. bicolor* can also induce systemic resistance.

The majorly studied MAMP from fungal cell walls is chitin. Chitin was chosen as the primary candidate for checking the role of fungal MAMPs in ISR. Chitin solution was prepared and pipetted onto the roots of Col-0 as explained in chapter 2.3.3. When the larvae were weighed after 7 days of feeding, it was observed that chitin treatment also significantly reduced *T. ni* weight gain by 38% ($p < 0.0001$; Figure 19; Vishwanathan et al., 2020). These data show that chitin can trigger systemic protection against herbivores in non-hosts. *T. ni* feeding experiments were also performed by inoculating Col-0 with another EMF, *P. involutus*. Though an ISR effect by the EMF is visible, more independent experiments are necessary to confirm the statistical significance (Supplementary figure 6.2.9).

If chitin perception is triggering the ISR in Col-0, then the chitin receptor, should also be involved in this ISR. Caterpillar feeding experiment was performed with *cerk1-2* mutant treated with live *L. bicolor*. The systemic protection phenotype against *T. ni* by *L. bicolor* treatment, which was observed in Col-0 was lost in the chitin receptor mutant *cerk1-2*. *L. bicolor* inoculation had no effect on the *T. ni* caterpillars feeding on *cerk1-2* plants (p = 0.92; Figure 19; Vishwanathan et al., 2020). Hence, chitin from *L. bicolor* was perceived by the CERK1 receptor kinase domain leading to the activation of ISR against *T. ni* caterpillars in the systemic leaves.

To further characterize the role of other receptor domains in *L. bicolor* induced ISR, caterpillar feeding experiments were performed in flg22 co-receptor mutants, *bak1-5* (impaired in immunity signalling) and *bak1-4* (impaired in immunity and BR signalling). *L. bicolor* inoculation on both *bak1* mutant lines did not result in reduced weight gain of *T. ni* larvae (p = 0.82 for *bak1-4* and p = 0.15 for *bak1-5*; Supplementary figure 6.2.10). The data indicates that the *L. bicolor* triggered negative effect on caterpillar growth is also dependent on flg22 co-receptors.

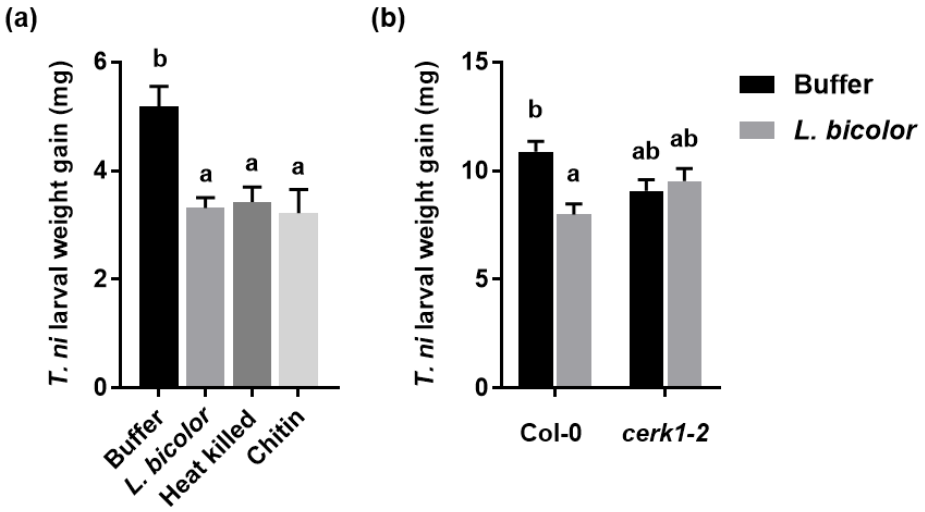


Figure 19. Chitin triggers systemic resistance in Col-0 against *T. ni*.

(a) Col-0 roots were inoculated with chitin, live or heat-killed *L. bicolor* and leaves were fed by *T. ni*. Data show that *L. bicolor*, heat-killed *L. bicolor* and chitin negatively affect *T. ni* weight gain. n = 4 experiments with at least 20 caterpillars/treatment (Vishwanathan et al., 2020). ANOVA was performed for determining statistical significance. (b) Chitin receptor mutant, *cerk1-2* roots were inoculated with *L. bicolor* and leaves were fed by *T. ni* larvae. Data show that ISR triggered by *L. bicolor* is dependent on CERK1. n = 3 experiments with at least 20 caterpillars/treatment (Vishwanathan et al., 2020). Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.4. Chitin triggers ISR against herbivory at the cost of susceptibility to biotrophic pathogen

3.4.1. Chitin and *L. bicolor* trigger ISS against *Pst* DC3000

Crosstalk between SA and JA pathways leading to antagonistic responses in biotic stress responses has been well documented (Leon-Reyes et al., 2010; Liu et al., 2016; Spoel et al., 2003; Van der Does et al., 2013). Chitin from *L. bicolor* depends on JA and SA signalling for negatively affecting *T. ni* weight (Figure 12). The SA-signalling transcriptional co-repressors, *NPR3* and *NPR4* significantly promoted *T. ni* larvae weight gain under *L. bicolor* treatment (Figure 13). These results hint to a stronger inhibition of SA responses by chitin. Therefore, it was hypothesized that *L. bicolor* or chitin treatment should make Col-0 plants more susceptible against the leaf foliar pathogen, *Pst* DC3000. To test this, Col-0 plants were treated with *L. bicolor* and challenged with *Pst* DC3000 as described in chapter 2.4.2.1. *L. bicolor* or chitin treatment in the roots resulted in significantly more *Pst* DC3000 CFU compared to the control ($p < 0.05$; Figure 20; Vishwanathan et al., 2020). This shows that chitin induces ISS against *Pst* DC3000 at the expense of triggering ISR against *T. ni*.

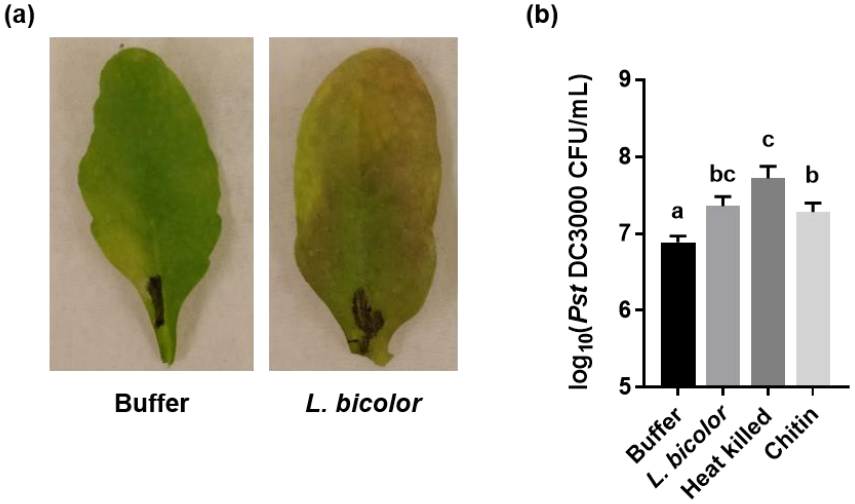


Figure 20. Chitin induces ISS against *P. syringae* DC3000.

(a) *Pst* DC3000 infected leaves from buffer and *L. bicolor* inoculated Col-0 plants. (b) Col-0 roots were inoculated with buffer, both live and heat-killed *L. bicolor* or chitin and leaves were infected with *Pst* DC3000. Chitin, live and heat-killed *L. bicolor* treatment in the roots increases colony count of Pst DC3000 in the systemic leaves. Data show $\log_{10}$ (*Pst* DC3000 (CFU/cm^2)) from n = 4 experiments with 12 leaves/treatment (Vishwanathan et al., 2020). Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

3.4.2. *L. bicolor* treatment did not influence ISR against Noco2

To elucidate the function of *L. bicolor* in defending Col-0 against biotrophic pathogen, the system was also challenged with Noco2 as described in chapter 2.4.2.2. The experiment was performed four times in an independent manner using pots filled with peat-soil substrate. L. bicolor was inoculated to the roots of Col-0 and the shoots were later infected with Noco2. The oomycete spores were counted after 5 days to determine the degree of infection. However, no significant difference was observed in Noco2 virulence between the control and *L. bicolor* treated seedlings (Supplementary figure 6.2.11).

4. DISCUSSION

4.1. Hosts and non-hosts in ISR: a paradox?

Even before 460 million years ago, symbiotic associations existed in the ecosystem (Martin et al., 2016). The origin of land plants led to the evolution of microbial communities for adaptive colonization (Martin et al., 2016). The diversification of symbioses altered the genetic architecture of both partners by gaining and losing certain traits. For instance, ectomycorrhizal clades emerged from saprotrophic ancestors by losing their ability to degrade plant cell walls (Bonfante, 2018). Adaptation of plants to diverse environmental conditions, also resulted in a few plant families to lose the common symbiotic signalling pathway for responding to symbiotic interactions with rhizobacteria and mycorrhizal fungi (Cope et al., 2019; Ehrhardt et al., 1996; Garcia et al., 2015). This paved the way for the plants to be closely associated with a certain class of microbes and also be characterized as host and non-hosts depending on the ability to engage in mutual symbiosis with a distinct microbe. Since majority of the land plants undergo symbiosis, secondary growth promotion and protection of a plant are majorly attributed to its interaction in the mycorrhizosphere (Giovannini et al., 2020). Compatibility of the host and the beneficial microbe was considered important for advantageous physiological traits of a plant including ISR. To test whether symbiosis is a requisite for ISR, Col-0-*L. bicolor* system was challenged with *T. ni*. The negative effect of this interaction on caterpillar weight gain showed that beneficial microbes can exert ISR phenotype independent of host adaptability (Figure 9).

4.2. MAMP-triggered systemic immunity

Plant roots have evolved to differentiate between beneficial microbes and pathogens in the rhizosphere. Nod factors from beneficial rhizobacteria is a key component in facilitating the molecular crosstalk during the initial stages of colonization (Stacey et al., 2014). Nod factors are the acylated form of chito-oligosaccharides (CO) called LCOs (Stacey et al., 2014). LCOs are also found in AMF and EMF as Myc factors and they too function as signalling molecules for mycorrhization of the host roots. Perception of Nod or Myc factors or LCOs is

a crucial component of the symbiotic toolkit as it facilitates differentiating mutualists from pathogens by the plant species (Fernández et al., 2019). Recognition of mutualists prevents the hosts from mounting basal PTI responses as observed with *Pseudomonas* spp. (Millet et al., 2010; Yu et al., 2019). However, *L. bicolor* did not suppress but rather activated MAPK cascades and oxidative burst in Col-0 (Figure 16 and 17).

The role of MAMPs/PAMPs in triggering local defense responses have been well characterized (Daudi et al., 2012; Millet et al., 2010; S. Wu et al., 2014). The overlapping genetic responses of Col-0 for WCS417r and MAMPs treatment suggests that conserved microbial patterns can also influence systemic defense (Stringlis, Proietti, et al., 2018). Lipopolysaccharides from the cell wall of ISR inducing *P. simiae* WCS417r can protect Col-0 foliar tissues against *Pst* DC3000 (van Peer & Schippers, 1992). But the influence of other MAMPs/PAMPs and the mechanism by which its perception in the roots can activate systemic immunity has not yet been clearly explained in detail. Chitin constitutes 15% of the fungal cell wall components, while the major cell wall component is β-1,3-glucan (Fesel & Zuccaro, 2016). Chitin is the most well characterized fungal cell wall MAMP/PAMP and its receptor kinase, CERK1 was also identified in Col-0 (Erwig et al., 2017; Lajunen et al., 2011; Miya et al., 2007). In addition to its role of chitin perception for triggering local PTI, AMF symbiosis in rice is also dependent on CERK1 (X. Zhang et al., 2015). Consistent with the speculation about MAMPs from *L. bicolor* in triggering ISR, chitin negatively affected *T. ni* larvae feeding and the ISR signaling pathway by the EMF was also dependent on CERK1 (Figure 19). Since chitin from *L. bicolor* is triggering ISR *via CERK1*, it was expected that *L. bicolor* treatment does not activate MAPK cascades in *cerk1-2* mutant. In contrast, the EMF treatment phosphorylated MAPK markers in *cerk1-2* seedlings (Supplementary figure 6.2.8). The three phosphorylated MAPKs are implicated in pathogen triggered resistance where MAPK6 is vital for elicitor-induced defense response, MAPK3 is involved in basal resistance against pathogens and MAPK4 negatively regulates ROS and SA accumulation (Galletti et al., 2011). Probably, other MAMPs present in the cell wall of *L. bicolor* can activate MAPK cascades and trigger ISR independent of CERK1. Supporting this hypothesis, *T. ni* feeding on flagellin co-receptor mutants, *bak1-4* and *bak1-5* inoculated with *L. bicolor* did not show a significant difference compared to mock inoculated plants (Supplementary figure 6.2.10). Therefore, other MAMPs in *L. bicolor* can also ISR responses against herbivory, independent of CERK1.

Unlike the single interaction system used in this research, plants are associated with a diverse microbial community in the natural environment. Constant local and systemic defense

activation after chitin perception in the rhizosphere will exert immense pressure on plant fitness. However, plants have developed mechanisms like priming to circumvent and balance growth-defense tradeoffs (Conrath et al., 1989; Martinez-Medina et al., 2016; Stringlis, Yu, et al., 2018). Also, plant-microbe and microbe-microbe interactions reduce pathogenic microbial load and restructure the mycorrhizosphere (Durán et al., 2018; Hacquard et al., 2017). In this way, plants have developed methods to control the microbial load it is exposed to in the soil. During this co-evolution, plants have also evolved receptors for recognizing beneficial symbionts (Stacey et al., 2014). Hence, it is rationale that plants may have also adapted to a specific microbial assemblage and dosage of MAMPs, which sets a threshold stimulus for activating ISR or SAR.

4.3. ISR seems to be a conserved mechanism of plants

JA/Et pathway is the major signalling pathway for ISR by most of the beneficial microbes (Martínez-Medina et al., 2013; Pangesti et al., 2016; Pieterse et al., 1998, 2014). *L. bicolor*-triggered ISR dependence on JA-Ile receptor, *COI1* and SA biosynthesis, *SID2* was also observed with the beneficial rhizobacteria *P. simiae* WCS417r (Figure 12) (Haney et al., 2017; Oosten et al., 2008; Pozo et al., 2008; Verhagen et al., 2004; Zamioudis et al., 2013). *SID2* encodes an isochorismate synthase which is required for generating the SA precursor isochorismate upon pathogen infection (Wang et al., 2011; Wildermuth et al., 2001) and it restricts the colonization of microbes in the roots

The antagonistic feature of SA on JA and vice versa has been well documented in biotic stress response (Kazan & Manners, 2008; Leon-Reyes et al., 2010; Millet et al., 2010; Pieterse et al., 2012; Vlot et al., 2009). The crosstalk between SA and JA is mediated endogenously by *NPR1* and also by exogenous application of ethylene (Leon-reyes et al., 2009; Spoel et al., 2003). The crosstalk was evident in the *T. ni* feeding experiments with SA, JA mutants and also in *L. bicolor*-triggered ISS against *Pst* DC3000 (Figure 12 and Figure 20). (Martínez-Medina et al., 2017). *NPR1* is the master regulator of SA mediated SAR pathway and is also involved in WCS417r triggered ISR pathway (Haney et al., 2017; Pieterse et al., 1998; Pozo et al., 2008; Verhagen et al., 2004; Zamioudis et al., 2013). However, the ISR signaling pathway triggered by *L. bicolor* looks partially dependent on or independent of *NPR1* (Figure 12). *NPR3* and *NPR4* are constitutively expressed in the plant to monitor and control the levels of SA by

suppressing *NPR1*-activated pathogenesis-related gene expression (Ding et al., 2018). In addition to relying on JA signaling, *L. bicolor* imparts ISR against *T. ni* by depending on the SA receptors and negative regulators, *NPR3* and *NPR4* (Figure 13). Rather than depending on *NPR1*, the SA-JA tradeoffs are modulated by *L. bicolor* for ISR against *T. ni* and ISS against *Pst* DC3000 by involving the SA negative regulators.

Poplar hosts upon perception of the volatiles from *L. bicolor*, reprogram its rooting mechanism by increasing lateral root density (Ditengou et al., 2015). This pre-colonization response by *L. bicolor* was also observed with the non-host Col-0 (Ditengou et al., 2015). Microbial or volatile perception activates iron deficiency responses necessary for triggering ISR (Martínez-Medina, Van Wees, et al., 2017; Zamioudis et al., 2014). Iron deficiency differentially regulates the transcriptome profile in the roots. Among all the differentially regulated genes, *FIT*, *bHLH38/39*, *FRO2*, *IRT1*, *BGLU42*, *MYB72*, *MYB10*, *F6'H1* are the genes necessary for iron homeostasis (Romera et al., 2019; Zamioudis et al., 2013). Of which, *MYB72* was also identified to be necessary but not sufficient for ISR triggered by beneficial micro-organisms (Martínez-Medina et al., 2017; Van Der Ent et al., 2008). The iron stress response regulator *MYB72* is also involved in *L. bicolor*- or chitin-triggered ISR (Figure 14). Hence, multiple stimuli from the invading beneficial microbe can exert ISR in plants. But it can be a possibility that the ISR signalling pathways triggered by volatiles or MAMPs/PAMPs or symbiotic mutualists could be the same.

4.4. Priming of defenses by *L. bicolor*

The primed defense responses are unleashed in a faster and stronger fashion to defend the plant against subsequent stresses (Martinez-Medina et al., 2016). Unlike SAR, minimal priming effect by beneficial microbes was observed only when challenged with a pathogen or herbivore and under controlled *in vitro* conditions (Haney et al., 2017; Mortel et al., 2012; Stringlis et al., 2018; Verhagen et al., 2004). Priming by *L. bicolor* was investigated with respect of defense gene expression, phytohormones and secondary metabolites.

Downstream of signalling pathways are defense genes which get transcriptionally activated upon biotic threats. The marker gene *PDF1.2* of the JA-ERF branch is stimulated not just against nectrorophic pathogens like *Alternaria brassicicola*, *Botrytis cinerea*, and herbivores like *Mamestra brassicae*, but also against the hemi-biotrophic pathogen *Pst* DC3000 (Leon-

Reyes et al., 2010; Pangesti et al., 2015; Spoel et al., 2003). Similarly, the expression of *VSP2* from the JA-MYC branch is enhanced when the plant is countered with wounding damage, herbivory and also infection by *Pst* DC3000 (Spoel et al., 2003). SA sensing by *NPR1* triggers the expression of the encoded PR1 protein which in addition to act against biotrophs, also exhibit limited antifungal properties (Maldonado et al., 2002; Pieterse et al., 1996). *PR1* genes are expressed *via NPR1* even when the plants are exogenously sprayed with functional analogs of SA (Hermann et al., 2013). Considering the ISR signaling pathway, the above mentioned defense marker genes should have been differentially regulated by *L. bicolor* treatment. Sample collection from a single time point post *T. ni* feeding and the variance associated with pooling samples from soil-grown plants restricted the thesis in identifying the defense gene transcripts contributing to ISR. Also, the moderate priming associated with ISR (~2.5 fold) (N. Pangesti et al., 2015b), renders it difficult to validate the priming effect in this multi-partite system.

Accumulation of plant hormones and secondary metabolites also contributes to inhibit pathogen infection or pest infestation (Durrant & Dong, 2004). In the case of SAR, SA levels are dramatically increased in the local and distal tissue upon pathogen infection (Wang et al., 2011; Zhang et al., 2010). ISR by beneficial rhizobacteria have not recorded an increase in concentration of any specific plant defense hormone (Pieterse et al., 2012). While *T. ni* had a strong effect on JA and its derivatives, *L. bicolor* treatment like other beneficial microbes, did not increase the concentrations of JA, SA, ABA and its derivatives (Figure 11; Supplementary figure 6.2.1). However, the analyses dropped a hint about the potential role of indolic glucosinolates in the ISR response (Supplementary figure 6.2.4). Glucosinolates constitute the major class of secondary metabolites against biotic stress in the *Brassicaceae* family (Bednarek, 2012; Ver et al., 2011). Aliphatic glucosinolates derived from methionine have been reported to defend the plants against *Pseudomonas syringae* and the pathogenic oomycete *Sclerotonia sclerotiorum* (Piasecka & Jedrzejczak-Rey, 2015). Indolic glucosinolates synthesized by the tryptophan pathway provide the host with broad spectrum resistance against various pathogens (Lipka et al., 2005). *CYP81F2/PEN2*, involved in the formation of indolic glucosinolates, are also involved in resistance against many fungal and oomycete pathogens like *Verticillum longisporum*, *Botrytis cinerea*, *Phytophthora brassicae*, *Plectoshpaerella cucumerina*, *Blumeria graminis* f. sp. *hordei* (Bednarek, 2012; Iven et al., 2012; Lipka et al., 2005; Piasecka & Jedrzejczak-Rey, 2015). Camalexin, the most abundant phytoalexin in *Arabidopsis*, is also synthesized in response to both biotic and abiotic stress (Glawischnig et al., 2004; Ver et al., 2011). Colonization of plant roots by beneficial and pathogenic microbes and also phosphorylation of

MAPK6/MAPK3 cascade leads to the accumulation of camalexin (Contreras-Cornejo et al., 2011; Iven et al., 2012; Ren et al., 2008). Similar to priming of camalexin by *L. bicolor* (Figure 15), JA/Et signalling triggered by WCS417r modulates camalexin and aliphatic gluosinolates synthesis in Col-0 (Pangesti et al., 2016). These reports and observations are therefore emphasizing a stronger role of the tryptophan pathway in ISR.

4.5. Conclusions & Outlook

The research experiments mentioned in this thesis were an effort taken to differentiate host and non-host interactions for ISR. The model system developed including *A. thaliana*, *L. bicolor*, *T. ni* and *Pst* DC3000 was beneficial in providing evidence that symbiotic association is not necessary for triggering systemic responses. The vast genetic resources available in *Arabidopsis thaliana* aided in the characterization of ISR signalling pathway triggered by *L. bicolor* or chitin. Plant genetic determinants in this ISR mechanism can be elucidated by screening other important mutants from different metabolite and phytohormone pathways. Characterization of the signalling pathway against *Pst* DC3000 infection can reveal information about the regulation of phytohormone crosstalk during ISR and ISS. A comparative analysis between ISR by *L. bicolor* in non-host Col-0 and the host poplar can clear our thoughts about the biased host/non-host classifications reported so far with respect to systemic defenses.

The scientific findings from this research work also highlights the role of chitin in modulating systemic defenses. However, the role of BAK1 in ISR also suggests that other MAMPs could also be involved in protecting systemic tissues from biotic threats. Testing other MAMPs can also help us determine whether induction of systemic immunity is conserved between a certain class of MAMPs or all MAMPs. Characterization of different MAMPs/PAMPs and their receptors for triggering ISR and ISS against diverse pathogen or insect model systems will unravel more information about this unexplored mechanism of plant immunity. Testing the potential of MAMPs and PAMPs in triggering ISR with other plant species will validate the consistency in this proposed mechanism.

Damage-associated molecular patterns (DAMPs) are endogenous plant cell wall breakdown products. Like MAMPs and PAMPs, DAMPs are perceived by receptors as danger signals leading to the activation of defense responses in the local tissue (Choi & Klessig, 2016; Ferrari et al., 2013; Mélida et al., 2018; Tanaka et al., 2014). Pathogen invasion, insect feeding

and root colonization or penetration by mutualists can trigger DAMPs synthesis and lead to perception by the intracellular receptors. Plant roots also secrete antimicrobial secondary metabolites like coumarin in a *MYB72*-dependent manner, upon colonization by beneficial rhizobacteria (Stringlis et al., 2018). Coumarins have also been reported to be secreted upon treatment of plant roots by chitosan (Conrath et al., 1989). These metabolites structure the microbiome in the rhizosphere by inhibiting pathogen growth without affecting beneficial mutualists (Schultheiss et al., 2019; Voges et al., 2019). With the gaining importance about DAMPs and our claim that ISR is a conserved mechanism of plants, it will be interesting to test the role of DAMPs and coumarin (specifically scopoletin) in triggering ISR in various plant systems.

5. REFERENCES

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*(5), 293–302. https://doi.org/10.1016/j.tplants.2012.02.006

Alström, S. (1991). Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads. *The Journal of General and Applied Microbiology*, *37*, 495–501. https://doi.org/10.2323/jgam.37.495

Audenaert, K., Pattery, T., Cornelis, P., & Höfte, M. (2002). Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: Role of salicylic acid, pyochelin, and pyocyanin. *Molecular Plant-Microbe Interactions*, *15*(11), 1147–1156. https://doi.org/10.1094/mpmi.2002.15.11.1147

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2010). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Bari, R., & Jones, J. (2009). Role of plant hormones in plant defence responses. In *Plant molecular biology* (Vol. 69). https://doi.org/10.1007/s11103-008-9435-0

Bednarek, P. (2012). Sulfur-containing secondary metabolites from *Arabidopsis thaliana* and other *Brassicaceae* with function in plant immunity. *ChemBioChem*, *13*, 1846–1859. https://doi.org/10.1002/cbic.201200086

Bennett, B. Y. R. N., & Wallsgrove, R. M. (1994). Tansley Review No . 72 Secondary metabolites in plant defence mechanisms. *New Phytologist*, *127*, 617–633.

Boller, T., & He, S. Y. (2009). Innate immunity in plants: An arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, *324*(5928), 742–744. https://doi.org/10.1126/science.1171647.Innate

Bonfante, P. (2018). Tansley review The future has roots in the past : the ideas and scientists that shaped mycorrhizal research. *New Phytologist*, *220*(4), 982–995. https://doi.org/10.1111/nph.15397

Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, *220*, 1108–1115. https://doi.org/10.1111/nph.14976

C van Loon, L., P J Geraats, B., & Linthorst, H. (2006). Ethylene as a modulator of disease resistance in plants. In *Trends in plant science* (Vol. 11). https://doi.org/10.1016/j.tplants.2006.02.005

Cao, H., Bowling, S. A., Gordon, A. S., & Dong, X. (1994). Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell*, *6*(11), 1583–1592. https://doi.org/https://doi. org/10.1105/tpc.6.11.1583

Champigny, M. J., Isaacs, M., Carella, P., Faubert, J., Fobert, P. R., & Cameron, R. K. (2013). Long distance movement of *DIR1* and investigation of the role of *DIR1*-like during systemic acquired resistance in *Arabidopsis*. *Frontiers in Plant Science*, *4*(230), 1–20. https://doi.org/10.3389/fpls.2013.00230

References

Chanda, B., Xia, Y., Mandal, M. K., Yu, K., Sekine, K., Gao, Q., Selote, D., Hu, Y., Stromberg, A., Navarre, D., Kachroo, A., & Kachroo, P. (2011). Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nature Genetics*, *43*, 421. Retrieved from https://doi.org/10.1038/ng.798

Cheng, Z., Li, J.-F., Niu, Y., Zhang, X.-C., Woody, O. Z., Xiong, Y., Djonović, S., Millet, Y., Bush, J., McConkey, B. J., Sheen, J., & Ausubel, F. M. (2015). Pathogen-secreted proteases activate a novel plant immune pathway. *Nature*, *521*, 213–216. Retrieved from https://doi.org/10.1038/nature14243

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., & Felix, G. (2005). The *Arabidopsis* receptor kinase *FLS2* binds flg22 and determines the specificity of flagellin perception. *The Plant Cell*, *18*, 465–476. https://doi.org/10.1105/tpc.105.036574

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L., & Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, *448*(7154), 666–671. https://doi.org/10.1038/nature06006

Choi, H. W., & Klessig, D. F. (2016). DAMPs , MAMPs , and NAMPs in plant innate immunity. *BMC Plant Biology*, *16*(232), 1–10. https://doi.org/10.1186/s12870-016-0921-2

Clavijo Mccormick, A., Irmisch, S., Reinecke, A., Boeckler, G. A., Veit, D., Reichelt, M., Hansson, B. S., Gershenzon, J., Köllner, T. G., & Unsicker, S. B. (2014). Herbivore-induced volatile emission in black poplar: Regulation and role in attracting herbivore enemies. *Plant, Cell and Environment*, *37*, 1909–1923. https://doi.org/10.1111/pce.12287

Clavijo McCormick, A., Unsicker, S. B., & Gershenzon, J. (2012). The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science*, *17*(5), 303–310. https://doi.org/10.1016/j.tplants.2012.03.012

Clay, N. K., Adio, A. M., Denoux, C., Jander, G., & Frederick, M. (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science*, *323*(5910), 95–101. https://doi.org/10.1126/science.1164627

Conrath, U. (2006). Systemic Acquired Resistance. *Plant Signaling & Behavior*, *1*(4), 179–184.

Conrath, U. (2011). Molecular aspects of defence priming. *Trends in Plant Science*, *16*(10), 524–531. https://doi.org/10.1016/j.tplants.2011.06.004

Conrath, U., Domard, A., & Kauss, H. (1989). Chitosan-elicited synthesis of callose and of coumarin derivatives in parsley cell suspension cultures. *Plant Cell Reports*, *8*, 152–155.

Contreras-Cornejo, H. A., Macías-Rodríguez, L., Beltrán-Peña, E., Herrera-Estrella, A., & López-Bucio, J. (2011). *Trichoderma*-induced plant immunity likely involves both hormonal- and camalexin- dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. *Plant Signaling and Behavior*, *6*(10), 1554–1563. https://doi.org/10.4161/psb.6.10.17443

Cope, K. R., Bascaules, A., Irving, T. B., Venkateshwaran, M., Garcia, K., Jawdy, S., Steigerwald, E., Setzke, J., Fung, E., Schnell, K. G., Wang, Y., Schlief, N., Strauss, S. H., Maillet, F., Jargeat, P., Division, B., Ridge,

References

O., & Ridge, O. (2019). The ectomycorrhizal fungus *Laccaria bicolor* produces lipochitooligosaccharides and uses the common symbiosis pathway to colonize *Populus* roots. *Plant Cell*. https://doi.org/10.1105/tpc.18.00676

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature*, *411*(6839), 826–833. https://doi.org/10.1038/35081161

Daudi, A., Cheng, Z., O'Brien, J. A., Mammarella, N. D., Khan, S., Ausubel, F. M., & Bolwell, G. P. (2012). The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *The Plant Cell*, *24*(1), 275–287. https://doi.org/10.1105/tpc.111.093039

Dewdney, J., Reuber, T. L., Wildermuth, M. C., Devoto, A., Cui, J., Stutius, L. M., Drummond, E. P., & Ausubel, F. M. (2000). Three unique mutants of *Arabidopsis* identify *eds* loci required for limiting growth of a biotrophic fungal pathogen. *The Plant Journal*, *24*(2), 205–218. https://doi.org/https://doi.org/10.1046/j.1365-313x.2000.00870.x

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X., & Zhang, Y. (2018). Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell*, *173*, 1–14. https://doi.org/10.1016/j.cell.2018.03.044

Ditengou, F. A., Mu, A., Rosenkranz, M., Felten, J., Lasok, H., Palme, K., Polle, A., Doorn, M. M. Van, & Legue, V. (2015). Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. *Nature Communications*, *6*, 6279. https://doi.org/10.1038/ncomms7279

Durán, P., Thiergart, T., Garrido-oter, R., Agler, M., Kemen, E., Schulze-lefert, P., & Hacquard, S. (2018). Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell*, *175*, 973–983. https://doi.org/10.1016/j.cell.2018.10.020

Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, *42*, 185–209. https://doi.org/10.1146/annurev.phyto.42.040803.140421

Ehrhardt, D. W., Wais, R., & Long, S. R. (1996). Calcium spiking in plant root hairs responding to rhizobium modulation signals. *Cell*, *85*, 673–681. https://doi.org/10.1016/S0092-8674(00)81234-9

Ellis, C., & Turner, J. G. (2002). A conditionally fertile *coi1* allele indicates cross-talk between plant hormone signalling pathways in *Arabidopsis thaliana* seeds and young seedlings. *Planta*, *215*, 549–556. https://doi.org/10.1007/s00425-002-0787-4

Erwig, J., Ghareeb, H., Kopischke, M., Hacke, R., Matei, A., Petutschnig, E., & Lipka, V. (2017). Chitin-induced and CHITIN ELICITOR RECEPTOR KINASE1 (CERK1) phosphorylation-dependent endocytosis of Arabidopsis thaliana LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE5 (LYK5). *New Phytologist*, *215*, 382–396. https://doi.org/10.1111/nph.14592

Felten, J., Kohler, A., Morin, E., Bhalerao, R. P., Palme, K., Martin, F., Ditengou, F. A., & Legue, V. (2009). The ectomycorrhizal fungus Laccaria bicolor stimulates lateral root formation in poplar and arabidopsis through auxin transport and signaling. *Plant Physiology*, *151*, 1991–2005. https://doi.org/10.1104/pp.109.147231

References

Fernández, I., Cosme, M., Stringlis, I. A., Yu, K., de Jonge, R., van Wees, S. C. M., Pozo, M. J., Pieterse, C. M. J., & van der Heijden, M. G. A. (2019). Molecular dialogue between arbuscular mycorrhizal fungi and the nonhost plant *Arabidopsis thaliana* switches from initial detection to antagonism. *New Phytologist*, *223*, 867–881. https://doi.org/10.1111/nph.15798

Ferrari, S., Savatin, D. V, Sicilia, F., Gramegna, G., Cervone, F., & Lorenzo, G. De. (2013). Oligogalacturonides : plant damage-associated molecular patterns and regulators of growth and development. *Frontiers in Plant Science*, *4*(49), 1–9. https://doi.org/10.3389/fpls.2013.00049

Fesel, P. H., & Zuccaro, A. (2016). β-glucan: crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genetics and Biology*, *90*, 53–60. https://doi.org/10.1016/j.fgb.2015.12.004

Feussner, K., & Feussner, I. (2019). High-throughput metabolomics: Methods and protocols. In *Methods in Molecular Biology* (Vol. 1978, pp. 167–185). https://doi.org/10.31826/9781463209094-015

Fu, Z. Q., & Dong, X. (2013). Systemic Acquired Resistance : Turning Local Infection into Global Defense. *Annual Review of Plant Biology*, *64*, 839–863. https://doi.org/10.1146/annurev-arplant-042811-105606

Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S. H., Tada, Y., Zheng, N., & Dong, X. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature*, *486*, 228. Retrieved from https://doi.org/10.1038/nature11162

Galletti, R., Ferrari, S., & De Lorenzo, G. (2011). *Arabidopsis* MPK3 and MPK6 play different roles in basal and oligogalacturonide- or flagellin-induced resistance against *Botrytis cinerea*. *Plant Physiology*, *157*, 804–814. https://doi.org/10.1104/pp.111.174003

Gange, A. C., Gane, D. R. J., Chen, Y., & Gong, M. (2005). Dual colonization of *Eucalyptus urophylla* S.T. Blake by arbuscular and ectomycorrhizal fungi affects levels of insect herbivore attack. *Agric For Entomol*, *7*, 253–263.

Garcia, K., Delaux, P. M., Cope, K. R., & Ané, J. M. (2015). Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. *New Phytologist*, *208*, 79–87. https://doi.org/10.1111/nph.13423

Garcion, C., Lohmann, A., Lamodiere, E., Catinot, J., Buchala, A., Doermann, P., & Metraux, J.-P. (2008). Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of *Arabidopsis*. *Plant Physiology*, *147*, 1279–1287. https://doi.org/10.1104/pp.108.119420

Gatz, C. (2013). From Pioneers to Team Players : TGA Transcription Factors Provide a Molecular Link. *Molecular Plant-Microbe Interactions*, *26*(2), 151–159.

Giovannini, L., Palla, M., Agnolucci, M., Avio, L., Sbrana, C., Turrini, A., & Giovannetti, M. (2020). Arbuscular Mycorrhizal Fungi and Associated Microbiota as Plant Biostimulants : Research Strategies for the Selection of the Best Performing Inocula. *Agronomy*, *10*, 1–14. https://doi.org/doi:10.3390/agronomy10010106

Glawischnig, E., Hansen, B. G., Olsen, C. E., & Halkier, B. A. (2004). Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis*. *PNAS*,

101(21), 8245–8250. https://doi.org/10.1073/pnas.0305876101

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, *43*(1), 205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923

Hacquard, S., Spaepen, S., Garrido-oter, R., & Schulze-lefert, P. (2017). Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology*, *55*, 565–589.

Haney, C. H., Riely, B. K., Tricoli, D. M., Cook, D. R., Ehrhardt, D. W., & Long, S. R. (2011). Symbiotic rhizobia bacteria trigger a change in localization and dynamics of the *Medicago truncatula* receptor kinase LYK3. *Plant Cell*, *23*, 2774–2787. https://doi.org/10.1105/tpc.111.086389

Haney, C. H., Wiesmann, C. L., Shapiro, L. R., Melnyk, R. A., O'Sullivan, L. R., Khorasani, S., Xiao, L., Han, J., Bush, J., Carillo, J., Pierce, N. E., & Ausubel, F. M. (2017). Rhizosphere-associated *Pseudomonas* induce systemic resistance to herbivores at the cost of susceptibility to bacterial pathogens. *Molecular Ecology*, *27*(8), 1833–1847. https://doi.org/10.1111/mec.14400

Hartley, S. E., & Gange, A. C. (2009). Impacts of Plant symbiotic fungi on insect herbivores: Mutualism in a multitrophic context. *Annual Review of Entomology*, *54*, 323–342. https://doi.org/10.1146/annurev.ento.54.110807.090614

Hartmann, M., Zeier, T., Bernsdorff, F., Reichel-deland, V., Kim, D., Hohmann, M., Scholten, N., Schuck, S., Bräutigam, A., Hölzel, T., Ganter, C., & Zeier, J. (2018). Flavin monooxygenase-generated N-Hydroxypipecolic acid is a critical element of plant systemic immunity. *Cell*, *173*, 456–469. https://doi.org/10.1016/j.cell.2018.02.049

Heijden, M. G. A. Van Der, Martin, F. M., & Sanders, I. R. (2015). Tansley review: Mycorrhizal ecology and evolution : the past , the present , and the future. *New Phytologist*, *205*, 1406–1423. https://doi.org/10.1111/nph.13288

Hermann, M., Maier, F., Masroor, A., Hirth, S., Pfitzner, A. J. P., & Pfitzner, U. M. (2013). The *Arabidopsis NIMIN* proteins affect *NPR1* differentially. *Frontiers in Plant Science*, *4*(88), 1–15. https://doi.org/10.3389/fpls.2013.00088

Hoffland, E., Pieterse, C. M. J., Bik, J., & Van Pelt, Johan, A. (1995). Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins. *Physiological and Molecular Plant Pathology*, *46*, 309–320.

Howe, G. A., Lightner, J., Browse, J., & Ryan, C. A. (2007). An octadecanoid pathway mutant (*JL5*) of tomato is compromised in signaling for defense against insect attack. *The Plant Cell*, *8*(11), 2067. https://doi.org/10.2307/3870413

Iven, T., König, S., Singh, S., Braus-Stromeyer, S. A., Bischoff, M., Tietze, L. F., Braus, G. H., Lipka, V., Feussner, I., & Dröge-Laser, W. (2012). Transcriptional activation and production of tryptophan-derived secondary metabolites in *Arabidopsis* roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum*. *Molecular Plant*, *5*(6), 1389–1402. https://doi.org/10.1093/mp/sss044

References

James, A. (2013). Plant immunity. Methods and protocols. *Annals of Botany*, *111*(1), viii–viii. https://doi.org/10.1093/aob/mcs272

Jander, G. (2012). Timely plant defenses protect against caterpillar herbivory. *PNAS*, *109*(12), 4343–4344. https://doi.org/10.1073/pnas.1201443109

Jiang, Y., & Yu, D. (2016). The WRKY57 transcription factor affects the expression of jasmonate ZIM-domain genes transcriptionally to compromise *Botrytis cinerea* resistance. *Plant Physiology*, *171*, 2771–2782. https://doi.org/10.1104/pp.16.00747

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323–329. https://doi.org/10.1038/nature05286

Jung, H. W., Tscaplinski, T. J., Wang, L., Glazebrook, J., & Greenberg, J. T. (2009). Priming in systemic plant immunity. *Science*, *324*, 89–91. https://doi.org/10.1126/science.1170025

Kaling, M., Schmidt, A., Moritz, F., Rosenkranz, M., Witting, M., Kasper, K., Janz, D., Schmitt-kopplin, P., Schnitzler, J., & Polle, A. (2018). Mycorrhiza-triggered transcriptomic and metabolomic networks impinge on herbivore fitness. *Plant Physiology*, *176*, 2639–2656. https://doi.org/10.1104/pp.17.01810

Kazan, K., & Manners, J. M. (2008). Jasmonate signaling : Toward an integrated view. *Plant Physiology*, *146*, 1459–1468. https://doi.org/10.1104/pp.107.115717

Kemppainen, M., Circosta, A., Tagu, D., Martin, F., & Pardo, A. G. (2005). Agrobacterium-mediated transformation of the ectomycorrhizal symbiont Laccaria bicolor S238N. *Mycorrhiza*, *16*, 19–22. https://doi.org/10.1007/s00572-005-0008-7

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., & Bücking, H. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, *333*, 880–882. https://doi.org/10.1126/science.1208473

Kloppholz, S., Kuhn, H., & Requena, N. (2011). Report a secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Current Biology*, *21*(14), 1204–1209. https://doi.org/10.1016/j.cub.2011.06.044

Koch, E., & Slusarenko, A. (1990). *Arabidopsis* is susceptible to infection by a downy mildew fungus. *The Plant Cell*, *2*(5), 437–445.

Koo, A. J. K., & Howe, G. A. (2009). The wound hormone jasmonate. *Phytochemistry*, *70*(13–14), 1571–1580. https://doi.org/10.1016/j.phytochem.2009.07.018.The

Kusch, S., Thiery, S., Reinstädler, A., Gruner, K., & Zienkiewicz, K. (2019). *Arabidopsis mlo3* mutant plants exhibit spontaneous callose deposition and signs of early leaf senescence. *Plant Molecular Biology*. https://doi.org/10.1007/s11103-019-00877-z

Lajunen, H. M., Gust, A. A., Bono, J.-J., Willmann, R., Newman, M.-A., Cullimore, J. V., Gotz, F., Erbs, G.,

References

Molinaro, A., Nurnberger, T., Katagiri, F., Kulik, A., Lipka, V., Tsuda, K., Fliegmann, J., Kolb, D., & Jehle, A. K. (2011). Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *PNAS*, *108*(49), 19824–19829. https://doi.org/10.1073/pnas.1112862108

Laluk. (2011). Necrotroph Attacks on Plants: Wanton Destruction or Covert Extortion? *The Arabidopsis Book*, 1–34. https://doi.org/10.1199/tab.0136

Lambers, H., & Teste, F. P. (2013). Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? *Plant, Cell and Environment*, *36*, 1911–1915. https://doi.org/10.1111/pce.12117

Lambert, B., Leyns, F., Rooyen, L. U. K. V. A. N., Gossele, F., Papon, Y., Swings, J., Systetns, P. G., Glent, B.-, Biosem, B. I., & Biologie, L. De. (1987). Rhizobacteria of maize and their antifungal activities. *Applied and Environmental Microbiology*, *53*(8), 1866–1871.

Lemarié, S., Robert-Seilaniantz, A., Lariagon, C., Lemoine, J., Marnet, N., Levrel, A., Jubault, M., Manzanares-Dauleux, M. J., & Gravot, A. (2015). Camalexin contributes to the partial resistance of *Arabidopsis thaliana* to the biotrophic soilborne protist *Plasmodiophora brassicae*. *Frontiers in Plant Science*, *6*, 539. https://doi.org/10.3389/fpls.2015.00539

Leon-Reyes, A., Du, Y., Koornneef, A., Proietti, S., Körbes, A. P., Memelink, J., Pieterse, C. M. J., & Ritsema, T. (2010). Ethylene signaling renders the jasmonate response of *Arabidopsis* insensitive to future suppression by salicylic acid. *Molecular Plant-Microbe Interactions*, *23*(2), 187–197. https://doi.org/10.1094/mpmi-23-2-0187

Leon-reyes, A., Spoel, S. H., Lange, E. S. De, Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F. F., Welschen, R. A. M., Ritsema, T., & Pieterse, C. M. J. (2009). Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiology*, *149*, 1797–1809. https://doi.org/10.1104/pp.108.133926

Leung, J., & Giraudat, J. (1998). Abscisic acid signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*, 199–222. https://doi.org/10.1146/annurev.arplant.49.1.199

Li, C., He, X., Luo, X., Xu, L., Liu, L., Min, L., Jin, L., Zhu, L., & Zhang, X. (2014). Cotton WRKY1 mediates the plant defense-to- development transition during infection of cotton by *Verticillium dahliae* by activating *JASMONATE ZIM-DOMAIN1* expression. *Plant Physiology*, *166*, 2179–2194. https://doi.org/10.1104/pp.114.246694

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S., & Schulze-lefert, P. (2005). Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. *Science*, *310*(November), 1180–1184.

Liu, J., Moore, S., Chen, C., & Lindsey, K. (2017). Crosstalk complexities between auxin , cytokinin , and ethylene in *Arabidopsis* root development : From experiments to systems modeling , and back again. *Molecular Plant*, *10*, 1480–1496. https://doi.org/10.1016/j.molp.2017.11.002

References

Liu, L., Sonbol, F., Huot, B., Gu, Y., Withers, J., Mwimba, M., Yao, J., He, S. Y., & Dong, X. (2016). Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. *Nature Communications*, *7*, 1–10. https://doi.org/10.1038/ncomms13099

Maldonado, A. M., Doerner, P., Dixonk, R. A., Lamb, C. J., & Cameron, R. K. (2002). A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature*, *419*(6905), 399–403. https://doi.org/10.1038/nature00962

Mammarella, N. D., Cheng, Z., Qing Fu, Z., Daudi, A., Bolwell, G. P., Dong, X., & Ausubel, F. M. (2015). Apoplastic peroxidases are required for salicylic acid-mediated defense against *Pseudomonas syringae*. *Phytochemistry*, *112*, 110–121. https://doi.org/10.1016/j.phytochem.2014.07.010.Apoplastic

Mandaokar, A., & Browse, J. (2009). *MYB108* acts together with *MYB24* to regulate jasmonate-mediated stamen maturation in *Arabidopsis*. *Plant Physiology*, *149*, 851–862. https://doi.org/10.1104/pp.108.132597

Mao, J. L., Miao, Z. Q., Wang, Z., Yu, L. H., Cai, X. T., & Xiang, C. Bin. (2016). *Arabidopsis ERF1* mediates cross-talk between ethylene and auxin biosynthesis during primary root elongation by regulating *ASA1* expression. *PLoS Genetics*, *12*(1), 1–20. https://doi.org/10.1371/journal.pgen.1005760

Martin, F., Kohler, A., Murat, C., & Veneault-fourrey, C. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, *14*, 760–773. https://doi.org/10.1038/nrmicro.2016.149

Martínez-Medina, A., Appels, F. V. W., & van Wees, S. C. M. (2017). Impact of salicylic acid- and jasmonic acid-regulated defences on root colonization by *Trichoderma harzianum* T-78. *Plant Signaling and Behavior*, *12*(8 e1344504), 1–4. https://doi.org/10.1080/15592324.2017.1345404

Martínez-Medina, A., Fernandez, I., Lok, G. B., Pozo, M. J., Pieterse, C. M. J., & Van Wees, S. C. M. (2017). Shifting from priming of salicylic acid- to jasmonic acid-regulated defences by *Trichoderma* protects tomato against the root knot nematode *Meloidogyne incognita*. *New Phytologist*, *213*(3), 1363–1377. https://doi.org/10.1111/nph.14251

Martínez-Medina, A., Fernández, I., Sánchez-Guzmán, M. J., Jung, S. C., Pascual, J. A., & Pozo, M. J. (2013). Deciphering the hormonal signalling network behind the systemic resistance induced by Trichoderma harzianum in tomato. *Frontiers in Plant Science*, *4*(206), 1–12. https://doi.org/10.3389/fpls.2013.00206

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C. M. J., Pozo, M. J., Ton, J., van Dam, N. M., & Conrath, U. (2016). Recognizing Plant Defense Priming. *Trends in Plant Science*, *21*(10), 818–822. https://doi.org/10.1016/j.tplants.2016.07.009

Martínez-Medina, A., Van Wees, S. C. M., & Pieterse, C. M. J. (2017). Airborne signals from *Trichoderma* fungi stimulate iron uptake responses in roots resulting in priming of jasmonic acid dependent defences in shoots of *Arabidopsis thaliana* and *Solanum lycopersicum*. *Plant Cell and Environment*, *40*(11), 2691–2705. https://doi.org/10.1111/pce.13016

Mazzotta, S., & Kemmerling, B. (2011). Pattern recognition in plant innate immunity. *Journal of Plant Pathology*, *93*(1), 7–17. https://doi.org/10.4454/jpp.v93i1.268

References

McConn, M., Creelman, R. A., Bell, E., Mullet, J. E., & Browse, J. (1997). Jasmonate is essential for insect defense in *Arabidopsis*. *PNAS*, *94*(10), 5473–5477. https://doi.org/10.1073/pnas.94.10.5473

Mélida, H., Sopeña-Torres, S., Bacete, L., Garrido-Arandia, M., Jordá, L., López, G., Muñoz-Barrios, A., Pacios, L. F., & Molina, A. (2018). Non-branched β-1,3-glucan oligosaccharides trigger immune responses in *Arabidopsis*. *The Plant Journal*, *93*, 34–49. https://doi.org/10.1111/tpj.13755

Millet, Y. A., Danna, C. H., Clay, N. K., Songnuan, W., Simon, M. D., & Ausubel, F. M. (2010). Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *The Plant Cell*, *22*, 973–990. https://doi.org/10.1105/tpc.109.069658

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., & Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *PNAS*, *104*(49), 19613–19618. https://doi.org/10.1073/pnas.0705147104

Mortel, J. E. Van De, Vos, R. C. H. De, Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., Loon, J. J. A. Van, Dicke, M., & Raaijmakers, J. M. (2012). Metabolic and transcriptomic changes induced in root *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiology*, *160*, 2173–2188. https://doi.org/10.1104/pp.112.207324

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I., & Yoshida, S. (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant Journal*, *33*, 887–898. https://doi.org/10.1046/j.1365-313X.2003.01675.x

Naznin, H. A., Kiyohara, D., Kimura, M., Miyazawa, M., Shimizu, M., & Hyakumachi, M. (2014). Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in *Arabidopsis thaliana*. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086882

Nickstadt, A., Thomma, B. P. H. J., Feussner, I., Kangasjärvi, J., Zeier, J., Loeffler, C., Scheel, D., & Berger, S. (2004). The jasmonate-insensitive mutant *jin1* shows increased resistance to biotrophic as well as necrotrophic pathogens. *Molecular Plant Pathology*, *5*(5), 425–434. https://doi.org/10.1111/J.1364-3703.2004.00242.X

Nürnberger, T., Brunner, F., Kemmerling, B., & Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. *Immunological Reviews*, *198*(1), 249–266. https://doi.org/10.1111/j.0105-2896.2004.0119.x

Oosten, V. R. Van, Bodenhausen, N., Reymond, P., Pelt, J. A. Van, Loon, L. C. Van, Dicke, M., & Pieterse, C. M. J. (2008). Differential effectiveness of microbially induced resistance against herbivorous insects in *Arabidopsis*. *Molecular Plant-Microbe Interactions*, *21*(7), 919–930. https://doi.org/10.1094 /MPMI -21-7-0919

Ossier, O. M. R., Engelnik, K. A. I. W., Ahn, K. A. H., & Onas, U. L. L. A. B. (1999). The *Xanthomonas Hrp* type III system secretes proteins from plant and mammalian bacterial pathogens. *PNAS*, *96*, 9368–9373.

Palmer, C. M., Hindt, M. N., Schmidt, H., Clemens, S., & Guerinot, M. Lou. (2013). *MYB10* and *MYB72* are

required for growth under iron-limiting conditions. *PLoS Genetics*, *9*(11). https://doi.org/10.1371/journal.pgen.1003953

Pangesti, N., Pineda, A., Dicke, M., & van Loon, J. J. A. (2015a). Variation in plant-mediated interactions between rhizobacteria and caterpillars: Potential role of soil composition. *Plant Biology*, *17*(2), 474–483. https://doi.org/10.1111/plb.12265

Pangesti, N., Pineda, A., Dicke, M., & van Loon, J. J. A. (2015b). Variation in plant-mediated interactions between rhizobacteria and caterpillars: Potential role of soil composition. *Plant Biology*, *17*(2), 474–483. https://doi.org/10.1111/plb.12265

Pangesti, Nurmi, Reichelt, M., Mortel, J. E. Van De, Kapsomenou, E., Gershenzon, J., Loon, J. J. A. Van, Dicke, M., & Pineda, A. (2016). Jasmonic acid and ethylene signaling pathways regulate glucosinolate levels in plants during rhizobacteria-induced systemic resistance against a leaf-chewing herbivore. *Journal of Chemical Ecology*, *42*, 1212–1225. https://doi.org/10.1007/s10886-016-0787-7

Park, J., Halitschke, R., Kim, H. B., Baldwin, I. T., Feldmann, K. A., & Feyereisen, R. (2002). A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. *The Plant Journal*, *31*(1), 1–12.

Park, S., Kaimoyo, E., Kumar, D., Mosher, S., & Klessig, D. F. (2007). Methyl salicylate is a critical mobile Signal for plant systemic acquired resistance. *Science*, *318*, 113–116. https://doi.org/10.1126/science.1147113

Pauwels, L., Barbero, G. F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A. C., Chico, J. M., Bossche, R. Vanden, Sewell, J., Gil, E., Gárcia-Casado, G., Witters, E., Inze, D., Long, J. A., Jaeger, G. De, Solano, R., & Goossens, A. (2010). *NINJA* connects the co-repressor *TOPLESS* to jasmonate signalling. *Naure Letters*, *464*, 788–793. https://doi.org/10.1038/nature08854

Pauwels, L., & Goossens, A. (2011). The JAZ proteins : A crucial interface in the jasmonate signaling cascade. *The Plant Cell*, *23*, 3089–3100. https://doi.org/10.1105/tpc.111.089300

Pellegrin, C., Daguerre, Y., Ruytinx, J., Guinet, F., Kemppainen, M., dit Frey, N. F., Puech-Pagés, V., Hecker, A., Pardo, A. G., Martin, F. M., & Veneault-Fourrey, C. (2019). *Laccaria bicolor MiSSP8* is a small-secreted protein decisive for the establishment of the ectomycorrhizal symbiosis. *Environmental Microbiology*. https://doi.org/10.1111/1462-2920.14727

Piasecka, A., & Jedrzejczak-Rey, N. (2015). Tansley review: Secondary metabolites in plant innate immunity : conserved function of divergent chemicals. *New Phytologist*, *206*, 948–964. https://doi.org/10.1111/nph.13325

Pieterse, C. M. J., Does, D. Van Der, Zamioudis, C., Leon-reyes, A., & Wees, S. C. M. Van. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, *28*, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pieterse, C. M. J., Wees, S. C. M. Van, Hoffland, E., & Pelt, J. A. Van. (1996). Systemic resistance in *Arabidopsis*

induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *The Plant Cell*, *8*, 1225–1237.

Pieterse, C. M. J., Wees, S. C. M. Van, Pelt, J. A. Van, Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J., & Loon, L. C. Van. (1998). A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *The Plant Cell*, *10*, 1571–1580.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Wees, S. C. M. Van, & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, *52*, 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., & Melton, S. J. (2014). Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. *PNAS*, *111*(22), 8299–8304. https://doi.org/10.1073/pnas.1322671111

Plett, J. M., Kemppainen, M., Kale, S. D., Brun, A., Kohler, A., Tyler, B. M., Pardo, A. G., & Martin, F. (2011). Report a secreted effector protein of Laccaria bicolor is required for symbiosis development. *Current Biology*, *21*, 1197–1203. https://doi.org/10.1016/j.cub.2011.05.033

Pozo, M. J., Van Der Ent, S., Van Loon, L. C., & Pieterse, C. M. J. (2008). Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *New Phytologist*, *180*, 511–523.

Ren, D., Liu, Y., Yang, K.-Y., Han, L., Mao, G., Glazebrook, J., & Zhang, S. (2008). A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*. *PNAS*, *105*(14), 5638–5643. https://doi.org/10.1073/pnas.0711301105

Riefler, M., Novak, O., Strnad, M., & Schmu, T. (2006). *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth , leaf senescence , seed size , germination , root development , and cytokinin metabolism. *The Plant Cell*, *18*, 40–54. https://doi.org/10.1105/tpc.105.037796.1

Rieske, L. K., Rhoades, C. C., & Miller, S. P. (2003). Foliar chemistry and gypsy moth, *Lymantria dispar* (L .), herbivory on pure american chestnut, *Castanea dentata* (Fam : Fagaceae), and a disease-resistant hybrid. *Environmental Entomology*, *32*(2), 359–365.

Roe, A. D., Demidovich, M., & Dedes, J. (2018). Origins and history of laboratory insect stocks in a multispecies insect production facility, with the proposal of standardized nomenclature and designation of formal standard names. *Journal of Insect Science*, *18*(3), 1–9. https://doi.org/10.1093/jisesa/iey037

Romera, F. J., García, M. J., Lucena, C., Martínez-Medina, A., Aparicio, M. A., Ramos, J., Alcántara, E., Angulo, M., & Pérez-Vicente, R. (2019). Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. *Frontiers in Plant Science*, *10*(March), 1–17. https://doi.org/10.3389/fpls.2019.00287

Santino, A., Taurino, M., Domenico, S. De, Bonsegna, S., Poltronieri, P., Pastor, V., & Flors, V. (2013). Jasmonate signaling in plant development and defense response to multiple (a)biotic stresses. *Plant Cell Rep*, *32*, 1085–1098. https://doi.org/10.1007/s00299-013-1441-2

References

Schechter, L. M., Roberts, K. A., Jamir, Y., Alfano, J. R., & Collmer, A. (2004). *Pseudomonas syringae* Type III secretion system targeting signals and novel effectors studied with a Cya translocation reporter. *Journal of Bacteriology*, *186*(2), 543–555. https://doi.org/10.1128/JB.186.2.543

Schultheiss, H., Conrath, U., Beesley, A., Rohmann, P. F. W., & Beyer, S. F. (2019). The *Arabidopsis* non-host defence-associated coumarin scopoletin protects soybean from Asian soybean rust. *The Plant Journal*. https://doi.org/10.1111/tpj.14426

Seyfferth, C., & Tsuda, K. (2014). Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. *Frontiers in Plant Science*, *5*(697), 1–10. https://doi.org/10.3389/fpls.2014.00697

Shoresh, M., Yedidia, I., & Chet, I. (2004). Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology*, *95*(1), 76–84. https://doi.org/10.1094/PHYTO-95-0076

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed.).

Spoel, S. H., Koornneef, A., Claessens, S. M. C., Korzelius, J., Van Pelt, Johan, A., Mueller, M. J., Buchala, A. J., Métraux, J.-P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., & Pieterse, C. M. J. (2003). *NPR1* modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *The Plant Cell*, *15*, 760–770. https://doi.org/10.1105/tpc.009159

Stacey, G., Liang, Y., Katalin, T., Cao, Y., Tanaka, K., Espinoza, C., & Stacey, G. (2014). Lipochitooligosaccharide recognition : an ancient story. *New Phytologist*, *204*, 289–296. https://doi.org/10.1111/nph.12898

Stringlis, I. A., Proietti, S., Hickman, R., Verk, M. C. Van, Zamioudis, C., & Pieterse, C. M. J. (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *The Plant Journal*, *93*, 166–180. https://doi.org/10.1111/tpj.13741

Stringlis, I. A., Yu, K., Feussner, K., Jonge, R. De, Bentum, S. Van, Verk, M. C. Van, Berendsen, R. L., Bakker, P. A. H. M., Feussner, I., & Pieterse, C. M. J. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *PNAS*, *115*(12), E5213–E5222. https://doi.org/10.1073/pnas.1722335115

Swiderski, M. R., Birker, D., & Jones, J. D. G. (2009). The TIR domain of TIR-NB-LRR resistance proteins is a signaling domain involved in cell death induction. *Molecular Plant-Microbe Interactions*, *22*(2), 157–165. https://doi.org/10.1094/mpmi-22-2-0157

Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., & Dong, X. (2013). Plant immunity requires conformational charges of *NPR1* via S-nitrosylation and thioredoxins. *Science*, *321*(5891), 1–9. https://doi.org/10.1126/science.1156970.Plant

Tanaka, K., Choi, J., Cao, Y., & Stacey, G. (2014). Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Frontiers in Plant Science*, *5*(446), 1–9.

https://doi.org/10.3389/fpls.2014.00446

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S. Y., Howe, G. A., & Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. *Nature*, *448*(7154), 661–665. https://doi.org/10.1038/nature05960

Ton, J., Ent, S. Van Der, Hulten, M. Van, & Pozo, M. (2009). Priming as a mechanism behind induced resistance against pathogens, insects and abiotic stress. *IOBC/Wprs Bull*, *44*(May 2014), 3–13. Retrieved from http://www.bio.uu.nl/pmi/publications/PDF/2009/IOBC-Ton-2009.pdf

Truman, W., Bennett, M. H., Kubigsteltig, I., Turnbull, C., & Grant, M. (2007). *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *PNAS*, *104*(3), 1075–1080.

Van der Does, D., Leon-reyes, A., Koomneef, A., Van Verk, M. C., Rodenburg, N., Pauwels, L., Goossens, A., Körbes, A. P., Memelink, J., Ritsema, T., Van Wees, S. C. M., & Pieterse, C. M. J. (2013). Salicylic acid suppresses jasmonic acid signaling downstream of *SCFCOI1-JAZ* by targeting GCC promoter motifs via transcription factor *ORA59*. *The Plant Cell*, *25*, 744–761. https://doi.org/10.1105/tpc.112.108548

Van Der Ent, S., Verhagen, B. W. M., Van Doorn, R., Bakker, D., Verlaan, M. G., Pel, M. J. C., Joosten, R. G., Proveniers, M. C. G., Loon, L. C. Van, Ton, J., & Pieterse, C. M. J. (2008). *MYB72* is required in early signaling steps of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Plant Physiology*, *146*, 1293–1304. https://doi.org/10.1104/pp.107.113829

van der Hoorn, R. A. L., & Kamoun, S. (2008). From Guard to Decoy: A New Model for Perception of Plant Pathogen Effectors. *The Plant Cell*, *20*, 2009–2017. https://doi.org/10.1105/tpc.108.060194

Van Etten, H. D., Mansfield, J. W., Bailey, J. A., & Farmer, E. E. (2007). Two classes of plant antibiotics: Phytoalexins versus "Phytoanticipins." *The Plant Cell*, *6*(9), 1191–1192. https://doi.org/10.2307/3869817

van Peer, R., Niemann, G. J., & Schippers, B. (1991). Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology*, *81*(7), 728–734. https://doi.org/10.1094/Phyto-81-728

van Peer, R., & Schippers, B. (1992). Lipopolysaccharides of plant-growth promoting Pseudomonas sp . strain WCS417r induce resistance in carnation to Fusarium wilt. *Neth. J. Pl. Path.*, *98*, 129–139.

Ver, E., Themaat, L. Van, Maddula, R. K., Bednarek, P., Pis, M., & Schulze-lefert, P. (2011). Conservation and clade-specific diversification of pathogen-inducible tryptophan and indole glucosinolate metabolism in *Arabidopsis thaliana* relatives. *New Phytologist*, *192*, 713–726. https://doi.org/10.1111/j.1469-8137.2011.03824.x

Verhage, A., Van Wees, S. C. M., & Pieterse, C. M. J. (2010). Plant immunity : It's the hormones talking, but what do they say? *Plant Physiology*, *154*, 536–540. https://doi.org/10.1104/pp.110.161570

Verhage, A., Vlaardingerbroek, I., Raaymakers, C., Dam, N. M. Van, Dicke, M., Van Wees, S. C. M., & Pieterse, C. M. J. (2011). Rewiring of the jasmonate signaling pathway in *Arabidopsis* during insect herbivory. *Frontiers in Plant Science*, *2*(47), 1–12. https://doi.org/10.3389/fpls.2011.00047

References

Verhagen, B. W. M., Glazebrook, J., Zhu, T., Chang, H., Loon, L. C. Van, & Pieterse, C. M. J. (2004). The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis. *MPMI*, *17*(8), 895–908.

Vernooij, B., Friedrichya, L., Reist, R., Kolditzjawhar, R., Ward, E., Uknes, S., Kessmann, H., & Ryals, J. (1994). Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *The Plant Cell*, *6*, 959–965.

Vishwanathan, K., Zienkiewicz, K., Liu, Y., Janz, D., Feussner, I., Polle, A., & Haney, C. H. (2020). Ectomycorrhizal fungi induce systemic resistance against insects on a non-mycorrhizal plant in a CERK1-dependent manner. *New Phytologist*. https://doi.org/10.1111/nph.16715

Vlot, A. C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, *47*(1), 177–206. https://doi.org/10.1146/annurev.phyto.050908.135202

Voges, M. J. E. E. E., Bai, Y., Schulze-lefert, P., & Sattely, E. S. (2019). Plant-derived coumarins shape the composition of an Arabidopsis synthetic root microbiome. *PNAS*, *116*(25), 12558–12565. https://doi.org/10.1073/pnas.1820691116

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V., Katagiri, F., & Glazebrook, J. (2011). *CBP60g* and *SARD1* play partially redundant critical roles in salicylic acid signaling. *The Plant Journal*, *67*, 1029–1041. https://doi.org/10.1111/j.1365-313X.2011.04655.x

Wei, G., Kloepper, J. W., & Tuzun, S. (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant-growth promoting rhizobacteria under field conditions. *Phytopathology*, *86*(2), 221–224.

Widemann, E., Miesch, L., Lugan, R., Holder, E., Heinrich, C., Aubert, Y., Miesch, M., Pinot, F., & Heitz, T. (2013). The amidohydrolases *IAR3* and *ILL6* contribute to jasmonoyl-isoleucine hormone turnover and generate 12-hydroxyjasmonic acid upon wounding in *Arabidopsis*. *The Journal of Biological Chemistry*, *288*(44), 31701–31714. https://doi.org/10.1074/jbc.M113.499228

Wildermuth, M. C., Dewdney, J., Wu, G., & Ausubel, F. M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, *414*(6863), 562–565. https://doi.org/10.1038/35107108

Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M. N., Maier, A., & Schwechheimer, C. (2007). The DELLA Domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A Gibberellin receptor of *Arabidopsis*. *The Plant Cell*, *19*, 1209–1220. https://doi.org/10.1105/tpc.107.051441

Woodward, A. W., & Bartel, B. (2005). Auxin: Regulation, action, and interaction. *Annals of Botany*, *95*, 707–735. https://doi.org/10.1093/aob/mci083

Wu, S., Shan, L., & He, P. (2014). Microbial signature-triggered plant defense responses and early signaling mechanisms. *Plant Science*, *0*, 118–126. https://doi.org/10.1016/j.plantsci.2014.03.001

Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., De Luca, V., & Després, C. (2012). The *Arabidopsis NPR1* protein is a receptor for the plant defense hormone salicylic acid. *Cell Reports*, *1*(6),

639–647. https://doi.org/10.1016/j.celrep.2012.05.008

Xue, M., & Yi, H. (2018). Enhanced *Arabidopsis* disease resistance against *Botrytis cinerea* induced by sulfur dioxide. *Ecotoxicology and Environmental Safety*, *147*, 523–529. https://doi.org/10.1016/j.ecoenv.2017.09.011

Yan, J., Zhang, C., Gu, M., Bai, Z., Zhang, W., Qi, T., Cheng, Z., & Peng, W. (2009). The *Arabidopsis CORONATINE INSENSITIVE1* protein is a jasmonate receptor. *The Plant Cell*, *21*, 2220–2236. https://doi.org/10.1105/tpc.109.065730

Yu, K., Liu, Y., Tichelaar, R., Savant, N., Lagendijk, E., van Kuijk, S. J. L., Stringlis, I. A., van Dijken, A. J. H., Pieterse, C. M. J., Bakker, P. A. H. M., Haney, C. H., & Berendsen, R. L. (2019). Rhizosphere-associated *Pseudomonas* suppress local root immune responses by Gluconic Acid- mediated lowering of environmental pH. *Current Biology*, *29*, 1–8. https://doi.org/10.1016/j.cub.2019.09.015

Zamioudis, C., Hanson, J., & Pieterse, M. J. (2014). b -Glucosidase *BGLU42* is a *MYB72*-dependent key regulator of rhizobacteria-induced systemic resistance and modulates iron deficiency responses in *Arabidopsis* roots. *New Phytologist*, *204*, 368–379. https://doi.org/10.1111/nph.12980

Zamioudis, C., Mastranesti, P., Dhonukshe, P., Blilou, I., & Pieterse, C. M. J. (2013). Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiology*, *162*, 304–318. https://doi.org/10.1104/pp.112.212597

Zhang, G.-B., Yi, H.-Y., & Gong, J.-M. (2014). The *Arabidopsis* ethylene/jasmonic acid-*NRT* signaling module coordinates nitrate reallocation and the trade-off between growth and environmental adaptation. *The Plant Cell*, *26*(10), 3984–3998. https://doi.org/10.1105/tpc.114.129296

Zhang, X., Dong, W., Sun, J., Feng, F., Deng, Y., He, Z., Oldroyd, G. E. D., & Wang, E. (2015). The receptor kinase *CERK1* has dual functions in symbiosis and immunity signalling. *Plant Journal*, *81*, 258–267. https://doi.org/10.1111/tpj.12723

Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y. T., He, J., Gao, M., Xu, F., Li, Y., Zhu, Z., Li, X., & Zhang, Y. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *PNAS*, *107*(42), 18220–18225. https://doi.org/10.1073/pnas.1005225107

Zhang, Yuelin, Cheng, Y. T., Qu, N., Zhao, Q., Bi, D., & Li, X. (2006). Negative regulation of defense responses in *Arabidopsis* by two *NPR1* paralogs. *The Plant Journal*, *48*(5), 647–656. https://doi.org/10.1111/j.1365-313X.2006.02903.x

Zhang, Yuelin, Tessaro, M. J., Lassner, M., & Li, X. (2003). Knockout analysis of *Arabidopsis* Transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. *Plant Cell*, *15*, 2647–2653. https://doi.org/10.1105/tpc.014894.Nawrath

Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology*, *12*(4), 414–420. https://doi.org/10.1016/j.pbi.2009.06.003

References

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., & Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor *EFR* restricts agrobacterium-mediated transformation. *Cell*, *125*(4), 749–760. https://doi.org/10.1016/j.cell.2006.03.037

6. APPENDIX

6.1. Supplementary tables

Supplementary table 6.1.1. List of defense marker genes and their primer sequences used for gene expression analyses (Vishwanathan et al., 2020)**.**

Candidate gene	Functionality	Forward and reverse primers	Reference
EIF4A	House-keeping gene	5′-GCAGTCTCTTCGTGCTGACA-3′ and 5′ TGTCATAGATCTGGTCCTTGAA-3′	(Mammarella et al., 2015)
PR1	SA pathway	5′-ACACCTCACTTTGGCACATC-3′ and 5′-GAGTGTGGAAAACGCAAAGA-3′	(Mammarella et al., 2015)
PR2	SA pathway	5′-CCTTCTCGGTGATCCATTCT-3′ and 5′-AGTGTGGAAAACGCAAAGACT-3′	(Mammarella et al., 2015)
GST6	SA pathway	5′-CCATCTTCAAAGGCTGGAAC-3′ and 5′-TCGAGCTCAAAGATGGTGAA-3’	(Mammarella et al., 2015)
MYC2	JA pathway	5’-AGATAAAACCGCCGGAGAAT-3’ and 5’-TACCGTTTGCTGGCTTTCTT-3’	(Haney et al., 2017)
VSP1	JA pathway	5′-CTCAAGCCAAACGGATCG-3’and 5′-TTCCCAACGATGTTGTACCC-3’	(Mammarella et al., 2015)
VSP2	JA pathway	5’-TCAGTGACCGTTGGAAGTTGTG-3’ and 5’-GTTCGAACCATTAGGCTTCAATATG-3’	(N. Pangesti et al., 2015a)
ERF1	JA/Et pathway	5’-ATTCTTTCTCATCCTCTTCTTCT-3’ and 5’-CGAATCTCTTATCTCCGCCG-3’	(Mao et al., 2016)
ORA59	JA/Et pathway	5’– AAGGGATAAGAGTGTGGCTTGGGA-3’ and 5’- CTTTCAAA-GCGAAAGCCGCCTGAT-3’	(G.-B. Zhang et al., 2014)
PR4	JA/Et pathway	5’-GAGAATAGTGGACCAATGCAG-3’ and 5’-GTAGACCGATCGATATTGACCT-3’	(Xue & Yi, 2018)
PDF1.2	JA/Et pathway	5’-AATGAGCTCTCATGGCTAAGTTTGCTTCC-3’ and 5’-AATCCATGGAATACACACGATTTAGCACC-3’	(Naznin et al., 2014)
MYB51	MAMP induced signaling	5′-CTTGTGTGTAACTGGATCAA-3’ and 5′-ACAAATGGTCTGCTATAGCT-3′	(Mammarella et al., 2015)
CYP71A13	MAMP induced signaling	5’-GCCCCGGGATA ATCTTG CT-3’ and 5’-TGTTGCATAGCATAACAAGGTGA-3’	(Lemarié et al., 2015)

Appendix

Supplementary table 6.1.2. Mass transitions and corresponding conditions used for determination of phytohormone and secondary metabolite concentrations in Col-0 leaves (Vishwanathan et al., 2020)**.**

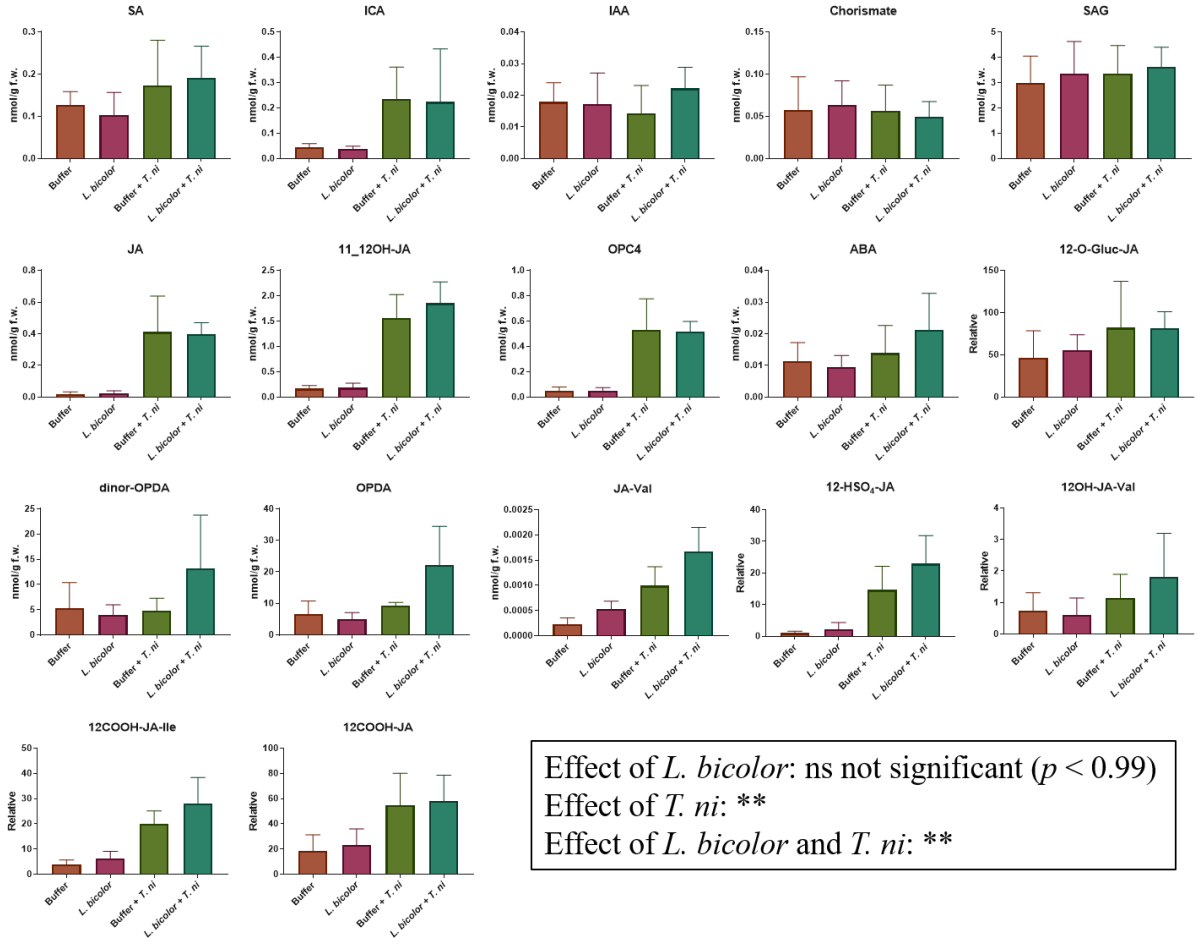
MRM Transitions		Analyte	DP [declustering potential]	EP [entrance potential]	CE [collision energy]
Q1	Q3				
137	93	SA	-25	-6	-20
141	97	D4-SA	-25	-6	-22
153	109	2,3-DHBA	-25	-9	-18
160	116	ICA	-40	-6.5	-22
162	58	RA	-15	-6	-14
174	130	IAA	-35	-9	-14
179	135	D5-IAA	-35	-9	-14
207	137	Chorismat-H2O	-20	-9	-16
209	59	JA	-30	-4.5	-24
214	62	D5-JA	-35	-8.5	-24
225	59	11,12-OH-JA	-35	-9	-28
237	165	OPC4	-45	-6	-24
263	153	ABA	-35	-4	-14
263	165	dinor-oPDA	-40	-5	-20
293	179	D6-ABA	-80	-10	-42
296	170.2	D5-oPDA	-65	-4	-28
299	137	SAG	-30	-4	-18
305	97	12-HSO_4-JA	-30	-4	-32
308	116	JA-Val	-45	-5	-28
322	130	JA-Ile/Leu	-45	-5	-28
325	133	D4-JA-Leu	-80	-4	-30
324	116	12OH-JA-Val	-45	-10	-30
338	130	12OH-JA-Ile	-45	-10	-30
352	130	12COOH-JA-Ile	-45	-10	-30
387	59	12-O-Gluc-JA	-85	-9	-59
425	263	ABA-GE	-30	-10	-16
447	97	Glucobrassicin	-45	-7	-40
477	97	4-M-glucobrassicin	-55	-5	-38

Supplementary table 6.1.3. GUS expression analyses of MAMP reporter lines after treatment with MAMPs, live and heat-killed *L. bicolor*.

CYP71A12p-, *MYB51p-*, *WRKY11p*-GUS seedlings were treated with flg22, chitin, live and heat-killed *L. bicolor* and later stained with GUS solution. Except flg22, other treatments did not induce defense expression in the roots. n = 3 experiments with 12 seedlings/treatment. (+ presence of GUS expression, - absence of GUS expression)

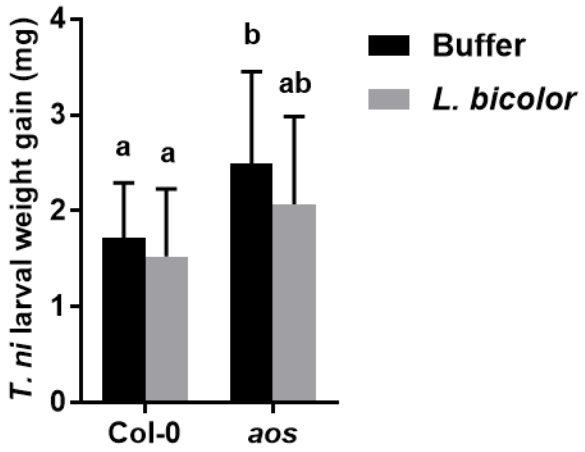
***CYP71A12p-, WRKY11p-, MYB51p*-GUS**	**GUS expression**
10 mM $MgSO_4$	-
flg22	+
Chitin	-
L. bicolor (OD_{600} = 0.1)	-
L. bicolor (OD_{600} = 0.1) + flg22	+
L. bicolor (OD_{600} = 0.1) + chitin	-
L. bicolor (OD_{600} = 0.2)	-
L. bicolor (OD_{600} = 0.2) + flg22	+
L. bicolor (OD_{600} = 0.2) + chitin	-
Heat killed *L. bicolor* (OD_{600} = 1)	-
Heat killed *L. bicolor* (OD_{600} = 1) + flg22	+
Heat killed *L. bicolor* (OD_{600} = 1) + chitin	-

6.2. Supplementary figures



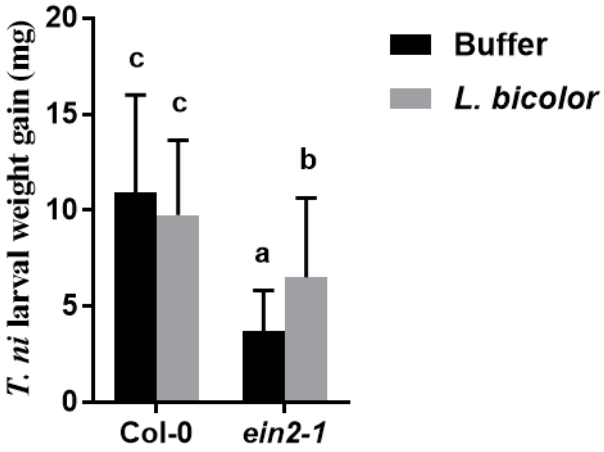
Supplementary figure 6.2.1. Accumulation of phytohormones in Col-0 leaves in response to *L. bicolor* inoculation and *T. ni* feeding.

Phytohormone levels in Col-0 leaves were quantified after root inoculation with/without *L. bicolor* and with/without foliar damage by *T. ni*. Data show absolute mean or concentrations of phytohormones and their derivatives relative to the endogenous concentration in the buffer treated plants. n = 6 experiments with 8 plants/treatment. Two-way ANOVA with Fisher's LSD test was performed for determing statistical significance (** $p < 0.01$, ns not significant).



Supplementary figure 6.2.2. *T. ni* feeding assay with JA biosynthesis mutant, *aos*, treated with and without *L. bicolor*.

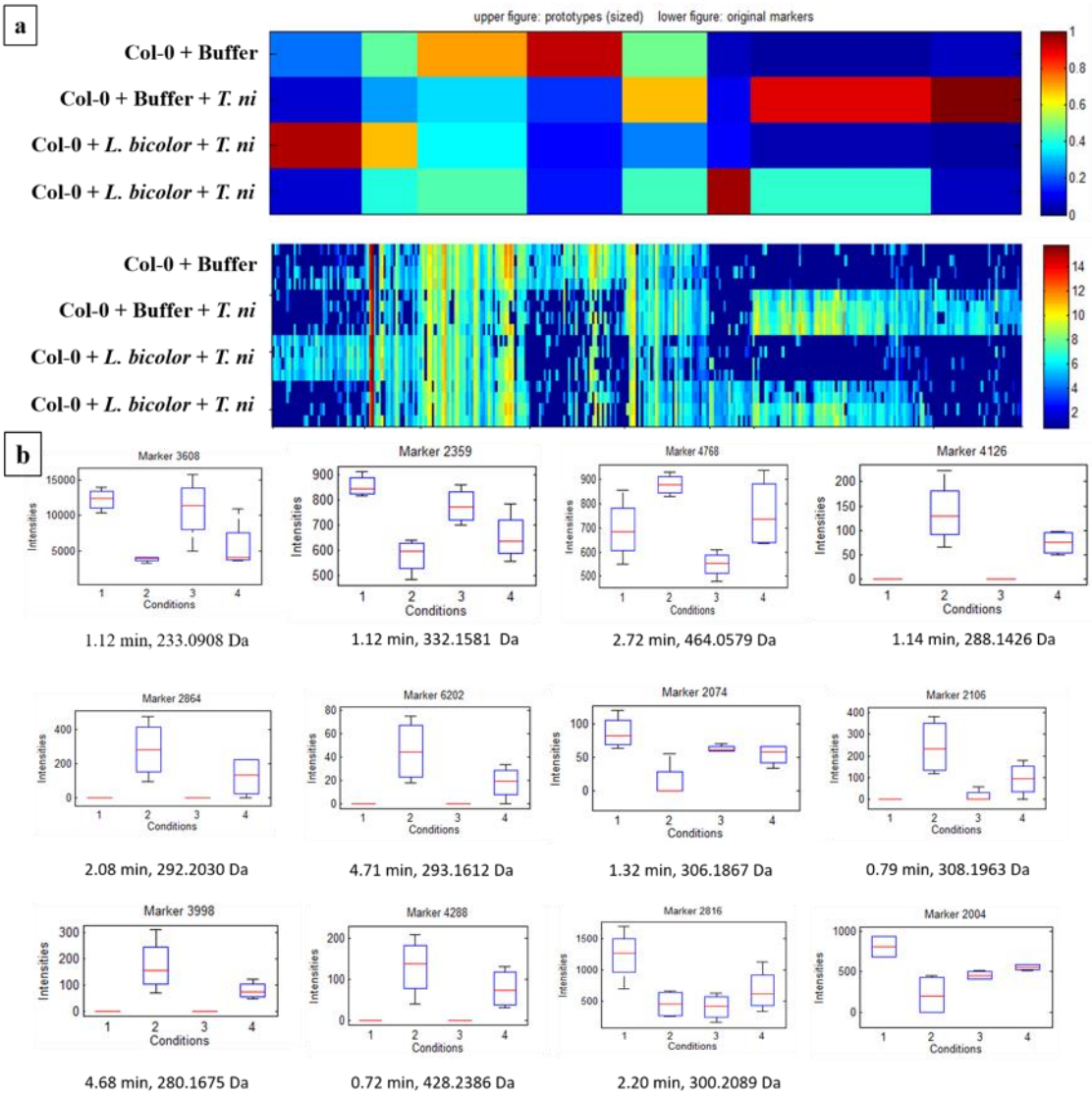
aos mutant roots were inoculated with buffer or *L. bicolor* and leaves were fed by *T. ni*. Data show mean of at-least 20 *T. ni* larvae/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.



Supplementary figure 6.2.3. Influence of *L. bicolor* treatment and subsequent larval feeding on *ein2-1* mutant.

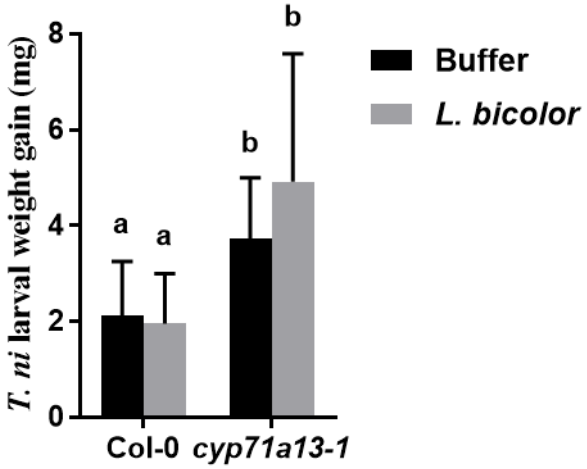
Roots of ethylene signalling mutant, *ein2-1* was inoculated with buffer or *L. bicolor* and leaves were fed by *T. ni*. Data show mean of at-least 20 *T. ni* larvae/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.

Appendix



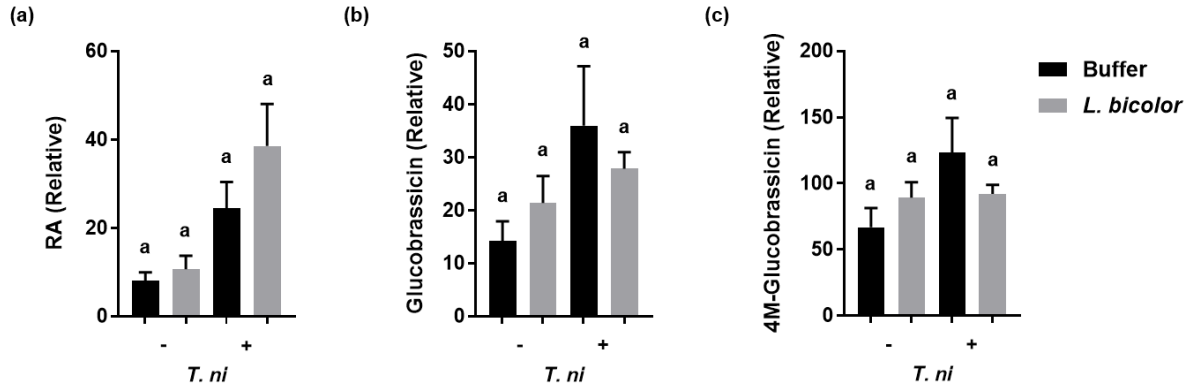
Supplementary figure 6.2.4. Non-targeted metabolite analysis of Col-0 leaves in response to *L. bicolor* root inoculation and caterpillar feeding.

Col-0 roots were inoculated with buffer or *L. bicolor* and leaves were fed by *T. ni* larvae. Leaves were harvested after 24 hours of *T. ni* feeding. Metabolome analysis of the samples was performed by Dr. Kirstin Feussner (Department of Plant Biochemistry, University of Goettingen). Data show (a) 1D-SOM representation of 309 feature ($p < 0.01$) (b) Selected features with tentative identities (obtained by data base search with exact mass information). n = 4 experiments with 8 leaves/treatment.



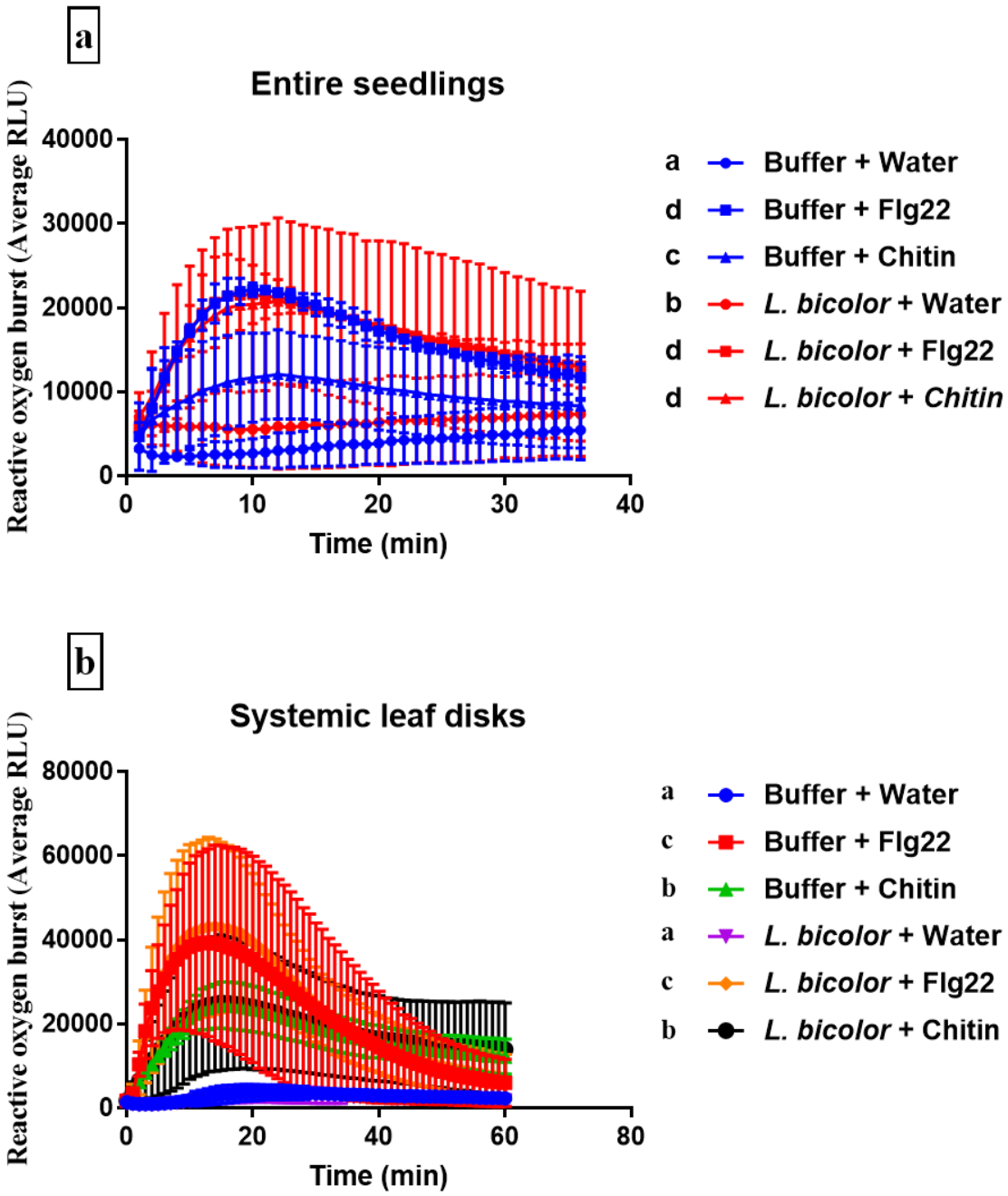
Supplementary figure 6.2.5. Effect of *L. bicolor* treatment on ISR against *T. ni* in camalexin biosynthesis mutant, *cyp71a13-1*.

cyp71a13-1 mutant roots were inoculated with buffer or *L. bicolor* and leaves were fed by *T. ni*. Data show mean of at-least 20 *T. ni* larvae/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.



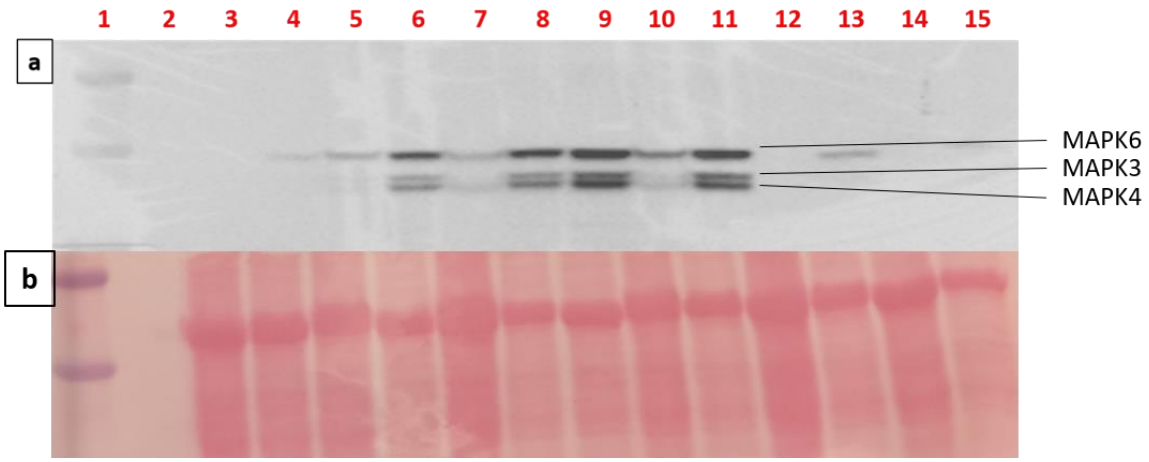
Supplementary figure 6.2.6. Influence of *L. bicolor* treatment and larval feeding on secondary metabolite concentrations in Col-0 leaves.

Leaf samples were harvested from Col-0 plants, inoculated with/without *L. bicolor* and exposed with/without *T. ni*. Targeted metabolite analysis was performed to quantify the accumulation of secondary metabolites in the samples as described in chapter 2.6.2. Data show concentrations of (a) Raphanusamic acid (RA) (b) Glucobrassicin (c) 4M-Glucobrassicin relative to the endogenous levels in buffer treated plants. n = 4 experiments with 8 leaves/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.



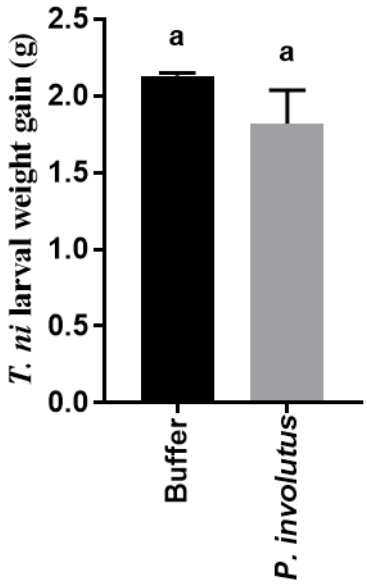
Supplementary figure 6.2.7. ROS burst responses in Col-0 seedlings to *L. bicolor* and elicitor treatments.

Experiment was performed using (a) entire seedlings (b) systemic leaf disks as described in chapter 2.9. Data show averages of RLU values of at least 2 independent experiments (n = 2) with 8 seedlings or leaf disks/treatment. Statistical analysis was performed as described in chapter 2.11 and error bars indicate the standard error. Different letters near the legends denote significant differences at $p < 0.05$.



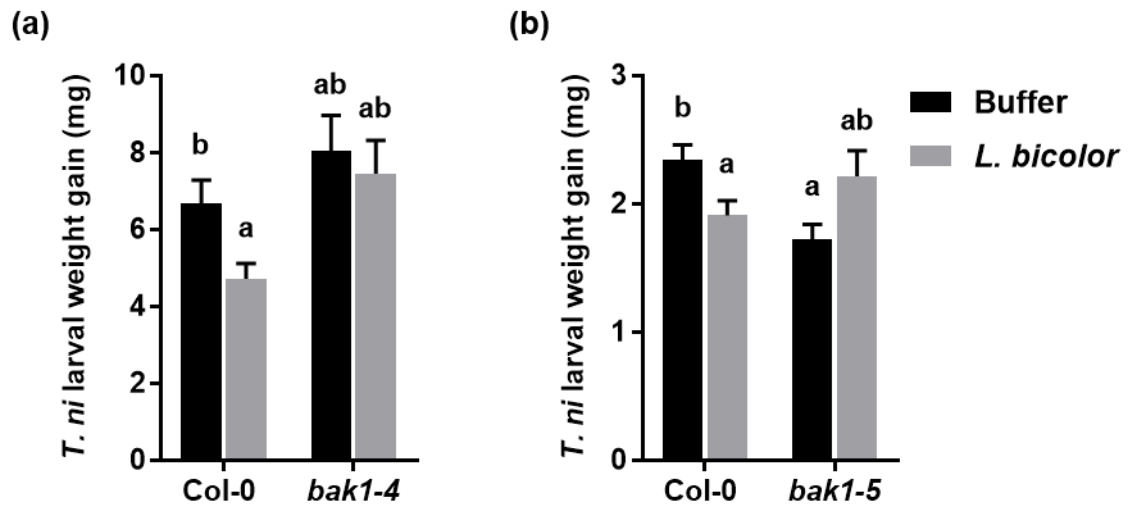
Supplementary figure 6.2.8. *L. bicolor* induced MAPK activation in *cerk1-2* mutant.

(a) Phosphorylation of MAPK6, MAPK3 and MAPK4 was analyzed in Col-0 and *cerk1-2* entire seedlings after treatment with chitin, flg22, live and dead *L. bicolor* for 15 min. Lanes: [1] marker, [2] empty [3] Col-0 + buffer, [4] Col-0 + *L. bicolor*, [5] Col-0 + dead *L. bicolor*, [6] Col-0 + chitin, [7] Col-0 + *L. bicolor* + chitin, [8] Col-0 + dead *L. bicolor* + chitin, [9] Col-0 + flg22, [10] Col-0 + *L. bicolor* + flg22, [11] Col-0 + dead *L. bicolor* + flg22, [12] *cerk1-2* + buffer, [13] *cerk1-2* + *L. bicolor*, [14] *cerk1-2* + chitin, [15] *cerk1-2* + *L. bicolor* + chitin. n = 2 experiments with 4 seedlings/treatment. (b) Proteins separated and blotted in PVDF membrane were stained with Ponceau reagent.



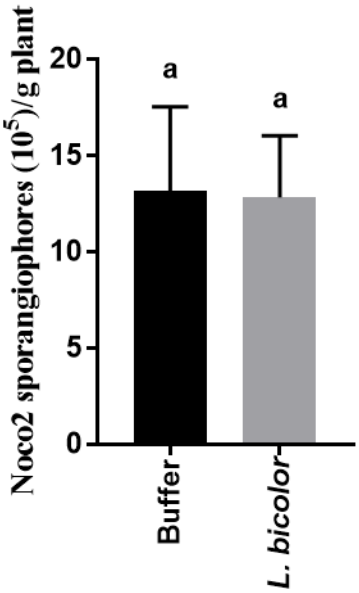
Supplementary figure 6.2.9. Effect of *Paxillus involutus* on ISR against *T. ni* in Col-0.

Col-0 roots were inoculated with *P. involutus* and later challenged with *T. ni*. Data show means of *T. ni* caterpillar weight gains from two independent experiments (n = 2) with at least 20 caterpillars/treatment. ANOVA was performed to determine statistical significance and different letters indicate significant differences at $p < 0.05$.



Supplementary figure 6.2.10. *L. bicolor*-triggered ISR depends on BAK1.

BAK1 mutants (a) *bak1-4* and (b) *bak1-5* were root inoculated with buffer or *L. bicolor* and leaves were fed by *T. ni*. Unlike Col-0, *L. bicolor* treatment did not negatively influence caterpillar weight gain in *bak1-4* and *bak1-5*. n = 2 experiments for *bak1-4* and n = 3 experiments for *bak1-5* with at least 20 caterpillars/treatment. Two-way ANOVA with Tukey's HSD test was performed for determining statistical significance with different letters indicating significant differences between treatments at $p < 0.05$ and the error bars representing SE.



Supplementary figure 6.2.11. Challenging Col-0-*L. bicolor* system with Noco2.

Col-0 roots were inoculated with buffer or *L. bicolor* and leaves were infected with Noco2. Data show means of Noco2 sporangiospores from n = 4 independent experiments with at least 90 Col-0 seedlings/treatment. Student's t-test was performed to determine statistical significance at $p < 0.05$.